trans2care

Transregional Network for Innovation and Technology Transfer to Improve Health Care
Rete transregionale per l'innovazione ed il trasferimento tecnologico per il miglioramento della sanità
Transregionalno omrežje za inovacijo in prenos tehnološkega znanja za izboljšanje zdravstva

# The Partners and the Objectives of Trans2Care, an Italy-Slovenia cross-border network of science and healthcare institutions

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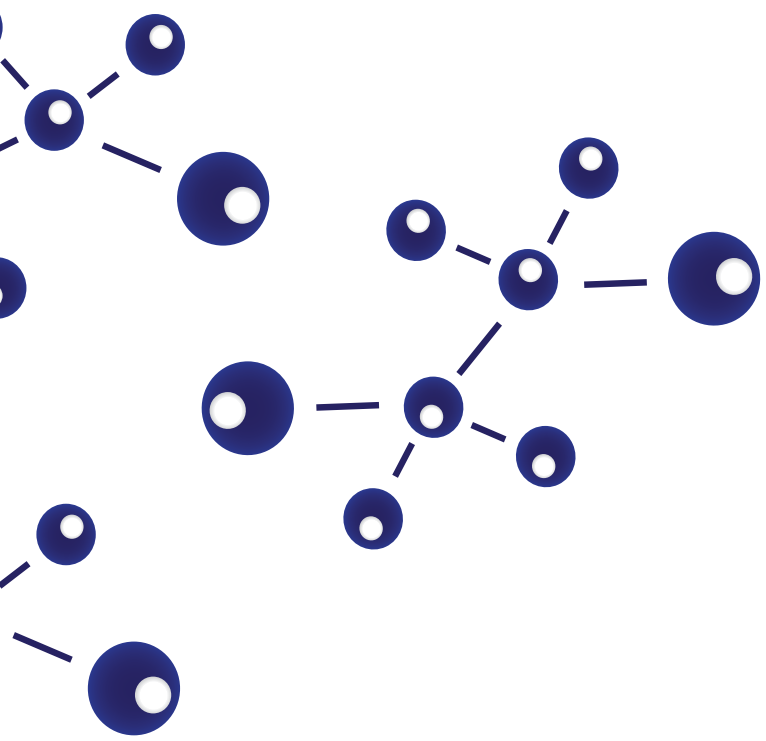

trans2care

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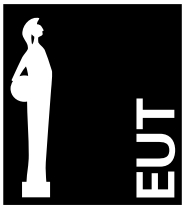

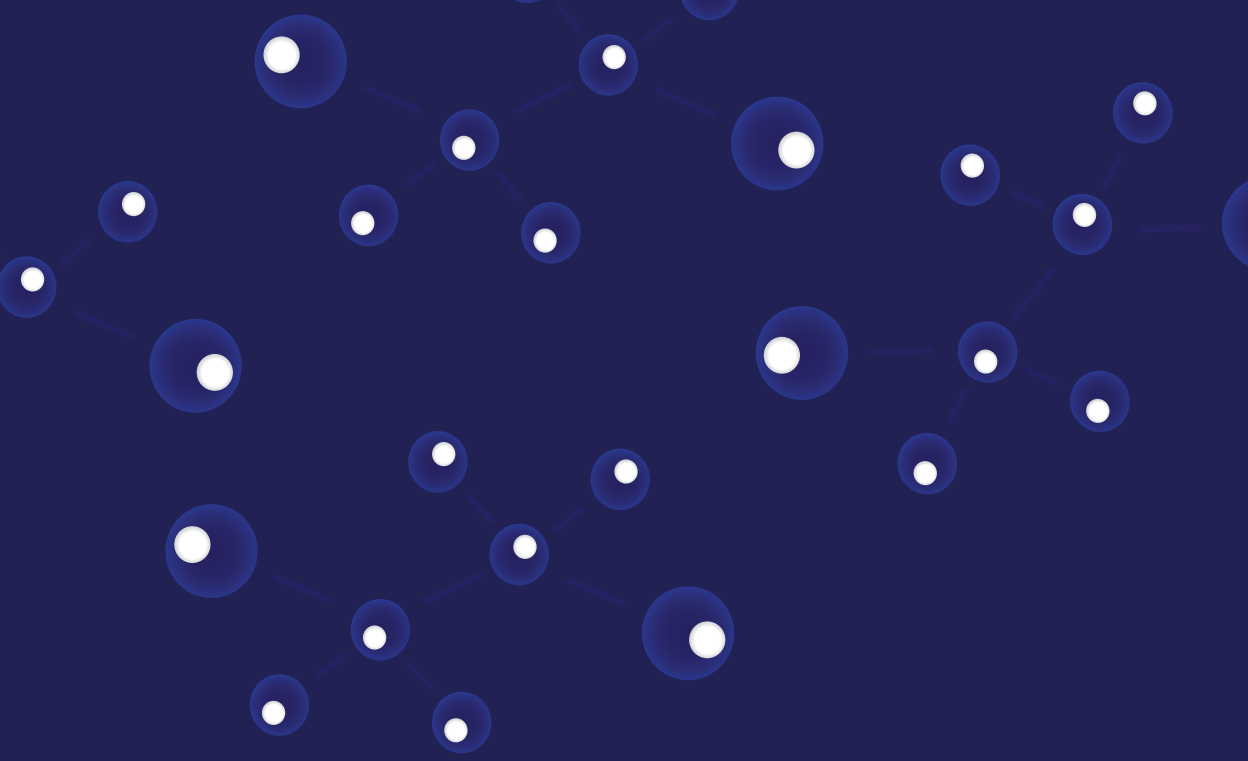

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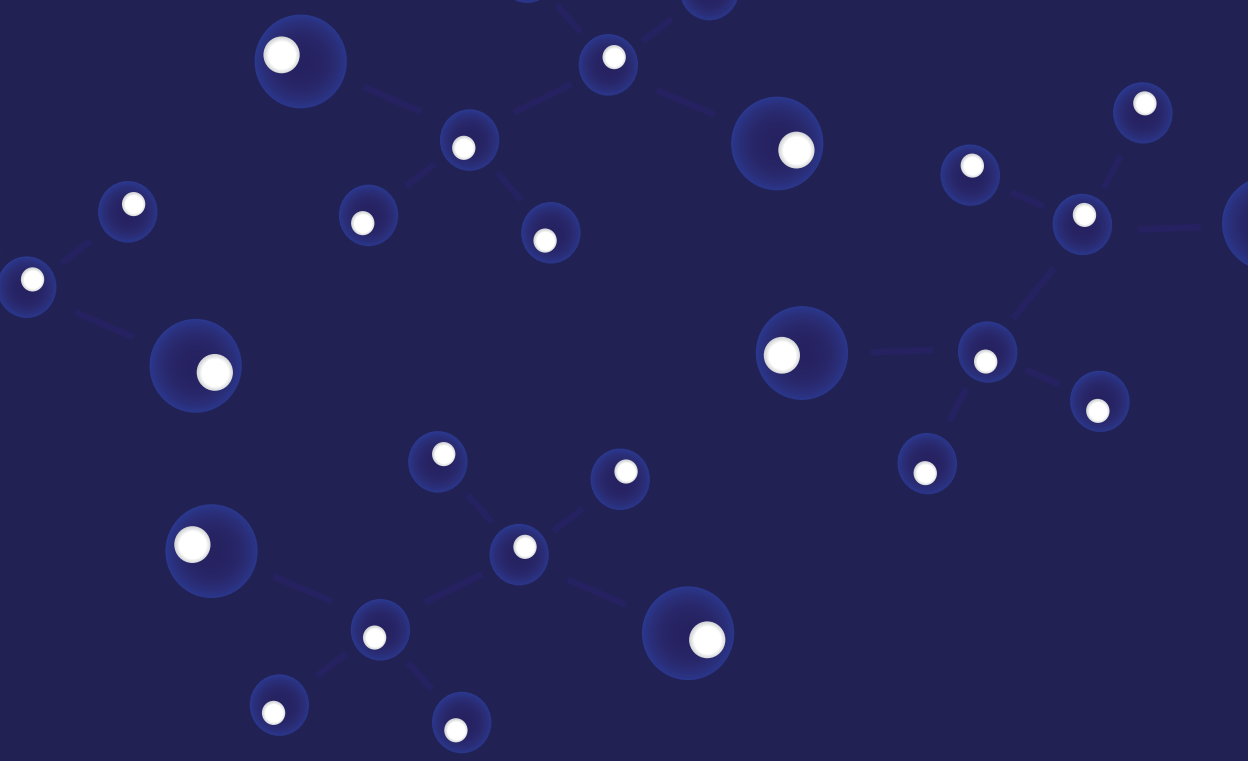


# Preface

Trans2Care project is an operation of the Cross-Border Cooperation Programme Italy-Slovenia 2007-2013, funded by the European Regional Development Fund and some national funds.

This project, started on 1st April 2011, will be implemented through a period of three and half years by a consortium of 13 Partners, lead by the University of Trieste. It aims at translating results of their biomedical research into innovative products and services to improve health.

The kick-off meeting of Trans2Care took place in Trieste on 21st-22nd November 2011. The venue was the Narodni Dom, i.e. the Trieste National Hall, a multimodal building of the Slovene minority in the city, which formerly included the Slovene theatre and a hotel (Hotel Balkan).

Narodni Dom was built on 1904 on the plan of Max Fabiani, one of the greatest Mittel-European architects. Burnt by the Italian fascists in 1920, it hosted later the Hotel Regina. Ultimately restored in 1990, it now hosts the Slovenski informativni center (Slovene information center) and the Advanced School of Modern Languages for Translators and Interpreters of the University of Trieste.

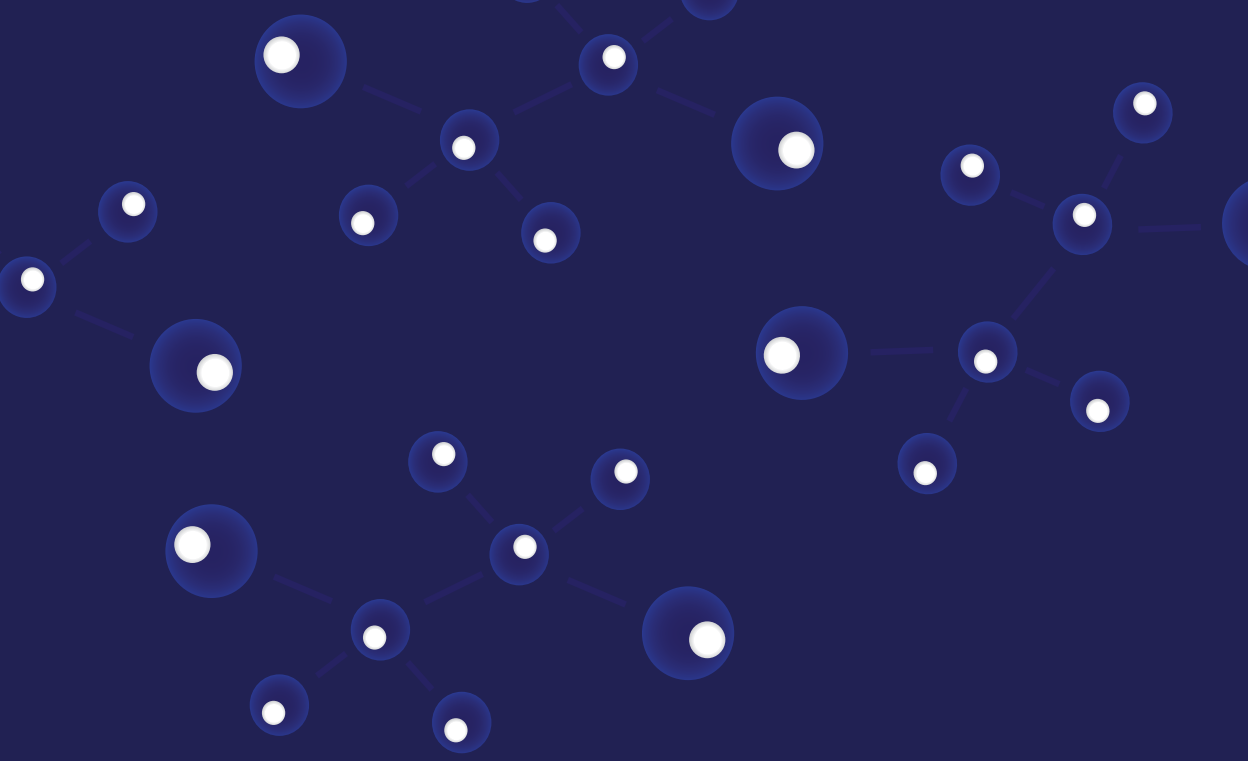
On 21st November, the project was presented to the Partners, the academic and public authorities, and the general public, as reported in Section I.

On 22nd November, the Partners had an internal meeting, aimed at laying down the scientific contents and objectives of their collaboration, described in the 21 chapters of Section II.

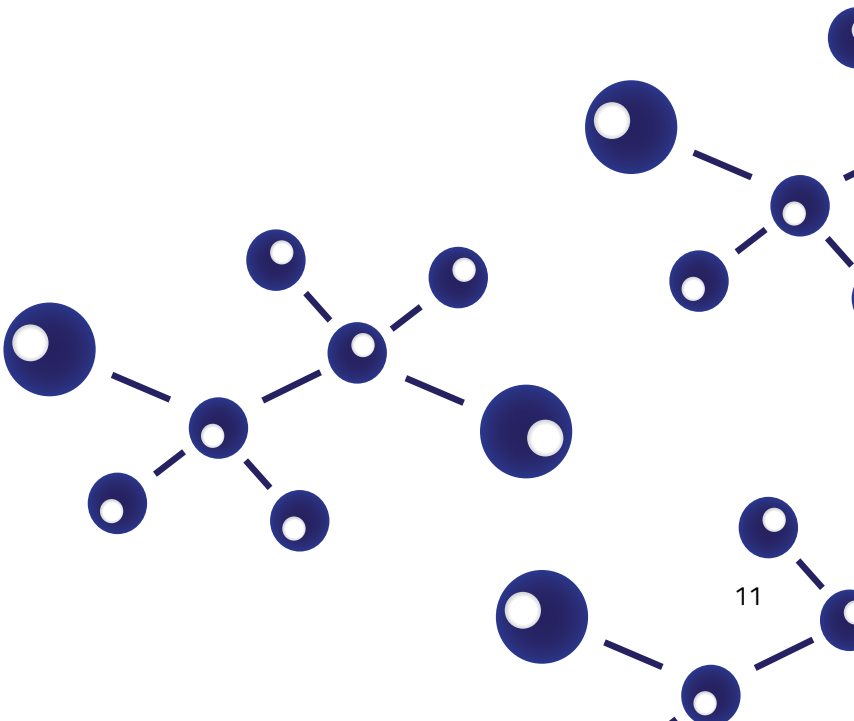
The project's proposal has had a unique trajectory during the 2-step evaluation process, recapitulated in Section III. The latter also hosts the points of view on the project by external observers.

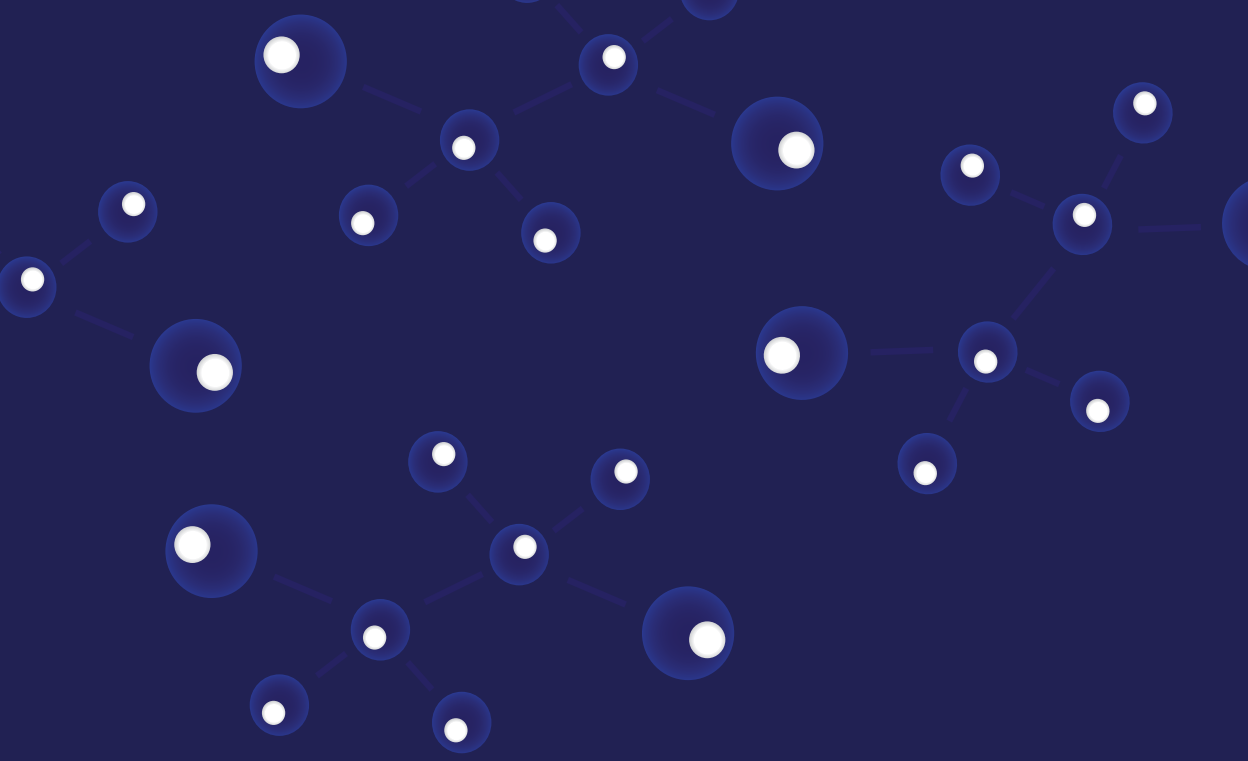
*Sabina Passamonti*
*team manager of the Lead Partner*

Trieste, December 2013



# PART I





# TRANS2CARE. Working plans: consciousness and perspectives

The project started on 1st April 2011 and will end on 30th September 2014.
The project received a budget of € 2,611,118 from the Italy-Slovenia 2007-2013 Cross-border Cooperation Programme.
Seven universities and research institutions, five hospitals and a center for technology transfer distributed over the Programme area constitute the 'Interregional network for innovation and technology transfer for health improvement", which will continuously develop new protocols and biotechnological devices for the prevention, early diagnosis and treatment of neurodegenerative, cardiovascular, orthopaedic and oncological diseases.

### WHY IS IT STRATEGIC?

The high and heterogeneous technical and scientific skills of team managers ensure the cohesion of the partnership and the project's quality in its three main phases: network set-up, network's operational implementation, its consolidation and enlargement.
The identification of targeted goals, which will at every working stage and level ensure the integrated action of partners, is of crucial importance. An increased efficiency depends on a considerable amount of management capacity. The partnership cohesion and its operational efficiency will attract new partners, including industrial, and new sources of funding. These elements will have a positive impact on the cross-border area, not only in socio-economic terms, but also in terms of an integration model to imitate.

## TRANS2CARE

The project started on 1st April 2011 and will end on 30th September 2014. The project received a budget of € 2,611,118 from the Italy-Slovenia 2007-2013 Cross-border Cooperation Programme. The project's objective is to set up an 'Interregional network for innovation and technology transfer for health improvement". The project can be summarized as follows:

# TRANS2CARE

- Lead Partner
  Università degli Studi di Trieste
- Project Partner 1
  Kemijski Inštitut Ljubljana
- Project Partner 2
  Scuola Internazionale Superiore
  di Studi Avanzati
- Project Partner 3
  Univerza v Novi Gorici
- Project Partner 4
  Università di Ferrara
- Project Partner 5
  Treviso Tecnologia
- Project Partner 6
  Splošna Bolnišnica Dr. Franca Derganca
- Project Partner 7
  Università Ca' Foscari di Venezia
- Project Partner 8
  Università di Udine
- Project Partner 9
  IRCCS Burlo Garofalo
- Project Partner 10
  Zavod Republike Slovenije
  Za Transfuzijsko Medicino
- Project Partner 11
  Ortopedska Bolnišnica Valdoltra
- Project Partner 12
  Univerza na Primorskem
  Fakulteta za Vede o Zdravju


Figure 1: List of project partners and their location in the Programme area.

# trans2care NETWORK

Figure 2: Roles within the partnership.

**5** healtcare institutions + **1** tech-transfer company + **7** research institutions

Zavod Republike Slovenije za transfuzijsko medicino
Blood Transfusion Centre of Slovenia

Treviso Tecnologia
AZIENDA SPECIALE PER L'INNOVAZIONE
Camera di Commercio Treviso

UNIVERSITÀ DEGLI STUDI DI TRIESTE

BURLO

università di ferrara
DA SEICENTO ANNI GUARDIAMO AVANTI.

SPLOŠNA BOLNIŠNICA
Dr. F. DERGANC
NOVA GORICA

Kemijski inštitut
National Institute of Chemistry Slovenia

Università Ca'Foscari Venezia

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|---|---|
| gastroenterology | applied chemistry |
| cardiology | biocompatible materials |
| orthopedy | biochemistry |
| immunology | molecular biology |
| cancer | genetics |
| | neurosciences |

# WORKING PLANS: CONSCIOUSNESS AND PERSPECTIVES

It is our intent to illustrate the 'structural" characteristics of the project, rather than its content. The structural characteristics analysis should help us to grasp its strategic nature, in order to apply it, if necessary, to other scientific or socioeconomic projects, which may widely differ from our TRANS2CARE.
The analysis is based on the following question: how does TRANS2CARE interpret the notion of strategic?
Besides the formal requirements of the public call, such as the number and geographical distribution of partners, duration and budget, are there any original operational aspects, which contribute to the strategic nature of the project?
Firstly, we will analyse the project's working plans in order to single out the expected strategic operational aspects.

## A) SETTING UP THE NETWORK

The preliminary stages of the network set-up were:

### 1 Study of the Italy-Slovenia 2007-2013 Operational Programme.

Under Section 2 the Operational Programme points out the reference framework:

- 'Research and development are key factors in the creation of an economic environment" (p. 32);
- 'In the Programme area, universities provide a wide range of courses, covering the major disciplines ... omissis ... it should not be difficult to develop cooperation projects between universities that teach the same subjects and deal with the same fields of interest and research projects. In this perspective, it is desirable that more or less recent universities of the Programme area are able to enhance the bilateral cooperation. '(p. 34);
- 'The health sector, both in Italy and in Slovenia, takes us a large proportion of GDP devoted to public spending." (p. 35).
- Under Section 4 - Programme strategy, several policy objectives of the Priority axis no. 2 - Competitiveness and knowledge-based society are identified; the University of Trieste and its partners could contribute to achieving them:
- 'The improvement of research and technological innovation and the strengthening of cooperation between universities, research centers and businesses are the prerequisite to enhance the competitiveness of the Programme area. Considering the rich potential of scientific and research- and innovation-oriented center in the Programme area, it is important to support the technology transfer between companies and research institutions, to promote the adoption of innovative 'highly technological" solutions and especially to foster the creation of networks between Italian and Slovenian R&TD." (p. 63);
- 'To ensure the development of valuable human resources in the Programme area, actions need to be supported by joint interventions of professional training aimed,

among other things, at retraining workers and promoting the integration of qualified personnel into the cross-border labour market. By doing so, it will be possible to optimally use the results of research and technological innovation activities, while contributing at the same time to prevent the so-called 'brain drain." (p. 64);
- 'The health and social sectors are the key elements for ensuring an increase in the quality of life in the Programme area." (p. 67).

2 Conceiving the project idea:

Create a cross-border network of universities, research centers, hospitals and company specialized in technology transfer with the objective of sharing technical-scintific skills and applying them to the development of innovative health products and services. The main actors of the project idea are supported by 14 highly qualified researchers.

3 Haring the project idea with colleagues, the so-called team managers, be longing to 13 different institutions, and two-stage networking: informal agreements between colleagues, and formal agreements between institutions.

## CONSCIOUSNESS

The data summarized in the table below chronologically mark the network achievements:

| Achievement | Phase | Date | Type of agreement |
|---|---|---|---|
| 1. Expression of interes | Call no. 1/2008 | 01.12.2008 | Letter of intent |
| 2. Project proposal | Call no. 1/2009 | 10.09.2009 | Letter of intent |
| 3. Admission to funding | List scrolling | 10.03.2011 | - |
| 4. Beginning of activities | Activities begin | 01.04.2011 | Informal agreement |
| 5. Signing of the partner-ship agreement | - | 22.04.2011 | Partnership agreement |
| 6. Delivery of first report | - | 10.11.2011 | - |
| 7. Kick-off event | - | 21.11.2011 | - |

It should be noted that without any funds, the network has been stable for twenty-nine months (from 01.12.2008 to 22.04.2011). In addition, partners informally agreed the start the activities (01.04.2011) before signing the partnership contract (22.04.2011).

Which are the factors of stability?

1. The quality of the people, expressed through various competences:
    a. High scientific and cultural skills;

b. Ability to share their scientific expertise, despite the fact that high specialization inevitably involves a lack in communication;
c. Willingness to interact with different people with different ethnic, linguistic, cultural, social background, or even with people not known before (curiosity as a tolerance factor for individual growth).

2. The quality of the project idea, due to two elements:
   a. Good correspondence to the Operational Programme: the project resisted the 'stress tests" of the multiple stages of evaluation;
   b. An optimal solution to overcome the inherent limit to the full utilization of the individual scientific-technological potential: sharing and complementary use of human resources and infrastructure, in order to build up a suitable operating mass to achieve otherwise unattainable goals;
   c. The team managers forecasted that by operating as a network, partners would achieve the project objectives (project feasibility).
3. A technical language: English. A century ago it was German, two centuries ago, Latin.

### PERSPECTIVES

If the network has resisted for such a long period of time without funds, it will probably be strengthened when funds will be allocated, not only to achieve the project objectives, but also to enable related initiatives, for example in first, second and third level training, or identifying new research contexts and promoting knowledge transfer.
Thus, quality and stability are two sides of the same coin, key reference features for other projects, other programmes, other contexts.

## B) OPERATION OF THE NETWORK

When the funds will be allocated the project will provide the following:

1. Networking: for sharing objectives, knowledge and skills through:
   a. Information and communication technologies (e-mail, website and content management system);
   b. Meetings (on average 1/month);
   c. Exchanges of researchers.
2. Training of researchers: to expand their technology skills and promote the acquisition of essential skills for self-sustainability of research and self-employment (continuous search for sources of funding, creation of employment opportunities);
3. Management: to ensure and/or increase productivity:
   a. Promptly perform the activities, which are necessary to achieve the objectives;
   b. Coordinate the activities in order to increase its efficiency and effectiveness.
4. Administration & reporting: to ensure traceability of project costs, and the correspondence between costs and their reimbursement to the partners.

## CONSCIOUSNESS

*The objectives*

At this stage the most critical task is to keep partners focused on the project's objectives. Specifically allocated funds may trigger a paradoxical reaction: funds may be used in a manner that is different from the purposes of the project and disconnected from the activities of the partners. This is obvious, since every partner has some ongoing activities and these indiscriminately need any available financial resources.

To avoid this risk, it is necessary to identify common goals that are achievable over a short period of time. One of the major factors of cohesion will be the achievement of results over a short period of time, as a result of the synergies created between workgroups. Thus, project activities must be carefully studied and their scientific and management aspects coordinated. Our project is born and grows with careful scientific planning, together with a careful management control.

*Management*

Among various university projects, this one stands out due to the strategic importance of its management. By entrusting the management to an external company we have given an added value to the project, since we have fostered the creation of an interface between the team managers, who work in publicly funded non-profit organizations, and the corporate funded company personnel responsible for the project management. This synergy of two completely different mentalities becomes a factor of success, because it creates a trans-disciplinary collaboration.

*The training of researchers*

The project's ambition is to instill in the minds of the newly-recruited researchers, who until recently have been committed to learning scientific concepts and methods, which are increasingly more sophisticated and intellectually challenging, the importance of acquiring complementary skills, mainly of a managerial nature, in order to optimize not only their technical and scientific productivity, but also to actually transfer their knowledge and technology to biomedical companies, which have to generate profits for their financial sustainability.

*Administrative and management tasks*

In the first seven months the administrative and reporting tasks absorbed enormous energies. Why? Albeit with a few exceptions, the scientific and health partners do not have the practical experience to manage a complex project in terms of partners, thoroughness and rigidity of financial reporting standards and obligation to implement unusual activity for scientific bodies, such as those of the Work Package no. 8 - Communication (to non specialized or sectoral areas).

The public funds granted to these institutions are generally insignificant and also sporadic. The scientific and cultural quality may have been marginally affected, because,

at the same time, the spread of the internet and the freedom of access to information and data, and the implementation of digital technologies for communication between colleagues (e-mail, video conferencing, etc.), has allowed to mitigate the damage: a certain productivity at no cost has been allowed by computer networks. The cohesion of the partnership during the months in which it did not receive any funding has been maintained thanks to these infrastructures.

However, these public bodies, which are constantly required to adopt a "corporate" mentality, are not at all prepared to leave their simple "cover-the-operating-costs" mentality, which requires minimum management and long-term vision skills.

By receiving additional funds, public bodies were given the opportunity to make much more challenging financial investments, not only from an administrative point of view, but also from an intellectual point of view, since they had to stake that these funds would have made them achieve important results: it was the first time for many of us.

We can start by analysing the most common tasks that university administrative departments usually tackle first: recruitment of contract personnel for "research support". Initially, the desired funds were intended to recruit highly qualified personnel not only for "research support" (in accordance with Italian Law n. 165/01), but also for the "future sustainability of research." This is a delicate operation. The collaboration lasts far more than normal (36 months) and the need for high qualification and motivation is correspondingly higher than normal. It comes to writing a public notice of selection (call) by adopting a completely different mindset, bearing in mind that qualified candidates can be attracted simply by writing only a couple of specific sentences. Paradoxically, the strategic approach of the project is implemented through simple acts, such as few simple sentences, and during the candidates' selection process, by focusing on the intuition of the selection committee.

In order to write down public notices for the selection of highly specialised external consultants, our administration department had to learn new skills and unusual administrative procedures, such as tenders.

Even more unusual was the creation of our own visual identity and website.

The involvement by gender

The table below summarizes the gender participation in the project:

| CATEGORY | FEMALE | | MALE | | TOT |
|---|---|---|---|---|---|
| | N | % | N | % | N |
| Team managers | 7 | 54 | 6 | 46 | 13 |
| Project managers | 1 | 33 | 2 | 66 | 3 |
| Researchers | 10 | 71 | 4 | 29 | 14 |
| Total | 18 | 60 | 12 | 40 | 30 |

The data indicate that women and men are equally balanced in the project, if we consider the senior members. Instead, in the category of researchers, there is a clear predominance of women, without any selection criteria beeing adopted for the benefit of women (as suggested by the OP, p. 114). This situation reflects the high level of expertise of young women and is in line with Eurostat data relating to the percentage rate of higher education among the population aged 30-34 years, recognized in 2010 by Eurostat[1] as shown in table below:

| Tertiary educational attainment by sex, age group 30-34 (% of group) | | |
|---|---|---|
| | ITALY | SLOVENIA |
| TOTAL | 19,8 | 34,8 |
| MALE | 15,5 | 26,4 |
| FEMALE | 24,2 | 44 |

Both in Italy and in Slovenia younger women are more educated.

## PERSPECTIVES

Actually, the most important perspective is not the achievement of the project objectives, which must be achieved in any case. The most important perspective is the achievement of the objectives in a shorter time than expected, thanks to a prudent selection of intermediate goals (which should be achievable) and the optimization of activities. By doing so, we understand that the planning of activities could free up time and human resources, which could in turn start identifying other elements for the innovation and consolidation of the network.
For this reason, the mix of administration and management and scientific-technological activities, which will fully affect the researchers, should provide them with the opportunity to develop the habit of planning work and forecasting results. This is precisely what defines a strategic approach.
Administration and management experiences are a typically strategic added value, which may attract new partners, new funding opportunities and thereby ensure the sustainability of the network and the applicability of this model in other areas.
This project contributes to balance the presence of women in the highly qualified labour market. If all female researchers, who have joined the network, will continue in their professional commitment, within 5 to 10 years after the end of the project they will have the qualifications and skills to meet leadership roles.

[1] http://epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&plugin=1&language=en&pcode=t2020_41

## C) CONSOLIDATION AND EXTENSION OF THE NETWORK

Once fully developed, the network will trigger new activities included in Work Package no. 7:

| | |
|---|---|
| **ACTIVITY NO.1** | Animation campaign aimed at including new members |
| **ACTIVITY NO.2** | New collaborations between industry, health professionals and academies |
| **ACTIVITY NO.3** | Programs for the integration of young researches into companies |
| **ACTIVITY NO.4** | Participation to tenders for the future support of the network |
| **ACTIVITY NO.5** | Spin-off/Start-up |

With these five activities, the network will try different approaches to ensure its existence in conditions of full efficiency. The network aims to become a reference point for attracting financial, human and technological resources.

### CONSCIOUSNESS

The biggest challenge is to cooperate closely with the biomedical industry. This is a double challenge, which involves: a) verification of the applicability and potential profitability of the basic knowledge generated within the network, b), verification of the (academic) basic research system's ability to intercept the innovation and competitiveness needs of industries and position itself as a reference that can offer scientific and technological solutions to health and sanitary needs.
This will not be a "deadly embrace", because from the activity plan we can expect the setting-up of (strategic) partnerships with other research institutes and universities, aimed at creating consortia to participate in public tenders that offer financial support to basic or applied research carried out at universities.
Within the network there is a strong awareness that knowledge base and study and research methodologies, and also strategies for the acquisition of financial resources, are the authentic socio-economic characteristics of university.

### PERSPECTIVES

These future collaborations will allow the optimal use of the knowledge and skills acquired within the network. In the terminal phase of the project, the collaboration with companies will be of fundamental importance for achieving an operational critical mass, as the setting up of the network with 13 project partners has been in the beginning. Therefore, we will achieve the objective of the Operational Programme, i.e. the transfer of highly qualified, university-trained human resources to the industrial sector, which will foster innovation by leveraging on human capital.

## CONCLUSIONS

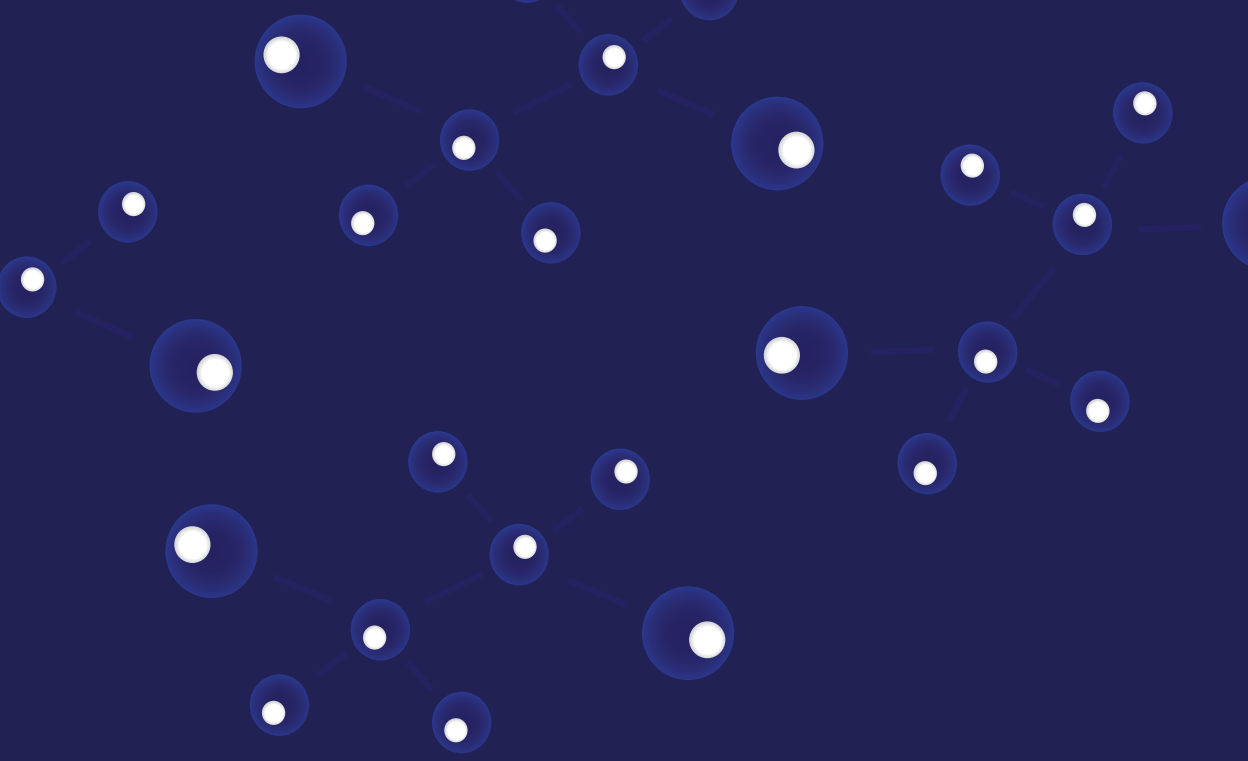
In reviewing the arguments exposed, the primordial strategic element is constituted by the competences of the participants and not by their study or cultural affinities. It is the competences that enable people with different cultural backgrounds to listen to each other, not the sound of familiar words or concepts or languages. At the same time, it is the diversity of skills that made us identify a distant, ambitious and unifying (thus strategic) goal: transfer our knowledge to young researchers and enable them to work. This objective is the only thing that brings together people, whose daily life is impossible to share, due to the strong specialization of their respective knowledge. The project idea is broad, strongly supported and in accordance with the Operational Programme, and has withstood the test of time without specific funding.

In the implementation phase, it appears strategic that two or preferably more partners jointly identify common and 'reachable" goals and achieve them in a short time, as expected when genuine synergies are being implemented. In the implementation phase we should reverse the perspective: only achievable objectives can demonstrate the value of sharing skills and maintaining the functionality of the network.

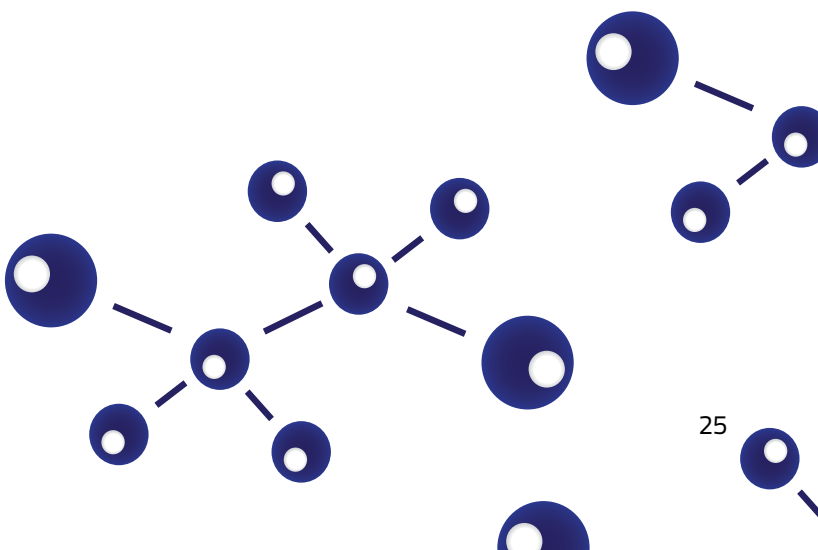
The introduction of shared methods for the management of project activities, ranging from the identification of objectives, synergies and perspectives, to the planning of activities, is new to the projects carried out by universities. The management objective is to optimize the complementarity of skills and infrastructures, promote time-planning and freeing up time for future planning and innovations testing.

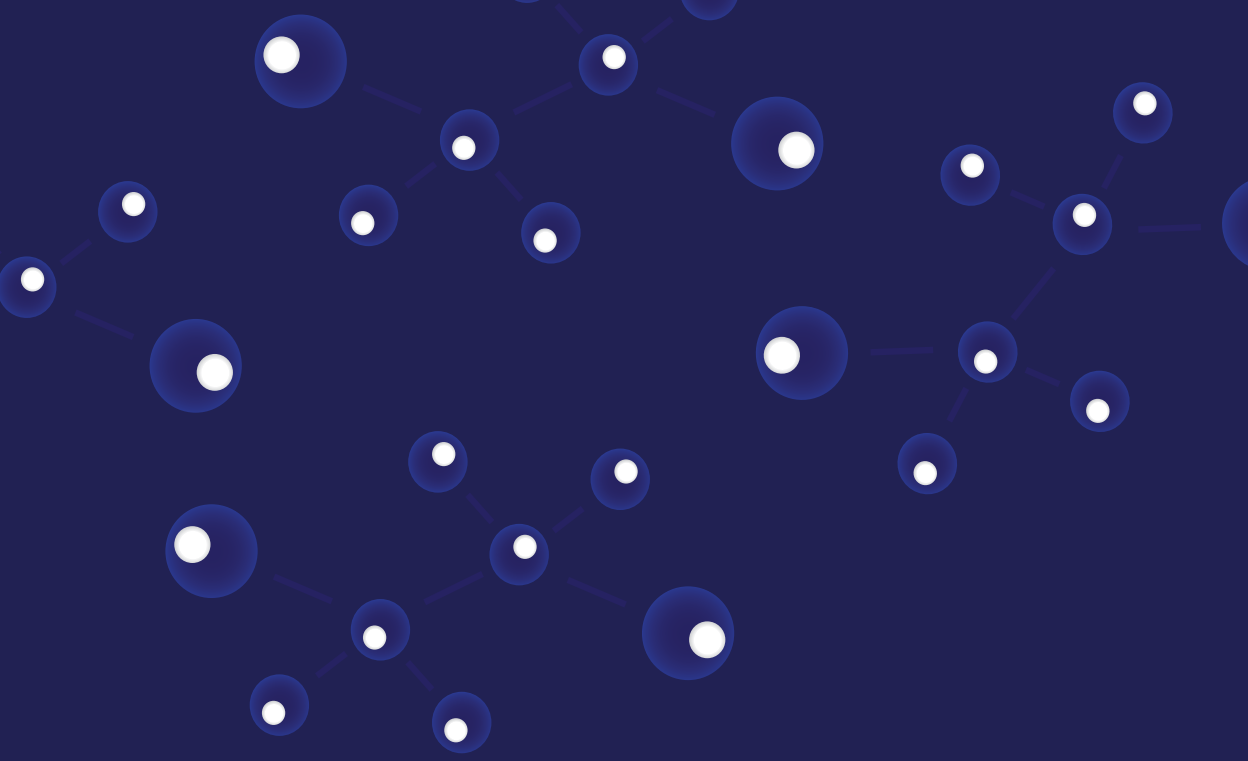
At the same time, by adopting new management and administrative skills the network will acquire essential operational competences to access additional sources of funding for future sustainability. These funds could be procured both through partnerships with companies and participation to public calls.

Finding financial resources is a strategic factor of cohesion for a partnership.



# PART II
# TRANS2CARE PARTNERS AND THEIR CONTRIBUTION TO BIOMEDICAL KNOWLEDGE





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trans2care
Transregional Network for Innovation and Technology Transfer to Improve Health Care

# Chemometrics: a versatile tool to explore large dataset

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Abstract — Chemometrics is the field of science covering the development and application of mathematical and statistical methods to identify important chemical information. It is indispensable in the evaluation of experimental results and suitable for exploration of large data sets. Within the Trans2Care project we intend to apply chemometrics methods in several areas related to the problems explored by the Project partners. In particular we shall investigate transmembrane protein transport mechanism with data-driven modelling approach and also applying biomolecular simulations. We'll combine our theoretical approach with experimental data provided by the Project partners, which will contribute to a better exploration of the available information in biomolecular systems studied, in the research of neurodegenerative diseases, in cardiovascular and pathohistological research. It will also intensify the collaboration, mobility of researchers and exchange of knowledge between partners.

Index Terms — chemometrics, data mining, predictive modelling, transmembrane proteins

## 1 THE NATIONAL INSTITUTE OF CHEMISTRY

The National Institute of Chemistry (NIC) was established in 1946 by the Slovenian Academy of Arts and Sciences (SAZU) as the Chemistry Laboratory of the SAZU, later it was renamed to the Chemistry Institute of the SAZU. Following Slovenian independence in 1992 the National Institute of Chemistry (NIC) became a public research institution. The NIC has 290 employees and they carry out research work in 15 laboratories and two infrastructure centers. More than 25% of the Institute's staff members represent young researchers, making NIC one of the leading Slovenian organizations for education of graduate students. Research activities of the Institute are oriented towards the development of new expertise, technologies and products,

which will help to ensure the long-term development of Slovenia. The Institute offers high-level research equipment including a Karl Zeiss Supra 35 VP Electronic Microscope with EDX analysis, a high resolution powder x-ray diffractometer, and an 800 MHz NMR spectrometer, allowing researchers to engage in advanced research challenges at the world level. Industry is also an important partner to the Institute; several Slovenian as well as foreign companies has established a close long-term cooperation with the Institute.

## 2 LABORATORY OF CHEMOMETRICS

In the early seventies the beginning of the chemometrics research started in Slovenia which was completely new field also in the world. At the National Institute of Chemistry prof. Dušan Hadži implemented chemometrics in his group in 1973 and in 1993 the group became an independent Laboratory of Chemometrics, first dealing with systems for identification of compounds based on infrared spectra, the study of algorithms, expert systems and modelling.

Today the Laboratory of Chemometrics has 14 associates, seven researchers, three PhD students, two post-doctoral students, one visiting professor and one professor emeritus. We are developing and applying chemometrics and statistical methods for solving problems in chemistry and related sciences, from visualization of many-dimensional data to analysis of biologically relevant information in proteomics and genomics. The research work is financed through national research programme schemes and several European projects. In 2010, the Laboratory of Chemometrics was involved in three EU projects (TRACE, CAESAR, IBAAC), bilateral projects with Turkey, Argentina and USA and made a strong collaboration with industry of asphalts (IMS-ADITOL). The laboratory has already established a strong long-term collaboration with University of Trieste and from 2010 we are partners in an international strategic project Trans2Care.

### 2.1 Research Areas

The researchers of the Laboratory of Chemometrics are engaged in various research activities, such as (i) introduction of chemometrics to the research and development, (ii) modelling of chemical properties and processes - QSAR and mechanistic models, (iii) development and application of artificial neural network methods in chemistry, (iv) application of discrete mathematics in structural chemistry, QSAR studies, proteomics, and in genomics, (v) development of methodologies and programme packages for mechanistic and empirical models, and (vi) development of 3D representations of chemicals structures for applications in QSAR.
We have successfully applied chemometrics methods in the field of traceability of food [1], in the prediction of toxic properties of various toxic data (developmental toxicity, mutagenicity, carcinogenicity, bio-concentration factor and skin sensitation) [2], in the optimization of pigment dyeing of polybenzimidazol fibres [3], in the optimization of bio-organic catalysts for stereo-selective reduction of prochiral ketones [4], in the research

of anti-tuberculosis drugs [5,6], in the optimization of gradient profiles in ion-exchange chromatography [7], in the investigation of a transport mechanism of a membrane protein, bilitranslocase [8,9], and in the research of structural characterization of transmembrane proteins [10]. In the field of proteomics we have recently published a review paper of Graphical representation of proteins [11].

## 2.2 Structural Elucidation of Transmembrane Protein, Bilitranslocase

In the collaboration with University of Trieste, Department of Life Science we have started the research project in which we address the problem of structural elucidation of transmembrane proteins. The experimental work performed at the Department of Life Science was the basis for the application of computational methods in the Laboratory of Chemometrics. In-silico methods are strongly dependent on experimental data available and a combination of experimental and theoretical approach can lead to a successful resolution of the specific problem. Experimental data are treated with computer algorithms, whose output are theoretical predictions of biological properties, and the predictions can be directly validated in additional experimental work. Below is a schematic presentation of a QSAR model for prediction of biological properties from structural data (Fig.1).

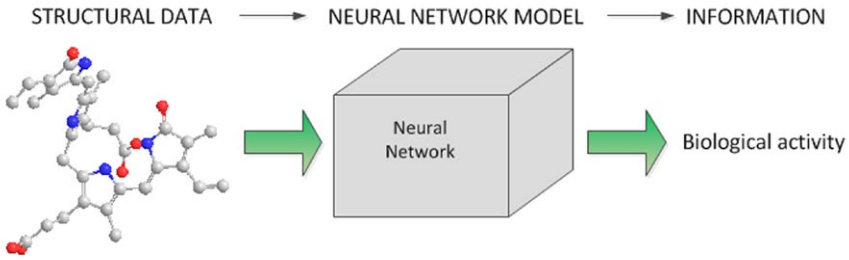


Fig.1. Schematic presentation of a QSAR model for prediction of biological property from structural data.

In the latest publication [12] we have presented an approach towards structure elucidation of bilitranslocase, the membrane protein which transports bilirubin from blood to liver cells. In this research we combined two approaches: first, the prediction of transmembrane segments of the protein based on the mathematical descriptors obtained from the information of membrane proteins of known 3D structure, and second, the information about the transport mechanism from experimentally tested set of small molecules for their competitive inhibition of bilitranslocase.
In the first approach the sequence and secondary structure information of transmembrane segments of proteins with known 3D structure available from public databases (PDB and PDBTP) was exploited to build a model for prediction of transmembrane segments of structurally unresolved target protein. The prediction error of the model for prediction of alpha transmembrane segments for the external

validation set was below 10%. The model was challenged with bilitranslocase and it proposed four transmembrane alpha helices, each containing around 20 amino acids, which is partially confirmed with experimental studies using particular antibodies corresponding to parts of amino acid sequences of bilitranslocase.
In the second approach we have tested a set of 89 non-congeneric compounds for their competitive inhibition constants in the investigated protein-substrate system. The information about 3D chemical structure of small molecules (represented by molecular descriptors) and the experimental data assessed by evaluating the kinetics of inhibition of bilitranslocase transport activity was used for development of a data-driven model using artificial neural networks.
QSAR models showed good predictive ability for bilitranslocase binding affinity. From the mechanistic interpretation of selected molecular descriptors, obtained with genetic algorithm, it was found that interactions between bilitranslocase and small molecules rely on the ability to establish hydrogen bonds, diminishing the involvement of charge interactions. The results of this work show that, contrary to dietary anthocyanins, most of dietary flavonols do not interact with bilitranslocase, whereas, some flavonol aglycones act as poor ligands of that carrier. In case of nucleobases and their derivatives the phosphate group in principle improved the transport ability by bilitranslocase.

# 3 CHEMOMETRICS IN TRANS2CARE

## 3.1 Competences or what can we offer to T2C

The long term collaboration with the University of Trieste in the field of transmembrane proteins is the basis for the research work within the T2C project. As described in the paragraph above, we have recently studied transmembrane protein, bilitranslocase, its transport activity and transmembrane segments. We will continue with the development of the prediction models, either for inhibition constants or for transmembrane alpha helices of beta sheets. We will also study the 3D structural model and the molecular dynamics simulations of bilitranslocase. A complementary study and characterization of the sequence of bilitranslocase isolated from plants will be another interesting research topic and will offer an interesting comparison with the bilitranslocase homologue obtained from rat liver. Furthermore, structure elucidation studies will be extended to other transmembrane transporters, such as SbmA transporter (protein present in the membrane of bacteria).

Chemometrics methods are suitable for exploration of large data sets, especially in the case when the information contained in the data is not obvious and the knowledge is not easily extracted. For this reason we intend to collaborate with the T2C partners whose role is a compilation of large amounts of data, not only in the research laboratories, but also in the hospitals, where a lot of data are collected during their everyday practise.

## 4 CONCLUSION

In Trans2Care project our priority will be to explore experimental data collected by several partners, from biochemical data on membrane transporters to various studies of the neurodegenerative disease on one side, and statistical analysis of different data-bases and registers on the other side. The interdisciplinary approach of our research work will offer a good opportunity for young researchers starting their research career. It will also straighten already established collaborations and connect the research institutes, universities and hospitals within the Project, having in mind a common goal, a network of participants for exchange of ideas, knowledge dissemination and technology transfer.


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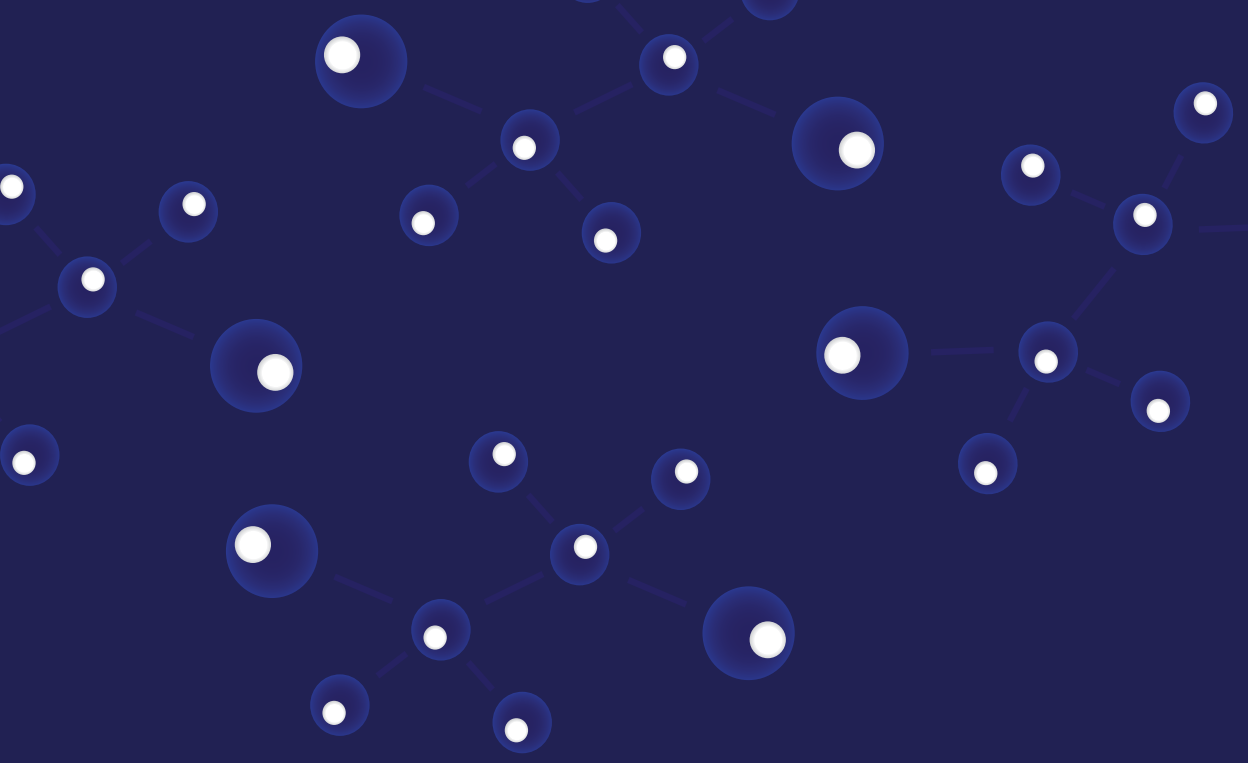

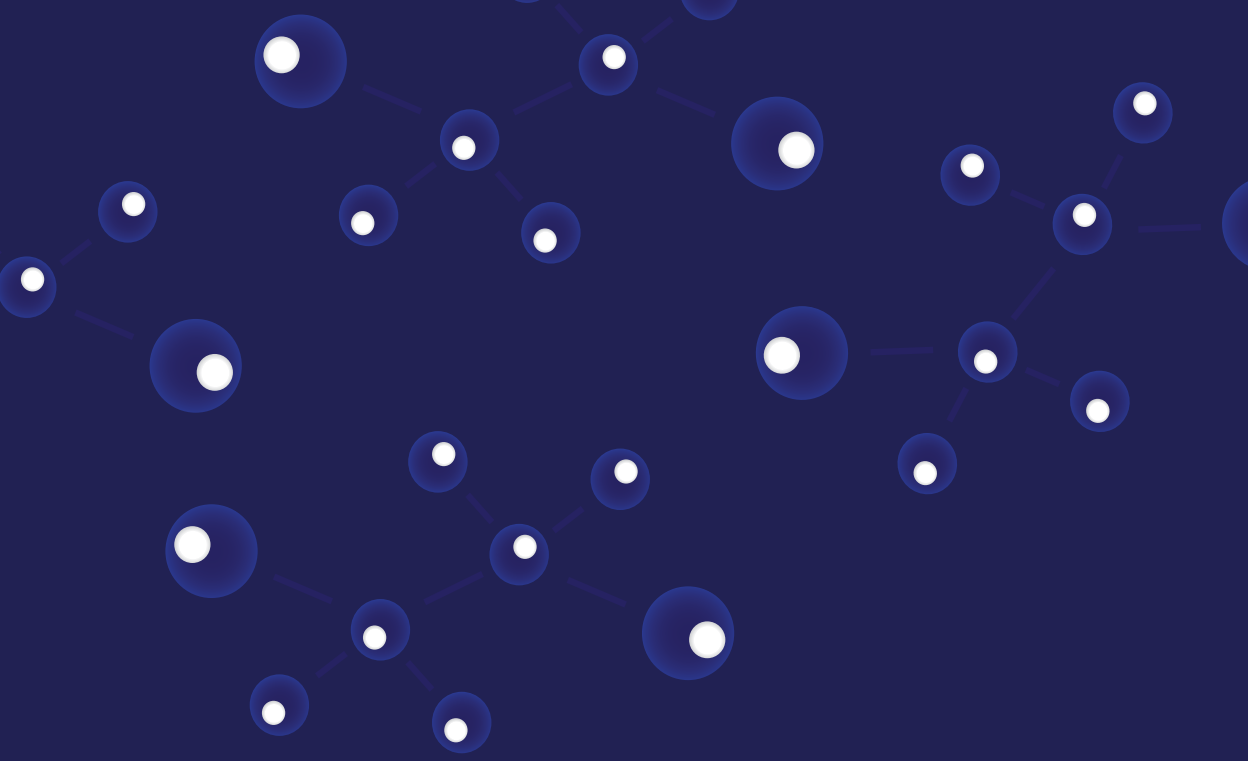

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# Transcriptome analysis of prion disease animal models

Maura Barbisin, Giuseppe Legname

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Abstract — Prion diseases are incurable and fatal neurodegenerative disorders that affect both humans and animals. The causative agent is an infectious protein called prion ($PrP^{Sc}$), which is the pathological form of a normal protein ($PrP^{C}$) present on the cell membrane. The molecular mechanisms underlying prion replication and subsequent degeneration of the Central Nervous System (CNS) are still poorly understood and therefore innovative approaches are needed to build diagnostic, therapeutic, taxonomic, and disease surveillance tools. We are going to adopt an unbiased genomic approach and conduct whole transcriptome analyses using microarray gene expression methods in brain and/or blood of infected animals versus healthy controls. We hope to identify a set of genes that can be used for early diagnosis and/or as targets for therapeutic strategies. Within the Trans2Care project we intend to promote collaboration and exchange of knowledge to facilitate all partners' research objectives, and possibly find a common way to accelerate the process aimed at improving our healthcare system.

Index Terms — prion, prion protein, neurodegeneration, gene expression, genomics.

## 1 SISSA, INTERNATIONAL SCHOOL FOR ADVANCED STUDIES

SISSA, International School for Advanced Studies was established in Trieste in 1978, and it is one of the leading scientific institutions in Italy for postgraduate training in Mathematics, Neuroscience and Physics. There are thirteen Ph.D. courses available, covering the three main research areas, three professional master's courses and, thanks to an agreement with University of Trieste and University of Trento, three joint curricula for master's degrees (laurea magistrale) in Mathematics, Physics and Neuroscience. Besides being a school, SISSA is also an international research center

with financial support granted by private and public entities (Friuli Venezia Giulia Region, Italian Government and European Research Council). In 2010-2011 alone, Italian funding amounted to some € 5.5 million, while international grants provided over € 10 million. The Neuroscience Department (hereafter Department) is built on Cognitive Neuroscience and Neurobiology. The Cognitive Neuroscience group focuses on how the brain generates behavior (language, perception, action) using methods from artificial neural networks to human neuropsychology. The Neurobiology component of the Department is devoted to research on the nervous system, using a combination of molecular, cellular and integrative approaches. One specialized division of the Neurobiology area is devoted to the study of neurodegenerative diseases (such as Alzheimer's, Parkinson's, Huntington's and Prion diseases), focusing on Functional and Structural Genomics. The Prion Biology Laboratory is part of the Genomics branch of the Department.

## 2 THE PRION BIOLOGY LABORATORY

The Prion Biology Laboratory currently includes one associate professor, four post-doctoral fellows, nine Ph.D. students and two undergraduate students. We have a Biosafety Level 2 facility with a cell culture room as well as protein expression and purification equipment. In addition, we have access to core facilities and technologies available in the Department such as an animal facility carrying transgenic animals, histology room, DNA sequencing, confocal microscopy, Real-Time PCR and Affymetrix micro-array platforms.
The laboratory is supported by various grants and organizations such as IIT, PRIN, FIRB, FP7, and so on. Just to mention one example, recently € 5 million were granted to our laboratory and another group in the same Department to identify the changes in the human genome leading to a number of incurable neurodegenerative diseases (FIRB-Programme agreements 2011; project title: Functional Genomics of Neurodegenerative Diseases). Within this same line of research we became partners of the international strategic project Trans2Care, FESR 2007-2013 for the cooperation between Italy and Slovenia.

### 2.1 Research Activities

The focus of the Prion Biology Laboratory is studying prion diseases, rare and fatal neurodegenerative maladies that affect humans and animals, for which there is no diagnostic tool, nor a cure [1, 2]. In mammals, prions reproduce by recruiting the normal, cellular isoform of the prion protein ($PrP^{C}$) and stimulating its conversion into the disease-causing isoform ($PrP^{Sc}$). $PrP^{C}$ and $PrP^{Sc}$ have the same amino acid sequence, but distinct conformations: $PrP^{C}$ is rich in α-helical content and has little β-sheet structure, whereas $PrP^{Sc}$ has less α-helical content and is rich in β-sheet structure (Fig. 1). The conformational conversion of $PrP^{C}$ to $PrP^{Sc}$ is the fundamental event underlying prion diseases, and it is still poorly understood. The main research lines of the laboratory are: therapy of prion disease [3], physiology of the prion protein [4], syntethic prions and molecular determinant to infectivity [5], structural biology

and biophysics of the prion protein [6], transcriptomics and neurodegeneration [7], molecular mechanisms of neurodegeneration [8].
The project funded by the Trans2care grant is focused on the analysis of the whole transcriptome of animal models affected by prion disease. The goal is identifying genes that can become potential targets for diagnostic and/or therapeutic approaches. Our collaborators identified some candidate genes by microarray gene expression analyses in brain tissue of a primate model of prion disease and they appear to be very promising. Our objective is validating these candidates using a more sensitive and accurate technology such as qRT-PCR. A second model we are using is cattle infected with BSE (Bovine Spongiform Encephalopathy or mad cow disease). In this case the tissue is blood and we are performing some QC tests of the samples before embarking in the microarray gene expression analysis and subsequent validation with qRT-PCR.
Recent findings support the idea that neurodegenerative diseases may all share a common mechanism that implies a prion-like behavior. Therefore, even though prion diseases are rare disorders, basic research on their mechanisms may be useful to explain all the neurodegenerative maladies, like Alzheimer's and Parkinson's disease, that affect large portions of the world population.

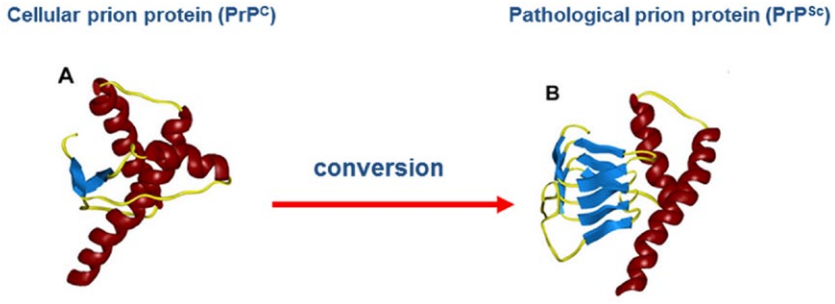


Fig.1. The conformational conversion of PrP$^{C}$ (A) to PrP$^{Sc}$ (B).

# 3 ROLES IN TRANS2CARE

## 3.1 What we can offer to T2C

We have focused our research projects on exploring the infection mechanisms of prions from various angles; therefore we have gained expertise in many fields, from protein expression and purification to animal models, as well as gene expression analyses. We can provide T2C partners with our knowledge to facilitate the exchange of expertise within the network, and at the same time we can take advantage of other partners' technological skills to further understand prion replication mechanisms and subsequent degeneration of the CNS. We have in fact an ongoing collaboration with ZTM - Blood Transfusion Centre of Slovenia (PP10) that we intend to exploit further.

So far PP10 has developed a panel of mAbs against different moieties of the prion protein and some of them can distinguish and differentiate between the wild type $PrP^{C}$ and its pathogenic form $PrP^{Sc}$. They will be tested in our laboratory mainly for the detection of PrP in immunohistochemical procedures (i.e. Western Blot, ELISA and IF), but they may also become important diagnostic and/or therapeutic tools. We are trying to expand this collaboration in the direction of targeted proteomics using Multiple Reaction Monitoring assays (MRM Proteomics). In this way markers obtained by transcriptomic analyses could be directly screened at the protein level in samples of either animal models or patients.
We are going to seek additional opportunities to collaborate with other project partners.

## 4. CONCLUSIONS

The aim of the Prion Biology Laboratory is conducting high level research in the field of neurodegeneration and in particular unraveling aspects of prion diseases that are still poorly understood. Within the Trans2Care framework, we intend to employ gene expression profiling methods to identify gene candidates that may become potential diagnostic biomarkers and/or therapeutic targets. The participation in the Trans2Care initiative can be very helpful in opening new collaborations with the partners or expanding existing ones that may lead to joint discoveries and publications. In addition, having the opportunity of interacting with such diverse partners ranging from basic research institutes to technology centers and clinics, may foster exchange of ideas that can lead to potential technology transfer opportunities to improve our healthcare system.

## ACKNOWLEDGEMENT

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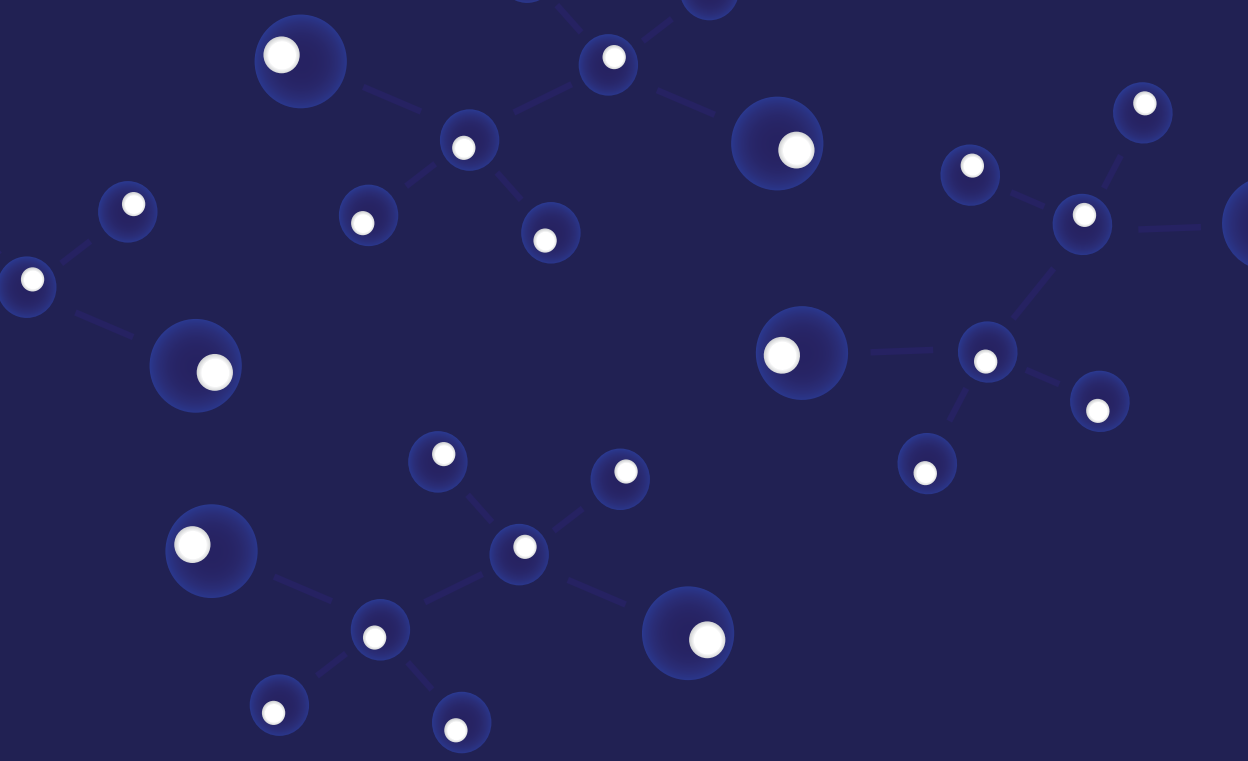

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# Novel laser-based photothermal methods of chemical analysis

Mitja Martelanc, Mladen Franko

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Abstract — University of Nova Gorica (UNG) provides access to knowledge, high quality education and incorporates nine different main research areas. One of them is covered by the Laboratory for Environmental Research which conducts basic and applied research in different areas of environmental sciences such as the investigation of photochemical and microbial degradation, and transport of pollutants in the environment; the development of laser-based methods, bioanalytical methods, and ecotoxicological tests for the identification and determination of toxic compounds and their effects on the environment; the development of new materials for applications in environmentally friendly technologies as well as the research in molecular biology and neurobiology. All in all, the main stream of our scientific work in TRANS2CARE project will be related to novel laser-based photothermal methods of chemical analysis coupled to bioanalytical assays and flow injection analysis.

Index Terms — Laser-based analytics, thermal lens spectrometry, flow-injection analysis, bioanalytical methods, chromatographic techniques

## 1 UNIVERSITY OF NOVA GORICA

Predecessor of the University of Nova Gorica (UNG) was the Faculty of Environmental Sciences, which was the first international postgraduate school in Slovenia. The Faculty had been founded on 24 September 1995 with an agreement from the Council of the Republic of Slovenia for Higher Education of 12 July 1995. It began operating in the 1995/96 study year. The founders of the Faculty were the Municipality of Nova Gorica and the 'Jožef Stefan" Institute from Ljubljana. The Faculty was reorganized into the Nova Gorica Polytechnic in 1998. UNG became a university institution in 2006 founded by Municipality of Nova Gorica, Municipality of Ajdovščina, the 'Jožef Stefan" Institute and the Research Center of the Slovenian Academy of Sciences and Arts

from Ljubljana. Currently UNG offers a broad range of academic programs - seven undergraduate programs, five second level programs (Master's), and seven third level programs (Doctoral). UNG has got 144 employees of whom 91 are Doctors of Science (80 of them are University professors), and 17 are young researchers (Ph.D. students). Over 32% of them are foreign scientists.

### 1.1 Research departments

The research activity at UNG is carried out in five research laboratories (Laboratory for Astroparticle Physics, Laboratory for Multiphase Processes, Laboratory of Organic Matter Physics, Materials research laboratory and Laboratory for Environmental Research), three research centers (Centre for Atmospheric Research, Centre for Systems and Information Technologies, Wine Research Centre) and one Research Institute (Institute for Cultural Studies). The Laboratory for Environmental Research, which will take part in TRANS2CARE project, is composed of five research groups focusing on: Organic substances in the environment (monitoring, transformation and effects), Materials for (photocatalysis) environmental applications, Molecular biology and biotechnology, Modeling of natural processes in forests, Assessment of environmental changes and Laser-based analytical methods. The latter group will be mostly engaged in TRANS2CARE project.

## 2 ACTIVITIES AND ROLES IN TRANS2CARE PROJECT

### 2.1 Laser-based methods for chemical analysis and characterization

Research related to laser-based methods for chemical analysis and characterization includes development of laser-based spectroscopic methods for qualitative and quantitative determination of various compounds present in environment, food and for studies of various chemical and biological processes. Highly sensitive novel analytical methods are being developed using thermal lens spectrometry (TLS) [1] coupled with bioanalytical techniques (acetylcholinesterase – AChE [2], transglutamninaze, and ELISA bioassays [3-4]), flow injection analytical (FIA) systems [5-6], liquid chromatography [5-6], or microscopic TLS (TLM) (shown on Fig.1) as detection technique for lab-on-a-chip chemistry and study of processes on microspace and in microfluidic systems [7]. Photothermal beam deflection spectrometry [8] is utilized to study thermal and optical properties of thin nanofilms of photocatalysts and organic semiconductors for photovoltaic cells as well as for carbon nanomaterials. Newly developed methods, which offer sensitivities over two orders of magnitude superior to those of conventional transmission mode spectrometric techniques were successfully applied for determination of various toxic compounds such as pesticides, allergens, biogenic amines, heavy metals or their species, and recently toxins, as well as essential and beneficial compounds like for example carotenoids, anthocyanins, and other physiologically relevant compounds (i.e. bilirubin) in foodstuffs, as well as in environmental and biological samples [9-10]. Recent applications include TLS detection of metal nanoparticles and exploitation of FIA-TLS for determination of toxic silver ions ($Ag^{+}$) [11].

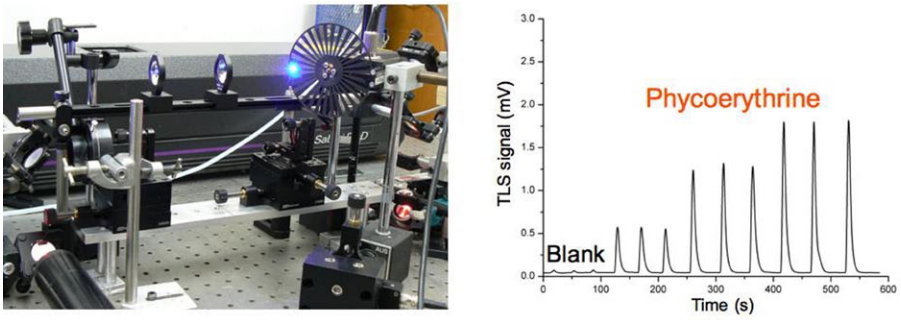


Fig. 1: Picture of a TLS microscope constructed at UNG by dr. Mingqiang Liu (left) and a sequence of TLS signals recorded by UNG PhD student Ambra Delneri during detection of phycoerythrine from cyanobacteria by the FIA-TLS technique (right) (triplicate injections of 200 µL samples: 5, 10, and 15 mg/mL).

## 2.2 Aims in TRANS2CARE project

The scope of our scientific contribution in TRANS2CARE project is the development of novel state of the art methods based on TLS detection in liquid flows such as in the case of high performance liquid chromatography (HPLC), capillary electrophoresis (CE), ion chromatography (IC), and FIA. These systems will enable the determination of various compounds (egg. free bilirubin, anthocyanins, glutene-derived peptides, and virus-like proteins) that might be in research focus of other TRANS2CARE partners. In addition, TLS itself will be offered as detection technique for already available bioanalytical assays such as ELISA, or those being developed by other project partners, which lack sufficient sensitivity. The research will focus also on applications of microscopic TLS relying on bioassays in combination with flow injection analysis on micro-chemical chips [4]. Such combinations provide ultra-high sensitivity, high sample throughput, reduced operational costs, simplicity and high reproducibility. It is also our aim to provide conditions for high quality training of young scientists, those employed by TRANS2CARE, as well as PhD students conducting research in fields related to this project, by enabling intensive contacts and exchange of expertise within the project consortium.

# 3 CONCLUSIONS

With an interdisciplinary approach and intensive collaboration of UNG with partners having expertise in fields other than analytical chemistry, TRANS2CARE project is expected to provide a stimulating environment, infrastructural conditions and opportunities for the development and application of novel state of the art analytical methods for the determination of various substances relevant in biomedical research and diagnostics. Other sectors beyond the scope of TRANS2CARE such as food quality and safety, environmental protection and others, shall also benefit from the outputs of the project. Finally, the industrial sector producing medical diagnostic tools shall benefit by the results of research conducted within the project and knowledge

transfer as well as by the availability of new highly trained young experts.

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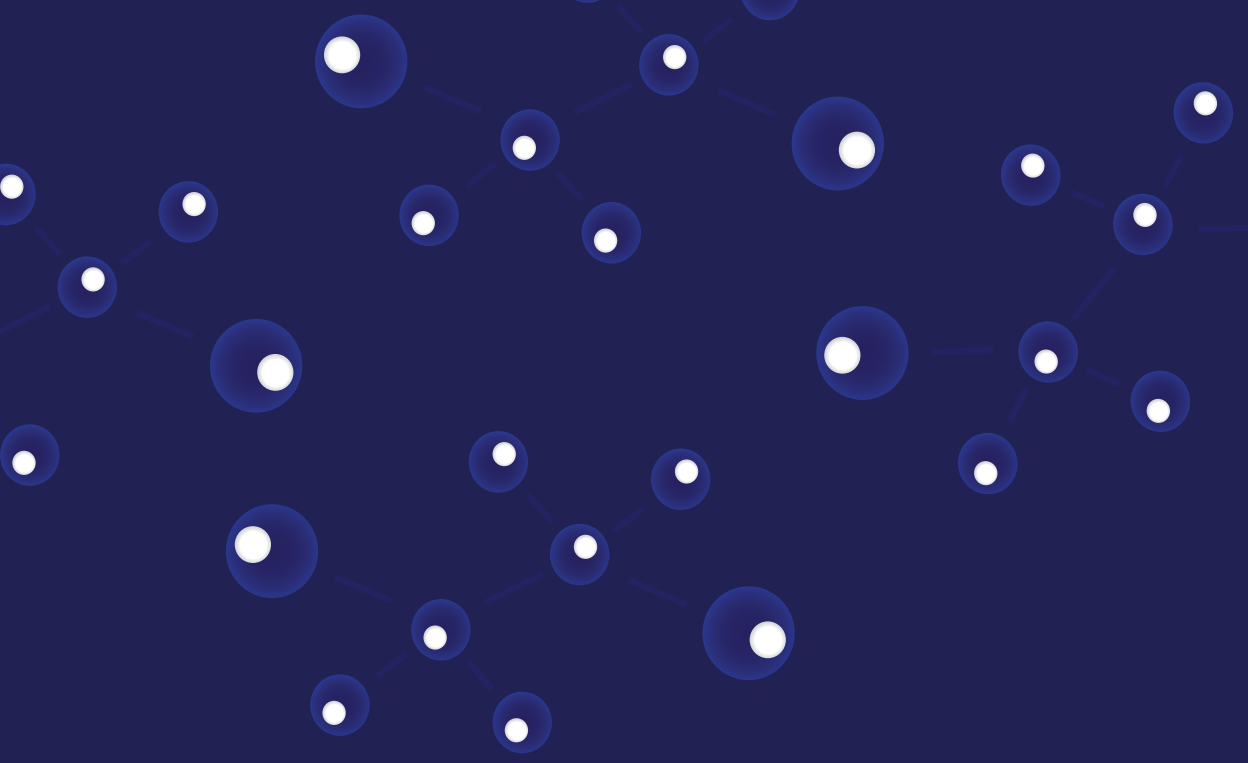

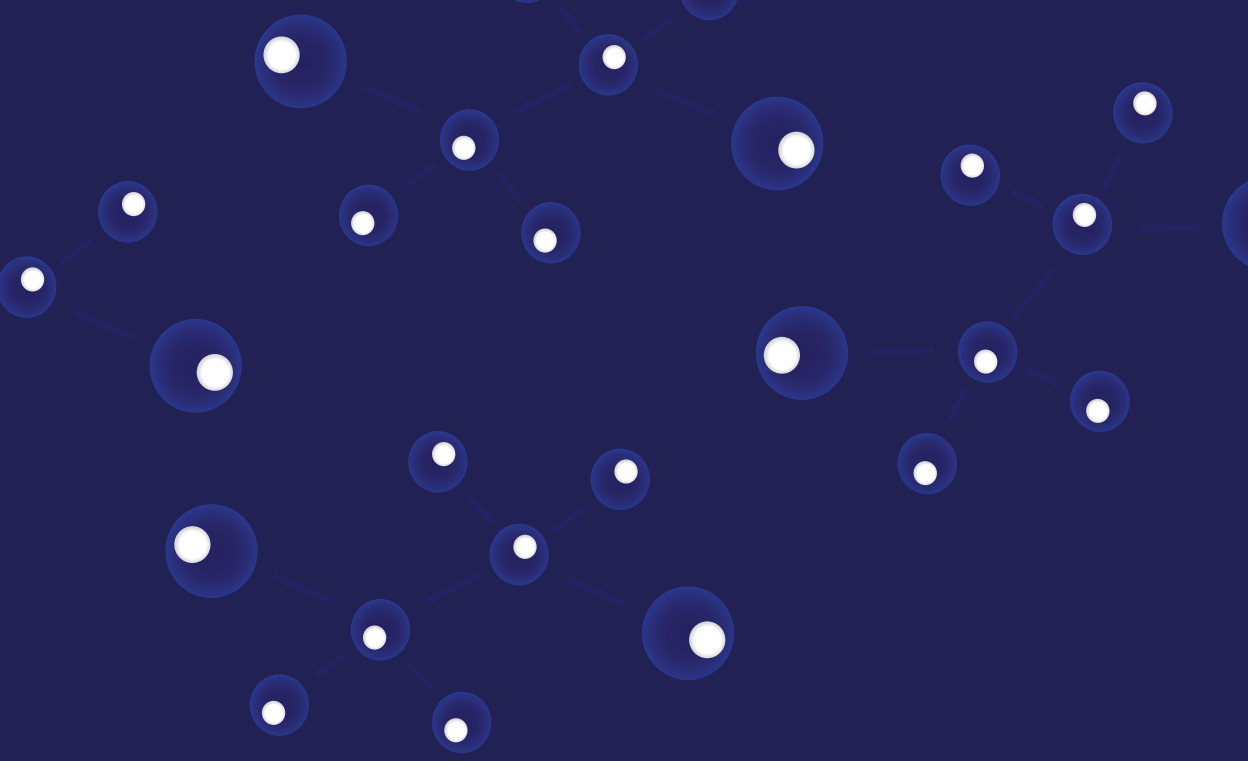

Passamonti S. (Editor)
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trans2care
Transregional Network for Innovation and Technology Transfer to Improve Health Care

# Studies of bone mineral density in children affected by dietary intolerances

Cristina Malaventura, Elisa Ballardini, Alessandro Baldan, Caterina Borgna-Pignatti

PP4-The Pediatric Department of the University of Ferrara

Abstract — The Pediatric Department of the University of Ferrara has developed a special expertise in the field of hemoglobinopathies and has also an interest in gastrointestinal diseases. It has a long standing collaboration with the Department of Genetics of the University of Verona. The main fields of interest are thalassemia, gluten intolerance, and osteoporosis in its various aspects. Using our previous research experience as a platform, we plan to study Vitamin D metabolism, bone mineral density, the FGF23 and Klotho axis in patients with thalassemia, in patients with adult type lactose intolerance, in patients treated with antiepileptic drugs and in those who suffer from gluten intolerance. Finally, we intend to cooperate with another group (PP9) of the Trans2Care project in order to clarify the role of tissue antitransglutaminases in seronegative patients with symptoms of gluten intolerance.

Index Terms — FGF23/Klotho, lactose intolerance, Bone metabolism, Thalassemia, gluten intolerance, pseudoxanthoma elasticum

## 1 INTRODUCTION

The University of Ferrara was founded by Alfonso I d'Este in 1321. It has been the Alma Mater to Paracelsus, Nikolaus Kopernikus, and Pico della Mirandola. Today, the Department of Pediatrics of the University of Ferrara is situated in the Sant'Anna Hospital in the city center centre. but It will be moved soon to a new facility 10 Km to the south where larger and newer spaces will be available. The staff comprises physicians, administrative personnel, one dietitian, and, with the support of Trans2Care, a new collaborator has just been enrolled. The Department has a

special expertise in the field of Thalassemia and other hemoglobinopathies, and in neurological disorders of childhood. In addition, part of our staff has an interest in gastrointestinal diseases.

## 2 ONGOING RESEARCH

### 2.1 Multicentre Study of Survival in Thalassemia major.

The team manager of PP4 has, for the past three decades, coordinated a cooperative study on survival, complications and diagnostic and therapeutic aspects of thalassemia. The results of the study on survival and complications have been regularly published [1], [2], [3], [4]. The most recent data, 20 years from the first collection, show that the prognosis of these patients has greatly improved. In fact, our results show a sharp decrease in mortality in the last few years, testifying to the efficacy of modern therapy. i.e. MRI for the quantification of iron in the heart [5] and liver, the oral iron chelators [6], [7], aggressive management of cardiac and hepatic complications.

### 2.2 Feasibility of a screening for hemoglobinopathies

In order to identify early the infants affected by hemogolobinopathies, in particular sickle cell anemia, we are collecting the cord blood from all the neonates who are born in Ferrara. In fact an early diagnosis of sickle cell anemia can prevent infection and death in the first years of life [8]. In the past year, 1600 samples were examined by HPLC and 19 were found to be abnormal.

### 2.3 Morbidity and Mortality according to sex

It is well documented that, female patients with thalassemia major survive longer than males [9]. In order to clarify the origin of this phenomenon, we have measured by magnetic resonance imaging the iron concentration in the heart of 776 patients (370 males) and have compared the results obtained in males and females, with and without cardiac failure or arrhythmias. As expected, cardiac disease was higher in males than in females, but no difference in cardiac iron content was observed according to sex [10]. Therefore, we concluded that males and females are at the same risk of accumulating iron in their hearts but females seem to tolerate iron toxicity better, possibly as an effect of reduced sensitivity to chronic oxidative stress. This study was conducted in collaboration with the Myocardial Iron Network in Thalassemia.

### 2.4 Vitamin D and Osteopenia in Thalassemia

Osteoporosis and osteopenia are frequent complications of thalassemia major and intermedia. In a cooperative study, [11] we found osteoporosis to be present in the great majority of patients with thalassemia intermedia and in 115/239 patients with thalassemia major. In thalassemia major, no association was found with

specific polymorphisms in candidate genes (vitamin D receptor, estrogen receptor, calcitonin receptor, and collagen type 1 alpha 1). Osteoporosis in female patients with thalassemia major was strongly associated with primary amenorrhea, while in male patients, hypogonadism was not significantly related to bone mineral density. Low bone mineral density was also associated with cardiomiopathy, diabetes mellitus, chronic hepatitis, and increased ALT.

### 2.5 Genetic of lactose intolerance

We studied the presence of a substitution of C to T- single nucleotide polymorphism (rs4988235, -13910C>T), at position -13910 bp upstream in the lactose gene (rs4988235), in a population of Italian children and their parents. The children were diagnosed as lactose intolerant on the basis of the breath hydrogen test [12]. The mutation, localized in a regulatory region, was found to be strongly associated with the lactase persistence phenotype in North-European population. We confirmed its presence in Southern Europeans. In fact, the correlation between the C/C genotype ( corresponding to lactose non-digesters) and positive breath test in unrelated family founders was significant. The genetic test compared to the breath test had a sensitivity of 95% and 91% and a specificity of 48% and 55% in adults and children, respectively.

### 2.6 Collaborations

We have a long standing collaboration with the Department of Life and Reproduction, Section of Genetics of the University of Verona, with the Section of Genetics of the Department of Clinical and Experimental Medicine of the University of Ferrara and with the Laboratory of Hematology & Clinical Chemistry of the University Hospital, Ferrara. Our Thalassemia Center is a part of the MIOT project, which is a network of 6 Magnetic Resonance Imaging apparatuses and 56 Italian Thalassemia Centers which share a common clinical and diagnostic database. In addition all the cooperative studies on Thalassemia and its complications are performed in collaboration with seven Italian Centers for the treatment of Thalassemia as listed in [2].

## 3 FUTURE PROJECTS AND TRANS2CARE NETWORKING

### 3.1 Mineral metabolism

In consideration of the work previously performed, as outlined above, we plan to clarify the bone mineral density, and the role of Vitamin D and the FGF23/Klotho axis in patients with thalassemia, in lactose intolerant children, in patients with gluten intolerance and in children treated with antiepileptic drugs. The components of the FGF23/Klotho axis have recently been recognized as important factors in the metabolism of calcium and phosphate. [13] Klotho functions as the obligate co-

receptor of FGF23. Mice lacking both FGF23 and Klotho develop identical phenotypes resembling premature aging syndromes, with hypogonadotropic hypogonadism, muscle and skin atrophy. The Klotho gene was originally identified as a putative age-suppressing gene in mice that extends life span when over-expressed. [14] In addition, Klotho functions as a humoral factor with pleiotropic activities including suppression of oxidative stress. [14] FGF23 regulates excretion of phosphates and synthesis of active Vitamin D in kidneys.[13]
Patients with thalassemia major exhibit many aging-like symptoms including hypogonadism, skin atrophy, muscle wasting osteopenia [15], reminiscent of the Klotho-deficient mice. Vitamin D metabolism and the FGF23/Klotho complex will therefore be studied in these patients and also in different populations of children affected by neurological and gastroenterological disorders.

Bone mineral density will be measured in patients by means of an ultrasonographic apparatus, acquired with Trans2Care funds. that spares the children studied the radiations inevitable with the more commonly used DEXA. [16].

### 3.2 Pseudoxanthoma elasticum in thalassemia

A subgroup of patients with thalassemia major or intermedia also suffer from Pseudoxanthoma elasticum, a condition in which widespread calcification of joints and arterial vessels develop without apparent cause unknown, [17] and in which the Klotho/FGF23 complex could be involved [18][19].
To clarify some of the aspects of this complication that (in the non-hereditary form) affects mainly patients with chronic hemolytic anemias and, in particular, patients with thalassemia major and intermedia, we plan to perform a PXE case-control study of the transcriptome using Next generation sequencing [20][21] of our patients with a and without PXE.

### 3.3 Tissue anti-transglutaminases in patients affected by seronegative gluten intolerance.

This part of the reaserch project will be conducted in collaboration with PP9. In fact, the pediatricians of the Burlo Garofalo Hospital in Trieste have already developed an expertise in this technique that we could use to plan a region-wide (Regione Emilia Romagna) study in seronegative symptomatic patients in whom gluten intolerance is suspected [22].

## 4 END SECTIONS

### 4.1 Acknowledgements

We wish to acknowledge the precious secretarial help of Ms Cinzia Tonioli. Most of the research projects cited were supported by funds provided by the Ministero Italiano dell'Università e della Ricerca scientifica

## 5 CONCLUSION

During the last few years, the Pediatric Department of the University of Ferrara has conducted research in the field of the hemoglobinopathies and in the field of gastroenterology. We intend to pursue the same lines of research, enriching them with the addition of bone ultrasonography and the study of the newly described FGF23/Klotho system that promises to clarify some of the mechanisms at the basis of osteoporosis and extra-osseous calcifications in various diseases. Interweaving with other groups of the Trans2Care net will hopefully allow us to obtain more detailed information on our fields of interest that will provide improvements in prevention, early diagnosis and treatment of chronic diseases and their complications. The training of the researcher recruited by the project will contribute to increase the education and the internationalization of this educated young scientist.

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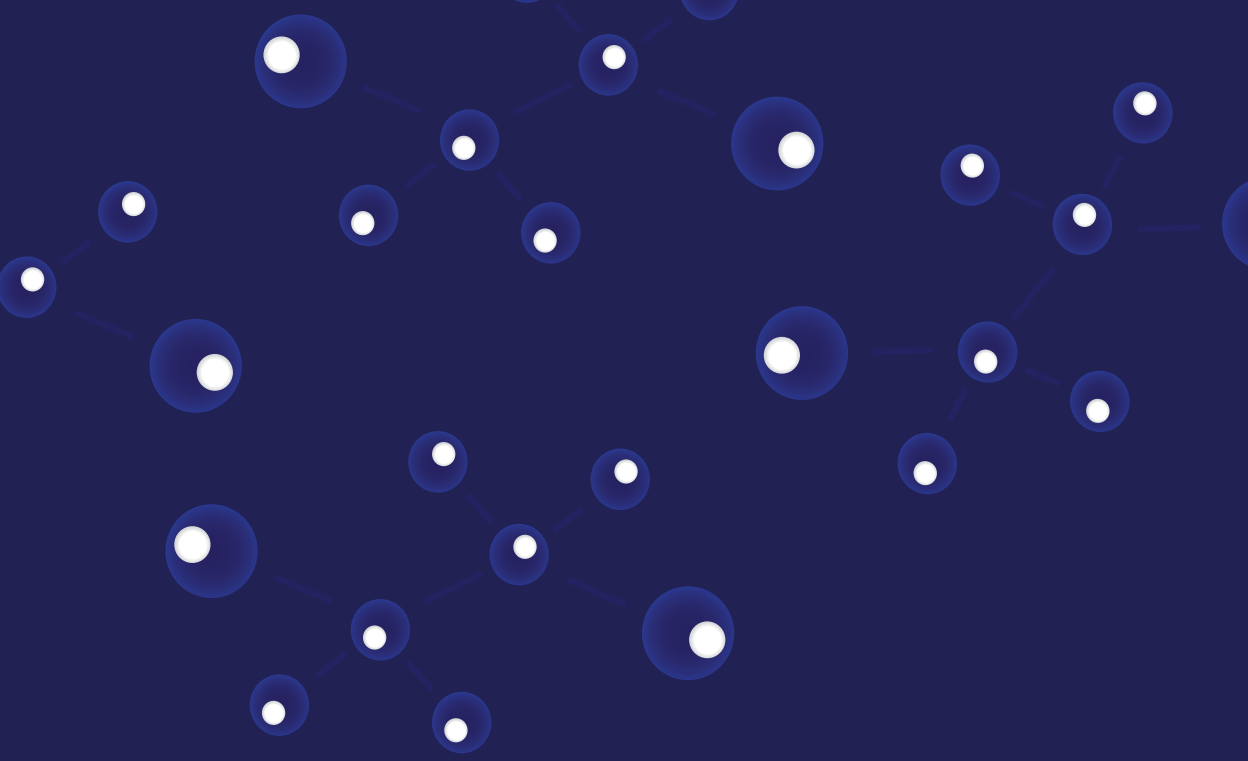

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Transregional Network for Innovation and Technology Transfer to Improve Health Care

# Facilitating collaboration, mobility of researchers and exchange of knowledge between Partners

Giorgia Favaro, Franca Bandiera

PP5-Treviso Technology

Abstract — Treviso Tecnologia is a non-profit entity which offers the market innovative high value-added services together with the development of projects financed on a local, national and European scale that allow a high degree of operational independence. It stands out as a facilitator offering information, education and developing services to promote the continuous dissemination of innovation with companies, leveraging the network between the world of research, university, the chambers of commerce and public institutions. Within the Trans2Care project we intend to apply this approach to innovation methods in several areas related to the problems explored by the Project Partners with the aim of promote collaboration, mobility of researchers and exchange of knowledge between Partners.

Index Terms — high value added services, projects, innovation, network, intellectual property

## 1 TREVISO TECNOLOGIA

Treviso Tecnologia, the Treviso Chamber of Commerce's special agency for innovation, was founded in 1989 to promote an entrepreneurial culture oriented towards innovation. With its workforce of 50 employees and collaborators, Treviso Tecnologia has always invested in the continuous updating of in-company skills in order to better face the need of companies and of local context that is constantly evolving. In this way, Treviso Tecnologia promotes the growth and development of an ecosystem favorable to innovation through the development of factors such as the quality of work, the improvement of managerial skills, the diffusion of ict in every economic sector (Digital Business Ecosystem, according to the EU) and the enhancement of

intellectual property as the currency of the new millennium. Furthermore, Treviso Tecnologia aims at supporting the territory in its ongoing transformation process through the high value-added, innovative services, technological infrastructures and international awards, programmes for the development of skills and professionalism, innovation and technology transfer supporting companies at any level (local, national and European) and finding resources whose final beneficiaries are companies and others. In addition to the corporate headquarters in Lancenigo di Villorba where the Training and Business Services are based, the company also has a product testing and certification centre (CERT) in Rustignè di Oderzo and its Neroluce Laboratory in the Fornace dell'Innovazione in Asolo.

## 2 SERVICES

Our offer of services is organized into four lines of activity:
Innovation and technology transfer: we promote the competitiveness of companies through the collaboration with centres of excellence in the science field within the framework of financed projects or specific projects;
Continuous development of advanced and innovative skills: we design and deliver educational pathways that connect education (high school and university) with the business world, in order to jointly support lifelong learning programmes for companies. These programmes are often financed by participating in local and international calls for tenders.
Delivery of services of excellence: we invest in infrastructures and competence to perform for products testing and certification, with high value-added services in the field of intellectual property protection and international technical regulations.
International network: through cross-functional networks and partnerships with outstanding parties on a European scale, we cooperate in actions and projects aimed at the development of research, innovation and technology. transfer.

### 2.1 Technology transfer activities

Specifically technology transfer activities can be organized in three lines of activities:

- Development and technology transfer: in this sense we conduct evaluation activities and development of Human-Centered design for the creation of high-innovative product concepts. Furthermore, some actions of market foresight, focus group with beneficiaries and technology intelligence activities aiming at let emerge potential development projects are carried out. Consequently a multi-disciplinary team and market orientation are the ingredients for a better competitiveness.
- Tests and product certifications: we offer test and certification services for analyses in the agri-food sector, the oenological sector, in particular, as well as in the industrial sector also identifying the presence of harmful or toxic substances through the Chemical Laboratory. We can evaluate and certify the reaction to fire and the energy classification of building, we can test and certify products including wood and other construction materials for EC marking and we can measure the presence of harmful or toxic substances in fabrics and leathers.

- High value added services: specifically in the field of Intellectual Property, the Patent Library Office offer high value added services for the protection and valorisation of Intellectual Property. The office offer priority searches on trademarks, patents and designs and communicate all information needed to understand and afford national and international application procedures. Beneficiaries can also receive technical assistance on the definition of claims as well as support and legal assistance for the developing of strategic and market solutions.

Some Tech Transfer Projects carried out by Treviso Tecnologia are the following:

| TITOLO | PROGRAMMA | SITO INTERNET | RUOLO | DATA CONCLUSIONE |
|---|---|---|---|---|
| EMUVE – Entrepreneur Multi-User Virtual Environment | Toi Leonardo da Vinci (Programma LLL) | www.emuve.euit/news.php | Capofila | 30/09/2012 |
| IP for SMEs - L'integrazione transfrontaliera nella gestione della proprietà intellettuale (IP) come leva di competitività regionale | InterReg 4c ITALIA - SLOVENIA 2007 -2013 | www.innoskills.net | Capofila | 31/4/2014 |
| LEONARDO REBASING - Research-based Competence Brokering | Toi Leonardo da Vinci (Programma LLL) | www.leonardorebas-ing.eu | Partner operativo | - |
| TRANS2CARE - Rete Transnazionale per l'Innovazione ed il Trasferimento Tecnologico per il miglioramento della sanità | InterReg 4c ITALIA - SLOVENIA 2007-2013 | www.trans2care.eu | Partner operativo | 30/09/2014 |
| IPR for SEE: intellectual Property Rights for SEE | Interreg SEE | www.iprforsee.eu | Partner operativo | 31/12/2012 |
| IP-SMEs - IP Awareness and Enforcement innovative services for Mediterranean SMEs | Interreg MED | www.ip-smes.eu | Partner operativo | 31/05/2013 |

| TITOLO | PROGRAMMA | SITO INTERNET | RUOLO | DATA CONCLUSIONE |
|---|---|---|---|---|
| RFID from Farm to Fork (RFID-F2F) | CIP | www.rfid-f2f.eu | Partner operativo | 31/08/2012 |
| TEMP Textile Excellence in EU-MED Partners | FP7 Kcitis Commissione Europea | www.temp-eumed.eu | Partner operativo | 30/11/2012 |
| Rapid open Innovation - speeding time to market (Rapid OI) | Cooperazione trans-frontaliera Italia-Austria | www.rapid-innova-tion.net | Capofila | 28/02/2015 |
| Sviluppo di un sistema di diagnosi dello stato per il riciclo attivo degli accumulatori del futuro | Cooperazione trans-frontaliera Italia-Austria | - | Partner operativo | 30/06/2014 |
| PIACE - Piattaforma intelligente, integrata, adattiva di microcogenerazione ad elevata Efficienza per usi residenziali | Industria 2015 | - | Partner operativo | 30/6/2013 (proroga ?) |
| PROsumer.NET | FP7 Cooperation | prosumernet.eu | Partner operativo | 01/06/2013 |
| R.I.ECO - Rete innovativa per la valutazione della ECOcompatibilità dei prodotti | POR Azione 1.1.1 | - | Capofila | 04/10/2012 |
| Reaorienting Patent Information Centres | EPO | - | Capofila | 01/11/2013 |
| SILE | Legge Regionale 9 | - | Partner operativo | 01/01/2012 |
| FAST FORWARD 2009 | Contributo Camerale di Treviso | - | Capofila | - |
| FAST FORWARD 2010 | Contributo Camerale di Treviso | - | Capofila | - |
| Trasferimento tecnologico, innovazione delle filiere produttive e rapporti con il mondo della ricerca universitaria | Fondo di perequazione | - | Partner operativo | 31/03/2011 |

## 3 ROLES IN TRANS2CARE

### 3.1 What offer to T2C

The role of Treviso Tecnologia within the Trans2care project is put our services at disposal to all Partners and share our knowledge on innovation and technology transfer. We would like to act as facilitator for the creation of a network that includes several actors like Universities, big industries, research centres, hospitals, tech transfer offices, public institutions, SMEs, laboratories and the market. Treviso Tecnologia, as Project Partner of Trans2care, intend to transfer its approach to innovation methods to the Partners and to the Project, that include the consideration of the importance of technology transfer activities, with the aim of promote cooperation with external partners, expedite mobility of researcher and foster the exchange of knowledge and expertise among Partners.

## 4 CONCLUSION

Through Trans2Care project, we intend to contribute with our expertise to reach the common Project goal, that is to establish interesting collaborations and connect the Project Partners in order to eventually improve their expertise and to promote exchange of ideas and technology transfer activities.

### ACKNOWLEDGEMENT

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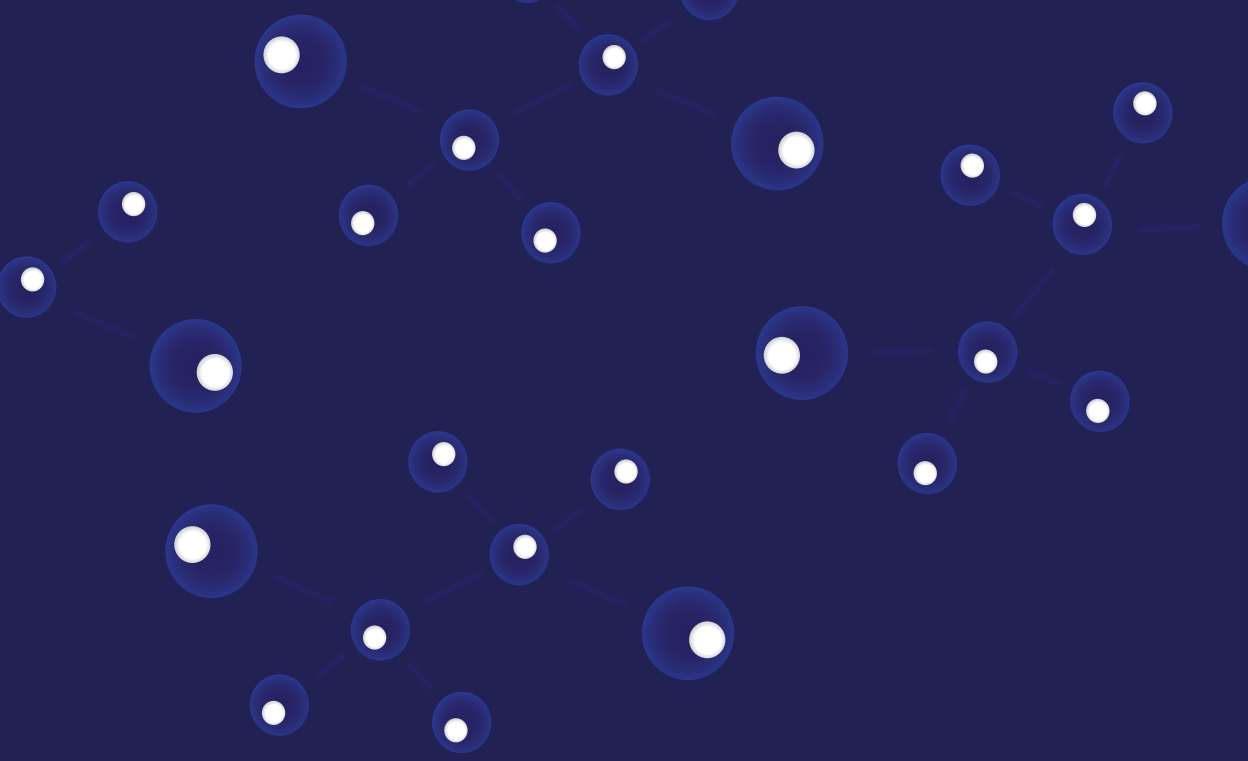

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# Early diagnosis of contrast media-induced nephropathy in intensive care

Matjaž Klemenc, Polona Likar

PP6-General Hospital of Nova Gorica

Abstract — At the Research Department in hospital of Nova Gorica we are faced with new cathlab practice and problems connected with it. Together with other project partners we intend to investigate contrast media induced nephropathy by measuring the release of NGAL, which is considered to be a good biomarker for detecting reduced kidney function. Our first priority is extensive study of NGAL and develop special detecting lab-on-a chip that should be small and simple to use at every day hospital practice. Our second priority is to develop new and intensify pre-existing collaborations and exchange of knowledge between project partners.

Index Terms — Catlab practice, biomarker, Neutrophil gelatinease-associated lipocalin (NGAL)

## 1 GENERAL HOSPITAL OF NOVA GORICA

General Hospital of Nova Gorica is the youngest general hospital in Slovenia, from the health care program is the medium size Slovenian hospital, which is characterized by rapid growth and large dynamic processes. From an entrepreneurial point of view, the hospital is a large company and one of the largest companies in Primorska region.

The hospital was founded in 1956. At the beginning it was located in old adapted buildings and had four main departments: internal medicine, surgery, paediatrics and gynaecology. In the year of 1965 the state began to build new hospital which was finished in 1985. The new 536-bed hospital has a surgery, internal medicine, gynaecology, paediatrics, ophthalmology, ENT, orthopaedic departments. It has a 750 employee, from which 82 specialists, 4 with PhD and 2 with BSc.

## 2 RESEARCH DEPARTMENT

The research department in Hospital Nova Gorica is located at the unit of intensive care and cardiology. The rapid medical technology development requires constant education and follow new developments. At our research department doctors and researchers are working together to investigate appearing medical questions and seeking for new knowledge and better solutions.

### 2.1 Research Areas

The intensive care unit and cardiology were engaged in various research fields, such as (i) arterial hypertension, (ii) autonomic nervous system activity, (iii) baroreflex sensitivity, (iv) diastolic dysfunction of the left ventricle, (v) and vascular elasticity (vi). The ICU and cardiology department has already established a strong collaboration with University of Nova Gorica and Physiology and Pathology Department at University of Trieste through various studies exploring human nervous system [1-6]. We are also connected with researchers of Faculty of Computing and Informatics Institute (FRI) at University of Ljubljana .The result of this collaboration is special device and software for analysis of changes in velocity of blood in the arteries, which are now used in the study of elastic arteries in hypertensive patients [7-15]. Furthermore, The ICU and cardiology department has also established collaboration with Texas Heart Institute. As mentioned above, establishing collaborations with different institutions to reach constant flow of knowledge and technology transfer has always been our priority. We intend to continue with building a network of our collaborations through Trans2care project.

### 2.2 Activities in Trans2care project

Catheterization laboratory practice and contrast media
Lately medical contrast technology continues to evolve dramatically. As scanning technology advances so does the requirements for contrast media. At the hospitals we are faced with fast development of various types of contrast media with different enhancement effect and also wide range of side effects. The most common types of contrast medium for enhancing x-ray based imaging methods are iodine and barium. Various sorts of iodinated contrast media exist, with variations occurring between the osmolality, viscosity, absolute iodine content and concentration of iodine in different media [16]. However, recent surveys are shown that High Concentration Contrast Media (HCCM) allows a greater maximum enhancement in a shorter period of time using the same volume of contrast medium [16]. While modern contrast media are generally safe to use [17] medical conditions can be caused by the administration of various contrast media. At the research department at Hospital of Nova Gorica we are focused on risk of contrast-medium induced nephropathy (CIN) in high risk patients, especially in patients with reduced kidney function. To diagnose the kidney failure the technology for diagnosis is required. For this reason we are interested in biomarkers for detecting early stages of CIN and supporting technology that should be small, simple, fast and with large data capacity.

### 2.3 NGAL as biomarker

The recent studies show that Neutrophil gelatinase-associated lipocalin or NGAL (also known as lipocalin 2, oncogene protein24p33 or uterocalin) is appropriate biomarker for acute kidney injury [19]. This is small, robust protein that belongs to the lipocalin family of the proteins. It is expressed by neutrophils and various epithelia, including the renal proximal tubules. Because of its small molecular size and resistance to degardation it is readily excreted and detected in the urine. While the functions of NGAL are not fully understood, it appears to be upregulated in cells under stress. However, NGAL levels rise significantly in both urine and blood as a response to kidney injury what makes it a useful marker of such injury [18]. The NGAL is mostly quantified by researchbased ELISA assays, which are impractical in the clinical setting. The development of small lab-on-a chip for simple and quick clinical measurement of plasma and urine NGAL would make it easier. It would also enable clinical investigators to assess the potential of this molecule as a diagnostic marker or a marker for response to use of contrast media.

## 3 HOSPITAL OF NOVA GORICA IN TRANS2CARE

Through Trans2care project we intend to study contrast induced release of NGAL. We will continue the collaboration with University of Nova Gorica to study the chemistry of NGAL and possibly develop specific lab-on-a-chip. We also see the possible connection with University in Trieste, where they can help us with specific studies of NGAL for instance reproducing in it vitro on endothelial cells, test NGAL on kidney cells (cytotoxicity assays). On the other hand we can offer other project partners data and knowledge of cardiovascular technology.

## 4 CONCLUSION

Through the initiated Trans2Care project we intend to contribute through the activities and studies aimed to a deeper understanding of NGAL function and developing detection technologies. That will help us at diagnostic of reduced kidney function and also at studies of contrast-media induced release of NGAL. On this way we can compare the safety of different types of contrast media. Our other goal is to connect with the research institutes, universities within the project partners to build the network of knowledge, innovation and technology transfer.

Acknowledgement
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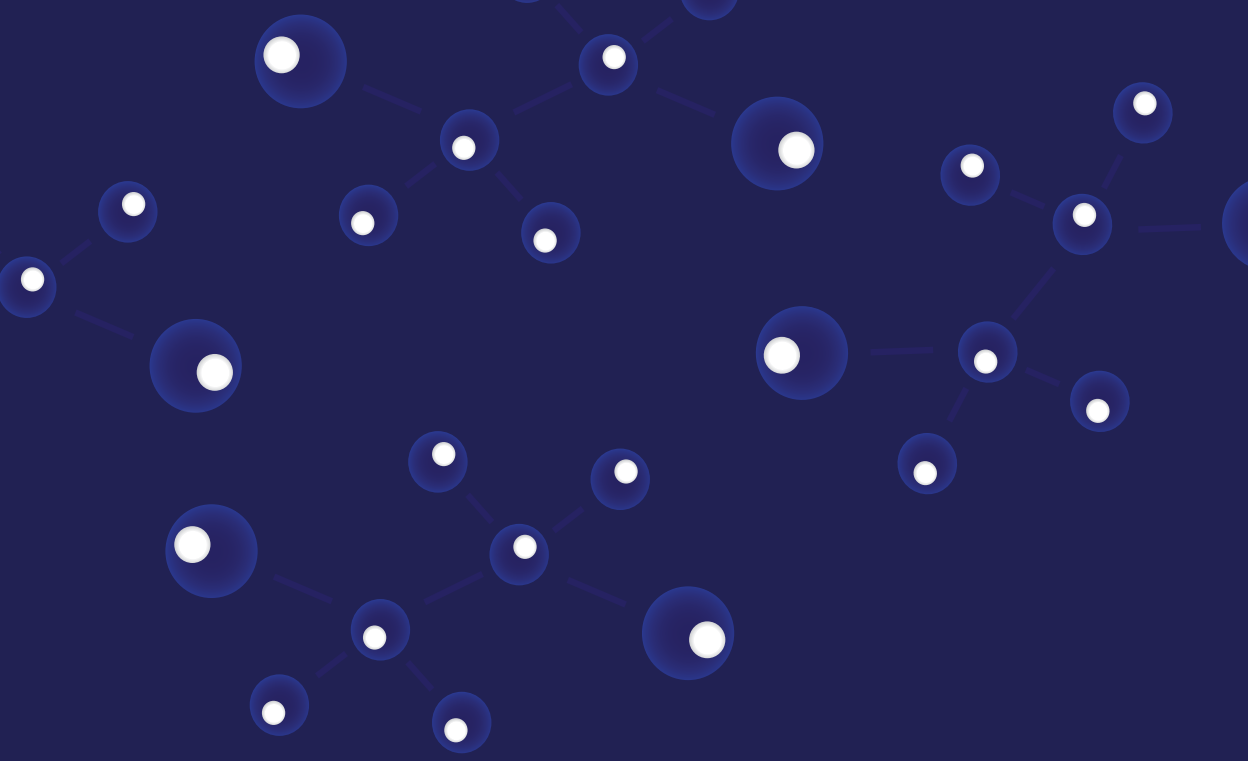

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# Molecular diagnostics with electrochemical biosensors and arrays

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Abstract — Biosensors are self-contained analytical devices in which a bioreceptor is integrated with a transducer. The interaction between the bioreceptor and a target analyte generates a signal suitable for analytical purposes. In electrochemical biosensors, a change in the redox state of the biorecognition/analyte system generates a change in an electrochemical quantity which can be monitored by electroanalytical techniques. Electrochemical sensors can be miniaturized using ultramicroelectrodes and nanoelectrodes and their arrays as transducers. These devices are characterized by high specificity and sensitivity and improved detection limits. Biosensors can be used by non-specialist operators at the point of care. For the above reasons, within the frame of the Trans2care project, the Laboratory of Electrochemical Sensors of the University Ca' Foscari of Venice will collaborate with the project partners to develop electrochemical sensors suitable for specific clinical needs.

Index Terms — electrochemistry, biosensors, affinity, proteins, analysis, electrodes, array.

## 1 INTRODUCTION

Biosensors are analytical devices in which a molecular recognition layer is integrated with a transducer. The immediate environment of the bioreceptor can change as a consequence of the interaction with the target analyte so generating a measurable signal. With a biosensor it is possible to measure the target molecule directly, without using any additional reagent.
In electrochemical biosensors a change in the redox state of the biorecognition layer produces a change in an electrochemical quantity (a Faradaic current or an electrical potential) which can be monitored by electroanalyticall techniques [1]. In this

research the focus will be on amperometric and voltammetric methods of detection. Interestingly, electrochemical systems can be miniaturized from the millimeter down to the sub-micrometer scale; moreover, it is possible to use individual micrometer or nanometer sized working electrodes as well as arrays of them. In particular, the use of arrays of ultramicroelectrodes (UMAs) or nanoelectrodes (NEAs) allows one to overcome problems related to the requirement for high signal amplification and careful shielding from electrical noise. Signals given by UMA and NEAs are indeed the summation (weighed by diffusion effects) of the signals generated at each single micro(nano)electrode [2]. UMAs and NEAs are characterized by very high signal/noise ratios, therefore they allow one to achieve very low detection limits, of the order of nanomolar concentrations or, as absolute quantity, few picomoles or even femtomoles.

In typical schemes used in electrochemical biosensors, a biorecognition layer is directly immobilized on the electrode surface and the signal is produced by exchange of electrons between this layer and the underlying electrode. Analytes which can be detected includes small redox molecules such as enzymatic co-factors, vitamins, oligopetides, enzyme substrates, antigens and antibodies, aptamers, oligo- and polinucleotides. Note that in the case of miniaturized electrodes, such as in the case of nanoelectrodes, the amount of immobilized biomolecules can be very small with obvious advantages in the case of expensive or difficult to purify bioreceptors.

Depending on the nature of the biorecognition layer, electrochemical sensors can be classified into two categories: biocatalytic and bio-affinity sensors.

In biocatalytic sensors, the biorecogniton layer is composed by an enzymatic layer, (typically an oxido-reductase or a dehydrogenase, immobilized on the electrode/array) which exchanges electrons with the metal surface of the electrode via a suitable redox mediator (see Figure 1).

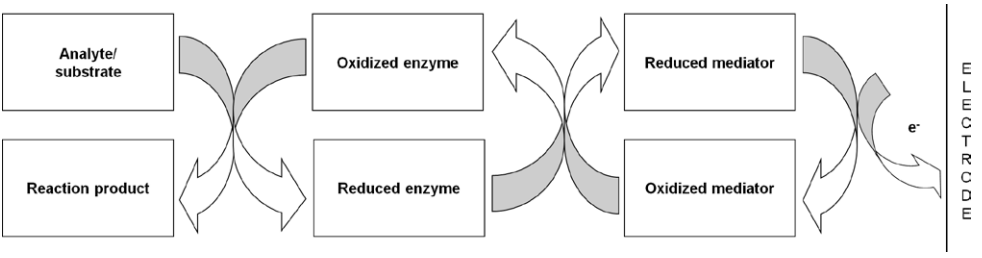


Figure 1. Recognition and signal generation in electrochemical enzymatic biosensor.

In bioaffinity electrochemical biosensors, the recogniton layer is obtained by immobiliation of antigen or antibody molecules; typically, redox enzymes are used as labels. The functioning scheme of some typical electrochemical immunosensors are summarized in Figure 2.

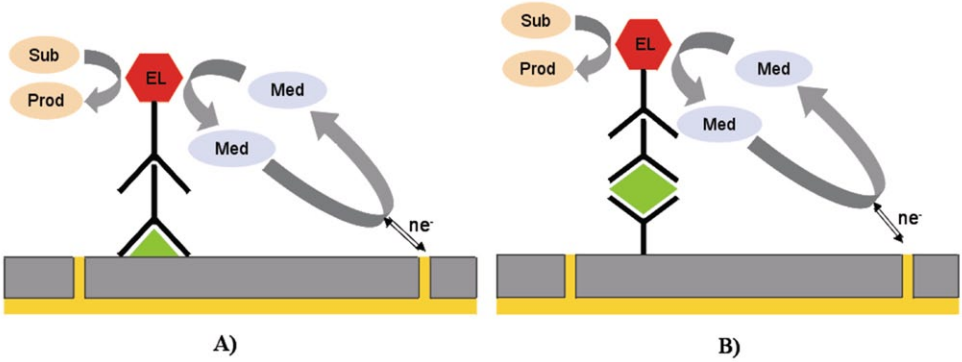


Figure 2: Schematic illustration of the two detection approaches used with electrochemical immunosensors: (A) the target protein is immobilized on the electrode/array, then it binds a primary antibody and secondary antibody with the enzyme label (EL); a soluble mediator (Med) shuttle electrons from the electrode to EL. (B) A primary antibody, specific for the target protein, is immobilized in order to capture the target protein. The other components follow as in scheme A.

The blood glucose sensor is a biocatalytic sensor which represents the most successful commercial biosensor developed so far. It employs amperometric detection and exploits the specificity of the enzyme glucose oxidase and a ferrocene-based redox mediator to produce a measurable current proportional to the blood glucose concentration. About 5-6 % of the population in western countries suffer from diabetes so that the glucose biosensor market growth is continuously growing.. According to a recent report [3], the global market for glucose biosensors and strips will reach 11.5 billion USD by 2012. It is obvious to expect that other kinds of biosensors will be developed to be ready for practical application in the near future. Note that biosensors can be used by non-specialist operators at the point of care and this allows for immediate action to be taken.

## 2 ACTIVITY AT THE UNIVERSITY CA' FOSCARI OF VENICE

### 2.1 The University Ca' Foscari of Venice

Established on August 6th 1868 as a Royal Business College, the University Ca' Foscari University of Venice actively participates in the city cultural life, organizing over 400 events every year, collaborating with other prestigious cultural institutions. The activity in Ca' Foscari is summarized by the following data:
4 main scientific-cultural areas: Economics, Humanities, Languages, Sciences;
15 First Cycle Degree Programmes;
29 Second Cycle Degree Programmes;
28 Specialist Master's Programmes;
1 Doctoral School, 16 Research Doctorate Programs;
9 Summer and Autumn Schools;
8 Departments, 6 Interdepartmental Schools;

1 Learning Centre Library,4 subject-related Libraries, 7 Departmental Libraries;
20,000 students, 4,000 new enrolments per year, 3,300 graduates per year
1,700 professors, lecturers, native language teachers and administrative staff.
Scientific research is carried out through Departments, Interdepartmental Schools and Doctoral Schools, which often work together on inter-disciplinary projects.
Ca' Foscari holds relationships with several associations and institutions through agreements for cooperation in the area of scientific information, teaching and research.
The University is a member of various research bodies including the Venice International University - VIU, Consorzio Venezia Ricerche and VEGA Science and Technology Park of Venice.

## 2.2 The laboratory of electrochemical sensors

In the last sixteen years the group of Electrochemical Sensors at the University Ca' Foscari of Venice, Department of Molecular Sciences and Nanosystems, has developed original know-how in the field of the fabrication and analytical application of ensembles and arrays of nanoelectrodes, nanostrutured electroactive membranes and polymer-based electrodes [2].
Among the other fabrication methods, the preparation of arrays of nanoelectrodes by controlled deposition of metal nanoelements within the pores of ultrafiltration membranes used as template, is an attractive and increasingly used nanofabrication procedure. Electroless deposition of gold in polycarbonate templates for producing NEAs was introduced some years ago by Menon and Martin [4] and was refined more recently in our laboratory [5]. Thanks to their peculiar geometry, NEAs are characterized by improved signal/noise ratios and by detection limits 2-3 orders of magnitude lower than those achievable with regular electrodes.
Among the bioanalytical applications of NEAs and nanostructured membranes developed in Venice it is worth listing:
-an electrochemical biosensor for the detection of nitrate which employs nitrate reductase as the biorecognition element [6];
-the direct electroanalysis of the redox enzymes such as cytochrome c with NEAs and with polymer coated electrodes [7, 8];
-an electrochemical sensor for glucose analyses based on NEAs which employs glucose dehydrogenase and a special redox mediator (a nitrofluorenone), developed by coworkers at the University of Bordeaux 1, which is very efficient in catalyzing NADH oxidation [9];
-the development of NEA-based electrochemical immunosensors applied, in collaboration with ICGB-Trieste (prof. G. Stanta) for the detection both of general model protein such as the single chain fragment variable (ScFV) protein [10], and of clinically relevant proteins such as the HER2 receptor [11]. The HER2 receptor is an important target protein for the identification of cancer that can be treated successfully with Herceptin (Trastuzumab) by the so called personalized therapies.
In recent studies carried out in collaboration with AB-Analitica srl (Padua), with CIVEN (Coordinamento interuniversitario veneto per le nanotecnologie; Porto Marghera-

Venezia) and with the Karlsrue Institute of Technology (group of Dr. Ljljiana Fruk), we developed a new procedure suitable for immobilizing oligonucleotides on NEAs aimed at using the biofuntionalized electrodes for the electrochemical detection of viruses.

## 3 ACTIVITY WITHIN THE TRANS2CARE PROJECT

The collaboration with other Trans2care partners will be finalized to the development both of biocatalytic and bioaffinty electrochemical biosensors, to detect suitable target analytes relevant for the partners involved in clinical and diagnostic practice.

IAs far as biocatalytic sensors are concerned, the development of an enzymatic electrochemical sensor for bilirubin analysis will be studied. Bilirubin is the breakdown product of the haem moiety of haemoglobin and other haemoproteins. Because of internal hydrogen bonding, bilirubin is water-insoluble and requires enzyme-mediated glucuronidation in the liver for biliary excretion. In normal circumstances, plasma bilirubin is mostly unconjugated and is tightly bound to circulating albumin. In cases of inherited or acquired deficiencies of bilirubin storage or excretion, both conjugated and unconjugated bilirubin accumulate in the plasma [9].The biosensor to be developed will be based on bilirubin specific enzymes such as bilirubin oxidase or biliverdin reductase, focusing on the discrimination between conjugated and unconjugated bilirubin. This work will be developed mainly in collaboration with the leader partner.

In the field of bioaffinty sensors, the focus will be on electrochemical immunosensors with capabilities to detect allergens, gluten and related antibodies, to be developed in collaboration with the leader partner and with project partner 2. The work can be potentially extended to the analysis of other proteins taken as biomarkers of different diseases, so involving also other project partners.

## 4 CONCLUSION

The know-how in the field of analytical application of electrochemical nano-biosensors acquired by the group at the University Ca' Foscari thanks to the present project can be extended and find practical application to the determination of clinically relevant molecules. Our participation in the Trans2Care project together with the other specialized partners, each bringing different know-how ranging from biochemistry to clinical practice, constitutes an important occasion and a challenge for new developments in the field of molecular diagnostics. Electrochemical biosensors can indeed be very helpful for obtaining quick and reliable analytical information thanks to their sensitivity, relative low cost, possibility of decentralized use and simple applicability.

## ACKNOWLEDGEMENT

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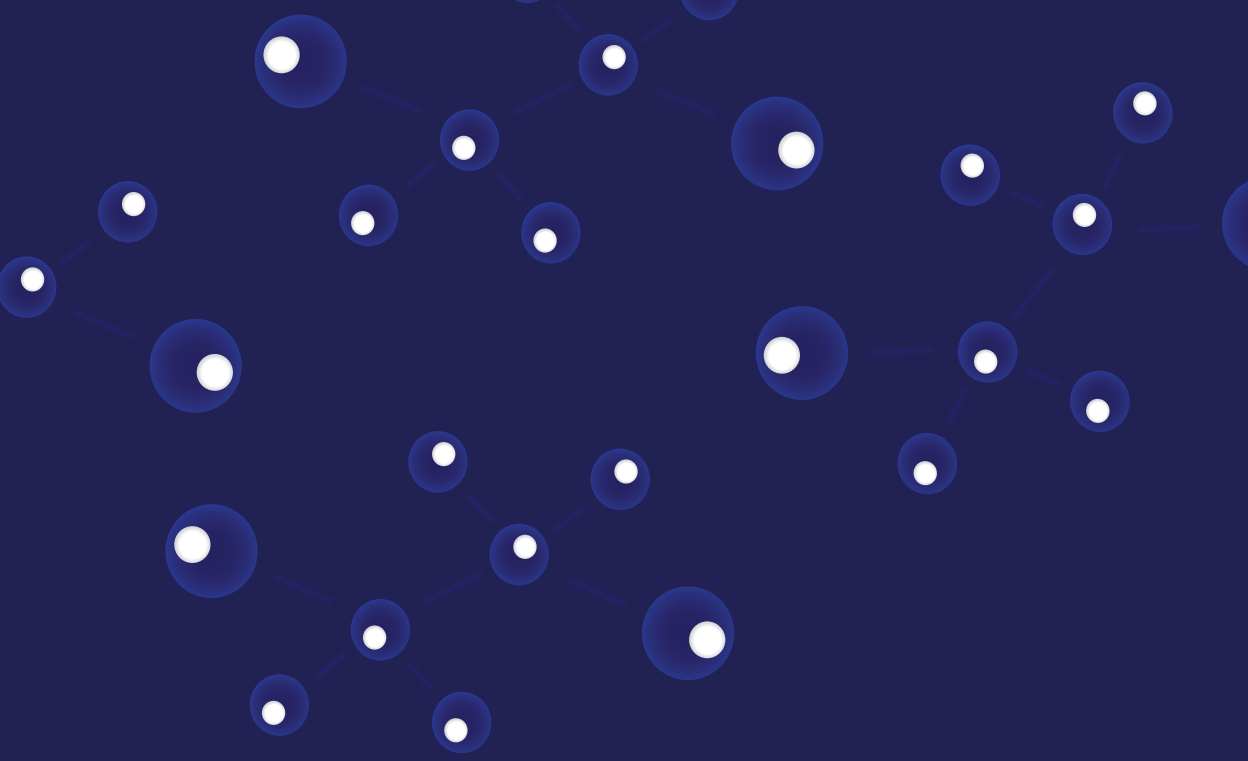

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# Exploring the biological properties and therapeutic potential of antimicrobial peptides

Francesca D'Este, Barbara Skerlavaj, Linda Tomasinsig, Michele Scarsini, Margherita Zanetti

PP8-University of Udine

Abstract — The researchers involved in the Trans2Care project at the Department of Medical and Biological Sciences of the University of Udine investigate the biological properties of the antimicrobial peptides (AMPs) of the immune system and their therapeutic potential for human and veterinary application. In addition to potent and broad-spectrum antimicrobial activities, some AMPs display anti-inflammatory and immunomodulatory effects and hold promise as novel antiinfective agents combining antibiotic and immunostimulating properties. A detailed knowledge of their physicochemical, biological and pharmacological properties and of their impact on clinical settings is an important prerequisite to this end. The Trans2Care project offers an invaluable opportunity to share knowledge, technical expertise and laboratory facilities to achieve a better understanding of the biological features and therapeutic potential of AMPs.

Index Terms — antimicrobial peptides, anti-infective drugs, antimicrobial activity, cytokine release, immunomodulatory activity

## 1 UNIVERSITY OF UDINE

The University of Udine is a public institution founded in 1978 as part of the reconstruction plan of Friuli after the earthquake in 1976. It is devoted to higher education, research and technology transfer. The University is organized into 10 faculties and 14 research departments with approx. 16000 enrolled students (a. y. 2009-2010). The University is actively involved in student and staff exchange projects with universities within EU and other non-EU countries and participates in many national and international research projects.

## 2 WORKING UNIT

The Udine working unit includes researchers interested in the characterization and development of plant-based models of drug transport (Department of Agricultural and Environmental Sciences), and others involved in the study of antimicrobial peptides (AMPs) as templates for the development of novel anti-infective drugs (Department of Medical and Biological Sciences).

## 3 ACTIVITIES AND ROLES IN TRANS2CARE PROJECT

### 3.1 Antimicrobial peptides (AMPs)

AMPs are small, evolutionarily conserved protein components of the innate immune system involved in first-line host defence against infection [1,2]. In mammals, two distinct families of such peptides, the defensins [3] and the cathelicidins [4], have been detected on epithelial surfaces and in circulating phagocytes. These peptides are either constitutively expressed or readily inducible upon infection, inflammation or injury [5]. AMPs are quite diverse by length, sequence and secondary structure. Common features of these peptides are a small size (12-50 amino acid residues), a net positive charge and an amphipathic character [1]. These features enable AMPs to interact with negatively-charged microbial membranes and affect the membrane integrity, leading to microbial killing [1,5]. Besides exerting direct antimicrobial activities, several AMPs have shown the ability to interact with host cells and influence cellular processes relevant to inflammation and immunity such as chemotaxis and cytokine and chemokine release, and to promote angiogenesis and wound healing [2,5].

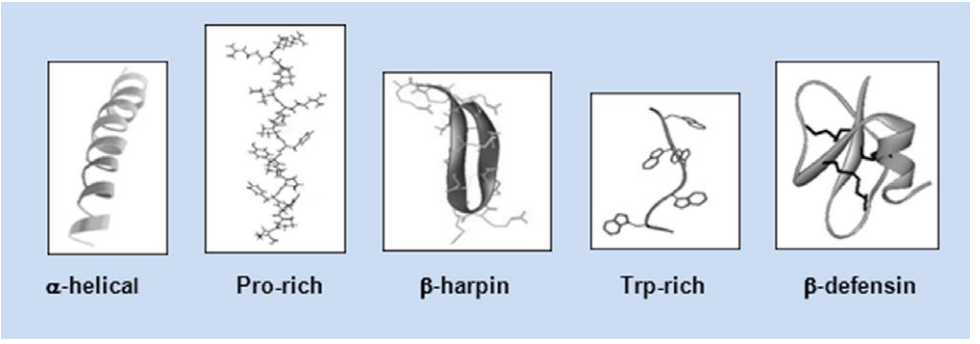


Fig. 1. Molecular diversity of mammalian AMPs.

### 3.2 AMPs as templates for the development of novel antiinfective drugs

Various AMPs have received attention as candidates for the development of novel anti-infective drugs [2,6] to be used in human and veterinary medicine. They show several attractive properties in this regard, including i) a potent antimicrobial

activity against a wide range of Gram-negative and-positive bacteria and fungi, also including multi-drug resistant clinical isolates [6,7]; ii) in vitro antimicrobial efficacy in the submicromolar range [6]; iii) low propensity to select resistant mutants [5,6]; iv) ability to neutralize proinflammatory microbial components such as the lipopolysaccharide and lipotheicoic acid [6,8]; v) ability to modulate host cell functions [2,5,7]. Our increasing awareness of the multiple activities of AMPs has encouraged studies aimed to evaluate the impact of these molecules on pathophysiological processes taking place at specific clinical settings, to define their anti-infective role and achieve a safe clinical application.

### 3.3 Ongoing research activities

The AMPs under study in our group are examined for i) antimicrobial activity against clinical isolates from epithelial infections; ii) immunomodulatory potential; iii) cell proliferation or cytotoxicity-inducing effects.

i) We are currently investigating the activities of selected AMPs against clinical isolates of Candida albicans from human vaginal infections and opportunistic yeast pathogens from other sources. The antifungal activity is determined as the minimum inhibitory and the minimum fungicidal concentration (MIC and MFC), according to the CLSI guidelines. The efficacy of AMPs is also examined using medically relevant fungal biofilms, implicated in increased fungal pathogenesis and resistance to drugs [9], and adhesion to biotic and abiotic surfaces [10]. Yeast viability is assessed by optical density, enumeration of colony forming units (CFU) and XTT tetrazolium salt-based assay. The effect of AMPs on yeast membrane integrity is quantified by flow cytometric and spectrofluorimetric analysis of the cellular uptake of the fluorescent dye propidium iodide (PI).

ii) The ability of AMPs to induce cytokine and chemokine gene expression in epithelial and macrophagic cells is assessed by qPCR and ELISA.

iii) Cell proliferation is assayed by a tetrazolium salt-based colorimetric assay. The cell membrane integrity is assessed by measuring the cellular uptake of PI and the extracellular release of the cytoplasmic enzyme lactate dehydrogenase. Apoptotic effects are evaluated by flow cytometry using the annexin V/PI assay.

## 4 CONCLUSION

A detailed knowledge of their physicochemical, biological and pharmacological properties is a basic prerequisite for translating native peptide molecules into innovative products for prevention of infectious/inflammatory diseases. The Trans2Care project offers an opportunity to share knowledge, technical expertise and laboratory facilities, that can be invaluable to increase our understanding of the biological features and therapeutic potential of AMPs. Critically important in this regard is the collaboration with Trans2Care partners involved in clinical activities, to set up experimental models of infection and inflammation and evaluate the role and efficacy of AMPs in specific clinical settings.

## ACKNOWLEDGEMENT

The AMP research in our group is supported by the Italian Ministry of Education, University and Research (Progetti di Ricerca di Interesse Nazionale 2007), Interuniversity Consortium for Biotechnology (CIB) and Regione Friuli Venezia Giulia (Grant art. 23 L.R. 26/2005). We greatly acknowledge the financial support of the Fondo europeo di sviluppo regionale (Evropski sklad za teritorialni razvoj) for the Trans2care project.

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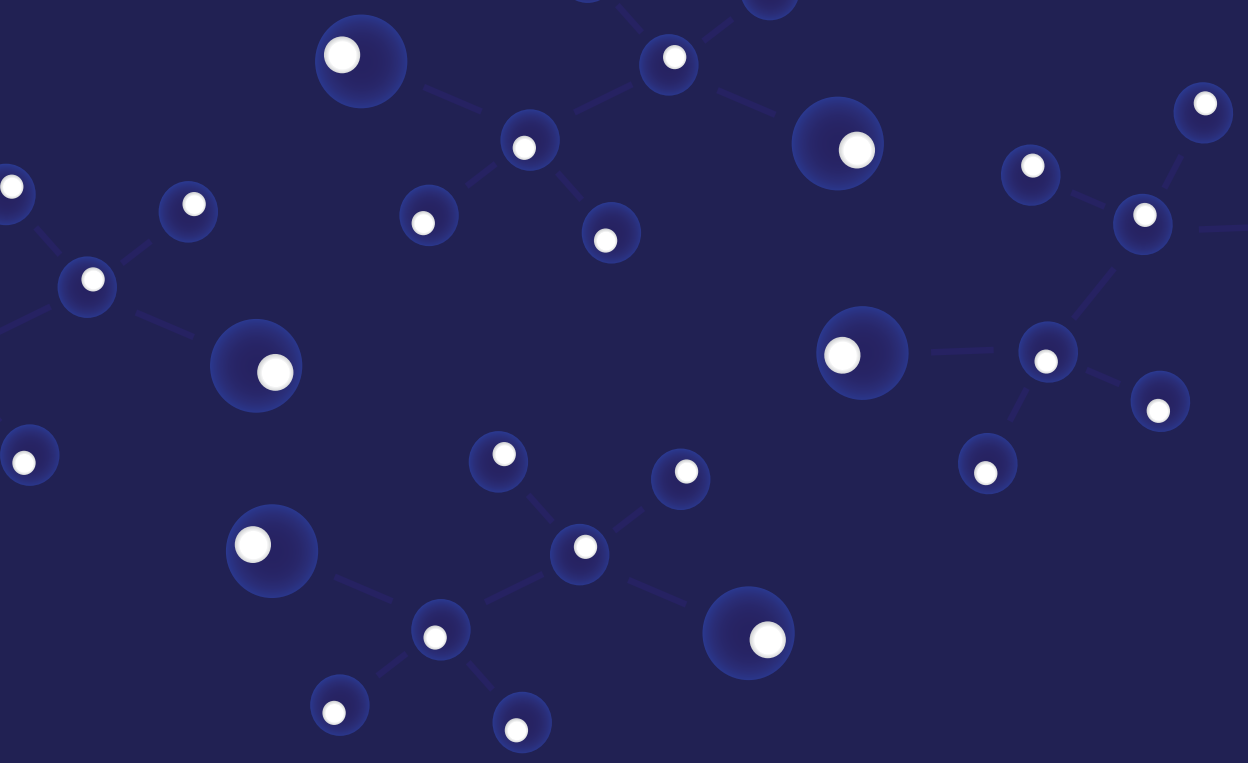

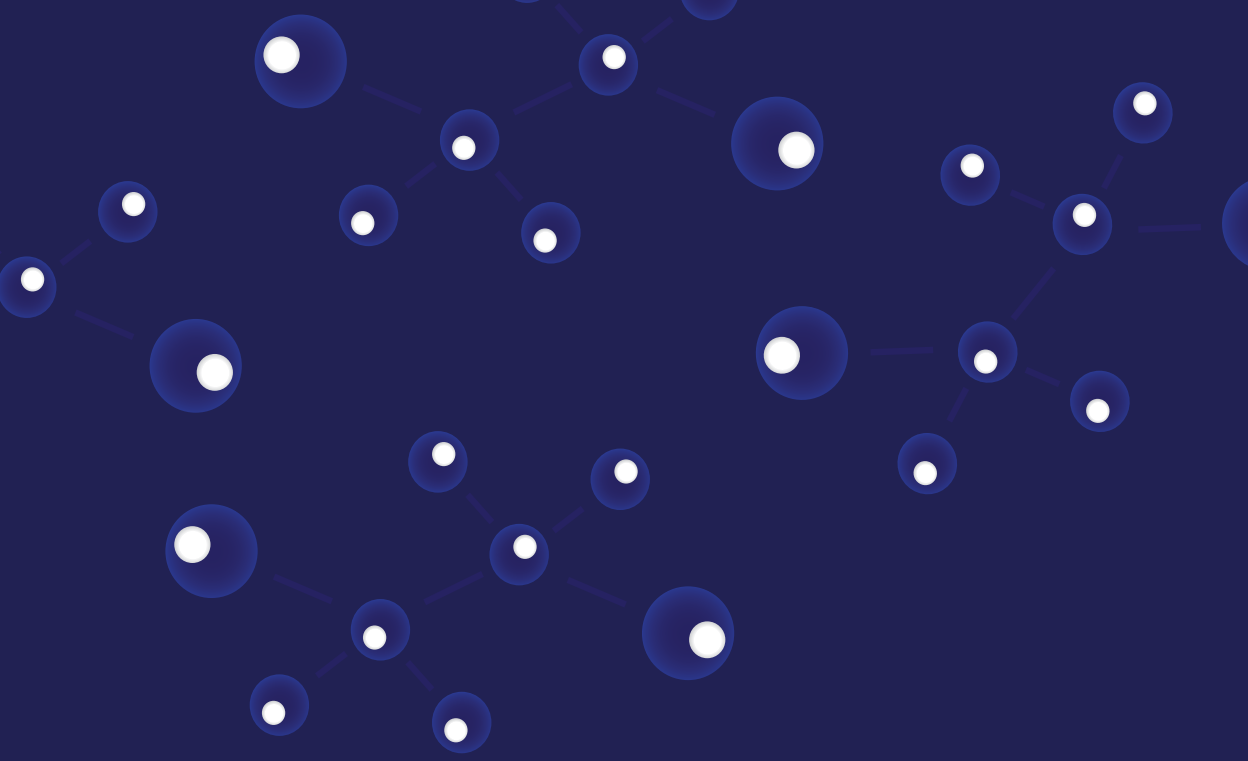

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# Biochemical and immunochemical similarities among mammalian bilitranslocase and a plant flavonoid translocator

Enrico Braidot, Elisa Petrussa, Carlo Peresson, Sonia Patui, Alberto Bertolini, Angelo Vianello, Marco Zancani

PP8-University of Udine

**Abstract** — Flavonoids are a large class of plant secondary metabolites, belonging to polyphenol family, which possess pharmacological and nutritional properties. Their synthesis takes place only in plants, while mammals can acquire them only with diet. It has been demonstrated that flavonoid uptake occurs in rat also by the activity of bilitranslocase, a carrier that is involved in anion transport in liver cell, vascular endothelium and gastric mucosa. A sequence of bilitranslocase interacting with flavonoid moieties is already known and characterized. Antibody raised against such protein epitope were shown to exhibit cross-reactivity against plant membrane proteins in tissues involved in flavonoid transport and accumulation, such as teguments of carnation petals and skin of grape berries. Further immunolocalization studies allowed to demonstrate the presence of cross-reacting protein not only at the level of tegumental tissues, but also associated to sieve elements and seed teguments in grape berries.

Index Terms — flavonoid accumulation, plant bilitranslocase homologue, protein biochemical and structural characterization, secondary metabolite transport.

## 1 UNIVERSITY OF UDINE

It is a recent University at the heart of Europe. Established by popular demand, the University of Udine has its reference point at the center of a region that has historically been a meeting place and crossroad of different worlds and cultures.
Since its origin, the University is strongly committed to the education of students

ready and able to face the challenges of a world, characterized by a global economy, that increasingly requires skills to be used at international level.
The internationalization represents the fourth mission of the University of Udine, alongside teaching, research and technology transfer. It grew through the constant increase and improvement of working relationships and partnerships with universities of Europe and other international institutions. This led, in particular, to the creation and offering of several graduate courses with international recognition of the certificate, masters, doctoral schools and European internships, as well as great development opportunities within the international mobility of students and teachers.
According to these aims, besides the traditional activities of higher education and scientific research, the University of Udine combines intensive transfer of innovative technologies and knowledge by serving the society and the world production and economics.

## 2 RESEARCH DEPARTMENT (UNIT)

The research unit consists of teachers/researchers involved in educational and research activities in Plant Biology. The most relevant field concerns some biochemical and plant physiology subjects focused on cell transport, mitochondrial respiration also in relation to oxidative stress and PCD manifestation. Approaches imply both membrane fraction as well as plant cell culture, grape in particular. The main task of such researches points to the acquisition of information about some fundamental metabolic plant process, as well as their role during stress responses.
The second field concerns taxonomical studies on higher plants and geobotany, as well as algal biology and ecology, with the aim to study both species diffusion and their dynamics in natural and anthropic environments.

## 3 ACTIVITIES AND ROLES IN TRANS2CARE PROJECT

Several components of the grape berry have a straight influence on wine quality. Among these, polyphenols and other secondary metabolites are the major compounds affecting taste and flavour. Polyphenols are currently under a lively attention by researchers, particularly flavonoids, because they are relevant to grape and wine quality as well as for their pharmacological properties protecting human tissues against activated oxygen species (AOS), thus reducing cardiovascular and cancer risk [1]. These compounds, particularly anthocyanins and proanthocyanidins, are synthesized through the biosynthetic pathway of flavonoids [2].
There are several experimental evidences about the existence of a mammalian protein, named bilitranslocase (BTL), capable of a secondary active transport. This carrier transports not only plasmatic catabolites, but also anthocyanins [3,4]. In a previous work [5], it was reported that antibodies raised against mammalian BTL epitopes do cross-react with proteins obtained from a microsomal fraction of carnation petals (*Dianthus caryophyllus* L.). Moreover, some kinetic studies showed remarkable

similarities between bilitranslocase-mediated transport activity in hepatocytes and gastric epithelial cells and the plant carrier, although the latter has shown a different pattern of affinity for the substrates tested. Preliminary data, obtained in previous studies [6,7], have shown that a cross-reaction also occurs between antibodies raised against mammalian BTL and the microsomal fraction from grape berries. By means of immunochemical techniques, the presence of a translocator in white and red grape thin sections has been demonstrated. Carrier localization together with its expression profile, in different developmental stages, have been evidenced in both skin and pulp tissues. Therefore, it is relevant to verify, in *Vitis vinifera* berries of red cultivars, if proteins similar to the mammalian BTL mediate the accumulation of polyphenols, especially anthocyanins and tannins, and which effect on transport activity could be exerted by some environmental factors such as water deprivation.

## 4 CONCLUSIONS

In this framework the purposes of the project could be widespread in several topics. First, the study of the phylogenetic links between mammalian bilitranslocase and similar plant proteins.
Second the transport mechanisms regarding secondary metabolites, such as flavonoid and chlorophyll degradation products (biline-like compounds) would be characterized. Third, investigation on metabolic and environmental factors able to increase flavonoid production in plant cell cultures, would be also necessary.
Fourth, research on plants, as a model living organisms, will represent an advantageous alternative to animal use, ethically acceptable in biological studies.
To obtain these goals, it is essential to establish a wide network among different partners able to share their specific skills and knowledge, in agreement with the main task of Trans2Care project.

## ACKNOWLEDGEMENTS

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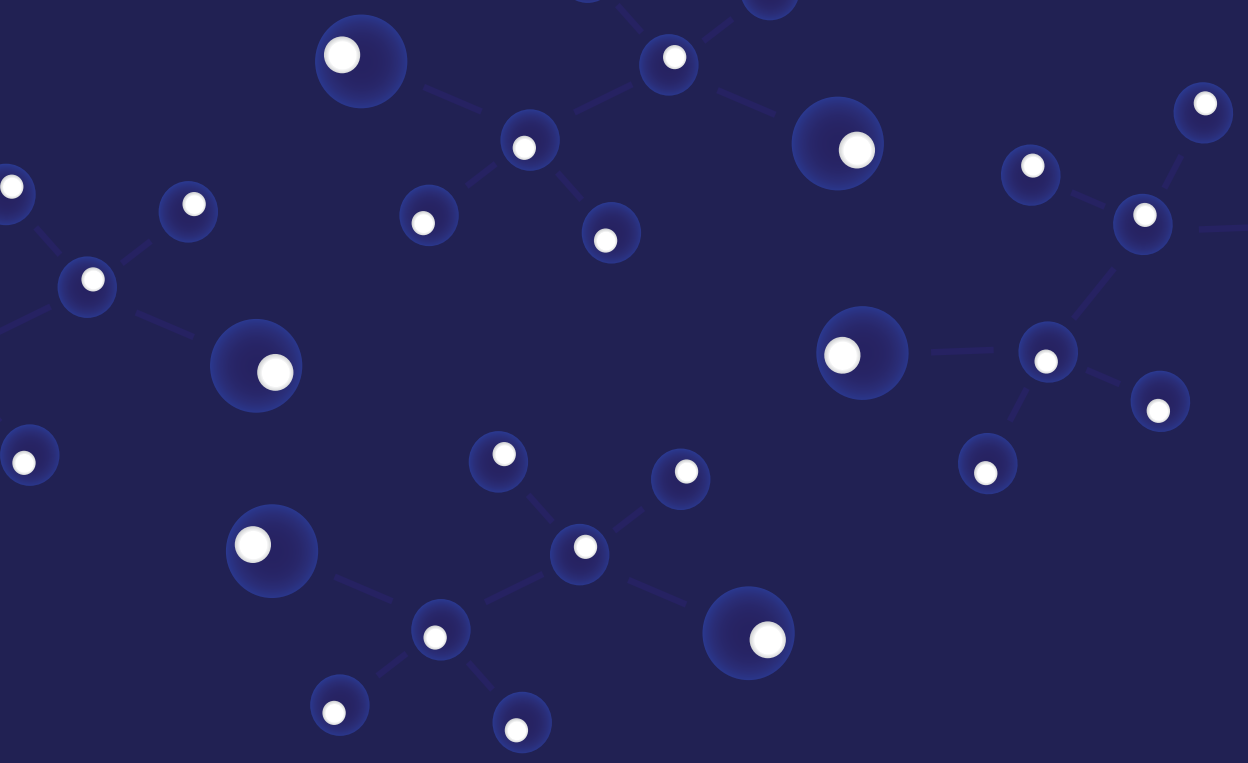

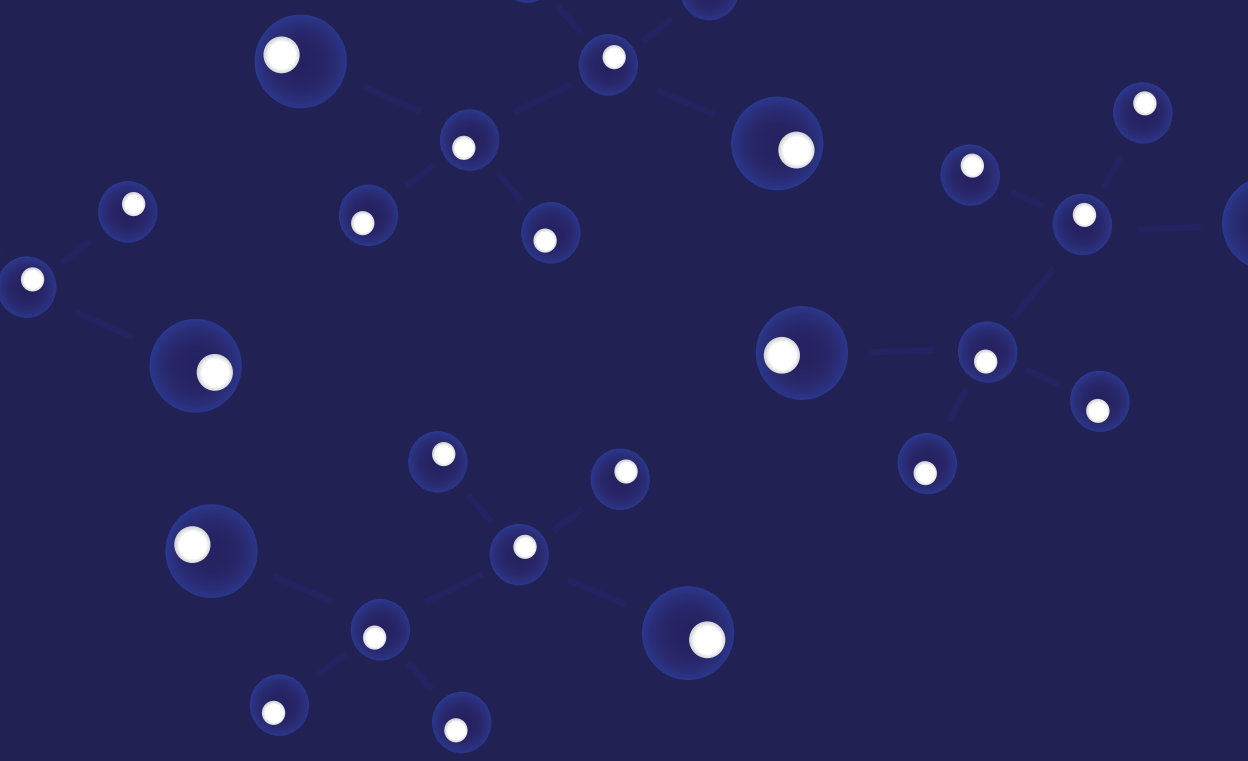

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# Early diagnosis of coeliac disease

Luigina De Leo, Tarcisio Not, Denise Zerial

PP9-Institute of Child Health IRCCS "Burlo Garofolo", Trieste

Abstract — At the Immunopathology Laboratory at the IRCCS Burlo Garofolo Hospital the research activity is based on autoimmune diseases, above all on celiac disease in order to diagnose it in an early stage. For this reason, we are collecting many serum samples and intestinal biopsies to analyse them with molecular (phage-display) and immunofluorescent (double staining and activated beads) assays. Within the Trans2Care project we intend to apply these methods in several areas related to the problems explored by the Project partners with the aim of promote collaboration, mobility of researchers and exchange of knowledge between partners.

Index Terms — autoimmune diseases, immunofluorescent assays, intestinal biopsies, phage-display analysis, serum samples

## 1 THE IRCCS BURLO GAROFOLO

The Burlo Garofolo Hospital was established on November 18th 1856, when the Spedale Infantile was instituted in order to grant free medical care to poor children. On 1968 the Institute was designated by the Ministry of Health as IRCCS. The Institute has promoted and implemented an innovative health culture, based on innovative policies aimed at reduction of hospital stay and humanization of medical care. The Hospital is the unique health care Institute for Mother and Child Health within the area the sourrounding region. The IRCCS Burlo Garofolo ensures clinical excellence in medical and surgical paediatric subspecialties, reproductive medicine and perinatology. The Institute offers graduate and post graduate courses and PhD programs. The Institute is a World Heath Organisation (WHO) Collaborating Centre for Maternal and Child Health. Fundamental, clinical, epidemiological and health services research are organised along 6 main subjects: Maternal and foetal medicine; neonatology; chronic diseases, including cancer, with onset in paediatric age; paediatric surgical and rehabilitation sciences; epidemiology prevention and quality of care; neuroscience in developmental age.

## 2 IMMUNOPATHOLOGY LABORATORY

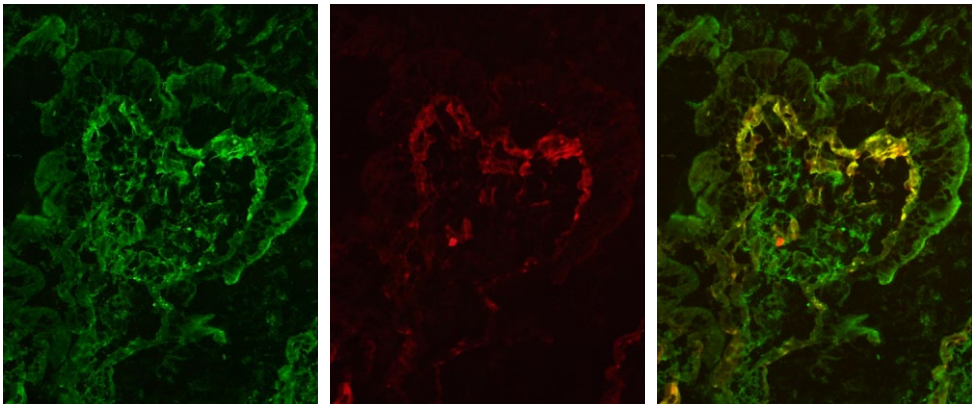
The Immunopathology Laboratory has one professor and one assistant professor in clinical pediatrics, four PhD researchers. We use and develop immunohistochemical and molecular techniques to study autoimmune diseases to understand its inflammatory cascades. Furthermore, we apply at the patient's bed side our assays to simplify the diagnosis of these pathologies. The research work is financed through national research programme schemes (NHS programme 2009: "Dilatative cardiomyopathy and gluten dependent autoimmunity"; Italian Ministry of University 2009: Anty-idiotypic network to anti-transglutaminase antibodies in the pathogenesis of celiac disease; XVII Executive programme of scientific and technology co-operation between Hungary and Italy 2010: LS17 Intestinal gluten-dependent immune response in the early stages of celiac disease) and from 2010 we are partners in an international strategic project Trans2Care.

### 2.1 Research Activities

Our interests are based on autoimmune disorders [1-3]. We organised a serum and tissue bio-bank from patients suffering from organ specific autoimmune disorders (e.g. type 1 diabetes, thyroiditis, celiac disease, rheumatoid arthritis) or from other inflammatory diseases (e.g. Crhon disease, eosinophyl-gastritis, ulcerative colitis) with a large samples stored at -80 C° (10000 serum samples and 1500 intestinal biopsies).

Our main goal is to study celiac disease (CD) [4-8], an autoimmune-mediated enteropathy characterised by gluten-triggered small bowel mucosal lesions in genetically susceptible individuals carrying the CD-related human leukocyte antigen (HLA) DQ2 or DQ8 haplotypes. The current diagnostic criteria for CD require intestinal mucosal villous atrophy and the presence of serum antitransglutaminase (anti-TG2) antibodies [9-13], even if many patients suffer from gluten-dependent gastrointestinal symptoms before the onset of villous atrophy and of anti-TG2 antibodies in serum. Anti-TG2 antibodies are synthesised by specific B lymphocytes in the small bowel mucosa and they are deposited in the morphologically normal small intestinal mucosa before they can be detected in the circulation. Starting from these evidences, we try to identify these antibodies working on intestinal specimens. Creating phage-antibody libraries against TG2, we observed that celiac-specific anti-TG2 antibodies are primarily comprised of the IGHV5-51 gene from the VH5 antibody variable gene family, indicating a possible preferential usage of this gene in the gluten-dependent autoimmune response to TG2. Indeed, using phage-antibody libraries against TG2, we demonstrated that a large proportion of HLA DQ2- or DQ8-positive relatives of CD patients produce anti-TG2 antibodies in the intestine as a response to gluten, even in the presence of normal intestinal morphology and when no anti-TG2 antibodies can ever be found in the serum [14].

We are also able to investigate IgA anti-TG2 in frozen biopsies by using two other assays easier and faster than phage assay: the double immunofluorescence staining (Fig.1) and the IgA anti-TG2 antibodies quantification through TG2 activated beads and flow cytometric analysis.



A B C

Fig.1. Criosections from a celiac intestinal biopsy: (A) green signal for IgA, (B) red signal for TG2, (C) yellow signal for IgA deposits co-localised with TG2.

## 3 ROLES IN TRANS2CARE

### 3.1 What offer to T2C

We have focused our research project in the diagnosis of celiac disease in an early stage using immunofluorescent assays and phage-display analysis. We provide our knowledge to T2C partners, in order to facilitate the exchange of expertise and to study tissue biopsies may also be different from intestinal ones.
As described in the paragraph above, we have an elevated number of biological samples (sera and intestinal specimens) suitable for exploration of large data sets.

## 4 CONCLUSION

Through Trans2Care project, we intend to contribute with our expertise in order to reach the common goal, that is to establish collaborations and connect the project partners to eventually improve their expertise and to promote exchange of ideas and technology transfer.

## ACKNOWLEDGEMENT

The financial support of the Fondo europeo di sviluppo regionale for the Trans2care project is greatly appreciated.

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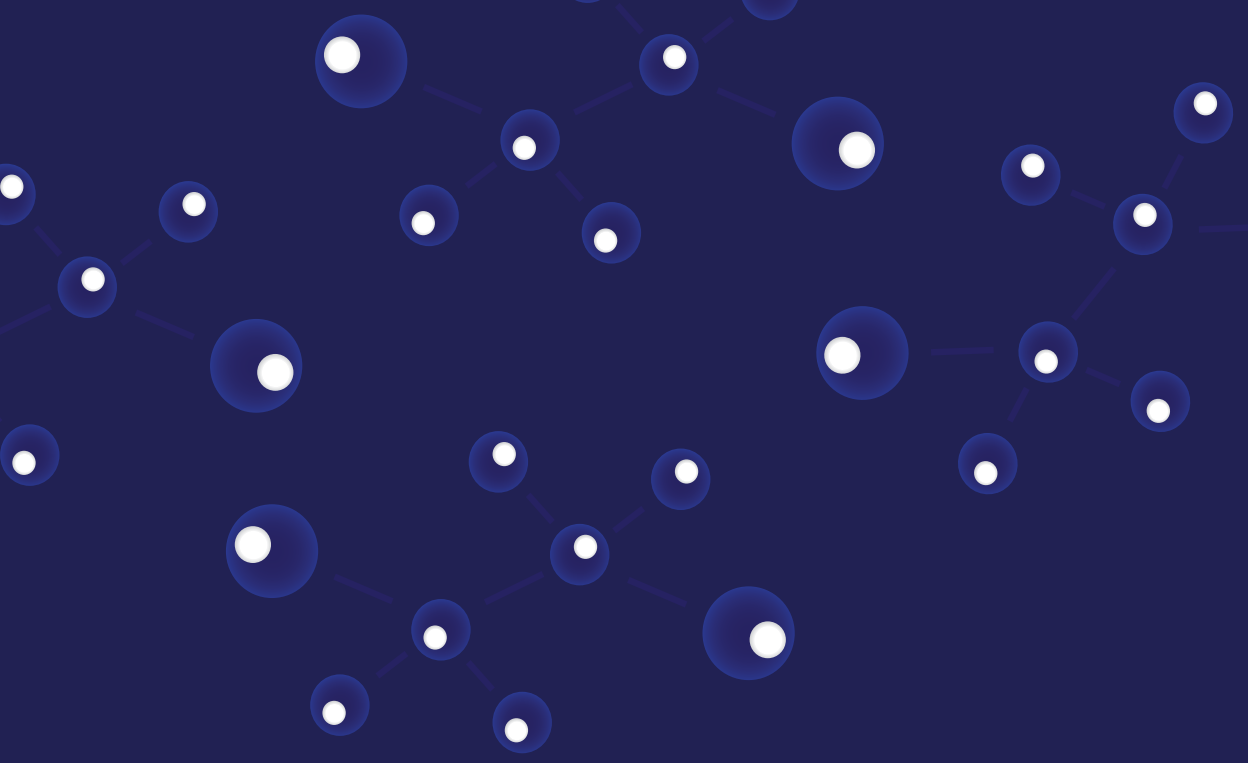

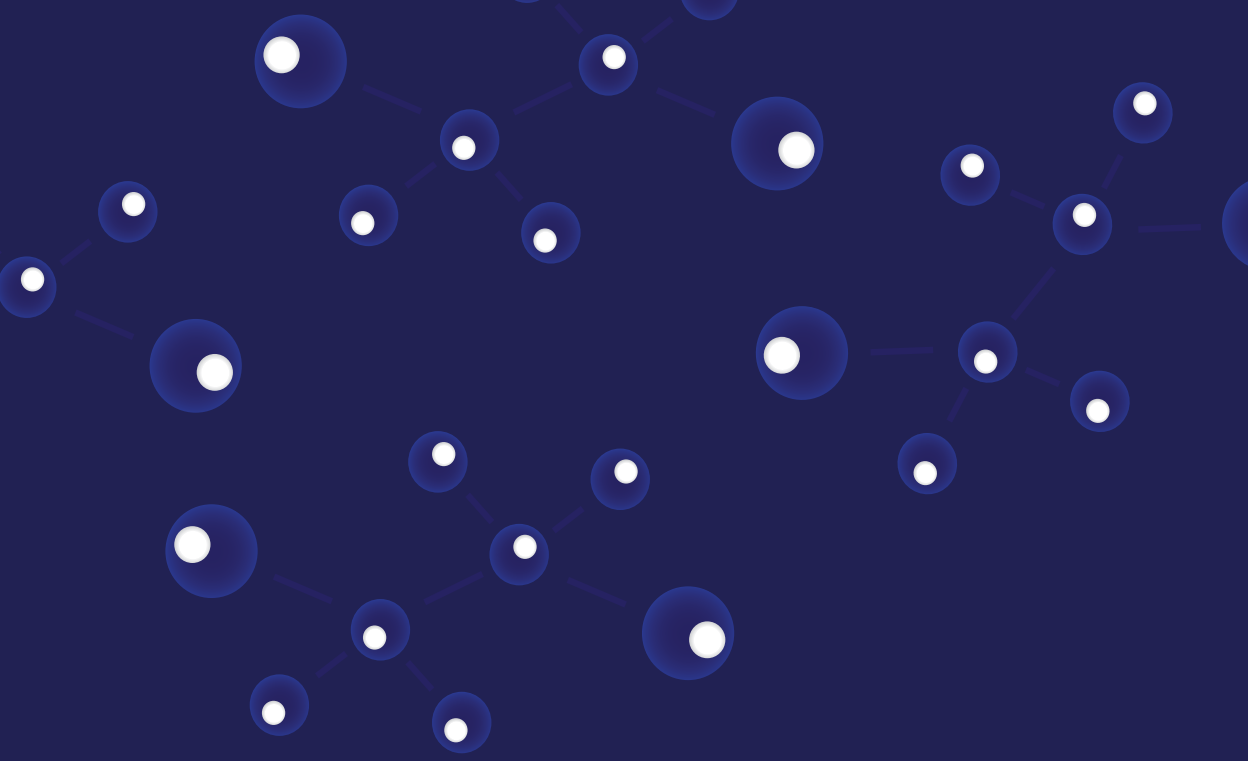

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# Novel immuno– and stem cell–based therapies

Sendi Montanič, Uroš Rajčevič and Vladka Čurin Šerbec

PP10-Blood Transfusion Centre of Slovenia, Ljubljana

Abstract — The main areas of the research at the Blood Transfusion Center of Slovenia are cell therapies, stem cells and new diagnostic reagents, based on monoclonal antibodies (mAbs). Our laboratories are equipped for cell-culture work (certified GLP; GMP in reconstruction), molecular biology, immunological techniques and biochemistry. In the course of our research we conducted studies and applied projects (the last mainly industrial) beside production of reagents, with common topic - the antibodies. They were prepared and used to optimise and validate ELISA tests for quality control assessment of the end-product (polyclonal antibodies, in production of drugs), to study protein structure, for immunodiagnostics and as potential immunotherapeutics. Among different mAbs, which we produced in the last decade, a panel of mAbs against prion protein (PrP) and mAbs against bilitranslocase (prepared in collaboration with the University of Trieste) will be studied with different project partners in the scope of Trans2Care. We are fully dedicated to translate the results of the basic research onto applied, possibly clinical level and to contribute to improved healthcare in a sense of advanced modes of immunotherapeutics development and cell based therapies.

Index Terms — human prion protein, prion, bilitranslocase, monoclonal antibodies, immunodiagnostics, immunotherapy, stem cells, cell therapy

## 1 INTRODUCTION

Blood Transfusion Centre of Slovenia (BTCS) is a national blood bank, responsible for the supply of safe blood and blood components in our country. Beside that, we are providing diagnostic and therapeutic services and blood-derived drugs to our hospitals. We are a strong partner of the University of Ljubljana, giving lectures and providing experimental work to students of graduate studies at Faculty of Medicine,

Faculty of Pharmacy, Faculty of Chemistry and Chemical Technology and Faculty of Health Sciences. We are also involved in postgraduate programs of Biomedicine and Biotechnology at the University of Ljubljana. The same personnel is engaged in research and development at BTCS, being members of three research groups: Tissue Typing Center, Transfusion Medicine and Biomedicine. As of 2004 BTCS has an ISO 9001 standard and is a WHO Collaborating Center and an EFI (European Federation of Immunogenetics) member.

## 2 RESEARCH AT THE BTCS

Research activities at the BTCS started as early as 1953, when the institution was registered for the research and development in medical biotechnology and medical sciences. In the course of next four decades, activities in this field intensified, especially in the research. During last 15 years the three forementioned research groups, conducted 33 national research projects, 3 national programmes, 6 industrial projects and 6 international projects, financed by Slovene Research Agency, by Ministry for Higher Education, Science and Technology, by industrial partners as well as the European Comission. 18 young researchers/PhD students were trained at the BTCS in the scope of these projects, covering medicine, life sciences and biotechnology. Our development is closely related to our routine operations and services (blood collection and processing, blood grouping, cell therapeutical services) and assures constant education and follow-up in transfusion medicine. The main areas of our research are cell therapies, stem cells and new diagnostic reagents, based on monoclonal antibodies (mAbs). Our laboratories are equipped for cell-culture work (certified GLP; GMP in reconstruction), molecular biology, immunological techniques and biochemistry. On the basis of our research, we were invited to take part of the educational programmes at the University of Ljubljana (see introduction).

### 2.1 Biomedicine research group

Our research group evolved from the department for the producion of diagnostic reagents, in charge of production of diagnostic reagents for blood grouping under GMP (Good Manufacturing Practice) conditions. Our first research project included the production of potent mouse IgM monoclonal antibodies (mAbs) against AB0 blood group system in early ninetees of the last century, which were introduced into routine work in the form of diagnostic reagents, registered in 1998. In consequent years, we conducted research and applied projects (the last mainly for the industry) beside production of reagents, with common topic - the antibodies. They were prepared and used to optimise and validate ELISA tests for quality control assessment of the end-product (polyclonal antibodies, in production of drugs), to study protein structure, for diagnostics and as potential therapeutics (mAbs). Among different mAbs, which we produced in the last decade, a panel of mAbs against prion protein (PrP) [1, 2, 4, 6, 9, 10] and mAbs against bilitranslocase (prepared in collaboration with the University of Trieste, started in 2006) [11] will be studied with different project partners in the scope of Trans2Care.

# 3 CONTRIBUTION TO TRANS2CARE

## 3.1 Bilitranslocase

Our current, ongoing collaboration with the T2C partners includes the research of mAbs against bilitranslocase in collaboration with University of Trieste (prof. S. Passamonti). This collaboration will further develop as we anticipate our first joint publications [11] .

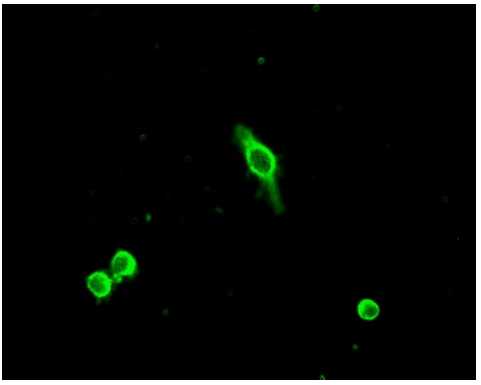

Figure 1: ICC staining of HepG2 cells with anti-bilitranslocase mAb 6E4/1F2 (40x, 2.4s).

## 3.2 Prion diseases

In our research on prion diseases we constructed and tested a panel of mAbs against prion protein (PrP) enabling us to distinguish and differentiate between the wild type PrPc and its pathogenic form PrPSc, which is of a great diagnostic value in Creutzfeld Jacob's disease (CJD). This research yielded EU and US patents for these antibodies as well as our common publications with T2C partners SISSA – prof. G. Legname and University of Trieste - prof. R. Gennaro [1, 6]. In the frame of T2C we are aiming at upgrading this collaboration in two possible directions: the use of proteomics for the identification of novel biomarkers of prion diseases using animal models of CJD as well as clinical samples of CJD. These biomarkers could help us elicit the molecular background of prion diseases as well as serve as potential diagnostic or therapeutic tools.

Alternatively we aim at using the expertise and specialized infrastructure (GMP laboratory standards) of the BTCS in the field of cell based therapeutics to reaserch a potential of neural stem cell based therapies for prion diseases using animal models of CJD.

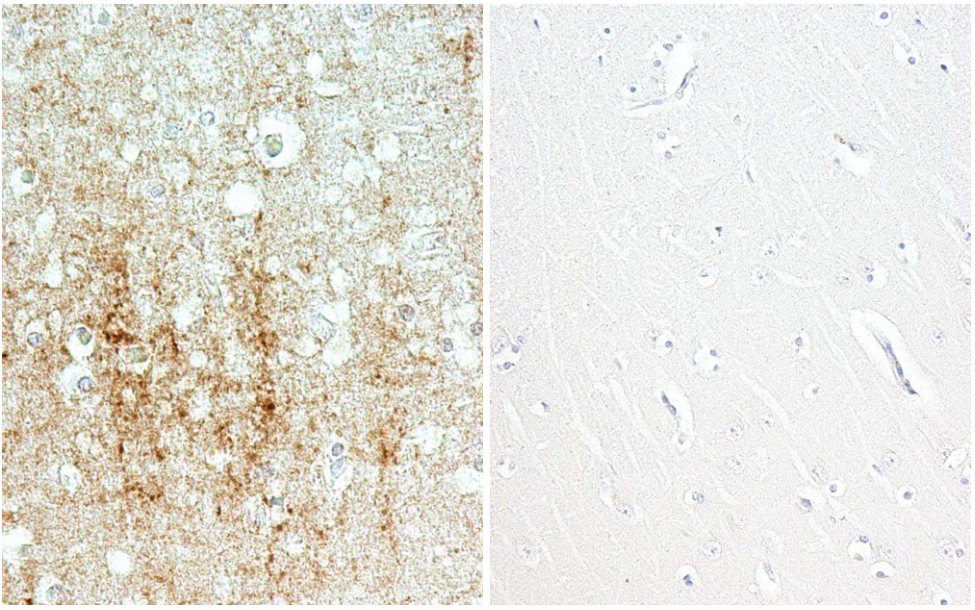

Figure 2: Immunohistochemistry of mAb V5B2 binding to the pathogenic form of PrPSc in CJD brain section, compared to normal brain section. (a) Diffuse PrPSc plaque depositions are visible in a synaptic section of cerebellum (40x). (b) PrPSc – negative staining of normal brain hypocampus (20x).

## 4 CONCLUSIONS

In the frame of T2C, we offer our above mentioned expertise to project partners, within the fields of development of immunotherapeutics, immunodiagnostics, cell based therapies and proteomics as well as whereever sinergies are anticipated. Thus far, we have proven our capacity of inter-regional collaborations and we are looking forward to expanding the ongoing and new collaborations with Slovene and Italian partners. We are fully dedicated to translate the results of the basic research onto applied, possibly clinical level and to contribute to improved healthcare in a sense of advanced modes of immunotherapeutics development and cell based therapies.

## ACKNOWLEDGEMENT

The financial support of the Fondo europeo di sviluppo regionale (Evropski sklad za regionalni razvoj) for the Trans2Care project is greatly appreciated. The financial support by the Slovene Research Agency through the research grant P4-0176 is acknowledged. We also acknowledge the contribution of the collaborators working on the projects in the past (please see the references) from within the BTCS and outside.

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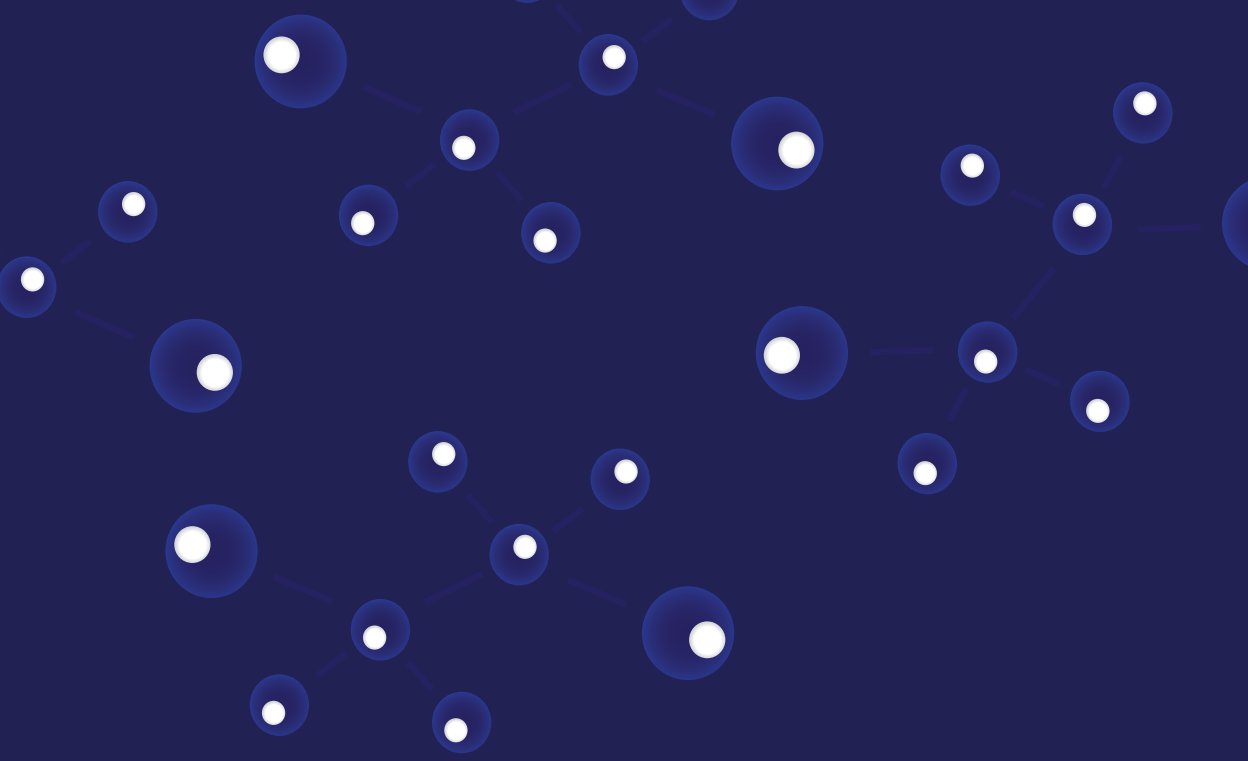

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# Pathophysiological mechanisms of joint implant loosening

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PP11-Valdoltra Orthopaedic Hospital, Ankaran

Abstract—Overthe past half-century, there have been many advances in the design, construction, and implantation of joint prostheses, resulting in a high percentage of successful long-term outcomes. One of the most common concerns of both patients and physicians is the problem of joint replacements becoming loose over time. Causes of failure include infections, aseptic loosening, dislocations, and fracture of the prosthesis or bone. Multidisciplinary research team studies are needed for an improvement in understanding in pathophysiological mechanisms of joint implant loosening and failure, which is the key point to improve implant survival and to minimize revisions.

Index Terms — diagnosis, infection, loosening, prosthetic joints

## 1 INTRODUCTION

As the average age of human population increases, the number of total joint arthroplasties performed is increasing dramatically. Nearly 4.500 such procedures are performed in Slovenia each year. Prosthetic joints improve the quality of life for many patients; however they may fail, necessitating a revision arthroplasty. It is believed that joint prostheses can reliably relieve pain and improve function in the majority of patients, with benefits lasting for a period of 15 to 20 years. However, approximately 14-28 % of prostheses need to be revised even before decade of service[1]. Causes of failure include infections, aseptic loosening, dislocations, and fracture of the prosthesis or bone. The incidence of revision surgeries almost doubled in the last 15 years and the revision frequency projections by 2030 are even more impressive [2].

Prosthetic joint infection (PJI), although uncommon, is the most serious complication, occurring in 0.8 to 1.9 % of knee arthroplasites and 0.3 to 1.7 % of hip arthroplasites [3]. Staphylococci account for more than half of all PJI cases. Since surgical treatment

of joint prosthesis loosening (septic or aseptic) is different, it is very important to establish the correct diagnosis of PJI, which is still a challenge in clinical practice. A misdiagnosed PJI has crucial consequences for the patients. Unfortunately, to date, there is no reliable preoperative or intraoperative test that is 100 % sensitive and specific for PJI diagnosis.

## 2 ACTIVITIES AND POSSIBLE ROLE IN TRANS2CARE PROJECT

The aim of our recent research project is to evaluate different intraoperative diagnostic tests, such as microbiological culturing, pathohistological analysis and molecular methods for PJI diagnosis.Ultrasonication of prostheses, followed by analysis of the dislodged material (sonicate) was suggested to improve the detection of prosthetic hip infections [4]. We therefore raised a question: how many cases diagnosed as aseptic failure are actually PJI?

Early diagnosis of PJI and a better understanding of biofilm production should lead to novel, effective treatment strategies and improved care and rehabilitation of patients with joint prostheses.

Currently, the most common cause of clinical failure of joint prostheses is aseptic loosening of the implant components [5]. Aseptic loosening of a joint prosthesis is hypothesised to be the result of a harmful combination of mechanical and biological events, which cause the destruction of the bond between implant and bone bed. In the long term,aseptic loosening is a significant clinical, as well as economic problem. The pathogenesis of prosthetic joint loosening continues to be a major focus of research in orthopaedics. The fibrous membrane that forms around the joint prosthesisis composed mostly from granulomatous tissue, namely macrophages, giant cells and also of immune cells (Figure 1). Although numerous descriptions of histological features of the fibrous membrane have been published [6, 7], the origin of this membrane and its role in progressive bone resorption, which is associated with prosthesis loosening, are still poorly understood. The gliding surfaces (counterfaces) of joint implants produce wear debris which stimulate macrophage activation[8]. Macrophages are generally recognized for their ability to phagocytise even immunologically non-opsonized wear particles and produce cytokines that stimulate osteoclast bone resorption. Our group has extensive expertise in pathohistological analyses of periprosthetic tissue obtained at revision surgery allowing as to be included in several national, as well as international multidisciplinary teams, involved in investigation of pathophysiological mechanisms of aseptic loosening.

Since wear debris produced in site of joint prosthesis and the resulting tissue reaction is the most important cause of prosthesis loosening, the idea of reducing wear debris is an important issue for improvement of long-term results of total joint replacement. It has in fact re-stimulated interest in evaluation of alternative bearing materials.

We are currently participatingin different international projects, in which our in vitro studies investigate biocompatibilities and toxicities of different bearing alloys and surface layer improvements, as well as biological response of different cell lines to biomaterials.

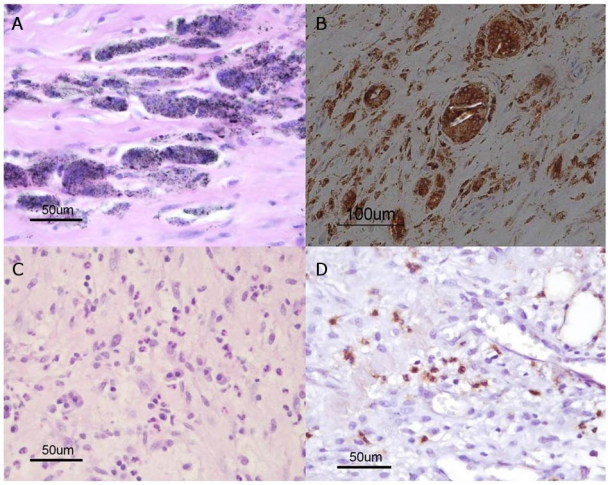


Fig.1.Histological analysis of periprosthetic tissue. A: Metal particles (black) phagocytosed by macrophages; B: Imunohistochemically stained slide under polarisation microscope with CD68 positive macrophages and giant cells (brown) with birefingent polyethylene particles (white); C: Infiltration with polymoprphonuclear leucocytes and plasma cells in periprosthetic tissue is a diagnostic for infection; D: Immunohistochemical staining with CD15 with positive granulocytes (brown) in tissue around infected joint prosthesis.

## 3 CONCLUSION

Total joint arthroplasty is one of the most successful orthopaedic surgery procedures; however, a number of joint replacements ultimately fail due tocomponent loosening. Because of the increasing need to implant joint prostheses in younger and more active patients, studies of interdisciplinary research teams are necessary to improve our understanding of the pathophysiology of joint implant loosening.

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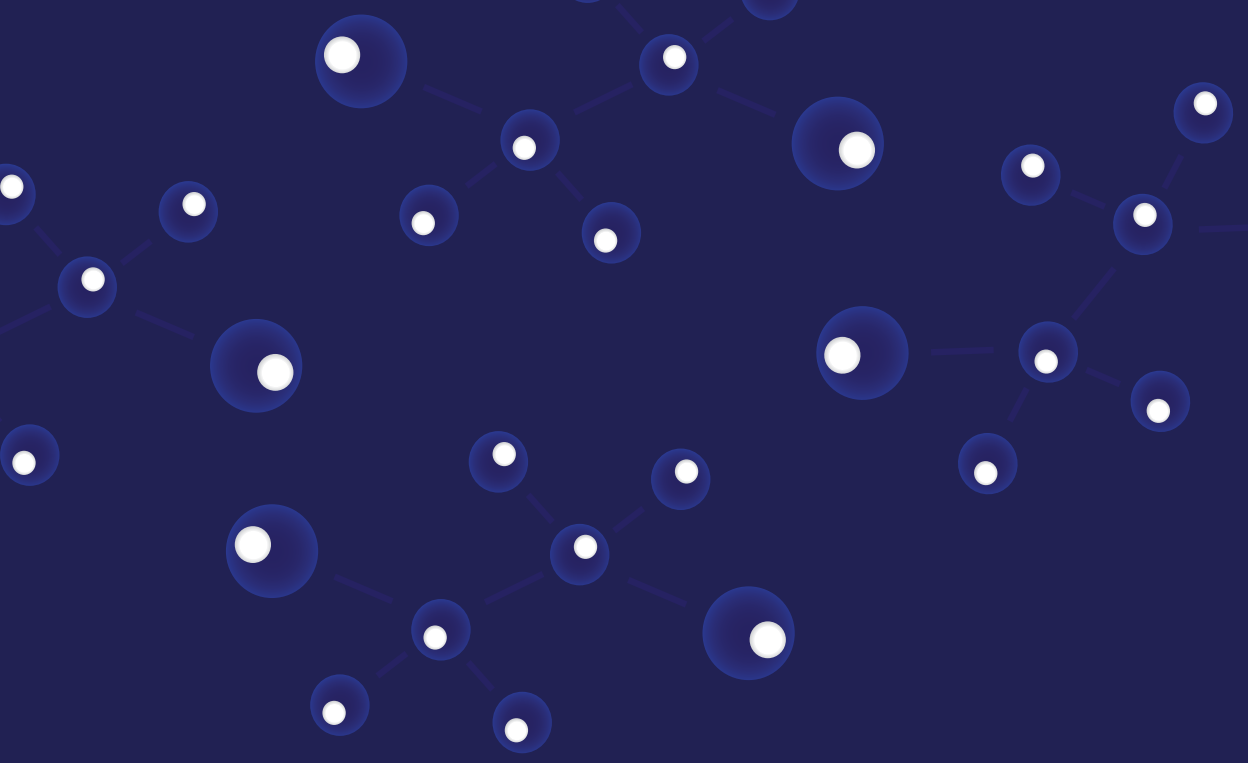

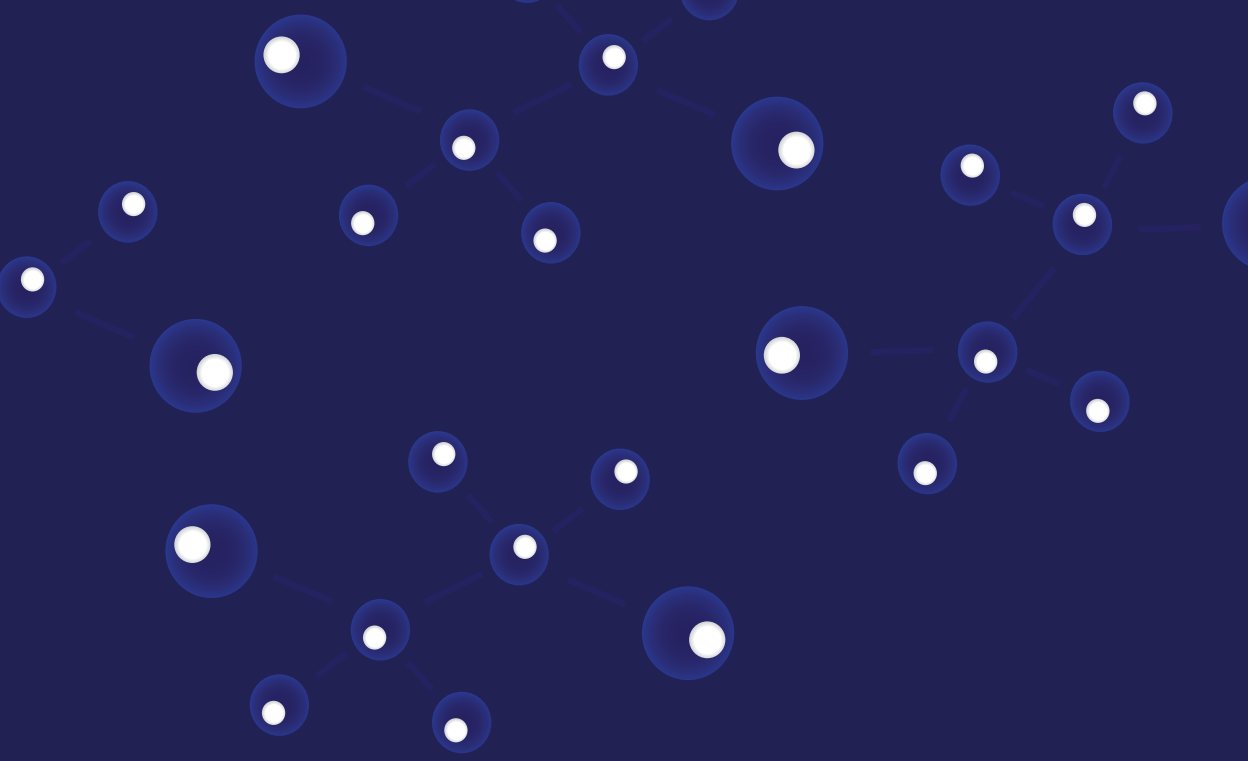

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# Mechanical, biological, material and clinical aspects of performance of joint prostheses

Ingrid Milošev, Vesna Levašič, Andrej Cör, Venčeslav Pišot

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Abstract — At the Research department at the Valdoltra Orthopaedic Hospital the surgeons and researchers are joined in an interdisciplinary team which performs research work and activities directed to the understanding of prosthesis performance from several aspects including mechanical, biological, material and clinical aspects. Studies are largely based on the hospital's Arthroplasty Register which represents a valuable source of information for data-based analysis of arthroplasty treatments. Our register is special in that it includes the implant retrieval program for explanted prosthetic components and periprosthetic tissue samples. Research is versatile and interdisciplinary, and is a challenge for young researchers searching for new knowledge and scientific results.

Index Terms — orthopaedic implants, survival, register, materials for biomedical applications, biological impact, statistics

## 1 VALDOLTRA ORTHOPAEDIC HOSPITAL

Valdoltra Orthopaedic Hospital has celebrated its 100th anniversary in 2009. Over the years the hospital has developed from a marine sanatorium for scrofulous diseases in ancient Austro-Hungarian monarchy to a modern orthopaedic institution today. Hospital is the oldest and largest specialized hospital in Slovenia, comprising more than 50% of Slovene orthopaedic activities with 2,600 surgeries, 25,000 orthopaedic examinations and 5,500 patients in hospital care. The Hospital collaborates with domestic and foreign orthopaedic institutions aimed at developing new surgical and therapeutic methods. The Hospital is equipped with modern operating rooms, sophisticated IT and diagnostic equipment including MR and CT, endoscopic technology and highly qualified medical staff. The hospital has around 300 employees, of whom more than 30 are MDs.

## 2 RESEARCH DEPARTMENT

Constant education and follow-up of the latest results of clinical studies has always been very important for everyday practice at the hospital. The Research department at the Valdoltra Orthopaedic hospital was formally founded in 2002. It is devoted to the research studies in orthopaedics, primarily to long-term performance of various prostheses. Our aim was to join the surgeons and researchers in an interdisciplinary team which would perform research work and activities directed to the understanding of prostheses performance from several aspects including mechanical, biological, material and, of course, clinical aspect. The research work proceeds through the projects within the hospital targeted to solve specific problems within the hospital practice, and through projects financed by the Slovenian Research Agency, i.e. basic and applied research projects, young researcher program and bilateral projects. The hospital is involved in two international strategic projects (e-health and Trans2Care).

## 3 ACTIVITIES AND ROLES IN TRANS2CARE PROJECT

### 3.1 Arthroplasty Register

Research studies at the hospital are largely based on the hospital's Arthroplasty Register which represents a valuable source of information for data-based analysis of arthroplasty treatments. Following the example of Scandinavian arthroplasty registries we have established the Arthroplasty Register of the Valdoltra Orthopaedic Hospital in 2002 in order to assure the results of arthroplasty treatment and long-term follow-up of clinical results. The Register comprises all primary and revision hip and knee operations. For that purpose, forms and date-base have been developed. Based on the analysis of the data given in the forms, annual reports are prepared. There are numerous advantages of Arthroplasty register, among them the most important are long-term follow-up of individual types of prostheses and the possibility of early recognition of possible deviations from the expected results and subsequent fast reaction, i.e. alarm.

### 3.2 Long-term survival of prostheses

Our goal is to carefully follow the long-term survival results of the implanted hip and knee prostheses and relate them to other clinical scores or indicators. In the last decade we have published several papers reporting mid- and long-term results of various prostheses [1-5]. We were especially interested in the effect of type of the bearing on the long-term performance of hip prosthesis. There are namely several types of bearings, so called traditional metal-on-polyethyene and ceramic-on-polyethylene bearings, and alternative metal-on-metal and ceramic-on-ceramic bearings, aimed for younger, more active patients. Polyethylene has been used as a bearing material for more than fifty years. It represents a golden standard in orthopedics. However, wear of polyethylene and consequent formation of wear particles are the key factor

in the mechanism of aseptic loosening of total hip replacements. Pathophysiology of loosening is a complex process. Osteoclasts and their precursors have crucial role in bone resorption. The differentiation of these multinucleated cells and activation is regulated with RANK/RANKL/OPG regulatory axis.

### 3.3 Retrieval studies

The specialty of our Register is that it includes the implant retrieval program for explanted prosthetic components and samples of periprosthetic tissue (Fig. 1). Collected samples are the basis for various research analyses aimed to reveal the changes at the surface of the component and in the periprosthetic tissue induced during in vivo functioning of the prosthesis [6-8]. Of special interest are mechanical, chemical and structural changes at the surface of retrieved metal and polyethylene components. For that purpose we use microscopic analysis of the surface by scanning electron microscopy combined with chemical analysis, computer coordinate machine for determination of wear, roughness measurements, and other analyses of interest. Histological analysis of periprosthetic tissue samples is performed in order to determine the biological impact of wear debris products. Wear debris particles are isolated from tissue samples in order to study their morphology and size distribution. Of special interest are also studies aimed to investigate incidence of infection of joint prosthesis and its mechanism.

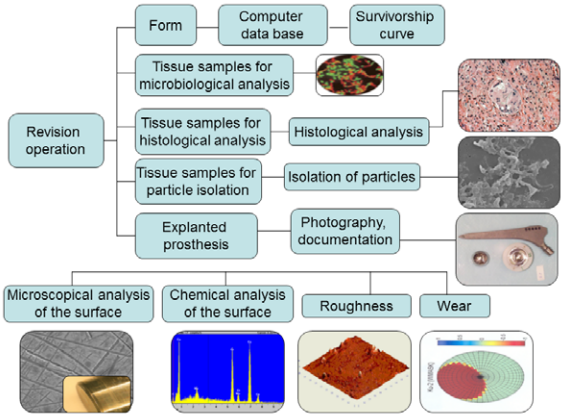


Fig. 1. Block-diagram of the Arthroplasty Register at the Valdoltra Orthoapedic Hospital.

## 4 CONCLUSION

Through the initiated Trans2Care project we intend at our hospital to contribute through the activities and studies aimed to a deeper understanding of prosthesis-biological environment interactions and, consequently, contribute to the improvement of approaches, materials, design and treatment selection, and eventually promote the prolongation of the life-time of prostheses in general.

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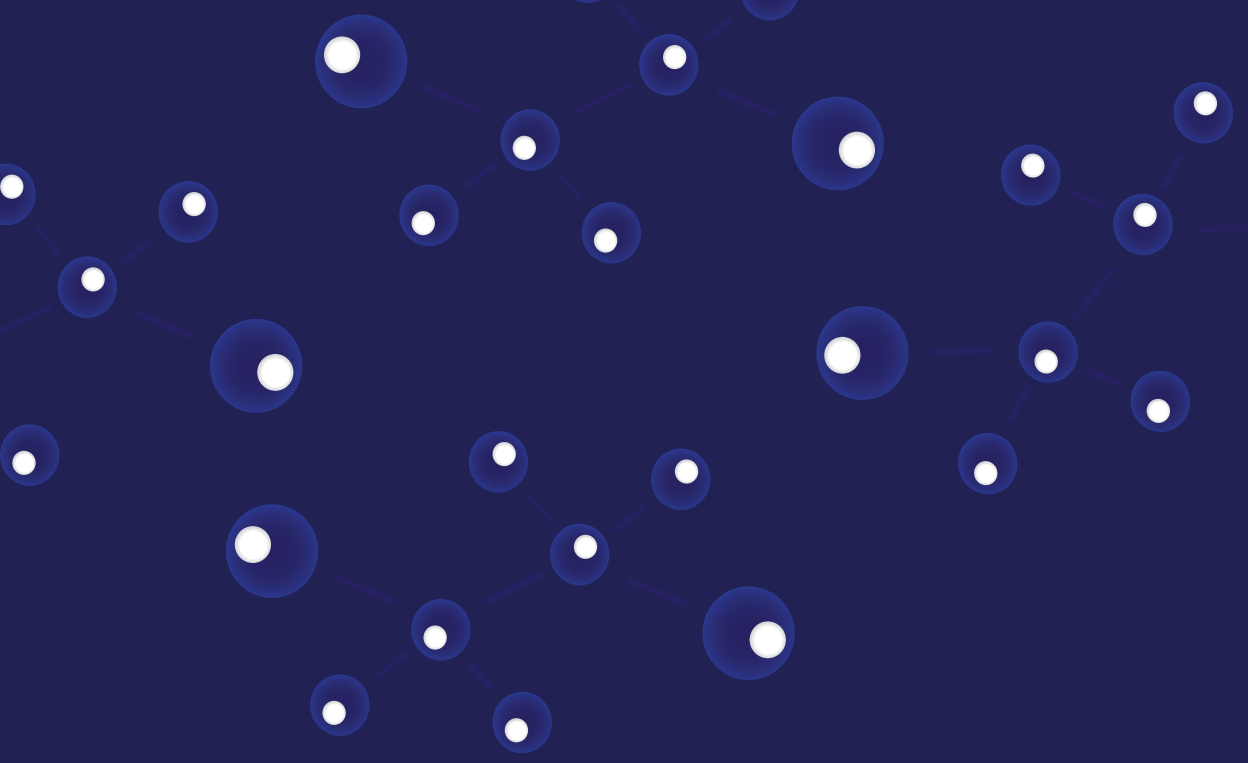

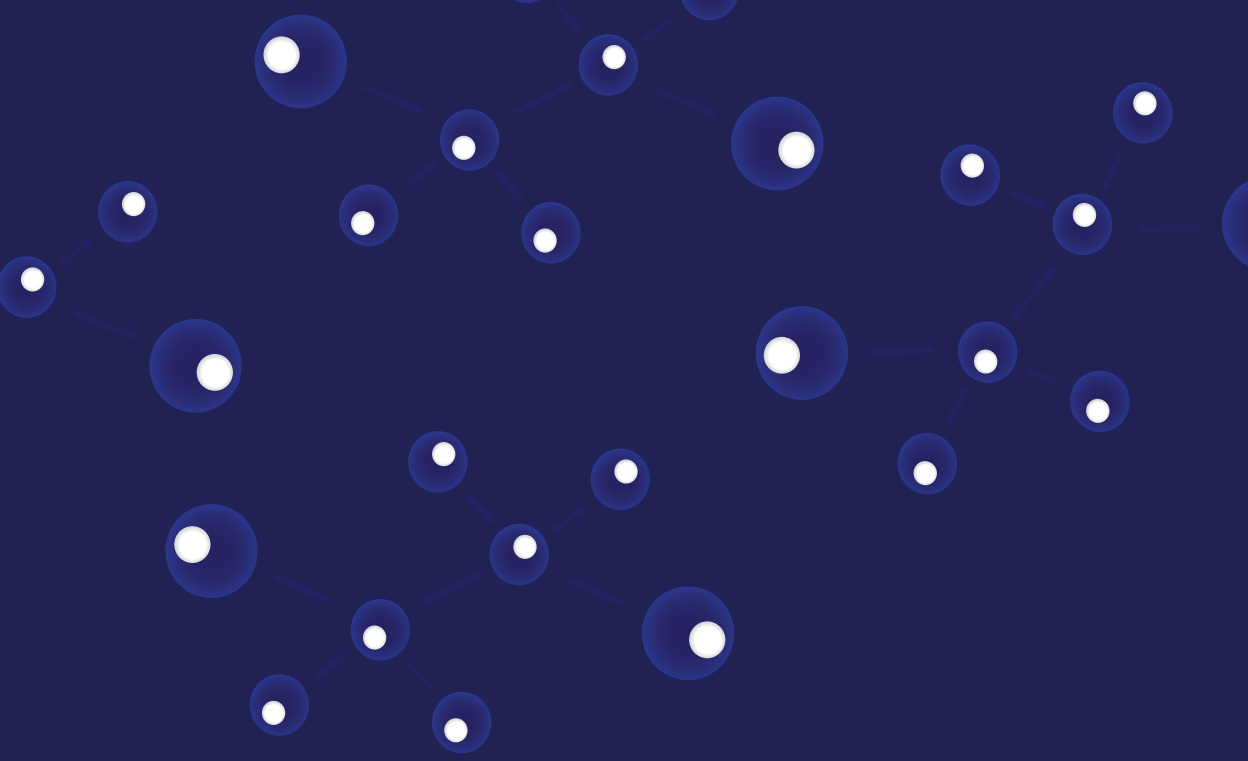

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# Nanocoatings for preventing orthopaedic implant–associated bacterial infections

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Abstract — The Research department of the Valdoltra Orthopaedic Hospital is determined to conduct more extensive research studies on modification of materials for orthopaedic implants. The starting research in the newly founded Research laboratory shall include basic and applied studies which results shall be potentially considered and eventually implemented in daily clinical practice of Valdoltra Orthopaedic Hospital. With an accurate inspection of the emerging medical needs in the field of orthopaedics we envisaged the urgent need to provide a long-term protection for orthopaedic prostheses. By means of using nano-engineering approaches for the functionalization of orthopaedic implant surfaces with suitable antimicrobial agents, it is possible to protect orthopaedic implants against harmful bacteria, which trigger the initiation of implant-associated bacterial infection. As the implant-associated bacterial infection can affect the longevity of the prosthesis, thus, the scientific and financial efforts, with the help of the project Trans2care, will be focused substantially on the design and fabrication of protective antibacterial coatings for orthopaedic implants.

Index Terms — research activity, bacterial infections, prevention, orthopaedic implant surface

## 1 RESEARCH LABORATORY

The Research Department takes an important role in the scientific activities of Valdoltra Orthopaedic hospital. The build-up of the new Research laboratory will be financially covered in great part by the hospital itself, whilst, the basic equipment and some necessary apparatus for research activities will be co-financed by the cross-border project Trans2care. Additionally, the laboratory will be equipped in a

modern fashion to provide all the comforts and ideal working environment to perform scientific research in the field of new biocompatible materials for orthopaedics. The financial plan shall include the acquisition of the following laboratory equipment and some apparatus, divided into two segments. Within the preparatory activities we will provide:

- Basic laboratory furniture, as benches and stools;
- Fume cupboard with safety storage cabinets for liquids and other reagents;
- Precision and Laboratory weights;
- Laboratory fridge and waste containers;
- General labware and consumables;
- Complete system for vacuum filtration;
- Magnetic stirrer with digital hotplate and vortex mixer;
- Shakers and ultra-sound bath;
- Centrifuges.

Within the analytical activities we plan to purchase:

- Laboratory microscope and
- Spectrophotometer with integrated measurement modes for absorbance, fluorescence and luminescence.

Till now, the majority of the research work at the Research department has been dedicated to the measurement and analysis of wear of implants surface and wear particles, histological analysis, as well as the analysis of periprosthetic tissue [1]. The wear of orthopaedic implants leads to loosening of the prosthesis, classified as »aseptic« and »septic« loosening. The septic loosening is always combined with the contamination of implant surface with bacteria (Figure 1).

## 2 ACTIVITIES AND ROLES IN TRANS2CARE PROJECT

### 2.1 Infections associated with orthopaedic implants

Implant-associated infections are the result of bacteria attachment to an implant surface and subsequent biofilm formation at the implantation site. Biofilm formation usually takes place in several phases, starting with rapid bacteria surface adherence, followed by multilayered cellular growth and intercellular adhesion in an extracellular polysaccharide matrix [2,3]. If bacteria adhesion occurs before tissue regeneration takes place, host defenses often cannot prevent surface colonization for certain bacterial species that are capable of forming a protective biofilm layer. Therefore, inhibiting bacterial adhesion is necessary to prevent implant-associated infection, because biofilm are extremely resistant to both the immune system and antibiotics [3]. The ability of the biofilm of detaching some individual bacteria into surrounding tissues and the circulatory system, it might lead to unavoidable spread of the inflammation thorough the body of the host [4]. Pathogenic bacteria,

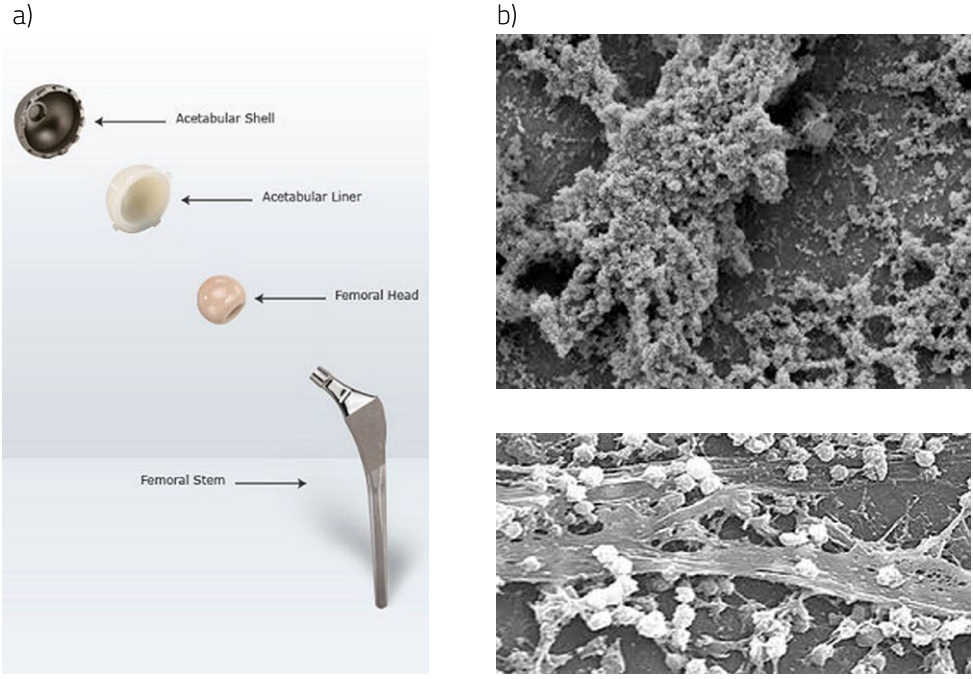


Fig.1. Components of a joint hip replacement: acetabular shell and liner, femoral head and femoral hip stem (a); surface of joint hip replacement infected by bacteria (b).

particularly gram(+) S. aureus and S. epidermidis, are found at the implantation site of approximately 90% of all implants [5]. Gram(-) bacteria, as E. coli and P. aeruginosa, have also been diagnosed in implant-associated infections [6]. The average infection rate has been reported to range between 0,5% and 3% for total hip replacement arthroplasties and the rate of reinfection after revision of infected hip prostheses is stated to be up even to 14% [7,8]. On the basis of these data, it is evident, that orthopaedic implants require better protection to avoid or at least diminish the incidence of prosthetic joint-associated bacterial infections, and consequently, the loosening of the implant with time might be prevented as well. All of these contribute to the extension of the longevity of an orthopedic implant.

## 2.2 Antimicrobial agents used in coating techniques

New strategies for the design of safer orthopaedic devices have been proposed to control and prevent bacterial contamination of implants. One of the promising approaches is to adjust, at the nano-meter scale, the antimicrobial capabilities of the implant surface [9]. This might be achieved by the utilization of different surface functionalization strategies in use, as the direct impregnation of implant surface with antibiotics or any other antimicrobial agent, as well as the coating technique with antimicrobial metals such as copper and silver. Titanium-oxide coatings deposited on

the implant surface have been also successfully examined [10]. However, the usage of certain coatings is still partially restricted to implants, thus, new coatings need to be developed to gain better protection against post-operative infection and provide a barrier to minimize metal ion release into the body.

### 2.3 Nanostructured coatings for better protection of orthopaedic implants

In comparison to conventional coatings, the advantages of using nanostructured coatings for orthopaedic implants are multiple. In addition to adding antibacterial properties to implants, it is possible, by the nanoengineering approaches, to attain other functionalities such as higher biocompatibility and mechanical stability as well as the ability of the implant to stimulate the process of new bone formation [11,12]. Moreover, due to higher surface area the modified implant surface with nano-scaled topography might provide additional available sites for protein adsorption, thereby enhancing cellular interactions directly at implantation site [12,13]. This is very positive, because even the implant acts against bacteria, at the same time, it influences the bone and the surrounded tissue to heal more rapidly and in a more proper way, respectively.

## 3 CONCLUSION

The working objectives of the new Research laboratory within the project Trans2care are to develop a new, multifunctional nanocoating with desirable properties such as, to be antibacterial and osteoinductive. We believe that the project goals shall be attainable also with the collaboration with other scientific and clinical partners, involved in the project network.


## ACKNOWLEDGEMENT

We greatly acknowledge the financial support of the Fondo europeo di sviluppo regionale (Evropski sklad za teritorialni razvoj) for the Trans2care project. The co-financing of research activities in the Research laboratory by the Valdoltra Orthopaedic hospital is fully appreciated.


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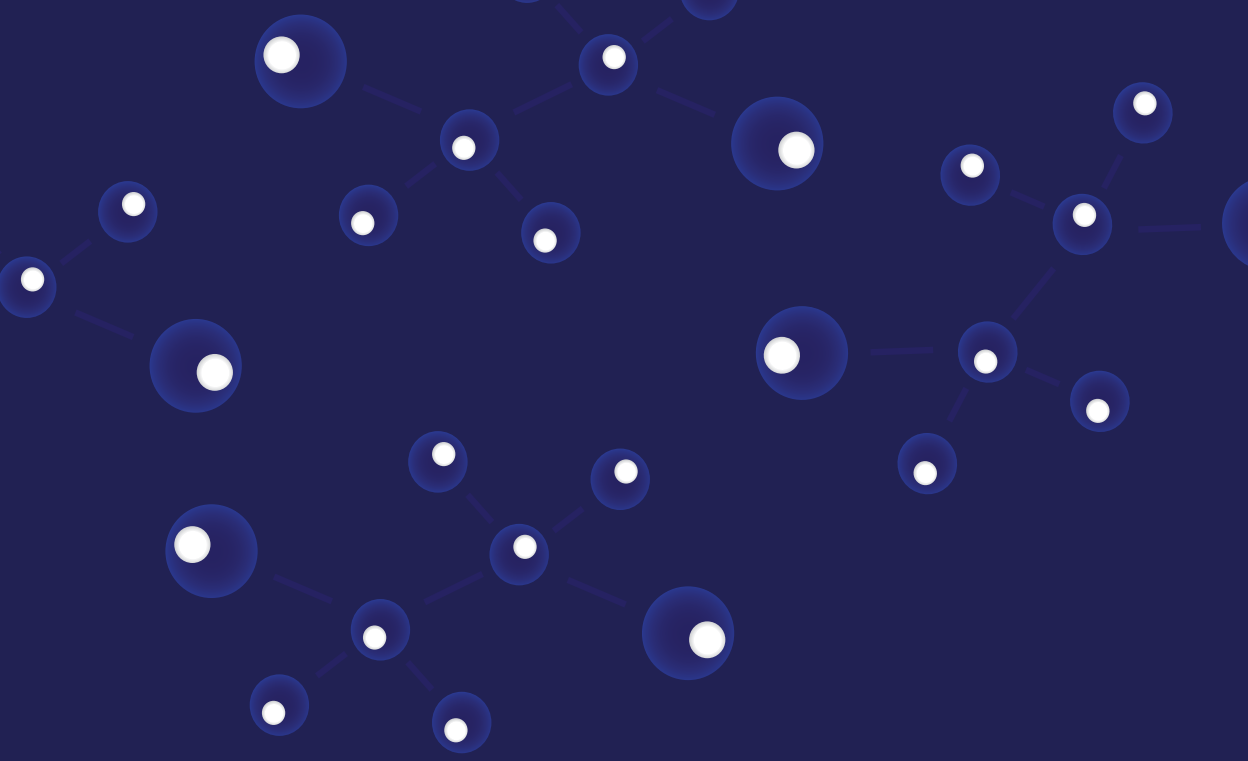

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# Gene – nutrient interactions

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Abstract — at the University of Primorska College of Health Care Izola the researchers are joined in an interdisciplinary team which will perform a research entitled 'A multidisciplinary approach in the treatment of obesity". Obesity and overweight pose a major risk for diet-related chronic diseases. So, understanding the role of obesity is of major importance in the prevention and treatment of these related diseases. It is known that genetic factors related to food intake, basal metabolic rate and adipocyte differentiation contribute, but also life style, psychological factors, and tradition, may provide to the etiology of obesity, that is why obesity will be studied from different aspects. The aim of the research is in addition to nutritional treatment also attempt to understand the regulation of the secretion of adipokines by different food components. Research is versatile and interdisciplinary, and is a challenge for researchers.

Index Terms — obesity, adipokines, gene-nutrient interactions, dietary fats

## 1 ACTIVITIES AND ROLES IN TRANS2CARE PROJECT

At the University of Primorska College of Health Care Izola interdisciplinary team of young researchers will use multidisciplinary approach in the treatment of obesity. Study corresponds to the trend of public-health problem. The goal is clear: to determine the appropriate diet to an individual, to improve the quality of life and maintain personal health. The project will be important also for the development of nutrigenomics at the University of Primorska College of Health Care Izola. Moreover, the knowledge obtained in this study will be useful also in the pedagogical processes that take place at the university of Primorska College of Health Care Izola.

### 1.1 Scientific background

The prevalence of overweight is increasing globally and has become a serious public health problem (1). Obesity is associated with low-grade chronic inflammation characterized by inflamed adipose tissue with increased macrophage infiltration.

This inflammation is now widely believed to be the key link between obesity and development of insulin resistance and metabolic disease development (2).
Adipose tissue is crucial for the inflammatory status associated with obesity, primarily because of macrophage infiltration (3). Adipocytes secrete both pro- and anti-inflammatory adipokines, including pro-inflammatory tumor necrosis factor- (TNF-), interleukin-6 (IL-6), and the anti-inflammatory adiponectin. Reduced adiponectin and increased C-reactive protein (CRP) concentrations are associated with cardiovascular diseases and type 2 diabetes (4).

## 1.2 Presentation of the problem

A reduction in inflammatory status may prevent the occurrence of disorders and diseases related to overweight. Many food compounds have been reported to have anti-inflammatory and/or antioxidant effects in various populations and the hypothesis is that these specific dietary components (polyunsaturated fatty acids (PUFAs), vitamin C, vitamin E, ...) are able to reduce low-grade inflammation as well as metabolic and oxidative stress. For example anti-inflammatory effects of n–3 fatty acids have been shown by reduced plasma concentrations of CRP, TNF-, and IL-6 (5). But the border between health and disease is often set by a complex equilibrium between two elements, genetics on one hand, and lifestyle (including diet) on the other (Figure 1). So, heterogeneity in circulating plasma concentrations of CRP, TNF-, IL-6, adiponectin, and other adipokines in response to specific dietary compounds may be due, in part, to genetics variations. Therefore at the same dietary intake of specific compound, their respective health effects may differ due to genetic differences (6).

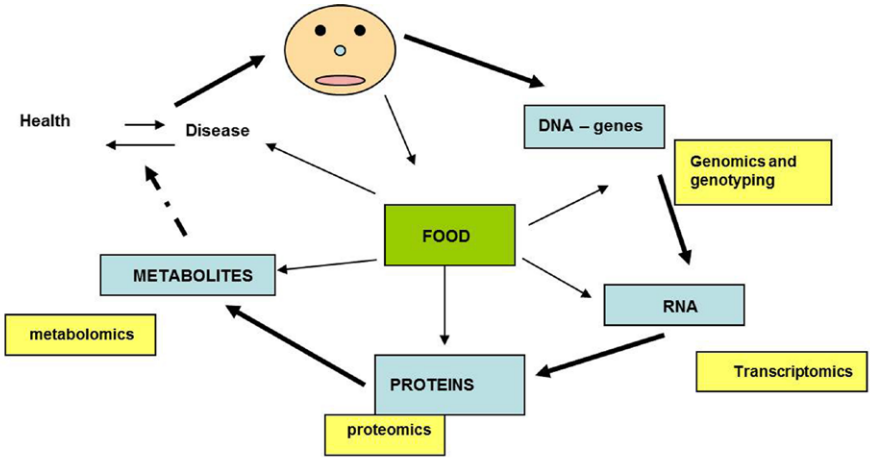


Fig. 1. Interactions between nutrients and biological macromolecules. Approaches to evaluate these interactions are genomics, transcriptomics, proteomics, and metabolomics.

### 1.3 Research objectives

In the study, we assume that changes in adipokines gene expression are caused by the ingestion of different kinds of nutrients and particularly of different types of fat. The objective of the study is to determine the relationship between TNF-, IL-6, adiponectin, resistin, and visfatin gene polymorphisms with obesity and investigate whether specific dietary compounds intake modulates these associations and how the specific dietary compound modulates adipokines secretion.

### 1.4 Methods

Different polymorphisms, and biochemical measurements, will be determined in the study of obese people and matched controls, comparable in age and sex. Dietary intake will be assessed on the basis of a food frequency questionnaire, adapted to Slovenian dietary patterns and with dietary record. Each participant in the study will be individually discussed. The nutritional status will be assessed, resting metabolic rate with indirect calorimeter will be measured and the proportion and distribution of fat will be determined with bioelectrical impedance analysis. DNA will be isolated from peripheral white blood cells and genotyping of different polymorphisms will be performed by using real time polymerase chain reaction. For the quantitative determination of adipokines in human plasma ELISA method will be performed. Moreover, all biochemical analysis will be done at the biochemical laboratory at Izola General Hospital following established procedures. In the end all results will be analyzed and evaluated using different statistical programs. Using various statistical analyses the interactions between food components and genes will be calculated.

## 2 CONCLUSION

Through the initiated Trans2Care project we intend at the University of Primorska College of Health Care Izola to contribute through the activities and studies to understand gene – nutrient interactions and, consequently, contribute to the improvement of the quality of life and to maintain personal health.


## ACKNOWLEDGEMENT

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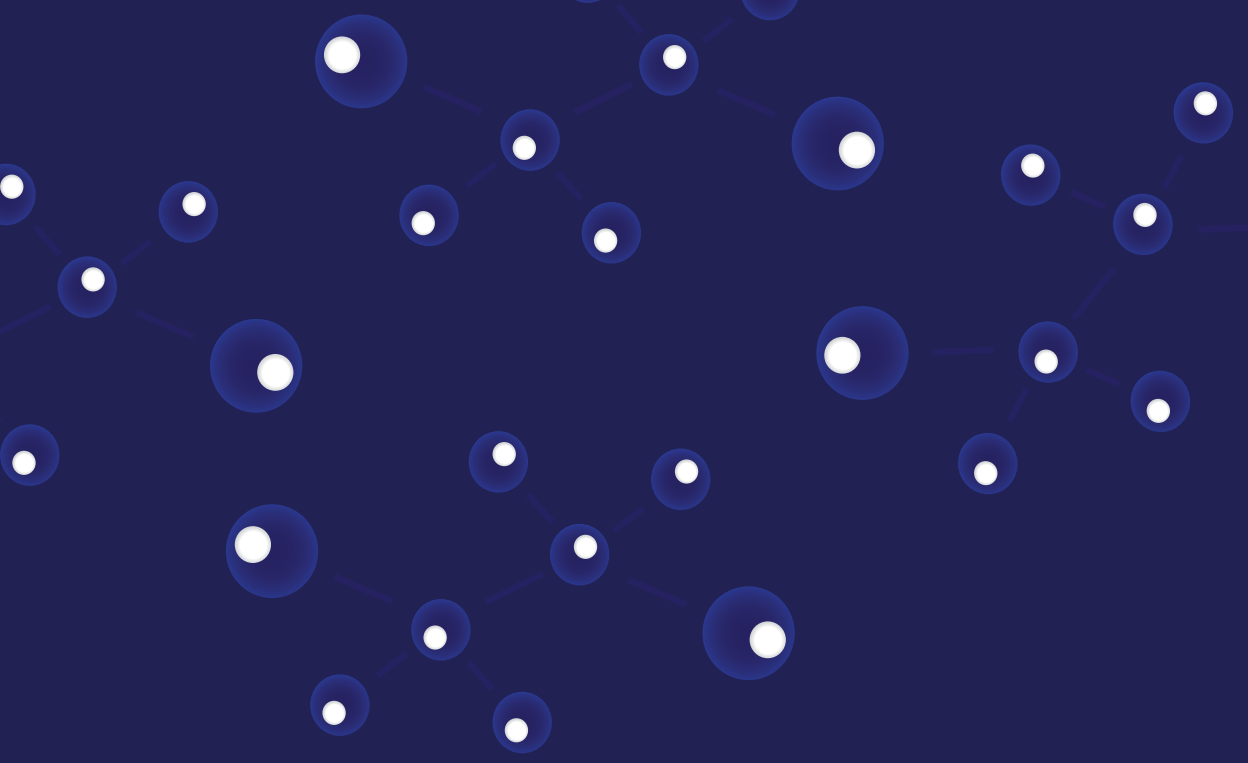

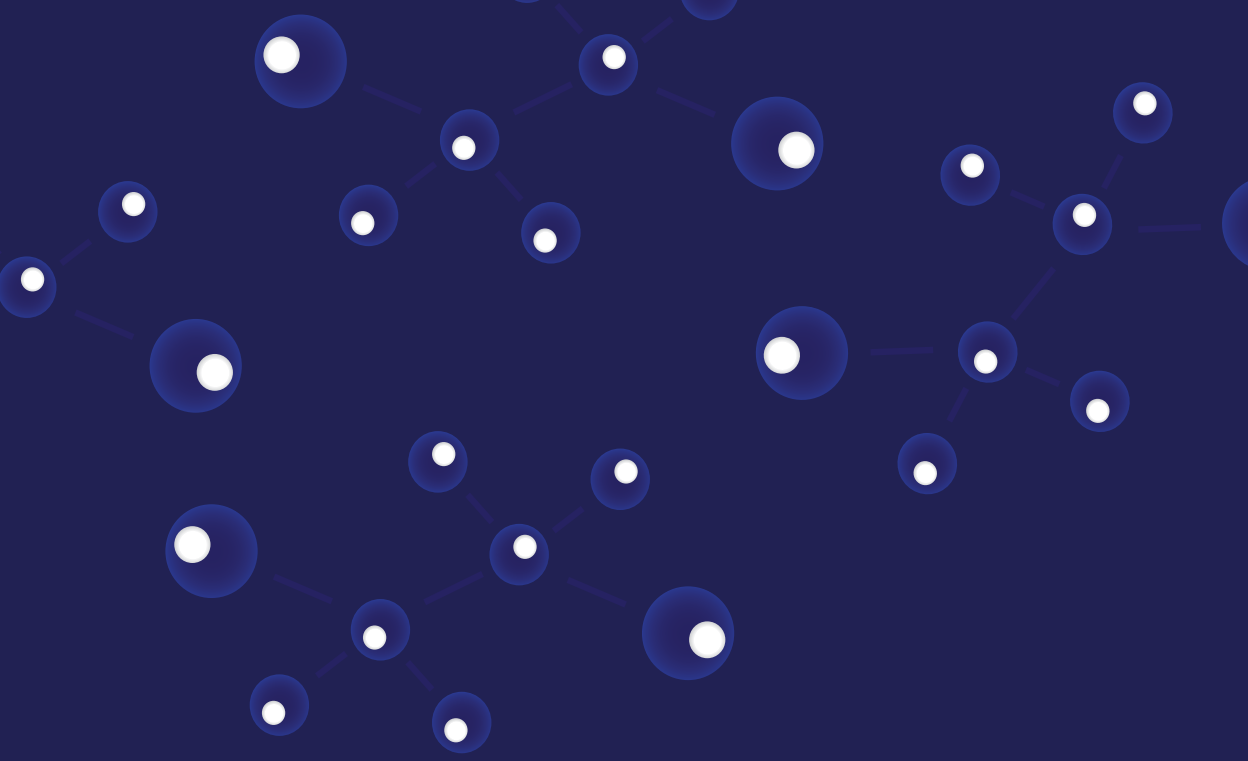

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# Biomedical application of electroporation: electrochemotherapy and electrogene therapy

Maja Čemažar, Vesna Todorović, Cécil W. Meulenberg, Nataša Tešić, Andrej Cör

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Abstract — Electroporation refers to exposure of cells to external electric field thatresults in transiently or permanently increased permeability of cell membranes. Cancer treatment, where local application of electroporation to tumor nodules is combined with chemotherapeutic drugs bleomycin or cisplatin is called electrochemotherapy. The antitumor effectiveness of electrochemotherapy is primarily based on direct killing of tumor cells due to the increased chemotherapeutic drug uptake, but other mechanisms, such as vascular disrupting action was also demonstrated. Our group is involved in elucidating the underlying mechanism of vascular disrupting action by studying the changes in cytoskeletal proteins after electrochemotherapy in endothelial cells by immunocytochemistry. In addition, metastatic potential and global changes in gene expression of melanoma cells that survived electrochemotherapy were also studied, demonstrating that metastatic potential of cells is not changed and that a very low percentage of genes are down or up regulated after electrochemotherapy, which further supports its safe use in the clinical setting. Another application of electroporation is delivery of plasmid DNA into the cells for gene therapy. Our group is involved in preparation of plasmid DNA with tissue specific promoters and without genes for antibiotic resistance, thus enabling safe use of gene therapy in line with USA Food and Drug Administration (FDA) and European medicines Agency (EMA) recommendations.

Index Terms — electrochemotherapy, electrogene therapy,electroporation, preparation of plasmids without genes for antibiotic resistance.

## 1 INTRODUCTION

Due to the physical nature of the method, all types of living cells (prokaryotic and eukaryotic) can be efficiently electroporated. Currently electroporation is used in food and biomass processing and as a cancer treatment. In food processing, the aim is to kill the microorganisms, while preserving the color, taste and the level of antioxidant in the processed food. Electroporation was successfully used for extraction of intracellular components from plant and has shown a potential in pretreatment of sludge and other substrates leading to increase in biogas production. Other applications, such as water treatment, extraction of oil from algae, sugar extraction from sugar beet are also being investigating. In cancer treatment, electroporation is currently used in combination with the chemotherapeutic drugs bleomycin and cisplatin for treatment of cutaneous and subcutaneous metastases of various histological types of tumors. Electroporation can also be used for introduction of genetic material - plasmid DNA - into the cells and therefore used for DNA vaccination and gene therapy of various diseases.

## 2 ELECTROCHEMOTHERAPY – EFFECTS ON ENDOTHELIAL CELLS

Electrochemotherapy consists of chemotherapy followed by local application of electric pulses to the tumor to increase drug delivery into cells. Drug uptake can be increased by electroporation only for those drugs whose transport through the plasma membrane is normally impeded. Among many drugs which have been tested so far, only bleomycin and cisplatin have transitioned from preclinical testing to clinical trials. In vitro studies demonstrated a several-fold increase in their cytotoxicity after electroporation of cells. In vivo, electroporation of tumors after local or systemic administration of either of the drugs, i.e. electrochemotherapy, proved to be an effective antitumor treatment. Several clinical studies were performed demonstrating that electrochemotherapy is effective in local tumor control of cutaneous and subcutaneous tumor nodules of different histology. So far, predominantly melanoma skin metastases have been treated, with ~70% long-lasting complete responses of the treated nodules [1]. Electrochemotherapy is also used in veterinary oncology, where it is used also for treatment of primary tumors and with similar results as in human medicine [2].

Besides membrane electroporation, which facilitates drug transport and its accumulation in the cell, other mechanisms that are involved in antitumor effectiveness of electrochemotherapy, have been described, potentiation of immune response, blood-flow modifying effect and vascular disrupting action. The application of electric pulses to tissues induces a transient but reversible reduction in blood flow. In vitro studies have shown that application of electric pulses to a monolayer of endothelial cells results in a profound disruption of microfilament and microtubule cytoskeletal networks, loss of contractility, and loss of cadherin-formed cell-to-cell junctions in the vascular endothelial lining immediately after electroporation, which recovered within 60 minutes after electroporation, without any significant loss of cell viability. The cytoskeletal effects of electroporation were paralleled by a rapid increase

in endothelial monolayer permeability, giving an indication of putative mechanisms responsible for the observed increase in permeability and cessation of blood flow in vivo[3]. When electroporation is combined with chemotherapeutic drugs the effects on vasculature are even more pronounced and resulted in electrochemotherapy with bleomycin in complete abrogation of blood flow in vivo. Currently, we are exploring the underlying mechanisms of this effect at the cellular level, by studying the effects of electrochemotherapy on adherent microvascular endothelial cells. We found that nuclear morphology changes as well as changes in microtubules and actin filaments network are more profound and long lasting after electrochemotherapy with bleomycin compared to changes induced by electroporation or bleomycin alone (Fig. 1). In addition, endothelial monolayer intactness was severely compromised after electrochemotherapy.

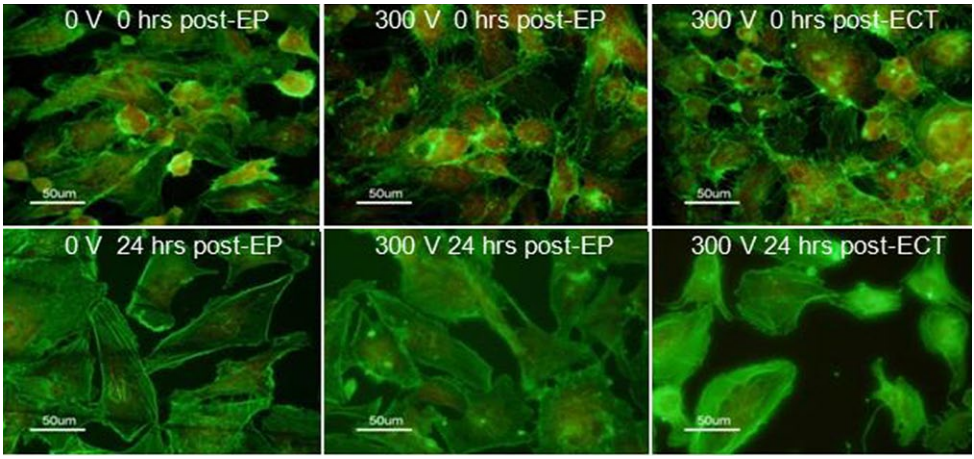


Fig. 1.Monolayers of Human Microvascular Endothelial Cells (HMEC-1) are differently affected by electroporation (EP) and electrochemotherapy (ECT).Monolayers stained for F-actin with FITC conjugated phalloidin. Note the honeycomblike appearance at 0 hrs for both 300V EP and ECT. Recovery is present at 24 hrs post-treatment, while in the presence of 300 nM bleomycin (ECT) cells start to die.

## 3 ELECTROCHEMOTHERAPY – EFFECTS ON METASTATIC POTENTIAL OF MELANOMA CELLS

Another topic of our research in the field of electrochemotherapy was to address the clinical relevant question i.e. if metastatic potential and global gene expression of melanoma cells that survive electrochemotherapy is changed. Namely, it is known that metastatic progression is a multi-step process that requires acquisition of many specific cell properties, each fulfilling a unique function in the metastatic cascade for successful establishment of metastases. Alterations in tumor cells and their microenvironment induced by therapy can play an important role in metastasis induction. We studied on viable cells 48 hours after electrochemotherapy

their ability to migrate and invade through Matrigel coated porous membrane. In addition, microarray analysis was used to detect changes in gene expression after electrochemotherapy. Cell migration and invasion were not changed in melanoma cells surviving electrochemotherapy. In addition, only a low number of tumorigenesis related genes was differentially expressed after electrochemotherapy (Fig. 2), demonstrating that the metastatic potential of melanoma cells was not affected by electrochemotherapy and confirming its safety in the clinics [4], [5].

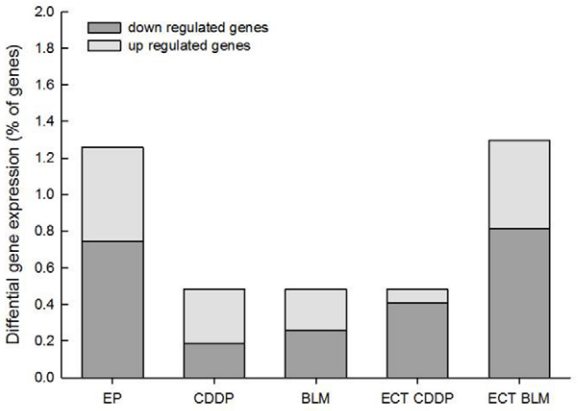


Fig. 2.Differentially expressed tumorigenesis related genes after electrochemotherapy. From 0,5% to 1,3% of all evaluated genes were differentially expressed after exposure to electroporation (EP), cisplatin (CDDP), bleomycin (BLM) or electrochemotherapy (ECT) with CDDP or BLM. Bars represent percentage of down regulated or up regulated genes of the total number of tested genes.

## 4 ELECTROGENE THERAPY – PREPARATION OF PLASMID DNA WITHOUT ANTIBIOTIC RESISTANCE GENE

Another application of electroporation is delivery of genetic material i.e. plasmid DNA into the cells. Due to the concerns connected to viral vectors, non-viral approaches for transfection are becoming very attractive [6]. One of the goals of researchers dealing with gene therapy or DNA vaccination is to develop safe and efficient systems for gene transfer into the target cells. Namely, this topic is still one the major hurdles in the progress of different approaches in gene therapy and is crucial factor for success of the therapy. Other factors that should be considered for development of gene therapy are the choice of appropriate therapeutic gene, regulation of its expression and administration route. Our group at the College of Health Care Izola in collaboration with Institute of Oncology Ljubljana, National Institute of Biology and University of Ljubljana Biotechnical faculty is planning to address two of these factors for the use in cancer gene therapy: safety of plasmid DNA by preparation of plasmid DNA without marker for antibiotic resistance and regulation of therapeutic gene expression by linking the therapeutic gene to cellular promoters to achieve either stress induced (p21 and radiation) or physiological regulation (tissues specific

promoters for endothelial and muscle cells and fibroblasts) of expression. We are planning to prepare plasmids encoding reporter or therapeutic genes under the control of tissue specific or inducible promoters using standard molecular biology techniques and plasmids without gene for antibiotic resistance according to the instructions of the producer of ORT® plasmid. The results of this on-going project, if successful, may have impact on design and execution of gene therapy trials.

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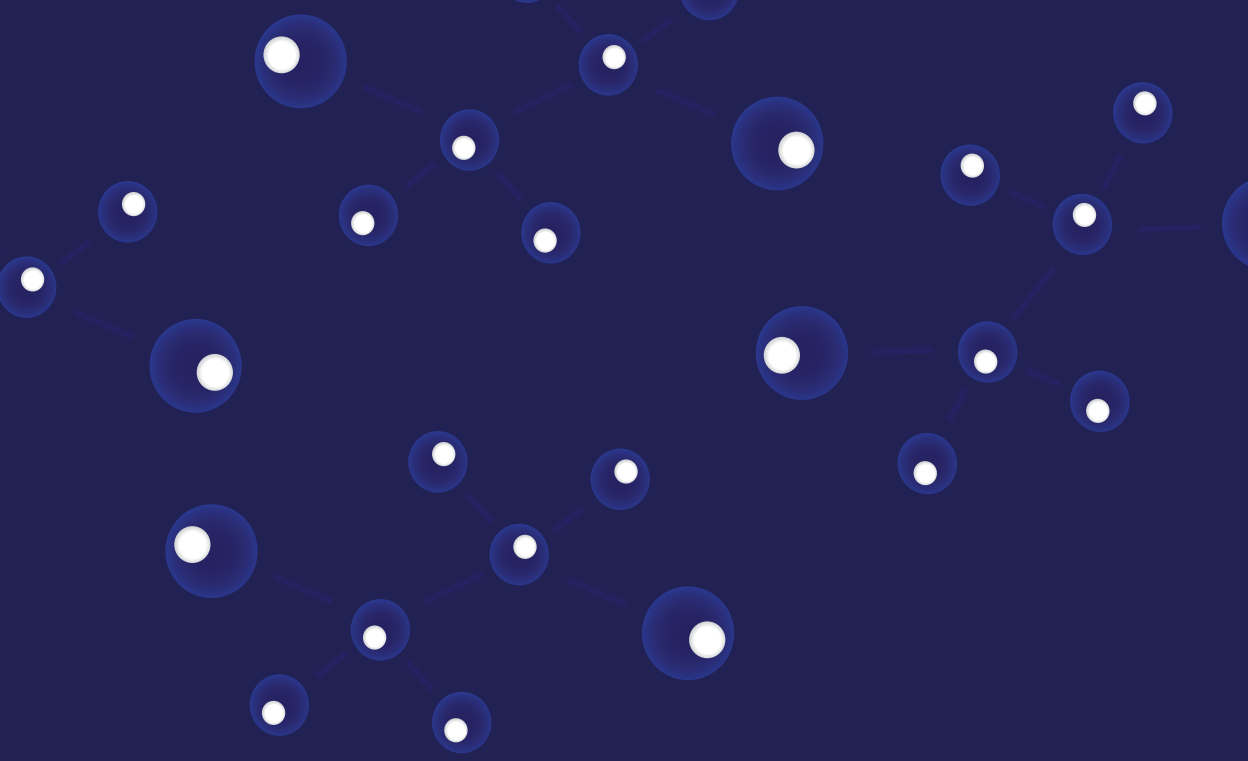

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# Real-time PCR applications in clinical research and diagnostics

Mitja Rak, Darja Barlič–Maganja, Julija Hmeljak, Vesna Todorović, Maja Čemažar, Andrej Cör

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Abstract — Polymerase chain reaction (PCR) has enabled enormous progress in the field of molecular biology in the last twenty-five years. It became popular due to its high sensitivity and specificity and was introduced to numerous scientific fields. Today many different variants of PCR exist, with real-time PCR being the most common. This method is being successfully used at our institution for a variety of different applications, among which gene expression analyzes and development of protocols for clinical diagnostics currently dominate. But the extreme flexibility and customizability of real-time PCR, coupled with our team's expertise, ensures efficient application of this technique to various new research projects.

Index Terms — real-time PCR, SYBR Green, TaqMan, quantification, gene expression

## 1 INTRODUCTION

### Principle of PCR reaction

Polymerase chain reaction (PCR) is a technique that has had a tremendous influence on development and research in molecular biology. Nowadays, it has become common and is an essential laboratory tool, on which the majority of molecular biological studies are based.

The primary purpose of PCR is rapid amplification of single or few copies of template DNA, generating thousands to millions of copies of the original (template) DNA sequence. Key components that enable this process are primers, deoxyribonucleotides, and a DNA polymerase. The first are short nucleotide sequences that specifically bind

to a region of the template DNA and enable selectivity of amplification and initiation of DNA synthesis. DNA polymerase is a thermostable enzyme that synthesizes a DNA strand complementary to the template. PCR reaction is composed of 30-40 cycles of alternated steps of heating and cooling, which are necessary for the separation of the two strands of DNA double helix, annealing of primers and for the DNA synthesis. Based on the detection method of PCR amplicons, two main PCR techniques exists: i) end point PCR, where the product is detected after the PCR reaction is completed, usually with gel electrophoresis, and ii) real-time PCR, where the product is detected during the reaction, as it is being

### Detection dyes in real-time PCR

Fluorescence-producing chemistries used in real-time PCR can be grouped into two main types: nonspecific and specific. The most commonly used nonspecific chemistry is SYBR® Green I, where the nonspecific dye emits fluorescence only when it reversibly binds to any double stranded DNA that is generated during PCR reaction. Therefore, distinguishing between specific and nonspecific products based only on the amplification curve is impossible. Post-amplification analyses are needed to confirm specificity of the amplicon with the use of dissociation-curve analyses.
The most commonly used specific chemistry is TaqMan® chemistry. What makes this chemistry appealing is that it integrates an additional level of specificity, obtained thanks to the use of specific oligonucleotide probes. A fluorescent reporter dye is bound to the 5'-end of the probe and a quencher dye is bound to the 3'-end. As long as the probe is intact, the quencher inhibits the fluorescence of the reporter dye. But when the probe hybridizes to its target sequence, the 5'-exonuclease activity of DNA polymerase degrades the probe, thereby separating the reporter and quencher dyes, which results in increased fluorescence of reporter dye. Since only specific products are detected that way, no post-amplification analyzes are needed. Nevertheless, TaqMan® has a unique drawback. In order for the chemistry to function as desired, one must pay special attention at the choice of DNA sequences of both primers and probe. A poorly chosen probe or mismatched annealing temperatures of all three oligonucleotides in the reaction can severely handicap detection of amplicons, producing a false result.
Moreover, labeling specific TaqMan® probes with different reporter dyes enables detection and quantification of different target sequences in the same reaction tube (multiplexing), which significantly reduces the reaction costs. SYBR® Green chemistry also enables multiplexing, but distinguishing between two different products is much harder, since there must be apparent differences in dissociation-curve peaks of the different multiple amplicons. On the other hand, when using TaqMan® chemistry, multiplexing is limited only by the choice of dyes with emission spectra compatible with the detection spectra of the real-time PCR machine.

## 2 OUR EXPERIENCE WITH REAL-TIME PCR

Our research facility is equipped with a 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). It is being used for a variety of different applications. Because PCR is a very sensitive method, designated areas and separate pipetting tools are used for PCR reaction set-up.

Not only that PCR is very sensitive, it also has high specificity, thus it can be used to distinguish highly similar template DNA sequences. This means that closely related species or even subspecies and strains, depending on the variability of nucleotide sequence being amplified, can be identified. Thus choosing an appropriate template gene and a region in that gene is essential for successful identification. At our institution, both TaqMan® and SYBR® Green I chemistries are currently being used for species detection and identification.

SYBR® Green I chemistry is used for detection and identification of bacteria and viruses in a variety of different clinical samples. Samples of periprosthetic tissue from patients who had undergone revision arthroplasty were analyzed with the use of broad-range primers for the presence of the 16S rRNA bacterial gene, while identification was done by sequencing of the amplified products. In clinical samples from patients with malignant pleural mesothelioma, species-specific primers were used for detection and identification of SV40 virus , thus dissociation-curve analyses in combination with appropriate controls were sufficient for confirmation of specificity of the amplified products.

The use of species-specific primers and sequencing of amplicons for identification can be overcome with the use of TaqMan® chemistry. An example of user-friendly TaqMan® chemistry is "Custom TaqMan gene expression assays" (Applied Biosystems), which include a validated and optimized mix of a fluorogenically labeled probe and primers. Besides analyzing gene expression (as the name implies), these assays can also be used for species identification, in our case bacteria. The advantage of these assays is that no post-amplification manipulation and analyses are needed for confirming the specificity of the fluorescence signal. No primer-probe validation needs to be performed by the user, which significantly reduces cost and labour.

Quantitative PCR analyses are also being performed at our institution. Currently, only TaqMan® chemistry is used for quantitative analyses. Gene expression was relatively quantified for analyzing transcriptional silencing of genes that are involved in cancer phenotype regulation . Residual amounts of therapeutic plasmids, containing the IL-12 gene, and used in electrogene therapy of canine skin tumors, were absolutely quantified using TaqMan® assays.

Standard detection and identification techniques in virology have already been replaced with modern molecular methods and used for diagnostic purposes enabling us to detect and subtype different viruses.

## 3 CONCLUSION

The use of the polymerase chain reaction (PCR) in molecular diagnostics has increased to the point where it is now accepted as the gold standard for detecting nucleic acids from a number of origins and it has become an essential tool in the research laboratory. Real-time PCR has engendered wider acceptance of the PCR due to its improved rapidity, sensitivity, reproducibility and the reduced risk of carry-over contamination. The technology has been applied to different areas of microbiology as well as studies of gene expression and genetic disease. Within our institution, real-time PCR method has been applied to various research fields, granting our team valuable expertise for implementation of this technique to several possible research projects in the future.

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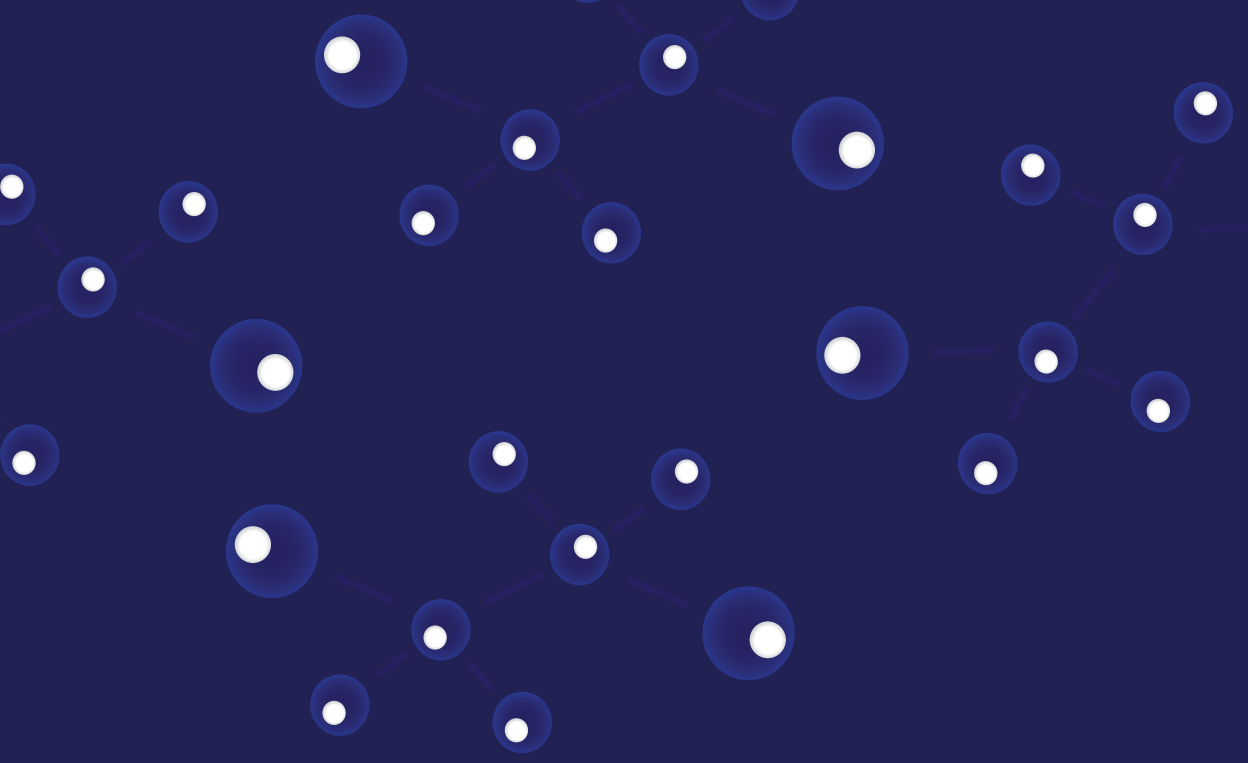

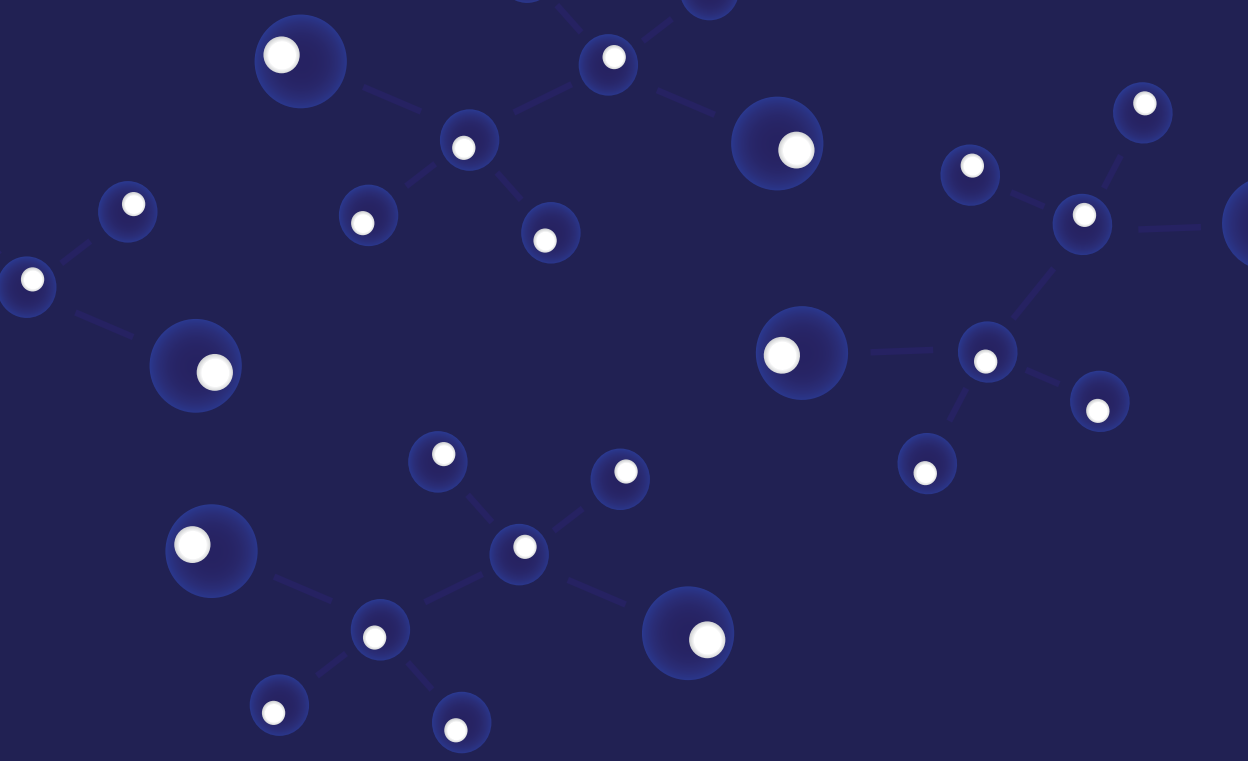

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# Medicine, Biology and Mathematics

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Abstract — Although mathematics finds large application in physical sciences, its use in the medical and biological field is less usual. Nevertheless, mathematical models can help in understanding many medical and biological phenomena and they can be a powerful tool in the designing process of many technological devices connected to the above mentioned phenomena. Of course, fundamental pre-requisite for building up a reliable mathematical model is the deep cooperation of different competences such as those of medical doctors, biologists, pharmacists and engineers. This paper deals with four different examples of mathematical models developed by our group and dealing with restenosis, enzyme action on two dimensional DNA brushes, oral delivery of drug in amorphous or nano-crystalline form and tumour cell behaviour.

Index Terms — Biology, Mathematical model, Medicine

## 1 INTRODUCTION

If it is well known that mathematics is largely employed in the engineering, physic and chemistry fields, less obvious is its utility in medicine and biology. This probably depends on both cultural and practical reasons. Indeed, the human necessity of knowledge categorising favoured, in the past, the formation of impermeable barriers among knowledge fields that hindered the diffusion of transversal tools such as mathematics. Accordingly, mathematics remained in its historical nest, i.e physical sciences field. In addition, the typical complexity of many medical and biological problems did not surely encourage crossing the above mentioned barriers [1]. Nowadays, fortunately, the diffusion of mathematics in the medical and biological sciences is well established and a huge variety of examples can be cited. For example, Freguglia [2] edited a book dealing with theoretical aspects of evolutionary biology, mathematical interpretation of Darwin's evolutionary theory, bacteria motion and heartbeat. Additionally, mathematics can find application, for example, in cardiovascular system, neuroscience, ecology, environment, evolution, immunology,

infectious diseases, tumor growth and therapy [3]. The spreading of mathematics is witnessed by the existence of many societies, such as the European Society for Mathematical and Theoretical Biology [4], the Society of Mathematical Biology [5] and the Japan Association for Mathematical Biology [6] that actively promote mathematics use in the medical and biological fields. Ultimately this is nothing more than a modern translation of the Leonardo da Vinci idea according to which "... niuna umana investigazione si può dimandare vera scienzia, s'essa non passa per le matematiche dimostrazioni ..." (no human research can be defined true science if it cannot be mathematically demonstrated) [7].
While the utility of mathematics in the medical and biological fields is obvious when thinking to the statistical analysis of experimental findings, the help that it can provide in the everyday research emerges also through the mathematical modelling activity. Indeed, the mathematical model, defined as a mathematical metaphor of some aspects of reality (drug systemic spreading after oral administration, for example) [8], consists of the theoretical representation (imitation or emulation) of something or in the visualisation of something that cannot be seen (a molecule, for instance). Consequently, mathematical modelling can be viewed as a cognitive activity of the human mind aimed to the description of how devices behave or phenomena and processes take place. This means that a reliable mathematical model can be built on condition that a clear idea of how a phenomenon develops is known. Thus, mathematical modelling means knowledge. Accordingly, it is evident the great advantage that can be gained by the use of mathematical models in the medical and biological field in order to support and to prove very different theories.
The aim of this paper is to show some examples of mathematical models developed by our groups and devoted to medical and biological problems. In particular, the attention will be devoted to restenosis, enzyme activity in self-assembled DNA layers, oral drug delivery and tumor cell behviour.

## 2 RESTENOSIS

Traditionally, coronary stenosis, a common atherosclerosis complication, has been matched by means of percutaneous transluminal coronary angioplasty (PTCA), a procedure leading to the enlargement of the stenotic portion of the coronary by means of an expanding balloon. Nevertheless, the high incidence of re-stenosis (30-40%) following PTCA [9], obliged to consider alternative approaches. The use of stents, rigid scaffolds positioned in correspondence of the coronary stenotic portion, emerged as an interesting therapeutic strategy due to the consistently reduction of re-stenosis occurrence with respect to the simple PTCA treatment [10]. However, stents did not completely solve the re-stenosis problem. Although they can really prevent the coronary wall early elastic recoil and late re-modelling, two known events leading to re-stenosis, they induce neointima iperplasia (In Stent Restenosis – ISR) due to the hyper-proliferation of vascular smooth muscle cells (VSMCs) [11]. At this purpose, the employment of drug eluting stents releasing anti-proliferative drugs (DES) substantially reduced the VSMCs iper-proliferation and the consequent

re-stenosis rate [12]. Unfortunately, however, recent studies evidence that the re-vascularization benefit with DES is attenuated in high-risk patients (diabetes; the acute coronary syndromes; smaller-diameter lesions and longer lesions; several stents or overlapping stents), compared to low risk patients [13]. Moreover, their use is connected to a higher incidence of late thrombosis (due to an incomplete endotelisation of the stented zone) in comparison with uncoated stents [14].
In the attempt of overcoming these problems, our group and other researchers proposed to use of new therapeutic agents such as, nucleic acid based drugs (NABD) that have been proved to hinder VSMCs hyper-proliferation by suppressing the expression of relevant cell cycle promoting genes [15, 16, 17]. The fragile nature and low cellular transfection attitude impose NABD complexation with proper delivery agents (DA; liposomes, polycations and fatty substances, for instance) in order to make this approach effective and reliable from the therapeutic point of view. In addition, NABD-DA properties (dimensions and physic-chemical characteristics) make their release from traditional DES problematic and alternative solutions need to be considered. A possible choice is represented by the endoluminal gel paving technique (EGP) [18], together with the implantation of a bare metal stent. EGP consists of the catheter application of a biocompatible polymer solution on the endoluminal vessel surface followed by in situ polymerization or crosslinking. The potential advantages of the gel paving-stent technique are an easy and safe complex loading within the gel matrix and the opportunity of creating a physical barrier between the damaged coronary wall and the overflowing thrombogenic and inflammatory elements present in the blood stream [19].
In the light of this frame, the developed mathematical model accounts for 1) NABD-DA diffusion out of the gel layer towards the coronary wall and the blood stream 2) NABD-DA convective motion in the coronary wall portion not shielded by the gel layer (this convective contribute is due to the radial hydrostatic pressure gradient between lumen and coronary wall) and 3) NABD-DA metabolism and internalisation into cells. In order to reduce the intrinsically three dimensional problem to a simpler two dimensional problem, the stent is schematised by a series of equally spaced rings that cannot be crossed by NABD-DA. In addition, with the aim of accounting for really occurring situations, two different stent positions with respect to the coronary wall are considered: stent strut totally embedded or totally out of the coronary wall [20]. The performed simulations make clear that in order to hinder all the stages leading to re-stenosis (thrombi deposition 0-10 days, VSMCs proliferation 2.5 – 25 days, late remodelling and inflammation 25 – 300 days) it is necessary to complex the NABD with at least two DA differing in size. Indeed, small DA guarantees a rapid diffusion of the NABD-DA complex that can hinder the fast re-stenosis phenomena (thrombi deposition and beginning of VSMCs proliferation). Big DA ensures a slow diffusion of the NABD-DA complex that can hinder the long occurring re-stenosis phenomena (late VSMCs proliferation, late remodelling and inflammation).

## 3 ENZYME

The immobilization of dense DNA structures on solid supports (ultra-flat gold surface), obtained by self-assembly and enzymatic manipulations, holds a central role as a tool for developing miniaturized devices with innovative applications, ranging from biosensing [21] to next-generation information technology [22]. For example, restriction enzymes, that cleave double-stranded (ds) DNA molecules, are widely utilized in cloning as well as in a number of DNA-based assays [23] because they target very specific dsDNA sites. Moreover, these enzymes have the ability to diffuse in one-dimension by sliding on the backbone of long dsDNA filaments during their site-searching process [24]. Recently, it was studied the behaviour of a restriction enzyme reaction (DpnII) with dsDNA oligomers packed into relatively large (and flat) surface-bound brushy matrices consisting of patches of monolayer with controlled density [25]. The enzyme action was followed by measuring the height reduction of the dsDNA brush because of cleavage using atomic force microscopy (AFM). It was found that the restriction reaction depends on the DNA density, and it was demonstrated the existence of a threshold above which the reaction is inhibited (essentially in a stepwise manner). This result, which has been later confirmed by other groups [26], suggested that the accessibility of dsDNA molecules in these surface-bound brushy matrices critically depends on the inherent steric hindrance. In particular, we saw that while enzyme cannot enter inside the dsDNA monolayer through the upper surface (i.e. that formed by dsDNA free end), the access is only allowed through the lateral dsDNA brush side. We explained these results by modelling the steric hindrance of the dsDNA brush in the upper and side surface [27]. This model was inspired by an established theory describing the diffusion of solutes inside polymer matrices [28]. Once this peculiar behaviour was elucidated, the attention was focussed on the mathematical description of enzyme diffusion and reaction inside the two dimensions dsDNA brush. In particular, the model was based on the idea that the 2D enzyme movement occurs according to Fick's law while enzyme reaction kinetics took place according to the Michaelis-Menten scheme where the reaction constants are those experimentally determined in bulk. On the basis of this model, it was then possible simulating the enzyme action (dsDNA cutting) on the two dimensions brush.

## 4 ORAL DELIVERY

Many pharmaceutical systems are essentially made up of a polymeric carrier hosting the active agent (drug) inside its three-dimensional network [29]. Especially in the case of oral administration, they are often prepared as particulate systems since these forms present remarkable advantages over the single unit devices. The easier dispersion inside the stomach reflects into an appreciable reduction of the local drug concentration which is usually responsible for gastric irritation. Moreover, they are very versatile and powerful as drug loading into cross-linked polymers can represent a profitable tool to increase the drug dissolution rate in aqueous media and, thus, the bioavailability of slightly water-soluble crystalline drugs [1]. Indeed, by means of different techniques such as solvent swelling, co-

grinding or supercritical CO2 it is possible loading drug nanocrystals and amorphous drug inside polymeric matrices [1]. The interesting aspect of nanocrystals is that their solubility increases with reducing their dimension up to the amorphous state that can be viewed as a crystal of vanishing dimension [30]. Thus, they are more bioavailable than bulk (macro) crystals [31] and their use implies dose reduction, with clear benefits for what concerns the reduction of side effects and costs. In order to theoretically study the in vivo behaviour of these 'activated" systems, we built up a mathematical model accounting for the simultaneous processes of drug release from the particulate systems and the drug spreading into the body. In particular, the release stage considered the phenomena concerning particle swelling, drug solubilisation and drug diffusion in the polymeric network. The spreading step, instead, accounted for drug absorption, partitioning among tissues and elimination/metabolism (ADME processes) [32]. This model proved to be a reliable tool to fit both in vitro and in vivo experimental data. Accordingly, it is a powerful mean to properly design delivery systems based on polymeric particles containing drug nanocrystals and amorphous drug.

## 5 TUMOUR CELL BEHAVIOUR

We have employed mathematical models also to describe the behaviour of different forms of tumour cells including prostate cancer cells. From the medical point of view, we are interested in studying the behaviour of prostate tumour cells as this kind of tumour is the main cause of cancer death in males in the Western World and also in Friuli Venezia Giulia (Registro Tumori 199-2003). By mathematical modelling, we could properly compare the growth rate of different types of prostate cancer cells and make interesting correlations with the expression genes (eEF1A1 and eEF1A2) representing potential markers for prostate cell transformation and/or of cancer progression [33]. Shortly we will use the mathematical approach developed to study the growth behaviour of prostate cancer cell upon the treatment by NABD (work in progress); this approach will allow us to shorten the experimental procedure providing a sharp picture of the effect of our molecules in different prostate cancer cell lines. Finally, it should be noted that a similar approach will be followed for NABD molecules directed against genes involved in the generation and development of hepatocellular carcinoma [34, 35], another relevant tumour pathology in Friuli Venezia Giulia.

## 6 CONCLUSIONS

Mathematical modelling can be a powerful tool for the understanding of many phenomena occurring in medicine and biology and, consequently, a powerful tool for the designing of devices connected to these phenomena. Of course, due to the complexity of the phenomena occurring in medicine and biology, a reliable mathematical model can be obtained on condition that many different competences concur in its building up. Accordingly, for example, the deep cooperation of medical

doctors, biologists, pharmacists and engineers is requested. We believe that this is the winning strategy for the challenges proposed by the modern research.

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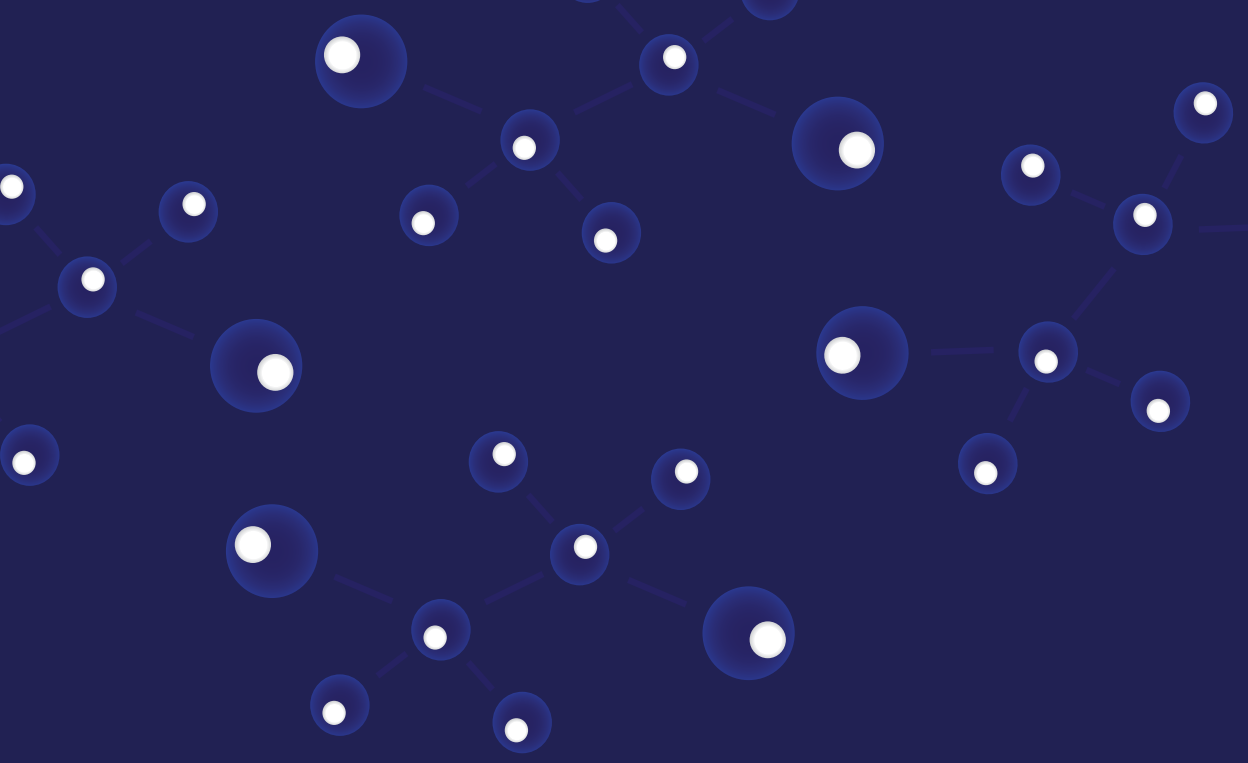

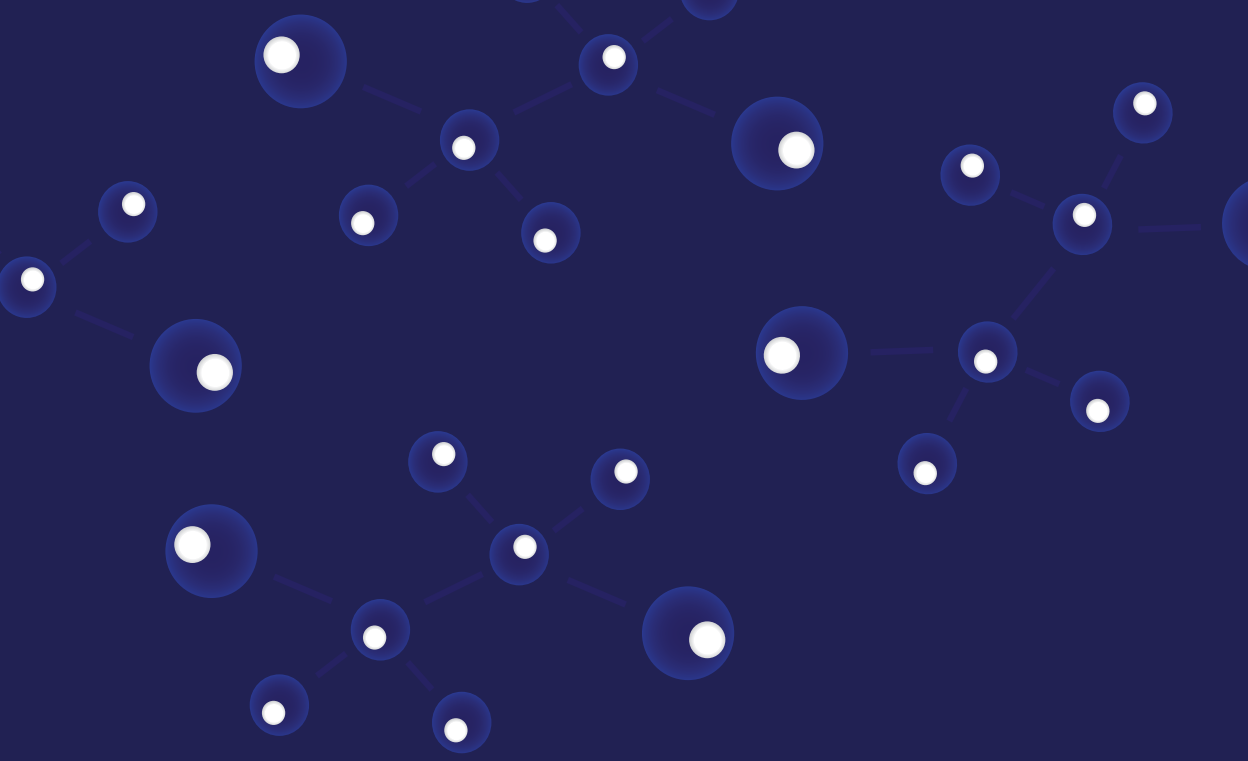

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Transregional Network for Innovation and Technology Transfer to Improve Health Care

# Elastin-based biopolymers for biomedical and biotechnological applications

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Abstract — Perhaps the most appealing opportunity is represented by gaining inspiration from nature for the precise tailoring of biomaterials with finely tuned unique functional properties. A very promising model is represented by Human Elastin-Like Polypeptides (HELP), repetitive artificial polypeptides based on penta- or hexa- peptidic motifs that characterize elastin. These protein polymers retain several peculiar biophysical properties as, the reversible inverse phase transition, changing the solubility and aggregation state in response to temperature variation. The smart nature of this class of compounds makes them attractive for many applications in the biomedical and biotechnological fields, in particular for biomaterial development. The Trans2Care project aiming to translating scientific and technological expertise into products for the biomedical field represents an exciting and challenging environment to best exploit the potential these biomimetic macromolecules.

Index Terms — recombinant protein, elastin, biomimetic strategy, advanced biomaterials

## 1 INTRODUCTION

### 1.1 Elastin, a model for protein-based artificial biopolymers

Bio-mimicry, the concept of taking inspiration from nature is perhaps among the most appealing strategies to create customized biomaterials with finely tuned peculiar features.

Elastomeric proteins, well represented among Vertebrates are among the components that received considerable attention. Elastin, one of the components

of the extracellular matrix, possesses rubber-like elasticity undergoing deformation without rupture and provides an important model for biomaterials design. Another peculiar extensively studied characteristic of elastomeric proteins is coacervation. Under appropriate conditions of concentration, ionic strength and increasing temperature, the protein is known to separate from solution as a second phase. It has been shown that this behavior is mainly due to the presence of the hydrophobic (VPGXG) pentapeptide, typical of the mammalian protein.
In 1974, Urry et al. first developed artificial polypeptides mimicking these repeated hydrophobic motifs characterizing bovine tropoelastin. [1].
In the human protein, the most structurally regular sequence is represented by the repetition of the (VAPGVG) hexapeptidic motif. It has been shown that the VAPGVG motif is biologically functional.
By a molecular biology approach, a regularly repeated domain from human tropoelastin was chosen as a basic modulus to obtain the Human Elastin-Like Polypeptides (HELPs). These artificial proteins can be employed in the production of innovative micro- and nano-structured biomaterials with a huge potential for employment in the biotechnological and biomedical fields.

## 1.2 HELP biopolymers family

In our lab the HELP macromolecule, based on the most structurally regular module of human tropoelastin was developed and expressed in bacteria [2]. The polypeptide is soluble in aqueous solution below a critical temperature, whereas it forms aggregates above this temperature. This process, known as "inverse" phase transition, is fully reversible.
A second synthetic gene was developed, and a polypeptide, named HELP1, based only on the most consistently repeated peptide sequence of human tropoelastin was obtained (Figure 1).

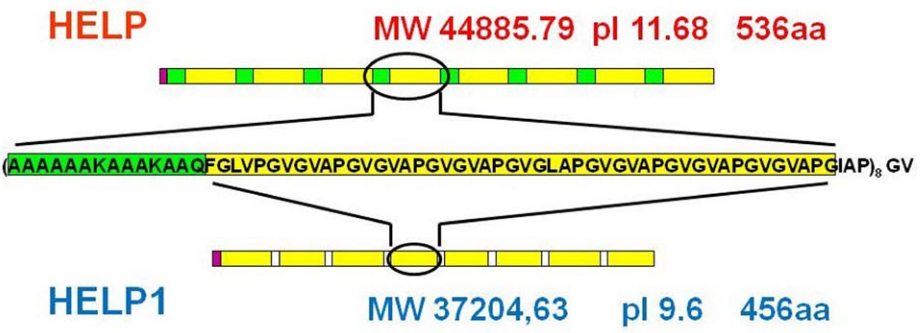


Figure 1. Schematic representation of the two HELP and HELP1 prototypes.

Overall, the work carried on in our lab is aimed at the production of a family of artificial polypeptides that exhibit different and variable physicochemical properties. The two prototype macromolecules HELP and HELP1 that differ in their primary structure have been characterized from the physic-chemical point of view, showing a different behaviour in relation to the different environmental conditions [3]. Both biopolymers are able to give reversible hydrogels under defined conditions by raising temperature (30-37°C).
Relatively large yields have been recently achieved upon optimization of biopolymer expression and of the purification steps, in the range of hundreds of milligrams of purified product per liter of bacterial culture. [4]
A key feature of these recombinant products is related to the modular structure of the synthetic genes that allows the tailoring of the macromolecule for a specific application.
In this light, the HELPs can be viewed as prototypes whose functionality can be implemented by simply fusing peptidic regions to the C-terminal part of the biopolymers. The addition of a bioactive domain of choice will result in the conferring specific function to the whole product.

## 2 BIOTECHNOLOGICAL AND BIOMEDICAL APPLICATIONS

Many recent examples show the potential of these polypeptides for a wide range of biomedical applications, and to build structures with highly controlled properties and behaviour [5].
The thermoresponsive self-assembly properties of these macromolecules are of particular interest to develop functional biomaterials, especially in the field of targeted delivery and controlled release of active molecules.
A method for preparation of 3D matrices with hydrogel features has been patented recently [6] and prosecution procedures are currently ongoing in Europe and USA (Figure 2).

Figure 2. Stable HELP hydrogel matrix.

This method represents a valuable option to preserve cell viability and functions as it avoids the use of harsh chemical reagents commonly used for preparation of matrices [7]. A huge potential for biomaterial development is foreseen. For example, one interesting feature is represented by the possibility to encapsulate cells in the gel maintaining their viability.
As shown in Figure 3, cells spheroids can grow within the gel.

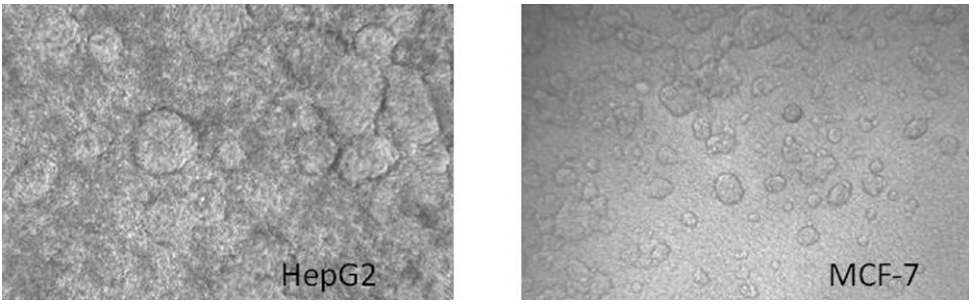


Figure 3. Viable cell encapsulation in 3D HELP matrix.

## 3 AIMS IN TRANS2CARE PROJECT

Proteinaceous material represents a promising alternative to chemically synthesised, traditional biomaterials still commonly employed in the biomedical field. HELPs represent promising innovative macromolecules and show high potential for a wide range of applications. Thus, we intend to exploit the Trans2Care partners' expertise and knowledge to:
a) exchange ideas between traditionally distinct research areas and bringing together the competences to devise the employment of HELP biopolymers for specific demand;
b) provide the HELP compounds to the partners to setup experimental work;
c) develop commercial applications of HELPs and biomaterials derived.
In particular, collaboration with:

- University of Nova Gorica (PP3), on development of innovative environmental biosensors;
- Treviso Tecnologia (PP5), on supporting technology transfer of existing and future HELP products;
- ZTM (PP10), on development of advanced cell growth and delivery systems.

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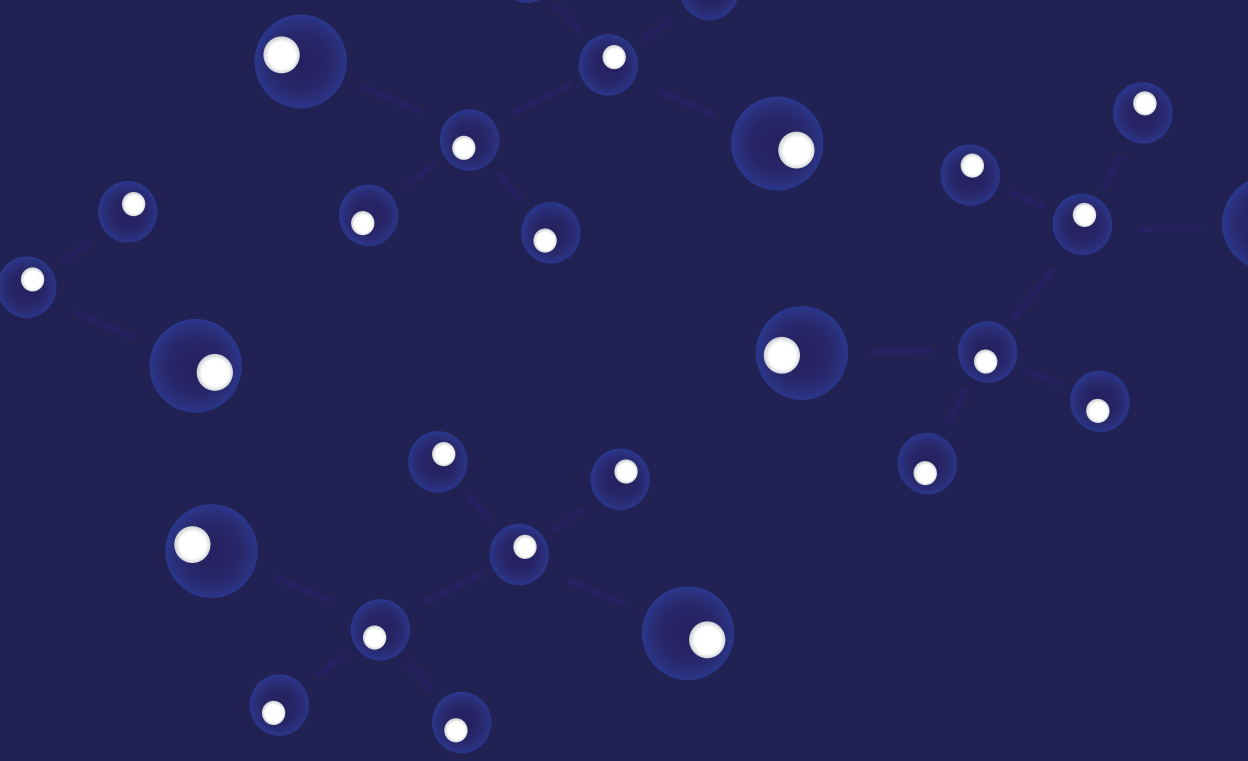

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# Bilitranslocase and anthocyanins role in the gastrointestinal tract

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Abstract —Bilitranslocase (BTL) is a organic anion transporter expressed in liver and in several extra-hepatic tissues. This membrane protein transports different substrates as pyrrolic molecules, nucleotides, flavonoids. Anthocyanins, one of the most represented flavonoids class are known to have antioxidant activity. They act as anti-inflammatory, anti-cancer and anti-proliferative molecules by interfering with different intracellular pathways. Some chronic colon diseases results in an increase in the pro-inflammatory machinery that are associated with a 5-fold increased risk of developing colon cancer. The expression and the role of BTL in the gastrointestinal tract in normal and pathological condition, as well as its role as anthocyanins transporter in colon cancer cells will be take into account in Trans2care project.

Index Terms — bilitranslocase, organin transporter, flavonoids, anthocyanins, colon cancer, chronic colon diseases

## 1 INTRODUCTION

### 1.1 Bilitranslocase (BTL)

Bilitranslocase (BTL) is a plasma membrane transporter of 340 amino acids that displays no homology with known proteins [1]. However, its coding sequence shares 96% identity with a segment of the antisense strand of ceruloplasmin. BTL was originally identified in rat liver, and subsequently found also in absorptive (stomach and intestine) and excretory (kidney) epithelia. Its sub-cellular localization is suitable for the uptake of its substrates from the gastro-intestinal lumen, suggesting its nutritional role. Concerning BTL expression in pathological conditions, preliminary data show reduction in its expression in kidney cancer cells. BTL has also been detected in colon cancer cell line Caco-2 where the transport activity is strongly

inhibited by anti-bilitranslocase antibodies [2]. However, there are no available data on the presence of this transporter in chronic colon diseases.
Bilitranslocase transports various polyaromatic compounds such as bromosulfophthalein (BSP) and indocyanine green, dyes used in the evaluation of hepatic function, but also bilirubin and nucleotides. BTL presence in the stomach [3] with the functional properties identical to those in the liver [4] might explain why flavonoids, as dietary anthocyanins for example, are absorbed a few minutes after their ingestion [5]. On the other hand, BTL high turnover might explain why flavonoids are rapidly metabolized and excreted. The detection of BTL on the luminal surface of the intestinal epithelium could also speak in favor of its role in flavonoid uptake since they are absorbed at this level as well.

### 1.2 Anthocyanins and chronic bowel diseases

Flavonoids are a large group of phytonutrients that may provide beneficial effects on human health. They are divided into several classes: flavanones, flavones, flavonols, flavanols and anthocyanins. The main dietary sources of flavonoids are fruits, vegetables and grains. Epidemiological, clinical and experimental studies have indicated that dietary intake of flavonoids confers protection against multiple chronic diseases [6]. Their possible role in cancer prevention and progression has gained increased attention over the last years. Anthocyanins, which are the most abundant flavonoid constituents of fruits and vegetables [7] might be of special interest in this respect. Recent data show that these compounds are excellent intracellular antioxidants even at very low concentrations [8] and it is their antioxidant activity that confers protection against carcinogenesis. However, in cells that have already undergone malignant transformation, anthocyanins, act as prooxidants and induce apoptotic death [9]. Various studies confirmed the antiproliferative and anticarcinogenic activities of anthocyanins in different cell lines originating from colorectal cancer [10, 11]. Colorectal cancer is the second most frequent cause of cancer-related death. A combination of various risk factors, including obesity, sedentary lifestyle, increased prevalence of smoking, excessive alcohol consumption, a diet rich in red and processed meat and low intake of fruits and vegetables, can trigger the carcinogenic process [12]. Anthocyanins have been shown to exert strong cytotoxicity on highly tumorigenic and metastatic Lovo/ADR colon cancer cell line while being less harmful to cells with low proliferation rate (Caco-2), which brings up their selective action [9]. Animal studies have also reported anticarcinogenic properties of anthocyanins. In induced rat colon cancer cell models they significantly decreased total tumors as well as aberrant crypts [13, 14]. Anthocyanins have also shown anti-inflammatory activity through targeting NF-κB pathway and COX-2 [15], which might have implications in tumorigenesis [16]. Even more, their ability to inhibit matrix metalloproteinase activity [17] could additionally contribute to both their anticancer and anti-inflammatory action. These activities, together with anthocyanins antioxidant properties, might also suggest their beneficial role in inflammatory conditions of the small intestine and colon, such as celiac disease and inflammatory bowel disease (IBD), yet there are no studies confirming this hypothesis.

Celiac disease is an immune-mediated disorder that affects primarily the small intestinal mucosa. It is characterized by chronic inflammation which occurs when gluten-activated Th1 cells start secreting cytokines (INF-γ and TNF-α) which in turn activate the release of enzymes such as metalloproteinases that can damage the intestinal mucosa leading to atrophy of intestinal villi. The progressive destruction of the small intestinal mucosa causes malabsorption, and a variety of clinical manifestations. Ulcerative colitis (UC) and Crohn's disease (CD) are the two main forms of inflammatory bowel disease. They are multifactorial conditions, where both genetic and environmental components play their parts. However, at the basis of UC and CD lays aberrant immune response and consequent inflammation regulated mainly by NF-κB. Both CD and UC are associated with a 5-fold increased risk of developing colon cancer [18].

## 2 AIMS IN TRANS2CARE PROJECT

Concerning the importance of diet on human health and the still scarce knowledge of the role of anthocyanins and bilitranslocase in both physiological and pathological conditions of the gastrointestinal tract, we will try to:

A) Set dietary guidelines (based on the molecular mechanisms underlying anthocyanins bioavailability: their membrane transport at the level of the epithelial barrier of the gastrointestinal mucosa, and the role of BTL in it) for the prevention of chronic disorders of the intestine ;
B) Study and exploit the drug transport potential of BTL in order to develop new orally-deliverable drugs on the basis of bilitranslocase substrate specificity;
C) Exploit BTL as a biomarker: it might be an indicator of cell health (intactness), whereas its expression might be lost in cancer. It would be interesting to see how early the loss of expression occurs in disease development.

To reach these aims we will work in collaboration with IRCCS Burlo Garofalo (PP9), taking advantage of their tissue bank and expertise in gut histopathology in order to study BTL expression and determine any changes depending on the state of mucosa; with University of Nova Gorica (PP3), who has at its disposal sophisticated techniques for anthocyanins separation and analysis in order to measure their uptake and transport across different cell monolayers; and with ZTM (PP10), who can help us to prepare a monoclonal antibody against BTL for immunohistochemistry procedures.

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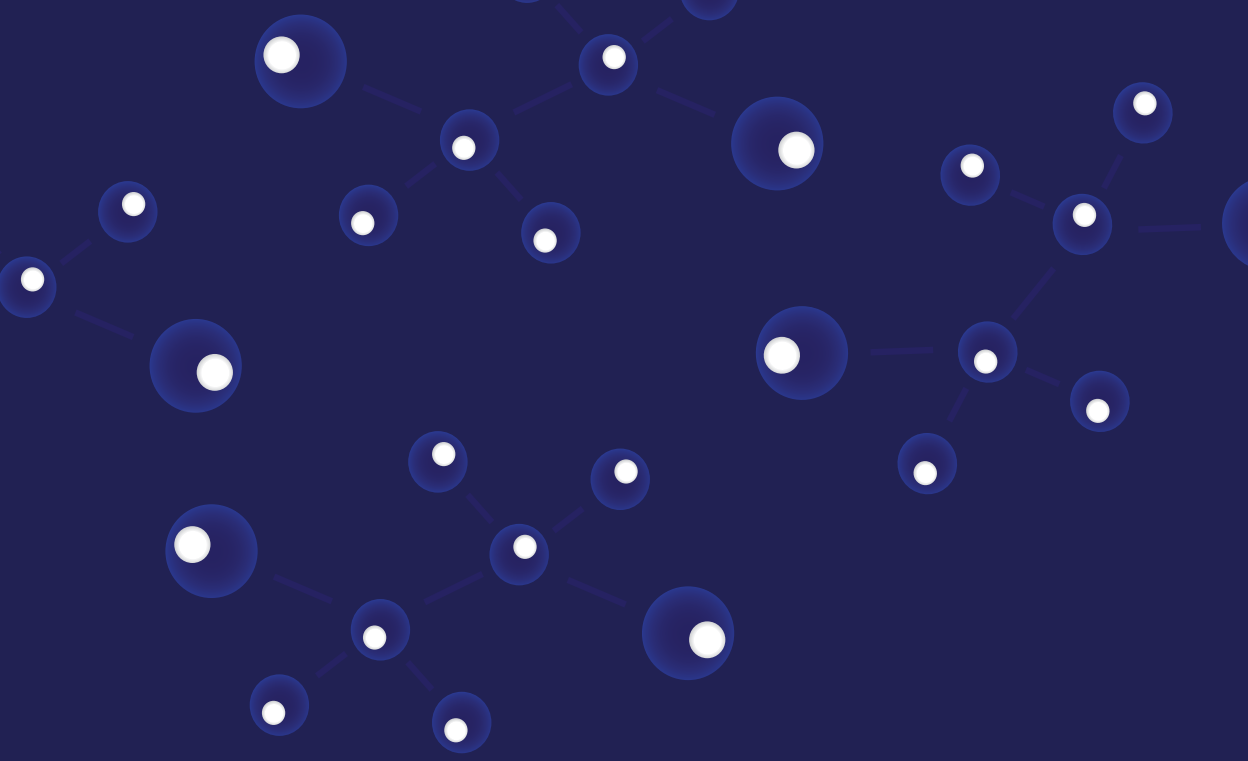

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# The enigma of flavonoids and bilitranslocase activity in the cardiovascular system

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Abstract — Numerous epidemiologic studies showed an inverse correlation between dietary flavonoid consumption and cardiovascular risk, but the exact mechanisms are still largely unknown. Flavonoids exhibit hormetic properties, where low concentrations activate adaptive cellular stress response pathways and thus lead towards cytoprotection, whereas high concentrations are cytotoxic. However, the limited bioavailability of dietary flavonoids doubts the relevance of effective flavonoid intracellular concentrations to induce bioactivity in endothelial cells. Therefore, translocation of flavonoids through the cell plasma membrane must occur via specific transporter proteins. Hereby, we describe the involvement of the membrane transporter bilitranslocase (TC #2.A.65.1.1) as the key underlying molecular mechanism for membrane transport, which might help resolve the enigma of flavonoids bioactivity.

Index Terms — bilitranslocase, flavonoids, cardiovascular health, hormesis, TRANS2CARE

## 1 FLAVONOIDS AND CARDIOVASCULAR HEALTH

Numerous epidemiological studies have shown that regular intake of flavonoids is associated with an improved cardiovascular prognosis [1,2]. In the setting of primary prevention, individuals with the highest flavonoid intake have modestly reduced risks for developing cardiovascular disease [3-5]. In addition, flavonoid intake benefits individuals with established cardiovascular disease. This can be seen from clinical signs like lowered blood pressure [6-8], improved endothelial function [9-11], inhibited platelet aggregation [7,12-14], decreased low density lipoprotein oxidation [15,16], and reduced inflammatory responses [17-19].

To promote the idea of healthy diet, it is now clear that the medical researcher must put big efforts in providing concise explanations how the potential beneficial effects of flavonoids can arise. Scientists have a responsibility to investigate this issue, and provide the general public evidence-based guidelines on a healthy diet. Even more, researchers must try to isolate the therapeutic lead molecules, and chemically improve them, to obtain new potential drugs for treating specific pathophysiological conditions. So far, exact mechanisms of how flavonoids act are still largely obscure, and thus represent a big challenge for research, whether in academia or in the pharmaceutical and the nutraceutical industry.

## 2 PHARMACOKINETIC CONCERNS RELATED TO THE FLAVONOIDS PHARMACODYNAMICS

The biggest pharmacological concern of dietary flavonoids can be attributed to their limited bioavailability, which results in very low plasma concentrations (0.1-1 μM) [20]. This questions whether intra-cellular concentrations attain levels that are of any relevance to the endothelial function [21]. In fact, it is not clear if flavonoids interact with any endothelial plasma membrane receptors and thus act by triggering an intracellular signal transduction cascade, or they penetrate into the cells and, consequently, bind to intracellular molecular targets. In the latter hypothetic pathway, translocation of flavonoids through the cell plasma membrane must occur via specific transport proteins [22].

On the other hand, high plasma flavonoid concentrations might have deleterious systemic effects, as it is suggested by some of theirs on the cardiovascular system [23]. Though still isolated, these observations warn against the potential alternative routes of administration, i.e. parenteral, which would avoid the low absorption and first-pass metabolism issues, or utilization of the enhanced absorption delivery systems.

It is widely accepted that flavonoids are good antioxidants when chemically tested in in vitro non-biological systems, but little emphasis is put on the fact that most of those studies showed effects in the supra-physiological concentration ranges, many magnitudes of order higher than reported in vivo. Thus, there is now a more plausible hypothesis that the in vivo beneficial effects of these phytochemicals are unlikely to be explained just by their per se antioxidant capability. Flavonoids may exhibit hormetic properties, by acting as `low-dose stressors' that may prepare cells to resist more severe oxidative stress, while high concentrations are cytotoxic [24]. In this biphasic manner, low concentrations of flavonoids induce cardiovascular protection, while high concentrations are cardiotoxic [23].

This is meaningful since low and transient concentrations of flavonoids in the plasma contribute little per se to the overall plasma antioxidant status, when compared to the relatively higher concentrations of other antioxidants in plasma, e.g. vitamin C, uric acid, bilirubin, etc. Taken altogether, the diverse chemical structures of flavonoids exhibit molecular mimicry with many mammalian endogenous molecules, thereby leading to manifold biological effects, i.e. altering intracellular signaling pathways and

enzyme activities. Compared to the specific activity of other plant-related chemicals, for example morphine's high affinity targeting of the opiate receptors, flavonoids have been always on the borderline between pharmaceuticals and xenobiotics. Since they don't have any specific cellular targets, as indirectly confirmed by all flavonoids-related studies with only partial inhibition and/or activation of numerous examined molecular targets, they should, in our opinion, fall in the category of xenobiotics.
From an evolutionary perspective, flavonoids are primarily produced by plants to prevent insects and other animals to eat plants, and this again speaks in favor that the observed bioactivity of flavonoids from animal and human studies is actually resulting from the activation of adaptive cellular stress response pathways [25,26]. On the other hand, we must also consider the plausibility that there do exist some conservative proteins in nearly all animals, who eat the flavonoid-rich plants, and there are also similar proteins found in both the plant and animal kingdoms. Many of these are still largely unknown in humans, but can profoundly influence both the pharmacokinetic or pharmacodynamics properties of flavonoids.

## 3 BILITRANSLOCASE TRANSPORTS FLAVONOIDS INTO THE CELLS

There is a big question in the science: how can flavonoids enter into the cells? A potentially involved carrier is bilitranslocase (TC #2.A.65.1.1) [27], a bilirubin-specific membrane protein that is also responsible for ATP-independent transport of flavonoids across cell membranes in various rat organs, e.g. on the epithelium of liver, gastrointestinal system, kidney, brain, lungs, etc., and also on the vascular endothelium [28]. Even though the investigation has not yet covered all mammalian tissues or other species, data already suggest that bilitranslocase might be a ubiquitous membrane protein, therefore playing a major role in biology.
Based on extensive transport activity assays performed on a battery of flavonoids subclasses, bilitranslocase can be considered as an anthocyanin-selective transporter, since other flavonoids are not effective ligands [27]. This selective interaction can be explained in terms of molecular mimicry between bilirubin and anthocyanins, as well as by specific structural characteristics of anthocyanins, such as the planar system of conjugated aromatic rings and pH-dependent tautomerism, which by protonation converts the quinoidal form into the phenolic form [29].
Bilitranslocase is expressed both in the vascular system, on the endothelium and vascular smooth muscle cells [28], and also in the cardiac system, on the endocardium and cardiomyocytes (data not yet published). This presence enables to understand some of the cardiovascular effects of flavonoids, which are summarized in the Table 1.

| | |
|---|---|
| 1. DIMINUTION OF OXIDATIVE STRESS | Direct scavenging of free radicals (antioxidant action in the narrow sense of the word) |
| | Metal interaction (iron and/or copper chelation) |
| | Inhibition of ROS producing enzymes, in particular xanthine oxidase, NADPH oxidase and lipoxygenases |
| | Stimulation of endogenous antioxidant defense mechanisms, increase in expression of eNOS, intracellular glutathione |
| 2. DECREASE IN THE EXPRESSION OF INFLAMMATORY SIGNALING MOLECULES | Inhibition of iNOS expression (endothelium) |
| | Inhibition of COX-2 expression (endothelium) |
| | Inhibition of leukocyte activation |
| 3. INHIBITION OF PLATELET AGGREGATION | Increase in platelet NO production |
| | Decrease in platelet production of superoxide anion |
| 4. DIRECT VASODILATORY ACTION | Activation of eNOS signaling pathway in the endothelium, leading to release of NO |
| | Activation of EDHF-mediated vasorelaxation in the endothelium |
| | Direct effects on ion channels |
| | Inhibition of cyclic adenosine monophosphate-dependent phosphodiesterase |
| 5. OTHER CARDIOVASCULAR ACTIVITIES | Isoflavones improve endothelial function through an effect on αestrogen receptor |
| | Positive/negative inotropic effects |
| | Activation of PPARγ |

Table 1. List of potential protective effects of flavonoids in the cardiovascular system. Adapted from [30].

## 4 ROLE OF BILITRANSLOCASE IN THE CARDIOVASCULAR SYSTEM

We have made a significant progress in our understanding of the functional role of the bilitranslocase protein in the cardiovascular system. Original approach towards studying the protein function was based upon the inhibition of the bilitranslocase activity by using anti-sequence bilitranslocase antibodies. They target distinct extracellular epitopes of the carrier, and thereby inhibit the transport of the substrates into the cytoplasm.

The data obtained from our experiments suggest that even low concentrations (in nM range) of flavonoids, which are comparable to post-absorption plasma levels, are rapidly (<1min) taken up into the endothelium via bilitranslocase (Ziberna, FRBM, under review). This rapid uptake into endothelial cells can be explained only by the

involvement of specific membrane transporters that catalyze the passage of polar substances, such as flavonoids, through the phospholipid bilayer.
Furthermore, bilitranslocase represents a key step in the flavonoids-induced endothelial cell-signaling cascade leading to the activation of intracellular PI3-kinase/Akt/eNOS pathway (Ziberna, not yet published), as schematically shown on Figure 1. Accordingly, bilitranslocase mediates the vasorelaxation responses induced by flavonoids [31].

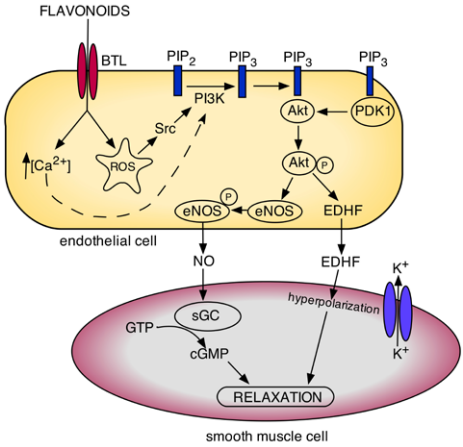


Figure 1. Scheme indicating that flavonoids are potent inducers of the endothelial formation of NO and EDHF with their corresponding different intracellular signaling pathways. BTL – bilitranslocase, EDHF – endothelium derived hyperpolarizing factor, NO – nitric oxide, eNOS – endothelial NO synthase, PI3K – phosphatidyl-inositol 3-kinase, PDK1 – posphoinositide-dependent kinase 1, ROS – reactive oxygen species, sGC – soluble guanylyl cyclase. Adapted from [30].

In the cardiac system, we introduced an innovative approach of retrograde coronary perfusion with the bolus of the bilitranslocase antibodies. Thus, specific immunocomplexes were formed on the surface of the coronary endothelium and in a lesser extent, if at all, on the endocardium of the left atrium and ventricle. Importantly, unbound immunoglobulins were removed by continuous perfusion. We found that bilitranslocase mediates the cardioprotective activity of flavonoids in ischemia-reperfusion model of the isolated heart, as observed by measuring coronary flow, cardiomyocyte lysis and duration of arrhythmias (Ziberna, FRBM, under review).

## 5 INTERPLAY BETWEEN FLAVONOIDS AND BILIRUBIN

Given the mutual metabolic interference between flavonoids and bilirubin, it can be speculated that the transient increase in the antioxidant capacity of endothelium following ingestion of flavonoids [32] might be, in part, ascribed to a transient and reversible decrease of bilirubin clearance by the endothelial cells. Furthermore, it can be speculated that flavonoids occupy the activity of bilitranslocase, thus decreasing the endothelial efflux of intracellular (endogenous) bilirubin, which is constantly produced by heme oxygenase-1 and biliverdin reductase. Since bilirubin is a strong

endogenous antioxidant, it can be speculated that even a very small increase in basal levels of free bilirubin can provide significant antioxidant protection of endothelium [33]. In fact, patients with Gilbert's syndrome, a hereditary condition characterized by slightly elevated bilirubin plasma levels and occasional mild and intermittent jaundice, show a decreased risk of cardiovascular diseases in comparison to normo-bilirubinemic populations [34,35].

## 6 CARDIOVASCULAR RESEARCH ON THE BILITRANSLOCASE WITHIN TRANS2CARE PROJECT

In the framework of TRANS2CARE project, we will continue to further elaborate our cardiovascular research on the role of bilitranslocase, jointly with our project partners:

- collaboration with PP1 – Chemical Institute of Ljubljana: in the field of computational modeling and chemometrics (QSAR);
- collaboration with PP3 – University of Nova Gorica: studying the metabolism and fast-uptake of flavonoids and bilirubin into endothelial cells;
- collaboration with PP10 – Blood Transfusion Center of Slovenia: development of novel monoclonal antibodies against bilitranslocase, which are crucial for our platform of functional studies, as well as having the potential to be used as biomarkers;

Furthermore, we will also expand our network, in accordance with WP7 - Enlargement and consolidation of the network, towards the research groups outside the network:

- collaboration with University of Trieste, Department of Materials and Natural Resources, CENMAT, contact person Alois Bonifacio: employing SERS Raman spectroscopy to detect low concentration of bilirubin in the extracellular and intracellular environment;
- collaboration with IASMA Research and Innovation Center, Food Quality and Nutrition Area, San Michele all'Adige, contact person Fulvio Mattivi: metabolomics experiments on the effects of flavonoids in cardiovascular tissues.

## 7 CONCLUSION

The knowledge presented is important for the better understanding of diet-based preventive medicine, which is an affordable and efficient way of improving worldwide health. Academic world must, therefore, provide the rationale for specific dietary guidelines about including flavonoids-rich food in the diet. And the people will have no hesitation to make excellent life choices in the direction of achieving significant health benefits.

## ACKNOWLEDGMENTS

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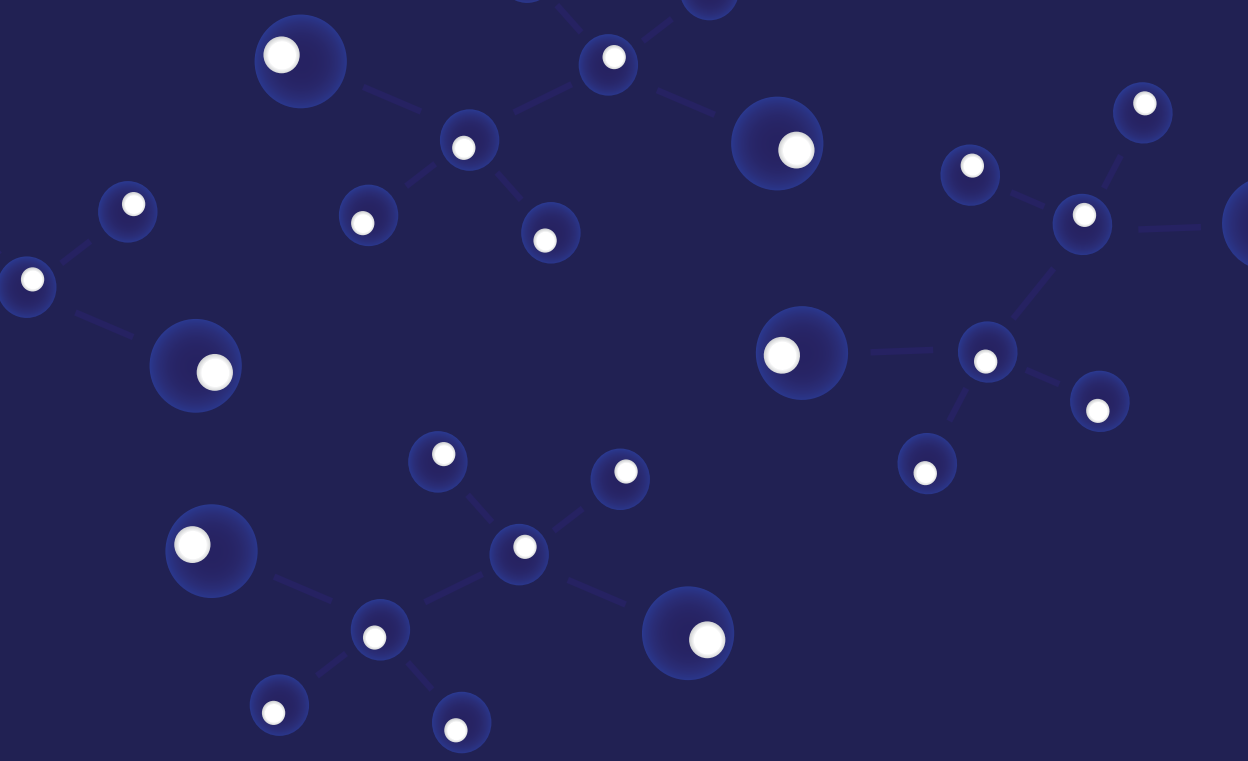
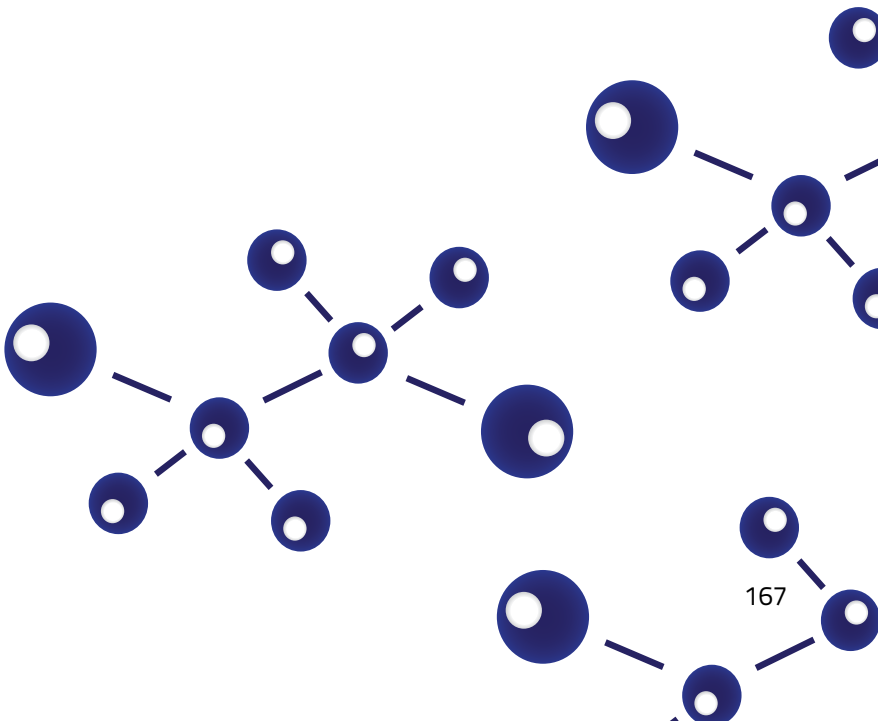
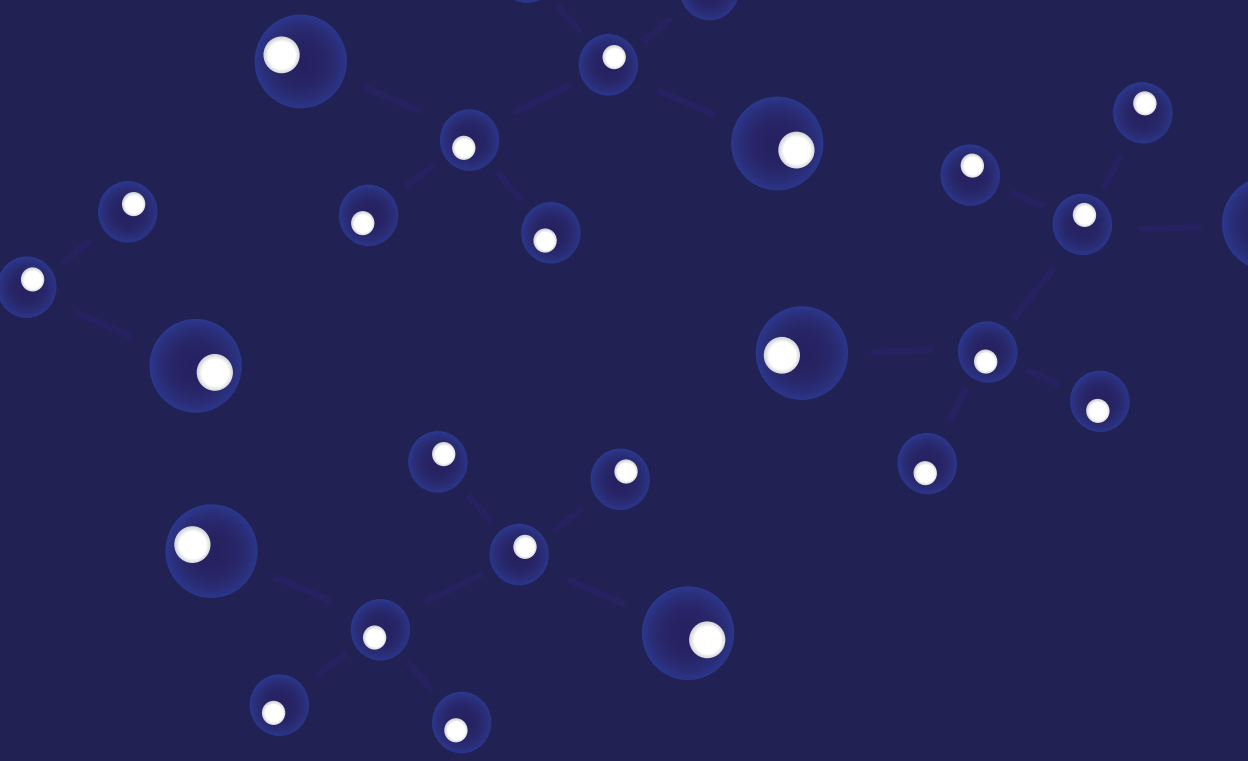


# PART III
# THE UNIVERSITY OF TRIESTE AND ITS STRATEGIC PROJECT TRANS2CARE





Passamonti S. (Editor)
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trans2care
Transregional Network for Innovation and Technology Transfer to Improve Health Care

# Entry of the University of Trieste in the Cross-Border Cooperation Programme Italy-Slovenia 2007-2013

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LP-University of Trieste

## The CBC Programme Italy-Slovenia 2007-2013 and the University of Trieste: correspondence between Programme's objectives for the European territorial cooperation and the institutional University mission

The CBC Programme Italy-Slovenia 2007-2013 (hereinafter, the Programme) aims at accomplishing the strategic European Union priorities set out by the Lisbon and Göteborg strategy, according to the other European Territorial Cooperation Programmes that are financed by the European Regional Development Fund (ERDF). The Programme aims at *'promoting knowledge and transfer of know-how, developing cross-border economic activities and the potential in the education, technical training and health sectors, as well as integrating the cross-border labour market and jointly manage both the environment and common risks. Where the grounds of cross-border cooperation have already been laid, the cohesion policy should be aiming mostly at actions that will bring added value to the cross-border activity, like for example the improvement of cross-border competitiveness through innovation, research and development, connecting intangible (services) or tangible networks (transport), ...*" [1]
In simple words, the main objective of the CBC Programme Italy-Slovenia 2007-2013, i.e. 'Enforcing the attractiveness and the competitiveness of the Programme area", can only be feasible, if the following specific objectives are pursued: 1) Guaranteeing a sustainable territorial integration; 2) Increasing competitiveness and the development of a knowledge-based society; 3) Improving communication and social, as well as cultural cooperation as to overcome the persistent obstacles. Each objective corresponds to a Priority Axis that is divided into precise operational objectives [2].
The Priority Axis 2, 'Competitiveness and knowledge-based society", includes four operational objectives, among which 'Promote research and innovation for the development of knowledge-based economy" and 'Improve and qualify employment potentials through coordinated higher-education and training".
Those operational objectives correspond to the most important institutional purposes of the University of Trieste, as stated in its first Social Responsibility Report 2008: The

[1] Operating Programme number CCI: 2007 CB 163 PO 036, page 44 (www.ita-slo.eu/documenti_di_riferimento/documenti_di_programma/).

[2] Therein, page 63.

University of Trieste is "*the promoter of the economic and social development in the territory. Its role is crucial for the success of the Nation in the knowledge economy. The University of Trieste must be involved in various action areas. Depending on the case, the University is seen as the actor, involved in the technology transfer, or background of intellectual capital ... a true territorial mission, carried out by the University of Trieste: an attractive function and promotion of cross-national scientific mobility that can help contrasting Trieste's population decline and regain a decisive role in the European integration process that has been steadily advancing in its ancient reference area.*" [3]
Given the common grounds of both the programme's objectives, on one side, and the institutional purposes, on the other side, and the a network of active links with public and private bodies, located in the Programme's Area, the University of Trieste has participated to the selection process of Call n. 1/2008 [4] , presenting an Expression of Interest. The EoI contains a project for setting up a "Transregional Network for Innovation and Technology Transfer to Improve Health Care-TRANS2CARE" that includes 13 research organisations, technology transfer bodies and health service organizations. The six partners from Slovenia are the National institute of Chemistry from Ljubljana, the University of Nova Gorica and the Primorska University, the Slovene national Institute of Transfusion medicine, the Orthopaedic hospital Valdoltra and the General Hospital Dr. Franc Derganc from Šempeter pri Novi Gorici. The six partners from Italy are the International School for Advanced Studies of Trieste, the University of Udine, the University Ca' Foscari of Venice and the University of Ferrara, the Scientific Research Institute and Hospital "Burlo Garofalo" of Trieste and Treviso Tecnologia, the Treviso Chamber of Commerce's special agency for innovation.

## Objectives of the TRANS2CARE project

The Project proposal aims at implementing the objectives of the Priority Axis 2. With regard to the knowledge economy, the Project would like to map the scientific, technological and industrial expertise of the *Partners*, as well as correlate it to the intangible or unmet needs of healthcare workers (unmet medical needs) by providing thematic group meetings (on neuropathologies, allergic diseases, cardiac diseases, and so forth); by creating and updating a database of innovative technological solutions (*Work package* n. 5); consultations with biomedical companies (*Work package* n. 6); by signing the agreements among the companies as to gain regional, national and European funds (*Work package* n. 7); and lastly by creating a spin off (*Work package* n. 7) that would enable a physiological continuation of the established cooperation and scientific-technological exchange. Such activities were set out mainly to overcome the critical state of the past years due to poor knowledge of research results among healthcare personnel and, viceversa, poor knowledge about healthcare issues among research personnel; weak integration among Public Health services, academic and industrial research and development activities; discontinuous cooperation among research bodies, health institutions and companies, that had as consequence poor

[3] Corporate Annual Report 2008, page 18
(www.openstarts.units.it/dspace/bitstream/10077/3560/1/BS_Units_sez_1.pdf).

[4] Published in the Official Bulletin of the Region n. 42 from 15th Oct 2008.

attractiveness of the Programme area for young researchers (pages 88-89 of the TRANS2CARE Project Application form).
With regard to the employment potentiality, the Project foresees recruiting, training and employing fourteen junior researchers with a doctoral degree that are to carry out *Technology Training* with the *Partners* (TRANS2CARE Project Application form, *Work packages* n. 3 and n. 4). At the end of the training period the Project foresees their employment in biomedical companies (*Work package* n. 7).
After evaluation, the Expression of Interest has scored maximum points (100/100) [5] and thus, the University of Trieste could present the complete project, qualified as a strategic project according to art. 3 of Call n. 1/2008, for a value of Euro 2,611,188,00 in response to Call n. 1/2009 ("Second phase Call notice") [6].

**Outcome of quality evaluation of the complete project proposal: funding denial**

In the classification of the project proposals published in the *Official Bulletin of the Region* n. 17 from 28th April 2010 and related integrations, published on the official web site of the Programme on the 3rd June 2010 [7], the project ranked fifth and first among the projects without funding. The outcome was thoroughly analysed as to understand why the funding was denied to the project. It seemed peculiar that funds were denied to a network of academic and research bodies, institutionally aiming at implementing *ante litteram* the Lisbon and Göteborg Strategies and carefully identified to accomplish the latter in the entire Programme area, as indicated in the Call.

The participants to the project TRANSCARE have been cooperating for a long time [8] and were aiming at setting a long-term and financially sustainable institutional cooperation that would outlive the time limit of the Programme [9]. The University of Trieste had invested substantial human and financial resources in preparing the project proposal and did, most of all, feel responsible to provide a rational self-analysis of the evaluation results to its *Partners*.
A request was presented to the Managing Authority, to access the documents, related to the evaluation procedure of the TRANS2CARE proposal and the strategic projects financed in the Priority Axis 2, as recommended by the European commission and granted by the regional, national and European legislation [10].

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[5] MoI classification after the quality evaluation (www.ita-slo.eu/progetti/bandi_pubblici/2009062509164240/).

[6] Published in the Official Bulletin of the Region n. 26 of 1st July 2009.

[7] www.ita-slo.eu/progetti/bandi_pubblici/2010060313454214/.

[8] Explicitly stated in the introduction, preceding the description of the project, under section B3 (State of the Art) of the Project application form (page 87) (www.trans2care.eu).

[9] Objective, included in one of the project activities, namely the work package 7 of the Project application form (page 115) and the strategic requirement under section C.6 (Sustainability and/or duplicability of the project) (page 143).

[10] Art. 42, Charter of Fundamental Rights of the European Union, EUR 21620 — the European Charter for Researchers. The Code of Conduct for the Recruitment. 2005. ISBN 92-894-9311-9. Law n. 241 of 7th Aug 1990 (Legge 7 agosto 1990, n. 241) "New provisions on administrative procedure and right to access to administrative documents." Regional law n. 7 of 20th March 2000 (Legge regionale 20 marzo 2000, n. 7) Single text of the provisions on administrative procedure and right to access.

**University of Trieste filing a case at the Regional administration court (T.A.R.) of the Friuli Venezia Giulia region**

After thorough analysis of the documents, the University of Trieste filed a complaint at the Regional administrative court of FVG on the 16th Sept 2010 against the Autonomous Region of Friuli Venezia Giulia, in the person of the President of the Regional Council, and the Programme's Managing Authorities, in the person of its legal representative, to suspend and cancel:

a) The final ranking list related to the Public Call n. 1/2009 and regarding the lists of Strategic Projects, which were either granted or denied financing in the Priority Axis 2;
b). All document and minutes of the comparative evaluation procedure, as well as the evaluation form with the scores assigned by the evaluators during the preliminary phase of 'Quality control";
c) Actions that lead to the appointment of the evaluators, briefly indicated with the abbreviations FVG ITA, VEN ITA, E-R ITA;
d) Art. 6 of the Public Call for the presentation of Strategic projects n. 01/2009 published in the Official Bulletin of the FVG region n. 26 of 1st July 2009, more specifically the part that sets the deadlines for presenting expenses and reports of the financed projects on the 30th Oct 2010, 30th Sept 2011 and 30th Sept 2012.

The complaint was withdrawn after the reply of the Friuli Venezia Giulia region.

**The reply of the Friuli Venezia Giulia region**

The letter dated 10th March 2011, sent by the Central Directorate for culture, sport, international and EU relations of the Friuli Venezia Giulia Region to the University of Trieste, stated: 'The Monitoring Committee of the Cross-border Cooperation Programme Italy-Slovenia 2007-2013 approved on the 8th March 2011 [...] the allocation of additional resources for the above mentioned Call [1/2009]. As a result of such decision, the project [...] TRANS2CARE qualifies for EU funding."

As specified in a press release of the Friuli Venezia Giulia Regional council dated 9th March 2011 [11], the agreement achieved in the Monitoring Committee'... shall bring EU funding (for a total of almost 10 million Euros) to three additional strategic projects, presented in the Public Call n. 01/2009 (the projects that ranked first among those denied funding for each Priority Axis)". The entire Programme funding amounts at about 136 million euros, meaning that the reprogramming reallocated the budged for a total of 7.35%.

The later press release of the Regional council[12] published some details of that considerable financial operation: the funding for strategic projects increased from 40 to

[11] Regional Council news, 09.03.2011 11:35, Cross-border cooperation: Programme Italy-Slovenia unblocked (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/&nm=20110309113521001).

[12] News from the Regional Council, 28.03.2011 19:29, FVG/SLO: De Anna-Pelikan, Additional 10 Millions of Euro for strategic projects (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/&nm=20110328192903010).

50 million euros (+25%), the funding for standard projects (Call n. 2/2009) stayed at 60 million euros, whereas the available funding for 'cross-border" projects (Call n. 3/2011 for partnerships between the Region of Friuli Venezia Giulia and Slovenia, considered as land border areas) and 'small projects" (Call to be issued) was reduced. Since the Call n. 3/2011 was granted funding of 17.5 million euros [13], rather then 22 million (-20%)[14] and 8.2 million euros have already been allocated to the Priority Axis 4-technical assistance, the residual funding for small projects is calculated at 0.3 million Euros together with any contingent 'funds from the 3 projects - assured the councillor De Anna"[15], justifying thus the deferment of the Call for small projects [16].

**From crisis to successes in the Programme Italy-Slovenia 2007-2013**

The CBC Programme Italy-Slovenia had been going through a tough period due to the disagreements among the Programme *Partners*.
By appointing Elio De Anna on the 15th October 2010 as Regional councillor for culture, sport, community and international relation[17] and relaunching the Mixed Commission between the Region of Friuli Venezia Giulia and Slovenia, approved by the President Renzo Tondo[18], the bilateral relations were restored after three years of disengagement: this European Community organ meeted in Trieste on the 26th January 2011 under the presidency of the Regional councillor Elio De Anna and the State Secretary Dr Boris Jesih of the Office for Slovenes Abroad[19]. The Mixed Commission met again in Brdo pri Kranju (Slovenia) on the 9th February 2011. During that occasion various Working Tables were started. One of them was dedicated to the 'scientific research and technology [...] sector where four strategic actions were identified. The first action concerns the mobility of qualified human capital through professional formation scholarships for graduates and young researchers that would involve research institutions [...] At the end of the meeting both the State Secretary at the Ministry of Foreign Affairs, Dragoljuba Benčina, and the Regional councillor, Elio De Anna, expressed their satisfaction" [20].

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[13] Regional Council news, 09.03.2011 11:35, Cross-border cooperation: Programme Italy-Slovenia unblocked.

[14] Regional Council news, 03.12.2010 15:37, International relations: De Anna in Ljubljana with Slovene Deputy Minister for Foreign Affairs (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/&nm=20101203153746005).

[15] Regional Council news, 28.03.2011 19:29, FVG/SLO: De Anna-Pelikan, Additional 10 Millions of Euro for strategic projects.

[16] Cross-border Cooperation Programme Italy-Slovenia 2007-2013. News-09.03.2011 Official implementation of the agreement among Programme partners (www.ita-slo.eu/notizie_ed_informazioni/notizie/20110309164724 57/).

[17] Regional Council news, 15.10.2010 16:53, the Region: new mandates for regional councillors (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/&nm=20101015165310013).

[18] Regional Council news, 26.11.2010 15:30, International relations: Tondo with the new Slovene ambassador in Italy (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/&nm=20101126153025007).

[19] Regional Council news, 21.01.2011 17:34, International relations: De Anna, minorities useful for EU competitiveness (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/&nm=20110121173434006).

[20] Regional Council news, 09.02.2011 18:21, International relations: Meeting at Brdo pri Kranju - Mixed Commission for the development of FVG-Slovenia (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/&nm=20110209182136010).

The renewed cohesion inspired the Monitoring Committee to achieve an overall agreement via a written procedure (without the college meeting) on various issues and thus decided to finance the Call n. 1/2009 with additional 10 million euros. By doing so the Programme implementation process was restarted, consisting of the passage to the quality evaluation phase of the standard projects (Call n. 2/2009) and issuing of the cross-border projects Call (Call n. 3/2010)[21]. The European Union expressed its satisfaction, since these actions have prevented the automatic disengagement of European funds due to the inability to meet the expenditure requirements [22].

### Uncertain truce or long-lasting peace?

The call for the strategic projects with beneficiaries spread over the entire Programme Area benefited enormously from the above mentioned cuts, but the funds cut hit the call for small projects, thus leaving out the small initiatives near the state border.
However, that Programme's agreement aimed at ensuring a uniform and wide-spread economic and social development at the cross-border level.
Some parameters show that the border still exists in a virtual form and is far from being abolished: according to the Eurostat data from 2010, 19.8% of the Italian population aged between 30-34 has a University degree in comparison to the 34.8% of the Slovene population[23]. Slovenia is ranked better than Italy in seven out of eight indicators taken into consideration for the 2010 Innovation score, placing thus Slovenia in the Innovation followers group, after the Innovation leaders group. Italy, on the other hand, is ranked in the Moderate Innovators group, just before the group, ranked as last - the Modest Innovators .
The Commission's Report 'State of the Innovation Union 2011" states that Slovenia is believed to improve its innovation performance, due to the fact that it has saved large part of its structural fund for research and development, in line with the political guidelines of the European Commission's Programme 'Innovation Union" , whereas Italy keeps decreasing its investments in this sector. 'There is clearly a risk of widening the innovation divide between the Member States": is the alarming message of the Report.

### The University of Trieste racing for innovation and development

The financing granted to the TRANS2CARE project diminishes the risk for the 2007-2013 period and lays the grounds for limiting it in the future programming period, when the Universities will be called to give their vital contribution to the economic and social development, well argued in the document 'Connecting universities to the regional growth" published by the DGRegio in September 2011 with the Universities to

[21] Regional Council news, 9.03.2011 11:35, Cross-border cooperation: Programme Italy-Slovenia and the Cross-border Cooperation Programme Italy-Slovenia 2007-2013 unblocked. News- 09.03.2011 Official implementation of the agreement among Programme partners (www.ita-slo.eu/notizie_ed_informazioni/notizie/20110309164724 57/).

[22] Regional Council news, 12.03.2011 11:23, International relations: EU plaudit for unblocking Interreg Italy-Slovenia funds (www.regione.fvg.it/rafvg/comunicati/comunicato.act?dir=/rafvg/cms/RAFVG/notiziedallagiunta/ nm=20110312112311003).

[23] epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&plugin=1&language=en&pcode=t2020_41.

be held responsible for the creation of synergies among various financial instruments , such as those of Horizon 2020 and those of the future Cohesion Policies.
It is more than obvious that without funding for the TRANS2CARE project, the distance between the university system of the Friuli Venezia Giulia Region and the Slovene system would have become even greater: with the Italian system on one side, not sufficiently expert for attracting and managing wide range innovation and technology transfer, and the Slovene system on the other side, projected in realizing these challenging operations, carrying forward constant communication with the public authorities and enterprises and ready to face the accession of Croatia to the European Union in terms of constant attraction of new European Structural and Research funds.
In this race towards Innovation and development the University of Trieste has nevertheless discovered the power of deep internal innovation - the power of a radical change of mentality.

### Change of mentality: from spectators to actors

Usually those responsible for the project proposals take unconditionally note of the assigned evaluation. The lack of understanding why a specific score was assigned to a project breaks the virtuous circle of criticism (and self-criticism), crucial for any innovative cultural activity (*learning by lessons*).
Through the analysis of the documents, related to the comparative evaluation procedure for strategic projects, it was possible to discover not only procedure faults, but also fault in the TRANS2CARE application form: the quality of the text, put in in the project application form, can be improved and provide a better way of bringing up the proposals, as well as better compliance with the objectives of the Operational Programme and the European Policy Documents.
By exercising its right to access the procedure documents, the University has shown its intention to compete for EU funds, based only on technical and meritocratic principles and refusing to opt for abnormal forms of two-sided interactions such as confidentiality, indiscretion, undeserved pressure (*lobbying*). Consistently with its orientation, the University of Trieste is a member in the EU Register that consists of a list of organisations and freelancers committed to processing and implementing EU policies, and openly express their opinions by participating to Public consultations.

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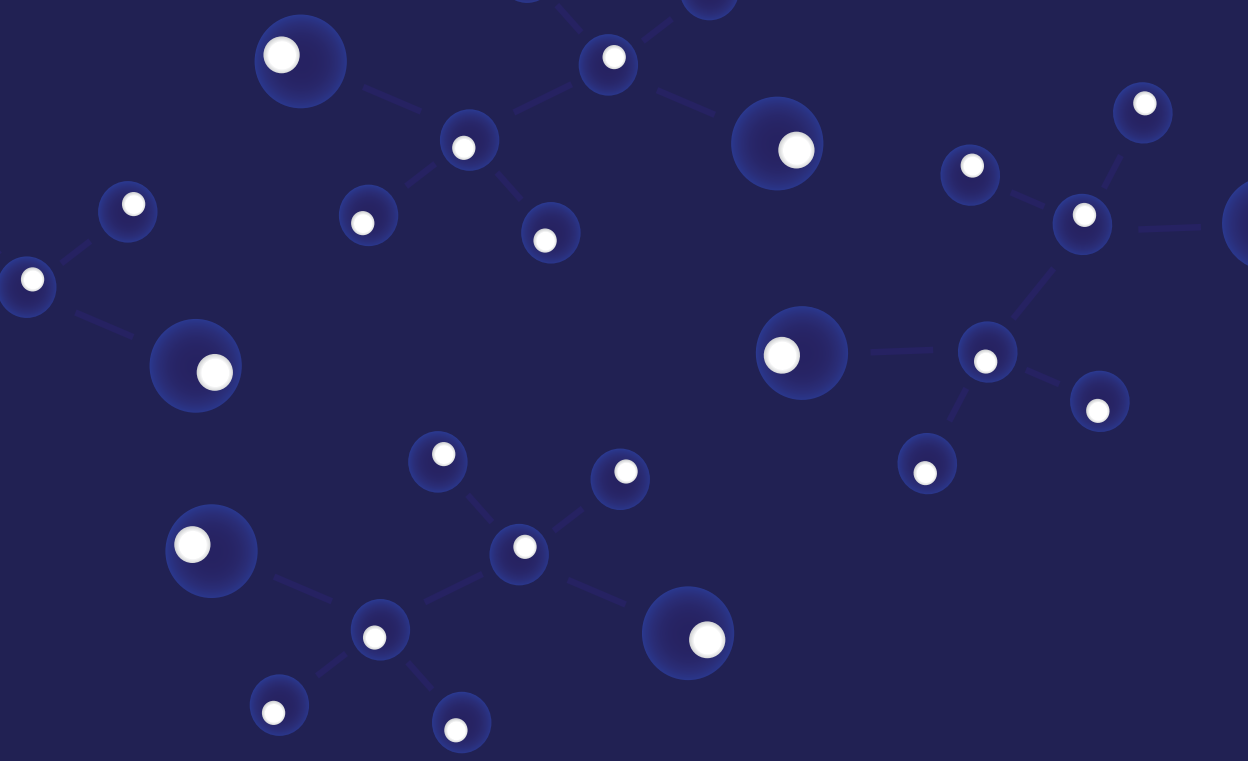

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Transregional Network for Innovation and Technology Transfer to Improve Health Care

# The contribution of Trans2Care to the European Cohesion Policy: not just an administrative fulfilment

Giorgio Tessarolo

Within the '2007-2013 European Territorial Cooperation" objective, on 20th December 2007, the European Commission issued a decision to adopt the 'Italy-Slovenia 2007-2013 Cross-border Cooperation Programme".
The program has a total budget of over € 136 million and is divided into four Priority axes: 'Environment, transport and sustainable territorial integration", 'Competitiveness and knowledge-based society", 'Social integration" and 'Technical support".
In Italy the Programme is implemented in the provinces of the Friuli Venezia Giulia Region (Trieste, Udine and Gorizia), in the Veneto Region (Venice, Rovigo, Padua) and in Ferrara and Ravenna in the Emilia Romagna Region. In Slovenia the project is implemented in the so-called NUTS 3 areas of Gorenjska, Goriška and Obalno-kraška. To a limited extent, the project may be implemented in the Italian provinces of Pordenone and Treviso and in Slovenia in the NUTS 3 areas of Osrednjeslovenska and Notranjsko-kraška.
The Operational Programme has its origin in the Community Strategic Guidelines on Cross-border Cooperation spelled out in the Regulations of 2006 which indicate that among the priorities laid down in the so-called 'Lisbon strategy", there is the need to promote 'innovation, entrepreneurship and the development of a knowledge-based economy through the development of research and innovation" including new information and communication technology.
This priority has been encompassed by the Italian and Slovenian National Strategic Frameworks. The Operational Programme stresses that Territorial Co-operation (in this case, cross-border) can effectively contribute to the general objective through the creation of scientific and technological cross-border networks and the enhancement of regional research and development, thus promoting the development of partnerships between producers of knowledge, increasing the degree of internationalization of the centers, developing new applied research skills and emphasizing existing excellence systems by making them available to local production systems.
That said, it is worth remembering what the overall objective of the Italy-Slovenia 2007-2013 Programme is: 'Strengthening the attractiveness and competitiveness of the Programme area".
When listing the main operational objectives of Axis no. 2, emphasis is given to joint actions promoted by business incubators, innovation centers, science and technology

parks, that can recognize the added value resulting from the sharing of experiences between Italy and Slovenia, in order to develop new capabilities to be made available to young entrepreneurs and researchers. The excellence of knowledge, the presence of experienced personnel and technical equipment and facilities should be shared within selected research themes and projects.
The Operational Programme foresees three types of projects: strategic projects, standard projects and small projects. All projects are selected through special calls.
Today, we will examine the characteristics of strategic projects, since TRANS2CARE belongs to this category.
Strategic projects should:

- contribute to the achievement of the objectives of the European strategies and Programme and have a significant sustainable impact on the Programme area, also through the identification of appropriate indicators;
- have a genuine cross-border character and meet the four requirements of Article 19 of the Regulation (EC) no. 1080/2006: joint development, joint implementation, joint staffing and joint financing;
- ensure that the cooperation continues even after the conclusion of the project through the creation of permanent cross-border networks and organizations;
- be designed and implemented by partners, who have an adequate level of expertise on project issues and are able to ensure the achievement of results.

This is the overall reference framework within which the TRANS2CARE project has been selected among those eligible for funding under the Call for strategic projects.
The project has a duration of three and a half years (until the 30th September 2014) and a budget of € 2,611,118. The partnership consists of thirteen partners which constitute an 'Interregional network for innovation and technology transfer for health improvement" in order to continuously develop new protocols and biotechnological devices for the prevention, early diagnosis and treatment of neurodegenerative, cardiovascular, oncologic and orthopaedic diseases.
Then, there are some characteristics that make this project original and unique: firstly, the mix of innovation, knowledge transfer and creation of new jobs for young and qualified researchers, who will be trained within the project.
Secondly, the project is built on a robust large partnership involving various subjects and possesses multiple skills and complementary and diversified knowledge since it involves universities and research institutions, hospitals and organizations for technology transfer.
In my opinion, the most important challenge TRANS2CARE will have to deal with is its consolidation beyond the time limits set by the Programme: as in Brussels they like to say, the project should lay the foundations for its future 'sustainability": this could be achieved by implementing its activities in the next 2014-2020 programming period, by expanding the current partnership and by creating a project spin-off, that will ensure a stable and structural employment to the fourteen young researchers. The project should be able to involve the companies operating in the sectors concerned, but it should also be able to acquire the necessary skills in order to obtain additional funds, both public and private, to support the future of the network.

In this regard, I would like to emphasize that TRANS2CARE already has the characteristics and meets the requirements that the European Commission has identified for future programming within its document entitled 'Europe 2020: a strategy for smart, sustainable and inclusive growth":

- smart, since it is aimed at developing an economy based on knowledge and innovation;
- sustainable, to promote a more efficient economy in terms of resources, and a more green and more competitive economy;
- inclusive, aimed at promoting an economy with a high rate of employment so as to promote the economic, social and territorial cohesion.

That said, a final thought is now required: what should we expect from the project results as not to reduce it to a mere fulfilment of administrative and accounting tasks?
This has always been the problem of all problems as community planning in concerned!
I believe it is important to remember, first of all, what is the purpose of the cohesion policy: promoting growth and economic development and, therefore, create wealth and additional employment.

## IS TRANS2CARE IN LINE WITH THIS "MISSION"?

At present, it certainly is, but the problem that will arise after 2014 will be the need to consolidate the current project in order to make it stable, both in terms of employment of researchers and reduction of welfare costs for the community, and structural enlargement of the partnership: if that happens, and there is no reason to doubt it, TRANS2CARE will have fully achieved the objectives for which it was designed and it will not be limited to a proper spending of the funds allocated by the European Commission, but it will have spent them EFFICIENTLY!

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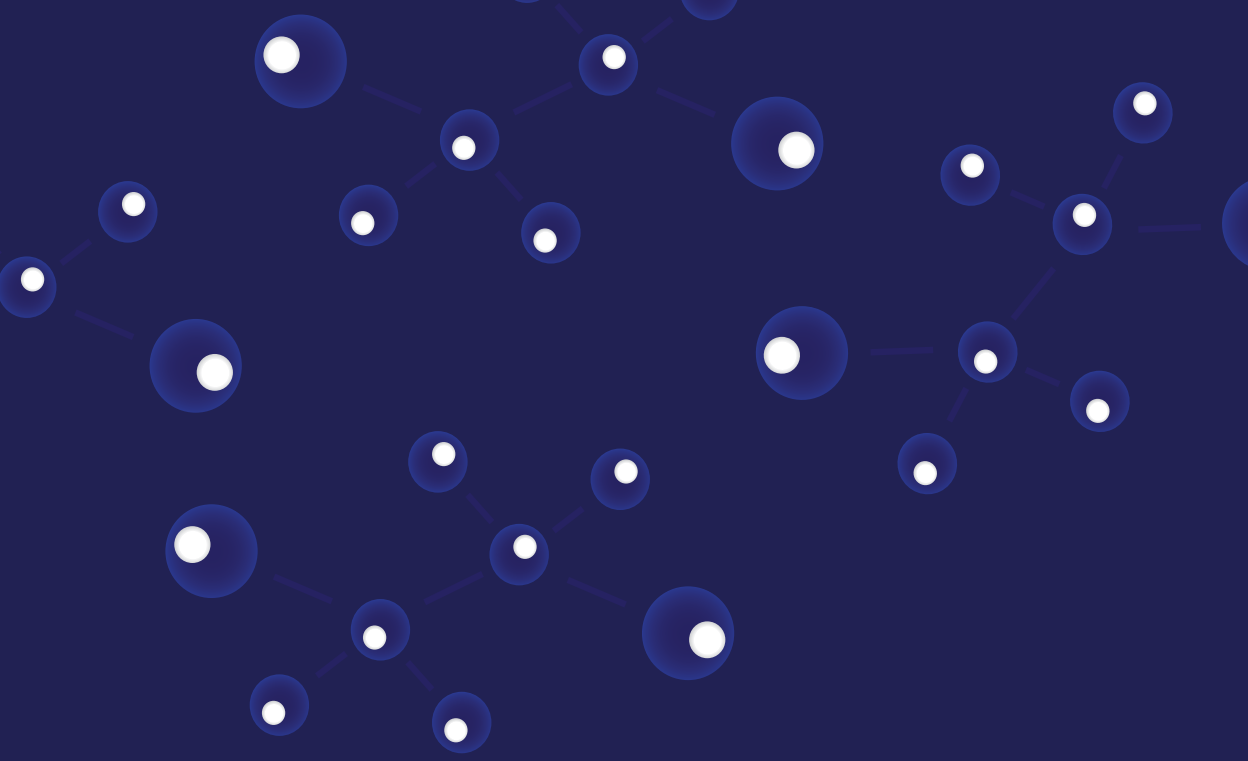

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# Trans2Care in pole position to implement Innovation Union

Giorgio Perini

*First of all, I would like to thank the University of Trieste in the person of Prof. Passamonti for this most welcomed invitation to attend the launch event of the Trans2Care project in my city, Trieste, where unfortunately I can only rarely return because of work commitments in Brussels.*
*I greet you, the University of Trieste and all the participants in the name of the Italian Ambassador to the EU, HE Ferdinando Nelli Feroci.*

## NOTES ON THE PERMANENT DIPLOMATIC REPRESENTATION OF ITALY TO THE EUROPEAN UNION

Let me begin with a brief presentation of the role of the Permanent Diplomatic Representation of Italy to the European Union, where I am honoured to serve as a regional expert, appointed by the Conference of Regions and Autonomous Provinces. The EU institutions cannot know the often-changing internal structure and internal distribution of powers of each Member State. For this reason the "Permanent Representations of the Member States" are a collector or a "funnel" for all EU communications at any level of the Member State government (central government, regions and municipalities). The same process happens in the other direction: the European institutions must be sure that official communications sent to them are attributable to the Member State and cannot be subsequently disregarded and the only way to achieve this is to send them through the permanent representations, usually through an electronically encrypted e-mail system. The Permanent Representation of Italy ensures this flow of official information in both directions (a different thing is of course the flow of unofficial communications, where many other actors are involved).
It is also worth mentioning, that the direct participation of the Regions in the ascending phase of Community law (the one where one decides the new "European laws") is now possible as a result of the reform of the Constitution and acts that have followed (the so-called La Loggia Law and the State-Regions agreement of 2007), but have not yet been implemented for Italy. Thus far the ascending phase is the exclusive prerogative of the Representation of Italy in Brussels, which is assisted by experts in various subjects, usually from ministerial background, which is why it is important to be there.

## THE 'TRANS2CARE" PROJECT

But let me immediately move on to the Trans2Care project for which we are here today: this project encompasses the virtuous model of the triple helix, where universities (higher education and research), public administrations and enterprises interact; it represents Europe2020 aimed at a smart, sustainable, supportive and inclusive growth, and of course there is the cohesion policy, in particular the territorial cooperation, presently under the Italy-Slovenia 2007-2013 Programme, but already projected in the next 2014-2020 programming period.
No wonder then that this project has been selected and I am sure it will be a big success: better health for longer periods, rationalization and sustainability (including financial) of health services, research and innovation spin-offs are also the goals of the Pilot partnership on healthy and active aging initiative launched by the Council of the European Union in February 2011 as part of the newly founded partnerships for innovation, in order to facilitate the achievement of the Union for Innovation, one of EU2020 flagship initiatives.

## THE STRATEGIC PLAN FOR THE IMPLEMENTATION OF THE 'PILOT PARTNERSHIP"

On November, 7th - exactly two weeks ago - the strategic plan for the implementation of this pilot partnership was approved. The plan aims to reduce barriers to innovation in the field of 'good" aging and promote a more efficient use of European funds, through a first series of concrete actions to be launched in 2012, among which I will mention only two:

- Disseminate and promote innovative models of integrated care of chronic diseases of the elderly, such as remote monitoring. A number of EU regions will take the lead in this field, according to the steering group which includes three Commissioners (for Health, ICT Technology and Research, development and innovation);
- Develop interoperable ICT solutions (in particular within the so-called AAL - Ambient Assisted Living) to help older people to stay independent, mobile and active for longer periods of time. In this regard, I would like to remember that the Friuli Venezia Giulia Region, through the Liaison Office in Brussels I was responsible for over the past five years, is part of CORAL or Community of Regions for Assisted Living.

Also in 2012, the European Commission will launch the calls for broadening stakeholders' involvement, beyond those already involved in the steering group, in order to implement with them the priorities and actions identified in the Plan. It goes without saying that 'Trans2Care" and its partnership should be in 'pole position" to play a leading role.

## THE OUTLOOK FOR THE 2014-2020 PROGRAMMING PERIOD

We have said that 'Trans2Care" was created in the current programming period, but in many ways it looks even more at the next 2014-2020 period. Therefore, we should briefly comment on the prospects for the next programming period on the basis of what we know today.

Firstly, we should keep in mind the increasing integration of European policies: EU2020, national reform plans, stability and convergence programs (and the so-called 'European semester"), review of the EU budget, regional policy, strategic framework for research, development and innovation (Horizon 2020), agricultural policy, development cooperation and financial perspectives are all strongly correlated with each other.

In particular, with regard to the future of the cohesion policy, there are some aspects of the proposal currently under discussion that we like more and among them the cohesion policy for all European regions as a catalyst for EU2020, preserving the competitiveness (thanks to the strong lobbying efforts at EU institutions - the European Commission in the first place - made by 174 European regions, including the FVG through the Liaison Office in Brussels), the proposed financial budget made by the European Commission, etc. But there are also some aspects that we like less, or rather we are concerned about, such as the ex-ante performance indicators (not the ex-ante conditionality), the external conditionality (or macroeconomic conditionality) linked to national performance with respect to the Stability and Growth Pact which is obviously beyond the control of individual regions; the partnership agreements to be signed with the EC (here the role of the regions is not clear). But there is also reason for concern given by the EU Financial Perspectives for the 2014-2020 period (i.e. the level of contribution of the Member States in terms of GDP percentage).

## MACRO-REGIONAL STRATEGIES

The Friuli Venezia Giulia Region, co-founder of a wide-area strategy ahead of its time - the ALPE ADRIA Working Community - could be left out from all macro-regional strategies, i.e. the Baltic (obviously), the Danubian, the Adriatic-Ionic and the Alpine, while our neighbours are already playing across the board.

Of the three no's that the European Commission needed to digest the macro-regional strategies (no new institutions, no new laws, no new funds), the last one is the one that convinces us less, since macro-regional strategies could play an important role in the next programming period, not only for the greater ability of virtuous regions to spend and perform, but also for their recognized role in territorial cooperation (the former Objective 3), in particular in transnational cooperation.

That's why we have begun to take action with respect to existing and future macro-regions with initiatives such as the promotion of the Baltic-Adriatic transport axis based on a partnership of 14 regions along the former Iron Curtain, which already has considerable success due to the inclusion of new Corridor no. 1 in the Trans-European transport networks), the promotion and visibility of the Conference of Rectors of Alpe Adria and Danube Universities for the development and implementation of the

third pillar of the Danubian strategy aimed at developing the Society and Economy of Knowledge, and finally a reorientation of the CPMR (Conference of Peripheral Maritime Regions) to the eastern Mediterranean - that is, the Adriatic-Ionian area - rather than just to the western Mediterranean, and in this context, a more equitable balance between a North-South and a West-East axis.

## EGTC (EUROPEAN GROUPING FOR TERRITORIAL COOPERATION)

It's hard not to mention it, seen the industry within which the 'Trans2Care" project is settled: health, which will probably become the priority area of cooperation and integration, together with transport, within the EGTC. It is important to emphasize that after 2013 non-EU subject will be able to participate to EGTCs. This will represent an opportunity to relaunch the Euroregion project called 'Without Borders," in which Trans2Care may already be part of.

## CONCLUSIONS

The Europe2020 strategy is not - and especially should not be - an unclear strategy for a selected group of experts in Brussels. It's a compulsory handbook for Europe and to become successful it needs to be known, understood, accepted and implemented at all levels.
Whether we like it or not (but we have to like it: we have no alternative, as it has become clear in recent months, when we thought we had seen it all, but we had not seen anything yet - think about the political earthquakes in Greece, Italy, Spain and even France), it will provide the guidelines for all European policies from now on to 2020. The project that begins here today goes exactly in this direction and I am sure we will illustrate it in Brussels as a best practice at European level and beyond.

*Brussels-Trieste, November 2011*

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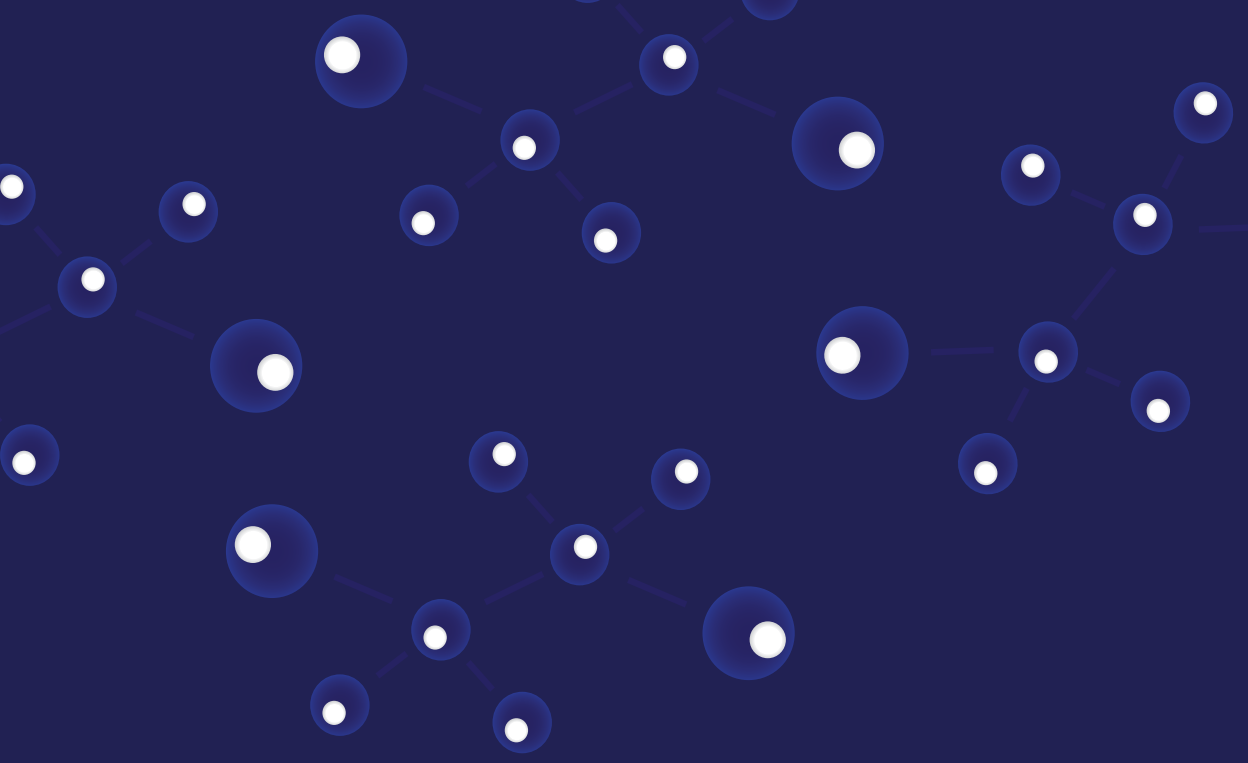

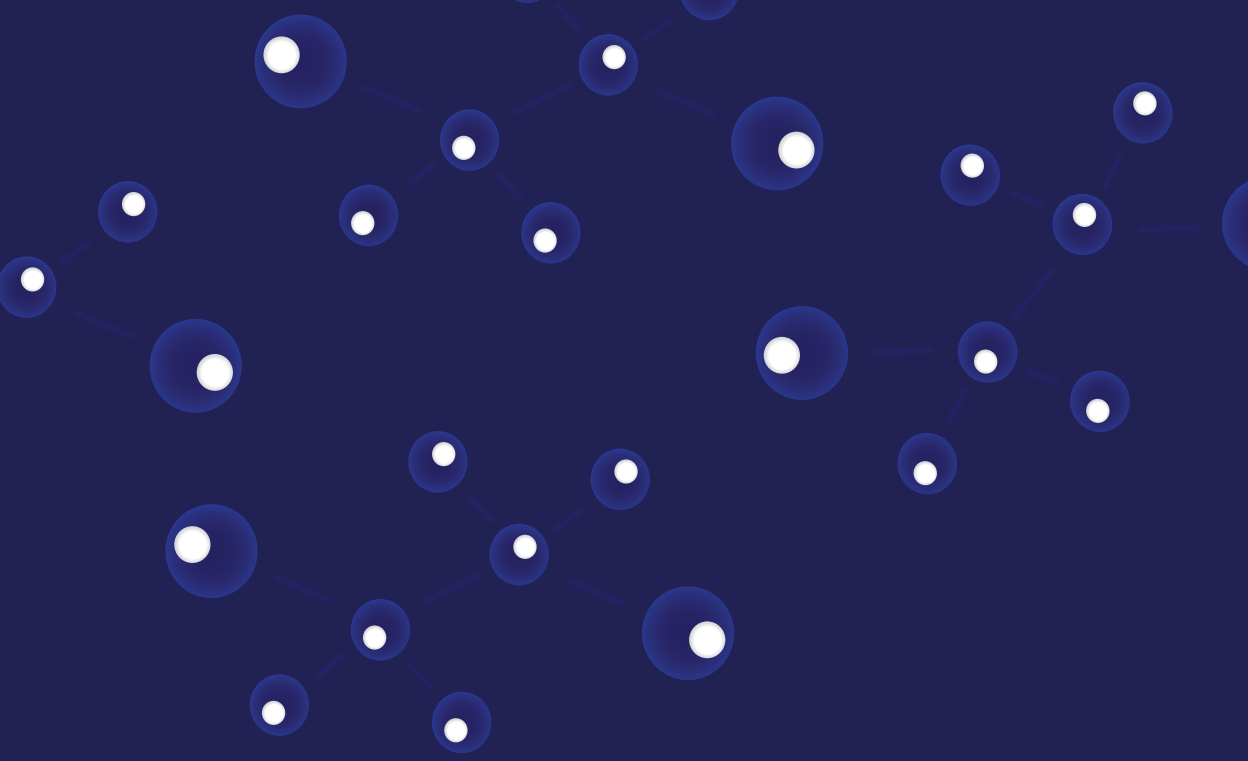

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# Translation of Trans2Care concept to the West-African countries: looking beyond tomorrow

Ayokunle O. Ademosun, Sabina Passamonti, Lovro Žiberna

Abstract — The regional partnership established between Italy and Slovenia under the TRANS2CARE Project aims at creating innovative products and services to improve public healthcare system through enhanced knowledge transfer between project partners and stakeholders. This framework of borderless partnership has potential to be translated to the West-African region to effectively utilize the available resources of member states. Such synergistic cooperation is needed to tap into the abundant ethnopharmacological potentials of the region to create an affordable healthcare system and economic advancement. Consequently, research networks and improved healthcare system in the region will drastically reduce braindrain of researchers and physicians.

Index Terms — European Union, knowledge translation, TRANS2CARE, West Africa

## 1 RESEARCH IN EUROPE: IS GLOBALIZATION HARMONY?

It has been said that arguing against globalization is like arguing against the laws of gravity. – Kofi Annan, Secretary General of UN

European Union research programs are quite complex, but enable researchers, research institutions and businesses to increasingly circulate, compete and co-operate across borders. The objective is to give them access to a Europe-wide open space for knowledge and technologies in which transnational synergies and complementarities are fully exploited.

While most research activities, programs and policies take place at regional and national levels, no single country offers sufficient resources to be competitive on the world scale. To strengthen the penetration of European research ideas, joint activities and policies are increasingly designed and operated from a transnational

perspective, including, where relevant, cross-border co-operation. It is now clear that transnational co-operation enables the most efficient and effective use of national and regional resources.

The success of globalization within the EU can be seen in many dimensions. In health care, all EU and some other European countries, offer their citizens a free European Health Insurance Card, which provides insurance for emergency medical treatment when visiting other participating European countries. Another important field is education, where EU strongly supports globalization of universities via the Bologna process, which brings comparable standards and compatible degrees across Europe, and the Erasmus Program, a university international exchange program, which is nowadays a symbol of European student life. However, although Europe's 4000 universities have big potential, most of it unfortunately dies instantly because of various rigidities and hindrances primarily due to conservative politics practiced by universities.

Scientific development in the EU is encouraged by various programs. Some are strictly researchoriented and based on scientific excellence, while others are funded through European regional development funds. The latter aim to strengthen economic and social cohesion between regions in the EU intending to underpin regional capacities for research and technological development. The ultimate goal is creating a more-or-less harmonious research space inside the EU. In this manner, EU trans-regional projects, such as TRANS2CARE, can function as a reference regarding science and health organization for developing countries.

Can we translate the EU's successful experience of globalizing science to another continent?

## 2 PROBLEMS IN WEST AFRICAN HEALTH SYSTEM

A replication of this kind of working relationship between countries in the West-African sub-region is long overdue as the health care system in this region has been in dire need of a boost. About 250 million people reside in the 15 independent states of West Africa and this is about a quarter of the whole of Africa [1]. According to the Sahel and West Africa Club of ECOWAS [2], the last major world region where mortality rates are still very high is Sub-Saharan Africa. The disease burden in West Africa is characterized majorly by malaria, which is the primary cause of morbidity and mortality. Furthermore, 100% of cases of trypanosomiasis and 90% of cases of yellow fever [3] are recorded in West Africa, while cholera and HIV/AIDS also present a serious threat. Others include schistosomiasis, meningitis, dracunculosis, onchocerciasis and leprosy. Furthermore, the average doctor to population ratio in Africa is 2.3 per 10,000 inhabitants and this does not compare favorably with 33.3 per 10,000 inhabitants in Europe [4]. More so, estimates show that about 70% of West Africa's doctors are found in Nigeria alone while countries such as Niger, Liberia and Sierra Leone have less than 1 doctor per 33,300 inhabitants [3]. Taken altogether, average life expectancy at birth in Africa is 54 years, while in EU is 75 years [4].

## 3 ETHNOPHARMACOLOGICAL POTENTIAL OF WEST AFRICA

It is not all bleak in this part of the world as the pharmaceutical potentials of African medicinal plants are immense, and West Africa has the human and natural resources to become an even greater producer of natural plant products of medicinal value [5]. Furthermore, in contrast to the 1:40,000 ratio for medical doctors to the rest of the population in sub-Saharan Africa, the ratio of traditional healers to the population is approximately 1:500. This means that there are 80 times as many traditional healers as biomedical doctors [6]. More specifically in West Africa, in the Kwahu district of Ghana the ratio of traditional healer to people is 1:224 compared to 1:21,000 for medical doctors. West Africa, like other regions in Africa and Asia, has had a long history of traditional medicine practice, however, unlike the Chinese and Indian medicinal plants, much of the phytochemical compositions and medicinal values of the natural products have not been fully researched and documented. Each community in West Africa has its unique approach to health and disease management. It can thus be argued that 'there are as many traditional medicines as there are communities" in the West African region [5]. The World Health Organization (WHO) has stated that traditional medicine is one of the strongest tools for taking 'total health care" to the world's unreached population [5]. Due to constraints such as paucity of funds, high cost of chemicals, lack of equipment and shortage of manpower, research oriented into the bioactive components of medicinal plants in West Africa has been hampered. Even more, in countries where research activities are on-going, they have become mere academic exercises, as there is no 'benchtobedside" transmission of research results.

To elaborate potentials, there is an immense challenge for the research community in studying traditional medicines used for hundreds of years. Specific climate conditions, such as high temperatures with intra-season drought and rainfall unpredictability, can lead to a plethora of novel, not yet isolated, plant chemicals specific to this region. Isolation, coupled with computational in silico molecular docking studies, can lead to the characterization of novel lead compounds with therapeutic potential, and possibly to the development of novel drugs.

## 4 TRANSLATION OF EUROPEAN NETWORKING TO WEST AFRICA

The concept of TRANS2CARE borderless partnership, as implemented in Europe between Slovenia and Italy, provides a 'light at the end of the tunnel" - serving as an excellent framework that should be copied by the countries in the West African region. The idea of Trans2care networking project can be summarized as 'aiming at establishing a permanent, bi-directional flow of knowledge transfer among the project partners, industry and other stakeholders, so to establish an environment generating innovative products and services for the public healthcare system. In particular, the project addresses questions about prevention, diagnosis and treatment of widespread diseases"[7]. As such, it appears appropriate for West Africa to start its implementation.

One obvious fact is that co-operation is needed among West-African countries to

fight the scourge of diseases and ensure efficient health care delivery through applied research on the abundant medicinal plants to be found in the region, training the numerous traditional healers, and development of phytomedical and nutraceutical industry. The latter is important for ensuring that the abundant natural products serve as raw materials and that these plants do not become over-exploited or extinct. Pharmaceutical production is normally capital-, knowledge- and technology-intensive, but ethno-pharmacological studies of indigenous plants will enhance the discovery of plant bioactive compounds that will improve the health of the population through nutrition. These studies will also help to identify plant bioactive compounds to open perspectives for the local pharmaceutical industry, especially for over-the-counter products, which would aid the economic development of the region. Networking of health care professionals and leading researchers amongst countries in West Africa can bring benefits to both the health care system and industry by promoting production of low-cost drugs. Primary production of pharmaceuticals in the region will also encourage technology transfer between academic research and industrial production [8,9].

Ensuring equal access and fair treatment for all interested parties across the West Africa can be a daunting task, which requires certain rules and procedures, as well as bringing attention to the need for better articulating the importance of such networking at a high political level. To improve research systems, a more favorable environment for transnational mobility throughout research career in West Africa must be created. Research should not be perceived as a closed system, but instead as an open and dynamic systems based on partnership and dialogue with researchers from all over the world, as already successfully implemented in the EU. Furthermore, removing obstacles to mobility can bring together countries in a community whose aim is to achieve integration via joint research activities with a commitment to economic expansion.

## 5 AFRICAN BRAIN GAIN – BRAIN DRAIN PROBLEM

A translation of this type of project to West Africa will not only create an environment that will attract foreign researchers for international (brain gain) collaboration, but will also stem the rising tide of brain drain. About six years ago, a New York Times' article [10] revealed that legal immigration into the United States by Africans had reached a similar magnitude to the days of the slave trade. Scientists, researchers and medical personnel are the worst hit by the brain drain. This was confirmed by French President Nicolas Sarkozy, who stated, during a visit to Senegal, that "there are more doctors from Benin (West Africa) in France then in Benin itself" [11]. Moreover, about ten years ago, it was observed that there were more Sierra Leonean (West Africa) medical doctors in Chicago than in Sierra Leone [12], while the WHO observed in 2006 that more than 25% of doctors trained in Africa work abroad [13]. However, it is anticipated that science students would choose to study and work in Africa if they had access to high-quality training [14]. There is therefore an increasing need for networking among West African countries to develop "centers of excellence", where

researchers can have access to modern research facilities, and where their research findings can be translated to products and services, which will improve the quality of service in healthcare centers.

## 6 CONCLUSION

Such a network will bring about a "pulling-together" of resources among member countries that will have a synergistic effect on health care delivery, new employment generation, pharmaceutical/nutraceutical product development, and training of medical personnel. Moreover, pragmatic cooperation that defies cultural, language, religious and economic barriers and transcends the political differences that have isolated member states is what will indeed give a birth to a united community of West African states.

A central objective of such joint activities is to establish the "fifth freedom": the freedom of movement of knowledge. Research and innovation arising from this will offer solutions to overcome the great challenges that face West African countries these days.

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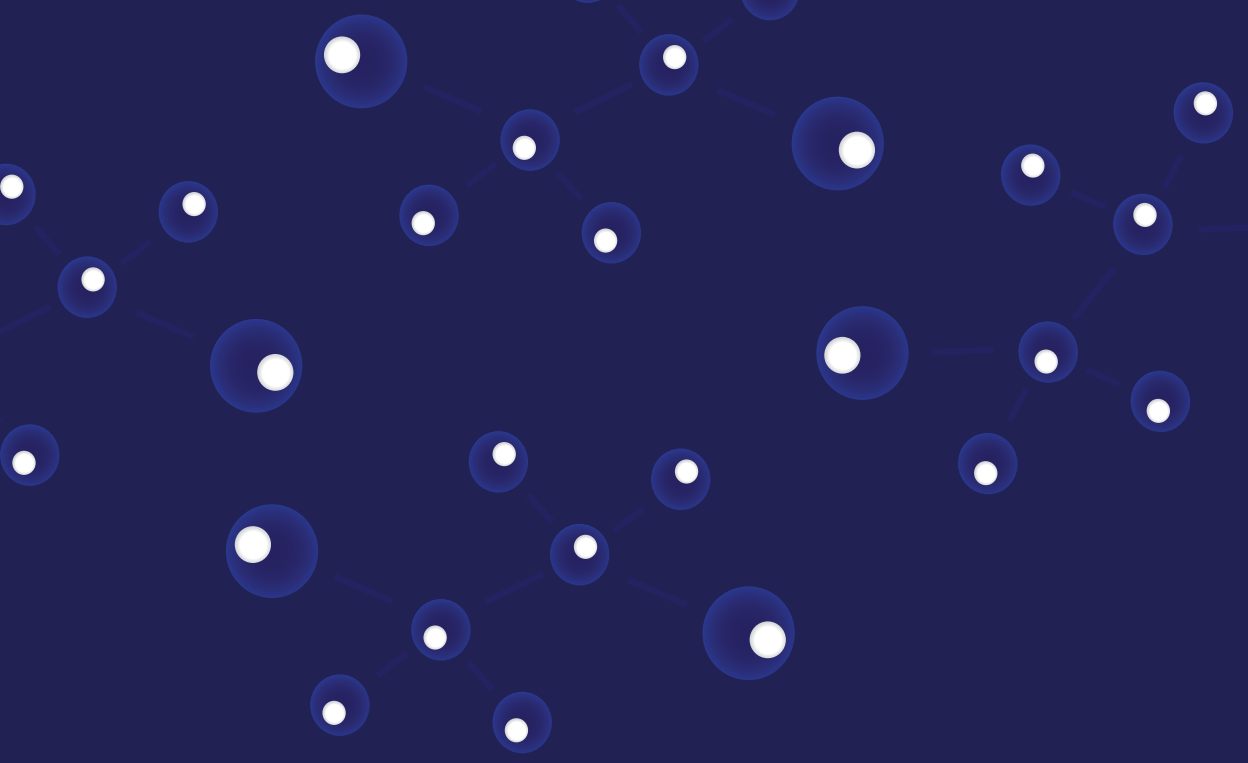

**La situazione**
La popolazione dell'Area Programma gode di elevata qualità e durata della vita. Nelle ultime decadi di vita, tuttavia, le esigenze di cura ed assistenza possono essere molto impegnative. È quindi necessario un continuo miglioramento delle tecniche di prevenzione, diagnosi precoce e cura di malattie croniche legate all'età.

**La soluzione/Gli obiettivi**
Mettere a punto una tecnostruttura transfrontaliera condivisa, dedicata allo sviluppo continuo di nuovi protocolli e dispositivi biotecnologici per l'efficace prevenzione e cura di malattie neurodegenerative, cardiovascolari, ortopediche e oncologiche.

**I risultati attesi**
TRANS2CARE ha una massa critica di infrastrutture, risorse umane e conoscenza sufficiente per realizzare innovazioni biomediche. Si prevedono tre principali impatti. A livello individuale, le persone fruiranno di migliori condizioni di salute (*healthy ageing*). A livello collettivo, ciò consentirà risparmi di spesa nel settore sanitario e dell'assistenza. Infine, l'ideazione di nuovi servizi e prodotti biotecnologici potrà favorire iniziative imprenditoriali nel settore delle biotecnologie e così aumentare l'occupazione di personale ad elevata competenza. Tutto ciò garantisce la forza, presente e futura, del progetto.

**Izhodiščno stanje**
Človeška populacija v okviru Programa uživa visoko kvaliteto življenja in dosega zavidljivo visoko življenjsko dobo. Sploh v zadnjih desetletjih slehernega življenja so potrebe po zdravstveni negi in pomoči povečane. Zato je potrebno tehnike na področju preventive, zgodnje diagnostike in zdravljenja kroničnih bolezni, značilnih za starost, neprestano izboljševati.

**Rešitve/Cilji**
Cilj projekta je postaviti trajno čezmejno organizacijsko strukturo, ki se zavzema za kontinuiren razvoj novih praks in biotehnoloških proizvodov za bolj učinkovito odkrivanje in zdravljenje nevrodegenerativnih, kardiovaskularnih, ortopedskih in rakavih obolenj.

**Pričakovani rezultati**
TRANS2CARE razpolaga z zadostnimi infrastrukturnimi zmožnosti, človeškimi viri in znanjem za uresničevanje inovacij v biomedicini. Uresničeni bodo trije glavni cilji. Na nivoju posameznika bodo osebki uživali izboljšanje v zdravju (*healthy ageing*). Na družbenem nivoju bo doprineslo k večjemu varčevanju v zdravstvenem sektorju. Navsezadnje bo prodor novih storitev in biotehnoloških produktov vzpodbudil razvoj novih poslovnih niš na biotehničnem področju in tako povečal obseg zaposlovanja visoko usposobljenega kadra. Vsa ta gonilna sila, sedanja in prihodnja, je združena v projektu.

**PARTNERS**
Università degli Studi di Trieste, Kemijski Inštitut Ljubljana, Scuola Internazionale Superiore di Studi Avanzati, Univerza v Novi Gorici, Università di Ferrara, Treviso Tecnologia, Splošna Bolnišnica Dr. Franca Derganca, Università Ca' Foscari di Venezia, Università di Udine, IRCCS Burlo Garofalo, Zavod Republike Slovenije Za Transfuzijsko Medicino, Ortopedska Bolnišnica Valdoltra, Univerza na Primorskem

Si prega di confermare la propria partecipazione a info@trans2care.eu

Prosimo, da potrdite svojo udeležbo na info@trans2care.eu

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Progetto Rete Transregionale per l'innovazione ed il Trasferimento Tecnologico per il Miglioramento della Sanità finanziato nell'ambito del Programma per la Cooperazione Transfrontaliera Italia-Slovenia 2007-2013, dal Fondo europeo di sviluppo regionale e dai fondi nazionali

Ministero dell'Economia e delle Finanze

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REPUBLIKA SLOVENIJA
SLUŽBA VLADE RS ZA LOKALNO SAMOUPRAVO IN REGIONALNO POLITIKO

DIPARTIMENTO DI SCIENZE DELLA VITA

UNIVERSITÀ DEGLI STUDI DI TRIESTE

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Transregional Network for Innovation and Technology Transfer to Improve Health Care

# RETE TRANSREGIONALE PER L'INNOVAZIONE ED IL TRASFERIMENTO TECNOLOGICO PER IL MIGLIORAMENTO DELLA SANITÀ

# TRANSREGIONALNO OMREŽJE ZA INOVACIJO IN PRENOS TEHNOLOŠKEGA ZNANJA ZA IZBOLJŠANJE ZDRAVSTVA

## *Kick-off meeting*

lunedì 21 novembre 2011
Ore 15.00

ponedeljek, 21. november 2011
Ob 15 h

2007-2013
cooperazione territoriale europea
programma per la cooperazione transfrontaliera
Italia-Slovenia
evropsko teritorialno sodelovanje
program čezmejnega sodelovanja
Slovenija-Italija

Scuola Superiore di Lingue Moderne per Interpreti e Traduttori - Narodni dom

Aula Magna - Konferenčna dvorana
Trieste - Trst, via Fabio Filzi 14

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Projekt sofinancira Evropski sklad za regionalni razvoj

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## Programma

ore 15.00 ***Indirizzi di saluto delle Autorità***
Modera *Nadine Celotti*
Preside della Scuola Superiore di Lingue Moderne per Interpreti e Traduttori

UNIVERSITÀ DEGLI STUDI DI TRIESTE
*Francesco Peroni*, Rettore
*Renato Gennaro*, Direttore del Dipartimento di Scienze della Vita, *Lead Partner*

COMUNE DI TRIESTE
*Roberto Cosolini*, Sindaco

PROVINCIA DI TRIESTE
*Maria Teresa Bassa Poropat*, Presidente

REGIONE AUTONOMA FRIULI VENEZIA GIULIA
*Elio De Anna*, Assessore regionale alla cultura, sport, relazioni internazionali e comunitarie

ore 16.00 ***Presentazione del progetto***
*Sabina Passamonti, team manager, LEAD PARTNER*, Dipartimento di Scienze della Vita
"TRANS2CARE. Piani di lavoro: coscienza e prospettive"

ore 16.20 ***Interventi***
Modera *Giorgio Tessarolo*, esperto di politiche comunitarie

AUTORITÀ DI GESTIONE DEL PROGRAMMA ITALIA-SLOVENIA 2007-2013
*Laura Comelli*

MINISTERO DELLO SVILUPPO ECONOMICO - DIPARTIMENTO PER LE POLITICHE DI SVILUPPO E COESIONE
*Marilena Barbaro*

REPUBBLICA DI SLOVENIA, Ministero della cultura e Ufficio del governo per gli sloveni all'estero
*Boštjan Žekš*, Ministro

Conclusioni, *Giorgio Tessarolo*

ore 17.30 ***Chiusura***

## Program

ob 15.00 ***Uvodni nagovor oblasti***
Moderator *Nadine Celotti*
Dekan Visoke šole modernih jezikov za tolmače in prevajalce

UNIVERZA V TRSTU
*Francesco Peroni*, Rektor
*Renato Gennaro*, Direktor Oddelka za znanosti o življenju, *Vodilni partner*

OBČINA TRST
*Roberto Cosolini*, Župan

POKRAJINA TRST
*Maria Teresa Bassa Poropat*, Predsednik

AVTONOMNA DEŽELA FURLANIJA JULIJSKA KRAJINA
*Elio De Anna*, Odbornik za kulturo, šport, mednarodne odnose in odnose z EU

ob 16.00 ***Predstavitev projekta***
*Sabina Passamonti, Vodja ekipe, Vodilni partner,* Oddelek za znanosti o življenju
"TRANS2CARE. Delovni načrt: zavest in perspektive"

ob 16.20 ***Predavatelji***
Moderator *Giorgio Tessarolo*, Strokovnjak s področja EU politike

ORGAN UPRAVLJANJA PROGRAMA ČEZMEJNEGA SODELOVANJA SLOVENIJA-ITALIJA 2007-2013
*Laura Comelli*

MINISTRSTVO ZA GOSPODARSKI RAZVOJ - ODDELEK ZA RAZVOJ IN KOHEZIJSKO POLITIKO
*Marilena Barbaro*

REPUBLIKA SLOVENIJA, Ministrstvo za kulturo in Urad vlade RS za Slovence v zamejstvu in po svetu
*Boštjan Žekš*, Minister

Sklepne misli, *Giorgio Tessarolo*

ob 17.30 ***Zaključek***

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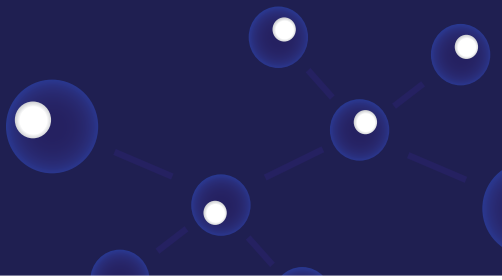

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REPUBLIKA SLOVENIJA
SLUŽBA VLADE REPUBLIKE SLOVENIJE ZA RAZVOJ
IN EVROPSKO KOHEZIJSKO POLITIKO