PROJECT FINAL REPORT

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Final publishable summary report

Executive summary

The consortium of 18 partners from 9 countries has successfully finalized work within the NaDiNe (Nanosystems for early Diagnosis of Neurodegenerative diseases) project. The project's main goal was to provide diagnostic tools to determine the onset of a neurodegenerative diseases (such as Alzheimer's disease or Parkinson's diseases) by using blood or serum samples, a procedure that is potentially amenable to regular screening of a certain segment of the population, as it is very little invasive, works on a small sample volume and can be performed at a cost level that is not prohibitive for public health systems. This was an extremely ambitious goal, as the concentration level of biomarker molecules indicative of these neurodegenerative diseases can be expected to be very low in the early (onset) stages of the diseases. Furthermore, to be able to distinguish between different types of neurodegenerative diseases and to improve the fidelity of the diagnosis, it is not sufficient to rely on a single biomarker, but on a group of biomarker, providing a signature or fingerprint.

During the project period, a wealth of approaches utilizing miniaturization and novel nanotechnologies have been developed and tested in combination with advanced biochemical protocols. It was a chosen strategy from the start to pursue several avenues, both to be able to explore different possibilities, but especially also to reduce the risk of failure of one single approach. In the project period, some of the chosen technologies (e.g., evanescent wave detection, self-assembled bar-coded particles) were demonstrated, but deemed to not provide sufficient performance or sensitivity to be useful for NaDiNe's main applications, but could certainly be used for other (medical) applications. In the end, novel magnetic nanoparticles with custom-made antibodies and fine-tuned assay protocols were used for an immunoprecipitation-based enrichment step, to be followed by either a protein array with fluorescence detection or a mass spectrometric approach with nanopillar arrays for further up-concentration. Also, in parallel, an electrochemical assay again using antibody-coated magnetic microparticles was used as a further risk-reducing measure. Further refinements are possible by incorporating miniaturized separation systems as well to aid in the fingerprinting. Microdevices were fabricated from polymer materials and operated using sophisticated fluidic control hardware and software, established by one of the project partners. This led ultimately to integrated lab demonstrator devices for the above-mentioned processes. These were fully functional, albeit not yet so user-friendly as to be used directly by the medical partners.

Instead, validation of the developed systems was performed in the labs of the developing partners, using non-clinical and clinical samples provided by the medical partners. Four developed technologies (microarray, micro droplet array, electrochemical assay, and mass spectrometric analysis) were tasked to determine diagnostic levels in these medical samples. The medical partners provided guidance for handling and preparation of the samples, and also joined in critically evaluating the data, together with bioinformatics experts. In particular, data from clinically relevant samples was compared to previously obtained results with the current gold standard method. Overall, while on a limited data set, the data produced by NaDiNe technology aligns very well with established methods, both in terms of (diagnostically relevant) limits of detection achieved as well as showing the ability to clearly distinguish between different diagnostics groups, and thus ultimately shows the potential to provide diagnostic answers with less sample, at earlier times in the disease stage and at significantly reduced costs. In conclusion, the results of the NaDiNe underline the significance of using sophisticated biochemistry combined with nanotechnology to provide next-generation medical diagnosis tools.

Summary description of project contents and objectives

From its inception, the NaDiNe project was designed to follow a structure, where the initial three years were earmarked to explore a variety of innovative nanotechnological approaches for the sensitive detection of relevant biomarkers for the early diagnosis of neurodegenerative diseases. The following (4^{th}) year was then planned to be used for consolidating the most promising technological bits and bringing them together in a prototype device, which, then, in the last (5^{th}) year, would be tested and validated in close collaboration with the medical partners on clinically relevant samples.

It was also already envisioned from the start, that not all technological tracks that the consortium initially had suggested would prove successful (because of the inherent risks associated with developing novel techniques and diagnostics approaches), but that, through iterative processes, technological solutions not sensitive enough or not robust enough would be pruned away, while others were further developed, improved upon and potentially used in conjunction with other developed bits (i.e., the combination of a novel sample preparation method with a highly sensitive detection or sensing method). This strategy of parallel development with regular checkpoints and decision points had always been deemed absolutely necessary, not least as a risk reducing effort as several of the initially suggested routes looked extremely promising (if successful) but also were associated with a high risk of failure.

Furthermore, apart from the overall time plan (development – consolidation and prototyping – testing and evaluation) and the parallel, risk-reducing strategy in the development phase, the final important design element for the project was to have a clear idea of the typically necessary steps in the workflow of a successful analysis and particularly a successful diagnosis. This entails a solid strategy for sample preparation and pre-treatment, highly sensitive detection or sensing approaches that are compatible with the sample preparation methods, instruments and instrumental control to automate processes and minimize operator mistakes, but, very importantly, also to involve bioinformatics tools to process data such that clinically meaningful results can be extracted and statistically validated. All these three “design elements” were reflected in the way the work packages for the NaDiNe project were put together, where they were placed in the project timeline, and how they were meant to provide input to each other.

The objectives of WP 1 were thus to focus in the preparation, characterisation, modification and testing of novel micro and nanoparticles. The use of such particles was important for both sample preparation purposes as well as detection and sensing purposes. In order to meet one of the key requirements of the NaDiNe project, namely to detect minute concentrations of biomarkers in the onset phase of the neurodegenerative disease, it was necessary to include sample pre-concentrations techniques into the overall workflow. Here, immune-labelled micro and nanoparticles provide a large surface when incubated with the sample, a high specificity when labelled with the best available antibodies, and the possibility to be manipulated by, e.g., magnetic forces inside microfluidic channel networks. Nanoparticles can also be made with special optical and electrochemical properties (e.g., quantum dots) making them very attractive for sensing platforms.

In WP 2, technologies for fabricating microfluidic devices and preparing them for use with biological matrices (blood, plasma, cerebrospinal fluid, …) were investigated along with ways to provide the basic fluidic handling elements (pumping, valving, connections, …) as well as strategies for integration and automation. Here, considerations for what parts of a final system should be re-usable and what parts should be disposable (because of direct contact with patient samples) were important. A lot of emphasis was put on working with polymer substrates to make the actual chips, but other materials were also investigated if necessary from, e.g., a detection point of view. As with the actual detection approaches themselves, material choices were tested, evaluated and adjusted during the course of the project.

WP 3 then focused on a range of different nanotechnological approaches to facilitate ultrasensensitive detection of selected biomarkers in biological matrices. The original selection included novel optical,

electrochemical and mass spectrometry-based approaches. Early on, it became obvious that the waveguide-based techniques, while inherently very elegant, would not provide the necessary sensitivity. The same became clear, albeit a bit later, for the quantum-dot approaches. The latter would probably have worked in combination with the sample pre-concentration approaches, but they were not selected for further development, as other techniques proved more promising. During the course of the project, a set of criteria (figures of merit, compatibilities, price, …) had been established to test and compare the various techniques, and this was used during the regular evaluation and decision meetings. Finally, an additional approach that had been developed in parallel for – initially – a different application, was adopted late in the project and proved successful for the detection of biomarkers for neurodegenerative diseases.

Further important techniques, which are not directly detection methods, were developed in WP4. This included in particular sample prep approaches, but also other techniques which were intended to be combined or hyphenated with detection, such as labelling, separation, compartmentalization, and immunocapturing. This WP also included some high-risk developments, such as the convective self-assembly of bar-coded particles, that were included as they potentially could provide highly novel functionalities, but it was expected that their potential to be fully developed to be included in a prototype device within the project period would be rather limited. One of the techniques planned in this WP was abandoned fairly early on, as it became clear – through discussions with the medical partners - that a clean-up of whole blood would not really be necessary as most clinical samples are available as plasma samples already.

Something that was discussed heavily already in the proposal writing phase of the project, but which was not possible to pin down clearly enough from the start was the selection of the actual biomarkers to be included. The choice of biomarkers – apart from being crucial for diagnostic success – influences almost all steps of the anticipated workflow: the choice and production of antibodies, the choices for sample enrichment, the design of the assay and all its protocols, the choice of materials and chemicals, the detection parameters and so on. Finding biomarkers for neurodegenerative diseases was not the objective of the NaDiNe project, but it was part of WP 5 to determine a roster of possible biomarkers that are clinically relevant and can help detecting both the onset of neurodegenerative diseases and also help in subtyping them. Alas, all throughout the project, it became apparent that the biomarker field was in constant flux and new potential biomarkers were described in the literature all the time. The temptation to use “better” biomarkers was obvious from earlier struggles with proteins and peptides that were very tedious to work with, but the amount of time necessary to, again and again, develop antibodies and optimize assays and detection protocols was prohibitive and this caused some frustration among the partners. It was probably here where the consortium should have made a clear decision early and stuck with it, instead of hoping for a “better” set of biomarkers to appear. This caused some of the delays that took from the prototyping and testing phase of the project.

Further tasks in WP 5 pertained to the optimization and comparison of the developed technologies, and to provide input for the prototype to be built in WP 6. In view of the obtained results, it became necessary to realistically assess and adjust the objectives after year 3, and to focus on a reduced and easier problem first, namely the detection of Abeta-peptides and ApoE in mainly cerebrospinal fluid samples. A panel of criteria was established and the most promising techniques were tested against these criteria for possible inclusion in the prototype. A final task in WP 5 was to develop and provide bioinformatics tools to a) improve biomarker selection through datamining of already published results, and b) statistically assess data provided by using the prototype for clinical samples. The latter had to be adjusted drastically as not enough data was produced in the end to require a more concerted biostatistical treatment.

The prototype itself was to be developed (and, potentially, iterated) in WP 6. Due to the delay in optimizing some of the most promising nanotechnologies, a true prototype device was not manufactured. Instead, several demonstrator units were provided demonstrating the usefulness of the chosen nanotechnologies. The distinction here being that a prototype would be tested at the clinic by trained, but non-technical operators, whereas a demonstrator, while fully functioning, is typically not very user-friendly yet and often only can be operated in a fully equipped laboratory. In the end, hybrids of these two cases were made

available, already with a fairly high degree of user-friendliness and automation as well as commercialization potential, but still being tested in a technical lab rather than a hospital setting. The main reasons for deviating from the original objectives are lying in longer development times for the nanotechnologies as breakthroughs are always “just around the corner”, and challenges with the biochemistry, where antibodies, functionalization protocols or assays just would not work reliably and/or with too much batch-to-batch variation.

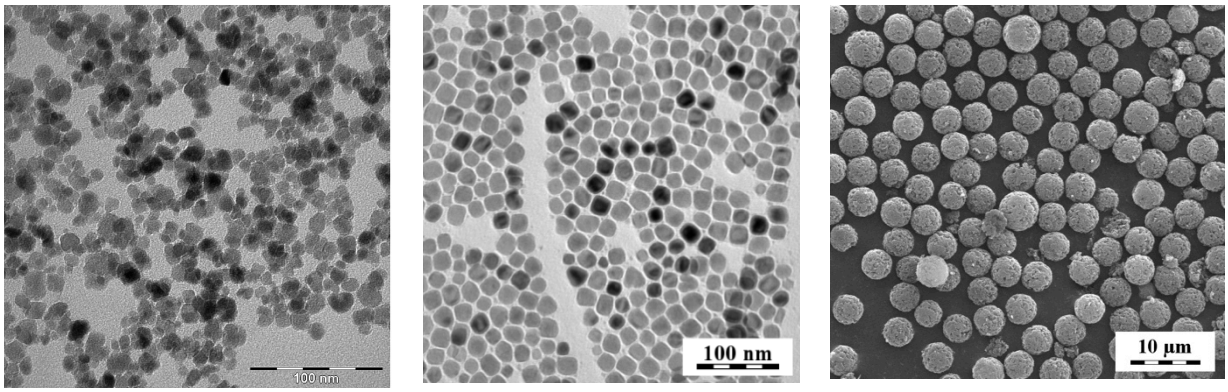
WP 7 was the workpackage where everything was supposed to come together, i.e., where clinical samples provided by the medical partners could be tested on the prototype developed in WP 6, and where these tests could be validated by, e.g., comparison with gold standard methods. This WP was reduced in time caused by the delays of the earlier WPs and the amount of testing done was consequently much less than anticipated. Also, instead of one prototype, four workflows including nanotechnological solutions from the partners were tested. Still, as described further below, excellent results were obtained that demonstrated that clinically relevant diagnostic results could be achieved.

Finally, WP 8 and 9 were predominantly concerned with administrative tasks and tasks related to dissemination and exploitation of results obtained throughout the project. It is noteworthy to mention that the inclusion of a partner almost exclusively in charge of designing and implementing risk management is something to be recommended for other projects. This both provided some valuable guidance in running the project and also forced the coordinator and the steering group to regularly take stock and adjust objectives and strategies as necessary. While not all issues could be anticipated and prevented, the awareness of potential risks and ways to react clearly contributed to the overall success of the project.

Main scientific and technological results (max 25 pages)

Task 1.1 - Innovative magnetic nanoparticles

We have developed **superparamagnetic maghemite nanoparticles** (γ-Fe_2O_3), 9 nm in size according to TEM, by coprecipitation of Fe^{2+} and Fe^{3+} chlorides with aqueous ammonia; the resulting magnetite was then oxidized with sodium hypochlorite to chemically stable maghemite (***Figure 1a***). The particles were subsequently coated with a shell of hydrophilic and biocompatible poly(*N,N*-dimethylacrylamide) (PDMAAm). The PDMAAm-γ-Fe_2O_3 nanoparticles were shown to be non-cytotoxic and they were intensively phagocytosized by the mammalian macrophages. High potential of PDMAAm-γ-Fe_2O_3 nanoparticles in diagnosis of phagocytary activity as well as in delivery of various biomolecules, such as specific proteins, can be predicted. Optionally, the γ-Fe_2O_3 nanoparticles were coated with *D*-mannose, poly(*L*-lysine), silica and its derivatives, poly(vinylpyrrolidone) (PVP), poly(ethylene glycol) (PEG) and poly(2-hydroxyethyl methacrylate) (PHEMA). The superparamagnetic γ-Fe_2O_3 nanoparticles are promising in cell applications as the cells labelled with the nanoparticles can be non-invasively monitored and easily magnetically separated and redispersed in water solutions on removing the external magnetic field.



a b c

Figure 1. *(a, b) TEM micrographs of (a) γ-Fe_2O_3 nanoparticles synthesized by coprecipitation and (b) Fe_3O_4 particles prepared by thermal decompositon of Fe^{3+} oleate in 1-octadecene. (c) SEM micrographs of magnetic PHEMA microspheres.*

Another approach for synthesis of monodisperse superparamagnetic iron oxide nanoparticles of controlled size consisted of thermal decomposition of organic iron compounds in different high-boiling solvents (1-octadecene, eicosane and trioctylamine) in the presence of oleic acid and/or oleylamine (***Figure 1b***). The compounds included Fe(III) oleate and mandelate, formed from $FeCl_3$ and the respective acids. The size of the nanoparticles was easily tuned to 8–27 nm by varying the experimental conditions. The nanoparticles were characterized using X-ray diffraction, transmission electron microscopy, and magnetization measurements. The hydrophobic coating of the particles was analyzed by thermogravimetric analysis and atomic absorption spectroscopy. To make the particles biocompatible and water dispersible, nontoxic hydrophilic poly(ethylene glycol) derivatives containing phosphonic and hydroxamic groups were synthesized and used for phase transfer of hydrophobic particles into water using a ligand-exchange procedure. Saturation magnetization of nanoparticles was between 40 and 95 Am^2/kg.

Our second main achievement during the project consisted of development of magnetic macroporous poly(glycidyl methacrylate) (**PGMA**) and poly(2-hydroxyethyl methacrylate) (**PHEMA**) microspheres containing carboxyl and amino groups by multi-step swelling and polymerization followed by precipitation of iron oxide inside the pores (***Figure 1c***). The functional groups enabled bioactive ligands of various sizes and chemical structures to couple covalently. Microspheres containing amino and/or carboxyl groups were then coated with α,ω-bis-carboxy PEG and amino-terminated PEG or α-methoxy-ω-amino PEG, optionally with sulfobetain. Adsorption of bovine serum albumin (BSA), γ-globulin, ^{125}I-BSA, pepsin, and

chymotrypsin on neat and PEGylated microspheres was determined by UV–VIS spectroscopy of supernatants and eluates or by measurement of radioactivity in an ionization chamber. The carboxyl groups of the magnetic PGMA microspheres were also conjugated with primary amino groups of mouse monoclonal DO-1 antibody using conventional carbodiimide chemistry. The efficiency of protein p53 capture and the degree of nonspecific adsorption on neat and PEG-coated magnetic microspheres were determined by western blot analysis. The applicability of these monodisperse magnetic microspheres in biospecific catalysis and bioaffinity separation was confirmed by coupling with the enzyme trypsin and IgG. The indisputable advantage of magnetic carriers consists in the ability to control their transport by applying a magnetic field. Magnetic microspheres modified by the above-described procedure can thus be utilized in standard immunoprecipitation techniques.

A nanobiosensor based on the use of porous magnetic microspheres as efficient capturing/pre-concentrating platform was presented for detection of Alzheimer's disease (AD) biomarkers (by partner ICN). These microspheres prepared by a multistep swelling polymerization combined with iron oxide precipitation afford carboxyl functional groups suitable for immobilization of antibodies on the particle surface allowing an enhanced efficiency in the capturing of AD biomarkers from human serum samples. The AD biomarker signalling was generated by gold nanoparticle (AuNP) tags monitored through their electrocatalytic effect towards hydrogen evolution reaction. Novel properties of porous magnetic microspheres in terms of high functionality and high active area available for enhanced catalytic activity of the captured AuNPs electrocatalytic tags were exploited for the first time. A thorough characterization by scanning transmission electron microscope in high angle annular dark field mode demonstrated the enhanced ability of porous magnetic microspheres to capture a higher quantity of analyte and consequently of electrocatalytic label, when compared with commercially available microspheres. The optimized and characterized porous magnetic microspheres were also applied for the first time for the detection of beta amyloid and ApoE at clinical relevant levels in cerebrospinal fluid, serum and plasma samples of patients suffering from AD.

Task 1.2 - Nanoparticles for signal amplification

Several types of **novel NPs** were synthesized: (i) different-sized gold nanoparticles (**AuNPs**) (5-80 nm) usable as labels in immunoassays and as blockers in nanochannels-based sensing systems, (ii) **CdS QDs NPs** (2 nm) with improved characteristics (size, stability and fluorescent/electrochemical properties) for electro-chemical/optical applications, (iii) iridium oxide nanoparticles (**IrO_2NPs**) (12 nm) with novel electrocatalytic detection method at pH 7 (through water oxidation reaction - WOR), and (iv) Prussian blue nanoparticles (**PBNPs**) (4 nm) usable as red-ox indicator in nanochannel-based sensing systems (***Fig. 2***). All these new nanoparticles and nanoparticle/biomolecule conjugates have been characterized using different optical techniques (TEM, Z-potential, UV-Vis spectroscopy) and their electrochemical behavior has also been successfully evaluated.

A new method for **oriented functionalization** of gold nanoparticles (AuNPs) with antibodies has been developed. It consists in taking advantage of oriented ionic attraction and strong covalent bond formation using EDC chemistry. The result is an oriented and strong functionalization, which creates an excellent label to be used in many different types of immunosensors.

Novel magnetic poly(2-hydroxyethyl methacrylate) (**PHEMA**) microspheres containing carboxylic groups (synthesized by partner **MACRO**) were used as platforms for the covalent and oriented immobilization of antibodies in a magnetosandwich immunoassay for the **detection of ApoE**, one of the AD biomarkers, using **AuNPs** (20 nm sized) as electrocatalytic labels.

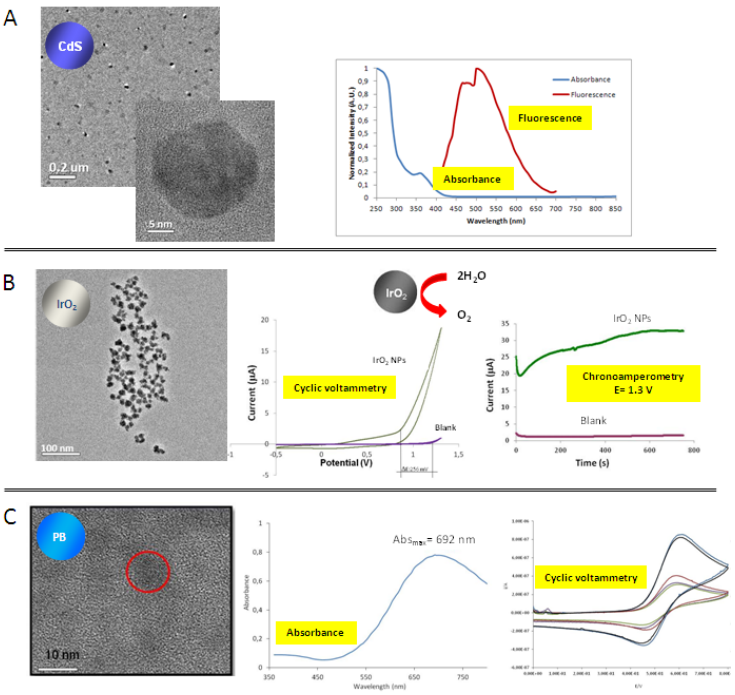


Figure 2. *(A) TEM images of the synthesized CdS Quantum dots and the corresponding optical characterizations. (B) TEM images of the synthesized IrO_2 nanoparticles and summary of their electrocatalytic activity on the water oxidation reaction in both voltammetric and chronoamperometric measurements. (C) TEM images of the synthesized Prussian Blue nanoparticles and the corresponding optical and electrochemical characterizations.*

ApoE detection by using porous magnetic microspheres and electrocatalytic gold nanoparticles were compared with detection of another AD biomarker, **Beta amyloid**. High porosity of magnetic microspheres (**PMMs**) allows a more efficient capturing of AD biomarkers in magnetoimmunoassays. AuNP tags and electrocatalytic hydrogen evolution (HER) were successfully used for biomarkers detection. Beta amyloid and ApoE in CSF, serum and plasma samples of patients suffering from AD were evaluated with achieving performances of clinical relevance.

The detailed study of the analytical parameters related to the **new catalytic nanoparticles** (LOD, linear range, etc.) and their **application** for the detection of AD biomarkers (i.e., ApoE) were performed also. ApoE detection through electrocatalytic water oxidation induced by iridium oxide nanoparticles was one such application. High catalytic effect of **IrO_2 NPs** tags towards the Water Oxidation Reaction (WOR) enables sensitive chronoamperometric quantification at pH 7. Sensitive method for the ApoE detection in human plasma (**LOD: 68 ng/mL**) following a magnetoimmunoassay format was developed. Both the IrO_2 NPs tags and electrocatalytic detection method present many advantages (simplicity, cost, etc.) compared with previously reported ones based on quantum dots (QDs) or Hydrogen Evolution Reaction (HER).

Task 1.3 – Biofunctionalisation

The objectives of the task have been successfully accomplished. Common and advanced procedures for surface modification of magnetic micro/nanoparticles with subsequent immobilization of specific IgG were applied to provide final functional immunosorbents to requesting partners (CI, DS, UPS). With the aim to minimize the rate of nonspecific adsorption and tendency of particles to self-aggregate and adhere to the walls of a microfluidic devices we successfully modified the surface of particles by poly(ethylene glycol) (PEG) derivatives and hyaluronic acid (HA) fragments. The list of modified magnetic micro/nanoparticles (commercial or newly developed (MACRO) and list of polymers used for modifications is given below (*Tab. 1*). Testing the behavior of modified particles in magnetic field and their ability to create self-organized columns in microfluidic systems were performed in cooperation with CI partner.

	List of tested carriers
Commercially available	Dynabeads M-270 Amine (Life Technologies, Carlsbad, CA, USA)
	SiMAG-Amine (Chemicell GmbH, Berlin, DE)
	Dynabeads M-270 Carboxylated
	SiMAG-Carboxyl (Chemicell GmbH, Berlin, DE)
	Sera-Mag-COOH (Seradyn, Indianapolis, USA)
Developed by MACRO	Hypercrosslinked polystyrene particles HPM
	P(GMA)-MOEAA -NH_2
	PHEMA-COOH
	PGMA-COOH
	P(GMA-EDMA)-HSA-COOH

	List of tested polymers
Type of HA	Oligo-HA 4 (776,66 Da, Sigma-Aldrich, St. Louis, USA)
	HA 10 kDa (Contipro Group, Dolní Dobrouč, ČR)
	HA 26kDa (Contipro Group, Dolní Dobrouč, ČR)
	HA 170kDa (Contipro Group, Dolní Dobrouč, ČR)
	Hyaluronan-Biotin Sodium Salt (700 kDa, Sigma-Aldrich, St. Louis, USA)
	Fluorescein-hyaluronic acid (> 800 kDa)
Type of PEG	Amine-PEG-Biotin (2000 Da, Laysan Bio Inc., Arab, USA)
	Amine-PEG-Carboxyl (3400 Da)
	Amine-PEG-Hydroxyl (3400 Da)
	Hydrazide-PEG-Hydrazide (3400 Da)

Table 1. *List of tested carriers and polymers used for surface modification.*

Various methods such as zeta potential measurement, infrared spectroscopy, scanning electron microscopy, laser diffraction, anti-PEG ELISA assay successfully proved the coating of particles. Grafting of specific antibodies (different clones of anti-Aβ, anti-Tau, anti-ApoE, anti-ubiquitin IgG molecules) in a proper way on magnetic micro/nanoparticles without adversely affected binding function was the major goal in the construction of an efficient immunosorbents. Different immobilization approaches were optimized and tested. Prepared carriers have been tested not only in batch, but mainly in the microfluidic systems prepared by partners, their colloidal stability was confirmed. Experiments with real biological samples (CSF, whole blood) were also successfully performed. Comparison of specific IgG immobilization efficiency using various particles is shown in *Figure 3*.

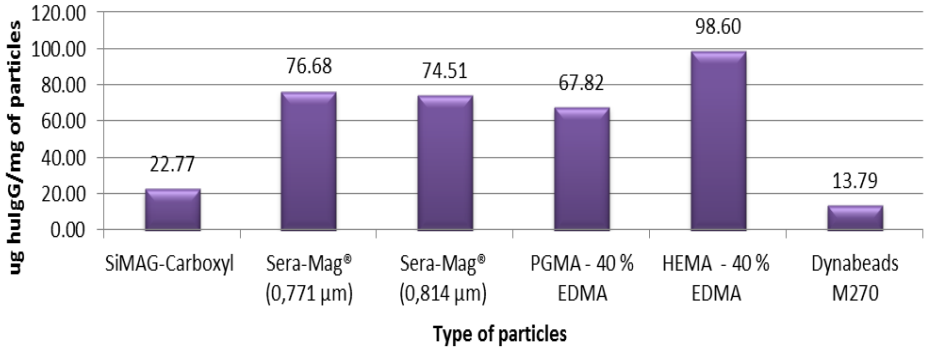


Figure 3. The *immobilization efficiency of IgG molecules to the various commercial and particles newly developed by partner MACRO*

Prepared immunosorbents with desired specificity (directed against Tau protein, Aβ peptides, ApoE, ubiquitin, etc.) were applied for immunocapturing of target proteins associated with AD from spiked/real complex biological materials such as human plasma, serum or CSF. Optimization of immunocapturing protocol was necessary (batch *vs.* µIP binding/elution conditions, subsequent detection method). As an example, we successfully detected Aβ peptides in CSF (***Fig. 4***). For affinity and specificity evaluation of applied antibodies we also developed dot-blot affinity test using NH_4SCN as chaotropic agent and magnetic beads-based epitope extraction technique.

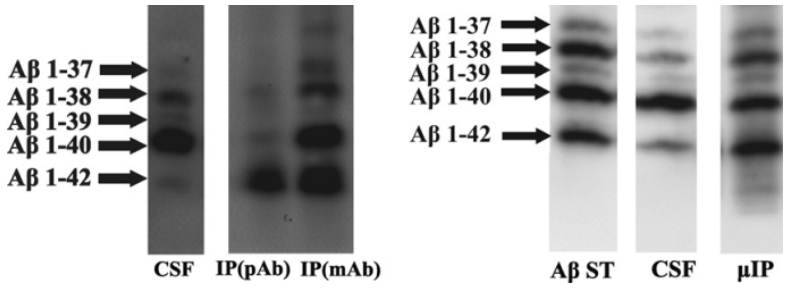


Figure 4. *Immunocapturing of Aß peptides confirmed by Western blot.*

In cooperation with partner ICN the quantum dots were coupled with specific antibodies to prepare conjugates for amplification of the detection signal and increasing the sensitivity of the analysis. Apolipoprotein E (ApoE) antigen (BioVision, USA) and specific polyclonal anti-ApoE antibody (partner MB) were used as a model system. Various strategies were tested and optimized. One of the innovative approaches is based on fixation of the antibody in the immunocomplex with specific antigen covalently coupled on the surface of magnetic particles wit subsequent QDs conjugation based on carbodiimide chemistry and elution of formed QDs-conjugate.

Task 1.4 - Nanoparticle characterization and safety

Nanostructural characterization, i.e., morphology, size and particle size distribution in the dry state were characterized by SEM and TEM microscopy; micrographs of cross-section of the microspheres provided information about interior of the particles. Dynamic light scattering determined hydrodynamic size (polydispersity) of particles in water and also the zeta-potential, values of which generally predict if the particles aggregate in water or not. ATR-FTIR spectra confirmed success of modification reactions, i.e., presence of functional groups on surface of the particles. Elemental analysis quantified the amount of functional groups, atomic absorption spectroscopy and thermogravimetric analysis then analyzed Fe content in the magnetic microspheres. Magnetic measurements, in particular the determination of hysteresis loops and saturation magnetization using magnetometer and measurement of temperature dependence of magnetic susceptibility, confirmed magnetic character of particles. Moreover, X-ray powder analysis enabled to differentiate between forms of iron oxides, Fe_3O_4 vs. γ-Fe_2O_3. Toxicity of the magnetic nanoparticles was evaluated in biological experiments, in particular, during engulfment of iron oxide nanoparticles by macrophages or mesenchymal stem cells. Non-toxicity (biocompatibility) of surface-modified iron oxide nanoparticles was thus confirmed if concentration of the particles was moderate. As the particles are contained in a colloidal fluid, there is no risk of inhalation. Hence, the potentially hazardous effects are effectively avoided.

Newly developed particles were delivered to the following partners: Univerzita Pardubice (UPCE), Institut Curie (CI), Institut Catalá de Nanotecnologia (ICN), Université Paris-Sud (UPS), Moravian-Biotechnology (MB), Diagnoswiss (DS), and Instituto di Chimica del Riconosciato Moleculare (ICRM).

Task 2.1 - Microfluidic flow control

The task consisted in developing an external fluid transport controller able to monitor reliably and accurately the transport of fluids in and between the modules. We have developed three solutions (combining hardware and software) to control transport of fluids in modules.

1. **A valving platform enabling the sequential injection of fluid in microfluidic system** (Figure 5): useful in WP4 and WP6 for partners CI and ICRM with the following specifications:

- Low internal volume to limit loss of samples
- Compatible with a large range of pressures
- Response time < 1s
- Compact, biocompatible, compatible with the software

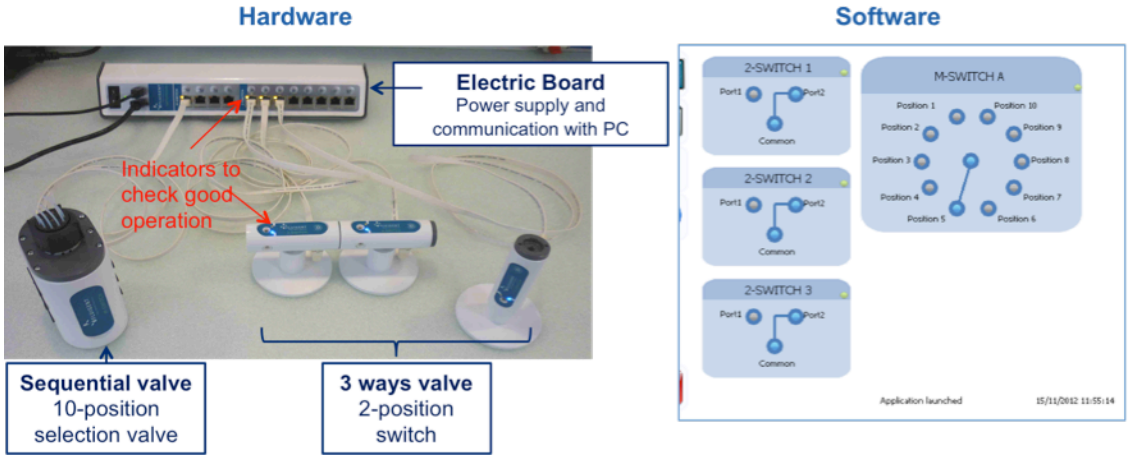


Figure 5: *Hardware and software of the valving platform.*

2. **Integrated electrodes** in pressurized reservoirs to apply electric fields in the module of pre-concentration (WP4, partner CI). The system of electrodes has been developed but the pre-treatment module has been abandoned in M12.
3. **Flow-rate control module**: useful in WP4 and WP6 for partners CI and ICRM

- Precise control of the flow: control of the flow-rate and/or the volume
- High stability of the flow
- Ability to perform long experiments

The flow-rate control module, patented during the project, is an algorithm that adjusts automatically the pressures to reach the flow-rate set points even in complex microsystems. It minimizes cross-talk between channels, compensates partial clogging, and allows stable and pulse-less flow (Figure 6).

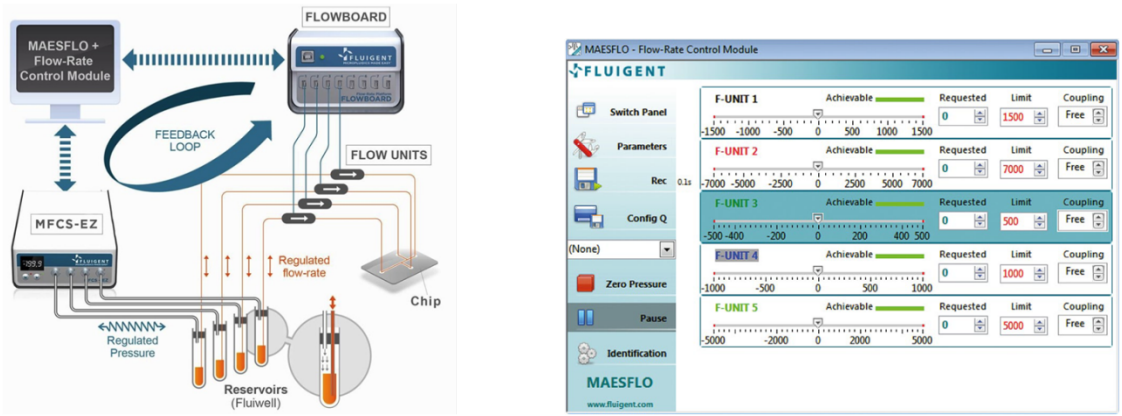


Figure 6: *Principle and software of the flow-rate control module.*

The contribution of the developments for the different partners and WPs are listed below:

- Fluidised bed (Institut Curie):
  - Integration of 3 valves (2way/3port): biocompatibility, low internal volume, allowing integration and automation
  - Use of flow-rate control module coupled with the pressure controller: feedback on flow-rate, preserve magnetic bead structure during protein capture, control of the volume injected in the system combining high flow stability and short response time.
- Microarray (ICRM):

- Integration of a rotative valve: biocompatibility, low internal volume, allowing the injection of 7 different solutions automatically, without cross contamination
- Use of flow-rate control module coupled with the pressure controller: feedback on flow-rate, control of the volume injected in the system combining high flow stability and short response time.

Task 2.2 - Low-cost microfabrication

For medical applications it is essential to work with materials that do not need to be cleaned after having been in contact with medical (patient) samples. Considering such factors as base cost of the material, ease of fabrication, fabrication costs, biocompatibility, and disposal, this points to polymer-based materials. A number of polymer materials have been considered, investigated and tested for use in this project.

Our first standard choice of material for fabrication needs in this project was poly methyl methacrylate (PMMA), as it is cheap, readily available and easy to machine. Certain parts of the prototype, which might come in contact with the sample were made in either polycarbonate (PC) or aluminum to facilitate more efficient cleaning.

It was decided to fabricate the microfluidic chip itself from poly dimethoxy siloxane (PDMS). That way DTU could simply supply the mould for the users of the platform to make their own chips on a daily basis. Furthermore, since PDMS is a soft rubber it is not necessary to bond the microfluidic part to the silicon chip, a simple clamping feature is sufficient to maintain leak proof operation.

It was immediately decided to go for a modular approach when fabricating the prototype. That way each individual part could easily be replaced and improved without affecting the other parts. The underlying intent was to have a setup consisting of several simple parts, which each could easily be adapted and refabricated to improve performance and handling.

Since the fluidic chip was to be made of PDMS, It was necessary to create a mould. The mould consists of three parts: bottom, frame and top. Each of these components serves a specific and separate purpose. The bottom part of the mould is the most delicate, as it carries the features of the microfluidic chamber and channels. The frame is simply a spacer used to define the thickness of the PDMS chip. The top part of the mould is where the in- and outlet positions are defined. Hence, in line with the modular approach, each part can be adapted individually and replaced if needed.

In order to be able to easily place the square silicon chip in the slide scanner for detection, a custom made aluminum slide was developed. The aluminum slide has similar dimensions as a regular glass slide except for a recess in one end, where the silicon chip fits in. The combined thickness of the slide and chip is just below 1 mm, which allows it to fit into the slide scanner. Aluminum was chosen as material for the slide due to its relatively high stiffness and chemical resistance, which allows the slide to be reused.

It is not enough to just place the PDMS chip on top of the silicon chip; in order to prevent leakage, a slight clamping force must be provided. Hence the chip holder platform and the chip holder top were developed. The platform has a simple recess to fit the aluminum slide, and a cavity to help removing the slide from the platform again. The top part of the holder has a window to prevent the clamping force from collapsing the microfluidic chamber. The top part also has three holes for metallic pins, which provides fool proof alignment between the window position and the chamber in the PDMS chip. The top part also features a threaded hole for connecting the reused fluidic tubings. The non-reusable tubings, such as the sample inlet and the outlet, are connected by squeezing the tubes into the PDMS. The top part is made from polycarbonate rather than PMMA as it needs cleaning with ethanol.

Task 2.3 - Surface treatment and characterization

The hydrophobic nature of disposable plastic, PDMS and thiol-ene devices presents a considerable drawback to microfluidics applications. Much effort has been devoted to modifying the surface of polymeric materials to adjust their wettability, adhesion and biocompatibility.

A simple approach to reduce dramatically the hydrophobicity for a wide group of materials including cyclic olefin copolymer (COC), polyethylene terephthalate (PET), polycarbonate (PC) and polytetrafluoroethylene (PTFE), polydimethylsiloxane (PDMS) and the newly introduced thiol-ene polymers was developed, to avoid protein unspecific binding and suppress electroosmotic flow in microchannel for microchip electrophoresis.

The process, easily implementable in any laboratory, including microfabrication clean room facilities, consists in coatings prepared via the "dip and rinse" approach by immersing the plasma-oxidized materials into an aqueous solution containing two different poly(dimethylacrylamide) based copolymers. The polymers, comprising a segment of poly(dimethylacrylamide) and incorporating a silane co-monomer, were functionalized with either *N*-acryloyloxysuccinimide (NAS), poly-(DMA-co-NAS-co-MAPS), or glycidyl methacrylate (GMA), (poly(DMA-co-GMA-co-MAPS). An extensive characterization of the hydrophilicity, binding capacity and resistance to unspecific protein adsorption of these coatings was performed. Polymers were distributed among the partners for use in their specific applications.

Task 2.4 - Disposable flow through microfluidic and nanoparticle based platforms

A flexible hybrid polydimethylsiloxane (PDMS)–polycarbonate (PC) microfluidic chip with integrated screen printed electrodes (SPE) was fabricated and applied for electrochemical quantum dots (QDs) detection. The developed device combines the advantages of flexible microfluidic chips, such as their low cost, the possibility to be disposable and amenable to mass production, with the advantages of electrochemistry for its facility of integration and the possibility to miniaturize the analytical device. Due to the interest in biosensing applications in general and particularly the great demand for labelling alternatives in affinity biosensors, the electrochemistry of cadmium sulfide quantum dots (CdS QDs) was evaluated. Square wave anodic stripping voltammetry (SWASV) was the technique used due to its sensitivity and low detection limits that can be achieved. The electrochemical as well as the microfluidic parameters of the developed system were optimized. The detection of CdS QDs in the range between 50 to 8000 ng/mL with a sensitivity of 0,0009 μA/(ng/mL) has been achieved.

Other significant results are related to the electrochemical detection of cadmium-selenide/zinc-sulfide (CdSe@ZnS) quantum dots (QDs) used as labeling carriers in an assay for apolipoprotein E (ApoE) detection. The immunocomplex was performed by using tosylactivated magnetic beads (2.8μm of diameter) as preconcentration platform into the same device. All the conjugation steps were performed in flow mode. ApoE was evaluated for its potential as biomarker for Alzheimer's disease detection. The limit of detection (LOD) achieved was ~12.5ng mL^{-1} with a linear range between 0 to 200ng mL^{-1}. Finally, dilutions from human plasma were assayed with high accuracy with respect to the calibration curve.

Task 3.1 - Electrochemical detection

At the start of the project, the technology concept for electrochemical detection in this task was already gathered in a semi-automatic prototype. During the Nadine project, this prototype was further developed to a more sensitive and robust approach and amenable to clinical routine. This objective has been reached through the development and production of two new electrochemical chips, a microwell (ImmuDrop-Chip) and a microchannel based sensor (ImmuChip), with dedicated manual (the ImmuDrop) and automatic (ImmuSpeed) operated systems, as well as specific detection methods and software (see deliverable 3.1 for details).

The production of industrial chips has been outsourced to local printed circuit board companies having the infrastructure and know-how required for the manufacture of these particular devices. The quality of the chips was verified and was in good agreement with the manufacturers specifications. Industrial prototypes of both ImmuSpeed and ImmuDrop systems have been designed and successfully fabricated. In addition they have been conceived so as to render them readily amenable to clinical practice, with control and traceability

features (bar codes, hardware key…). The two approaches showed to be applicable to immunoassays. Regarding assay protocols, the ImmuDrop system requires larger amount of reagents and the detection takes longer before stabilization of the signal compared to ImmuSpeed system. In addition, the ImmuDrop-chips cannot be regenerated which is the case for the ImmuChips. The ImmuSpeed system has been therefore used for the assay development and the dosage of Aolipoprotein E and Amyloid beta 1-42 AD biomarkers (see WP 6&7).

Task 3.2 - Integrated optical waveguides for various detection schemes

The objective for task 3.2 was "to develop waveguides, integrated with separation chips for sensitive on-line detection". Because of the many promising properties of thiol-ene, including the initial assumption that the refractive index would change proportional to the sulfur concentration, it was decided that the device would be based on a thiol-ene chip. During the early work in the NADINE project it had already been published that thiolene chips support an electroosmotic flow (EOF). One of the advantages of using thiol-ene is that during the chip fabrication the same material can be used as both substrate, channel/waveguide layer, and lid. The design of the channel/waveguide layer is shown in Fig. 7. The chip features a standard injection cross in one end and a set of 200 µm wide waveguides just before the outlet in the other end. The channel is 30 µm wide and measures 48 mm from injection cross to detection region.

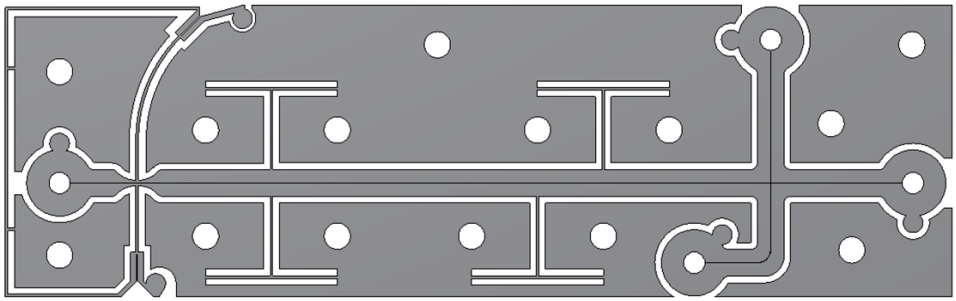


Figure 7: *The design of the chip features a curved input waveguide (top left) to prevent unguided light from reaching the detection region. The white circles serve as support beams during the chip moulding process. The small grey circles are the in/outlets to be used during the moulding of the chip. The T-shaped channels serve to guide excess thiol-ene and air bubbles away from the more important parts of the chip.*

The standard method for making thiolene chips is to pour the liquid thiolene mixture into a PDMS mould and close it with a lid (i.e., a glass slide). The mould is then exposed to UV light to cure the thiolene (see Fig. 8 - moulding). This method does, however, often leave a residual layer behind after curing. The residual layer is rarely a problem when the chip only contains features such as channels. However, when making waveguides, which are ridges and not recesses like channels, a residual will form. The residual layer adjacent to the waveguide will allow light to escape and thereby lower the quality of the waveguide. So, to avoid creating a residual layer, a new moulding method was developed. In the new method, the PDMS mould is completely assembled prior to adding the thiolene mixture. Using a syringe with a needle the thiolene is then injected into the mould through venting holes, the same holes allow the trapped air to escape (see Fig. 8 - Injecting). When using the injection method no residual layers were created, but the method does leave another unintended feature. At each venting hole there is a chance that excess thiolene will form little pillars. This is potentially critical, but if the mould is designed carefully the venting holes can be positioned in areas where the left over pillars will not interfere with the chip's performance.

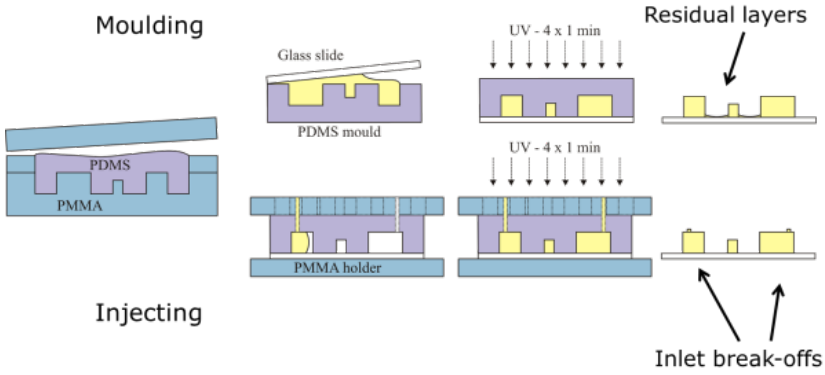


Figure 8: *The two different methods developed to fabricate thiolene chips. The moulding method (top) is good when making channels, but leaves a residual layer. The injection method (bottom) is preferred when making waveguides. In cases where waveguides or other high precision features are necessary, the bottom part of the PMMA holder can be replaced by a cleanroom fabricated master.*

In order to obtain the highest possible quality of the waveguides the chip was originally designed to be made by the injection method. However, due to the issue with non-changing refractive indices the loss in the waveguides would become too large if the bottom substrate was not changed to a different material (glass). Also, the lid of the chip could not be the same material as the waveguides, but this could easily be fixed by simply cutting holes in the lid above the waveguides, thereby changing the cladding material to air. If the injection method was kept while the bottom substrate was changed to glass, the waveguides would be perfect but the bottom of the channel would now be a completely different material from the remaining three walls. So, by switching to the regular moulding method, a residual layer will cover the entire channel floor, thus restoring the situation. However, now the quality degrading residual layer will also be present next to the waveguides. By relating the 120 µm height of the waveguide to the assumed, less than 10 µm thick, residual layer this was considered to be an acceptable configuration.

The final step in the chip fabrication process is to attach little glass reservoirs with UV-glue to each of the four in/outlets of the chip.

Task 3.3 - Evanescent wave optical detection

Due to a combination of the resulting (low) quality waveguides from T3.1 and the particular chip design, the optical signal from the analytes could not be collected and detected by waveguides as originally planned. However, the result presented below (see Fig. 9) does use the thiol-ene waveguide to excite the fluorophores, while the collection of emitted light was done through a microscope.

Waveguide assisted fluorescence detection of CE-analytes was achieved on a thiol-ene device with the exception that emitted light was collected through a microscope rather than the output waveguide. Excitation through the thiol-ene waveguide was working satisfactorily despite the loss caused be the residual layer. The results shown in Figure 9 demonstrate that the chip was capable of single analyte injections, inverse injection, and separation of multiple analytes.

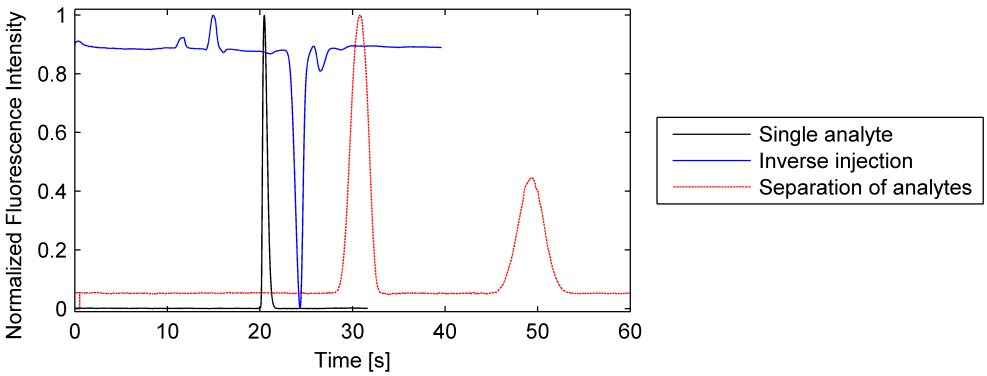


Figure 9: *Examples of detection of CE-analytes. All experiment were conducted at approximately 550 V/cm.*

In order to investigate the band broadening in the chips, a series of single analyte injections was performed at field strengths varying from 200 V/cm to 720 V/cm using an injection time of 0.5 seconds. The retention times and the full widths at half height (FWHM) of each peak were determined and used to calculate the "plate height" as a figure of merit to evaluate the quality of the separation system. All data points fall around a linearly increasing trend line between 3 µm and 14 µm. This tells us that in the parameter space investigated the longitudinal diffusion is not significant and does not become large enough to influence the plate height and thus the performance. Since the chip design in this case features rather big channels (30 x 120 μm^2) it is reasonable to assume that the fairly steep slope is mainly due to significant Joule heating.

A re-designed chip clearly demonstrated the feasibility of evanescent-based detection, but also pinted to a number of inherent issue with this mode of detection. Results of these experiments were published, but the approach was deemed not sensitive enough for the particular challenges in this project.

A modified and further developed version of this chip has later also been used by partner UPS in WP 4.

Task 3.4 - Nanotarget mass spectrometry

In this task, we have designed and fabricated chip-based pillar-structured targets for MALDI mass spectrometry. The pillars were made in various sizes, ranging from 500 down to 5 µm in diameter. The particular features of the new targets are a strong confinement of the sample volume and a concentration of the analytes onto a very small surface area.
To be able to load the samples onto the pillars, we designed and constructed a robotic system, which made it possible to handle volumes down to 1 picoliter. The new concept was evaluated with a set of model peptides, and a sensitivity of 10 zeptomol was routinely obtained. This is at least a thousand-fold better than results obtained with conventional target structures.

In a subsequent stage, we tested the new concept for the analysis of Aβ-peptides in cerebrospinal (CSF) fluid. After performing an immunoprecipitation (IP) and concentration of the analytes, clear MS signals for a set of Aβ-peptides (including Aβ 1-42) were obtained, even when only 0.1% of the final sample volume was loaded on a micropillar. Using the same IP protocol, we were also able to detect the Aβ-peptides in blood plasma. Additionally, we successfully performed an Aβ- peptide analysis using CSF from mice, where the amount of CSF was only 10 – 15 µL.

The IP protocol was further optimized, and Aβ-peptide analyses, using a set of 40 clinical CSF samples (20 Alzheimer disease (AD) patients and 20 non- AD patients) was performed. From the mass spectra obtained, we calculated the ratio between the integrated peak areas for Aβ-1-40 and Aβ-1-42. It was shown that there was a significant difference for this ratio between the AD patients and the non-AD patients. It is therefore not unlikely, that this method could be utilized as a clinical tool for detecting AD.

Compared to traditional methods, based on single component immunoassays, mass spectrometry has

several advantages. Apart from the fact that data for basically all Aβ-peptides are obtained, possible post translational modifications and/or truncated variants can also be detected. Specific antibodies for performing an immunoassay of such components are normally not available. It is believed, that these components can contribute to a better understanding of the cause for onset of AD.

It should be pointed out that it would be feasible to perform the IP process as well as the deposition of the analyte concentrate on the MALDI targets in parallel mode, by using a robotic work station. In comparison with the time-consuming traditional ELISA methods, the actual MALDI-MS analysis only takes a few seconds, and such a setup would be both time-saving and cost-effective, when dealing with a large number of samples.

Task 3.5 - Signal amplifications with nanoparticles

Gold nanoparticles (AuNPs) have been successfully used as signal enhancers in both electrochemical and optical biosensing systems. They have been proposed as blocking agents in nanochannels-based electrochemical approaches for the sensitive (at ng/mL levels) detection of protein biomarkers (CA15-3 breast cancer biomarker and thrombin) in whole human blood (Figure 1, left). On the other hand, AuNPs properties have been approached for their use as carrier of enzymes for the amplified optical detection (at ng/mL levels) of model proteins (human IgG) in lateral-flow immunoassays (Figure 10, right).

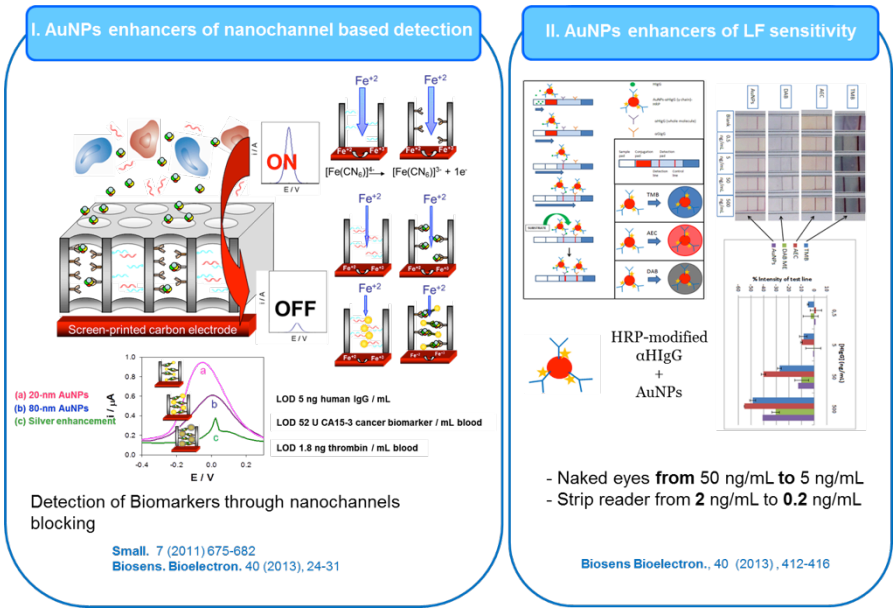


***Figure 10**. Summary of the applications of AuNPs for signal amplification in nanochannels (left) and lateral-flow (right) based biosensing systems.*

In parallel, cadmium-selenide/zinc-sulfide (CdSe@ZnS) quantum dots (QDs) have proved to be highly effective reporters in fluorescent microarrays. QDs are five times more sensitive than the Alexa dye in microarray format and seven times more than the corresponding ELISA for ApoE biomarker screening (at pg/mL levels). Real (clinical) samples (serum and plasma) have also been assayed (in collaboration with UULM) with acceptable accuracy and precision (Figure 11).

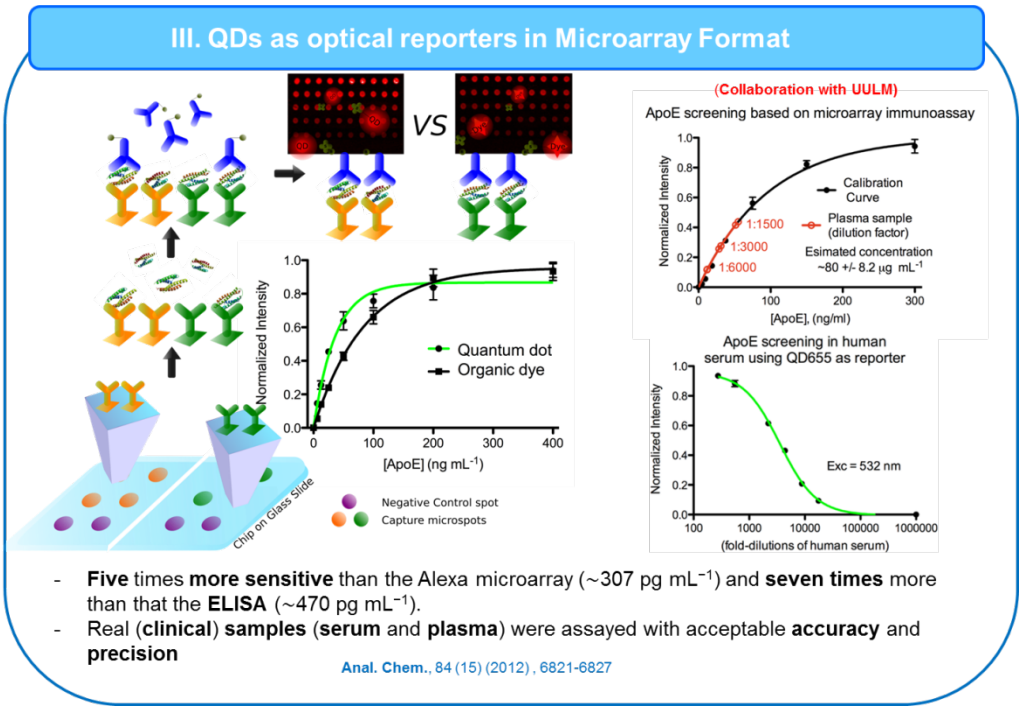


***Figure 11**. Summary of the use of QDs for signal amplification in microarray format.*

Task 3.6 - Chemically compartmentalized detection

The objective of Task 3.6 was to seek for ultimate sensitivity in protein microarray on chips. This was intended as a contingency strategy, if the sensitivity obtained in the other tasks of WP3 was found not sufficient (at the price of a reasonable increase in complexity, development and fabrication cost).

Different strategies were first considered, but at we finally focused on droplet based microfluidic technology. This new platform aimed at bringing to the clinical world the advantages of droplet microfluidics relevant to the specific challenges of diagnosis, i.e., reliability, robustness to contamination, high multiplexing potential, user-friendliness and cost reduction, while maximizing the compatibility and interoperability with currently validated and used protocols and workflows. The front-end of this platform is a pipetting robot, which allows the direct sampling of samples and reagents from standard microtiter plates (MTP), for full compatibility with current sample storage strategies and equipment. As a main difference as compared to conventional droplet approaches, all droplets are kept equally spaced by oil in a highly confined state (a format also called “plug microfluidics” in literature). This way, inter-droplet contamination risk is reduced by orders of magnitude, and the “identity” (originating well) of each droplet, built in the sequence of droplets all along the protocol, is known without need for internal tags. These differences alleviate several current limitations of droplet microfluidics. The second original feature of the platform is the “magnetic tweezer” concept (Fig. 12). This allows the transfer of functionalized magnetic particles between subsequent droplets, bringing into the droplet microfluidics world the power of solid-state extraction and the flexibility of magnetic beads-based protocols. In particular, we demonstrated the potential of this technology for protein detection (ELISA for TSH analysis) and in the frame of WP8 its ability to be used for nucleic acid analysis (HER2 overexpression).

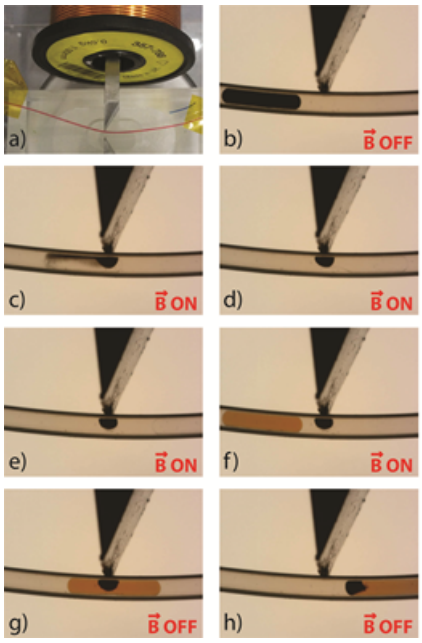


Figure 12: *Magnetic tweezers technology a) Picture of the magnetic tweezers with the capillary (highlighted with a red liquid). A second passive magnetic tip, placed opposite to the first with regards to the capillary contributes to field lines shaping, and to the optimization of the magnetic force. b-h) Sequence of images showing the extraction and the redispersion of magnetic beads from one droplet to another one (colored in orange), switching ON and OFF the electric current in the coil.*

Task 3.7 - Digital ELISA towards Abeta and neurofilament biomarkers

A new digital platform for immunoassays like ELISA was developed and applied for Abeta detection. The platform is based on single molecule counting where a sample is automatically divided into millions of femtoliter droplets ordered in an array on a surface. If target molecules are present in a sample, they will be trapped in the droplet and elicit a signal. Quantification is obtained by simply counting the positive droplets. The technology was tested by detecting pure ApoE3 and the results showed that the detection limit was in the upper aM range. This was several orders of magnitude more sensitive than the corresponding ELISA, and enables blood based diagnostics when biomarkers exist.

The platform was used to detect Abeta 1-42. The detection limit was in the fM range and thereby poorer than the corresponding ApoE3 assay. The explanation was probably a relatively poor antibody pair to detect Abeta1-42. However, fM detection limit was sufficient to analyze CFS samples and more detailed results will be reported in WP 7. It was not possible to find suitable antibodies for neurofilaments.

In addition, T3.7 involved prototyping an instrument. The instrument was designed and built and can process semi automatically 16 samples in parallel and detect the positive droplets. The instrument demonstrates that the technology can be packaged into a smaller instrument, less complex than separate large peristaltic pumps and extensive microscopes.

Task 4.1 - Sample pre-treatment

The initial deliverable was the development of an automated system able to get plasma from total blood before analysis. The specifications of the module have been defined and a first design based on tangential filtration was proposed, see Figure 13.

During the first period (M1-M18), the need for the deliverable has changed. Partners have stated that it was more relevant to deal with serum sample rather than raw blood. Indeed, it is easier to manipulate serum than blood: no need for specific agreement, large banks available. Consequently, the pre-treatment module was not necessary anymore.
After the decision of analyzing serum sample, work was done towards integration and automation of the concentration module developed by partner Institut Curie (task 4.2).

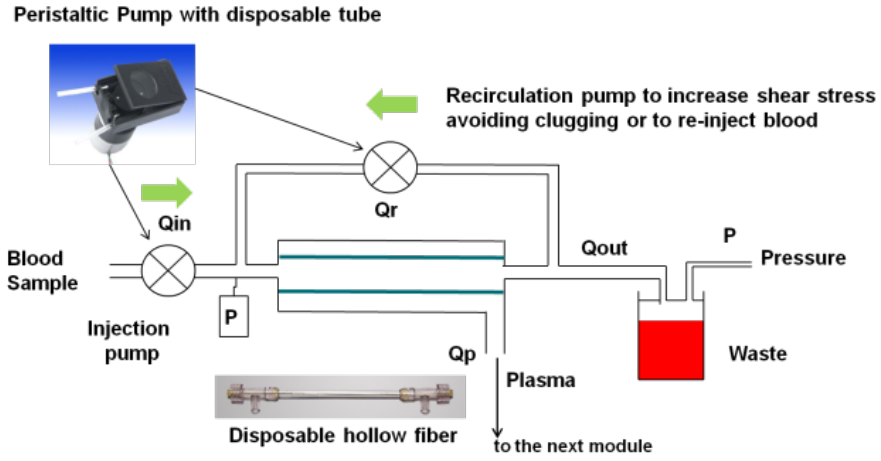


Figure 13: *Principle and software of the flow-rate control module.*

Task 4.2 - Dynamic magnetic immunocapturing

Task 4-2 is aimed at capturing low-abundance peptides from the plasma using micro- or nanoparticles (issued from Task 1.1) and releasing them as a concentrated plug, for downstream analysis by CE on chip (Task 4.3). This preconcentration is mandatory for reaching pM sensitivity, even in combination with online labelling (Task 4.4).

In this task, we developed a completely new microfluidic technology relying on the concept of fluidized bed. Fluidized beds are an appealing strategy to enhance the kinetics of liquid-solid processes in microfluidics, but until recently their implementation was hindered by the weakness of gravitational forces at the microscale. In the context of the NaDiNe project, we introduced magnetically-driven fluidized beds. The design of the microfluidic device was optimized in order to compensate for the decaying intensity of the magnetic field (Fig. 14). A particle image velocimetry (PIV) analysis has shown that beads undergo a continuous recirculation. Indeed, a higher velocity at the centre of the bed is observed, followed by a perpendicular circulation to the borders of the chamber when arriving to the end of the bed, and a backflow near the borders. Playing with the flow rate applied using partner FG's fluidic control equipment, we have been able to control finely the bed porosity. The microfluidic fluidized bed presents specific features regarding

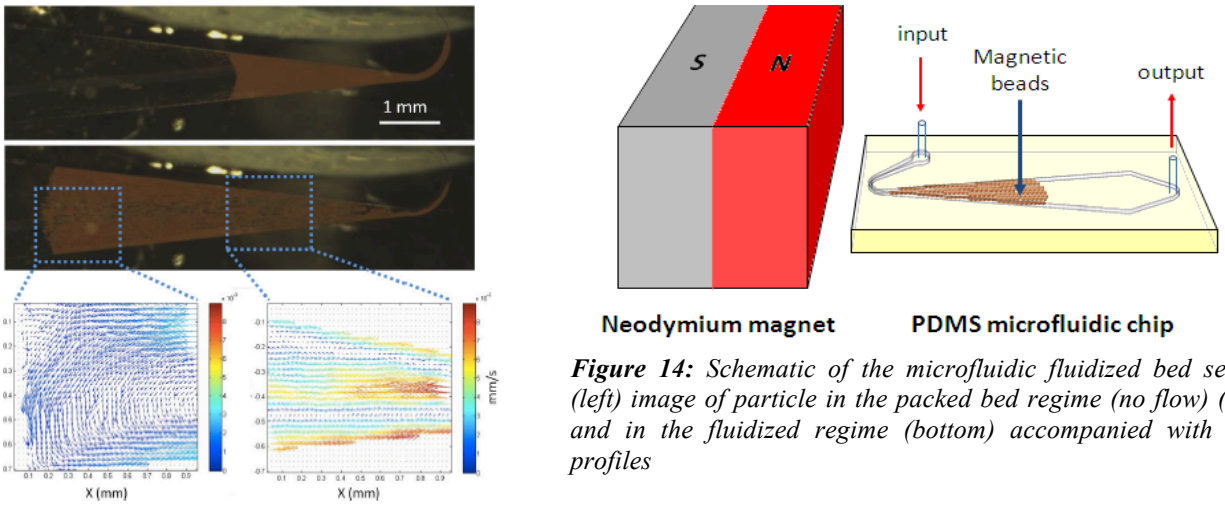


Figure 14: *Schematic of the microfluidic fluidized bed set-up (left) image of particle in the packed bed regime (no flow) (top) and in the fluidized regime (bottom) accompanied with PIV profiles*

biomarkers extraction and preconcentration: a low backflow pressure allowing to sample a large volume, and a continuous recirculation while maintaining a high beads density.

This new technology was successfully applied to Ab extraction. We demonstrated that a high preconcentration factor could be achieved with commercial peptides ranging from 700 to 1000 with high

selectivity. Besides the potential of this technology for other applications has also been considered especially regarding bacteria extraction and detection.

Task 4.3 - On-line fluorescent labelling

An on line-derivatization method to analyse ubiquitin in CSF has been developed. The method allows ubiquitin to be fluorescently labeled (without the need of antibodies), during the capillary electrophoresis separation process. The linearity of the CE assay was demonstrated in the range of physiological concentrations expected in CSF, as well as the reproducibility of the method. The method has a limit of detection around 15 nM, which is compatible with its detection in CSF with a preconcentration step, which is performed to desalt and preconcentrate three times the sample. The electrophoretically mediated microanalysis method could be applied to the detection and quantification of ubiquitin in CSF samples (Fig. 15).

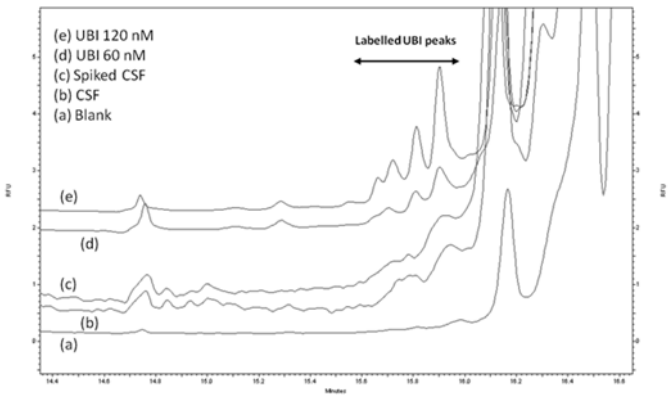


Figure 15: *Quantification of UBI in desalted CSF using electrophoretically mediated microanalysis. Samples: Derivatization blank (a), CSF (b), CSF spiked with 16 nM of UBI (c), standard solution of UBI at 60 nM (d), at 120 nM (e). Before injection, samples are diluted in DMSO (2/1 v/v).*

An innovative isotachophoresis preconcentration method compatible with alkaline conditions has been developed for the first time. This strategy allows to achieve the detection of Aβ 1-40 at a concentration of 30nM without derivatization, which is an unprecedented sensitivity for UV detection.

We developed a new methodology called “multiple ITP”, to preconcentrate abeta peptides from CSF. Starting from standard solution, we can reach the limit of quantification (LOQ) of 50 nM with normal UV detection (at 200 nm wavelength), which is much improved compared to the LOQ obtained with CE-UV without electrokinetic preconcentration (2000 nM).

We also demonstrated that immunocapture on magnetic beads together with derivatization of captured peptides may be performed in one step and lead to both efficient capture and high sensitivity of detection. We have successfully detected A-beta peptides, at 1-38 (1-2 nM), 1-40 (6-8 nM) and 1-42 in CSF (0.5 nM) after this pre-treatment using capillary electrophoresis coupled to LIF detection for the off-line analysis of the eluted fractions. We obtained an enrichment factor of 100.

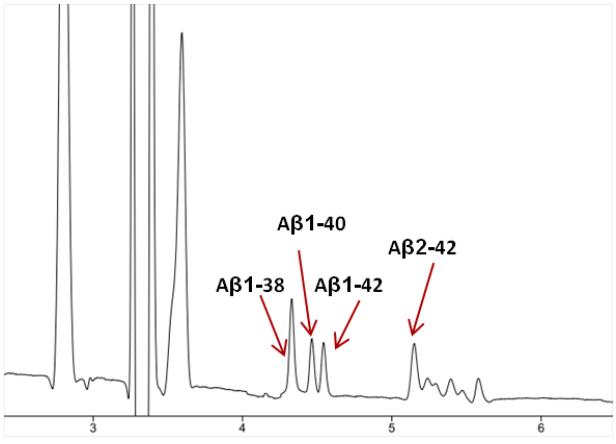


Figure 16: *Electropherogram corresponding to electrokinetic separation of a mixture of Aβ 1-38, Aβ 1-40, Aβ 1-42, Aβ 2-42, on EpDMA coated glass microchip. The peptides concentration is around 40 µM in the mixtures. LOD was estimated to 1-10µM.*

Task 4.4 - High resolution electrophoretic separation on chip

A highly resolving electrophoretic method for the separation of different *Aβ*-amyloid peptides on coated glass microchips has been developed by combining polymeric surface treatment (input from partner ICRM) and their (off-line) derivatization by Fluoprobes 488. The method led to good resolution between close migrating species with very satisfactory RSD for EOF, migration times and peak areas. This high resolutive electrophoretic method has been applied to the separation of a mixture of Aβ 1-38, Aβ 1-40, Aβ 1-42, Aβ 2-42 on EpDMA coated glass microchip (Figure 16).

Alternatively, thiolene chips have been designed and microfabricated by partner DTU. We performed a deep investigation on the potential of thiolene microchips (with partner DTU) for the electrokinetic separation of proteins, measuring the EOF, searching for a new coating (with partners ICRM and DTU). We have now found a stable coating over a wide pH range, an efficient coating procedure, demonstrated the efficient hydrophilisation of the channel surface as well as the high ability of this coating to suppress protein adsorption. We have applied this system successfully to the analysis of acidic and alkaline proteins (see Fig. 17).

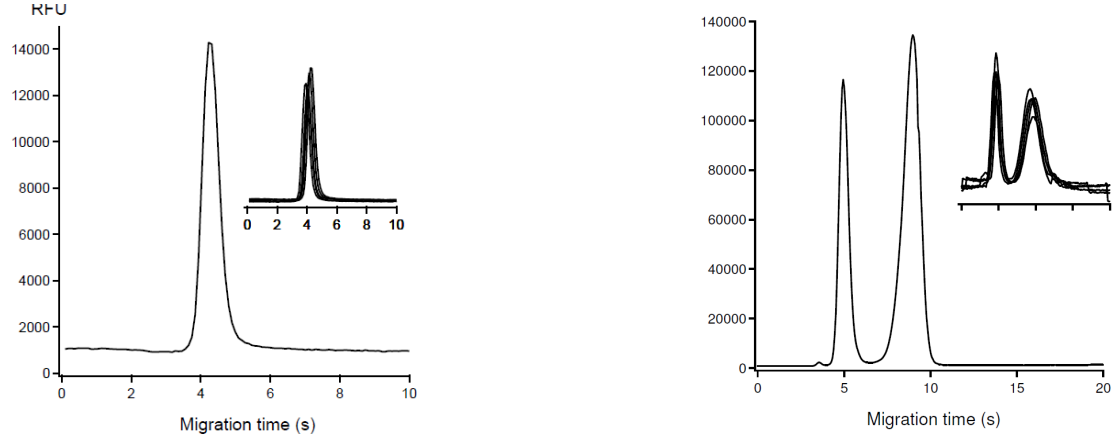


Figure 17: *Electropherograms obtained from the separation of two acidic fluorescently labeled proteins (left) and of Histone-Alexa 488, an alkaline fluorescently labeled protein (right) on thiolene chip (20 % excess of thiols, coated with the terpolymer DMA-PMA-MAPS). The insert in the figures displays electropherograms from five repetitive analyses*

Task 4.5 - Spotted proteins and peptide microarrays

Antibody microrarrays for high-sensitivity immunoassays were developed. The microrarray platform consists in polymer coated silicon slides. Due to constructive interference, Si/SiO_2 layered slides allow enhancement of the fluorescence intensity on the surface with significant improvements in sensitivity of detection.

Silicon oxide surfaces coated by copoly(DMA-NAS-MAPS) have been extensively characterized by atomic force microscopy (AFM) and dual polarization interferometry (DPI) showing a uniform and smooth polymeric layer with a thickness of 1.89 nm in dry conditions swelling up to 15.7 nm in buffer. Model experiments on the detection of 5 inflammation markers (Interleukin 6 and 10, PTX3, CRP and TNF-alpha) were used to optimize spotting and incubation protocols and demonstrated the feasibility of fluorescence microarrays on copoly(DMA-NAS-MAPS) coated silicon oxide for the detection of a panel of biomarkers with femtomolar sensitivity.

Highly sensitive fluorescence immunoassays for the detection of neurodegenerative diseases biomarkers amyloid-beta 1-42 and 1-40 (Aβ42), (Aβ40), and apolipoprotein E (ApoE) were realized based on a label/label-free microarray platform that utilises silicon/silicon oxide (Si/SiO_2) substrates. Limits of detection in the order of pg/mL were achieved.

Integration of the developed microarrays with microfluidics (WP6) allowed a reduction in the assay time and semi-automation of the assay. A validation of the proposed method using human CSF samples was performed within WP7.

Task 4.6 - Microfluidic convective self-assembly for microarrays

This task was planned by partner CI as an alternative approach, in which the different biocapture elements should be prepared in a high throughput fashion in a batch process by stop-flow lithography, and then self-assembled microfluidically in the microfluidic chip.

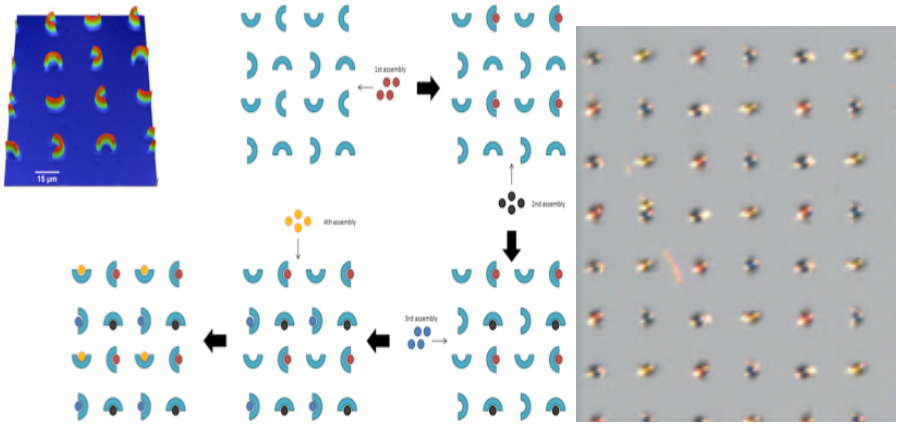


Figure 18: *Successive capillary assembly steps in selective traps from schematics to experiments*

The objective we have defined is to develop an alternative approach to overcome the limitations of conventional antibody spotting approaches used to elaborate protein microarray. We have mainly focused our work on the self-assembly by fluidic means of commercial particles or photo-polymerized hydrogel particles for the creation of high-density protein arrays directly integrated in the detection chamber of microfluidic devices. This work is based on a technology pioneered by L. Malaquin that allows a high multiplexing capability of beads self-assembly with high accuracy (Fig. 18). Two approaches have been successfully investigated and applied in the NaDiNe project namely convective and capillary self-assembly that allows either to create 2D dense layers of particles or to induce a deterministic positioning of beads of different nature. We have demonstrated the ability to assemble protein labelled beads with various sizes (ranging from 500 nm to 5 µm) while maintaining the ability of antibodies grafted on the beads surface to bind their targeting antigens. This was demonstrated by capturing cells by antibody-antigen interaction.

Finally, by optimizing the device microstructures as well as the wetting properties of the substrate, we have successfully demonstrated the multiplexing capabilities of the methods: dense arrays composed of four different types of functionalized beads were precisely assembled on PDMS substrate with a placement accuracy <2µm and an assembly yield >95%.

Task 4.7 - Hyphenation with mass spectrometry

We have developed a system for preparative fraction collection from a CE capillary for subsequent matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Initially, a direct coupling of CE to a mass spectrometer via an electrospray interface (ESI) was tested, but there is too much incompatibility between the crucial criteria for obtaining a good ESI and the optimal operating parameters for CE of our target molecules (Tau-fragments). An additional advantage of MALDI-MS is that it offers a much higher sensitivity than ESI-MS. A schematic of the developed preparative system is shown in the figure 19.

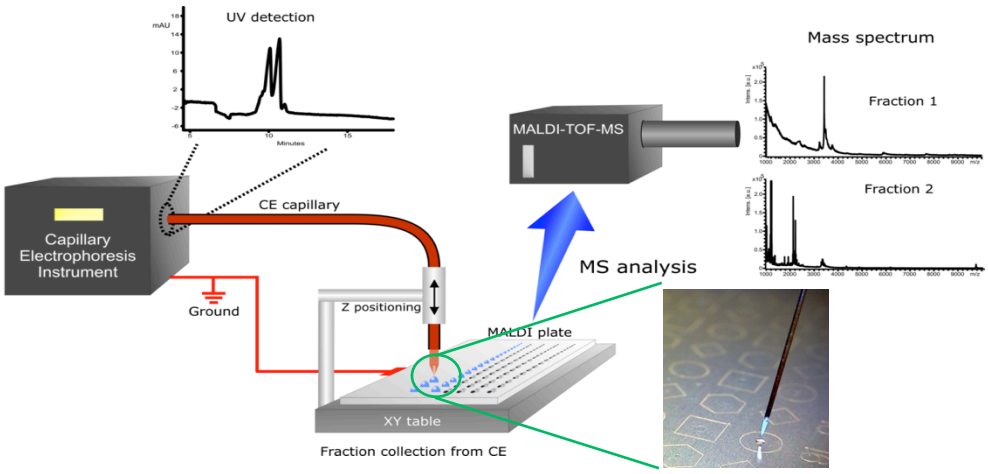


Figure 19: *Schematic of the preparative systems for sampling from a CE capillary to a MALDI target*

It includes an in-line UV detector, to monitor the content of the effluent flow from the CE-capillary, and a XYZ robotics system, which deposits the effluent flow onto an array of MALDI targets (such as silicon pillars).

A successful MALDI analysis of CNBr fragments of the important Alzheimer's disease biomarker Tau-441 was accomplished. All of the expected (6) fragments were detected. With the new preparative separation system, 5 of the 6 expected fragments could be detected.

Task 5.1 - Biomarker panel elaboration

Potentially interesting biomarker candidates have been evaluated. In addition to the biomarker candidates S-100B, GFAP, ERK1/2, progranulin, ubiquitin, which had been selected already within the initial phases of the project, partners UULM and UKES have started efforts towards the study of charge-isoforms of Serpin and DJ-1 in CSF samples by novel capillary isoelectric focusing immunoassays. These are currently being established as novel applications of the NanoProTM technology (ProteinSimple). Additionally, the application of this technology for the detection of oligoclonal IgG bands in CSF has been successfully tested.

To further address the potential prognostic and diagnostic value of measuring total ERK1/2 in CSF in early stages of Alzheimer's disease and mild cognitive impairment, partners UKES and UEF have selected a suitable clinical cohort. Pilot experiments have been done successfully so that the clinical study will be launched soon. Partner MB has produced polyclonal and monoclonal antibodies against different phosphorylated and unphosphorylated forms of ERK1/2, which were evaluated by partner UKES by Western blot and capillary isoelectric focusing immunoassay. While reasonably good antibodies were identified against diphosphorylated ERK1/2 (serum 78), the generation of antibodies against monophosphorylated ERK epitopes was apparently not successful. Partner UKES established a capillary isoelectric focusing immunoassay for the measurement of amino-terminal variants of Aβ peptides.

Task 5.2 - Antibody production

We have developed and characterized a number of polyclonal and monoclonal antibody reagents recognizing Aβ-peptides (DAEFRHDSGYE; AEFRHDSGYEVHH; EVHHQKLVFFAED; KLVFFAEDVGSNK) generated from Aβ 1-42 peptide. 19 hybridoma clones (AEF1.1-AEF19.1) originating from the extreme N-terminal part of Aβ 1-42 peptide recognize both Aβ 1-42 and Aβ 2-42 isoforms in different approaches including ELISA. We also finalised the development of five new monoclonal antibodies to the central part of Aβ 1-42 peptide. Three of these monoclonal antibodies recognize the sequence KLVFFAEDVGS within the full length Aβ 1-42 peptide. These antibodies are therefore useful for combination with N-terminal Aβ 1-42 peptide specific antibodies AEF to improve sensitivity and specificity in different applications, including two-site ELISA. The

requirement to develop antibodies that will specifically recognize only Aβ 2-42 was achieved using affinity purified polyclonal serum 77 that binds strongly to Aβ 2-42 peptide with very low cross-reactivity with Aβ 1-42 peptide. To remove this low level cross-reaction and improve sensitivity and specificity, we developed a new method for affinity purification of rabbit sera to isolate only antibodies recognizing Aβ 2-42 peptide and not Aβ 1-42 peptide (one amino acid difference). We also developed and affinity purified rabbit serum 76 that specifically recognizes Aβ 1-42 peptide and does not recognize Aβ 2-42. Additionally, we developed a new methodology to prepare Fab fragments of IgG from monoclonal antibodies AEF-4.1, AEF14.1 and AEF-16.1. These fragments were also conjugated with biotin in order to immobilize them on paramagnetic microparticles.

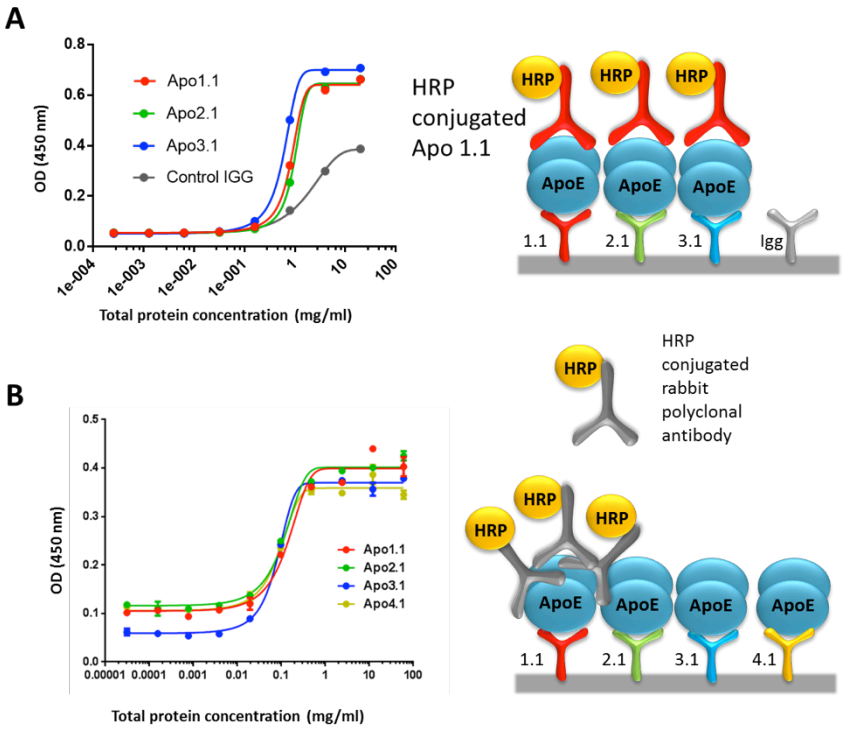


Figure 20: *Development of ELISA test for detection of ApoE in human sera. (A) Sandwich ELISA was coated using different mouse monoclonal antibodies and detected by HRP conjugated mouse monoclonal antibody Apo1.1. (B) Sandwich ELISA using combination of mouse monoclonal antibodies and HRP conjugated rabbit polyclonal antibody.*

We also concentrated on antibody development for the human ApoE3 protein. We have developed five high quality monoclonal antibodies (Apo1.1 - Apo5.1) and one rabbit polyclonal sera number 33 that recognize both the native and denatured forms of ApoE3, either in purified form or in the endogeneous form in serum. We developed the two site ELISA method using our new set of antibodies, which is able to recognize ApoE3 protein in human serum, supporting their use for NaDiNe partners for development of original two-site ELISA methodology for detection of ApoE3 in serum or other body fluids (**Figure 20**).

Within the project we additionally developed ubiquitin specific antibodies (UBI3.1, UBI4.1, UBI5.1) recognizing mono-ubiquitin and one affinity purified rabbit polyclonal serum (number 37) specific to mono and poly-ubiquitin and we also developed and characterised new monoclonal antibodies to poly-ubiquitin (peptide CTLEVEPSDTIENVK), three of which also recognize full length poly-ubiquitin.

In summary, we have developed several monoclonal antibodies recognizing different regions of Aβ peptide, ApoE3 and ubiquitin, and sets of polyclonal antibodies recognizing newly discovered isoforms of Aβ peptide, including a method of affinity purification of these isoforms. We have also developed a method for preparation of Fab fragments of these antibodies and their immobilization on solid surfaces such as magnetic microparticles. These Fab fragments will be useful for downstream applications involving either diagnostic kits or analytical techniques based on MALDI-TOF.

Full details of antibody production and the testing of these reagents using a variety of immunochemical methods are available on the Moravian Biotechnology website (http://www.moravian-biotech.com/sql). A presentation of the results is also available on www.moravian-biotech.com.

Task 5.3 - Optimization with model samples and clinical samples

The objective of this task has been to prepare a "catalogue" of potential technologies or combination of technologies for prototyping. The technologies will be optimised and compared by tests with model samples and clinical samples.

During the first 30 months, the NaDiNe partners have been developing technologies based on different approaches (electrokinetic separation, electrochemical detection, microarray, fluidized bed, mass spec…). To achieve a sufficient maturity of the technologies developed in the framework of the NaDiNe project, the decision on the candidate technologies has been slightly delayed. A specific meeting focused on this topic has been held in Paris in January 2013. The objective of this meeting was to select the different technologies for further prototyping. A methodology combined to a set of criteria has been established to compare these different approaches in the most objective way possible. The technologies comparison has been performed based on a set of two biomarkers. The analysis has been, in a first approach, restricted to ApoE and Ab peptides, in either CSF or serum/plasma. If a technology can provide distinction of isoforms of A-beta, then ratios of certain isoforms should be considered for improved sub-typing. As expected, serum and CSF samples have been provided by partners UULM, UKES and UEF. Based on the results obtained during this comparative study, different technologies have proven their potential mainly for CSF sample analysis and have been identified as candidate technologies for prototyping. Besides the technologies developed by the NaDiNe partners, partner UULM has been working on a commercial apparatus (NanoPro system) that will be considered as a commercial benchmark. During the Paris meeting, the different technologies have been compared and different approaches have been selected (Figure 21)

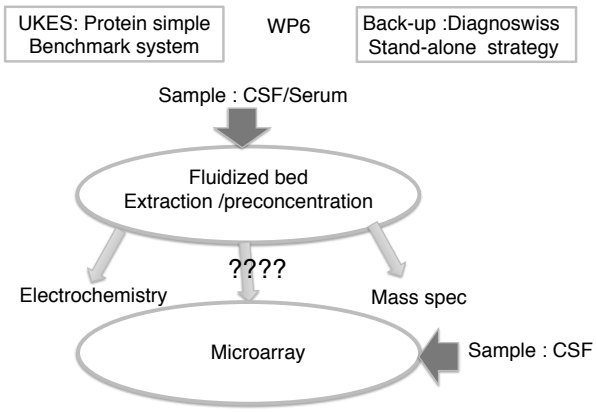


Figure 21: *Potential workflows and suitable technologies.*

Task 5.4 - Data analysis strategies and bioinformatics

To prepare a bioinformatics strategy to build a multi-marker diagnostics platform, we performed a systematic study and benchmark of several statistical and machine learning methods for data-driven feature selection, the goal being to extract a robust and accurate prognostic signature from a limited number of samples. We identified the best strategies and reported the results in the following publication:
A.-C. Haury, P. Gestraud and J.-P. Vert, "The influence of feature selection methods on accuracy, stability and interpretability of molecular signatures", PLoS ONE, 6(12):e28210, 2011.To help in the selection of biomarkers, we re-analysed the seminal work of Ray et al. (Nature Medicine, 2(9):e4141, 2007) who identified a multi-markers signature of 18 proteins for AD diagnosis, and the later re-analysis of Ravetti et al. (PLoS One, 3(9):e3111, 2008) who shortened the signature to 5 proteins. We found flaws in the second analysis, the results of which should therefore not be trusted for our own selection of markers.

Task 6.1 - Instrument specification and design

A first step here was to determine which techniques would be selected to be integrated in the instrument prototype (based on input from T5.3) in order to draw the instrument specifications and design.

The planned functional diagram for the instrument is shown on Fig. 22.

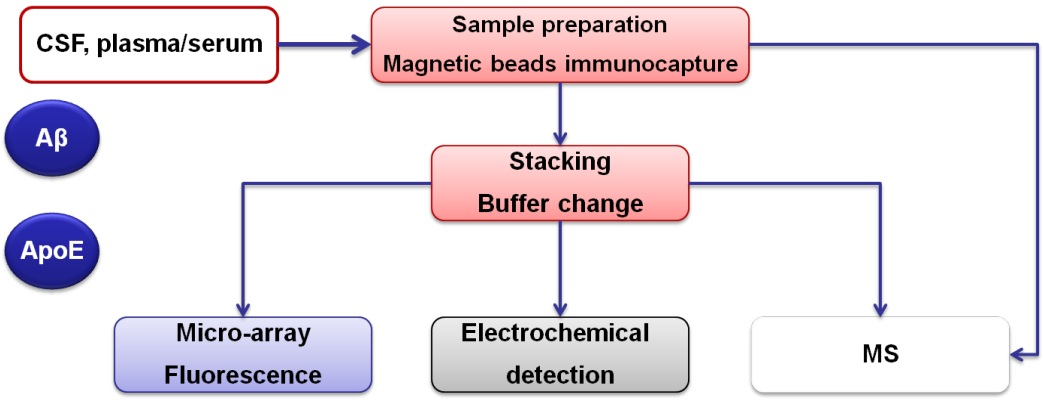


Figure 22: *First version of the instrument functional diagram*

The work has then mainly been focused on the automation of the fluidized bed and the "fluidic" microarray approach, and on the achievement of integrated platforms.

Task 6.2 - Prototype instrument development

The work on the development of the prototype instrument was divided into 2 parts: the electronic integration and the fluidic integration. This development was motivated by different aspects: ease of use, volume, robustness, compactness and integration.

In line with the needs of the Nadine project, a compact module has been developed allowing the fluid handling of the fluidized bed and the fluidic microarray. In Figure 23, we see the electronic board integrating all the different parts needed. Further, in Figure 24, we see the fluidic platform developed for the fluidized bed and the microarray. The fluidic platform integrates flow sensor, valves, and reservoirs.

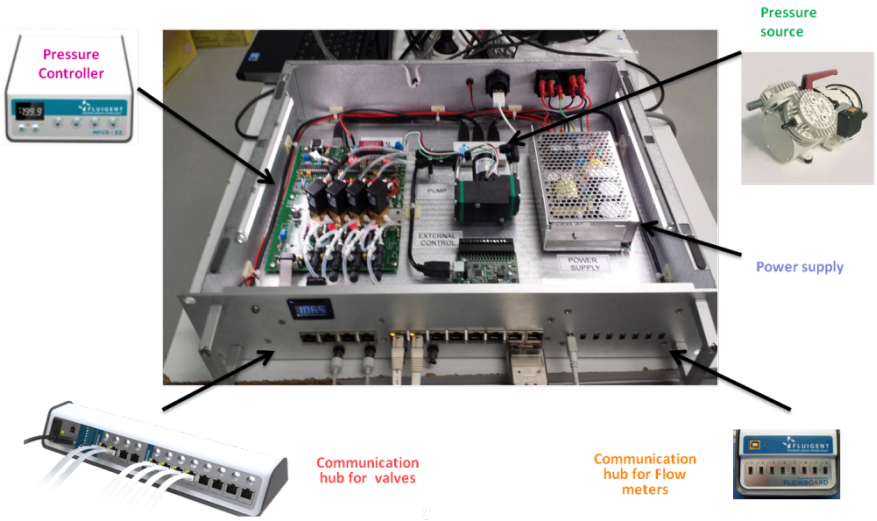


Figure 23: *Electronic platform.*

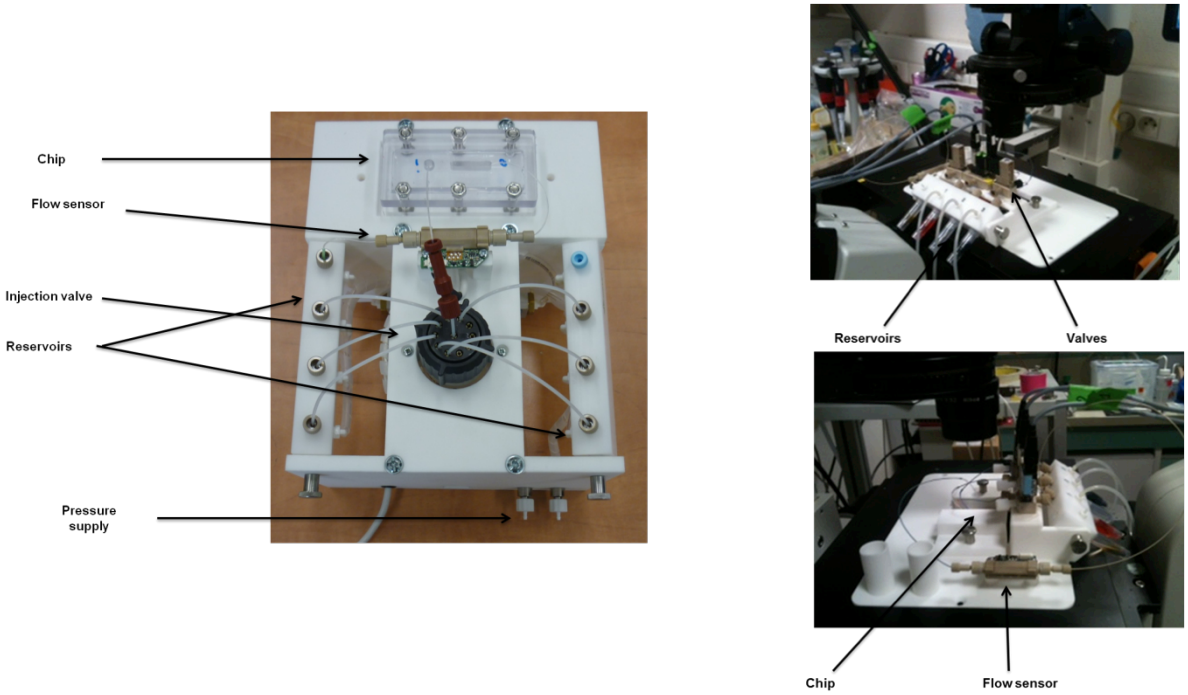


Figure 24: *Fluidic platform for the sample preparation (left) and for the fluidized bed (right)*

Task 6.3 - Software for instrument control

At M36, prototypes of the different selected modules were developed to progress towards the prototype instrument. The software developed in this task enables the control of all the flow-control tools developed in other WPs and a lot of effort was put into the programming of a software architecture based on a modular and powerful approach. This version is adaptive depending on the connected devices: the user can choose which elements he wants to display (Figure 25). This approach makes all the more sense to match precisely the different needs of the integrated modules.

Additionally, a scripting tool to automate the two selected technologies and a SDK (Software Development Kit) to allow the integration in global software has been developed.

Task 6.4 - Software for analysis of results

A prototype of software to learn a predictive multi-marker signature from the device measurements has been implemented in the R language. This prototype, based on preliminary development performed in Task 5.4, systematically compares different methods for feature selection (univariate tests, lasso regression, random forests) and predictive modelling (logistic and linear regression, support vector machines, random forests) and selects the best one, as evaluated by cross-validation, to form the final signature. In addition to automatically learning a predictive signature, the software prototype comes with various visualization options to control the distribution of measurements over a cohort of samples. It also implements functions to quickly visualize the performance of different methods to train a predictive signature.

However, due to unavailability of a fully functional demonstrator tested on a collection of sample, this prototype software has not been implemented yet to directly process the outputs of the demonstrators, which will require additional functionalities to process and normalize the measurements before running the prototype to learn a signature.

Task 6.5 - Optimisation of prototype with model samples

The task involves some optimizations on the prototypes developed earlier in the project. From the realisation of the first prototype until the end of the project some optimisations and improvements have been achieved for the fluidized bed: selection of a suitable immunosorbent, Ab grafting method, selection of

microchip material (COC), surface treatment, flow-rate, reduction of the manual steps. Similarly, the microfluidic microarray were optimized on a number of parameters: microfluidics layout, the chip clamping method, the flow-rate platform, the surface treatment, the software. The results show that the optimisations achieved on the prototypes allow a good efficiency compared with the static method, are time saving, and are amenable towards automation.

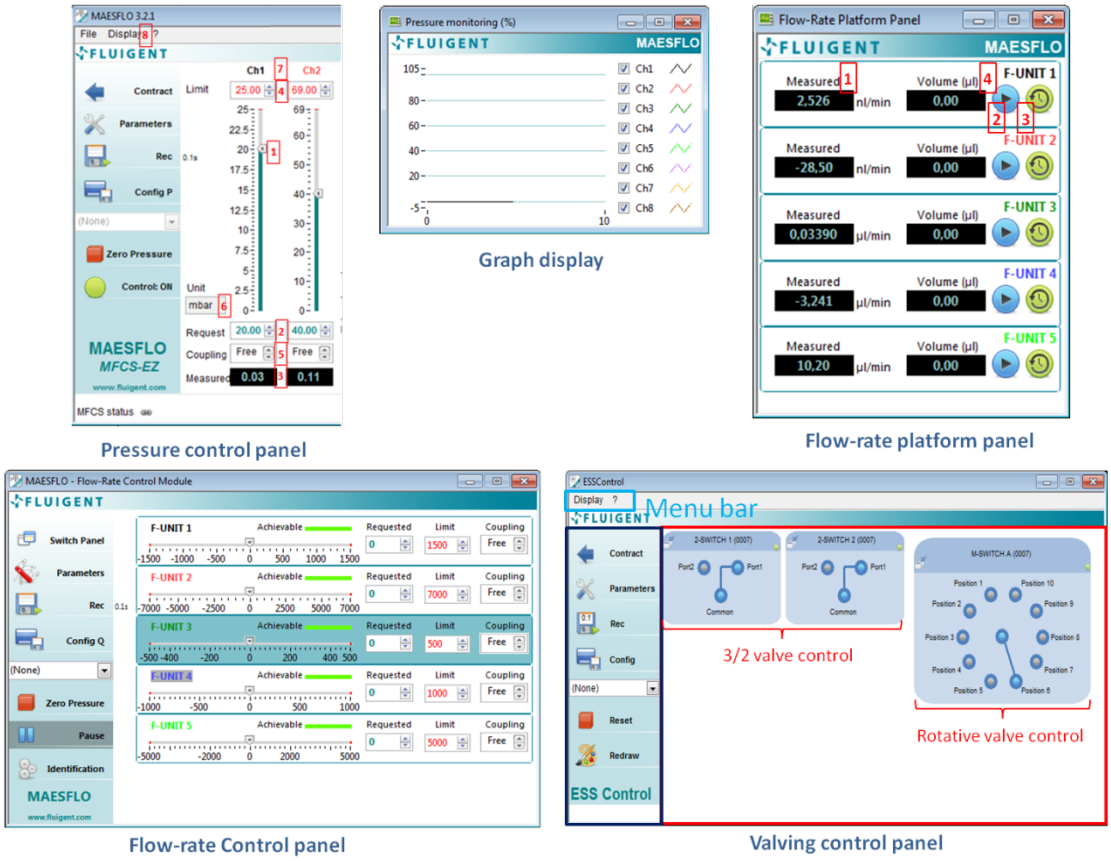


Figure 25: *Software panels*

For the parallel approach on the equipment from partner DS, different assay protocols were successfully developed for ApoE and Ab1-42 for the different matrices. The Apo E assay takes less than 1.5 h and requires low volumes (<1ml) of samples since CSF must be diluted by a factor of 300, and plasma and serum by a factor of 3'000 for the assay. The Ab1-42 assay takes about 2h and requires 10ml of CSF maximum to be tested. It was shown to be difficult to measure Ab1-42 in plasma with classical dilution approach. A specific standard-addition method has therefore been developed but required larger amount (~100ml) of samples compare to the classical dilution method. Both assays showed to give comparable results as compared with commercial ELISA but with shorter time to result (2h instead of 4h in general) and with lower reagents and sample consumption (~1/10th).

Task 6.6 - Risk assessment and regulatory issues

Any medical device put into the market needs CE marking and approval, where a full risk assessment will have to follow EN14971, which covers risks through the entire life-cycle of the device. A full risk assessment according to EN14791 has not possible as even the integration of the various steps into an integrated device is still work to come. Hence, an analysis has been carried out at the final stage of the prototypes. The study concerned risk assessment in relation to human safety when handling the LAB prototype. Moreover, the study has provided inputs to a potential future integrated instrument. The analysis has covered all four envisioned prototypes: "Fluidized Bed", "Automated microfluidic Elisa with electrochemical detection", "Micro-array for fluorescence detection module" and "Lab prototype microarray". For each prototype, all steps in the process as relevant for the LAB prototype have been described, and the specific process has

been briefly described for each step, and the potential hazards has been identified and listed. Hazard causes have been included where relevant, and barriers reducing the frequency and/or the consequences of the hazard have been listed. The assessment concluded that for all the four prototypes hazards and relevant barriers have been identified, and at the present stage all of the prototypes have an acceptable safety level. Furthermore, there were no indications that the prototypes cannot be taken to commercials products that could not be approved (in accordance with EN14971).

Task 7.1 - Clinical characterization of patients and biobanking

The three clinical partners UEF, UULM and UKES have continued to assemble the patient collective as defined in the NADINE WP7 goals and milestones. Altogether the three clinical partners have collected a sufficient number of CSF and EDTA-plasma samples for each diagnostic subgroup and a control cohort. The total number of available samples is > 1500. CSF and plasma samples can be provided to all partners on request to support the technology development within other NaDiNe work packages.

Task 7.2 - Pre-clinical testing on model samples

The objective of Task 7.2 was to perform a first validation of the project's prototypes, on samples containing well defined concentrations of analytes, and thus to provide an assessment on a technical performance, rather than clinical, basis. The intention was to provide quantitative references for clinical testing.

This task has been performed with two prototypes technologies:

- The automated version of the microfluidic fluidized bed has been applied to immunoextraction with commercial Ab peptides. The peptides have been spiked at different concentrations in PBS and high preconcentration factors have been achieved (around 1000 depending on eluting buffers).

- The prototype of integrated microarray has been applied to model samples of ApoE and Ab peptides analyses. The efficiency of the prototype has been proven with ApoE (for concentrations between 10 ng/mL and 200 ng/mL) and with Ab-42. The results show that the optimisations achieved on the prototype allows a good efficiency compared with the static method, are time saving, and support automation approaches.

Task 7.3 - Clinical testing on samples from humans (Task 7.4 and 7.5)

• The three clinical partners UEF, UULM, UKES have collected a large number of CSF and blood plasma samples from well characterized patients with neurodegenerative diseases and suitable control groups with other diseases (disease controls). In total, more than 1500 CSF and blood plasma samples are potentially available for method development, assay validation and biomarker research within or related to the NaDiNe project.
• A reference method (gold standard) for measuring Aβ peptides in CSF has been selected and validated.
• Fluorescence microarray immunoassay (partner ICRM), microfluidic ELISA (partner DS) and droplet array (partner DTU) were demonstrated by the respective partners to be able to detect Aβ42 in human CSF and to differentiate preselected diagnostic groups.
• Anti-Aβ immunoprecipitation followed by mass spectrometry was successfully applied by partner KTH to determine Aβ42/40 ratios in human CSF samples and to differentiate preselected diagnostic groups.

Microfluidic ELISA (DS): The analytical performances of both ApoE and Ab1-42 assays was extensively studied and showed high performances with very low limit of detection in the picomolar range (respectively 0.4 and 3 pM) and reproducibility below 15%. In CSF both assays showed to give linear results with recovery percentages within the 80-120% acceptance range. Ab1-42 was further investigated in a Case/Control and in a blinded study with samples with low and high levels of Ab1-42. The results were highly correlated with commercial ELISA and a clear separation of clinical CSF samples with either high or low levels of Aβ42 was achieved (statistical significance). We conclude that, as a next step, our electrochemical micro ELISA should be applied to a larger clinical sample under routine laboratory conditions.

In conclusion, we have demonstrated that our approach was able to measure with a high reproducibility and repeatability the targeted biomarker in complex human samples. In addition the assay was applied to the dosage of two sample cohorts and demonstrated statistical power to distinguish between non-AD and AD samples. Finally the ImmuSpeed ™ prototype is directly amenable to clinical routine. This is in line with the objective of DiagnoSwiss' contribution in the NaDiNe project.

Microarray based analysis (ICRM): The microarray test was performed using standard Amyloid Beta 1-42 peptide freshly dissolved and diluted in ACSF as described in (Gagni et al. 2013). Calibration curves showed a limit of detection (LOD) of 200pg/mL. However, analysing clinical samples required additional optimization and control of CSF dilution (4-fold dilution was optimal) and storing and shipping conditions of samples. Due to unforeseen difficulties with antibodies to detect A Beta 1-42, only five patients were analysed. The results showed however that the method could separate AD patients from controls.

Droplet array digital analysis (DTU): For the work performed by partner DTU: Patient samples were analyzed on the droplet microarray using 20 hCSF samples shipped from UULM. Briefly, 10-fold diluted CSF samples was flushed over droplet array substrates contains spots of Abeta 1-42 capture antibody. After incubation with a biotinylated detection antibodies and horse reddish peroxidase conjugated streptavidin, droplets with detection substrate was created. Fluorescent imaging and image analysis allowed for counting positive droplets where one spot correspond to one capture Abeta 1-42 peptide. The patient samples could be sorted in two groups based on number of positive spots where healthy individual had a mean spot count of 1623 (SD=570) while AD patients had spot counts of 685 (SD=165). The data suggest that the droplet array technology is a convenient and robust method to diagnose AD patients.

Mass spectrometry based analysis (KTH): This report describes the evaluation of the performance of a platform, comprising immunoprecipitation (IP) and MALDI-Mass spectrometry (MS). This platform was developed in task 3.4, and was utilized to determine the area ratio between Amyloid beta (Aβ) 1-42 and 1-40 peptides in the cerebrospinal fluid (CSF) from Alzheimer and non-Alzheimer disease (AD) patient samples. The IP-MALDI platform included a parallel batch immunoprecipitation (IP)-procedure using magnetic beads, which allowed a simultaneous analyte concentration determination from several samples. The concentrates were deposited on microchip-based MALDI-MS targets by means of a computer-controlled robotic device. The mass spectra obtained provided the possibility for an unambiguous identification of all visible Aβ-peptides. Subsequently, ratios between different Aβ-peptides could be calculated. Moreover, post-translational modifications were also detected.

The developed IP-MALDI-MS platform reached a satisfactory analytical performance regarding precision, with coefficients of variation (CV) below 18%. Measurements, using a set (20 + 20) of CSF patient samples showed that our IP-MALDI-MS approach was able to distinguish between samples from AD and non-AD patients, with a clear statistical significance

Golden standard (UKES/UMG): As a reference method, the commercially available electro-chemiluminescence Aβ-multiplex assay (Mesoscale Discovery Aβ Peptide Panel 1 (6E10) V-Plex was selected as a "gold standard" for comparisons. It allows for the simultaneous immunological measurements of the Aβ peptides Aβ38, Aβ40 and Aβ42 in a 96 well assay. The MSD V-Plex assay was subjected to a partial, "fit for purpose" assay validation program addressing the lower limits of detection, the lower limits of quantification, impact of sample dilution and parallelism, precision and analytical spike recoveries. In general, the assay performed very well in our hands, and it passed defined acceptance criteria regarding intra- and inter-assay reproducibility, parallelism and spike recoveries. The clinical sample under investigation included 62 patients diagnosed as probable dementia due to Alzheimer' disease (AD) and 90 subjects categorized as improbable AD (non-demented disease controls, nDC). We performed Receiver-Operator-Characteristics (ROC) analyses for Aβ42 and the Aβ42/40 ratio according to the MSD-assay and calculated cutpoints (maximum Youden indices) of < 481.6 pg/ml for CSF Aβ42 and < 0.0635 for the Aβ42/40 ratio for the discrimination between the two diagnostic groups. In summary, we consider the MSD V-Plex assay suitable for routine measurements of Aβ38, Aβ40 and Aβ42.

Impact and dissemination

Introduction

NaDiNe has addressed areas of technologic development and diseases with continuously increasing market forecasts. The achievements of the project have an important economic impact in: 1) analytical instrumentation; 2) diagnostics and monitoring of dementia patients. The achievement in the NaDiNe project is summarized in Fig. 1 below. The graph shows an intricate network of output in relation to R&D and wider societal impact. Overall the NaDiNe project has mostly worked in the R&D field with small activities in commercialization and end user product development. A number of new techniques developed under NaDiNe are sensitive enough to detect very low expressed biomarkers in blood such as those expected for neurodegenerative disease. Unfortunately, we did not find suitable biomarkers for diagnosing Alzheimer's disease (AD) in blood. However, neurofilament measured in blood provided diagnostics for amyotrophic lateral sclerosis (ALS). The NaDiNe project has achieved a large knowledge base for various kinds of diagnostics and future advanced diagnostics devices. These techniques can be used for ultrasensitive detection of, e.g., cancer and infections in humans and animals. The technologies are also applicable to environmental control such as analysis of drinking water.

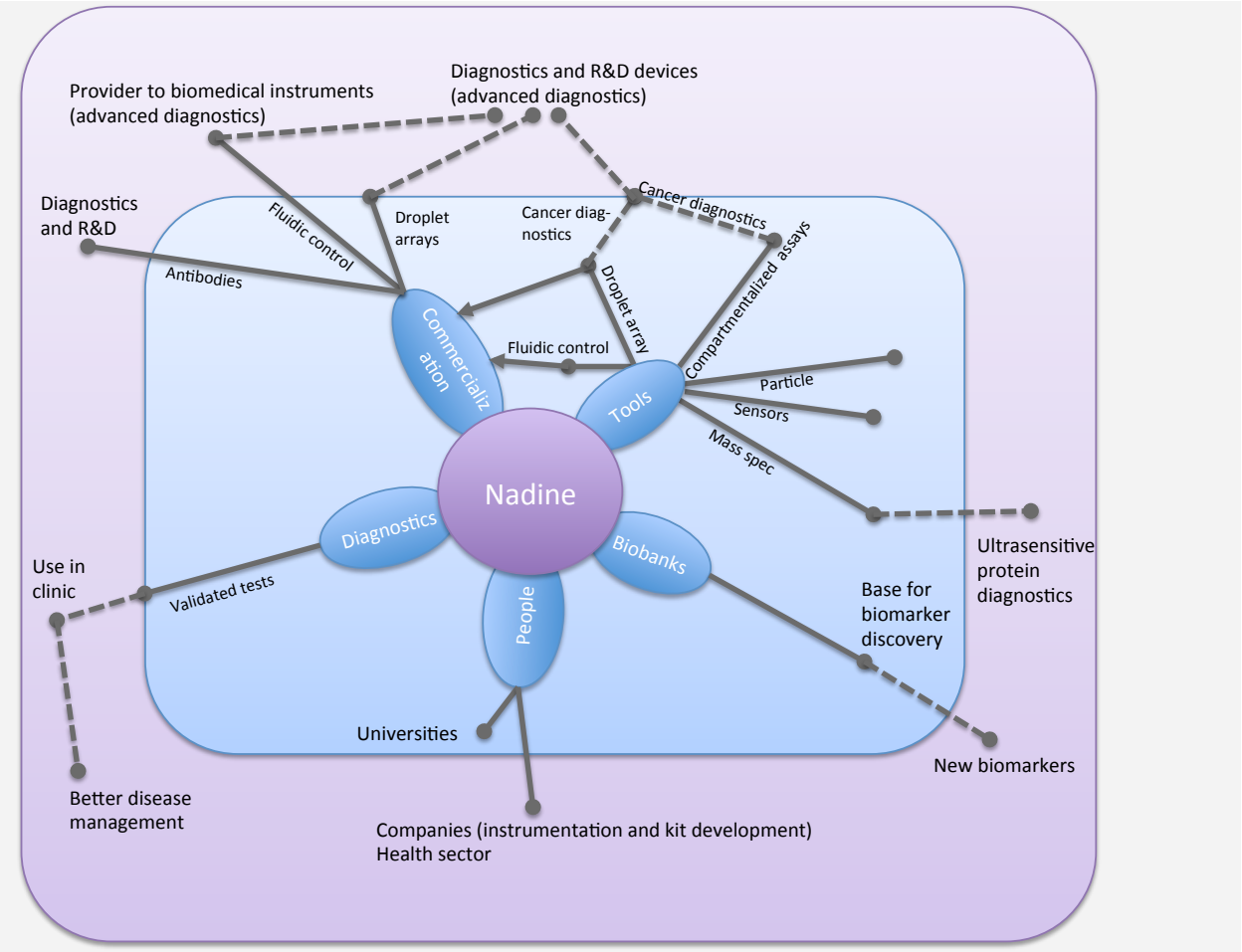


Figure 1. Overview of impact of the NaDiNe project. NaDiNe has been firmly planted in the R&D sphere (light blue) with a few activities going into the general societal sphere (commercialization or usage in the clinic). Dashed lines indicate projections with further additional R&D funding.

Impact of better diagnostics provided by NaDiNe and spinoffs

Neurodegenerative disease.

According to the World Health Organization, the number of people with dementia worldwide in 2010 is estimated at 35.6 million and is projected to nearly double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050. The total number of new cases of dementia each year worldwide is nearly 7.7 million. Treating and caring for people with dementia currently costs the world more than US$ 604 billion per year. AD is the most common form of dementia and thus one of the main causes of disability and dependence in elderly people, with great socioeconomic impact. On this ground, AD has been recognized as a public health priority by WHO which has recently recommended the development of national programs to address dementia, focused on improving early diagnosis and disease management, and providing better support to caregivers.

NaDiNe supports this societal need fully by proving validated assays, biobanking for future biomarker discovery, and a whole range of viable technologies for detecting biomarkers in CSF and blood. Some of these technologies (microfluidics, nanoparticles, microarray, sensing) can be packages in a point of care device serving developing countries, while developed countries may opt for automated processes like the mass spectroscopy methods developed in NaDiNe. The foundation is thereby laid in NaDiNe to improve disease management of neurodegenerative diseases. Even small improvements of disease management may have large positive economic consequences.

Cancer.

Some of the developed techniques in NaDiNe proved to be so sensitive and powerful that they are better used for cancer diagnostics as compared to neurodegenerative diagnostics. Preliminary tests performed in the NaDiNe project indicated that some of the technologies could be converted to cancer diagnostics tests for breast cancer and leukemia. Oncology is the second fastest growing segment of the molecular diagnostics market (infectious diseases being the fastest growing). The technologies proposed here is equally ground breaking for infectious diseases, as they also typically require long procedures in order to amplify few infectious agents from a large volume of patient samples. Furthermore, other preliminary experiments in the NaDiNe project showed ultra sensitive detection of bacteria in milk using a highly efficient sample preparation method. Hence, the proposed technology under NaDiNe could carve a large share of the molecular diagnostics market after future maturation (Fig 1).

The total economical impact on cancer treatment of NaDiNe achievements is likely much larger than just getting a part of the diagnostic market as cancer-related costs in the EU were about 126 billion Euro in 2009. 40% were directly associated with health care costs while the rest were lost work, care leaves for relatives etc. The cancer burden is projected to increase just like AD over time given EU's ageing population. Diagnosing disease efficiently leading to more efficient treatment will have a large economic impact. A 10% efficiency boost would give the EU about 12 billion Euro annually in savings (and 120 000 saved lives per year) using 2009 annual cancer cost numbers. NaDiNe has provided state of the art technologies and knowledge to strengthen Europe in a highly competitive and large market, and the potential products are poised to reduce the economic burden of cancer.

Molecular diagnostics.

Digital analysis of samples has a strong future because of its sensitivity and ability to process large sample volumes. Some digital analysis was explored in the NaDiNe project and a so-called droplet array was the most successful. It is expected that digital analysis will have a large impact on molecular diagnostics.

Sales of molecular diagnostics totaled $4.1Billion (app. €3B) in 2010 and $4.5B (app. €3.4B) in 2013 (http://www.darkdaily.com/frost-sullivan-report-identifies-molecular-diagnostics-as-fastest-growing-sector-

of-clinical-pathology-laboratory-testing-03192012#axzz3Aev8WjxQ),
http://www.grandviewresearch.com/industry-analysis/molecular-diagnostics-market,
http://www.marketsandmarkets.com/PressReleases/molecular-diagnostic.asp). Estimates of annual growth rates vary from 8.7 to 11%. This shows that NaDiNe provides tools and technology into a fast growing field.

Much of molecular diagnostics is driven by polymerase chain reaction (PCR) and protein assays. Due to its ability to screen large sample volumes, the droplet arrays platform can compete efficiently with PCR for a number of applications including cancer diagnostics and infectious disease diagnostics. This indicates that the platform has the possibility to take a significant part of the diagnostics market as droplet arrays have a number of practical, economical and analytical advantages compared to PCR. When super sensitive detection is needed for identification of protein markers in blood, droplet arrays will be useful and have a chance to compete with existing de facto gold standards, such as enzyme linked immune assays (ELISA) as well as existing digital protein analysis platforms (Qanterix). Therefore, we strongly believe that droplet arrays will produce job opportunities in the EU as well as enable better disease management through streamlined and highly sensitive assays.

Point of care diagnostics encompasses many variants and implementation modes, but most techniques are typically based on simple flow control. These devices are not compatible with advanced diagnostics such as those demonstrated in NaDiNe. The prototypes developed under NaDiNe demonstrate that advanced molecular diagnostics can be packaged into devices that can be further developed into point of care devices. We expect that these types of advanced diagnostics devices will enable new types of diagnostics in medicine but also in other areas.

Providing a highly skilled and educated work force to relevant industries

It is without doubt that future molecular diagnostics devices will be very complex, but at the same time, as easy to operate for the users as modern mobile phones. In the NaDiNe project we have had numerous students working with the development of advanced systems and characterization and usability of potential biomarkers. NaDiNe has therefore provided Europe with a number of people that are knowledgeable in instrument development, usage of these instruments, developing molecular diagnostic kits, and their application in the clinical setting.

Bio banking

NaDiNe has assembled a large biobank for the research in the project. However, the biobank is equally usable in the future for further studies. Having a large available biobank is really important for rapid biomarker discovery. The concrete impact of the biobank is not easy to predict and depends very much on the research it is going to enable. There are, however, numerous examples where biobanks provide competitive advantages in research for biomarkers. In this context, it is noteworthy that any new biomarkers together with suitable detection technologies are patentable intellectual properties. We thus see this effort as an investment for the future.

Analytical tools and techniques developed in the project.

The technological development in NaDiNe lays the foundation for further studies and later possible commercialization.

Particles

There has been a large development of innovative nanoparticles in the NaDiNe project. Specifically, we specialized on their biofunctionalization. All the achievements obtained in this project constitute the starting point for further inventions in the field of preparation and application of biofunctionalized magnetic particles. Most of the developed approaches are versatile and applicable in variety of therapeutic and diagnostic contexts. A large part of existing (and, arguably, future) biomedical methods are particle-based,

and thus there is a substantial commercial interest. Particles have furthermore large binding capacity and can be extended to other usages, such as, for instance, nano particle cleanups and other environmental applications.

Microfluidics

Many of the microfluidics techniques developed in NaDiNe are modules that are going to be used in compound instruments or work flows. For instance, a - microfluidic fluidized bed was developed to extract and pre-concentrate analytes from large samples volumes (1-1000µL). This is important and useful for a number of down-stream analytical techniques such as mass spectrometry, protein microarrays (both tested in NaDiNe), but also other detection or identification techniques. There is an unmet need for highly automated and miniaturised sample pre- concentration in order to improve detection limits and specificity of a range of analytical instruments existing in labs today. This technology has therefore a large commercial potential if connected to down stream analysis equipment.

Detection technologies

NaDiNe has explored a whole range of different detection technologies including electrochemistry, quantum dots enhanced detection, enzymatic amplifications, fluorescence and absorbance. The detection methods were furthermore combined with various techniques to obtain identity of the biomarker such as on chip capillary electrophoresis, isoelectric focusing and sandwich immune assays. In the end common fluorescent based microarrays, enzymatic amplified droplet array detection were superior combinations. However, the other techniques and combination may have suitable application is other areas where low detection limit is not required. The importance of this exhaustive effort is the knowledge, which are the viable alternatives for future instrumentations.

Mass spectrometry

The new concepts for ultra-sensitive matrix-assisted laser desorption ionization (MALDI) mass spectrometry, as developed in this project, could have a considerable impact for the discovery of unknown biomarkers, not only in relation to Alzheimer's disease, but also to other diseases such as different forms of cancer for which the number of reliable clinical diagnostic tools is very limited or not available yet.

Therefore, a scenario could be foreseen that the technology platform developed in this project could have a large socio-economic impact, and a great benefit for society as a whole. However, it should be pointed out that thus far, the project has only shown the feasibility of the concepts. A more focused, large effort is required to develop the technology into a clinical tool for routine use (Fig. 1). Apart from further basic improvements, such work should include the development of automated handling of patient samples, as well as a sample work-up system, using a high throughput robotized system.

Biomarkers

Especially with the recently investigated markers Ubiquitin, Neurofilaments and SerpinA1 disease management of an individual patient can be better organized. This is far beyond a proof of principle study, but will have a direct differential diagnostic value. For markers like tau and abeta1-42 the diagnostic value was already established. This was not the case for the recently investigate markers. Ubiquitin will directly help us to define subtypes of AD patients and may help to restratify therapeutic trails. The same hold true for SerpinA1. This is the first neurochemical marker, which helps to predict the development of a dementia in Parkinsons´s disease. The measurement of NF has the impact to be included in the diagnostic criteria for Amytrophic lateralsclerosis (ALS). Instead of waiting until all clinical criteria are fulfilled, patients under the differential diagnosis of an ALS can be directly treated if NF levels are elevated or included in therapeutic trail at a time point which a therapeutic effect is more realistic. The economic impact must be evaluated in further studies and is not within the scope of NaDiNe.

Commercialization

Fluigent – fluidics control instruments.

Three different products have emerged from the NaDiNe project and are now commercialized by Fluigent:

- The Easy Switch SolutionTM platform: a product combining hardware and software to be able to inject sequentially different fluids into a microfluidic system.
- The Flow-Rate Platform: a product enabling the monitoring of flow-rates generated by pressure pumps.
- The Flow-Rate Control Module: an algorithm to be able to control the flows by flow-rate using pressure actuation of fluids.

The different products enable the company to increase its sales revenue, its market share and also to penetrate new markets. One such market is microfluidics driven by syringe pumps, the most popular solution for microfluidics at research labs. The thousands of microfluidics labs constitute a large commercial opportunity. However, Fluigent also developed an application programming interface (API) which significantly broadens Fluigent's potential customer base.

Already today, almost all instrumentation in the general practitioner's office is based on microsystems and some are even microfluidics based. With the NaDiNe project, Fluigent is well positioned to go into further collaboration to solve the next step in diagnostics – namely molecular diagnostics that is usually not found in the practitioner's office (or in bed side diagnostics) yet. In fact, the prototype developed by Fluigent is capable of controlling automated and highly advanced molecular analyses, as was successfully demonstrated in the project. The billion USD molecular diagnostics market will likely increasingly incorporate more miniaturized analysis systems. The NaDiNe project therefore has strengthened Fluigent in particular and Europe in general to address this fast growing market.

AK Diagnostics, a new NaDiNe spinoff company.

One of the techniques developed in the project was the droplet array digital platform where very small concentrations of biomarkers could be detected. The platform has a potentially large commercial value in molecular diagnostics in general. Therefore, we have investigated patenting and commercialization. The result is a Danish spinoff company (AK-Diagnostics), possibly a patent application (still in preparation) and, at a later stage, scientific journal publications. The goal of the company is molecular diagnostics on serum samples. Areas of interest are cancer and infection diagnostics. AK Diagnostics is looking into point of care diagnostics devices but the technology is also highly suitable for single cell proteome or transcriptome analysis. Recently, talks with a medium sized European company have been initiated to explore collaborations to put the technology into research instruments for various purposes such as epigenomics.

Diagnoswiss

Diagoswiss has had the opportunity to develop assays for neurodegenerative diseases, which will be considered to be commercialized. They further improved upon their equipment (both from a hardware and software point of view) and, together with the new assays, are now able to offer their customers advanced solutions for medical diagnostic applications.

Moravian Biotech - Antibodies

In a broad sense, the reagents we have produced are expected to contribute to further understanding of the (neurodegenerative) diseases, allowing for improved diagnostics and potentially identifying novel therapeutics, with the corresponding socio-economic benefits. With specific respect to Moravian Biotech (MB), the reagents that have been produced will be further commercialised to allow their wider use across the scientific community, also providing MB with a stable income. This future impact relies on the dissemination

of data relating to the reagents, which is available on the MB website and is accessible through the reports and the website of the NaDiNe project.

Dissemination

The NaDiNe project has the potential to be of larger than average public interest due to the fact that Alzheimer´s disease is widespread and frequently covered in the media. However, the NaDiNe project's technical and medical results are, while important, challenging to communicate to a wider audience. We have therefore prepared a dissemination manual based on this experience. Instead of a wide outreach, NaDiNe has focused its dissemination mostly to researchers. The NaDiNe project has resulted in >90 peer reviewed journal papers and >150 conference and lecture invitations during the last 5 years indicating a large academic impact. The key results of NADINE are presented on NaDiNe's home page (http://www.fp7nadine.eu, Figure 3 with all partner listed). Extensive consortium networking has taken place and numerous PhDs and postdocs have accessed a large multidisciplinary research community. An outline of NaDiNe's dissemination activities is given in Figure 2.

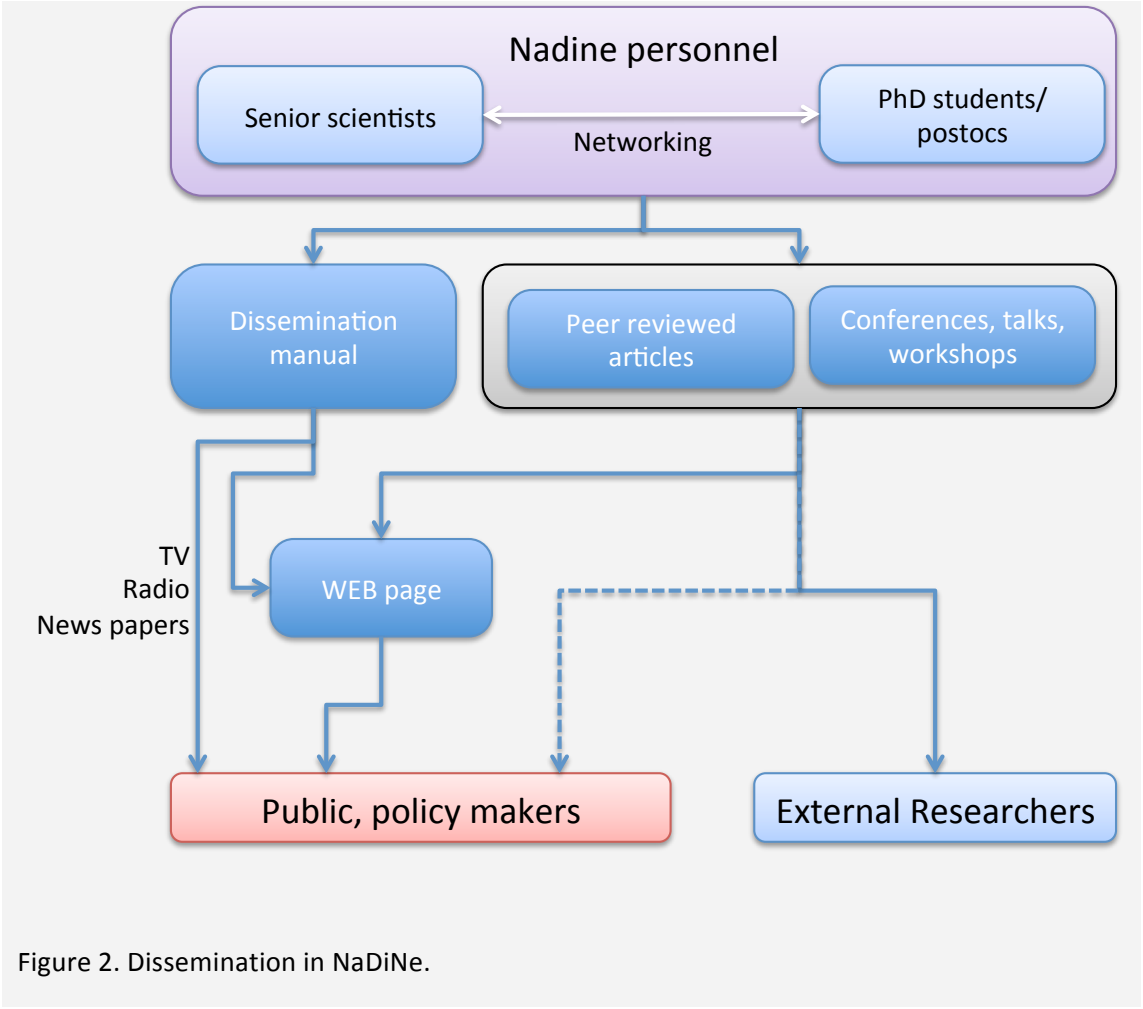


Figure 2. Dissemination in NaDiNe.

Networking

The NaDiNe consortium was highly cross-disciplinary with researchers from physics, engineering, chemistry, biology and medicine all working together to reach the goal of the project. This cross-disciplinary consortium also provided large possibilities to learn form each other.

During the project we had extensive flow of people between different labs and countries. Furthermore, we had two in-person project meetings per year where principle investigators as well as students attended, as well as numerous teleconferences with the entire project team, or, more frequently, focused subsets of the project team. The opportunity to collaborate and exchange experiences with many excellent partner institutions within the project enabled researchers to broaden their existing knowledge and skills, which can be applied in further work. Having been part in the FP7 framework NaDiNe project serves as a reference point for future potential partners in similar projects, as well as improving the ability to interact with and provide additional resources to collaborators, both current and coming.

Articles, Conferences and talks

>90 articles and >150

Website and communication plan

DTU has set up a website, www.fp7nadine.eu from the beginning of the project. The webpage has been the project's public face throughout the project, where publications and conference presentations have been updated regularly. The web page will be updated for the next 6 months to include the last results of the project. The web site will be publically accessible for the next three years.

A communication plan for the partners how to disseminate their results to the broader public. It has been sent to all partners. The plan includes for example information on

- The identification of different target groups such as scientific community, general public and external stake-holders (industry and patients groups).
- Identification of primary and secondary stories
- Different means to promote the results
- Individual story placement and press stories
- Creating attention in the press

Demo events and training.

Three demo events has been organised during the project course

- At the partner meeting on 5-6 March 2015 at DTU, Fluigent organized demo and training (with the help of other partners, ICN/ICRM/Curie). Fluigent brought the flow control demonstrator and operated typical experiments using colored water. In addition, DTU demonstrated the droplet array platform. The objectives were to show the demonstrator to the clinicians and to explain them how they can use it. Number of participants: 30

- The mass spectrometry was demonstrated for the clinicians at a separate meeting in Stockholm on 24 February 2015. Number of participants: 6

- On 3 July 2015, Fluigent organized a workshop in their facilities. This event was divided in lectures and training sessions and was not only addressed to members of the Consortium but also external stakeholders and the pharmaceutical industry. Number of participants: 18

Project website and contact information

Figure 3. NaDiNe homepage.

Use and dissemination of foreground

- Section A (see end of document)
- Section B (see end of document)

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Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

See end of report for tables A1 and A2

Section B (Confidential[2] or public: confidential information to be marked clearly)

Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[3]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	YES		EP20120290339	Flow-rate calibration and control in a microfluidic device	Fluigent SA

[2] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[3] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Please complete the table hereafter:

Type of Exploitable Foreground[4]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application[5]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Commercial application of R&D	*New digital analysis platform*	*Yes*	*31/12/2016*	*Chips and analysis platforms*	*1. Medical 2. Biomedical research*	*2016*	*A methods patent is planned for 2016*	*DTU*
MOUSE MONOCLONAL ANTIBODIES	*Monoclonal antibodies recognizing new epitopes of Aβ–peptide,ubiquitin and ApoE3*	**No**	Not applicable	*Mouse IgG*	*1. Medical (potentially) 2. Basic and translational research*	*Antibodies are ready for licensing and commercialisation (products are finalised)*	*For antibodies patents are not required*	*MB and members of NADINE consortium. In case of future commercialisation other research laboratories working in Alshaimer disease*
RABBIT POLYCLONAL ANTIBODIES	*Polyclonal antibodies developed against new peptides or epitopes by antigen affinity purification technique*	**No**	Not applicable	*Rabbit IgG*	*2. Basic and translational research* ***H***	*Antibodies are ready for commercialisation (products are finalised)*	*For antibodies patents are not required*	*MB and members of NADINE consortium. In case of future commercialisation other research laboratories working in Alshaimer disease*
Microfluidic fluidized bed	*New microfluidic technolgy for biomarkes extractiona dn*	*YES*		*Microfluidic device*	*1. Medical 2. Environmental*		*Patent*	*Viovy Malquin Descroix*

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[5] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

Type of Exploitable Foreground[4]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application[5]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	preconcentration							
MAGNETIC PARTICLES MANIPULATION IN DROPLET	**NEW DROPLET BASED PLATFROM FOR COMPLEX BIOASSAY**	**YES**		**BIOASSAY ROBOT**	**MEDICAL ANALYTICAL**		**PATENT**	**CNRS**

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

- Its purpose
- How the foreground might be exploited, when and by whom
- IPR exploitable measures taken or intended
- Further research necessary, if any
- Potential/expected impact (quantify where possible)

Digital analysis platform.
The foreground describes chip and microfluidics system to operate that chip to perform bioassays. DTU has done a news search and need to find a strategy to patent the chips and system. The technology has resulted in a spin off company from DTU with the name AK diagnostics. We have furthermore started negotiations with medium sized european Biotech company that want to use the technology for cancer diagnostics and chromatin immunoprecipitations within research. Expected time line is three years. The potential impact is cheaper, much more sensitive cancer diagnostics compared to current technology which leads to more precise treatment and hopefully survival. We expect that this leads to several hundreds of million Euro saving per year in costs. The platform can be used in lots of diagnostics and research situations and the diagnostic market alone is expected to be worth 8 billion USD in 2018. The technology ahs the potential to take a large share of this market as the technology allows for hyper sensitive immunoassasy and PCR free detection of DNA and RNA with at least equal sensitivity.

Antibodies:

Moravian-biotechnology spol. s r. o. (MB) developed set of monoclonal antibodies and rabbit polyclonal sera to different proteins including isoforms of Ab-peptide, ApoE3 protein and ubiquitine. The antibodies to Ab-peptide, ApoE3 protein and ubiquitine are ready for commercialisation through third parties and can be also offer through MB website. MB has ready large scale production and purification methodology including labelling technologies so we can offer these antibodies in different formats for different methodological approaches directly for sale if there will be interest from third commercial parties. The potential impact is that antibodies will be cheaper and also well characterise and each batch will be under control of our company that will ensure reproducible results using these antibodies. Expected time line for commercialization is about two years. We hope the antibodies can save money to researchers as they have to be cheaper that other commercially available antibodies as we can establish real price to commercial partners.

Report on societal implications

Replies to the following questions will assist the Commission to obtain indicators on societal and socio-economic issues addressed by projects. The arranged in a number of key themes. As well as producing certain statistics, t also help identify those projects that have shown a real engagement with wider and thereby identify interesting approaches to these issues and best practices. individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreeme** entered.*

Grant Agreement Number:	GA246513
Title of Project:	NaDiNe
Name and Title of Coordinator:	Associate Professor Martin Dufva, DTU

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)?

- If Yes: have you described the progress of compliance with the rele Review/Screening Requirements in the frame of the periodic/final project reports?

Special Reminder: the progress of compliance with the Ethics Review/Screening Requiremer described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achie*

2. Please indicate whether your project involved any of the following iss box) :

RESEARCH ON HUMANS
• Did the project involve children?
• Did the project involve patients?
• Did the project involve persons not able to give consent?
• Did the project involve adult healthy volunteers?
• Did the project involve Human genetic material?
• Did the project involve Human biological samples?
• Did the project involve Human data collection?
RESEARCH ON HUMAN EMBRYO/FOETUS
• Did the project involve Human Embryos?
• Did the project involve Human Foetal Tissue / Cells?
• Did the project involve Human Embryonic Stem Cells (hESCs)?
• Did the project on human Embryonic Stem Cells involve cells in culture?
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Emb
PRIVACY
• Did the project involve processing of genetic information or personal data (eg. h lifestyle, ethnicity, political opinion, religious or philosophical conviction)?
• Did the project involve tracking the location or observation of people?
RESEARCH ON ANIMALS
• Did the project involve research on animals?
• Were those animals transgenic small laboratory animals?
• Were those animals transgenic farm animals?

• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	0 Yes 0 No
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator		2
Work package leaders	2	7
Experienced researchers (i.e. PhD holders)	25	41
PhD Students	21	11
Other	20	9

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**N/A due to lack of info.**
Of which, indicate the number of men:	N/A due to lack of info.

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

X	Yes
○	No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
X	Design and implement an equal opportunity policy	○	○	X	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
X	Actions to improve work-life balance	○	○	X	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

X Yes- please specify

AD is more common in females than men

○ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

X No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

X Yes- please specify

Summer schools, educational animations

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

X Main discipline[6]: 1, 2, 3

○ Associated discipline[6]:1.3, 1.5, 2.2, 2.3, 3.1, 3.2

○ Associated discipline[6]:

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	○ X	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[6] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**115**
To how many of these is open access[7] provided?	**6**
How many of these are published in open access journals?	**6**
How many of these are published in open repositories?	**No information**
To how many of these is open access not provided?	**109**
Please check all applicable reasons for not providing open access:	
X publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available X no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[8]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**1**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**2**
	Registered design	
	Other	
17. How many spin-off companies were created / are planned as a direct result of the project?		**1**
Indicate the approximate number of additional jobs in these companies:		**1**

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	X	In small & medium-sized enterprises
X	Safeguard employment, or	X	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*

[7] Open Access is defined as free of charge access for anyone via Internet.
[8] For instance: classification for security project.

Difficult to estimate / not possible to quantify	X

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes X No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

X Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

❑	Press Release	X	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	X	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
❑	Brochures /posters / flyers	X	Website for the general public / internet
❑	DVD /Film /Multimedia	X	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	X	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as

geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

Section A (public)

This section includes two templates

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES:										
NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[1] (if available)	Is/Will open access[2] provided to this publication?
1	A nanochannel/nanoparticle-based filtering and sensing platform for direct detection of a cancer biomarker in blood	A. de la Escosura-Muñiz and A. Merkoçi,	Small	7	Willey Online Library		2011	675-682	DOI: 10.1002/smll.201002349	No
2	Immunomagnetic sulfonated hypercrosslinked polystyrene microspheres for electrochemical detection of proteins	Šálek P., Korecká L., Horák D., Petrovský E., Kovářová J., Metelka R., Čadková M., Bílková Z.	J. Mater. Chem.	21	Royal Society of Chemistry		2011	14783-14792	DOI: 10.1039/C1JM12475G	No

[1] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[2] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

3	Magnetic poly(propargylacrylamide) microspheres: Preparation by precipitation polymerization and use in model click reactions	Macková H., Proks V., Horák D., Kučka J., Trchová M.	J. Polym. Sci. A, Polym. Chem.	49	Wiley		2011	4820–4829	DOI: 10.1002/pola.24930	No
4	Size-dependent direct electrochemical detection of gold nanoparticles: application in magnetoimmunoassays	A. de la Escosura-Muñiz, C. Parolo, F. Maran and A. Merkoçi	Nanoscale	3	Royal Society of Chemistry		2011	3350-3356	DOI: 10.1039/C1NR10377F	No
5	Surface-initiated polymerization of 2-hydroxyethyl methacrylate from heterotelechelic oligoperoxide-coated g-Fe2O3 nanoparticles and their engulfment by mammalian cells	Horák D., Shagotova T., Mitina N., Trchová M., Boiko N., Babič M., Stoika R., Kovářová J., Hevus O., Beneš M., Klyuchivska O., Holler P., Zaichenko A.	Chem. Mater.	23	ACS		2011	2637–2649	DOI: 10.1021/cm2004215	
6	THIOL-ENE BASED POLYMER WAVEGUIDES FABRICATED BY UV-ASSISTED SOFT LITHOGRAPHY FOR OPTOFLUIDIC APPLICATIONS	Guisheng Zhuang, Thomas G. Jensen, and Jörg P. Kutter	ISBN: 978-0-9798064-4-5	15th	Chemical and Biological Microsystems Society	International Conference of Miniaturized Systems for	2011	1989-1991		No

						Chemistry and Life Sciences				
7	Voltammetric Detection of Ovalbumin at Screen-Printed Electrodes in Combination with Immunomagnetic Particles	M. Cadková, L. Korecká, P. Šálek, R. Metelka and Z. Bílková	Sensing in Electroanalysis	6	University Press Centre	Pardubice	2011	347-356	https://dk.upce.cz/handle/10195/42518	no
8	Anti-stiction coating of PDMS moulds for rapid microchannel fabrication by double replica moulding	Guisheng Zhuang and Jörg P Kutter	Journal of Micromechanics and Microengineering				2011	21, 1-6	10.1088/0960-1317/21/10/105020	no
9	Aminoterminally truncated and oxidized amyloid-beta peptides in the cerebrospinal fluid of Alzheimer's disease patients	M. Bibl	J Alzheimers Dis	2012, 29(4)	IOS Press		2012	809-816	doi: 10.3233/JAD-2012-111796.	
10	Carbon nanotubes and graphene in analytical sciences	B. Pérez-López, A. Merkoçi	Microchimica Acta	179	Springer International Publishing AG		2012	42370	10.1007/s00604-012-0871-9	no

11	Cerebrospinal fluid amyloid-beta 2-42 is decreased in Alzheimer's, but not in frontotemporal dementia	M. Bibl	J Neural Transm	2012 Jul;119(7) : (2012)	Springer	Vienna	2012	805-8013	doi: 10.1007/s00702-012-0801-3	
12	Detection of circulating cancer cells using electrocatalytic gold nanoparticles	M. Maltez-da Costa, A. de la Escosura-Muñiz, C. Nogués, L. Barrios, E. Ibáñez and A. Merkoçi	Small	8	Willey Online Library		2012	3605-3612	DOI: 10.1002/smll.201201205	No
13	Development of a magnetic immunosorbent for on-chip preconcentration of amyloid beta isoforms: Representatives of Alzheimer's disease biomarkers	Z. Svobodova,	Biomicro fluidics	6, 024126 (2012)	AIP Publishing		2012	24126-1 - 24126-12	doi: 10.1063/1.4722588.	
14	Fabrication of thermoplastics chips through lamination based techniques. .	S. Miserere	Lab Chip		RSC		2012			
15	Graphene Oxide as an Optical Biosensing Platform	E. Morales-Narváez and A. Merkoçi	Advanced Materials	24	Willey Online Library		2012	3298-3230	DOI: 10.1002/adma.201200373	No

16	Microchip electrophoresis, with respect to "profiling of Aβ peptides in the cerebrospinal fluid of patients with Alzheimer's disease"	Mohamadi MR	Methods Mol Biol		springer		2012	173-84		
17	Nanochannels preparation and application in biosensing	A. de la Escosura-Muñiz and A. Merkoçi	ACS Nano	6	American Chemical Society		2012	7556-7583	DOI: 10.1021/nn301368z	No
18	Nanomaterials and lab-on-a-chip Technologies	M. Medina-Sánchez, S. Miserere and A. Merkoçi	Lab on a Chip	12	Royal Society of Chemistry		2012	1932–1943	DOI: 10.1039/C2LC40063D	No
19	Nanoparticles for proteins and cells detection. Novel tools for clinical Diagnostics	A. de la Escosura-Muñiz and A. Merkoçi	G.I.T. Laboratory Journal	42036			2012	21-23		Yes
20	Neurochemical biomarkers in Alzheimer's disease and related disorders	M. Bibl	Ther Adv Neurol Disord	2012 Nov;5(6)	SAGE Publications LTD		2012	335-48	doi: 10.1177/1756285612455367.	

21	New monodisperse magnetic polymer microspheres biofunctionalized for enzyme catalysis and bioaffinity separations	Horák D, Kučerová J, Korecká L, Jankovičová B, Palarčík J, Mikulášek P, Bílková Z	Macromolecular Bioscience	12(5)	Wiley	Weinheim, Germany	2012	647-655	doi: 10.1002/mabi.201100393	no
22	On chip electrochemical detection of CdS quatum dots using normal and multiple recycling flow through modes	M. Medina-Sánchez, S. Miserere, S. Marín, G. Aragay and A. Merkoçi	Lab on a Chip	12	Royal Society of Chemistry		2012	2000-2005	DOI: 10.1039/C2LC00007E	No
23	Programmable magnetic tweezers and droplet microfluidic device for high-throughput nanoliter multi-step assay.	A. Ali Cherif	Angewandte Chemie International		wiley		2012	10765-9		yes/no
24	RAPID PHOTOCHEMICAL SURFACE PATTERNING OF PROTEINS IN	Josiane P. Lafleur, Radoslaw Kwapiszewski, Thomas G. Jensen, Jörg P. Kutter	ISBN: 978-0-9798064-5-2	16th	Chemical and Biological Microsystems Society	International Conference of Miniaturized Systems for Chemistry and Life Sciences	2012	1258-1260		No

25	Rapid photochemical surface patterning of proteins in thiol-ene based microfluidic devices.	Josiane P. Lafleur, Radoslaw Kwapiszewski, Thomas G. Jensen and Jörg P. Kutter	DOI: 10.1039/c2an36424g	138(2013)	RSC Publishing	Analyst	2012	845-849		no
26	Signal Enhancement in antibody microarrays using Quantum Dots nanocrystals: application to potential Alzheimer's disease biomarker screening	E. Morales-Narváez, H. Montón, A. Fomicheva and A. Merkoçi	Analytical Chemistry	84	American Chemical Society		2012	6821-6827	DOI: 10.1021/ac301369e	No
27	Simple Förster resonance energy transfer evidence for the ultrahigh quantum dot quenching efficiency by graphene oxide compared to other carbon structures	E. Morales-Narváez, B. Pérez-López, L. Pires and A. Merkoçi	Carbon	50	Elsevier		2012	2987-2993	DOI: 10.1016/j.carbon.2012.02.081	No
28	Simple monitoring of cancer cells using nanoparticles	M. Maltez-da Costa, A. de la Escosura-Muñiz, C. Nogués, L. Barrios, E. Ibáñez and A. Merkoçi	Nano Letters	12	American Chemical Society		2012	4164-4171	DOI: 10.1021/nl301726g	No

29	The use of hydrophilic poly(N,N-dimethylacrylamide) grafted from magnetic g-Fe2O3 nanoparticles to promote engulfment by mammalian cells	Zasonska B.A., Boiko N., Horák D., Klyuchivska O., Macková H., Beneš M., Babič M., Trchová M., Hromádková J., Stoika R.	J. Biomed. Nanotechnol.	9	ACS		2012	479-491	DOI: 10.1021/cm2004215	
30	Thiol-Ene Waveguides As Promising Components Of Optofluidic Microsystems	Radoslaw Kwapiszewski, Thomas G Jensen, Klaus B Mogensen, Zbigniew Brzozka, Jörg P Kutter	ISBN: 978-0-9798064-5-2	16th	Chemical and Biological Microsystems Society	International Conference of Miniaturized Systems for Chemistry and Life Sciences	2012	1900-1902		No
31	Use of magnetic hydrazide-modified polymer microspheres for enrichment of Francisella tularensis glycoproteins	Horák D., Balonová L., Mann B.F., Plichta Z., Hernychová L., Novotny M.V., Stulík J.	Soft Matter	8	Royal Society of Chemistry		2012	2775-2786	DOI: 10.1039/C2SM07036G	No
32	Analytical Miniaturization and Nanotechnologies	A. Merkoci, J. P. Kutter,	Lab on a Chip		Royal Society of Chemistry		2012	12(11), 1915-1916	10.1039/C2LC90040H	no

33	A low cost and high throughput magnetic bead-based immuno-agglutination assay in confined droplets	B. Teste	Lab on chip		RSC		2013	2344-9.		
34	Analysis of Amino-Terminal Variants of Amyloid-β Peptides by Capillary Isoelectric Focusing Immunoassay	U. Haussmann	Analytical Chemistry	2013 Sep 3;85(17)	ACS publications	Washington, DC 20036	2013	8142−8149	doi: 10.1021/ac401055y	
35	Design, preparation and evaluation of a fixed-orientation antibody/gold nanoparticle conjugate as immunosensing label	C. Parolo, A. de la Escosura-Muñiz, E. Polo, V. Grazu, J.M. De La Fuente, A. Merkoçi	ACS Applied Materials and Interfaces	5	American Chemical Society		2013	10753–10759	10.1021/am4029153	no
36	Development of a capillary isoelectric focusing immunoassay to measure DJ-1 isoforms in biological samples	D. Besong Agbo	Analytical Biochemistry	2013 Dec 15;443(2):	Elsevier		2013	197-204	doi: 10.1016/j.ab.2013.09.013	
37	Development of a high-sensitivity immunoassay for amyloid-beta 1-42 using a silicon microarray platform	Paola Gagni	Biosensors and Bioelectronics	(2013) N° 47	Elsevier	Amsterdam	2013	pp. 490-495	doi:10.1016/j.bios.2013.03.077.	no

38	Dot-ELISA affinity test: An easy, low-cost method to estimate binding activity of monoclonal antibodies	Svobodova Z., Jankovicova B., Horak D., Bilkova Z.	J. Anal. Bioanal. Tech.	4 (3)	OMICS International		2013		doi:10.4172/2155-9872.1000168	Yes
39	Enhanced lateral flow immunoassay using gold nanoparticles loaded with enzymes	C. Parolo, A. de la Escosura-Muñiz and A. Merkoçi	Biosensors and Bioelectronics	40	Elsevier		2013	412-416	DOI: 10.1016/j.bios.2012.06.049	No
40	Fabrication and bonding of thiol-ene-based microfluidic devices	Tiina M Sikanen, Josiane P Lafleur, Maria-Elisa Moilanen,Guisheng Zhuang, Thomas G Jensen and Jörg P Kutter	doi:10.1088/0960-1317/23/3/037002	23 (2013) 037002	IOP PUBLISHING	J. Micromech. Microeng.	2013	42186		no
41	Fabrication and characterization of tosyl-activated magnetic and non-magnetic monodisperse microspheres for use in microfluic-based ferritin immunoassay	Reymond F., Vollet C., Plichta Z., Horák D.	Biotechnol. Progr.	29	Wiley		2013	532-542	DOI: 10.1002/btpr.1683	No

42	Nanochannels for diagnostic of thrombin-related diseases in human blood	A. de la Escosura-Muñiz, W. Chunglok, W. Surareungchai, A. Merkoçi	Biosensors and Bioelectronics	40	Elsevier		2013	24-31	DOI: 10.1016/j.bios.2012.05.021	No
43	Nanoparticles Based Electroanalysis in Diagnostics Applications	A. Merkoçi	Electroanalysis	25	Willey Online Library		2013	15-27	10.1002/elan.201200476	no
44	New non-covalent strategies for stable surface treatment of thermoplastic chips	K. Perez Toralla	Lab on Chip		RSC		2013	4409-18		
45	Novel fluorescent microarray platforms: a case study in neurodegenerative disorders	Marina Cretich	Expert Review of Molecular Diagnostics	(2013) N°13	Taylor & Francis	Oxford	2013	pp. 863-73	doi:10.1586/14737159.2013.849574.	no
46	Optimisation of BACE1 inhibition of tripartite structures by modification of membrane anchors, spacers and pharmacophores - development of potential agents for the	P. Linning	Org Biomol Chem.	2012 Oct 3;10(41)	RSC Publishing		2013	8216-35	doi: 10.1016/j.ab.2013.09.013	

	treatment of Alzheimer's disease									
47	Paper-based nanobiosensors for diagnostics	C. Parolo, A. Merkoçi	Chemical Society Reviews	42	Royal Society of Chemistry		2013	450-457	DOI: 10.1039/C2CS35255A	no
48	PEG-modified poly(glycidyl methacrylate) and poly(2-hydroxyethyl methacrylate) microspheres to reduce nonspecific protein adsorption	Hlídková H., Horák D., Proks V., Kučerová Z., Pekárek M., Kučka J.	Macromol. Biosci.	13	Wiley		2013	503–511	DOI: 10.1002/mabi.201200446	No
49	Simple paper architecture modifications lead to enhanced sensitivity in nanoparticle based lateral flow immunoassays	C. Parolo, M. Medina-Sánchez, A. de la Escosura-Muñiz and A. Merkoçi	Lab on a Chip	13, 2013	Royal Society of Chemistry		2013	pp. 386-390	DOI: 10.1039/C2LC41144J	No
50	Surface immobilized hydrogels as versatile reagent reservoirs for microarrays	Laura Sola	Journal of Immunological Methods	(2013) N° 391	Elsevier	Amsterdam	2013	pp. 95-102	doi:10.1016/j.jim.2013.02.013.	no

51	Fabrication and bonding of thiol-ene-based microfluidic devices,	Tiina M Sikanen, Josiane P Lafleur, Maria-Elisa Moilanen, Guisheng Zhuang, Thomas G Jensen and Jörg P Kutter,	Journal of Micromechanics and Microengineering		iopscience		2013	23, 1-7 (2013)	-	no
52	Rapid photochemical surface patterning of proteins in thiol-ene based microfluidic devices	Josiane P. Lafleur, Radoslaw Kwapiszewski, Thomas G. Jensen and Jörg P. Kutter	Analyst		Royal Society of Chemistry	Cambridge	2013	138, 845-849	10.1039/C2AN36424G	no
53	Fabrication and bonding of thiole-ene-based microfluidic devices	T. M. Sikanen, J. P. Lafleur, M.-E. Moilanen, G. Zhuang, T. G. Jensen,J. P. Kutter	Journal of Micromechanics and Microengineering				2013	23(3), 037002		no
54	Alzheimer Disease Biomarker Detection Through Electrocatalytic Water Oxidation Induced by Iridium Oxide Nanoparticles	L. Rivas, A. de la Escosura-Muñiz, J. Pons, A. Merkoçi	Electroanalysis	26	Willey Online Library		2014	1287–1294	DOI: 10.1002/elan.201400027	no

55	An Inkjet-Printed Field-Effect Transistor for Label-Free Biosensing	M. Medina-Sanchez, C. Martínez-Domingo, E. Ramon, A. Merkoçi	Advanced Functional Materials	20	Willey Online Library		2014	6291–6302	DOI: 10.1002/adfm.201401180	no
56	Characterization of a new fluorescence-enhancing substrate for microarrays with femtomolar sensitivity	Marina Cretich	Sensors and Actuators B	(2014) N° 192	Elsevier	Amsterdam	2014	pp. 15-22	doi:10.1016/j.snb.2013.09.119	no
57	High-fat diet induced isoform changes of the Parkinson's disease protein DJ-1.	G. Poschmann	J Proteome Res	2014 May 2;13(5)	ACS Publications	Washington, DC	2014	2339-51	doi: 10.1021/pr401157k	
58	Magnetic microparticles post-synthetically coated by hyaluronic acid as an enhanced carrier for microfluidic bioanalysis	L. Holubova, P. Knotek, J. Palarcik, M. Cadkova, P. Belina, M. Vlcek, L. Korecka, Z. Bilkova	Materials science & engineering. C, Materials for biological applications	44	Elsevier	Amsterdam	2014	345-351	doi: 10.1016/j.msec.2014.08.039	no

59	Magnetic poly(glycidyl methacrylate) microspheres for capture of proteins	Koubková J., Müller P., Hlídková H., Plichta Z., Proks V., Vojtěšek B., Horák D.	New Biotechnol.	31	Elsevier		2014	482-491	DOI:10.1016/j.nbt.2014.06.004	No
60	Micromotor Enhanced Microarray Technology for Protein Detection	E. Morales-Narváez, M. Guix, M. Medina-Sánchez, C. C. Mayorga-Martinez, A. Merkoçi	Small	10	Willey Online Library		2014	2542–2548	DOI: 10.1002/smll.201303068	no
61	Monodisperse carboxyl-functionalized poly(ethylene glycol)-coated magnetic poly(glycidyl methacrylate) microspheres: Application to the immunocapture of ß-amyloid peptides	Horák D., Hlídková H., Hiraoui M., Taverna M., Proks V., Mázl Chánová E., Smadja C., Kučerová Z.	Macromol. Biosci.	14			2014	1590–1599	DOI: 10.1002/mabi.201400249	No
62	Monodisperse superparamagnetic nanoparticles by thermolysis of Fe(III) oleate and mandelate complexes	Patsula V., Petrovský E., Kovářová J., Konefal R., Horák D.	Colloid Polym. Sci.	292	Springer		2014	2097-2110	DOI: 10.1007/s00396-014-3236-6	No

63	On-chip magneto-immunoassay for Alzheimer's biomarker electrochemical detection by using quantum dots as labels	M. Medina-Sánchez, S. Miserere, E. Morales-Narváez, A. Merkoçi	Biosens. Bioelectron	54	Elsevier		2014	pp. 279-284	DOI: 10.1016/j.bios.2013.10.069	no
64	PEGylation of magnetic poly(glycidyl methacrylate) microparticles for microfluidic bioassays	J. Kučerová, Z. Svobodová, P. Knotek, J. Palarčík, M. Vlček, M. Kincl, D. Horák, J. Autebert, J.-L. Viovy, Z. Bílková	Materials science & engineering. C, Materials for biological applications	40	Elsevier	Amsterdam	2014	308–315	doi: 10.1016/j.msec.2014.04.011	no
65	Poly(glycidyl methacrylate)/silver nanocomposite microspheres as a radioiodine scavenger: Electrophoretic characterization of carboxyl- and amine-modified particles	Macková H., Oukacine F., Plichta Z., Hrubý M., Kučka J., Taverna M., Horák D.	J. Colloid Interface Sci.	421C	Elsevier		2014	146-153	doi:10.1016/j.jcis.2014.01.042	No
66	Protein microarrays technology: how far off is routine diagnostics?	Marina Cretich	Analyst	(2014) N° 139 (3)	Royal Society of Chemistry	Cambridge	2014	pp. 528 - 542	doi:10.1039/c3an01619f.	no

67	Quality Evaluation of Monoclonal Antibodies Suitable for Immunomagnetic Purification of Native Tau Protein	B. Jankovičová, L. Hromádková, R. Kupčík, J. Kašparová, D. Řípová, Z. Bílková	Scientific Papers of the University of Pardubice, Series A, Faculty of Chemical Technology	20	University of Pardubice	Pardubice	2014	147-163	-	no
68	Surface functionalized thiol-ene waveguides for fluorescence biosensing in microfluidic	Nikolaj Agentoft Feidenhans'l, Josiane P. Lafleur, Thomas Glasdam Jensen and Jörg P. Kutter	Electrophoresis				2014	35(2-3):282-8	10.1002/elps.201300271	no
69	Alzheimer's disease biomarkers detection in human samples by efficient capturing through porous magnetic microspheres and labelling with electrocatalytic gold nanoparticles	de la Escosura-Muñiz A., Plichta Z., Horák D., Merkoçi A	Biosens. Bioelectron.	67	Elsevier		2015	pp. 162-169	DOI: 10.1016/j.bios.2014.07.086	No

70	An Integrated Microfluidic Chip for Immunocapture, Preconcentration and Separation of β-amyloid Peptides	Mohamadi MR	Biomicro fluidics		aip		2015	accpeted		
71	Application of trypsin core/shell nanoparticles Fe3O4@SiO2 for protein digestion	M. Slováková, M. Sedlák, B. Křížková, R. Kupčík, R. Bulánek, L. Korecká, Č. Drašar, Z. Bílková	Process Biochem istry	Available online 3 Septembe r 2015 (In Press, Corrected Proof — Note to users)	Elsevier	Amsterda m	2015	-	doi:10.1016 /j.procbio.2 015.09.002	no
72	Benefits of Immunomagnetic Separation for Epitope Identification in Clinically Important Protein Antigens: A Case Study Using Ovalbumin, Carbonic Anhydrase I and Tau Protein	B. Jankovicova, Z. Svobodova, L. Hromadkova, R. Kupcik, D. Ripova, Z. Bilkova	Univers al Journal of Biomedi cal Enginee ring	3(1)	Horizon Researc h Publishi ng	Alhambra, CA, USA	2015	42217	doi: 10.13189/uj be.2015.03 0101	yes
73	Calf thymus histone-conjugated magnetic poly(2-oxoethyl methacrylate) microspheres for affinity isolation of anti-histone IgGs from blood serum of patients with systemic lupus erythematosus	Horák D., Plichta Z., Starykovych M., Myronovskij S., Kit Y., Chopyak V., Stoika R.	RSC Advance s	5	Royal Society of Chemist ry		2015	63050-63055	DOI: 10.1039/C5 RA09280A	No

74	Capillary isoelectric focusing immunoassay as a new nanoscale approach for the detection of oligoclonal bands	Poschmann	Electrophoresis	2015			2015	355-62	10.1002/elps.201400339	No
75	Detection and Differentiation of Threonine- and Tyrosine-Monophosphorylated Forms of ERK1/2 by Capillary Isoelectric Focusing-Immunoassay	I. Kraus	Scientific Reports	2015 Aug 3;5:12767	Nature Publishing Group		2015	5:12767 | DOI: 10.1038/srep12767	doi: 10.1038/srep12767	Yes
76	Digital detection of biomarkers assisted by nanoparticles: application to diagnostics	Marina Cretich	Trends in Biotechnology	(2015) N° 33 (6)	Elsevier Science Publishers	Barking	2015	pp 343-351	doi:10.1016/j.tibtech.2015.03.002.	no
77	Magneto-immunocapture with on-bead fluorescent labeling of amyloid-β peptides: towards a microfluidized-bed-based operation.	Duc Thanh Mai	Analyst	140(17):5891-900.	Royal Society of Chemistry	U.K.	2015	pp. 151 - 167	http://pubs.rsc.org/en/Content/ArticleLanding/	no
78	Nanochannel array device operating through Prussian blue nanoparticles for sensitive label-free immunodetection of a Cancer biomarker	M. Espinoza-Castañeda, A. de la Escosura-Muñiz, A. Chamorro, C. de Torres, A.	Biosensors & Bioelectronics	67	Elsevier		2015	107-114	doi:10.1016/j.bios.2014.07.039	no

		Merkoçi								
79	Nanoparticles-based nanochannels onto a plastic flexible substrate for label-free immunosensing	A. de la Escosura-Muñiz, M. Espinoza-Castañeda, M.Hasegawa, L. Philippe, A. Merkoçi	Nano Research	8	Springer International Publishing AG		2015	1180–1188	DOI: 10.1007/s12274-014-0598-5	no
80	Neurofilaments in the diagnosis of motoneuron diseases: a prospective study on 455 patients	Steinacker, P., E. Feneberg, J. Weishaupt, J. Brettschneider, H. Tumani, P. M. Andersen, C. A. von Arnim, S. Bohm, J. Kassubek, C. Kubisch, D. Lule, H. P. Muller, R. Muche, E. Pinkhardt, P. Oeckl, A. Rosenbohm, S. Anderl-Straub, A. E.	Neurol Neurosurg Psychiatry	2015			2015		10.1136/jnnp-2015-311387	No

		Volk, P. Weydt, A. C. Ludolph and M. Otto								
81	Neurofilaments levels as biomarkers in asymptomatic and symptomatic familial ALS	Weydt, P., P. Oeckl, A. Huss, K. Müller, A. Volk, J. Kuhle, A. Knehr, P. Andersen, J. Prudlo, P. Steinacker, J. Weishaupt, A. Ludolph and M. Otto	Ann Neurol	2015			2015	Accepted		No
82	Selective handling of droplets in a microfluidic device using magnetic rails.	B. Teste	Micro and Nanofluidics		Springer		2015			
83	Tuning capillary surface properties by charged polymeric coatings	Laura Sola	Journal of Chromatography A	(2015) N° 1414	Elsevier	Amsterdam	2015	pp 173-181	doi:10.1016/j.chroma.2015.08.032.	no

84	Advanced antibody quantum dot-based labeling methodology	V. Dvorakova, M. Cadkova, V. Datinska, L. Korecka, K. Kleparnik, F. Foret, Z. Bilkova	-	in progress	-	-	-	-	-	no
85	Difficulties associated with structural analysis of proteins susceptible to form aggregates: a case of Tau protein as a biomarker of Alzheimer's disease	L. Hromadkova, R. Kupcik, B. Jankovicova, T. Rousar, D. Ripova and Z. Bilkova	Journal of Separation Science	submitted (after revisions - September 2015)	Wiley	Weinheim, Germany	-	-	-	no
86	Efficient CE separation of Tau protein fragments for MS profiling of clinically significant peptides, representatives of all 6 Tau isoforms	B. Jankovicova, J. Jacksen, M. Taverna, C. Riviere, M. Slovakova, P. Ek, D. Bohoyo, J. Roeraade, Z. Bilkova, I. Le Potier	-	in progress	-	-	-	-	-	no
87	ERK and GSK-3 kinases immobilized on magnetic beads for multiply phosphorylation of peptides and proteins	Slováková M., Kupčík R., Hromádková L., Charvátová A., Vajrychová M., Řípová D., Bílková Z.	-	in progress	-	-	-	-	-	no

88	Native polyacrylamide gel electrophoresis for preparation quantum dot-based bioconjugates analysis	V. Dvorakova, M. Cadkova, L. Korecka, Z. Bilkova	-	in progress	-	-	-	-	-	no
89	Naturally occuring anti-tau antibodies in IVIG: identification, purification and characterization	L. Hromadkova, M. Kolarova, B. Jankovicova, A. Bartos, J. Ricny, Z. Bilkova, D. Ripova	Journal of Neuroimmunology	submitted (after revisions - September 2015)	Elsevier	Amsterdam	-	-	-	no
90	Selective isolation of hydrophobin SC3 by solid-phase extraction with polytetrafluoroethylene microparticles and its proteomic analysis	R. Kupčík, M. Zelená, P. Řehulka, Z. Bílková, L. Česlová	Journal of Separation Science (Special Issue)	submitted (after minor revisions - September 15, 2015)	Wiley	Weinheim, Germany	-	-	-	no
91	Using of Immobilized Kinases as a Tool for Preparation of High Purity Phosphorylated Tau	Hromadkova L., Kupcik R., Jankovicova B., Ripova D., Bilkova Z., Slovakova M.	-	in progress	-	-	-	-	-	no

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	Type of activities[1]	Main leader	Title	Date/Period	Place	Type of audience[2]	Size of audience	Countries addressed
1	Invited talk,DATE (Design Automation and Test Conference)	A.Merkoçi	"Nanomaterials-based biosystems for diagnostics applications"	9-13 March 2015	Grenoble, France	Scientific Community		
2	Keynote Talk, Nanoscience and Nanotechnology International Conference	A.Merkoçi	"Nanobiosensors and applications in diagnostics"	11-13 February 2015	Porto, Portugal	Scientific Community		

[1] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[2] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

3	Plenary Lecture, 9èmes JOURNEES MAGHREB-EUROPE, MATERIAUX ET APPLICATIONS AUX DISPOSITIFS ET CAPTEURS, MADICA 2014	A.Merkoçi	"Nanomaterials-based platforms for biosensing applications"	5-7 November 2014	Mahdia, Tunisie	Scientific Community		
4	Poster presentation,XIX Trobada Transfronterera sobre Sensors i Biosensors	A. de la Escosura-Muñiz, Z. Plichta, D.I Horák, A.Merkoçi	Porous magnetic microspheres as efficient capturing/pre-concentrating platforms for detection of Alzhemier disease biomarkers using electrocatalytic gold nanoparticle tags	25-26 September 2014	Bellaterra, Spain	Scientific Community		
5	Poster presentation,XIX Trobada Transfronterera sobre Sensors i Biosensors	E.Morales-Narváez, A. Merkoçi	Micro/Nanomaterials and microarray technology	25-26 September 2014	Bellaterra, Spain	Scientific Community		
6	Poster presentation,XIX Trobada Transfronterera sobre Sensors i Biosensors	M. Medina-Sánchez, S. Miserere, Miquel Cadevall, A. Merkoçi	Enhancement of in- chip quantum dots labelled protein electrochemical analysis through the use of an in-situ bismuth modified detector	25-26 September 2014	Bellaterra, Spain	Scientific Community		

7	Poster presentation,XIX Trobada Transfronterera sobre Sensors i Biosensors	S. Miserere, M. Medina-Sánchez, Miquel Cadevall, A. Merkoçi	Magnetic plug-based platform modelling and application in a microfluidics/quantum dots based electrochemical biosensing system	25-26 September 2014	Bellaterra, Spain	Scientific Community		
8	Invited talk,Workshops - WAM-NANO2014,	S. Miserere, M. Medina-Sanchez and A. Merkoçi.	On-chip magneto-immunoassay for Alzheimer's biomarker electrochemical detection by using QDs as labels	23-24 June 2014	Copenhagen, Denmark	Scientific Community		
9	Poster presentation, Biosensors 2014	M. Medina-Sánchez, S. Miserere, M. Cadevall and A. Merkoçi	Enhancement of in-chip QDs labelled protein electrochemical analysis through the use of an in-situ bismuth modified detector	27-30 May 2014	Melbourne Australia	Scientific Community		
10	Poster presentation, Biosensors 2014	S. Miserere, M. Medina-Sánchez, Miquel Cadevall and A. Merkoçi	Magnetic plug-based platform modelling and application in a microfluidics QDs based electrochemical biosensing system	27-30 May 2014	Melbourne Australia	Scientific Community		
11	Oral presentation, Biosensors 2014	E.Morales-Narváez, A. Merkoçi	Integration of micro/nanomaterials into microarray technology: boosting biosensing platforms	27-30 May 2014	Melbourne Australia	Scientific Community		

12	Poster presentation, Biosensors 2014	A. de la Escosura-Muñiz, Z. Plichta, D.Horák, A. Merkoçi	Novel porous magnetic microspheres as enhanced capturing platforms for the detection of Alzheimer disease biomarkers in human samples using electrocatalytic gold nanoparticle tags	27-30 May 2014	Melbourne Australia	Scientific Community		
13	Oral presentation, Biosensors 2014	M.Espinoza-Castañeda, A. Chamorro, C. de Torres, A. Merkoçi, A. de la Escosura-Muñiz	Nanochannel array devices for sensitive label-free immunodetection of cancer biomarkers	27-30 May 2014	Melbourne Australia	Scientific Community		
14	Poster presentation,MicroTAS2013: 17th International Conference on Miniaturized Systems for Chemistry & Life Sciences	M. Medina; S. Miserere; E. Morales; A.Merkoçi.	Lab-on-a-chip for electrochemical magneto-immunoassay for Alzheimer's biomarker detection	27-31 October 2013	Freiburg, Germany	Scientific Community		
15	Poster presentation, Workshop on Bioinspired-Nanotechology for Biosensing (COST Action TD 1003)	A. de la Escosura-Muñiz, A. Merkoçi,	Solid-state nanochannel arrays for electrochemical biosensing	16 May 2013.	Sitges (Spain)	Scientific Community		

16	Oral presentation, 3rd International Conference on Bio-sensing Technology	A. de la Escosura-Muñiz, A. Merkoçi	Solid-state nanochannel arrays for electrochemical biosensing	12-15 May 2013	Sitges (Spain)	Scientific Community		
17	Poster presentation, Advances in Biodetection and biosensors	Sandrine Miserere, Mariana Medina, Alfredo de la Escosura and Arben Merkoçi	Human IgG electrochemical detection in COC microfluidic device	5-6 March 2013	Barcelona, Spain	Scientific Community		
18	Poster presentation,Advances in Biodetection and biosensors	C. Parolo; A. de la Escosura; M. Medina; L. Rivas; A. Merkoçi	Lateral flow immunoassay designs with improved sensitivities using gold nanoparticles	5-6 March 2013	Barcelona, Spain	Scientific Community		
19	Invited Lecture, 1st International Conference on Nanomaterials: Fundamentals and Applications, NFA 2012,	Prof. Arben Merkoçi	Nanomaterials-based platforms in biotechnological applications	3-6 October 2012	Strbske Pleso, Slovakia.	Scientific Community		
20	Poster presentation,XVIIèmes Rencontres Transfrontalières Capteurs et Biocapteurs	A. De la Escosura, A. Merkoçi	Nanoporous membranes for biomarkers detection in human blood	20-21 September 2012	Tarragona, Spain	Scientific Community		

21	Poster presentation, XVIIèmes Rencontres Transfrontalières Capteurs et Biocapteurs	S. Miserere, M. Medina-Sánchez, A. De la Escosura, A. Merkoçi	Human IgG electrochemical detection in COC microfluidic device	20-21 September 2012	Tarragona, Spain	Scientific Community		
22	Invited Lecture,5th Edition of the Cell Model Systems Summer School. 5th Edition of the Cell Model Systems Summer School.	A. Merkoçi	Nanomaterials based biosensors	2012	Rome, Italy	Scientific Community		
23	Invited Lecture,14th International Conference on Electroanalysis (ESEAC 2012)	Prof. Arben Merkoçi	Electroanalysis using Nanomaterials	3-7 June 2012	Portorož, Slovenia	Scientific Community		
24	Invited Lecture,IMCS 2012 - The 14th International Meeting on Chemical Sensors	Prof. Arben Merkoçi	Nanomaterials-based Biosensors	2012	Nurenberg, Germany	Scientific Community		

25	Oral presentation, IPN-UPIITA	E. Morales-Narváez, A. Merkoçi	Graphene and biosensors	31 August 2012.	Mexico	Scientific Community		
26	Oral presentation,IPN-UPIITA	E. Morales-Narváez, Arben Merkoçi	Biosensors based on nanotechnology	30 August 2012.	Mexico	Scientific Community		
27	Poster presentation, WAM-NANO 2012	M. Medina-Sánchez, S. Miserere, S. Marín, G. Aragay, and A. Merkoçi	On-chip electrochemical detection of CdS quantum dots usinng normal and multiple recycling flow through modes	11-12 June 2012	Barcelona-Spain	Scientific Community		
28	Poster presentation,WAM-NANO 2012	S. Miserere, M. Medina-Sánchez, A. De la Escosura, A. Merkoçi	Human IgG electrochemical detection in COC microfluidic device	11-12 June 2012	Barcelona-Spain	Scientific Community		
29	Poster presentation,WAM-NANO 2012	Alfredo de la Escosura-Muñiz, Arben Merkoçi	In-nanochannel diagnostics	11-12 June 2012	Barcelona-Spain	Scientific Community		

30	Poster presentation,WAM-NANO 2012	Claudio Parolo, Alfredo de la Escosura-Muñiz, Sergio Gassò, Arben Merkoçi	Enhanced lateral flow immunoassay based on enzyme-gold nanoparticle dual label	11-12 June 2012	Barcelona-Spain	Scientific Community		
31	Poster presentation,21th Biosensors World Congress	E. Morales-Narváez; H. Montón; A. Fomicheva; A. Merkoçi	QDs versus fluorescent dyes in microarray based biomarkers screening.	15-18 May 2012.	Cancún (Mexico)	Scientific Community		
32	Poster presentation,21th Biosensors World Congress	C. Parolo, A. de la Escosura-Muñiz, G. Aragay, S. Gassó, A. Merkoçi.	Enhanced lateral flow immunoassay using gold nanoparticles loaded with enzymes.	15-18 May 2012.	Cancún (Mexico)	Scientific Community		
33	Poster presentation,21th Biosensors World Congress	A. De la Escosura, A. Merkoçi	Nanochannels for diagnostics	15-18 May 2012.	Cancún (Mexico)	Scientific Community		
34	Poster presentation, Graphene International Conference	E. Morales-Narváez, B. Pérez-Lopez, A. Merkoçi	Optical biosensors based on graphene	11-13 April 2012.	Brussels, (Belgium)	Scientific Community		

35	Oral presentation,NanoSpain 2012	E. Morales-Narváez, B. Pérez-Lopez, A. Merkoçi	Optical biosensors based on graphene	27 Feb - 1 March 2012	Santander, Spain	Scientific Community		
36	Poster presentation,NanoSpain 2012	B. Pérez-López, L. Baptista-Pires, S. Miserere, A. Merkoçi	Graphene for electrochemical biosensing platforms	27 Feb - 1 Mar 2012	Santander, Spain	Scientific Community		
37	Poster presentation,	A. de la Escosura-Muñiz, C. Parolo, M. Maltez-da Costa, A. Merkoçi	"Nanomaterials based biosensors for rapid and cost effective diagnostic of biomarkers"	21-23 November 2011	Barcelona, Spain	Scientific Community		
	ICREA Conference on Network Medicine Approaches to Human Disease							
38	Poster presentation, "Evolving Challenges in Promoting Cardiovascular Health,"	Claudio Parolo, M. Maltez-da Costa, A. de la Escosura-Muñiz, A. Merkoçi	Nanomaterials based biosensors for rapid and cost effective diagnostic of biomarkers	4-5 November 2011	Barcelona, Spain.	Scientific Community		

39	Lectures: Bioanalytical Nanotechnology School	A.Merkoçi	Building nanoblocks (I). Metal nanoparticles (MNP). Quantum dots. Carbon nanotubes. Applications in electrochemical and optical methods	1-6 October 2011	México D.F., México	Scientific Community		
40	Lectures: Bioanalytical Nanotechnology School	A. de la Escosura	Bioanalytical nanosystems. Catalytic nanoparticles and nanochannels. Applications in electrochemical and optical methods	3-6 October 2011	México D.F., México	Scientific Community		
41	Poster presentation,XVIèmes Rencontres Transfrontalières « Capteurs et Biocapteurs »	I. X.Cantarelli, G. Aragay, H.Montón, S. Marin, C. Parolo, F.Maran, A.Merkoçi	Protein Electrochemical Detection based on a Sandwich System formed by ZnS-thioglycerol Quantum Dots and Magnetic Nanoparticles	29-30 September 2011	Toulouse, France	Scientific Community		
42	Oral presentation, XVIèmes Rencontres Transfrontalières « Capteurs et Biocapteurs »	L. Rivas, A. de la Escosura-Muñiz, G. Aragay, T. Placido, L. Curri, J. Pons, A. Merkoçi	Synthesis of gold based nanostructures for biosensing applications	29-30 September 2011	Toulouse, France	Scientific Community		

43	Poster presentation, XVIèmes Rencontres Transfrontalières « Capteurs et Biocapteurs »	M. Maltez-da Costa, A. de la Escosura-Muñiz, A. Merkoçi	Diagnostics using electrochemical sensing platforms and nanomaterials	29-30 September 2011	Toulouse, France	Scientific Community		
44	Oral presentation,XVIèmes Rencontres Transfrontalières « Capteurs et Biocapteurs »	Eden Morales-Narváez, Arben Merkoçi	Alzheimer biomarker screening using microarrays	29-30 September 2011	Toulouse, France	Scientific Community		
45	Poster presentation,XVIèmes Rencontres Transfrontalières « Capteurs et Biocapteurs »	S. Miserere, M. Medina-Sánchez, A. Merkoçi	Integration of electrochemical détection in COC microfluidic Platform for biosensing applications	29-30 September 2011	Toulouse, France	Scientific Community		
46	Invited talk,Conference - Potsdam Days On Bioanalysis 2011	Prof. Arben Merkoçi	Nanomaterials applications in biosensing platforms	9-11 November 2011	Potsdam, Germany	Scientific Community		
47	Invited talk,Workshops - International Workshop on Graphene Nanostructures,.	Prof. Arben Merkoçi	Carbon nanotube and graphene based biosensing platforms	28-30 September 2011	Regensberg, Germany	Scientific Community		

48	Invited talk,Conference - 4th annual Advances in Biodetection & Biosensors conference and exhibition	Dr. Alfredo de la Escosura-Muñiz	Diagnostics Using Nanobioelectronics Based Sensing Systems	30 June - 1 July 2011	Hamburg, Germany	Scientific Community		
49	Poster presentation,Focus on Microscopy 2011	H. Montón, S. Marín, G. Aragay, A. de la Escosura, M. Guix, C. Nogués, A. Merkoçi	Confocal Laser Scanning Microscopy In Nanobiosensing	17-20 April 2011	Konstanz, Germany	Scientific Community		
51	Lectures,Bioanalytical Nanotechnology School	Prof. Arben Merkoçi	Bioanalytical nanosystems. Building nanoblocks (I) and (II). Metal nanoparticles (MNP). Quantum dots. Carbon nanotubes. Applications in electrochemical and optical methods	31 January - 4 February 2011	University of Santo Tomas, Manila, Philipines	Scientific Community		
52	Lectures,Bioanalytical Nanotechnology School	Dr. Alfredo de la Escosura-Muñiz	Bioanalytical nanosystems. Building nanoblocks (III). Metal nanoparticles (MNP). Quantum dots. Carbon nanotubes. Applications in electrochemical and optical methods	31 January - 4 February 2011	University of Santo Tomas, Manila, Philipines	Scientific Community		

53	Invited talk,Conference - International Symposium of Materials on Regenarative Medicine (ISOMRM) 2010	Arben Merkoçi	Nanoparticles Based Biosensors for Diagnostics Applications	3-5 November 2010	Taiwan	Scientific Community		
54	Invited talk,Conference - Congreso Internacional de Docencia e Investigación en Química	Arben Merkoçi	Nanoparticles for DNA, protein and cell sensors	40477	Ciudad de México, México	Scientific Community		
55	Invited talk,Conference - SLONANO 2010	Arben Merkoçi	Nanoparticles for DNA, protein and cell sensors	20-22 October 2010	Ljubljana, Slovenia	Scientific Community		
56	Invited talk,Concateno (company)	Arben Merkoçi	Nanoparticles for DNA, protein and cell sensors	40463	Oxford, UK	Scientific Community		
57	Poster presentation,6th Workshop on Scanning Electrochemical Microscopy	M. Espinoza-Castañeda, A. de la Escosura-Muñiz, W. Cantanhêde, A. Merkoçi.	Scanning electrochemical microscopy studies of nano and microstructurated platforms with interest for biosensing applications	3-7 October 2010	Frejus, France	Scientific Community		

58	Poster presentation,XV Trobada Transfronterera sobre Sensors i Biosensors,	C. Parolo, A. de la Escosura-Muñiz, A. Merkoçi	Effect of gold nanoparticles size on electrochemical immunosensing	16-17 September 2010	Sant Carles de la Ràpita (Spain)	Scientific Community		
59	Poster presentation,XV Trobada Transfronterera sobre Sensors i Biosensors	B. Pérez-López, A. Merkoçi	“On-off” Magneto-switchable Biosensor Based on Hybrid Nanobiomaterials	16-17 September 2010	Sant Carles de la Ràpita (Spain)	Scientific Community		
60	Oral presentation,XV Trobada Transfronterera sobre Sensors i Biosensors	M. Espinoza-Castañeda, A. de la Escosura-Muñiz, W. Cantanhêde, A. Merkoçi	Scanning electrochemical microscopy studies of nano and microstructurated platforms with interest for biosensing applications	16-17 September 2010	Sant Carles de la Ràpita (Spain)	Scientific Community		
61	Oral presentation,XV Trobada Transfronterera sobre Sensors i Biosensors,		Non-toxic ZnS nanoparticles as electrochemical tags in biosensing	16-17 September 2010	Sant Carles de la Ràpita (Spain)	Scientific Community		
62	Oral presentation,XV Trobada Transfronterera sobre Sensors i Biosensors	W. Chunglok, A. de la Escosura-Muñiz, A. Merkoçi	Electrochemical aptasensor using nanoporous platforms	16-17 September 2010	Sant Carles de la Ràpita (Spain)	Scientific Community		

63	Conference	Claire Smadja	Microscale Bioanalysis 2015	2015	China (Shangai China)	Scientific community	500	Worldwide
64	Conference	Duc Thanh Mai	1st Caparica Christmas conference on sample treatment	2014		Scientific community	130	Worldwide
65	Conference	Myriam Taverna	Microscale Bioanalysis MSB 2014	2014		Scientific community	500	Worldwide
66	Thesis	Kiarach Mesbah		2014	Faculté of pharmacy-Chatenay malabry	Local Scientific community	20	France
67	Poster, Scientific and Clinical Applications of Magnetic Carriers	Horák D., Hlídková H., Hiraoui M., Taverna M., Smadja C.	"Novel monodisperse carboxyl functionalized poly(ethylene glycol)-coated magnetic poly(glycidyl methacrylate) microspheres: Application to the	2014	Dresden, Germany	Scientific Community		

			immunocapture of ß-amyloid peptides"					
68	Poster, Frontiers of polymer colloids: From Synthesis to Macro-Scale and Nano-Scale Applications	Macková H., Oukacine F., Plichta Z., Hrubý M., Kučka J., Taverna M., Horák D.	"Preparation and characterization of radioiodine scavenger based on poly(glycidyl methacrylate)/silver nanocomposite microspheres"	2014	Prague, Czech Rep.	Scientific Community		
69	Poster, IPCG Polymer Colloids Conference 2015	Koubková J., Horák D.	"RAFT of sulfobetaine for modifying poly(glycidyl methacrylate) microspheres to reduce nonspecific protein adsorption"	2015	Durham, New Hampshire	Scientific Community		
70	Invited talk,14th International Congress on Amino Acids, Peptides and Proteins (conference abstract in Amino Acids, 2015, 47(8), p. 1646, ISSN: 0939-4451, IF 3,877)	M. Slováková, L. Hromádková, R. Kupčík, A. Charvátová, P. Přikryl, B. Jankovičová, M. Vajrychová, D. Řípová, Z. Bílková	Kinases-superparamagnetic beads for hyperphosphorylation of peptides/proteins	3 – 7 August 2015	Vienna, Austria	Scientific Community		

71	Poster presentation,14th International Congress on Amino Acids, Peptides and Proteins (conference abstract in Amino Acids, 2015, 47(8), p. 1638, ISSN: 0939-4451, IF 3,877)	L. Hromadkova, R. Kupcik, B. Jankovicova, Z. Bilkova and D. Ripova	Relationship of PHF6 hexapeptide motif and abnormal sticky behaviour: complication in structural analysis of Tau protein at the peptide level	3 – 7 August 2015	Vienna, Austria	Scientific Community		
72	Poster presentation,3rd Student Scientific Conference of Third Faculty of Medicine,Charles University in Prague (SVK2015) (abstract in book of abstracts, ISBN 978-80-87878-14-9, pp. 143-144) - price of the dean	M. Kolářová, L. Hromádková, B. Jankovičová, A. Bartoš, J. Říčný, D. Řípová, Z. Bílková	Naturally Occurring Antibodies against Protein Associated with Alzheimer Disease	19 May 2015,	Prague, Czech Republic	Scientific Community		
73	Poster presentation,63rd ASMS Conference on Mass Spectrometry and Allied Topics (abstract in book of abstracts - p. 116)	R. Kupcik, J. Macak, P. Krulisova, P. Rehulka, Z. Bilkova	A Novel Carrier Based on TiO2 Suitable for Isolation of His-tagged Recombinant Proteins and Peptides	31 May – 4 June 2015	St. Louis, Missouri, USA	Scientific Community		
74	Poster presentation,European Industrial Doctoral School Summer Workshop 2015	R. Kupčík, J. Macák, P. Řehulka, P. Krulišová, J. Kašparová, Z. Bílková	Improved enrichment of multiphosphorylated peptides suitable for tau protein analysis	26 April – 2 May 2015	Aveiro, Portugal	Scientific Community		

75	Lecture, Karolinska Institute	Z. Bílková	Magnetic beads-based microfluidic systems: the past and the future	2015	Stockholm, Sweden	Scientific Community		
76	Poster presentation,12th International Congress of Neuroimmunology (abstract in Journal of Neuroimmunology, 275(1-2), Proceedings of the XII. International Congress of Neuroimmunology, p. 6, ISSN 0165-5728, IF: 2.467)	L. Hromadkova, M. Kolarova, B. Jankovicova, A. Bartos, J. Ricny, Z. Bilkova and D. Ripova	Immunoaffinity Purification and Characterization of Specific Antibodies against Recombinant Tau Protein from Intravenous Immunoglobulin Product	9 – 13 November 2014	Mainz, Germany	Scientific Community		
77	Oral presentation,11th International Interdisciplinary Meeting on Bioanalysis (CECE 2014) (abstract in book of abstracts, ISBN 978-80-904959-2-0, Institute of Analytical Chemistry AS CR, pp. 77-8 0)	Svobodová Z., Jankovičová B., Kučerová J., Bílková Z.	Magnetic beads-based immunocapture of clinical biomarkers in microfluidic devices: from peptides to whole cells	20 – 22 October 2014	Brno, Czech Republic	Scientific Community		
78	Poster presentation,13th Human Proteome Organization World Congress (abstract in book of abstracts - p. 152)	R. Kupčík, J. Macák, P. Řehulka and Z. Bílková	Improved detection of multiphosphorylated peptides using novel TiO2 based nanomaterials	5 – 8 October 2014	Madrid, Spain	Scientific Community		

79	Poster presentation,20th International Symposium on Separation Sciences (abstract in book of abstracts, ISBN 978-80-7395-777-3, p. 147) - Best Poster Award	B. Jankovicova, Z. Svobodova, L. Hromadkova, R. Kupcik, D. Ripova, Z. Bilkova	Benefits of Immunomagnetic Separation for Epitope Identification in Clinically Important Protein Antigens	30 August – 2 September 2014	Prague, Czech Republic	Scientific Community		
80	Poster presentation,European Industrial Doctoral School Summer Workshop 2014 (abstract in Compendium, ISBN 978-80-7395-779-7, pp. 53 -54)	R. Kupčík, L. Hromádková, P. Řehulka, B. Jankovičová, J. Stulík, D. Řípová, Z. Bílková	Mass Spectrometric Analysis of Phosphopeptides from in vitro Phosphorylated Tau Protein	26 – 30 May 2014	Pardubice, Czech Republic	Scientific Community		
81	Poster presentation,Advances in Chromatography and Electrophoresis & Chiranal 2014 (abstract in book of abstracts, pp. 147 -148, ISBN 978-80-244-3950-1)	R. Kupčík, L. Hromádková, P. Řehulka, B. Jankovičová, J. Stulík, D. Řípová, Z. Bílková	Improved microscale RP LC separation of phosphopeptides from in vitro phosphorylated tau protein	10 – 14 February 2014	Olomouc, Czech Republic	Scientific Community		
82	Oral presentation,9th Seminar/Workshop on Sensing in Electroanalysis	Čadková M., Dvořáková V., Korecká L., Metelka R., Bílková Z.	New trend in electrochemical immunomagnetic biosensors for protein detection	13 – 16 November 2013	Pardubice, Czech Republic	Scientific Community		

83	Oral presentation,10th International Interdisciplinary Meeting on Bioanalysis (CECE 2013) (abstract in Chemické listy, 107(3), ISSN: 0009-2770, IF: 0.196, pp. 299-300)	Čadková M., Dvořáková V., Korecká L., Metelka R., Bílková Z.	New approach in electrochemical immunomagnetic biosensors for protein detection	12 – 13 November 2013	Brno, Czech Republic	Scientific Community		
84	Poster presentation,3rd Conference of the Czech Society for Mass Spectrometry (abstract in book of abstracts, ISBN 978-80-905045-3-0, p. 56)	R. Kupčík, L. Hromádková, B. Jankovičová, P. Řehulka, M. Slováková, J. Stulík, D. Řípová, Z. Bílková	Characterization of phosphorylation sites on recombinant Tau protein after in vitro phosphorylation with soluble and immobilized forms of kinases	16 – 18 October 2013	Hradec Králové, Czech Republic	Scientific Community		
85	Poster presentation,3rd Conference of the Czech Society for Mass Spectrometry (abstract in book of abstracts, ISBN 978-80-905045-3-0)	Staněk V., Jankovičová B., Kupčík R., Konečná M., Bílková Z.	Využití MALDI-MS a CZE pro analýzu fosforylovaných a defosforylovaných peptidů	16 – 18 October 2013	Hradec Králové, Czech Republic	Scientific Community		
86	Oral presentation,7th Central and Eastern European Proteomics Conference on Proteomics Driven Discovery and Applications (abstract in book of abstracts, pp. 47 – 48)	R. Kupčík, P. Řehulka, J. Stulík, Z. Bílková	Phosvitin as a standard protein for optimization of enrichment of multiply phosphorylated proteins	13 – 16 October 2013	Jena, Germany	Scientific Community		

87	Poster presentation,13th International Congress on Amino Acids, Peptides and Proteins (ICAPP) (conference abstract in Amino Acids 45 (3) (2013) 585)	Krulisova P., Jankovicova B., Kucerova J., Bilkova Z.	Optimization of magnetic bead enzyme-linked immunosorbent assay for detection of specific antibodies against Amyloid beta representing biomarkers of Alzheimer's disease	5 - 7 October 2013	Galveston, Texas, USA	Scientific Community		
88	Poster presentation,13th International Congress on Amino Acids, Peptides and Proteins (ICAPP) (conference abstract in Amino Acids 45 (3) (2013) 585)	B. Jankovicova, P. Muller, J. Lakota, B. Vojtesek, Z. Bilkova	Immunodominant epitope of carbonic anhydrase I identified by phage display peptide library	5 - 7 October 2013	Galveston, Texas, USA	Scientific Community		
89	Poster presentation,FENS Featured Regional Meeting(abstract in book of abstracts, ISBN 978-80-260-4881-7, p. 187)	L. Hromadkova, M. Vajrychova, M. Slovakova, R. Kupcik, B. Jankovicova, Z. Bilkova, D. Ripova	In vitro Tau phosphorylation performed by soluble and immobilized forms of MAPK and GSK-3β	11 - 14 September 2013	Prague, Czech Republic	Scientific Community		
90	Oral presentation,19th international conference „Analytical Methods and Human Health“(peer-reviewed contribution in proceedings book, ISBN 978-80-971179-1-7, pp. 48-49)	Z. Bílková, B. Jankovičová, Z. Svobodová	Nové diagnostické postupy na bázi mikrofluidních systémů a magnetických částic	24 – 27 June 2013	Rajecké Teplice, Slovakia	Scientific Community		

91	Poster presentation,19th international conference „Analytical Methods and Human Health“peer-reviewed contribution in proceedings book, ISBN 978-80-971179-1-7, pp. 237-238)	V. Staněk, M. Konečná, B. Jankovičová, R. Kupčík, Z. Bílková	Analýza fosforylovaných a defosforylovaných peptidů pomocí CZE a MALDI-MS	24 – 27 June 2013	Rajecké Teplice, Slovakia	Scientific Community		
92	Poster presentation,3rd practical EMBO course in "Phosphoproteomics"	R. Kupcik, P. Rehulka, B. Jankovicova, J. Stulik, Z. Bilkova	Enrichment of phosphopeptides from Francisella tularensis	14 – 19 April 2013	Odense, Denmark	Scientific Community		
93	Poster presentation,4th International Congress Nanotechnology, Medicine and Biology (BioNanoMed 2013) (abstract in book of abstracts)	Čadková M., Holubová L., Metelka R., Bílková Z., Korecká L.	Electrochemical magnetic immunosensor as a versatile device for high specific and sensitive biomarker detection	13 – 15 March 2013	Krems, Austria	Scientific Community		
94	Poster presentation,4th International Congress Nanotechnology, Medicine and Biology (BioNanoMed 2013) (abstract in book of abstracts)	Holubová L., Čadková M., Knotek P., Palarčík J., Svobodová Z., Horák D., Bílková Z., Korecká L.	Advanced hyaluronan-modified superparamagnetic particles with defined features desired for diagnostic devices	13 – 15 March 2013	Krems, Austria	Scientific Community		
95	Poster presentation,The 11th International Conference On Alzheimer´s & Parkinson´s Diseases (AD/PDTM 2013) (abstract in Alzheimer's and	B. Jankovicova, L. Hromadkova, M. Kolarova, R. Kupcik, A. Bartos, J. Ricny, D. Ripova, Z. Bilkova	Qualitative parametrization of anti-Tau antibodies for evaluation of autoimmune process in Alzheimer disease	6 – 10 March 2013	Florence, Italy	Scientific Community		

	Parkinson's Diseases: Mechanisms, Clinical Strategies, and Promising Treatments of Neurodegenerative Diseases. Neurodegener. Dis. 2013; 11(suppl 1):1, ISBN 978-3-318-02391-6, IF: 3.511)							
96	Oral presentation,8th Seminar/Workshop on Sensing in Electroanalysis	Čadková M., Korecká L., Metelka R., Bílková Z.	Signal amplification of magnetic immunosensor for protein detection	14 – 17 November 2012	Pardubice, Czech Republic	Scientific Community		
97	Oral presentation,9th International Interdisciplinary Meeting on Bioanalysis (CECE Junior 2012) (abstract in book of abstracts, ISBN: 978-80-904959-1-3, pp. 69 – 73)	R. Kupcik, P. Rehulka, B. Jankovicova, Z. Svobodova, L. Hernychova, J. Klimentova, J. Stulik, Z. Bilkova	Microchip isolation combined with MS-identification of phosphoproteins from Francisella tularensis	31 October – 2 November 2012	Brno, Czech Republic	Scientific Community		
98	Poster presentation,63rd Annual Meeting of the International Society of Electrochemistry (abstract in book of abstracts)	M. Cadkova, L. Korecka, P. Salek, R. Metelka, Z. Bilkova	Improving the sensitivity of electrochemical immunosensor for protein detection	19-24 August 2012	Prague, Czech Republic	Scientific Community		

99	Poster presentation,Colloids and Nanomedicine 2012 (abstract in book of abstracts)	Čadková M., Korecká L., Kuchař L., Černá V., Bílková Z.	Immobilized Sphingolipid ceramide N-deacylase as tool for simple semisynthesis of C17:0 sphingolipid isoforms	15 - 17 July 2012	Amsterdam, Netherlands	Scientific Community		
100	Poster presentation,International Conference „Colloids and Nanomedicine 2012“ (abstract in book of abstracts)	Holubová L., Palarčík J., Knotek P., Bělina P., Korecká L., Bílková Z.	Surface Modification of Magnetic Particles with Hyaluronic Acid for Minimalization of Non-Specific Sorption	15 - 17 July 2012	Amsterdam, Netherlands	Scientific Community		
101	Poster presentation,12th International Stockholm/Springfield Symposium on Advances in Alzheimer Therapy	B. Jankovicova, M. Link, R. Kandar, P. Drabkova, A. Bartos, M. Slovakova, L. Korecka, J. Stulik, Z. Bilkova	HPLC-separation of Tau protein fragments for subsequent analysis of immunoreactive domains	9 -12 May 2012	Stockholm, Sweeden	Scientific Community		
102	Oral presentation,XIV. conference „Monitorování cizorodých látek v životním prostředí“ (contribution in proceedings book, ISBN: 978-80-7395-525-0, pp. 5-13)	Čadková M., Korecká L., Kuchař L., Bílková Z.	Příprava nosiče s imobilizovanou sfingolipid ceramid N-deacylasou pro specifickou modifikaci sfingolipidů	17 – 19 April 2012	Ovčárna pod Pradědem	Scientific Community		
103	Oral presentation,XIV. conference „Monitorování cizorodých látek v životním prostředí“ (contribution in proceedings book, ISBN: 978-80-7395-525-0, pp. 47-56)	Holubová L., Korecká L., Palarčík J., Bělina P., Bílková Z.	Modifikace povrchů syntetických nosičů kyselinou hyaluronovou	17 – 19 April 2012	Ovčárna pod Pradědem	Scientific Community		

104	Oral presentation,7th Seminar/Workshop on Sensing in Electroanalysis (contribution in proceedings book, ISBN: 978-80-7395-434-5, pp. 347-356)	Čadková M., Korecká L., Šálek P., Metelka R., Bílková Z.	Voltammetric detection of ovalbumin at screen-printed electrodes in combination with immunomagnetic particles	14 – 17 November 2011	Pardubice, Czech Republic	Scientific Community		
105	Oral presentation,conference „Experimental research in medicine and its clinical applications" (abstract in book of abstracts, ISBN: 978-80-7177-993-3, p. 42)	Holubová L., Korecká L., Palarčík J., Bělina P., Bílková Z.	Povrchová modifikace anorganických nosičů kyselinou hyaluronovou pro aplikace in vivo	2011	Plzeň, Czech Republic	Scientific Community		
106	Poster presentation,Fifth International Workshop on"Biosensors for Food Safety and Environmental Monitoring"	L. Korecká, M. Čadková, P. Šálek, R. Metelka, Z. Bílková	Combination of innovative magnetic particles and electrochemical immunosensor for ovalbumin detection	6 – 8 October 2011	Ouarzazate, Marocco	Scientific Community		
107	Oral presentation, 18th Young Investigators' Seminar on Analytical Chemistry (YISAC 2011) (abstract in book of abstracts)	Holubová L., Korecká L., Bílková Z.	Biofunctionalization of carriers and possibilities of their utilization	28 June – 1 July 2011	Novi Sad, Serbia	Scientific Community		
108	Poster presentation,18th Young Investigators' Seminar on Analytical Chemistry (YISAC 2011) (abstract in book of abstracts)	B. Jankovicova, Z. Svobodova, M. Slovakova, L. Korecka, C. Riviere, H. Klafki, I. Le Potier, M. Taverna, Z. Bilkova	Immunoprecipitation of peptide fragments common to all Tau protein isoforms using phospho-insensitive magnetic immunosorbents for	9 – 13 March 2011	Barcelona, Spain	Scientific Community		

			diagnosis of Alzheimer´s disease					
109	Poster presentation,10th International Conference On Alzheimer´s & Parkinson´s Diseases (AD/PD 2011)	L. Korecka, M. Slovakova, B. Jankovicova, Z. Svobodova, C. Riviere, P. Prikryl, Z. Kucerova, L. Hernychova, I. Le Potier, M. Taverna, Z. Bilkova	Bioactive magnetic microreactors for structural modifications of Tau protein molecules usable in the study of phosphorylation process and diagnosis of Alzheimer´s disease	9 – 13 March 2011	Barcelona, Spain	Scientific Community		
110	Conference	American Chemical Society	American Society for Mass Spectrometry (ASMS)	20-24 May, 2012	Vancouver, Canada	Scientific Community, Industry	Ca 7000	All countries
111	Workshop	Institut Catalá de Nanotecnologia	3rd workshop on Analytical Instrumentation and Nanotechnologies	June 11-12, 2012	Barcelona, Spain	Scientific community	Ca 50	Mainly European

112	Conference	University of Pécs, Hungary	10th International Meeting on Bioanalysis (CECE)	April 25-27, 2013	Pécs, Hungary	Scientific Community	Ca 150	All countries
113	Conference	American Chemical Society	American Society for Mass Spectrometry (ASMS)	15-17 June 2014l	Baltimore, USA	Scientific Community, Industry	Ca 7000	All countries
114	Conference	Swedish Chemical Society	Analysdagarna	2014	Djuronäset, Sweden	Scientific Community. Industry	Ca 400	Mainly Sweden
115	Conference	American Chemical Society	American Society for Mass Spectrometry (ASMS)	2015	St. Louis, USA	Scientific Community. Industry	Ca 7000	All countries
116	Poster presentation at the Alzheimer's Association International Conference (AAIC) 2012 July 14 - 19, 2012; Vancouver, British Columbia	U. Haussmann	Modifications to Urea SDS Aβ-PAGE facilitate the resolution of N-terminally truncated Aβ peptides and of Aβ forms longer than 42 residues	July 14 - 19, 2012	Vancouver, British Columbia	Researchers		international

117	Poster presentation at the 11th International Conference on Alzheimer's & Parkinson's Diseases, Florence, Italy, March 6-10 (ADPD 2013)	U. Haussmann	Targeted BACE1 inhibition by optimised Tripartite structure inhibitors	March 6-10, 2013	Florence, Italy	Researchers		international
118	Posterpresentation at the DGPPN Kongress Berlin, 27.-31.11.2013 (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN))	R. Schnitzler	Differentieller Effekt von thermischem Stress auf MAP-Kinasepathways und Tau-Phosphorylierung in aviären Telenzephalonneuronen und Astrozyten	Nov. 27-31, 2013	Berlin, Germany	Clinicians and Researchers		Germany
119	Posterpresentation at the DGPPN Kongress Berlin, 27.-31.11.2013 (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN))	J. Genius	Induktion abnormer Tauphosphorylierung durch intermittierenden mechanischen Stress in Telenzephalonneuronen des Hühnchens. – Ein neues Modell für die Alzheimerdemenz und die chronisch-traumatische Enzephalopathie?	Nov. 27-31, 2013	Berlin, Germany	Clinicians and Researchers		Germany
120	Posterpresentation at the DGPPN Kongress Berlin, 27.-31.11.2013 (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN))	I. Kraus	Untersuchungen zur subzellulären Verteilung von mono- und diphosphorylierten Formen der MAP Kinasen ERK1 und ERK2 in SH-SY5Y und	Nov. 27-31, 2013	Berlin, Germany	Clinicians and Researchers		Germany

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121	Posterpresentation at the DGPPN Kongress Berlin, 27.-31.11.2013 (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN))	U. Haussmann	Gezielte β-Sekretase-Inhibition mit optimierten membranverankerten Tripartite-Inhibitoren	Nov. 27-31, 2013	Berlin, Germany	Clinicians and Researchers		Germany
122	Posterpresentation at the DGPPN Kongress Berlin, 27.-31.11.2013 (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN))	C. Janßen	Auftrennung und Detektion aminoterminal verkürzter Aβ-Peptide mittels kapillarbasierter isoelektrischer Fokussierung (CIEF) mit Immundetektion	Nov. 27-31, 2013	Berlin, Germany	Clinicians and Researchers		Germany
123	Conference Posters (2) and conference proceedings (2)	ICRM CNR	NSTI nanotechnology Conference & Expo - Nanotech 2013	May 12-16 2013	Washington DC, USA	Scientific community	1000	all
124	Conference Posters (2) and conference proceedings (2)	ICRM CNR	18th International Conference on Miniaturized Systems for Chemistry and Life Sciences	October 26-30, 2014	San Antonio TX, USA	Scientific community	1000	all

125	Conference Poster	ICRM CNR	WAM-NANO2012, III international Workshop on Analytical Miniaturization and Nanotechnologies	June 11,12 2012	Barcelona, Spain	Scientific community	100	all
126	Conference Poster	ICRM CNR	2nd International Conference on Alzheimer's Disease and Dementia	September 23-25, 2014	Valencia, Spain	Scientific community	300	all
127	Oral communication	ICRM CNR	AMT 2013 Adavances in Microarray Technology	March 5-6 2013	Barcelona, Spain	Scientific community	300	all
128	Oral communication	ICRM CNR	AMT 2014 Adavances in Microarray Technology	March 10-11 2014	Berlin, Germany	Scientific community	300	all
129	Oral communication	ICRM CNR	WAM NANO 2014 Fourth international workshop on Analytical Miniaturization and Nanotechnologies	23-24 giugno 2014	Copenhagen. Denmark	Scientific community	100	all

130	Oral communication	ICRM CNR	XXX International Symposium on MicroScale Bioseparations	April 27th-May 1st 2014	Pécs, Hungary	Scientific community	200	all
131	Oral communication	ICRM CNR	28th International Symposium on MicroScale Bioseparations and Analyses	October 21-24, 2012	Shanghai China	Scientific community	300	all
132	Oral communication	ICRM CNR	Multiplex Assays: Science Facts &Science Fiction, Workshop.	September 15-16, 2011	Berlin, Germany	Scientific community	200	all
133	Oral communication	ICRM CNR	Gordon Research Conference in Microfluidics Physics and Chemistry	une 26th - July 1st 2011	Waterville Valley, NH, USA	Scientific community	300	all
134	Oral communication	ICRM CNR	amic 2010, Synthesis and Methodologies in Inorganic Chemistry.	November 28th – December 1st	Bressanone (Italy)	Scientific community	100	all

135	Conference	Malaquin	MicroTAS 2011	October 2011	Seatle USA	Scientific community		international audience
136	Conference	Descroix	MSB	January 2012	Geneva, Switzerland	Scientific community		international audience
137	Invited Conference	Descroix	ITP	September 2012	Baltimore, USA	Scientific community		international audience
138	Invited Conference	Viovy	EMBL, Microfluidics	July, 2012	Heidelberg, Germany	Scientific community		international audience
139	Conference	Malaquin	MicroTAS 2012	October 2012	Okinawa, Japan	Scientific community		international audience

140	Conference	Viovy	Nanobiotech	November 2013	Montreux, Switzerland	Scientific community		international audience
141	Invited Conference	Descroix	Chiranal	February 2014	Olomouc, Czech Republic	Scientific community		international audience
142	Invited Conference	Descroix	WAM Nano	June 2014	Copenhaguen, Denmark	Scientific community		international audience
143	Conference	Viovy	EMBL, Microfluidics	Juky 2014	Heidelberg, Germany	Scientific community		international audience
144	Conference	Descroix	Innovation in Paris Hospital	November 2014	Paris, France	Scientific community		national audience Medical doctors

145	Poster:Functionalization of embedded thiol-ene waveguides for evanescent wave-induced fluorescence detection in a microfluidic device	Nikolaj A. Feidenhans'l, Thomas Glasdam Jensen, Josiane P. Lafleur and Jörg P. Kutter,	MicroTAS 2013	2013	Freiburg,Germany	Scientific community	>2000	International
146	Poster, Thiol-EneWaveguides As Promising Components Of Optofluidic Microsystems	Radoslaw Kwapiszewski, Thomas G Jensen, Klaus B Mogensen, Zbigniew Brzozka and Jörg P Kutter	MicroTAS 2012	2012	Okinawa, Japan	Scientific community	>2000	International
147	Poster, Thiol-ene based polymer waveguides fabricated by uv-assisted soft lithography for optofluidic applications,	Guisheng Zhuang, Thomas G. Jensen, and Jšrg P. Kutter	MicroTAS 2011	2011	Seattle, USA	Scientific community	>2000	International