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ECXX_LABYY_ NOMChercheur_ Numer
ECXX = ECLi, ECL, ECM, ECN, CS
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PhD Proposal 2017

School: Ecole Centrale de Lyon	
Laboratory: INL	**Web site: http://inl.cnrs.fr/fr**
Team: Chimie et Nanobiotechnologies	**Head of the team: Dr. Yann Chevolot**
Supervisor: Pr. Magali Phaner-Goutorbe Dr. Christelle Yeromonahos	**Email: christelle.yeromonahos@ec-lyon.fr**
Collaboration with other partner during this PhD: In France: CEA LETI (Dr. Ali Bouamrani) Synchrotron SOLEIL (Dr. Jean-Jacques Gallet)	**In China:**

Title: Bio-functionalized nanoporous Silicon arrays for metabolites based diagnostic at the bedside
Scientific field: Nanobiotechnologies
Key words: biofunctionalized nanoporous Si, metabolites, human biological fluids, (NAP)-XPS, FTIR, PM-IRRAS, mass-spectrometry, AFM, molecular dynamics, statistical analysis, diagnostic

Details for the subject:

(Maximal length of 2 pages, including images, list of reference, …The pdf file should not exceed 1Mo)

Background, Context:

The accurate annotation of human pathological fluids for early disease detection, therapeutic response prediction, and disease progression evaluation is a medical priority. However, the intrinsic complexity of the samples limits the detection of metabolites. Metabolites are Low Molecular Weight (LMW) disease markers of hypertension, brain damages, presbycousis…

They are present in trace amounts within a background of abundant non-relevant molecules. Additionally, the detection of these LMW species remains a challenge due to experimental variability, their low stability at a few minutes scale, and generation of artifacts as a consequence of unreliable experimental procedures.(Yeromonahos 2014) In the clinical field, there is an urgent need for developing techniques to detect intact metabolites from complex human fluids as urine or plasma.

Methods combining mass spectrometry and fast sample preparation strategies based on nanoporous Silicon or organosilicate thin films are emerging. Nanoporous Silicon or nanoporous SiOCH thin films with tunable features at the nanoscale (wettability, C content, pore size, pore density…) are fabricated on Silicon substrates by electrochemical techniques (Morin 2014) or by plasma enhanced chemical vapor deposition.(Yeromonahos 2015) A few microliters of urine or plasma are spotted onto the nanoporous millimeter sized chip allowing metabolites to be trapped in the pores while larger species are removed from the surface of the chip. Finally mass spectra of sample fractionated on the nanoporous chip result both in a significant improvement of metabolites detection, and in metabolites stabilization. About 1000 metabolites can be detected simultaneously. Blind statistical analysis (PCA) of such mass spectra can discriminate groups of highly pathological samples from groups of wealthy samples (*e.g.* cardiovascular disease combined with hypertension, neurotoxin in concentrations which cause brain damages, presbycousis…).(Yeromonahos 2015)

Nevertheless discrimination of the many intermediate disease states is currently impossible and reflects a lack of sensitivity in metabolites harvesting and detection. So, adequate sample preparation strategies remain an extraordinary technical challenge.

To address the sample preparation challenge that limits the use of mass spectrometry in biomarkers detection, we propose to functionalize nanoporous Silicon chip for specific metabolites harvesting. To identify for each sub-class of metabolites the most appropriate functionalization molecule(s), we will characterize and model interactions at the interface between metabolites and functionalized surface.

Research subject, work plan:

The project concerns the bio-functionalization of porous Silicon for the specific harvesting of metabolites. For this purpose interactions between metabolites and chemically modified porous silicon will be modeled. Different functionalization molecules, organized in self-assembled monolayer on the Silicon substrate, will be evaluated. These molecules will be composed of a silane (link to the substrate), an alkyl chain (different lengths will be investigated as well as the presence of a cross-linker), and a functionalization head (carboxyl, diol, hydroquinone, amine, vinyl, methyl). (Palazon 2014)

Substrates will be multi-functionalized, at INL, in the form of milli-arrays for parallel screening of dozens of interactions. Interactions between purified metabolites and functionalization molecules will be systematically characterized at INL by infrared spectrometry in liquid phase (PM-IRRAS), XPS, fluorescence.(Cloarec 2014) Interactions will be characterized by mass spectrometry at CEA (Grenoble, France). Also, interactions between selected metabolites / bio-functionalization molecules systems will be characterized in liquid phase at different pH by Near-Ambient-Pressure XPS on synchrotron (SOLEIL, TEMPO beam-line, France) and by Single Molecule Force Spectroscopy a spectroscopic mode of the Atomic Force Microscopy(Phaner 2016) at INL. Molecular Dynamic simulations(Huang 2011, Emami 2014) and statistical analysis (Yeromonahos 2015) will be developed and coupled to experimental characterizations to model and to predict interactions and to identify for each sub-class of purified metabolites the most appropriate functionalization molecule(s). Then, the case of complexes samples (urine, plasma) will be evaluated. Finally, the ability of the technique to discriminate different stages of a cardiovascular disease will be evaluated.

Three main tasks are identified:

1- Development of a characterization and modeling platform to identify the best functionalization molecule(s) to optimize harvesting of each sub-class of purified metabolites. Substrate will be plain Silicon.
2- Expansion of the characterization and modeling platform (from task 1) to the study of interactions at functionalized porous Silicon interfaces. Identification of the best functionalization molecule(s) to optimize harvesting of each sub-class of purified metabolites on porous Silicon.
3- Evaluation of functionalized porous Silicon (from task 2) as a diagnostic tool.

Expected results:

Our overall objective is the development of a low-cost and manufacturable platform, based on chemically modified nanoporous Silicon, to offer a significant enhancement in selectivity and sensitivity of metabolites recovery from complex biological fluids for mass spectrometry analysis. This platform will offer simple and fast sample acquisition and simple sample long-term storage protocol, and is expected to provide enormous enhancement for LMW biomarker discovery, diagnostic and prognostic.

In addition this project will generate a database classifying affinities between metabolites and molecules of functionalization.

Also, this project will lead to the development of an innovative and state of the art experimental and modeling characterization platform for the general study of biointerfaces.

The PhD candidate will work at INL in the Chemistry and Nanobiotechnologies group at Ecole Centrale de Lyon in France. This group has a strong background in surface bio-functionalization techniques and protein detection by fluorescence, SPR, and PM-IRRAS. XPS equipment and a cluster to run simulations are also available at INL for this project. The candidate will work in strong interaction with the different partners involved in the project: The group of Dr. Bouamrani (CEA France) for mass spectrometry experiments and the realization of the task 3, and Dr. Gallet (Synchrotron SOLEIL TEMPO beam line France) for NAP-XPS experiments through proposal applications.

A background in chemistry, physics or materials is required for the PhD candidate. The candidate must like experimental work and computational biochemistry. The knowledge of experimental techniques (such as infrared spectroscopy, XPS and AFM) and / or of simulations (such as molecular dynamics, Monte Carlo, DFT) will be appreciated. The candidate must be able to work at the interface of several domains (chemistry, physics and biology). He/she must speak English fluently in order to communicate the scientific results during the thesis and learn French during his/her stay in France.

References:

(Yeromonahos 2014) C. Yeromonahos et al, Nanoporous organosilicates chips for selective enrichment of metabolites. May 2014. Oral Communication. Biosensors Annual Meeting, Melbourne, Australia.

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(Palazon 2014) F.Palazon, Surface functionalization of heterogeneous gold/silica substrates for the selective anchoring of biomolecules and colloids onto LSPR biosensors, *PhD Thesis*, Ecole Centrale de Lyon – Université de Lyon, 2014.

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(Huang 2011) D. Huang et al, Small molecules binding to proteins: affinity and binding / unbinding dynamics from atomistic simulations, *ChemMedChem*, 2011, 6, 1578 – 1580.

(Emami 2014) F. Emami et al, Force Field and a Surface Model Database for Silica to Simulate Interfacial Properties in Atomic Resolution, *Chem. Matter*, 2014, 26, 2647– 2658.