



# Chemical Complexity & Biology

## Opening lecture

**Jean-Marie LEHN**

*ISIS, Université de Strasbourg, France*

## Keynote speakers

**Gonen Ashkenasy**

*Ben Gurion University of the Negev, Israel*

**Emilie Chouzenoux**

*Université Paris-Est, Marne-la-Vallée, France*

**Camille Couprie**

*IFP Energie Nouvelle, Rueil Malmaison, France*

**Philippe Dumas**

*IBMC, Université de Strasbourg, France*

**Vincent J. Hilser**

*Johns Hopkins University, Baltimore, USA*

**Robert Pascal**

*Université Montpellier, France*

**Douglas Philp**

*University of St-Andrews, UK*

**Christian Rolando**

*Université de Lille 1, France*

**Jean-Pierre Simorre**

*IBS, Université Joseph Fourier, Grenoble, France*

**Carine van Heijenoort**

*ICSN, Université Paris-Sud Orsay, France*

**Roman A. Zubarev**

*Karolinska Institute, Stockholm, Sweden*

**January 19<sup>th</sup>-20<sup>th</sup> 2015**

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**Inserm**





# Chemical Complexity & Biology

19<sup>th</sup> Jan 2015

Dear participant to the **Chemical Complexity & Biology** symposium,

Life is a complex phenomenon indeed and the purpose of this meeting is to explore these complex systems at the molecular level, in their theoretical as well as experimental aspects. On these aspects, there are many convergences between supramolecular chemistry and biochemistry, measure and big-data, modeling and mathematical analysis, etc. Mathematical is the natural language of science and chemistry the language of matter, the idea behind this symposium is to build some bridges between these different realms.

The purpose of the meeting is to invite scientists from various fields in order to have a rich and fruitful discussion on these topics, and to show to the communities of biologists, chemists and analysts, the links that exist between all these approaches. Complexity is a buzzword these days, but we gather not to follow a fashion trend but because this complexity is the engine which drives this world and the very foundation of our interest in sciences.

We want to thank the many talented scientists, coming from fields as diverse as Chemistry, Mathematics, Spectroscopy or Biology, who have accepted to present here their deeper ideas and finest results on these aspects.

This two-day symposium has been made possible by the joint efforts of two groups: the MeasureHD CNRS action, which gather experimentalists and theoreticians on the aspects of the measure of the large and complex objects studied in biophysics, and the ReAd European ITN network working and training early researchers on the aspects of complex interacting molecular networks.

We want to thank also Céline and Julia as well as all the IGBMC staff who have permitted this symposium to take place, as well as our numerous sponsors, thanks to whom this meeting is organized in the best possible manner.

Marc-André Delsuc & Bruno Kieffer  
Co-chairs

***Program***

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# Chemical Complexity & Biology

January, 19<sup>th</sup> - 20<sup>th</sup> 2015

## Program

### Day 1: Monday, January 19<sup>th</sup>

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08.30 – 09.00	<i>Registration</i>	
09.00 – 09.15	Welcome	<b>Marc-André Delsuc</b>
09.15 - 10.15	<b>Opening lecture</b> Towards adaptive chemistry - constitutional dynamic chemistry and networks	<b>Jean-Marie Lehn</b>
10.15 – 10.45	Theoretical and experimental challenges in the Complex Systems Digital Campus	<b>Paul Bourguine</b>
10.45– 11.15	<i>Coffee break</i>	
11.15 – 11.55	Molecular interactions with the bacterial cell wall by liquid state, standard and DNP solid state NMR	<b>Jean-Pierre Simorre</b>
11.55 – 12.35	Parallel tuning of activation and repression in intrinsic disorder-mediated allostery	<b>Vincent J. Hilser</b>
12.35– 14.00	<i>Lunch buffet and poster session</i>	
14.00 – 14.40	Proximal methods: tools for solving inverse problems in a large scale. Application to biophysics measurements processing	<b>Emilie Chouzenoux</b>
14.40 – 15.00	Monitoring protein-ligand interactions in human carbonic anhydrase by long-range pseudo contact shift NMR	<b>Daniel Häussinger</b>
15.00 - 15.40	2D FT-ICR MS: from a laboratory curiosity to an analytical tool	<b>Christian Rolando</b>
15.40– 16.10	<i>Coffee break</i>	
16.10 – 16.50	How scarce sequence elements control the function of single $\beta$ -thymosin/WH2 intrinsically disordered domains in actin assembly	<b>Carine van Heijenoort</b>
16.50 – 17.10	m1A modification in tRNA	<b>Carine Tisné</b>
17.10 – 17.30	Prion quasi-species and molecular basis of auto-perpetuation of Prion structural information	<b>Human Rezaei</b>



## Day 2: Tuesday, January 20<sup>th</sup>

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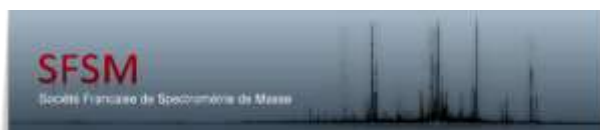
09.00 – 09.40	Dr. Ehrich's magic bullet: is perfect anticancer drug possible?	<b>Roman A. Zubarev</b>
09.40 – 10.20	Identifying the conditions needed for stability and complexity in living systems	<b>Robert Pascal</b>
10.20 – 10.40	Study of hetero-association between tetracycline and caffeine by means of diffusion-ordered NMR spectroscopy	<b>Anatoly Buchelnikov</b>
10.40 – 11.10	<i>Coffee break</i>	
11.10 – 11.50	Unnatural Information-containing Macromolecules	<b>Jean-François Lutz</b>
11.50 – 12.10	Chemical cross-linking and mass spectrometry to determine the interaction network of protein complexes	<b>Nha-Thi Nguyen-Huynh</b>
12.10 – 12.30	Sensing of analytes using fluorescent amphiphiles	<b>Ziya Kostereli</b>
12.30 – 14.00	<i>Lunch buffet and poster session</i>	
14.00 – 14.40	Gene regulatory network inference using structural biological a priori	<b>Camille Couprie</b>
14.40 – 15.00	Solid-state NMR as an emerging technique to determine supramolecular interactions in biological and chemical complexes	<b>Antoine Loquet</b>
15.00 – 15.30	<i>Coffee break</i>	
15.30 – 15.50	Membrane structure and interactions of the amino-terminus of huntingtin and its regulatory role in poly-glutamine aggregation	<b>Burkhard Bechinger</b>
15.50 – 16.30	Kinetic mechanism of RNA-mediated genetic regulation	<b>Philippe Dumas</b>

*End of the symposium*

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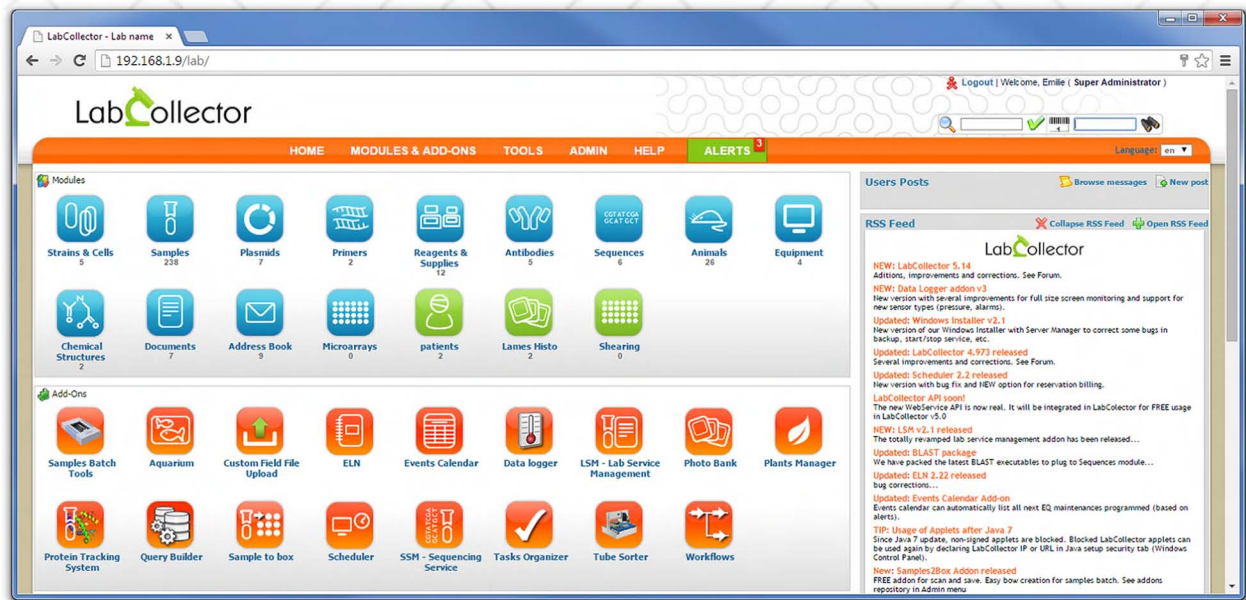
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## ***Speakers abstracts***

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# Steps towards Complex Matter: from Supramolecular Chemistry to Adaptive Chemistry

Jean-Marie Lehn

ISIS, Université de Strasbourg, France

*Supramolecular chemistry* lies beyond molecular chemistry and aims at constructing highly complex chemical systems from components held together by non-covalent intermolecular forces. It explores systems undergoing *self-organization*, capable of generating well-defined functional supramolecular architectures by *molecular information controlled* self-assembly from their components, thus behaving as *programmed chemical systems*.

Supramolecular chemistry is intrinsically a *dynamic chemistry* in view of the lability of the interactions connecting the molecular components of a supramolecular entity and the resulting ability of supramolecular species to exchange their components. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form and break reversibly, so as to allow a continuous change in constitution by reorganization and exchange of building blocks. These features define a *Constitutional Dynamic Chemistry* (CDC) on both the molecular and supramolecular levels.

CDC introduces a paradigm shift with respect to constitutionally static chemistry. It takes advantage of dynamic diversity to allow for variation and selection. It opens to *self-organization with selection* and operates on dynamic constitutional diversity in response to either internal or external factors to achieve *adaptation*.

In the process of reaching higher levels of self-organisation, CDC gives access to the generation of networks of dynamically interconverting constituents connected either structurally (molecular and supramolecular arrays) or reactionally (set of connected reactions) or both. They define a class of *constitutional dynamic networks* (CDNs), presenting *agonistic* and *antagonistic* relationships between their constituents, that may couple to thermodynamic or kinetic processes and respond to perturbations by physical stimuli or to chemical effectors.

These concepts and their implementation in biological systems as well as in materials science will be described.

The merging of the features: - information and programmability, - dynamics and structural diversity, - constitution and selection, points to the emergence of *adaptive* and *evolutive chemistry*, towards *systems of increasing complexity* and a *science of complex matter*.

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Lehn, J.-M., *Supramolecular Chemistry: Concepts and Perspectives*, VCH Weinheim, 1995.

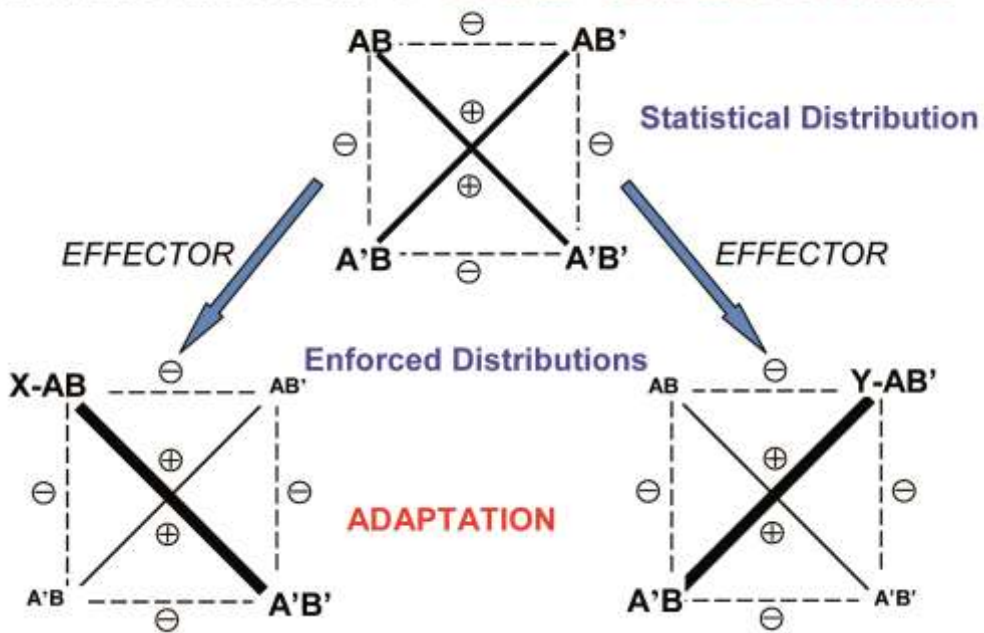
Lehn, J.-M., Toward complex matter: Supramolecular chemistry and self-organization, *Proc. Natl. Acad. Sci. USA*, **2002**, 99, 4763.

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**DYNAMIC NETWORKS : Towards COMPLEX SYSTEMS**



# Theoretical and experimental challenges in the Complex Systems Digital Campus"

Paul Bourguine

*ISC-PIF, 113 rue Nationale, 75013 Paris*

The main aim of the Complex Systems Digital Campus (a UNESCO UniTwin network comprising more than 100 universities and research institutions) is to be the place for sharing all necessary research and educational resources for dealing with the trans-disciplinary challenges of complex systems. The CS-DC is developing two kinds of international e-departments depending of two main ways for launching trans-disciplines:

- Each **theoretical** e-department is starting with one main theoretical challenge. It is looking, with the help of its e-laboratories, at the theories, methods and algorithms for dealing with this challenge through the different classes of complex systems. This starting point is Complex Systems Science.
- Each **experimental** e-department is starting with the experimental challenge of one main class of complex systems. It is looking, with the help of its e-laboratories, at the different main theoretical challenges for its class of complex systems. This starting point is the one of the integrative and predictive sciences, like Complex Matter Physics or Chemistry, integrative Biology or Ecology, integrative Cognitive Science and Social Science, Socio-technical Network Science, ...

The scientific dynamics from these two starting points are dual with each other, covering the same open questions and complex systems classes at the intersections of the same matrix. Each intersection is the place for a project-team with its double bottom-up link with at least one theoretical e-laboratory and one experimental e-laboratory. Such project-teams are the main instruments for organizing permanently the natural convergence between theoretical and experimental challenges. This "natural convergence" is of the same kind as the reciprocal co-evolution of mathematics and physics in the last centuries.



# Molecular interactions with the bacterial cell wall by liquid state, standard and DNP solid state NMR.

<sup>¶</sup>C. Bougault, <sup>¶</sup>C. Laguri, <sup>¶</sup>N. Jean, <sup>+</sup>S. Hediger, <sup>+</sup>H. Takahashi, <sup>#</sup>M. Arthur, <sup>§</sup>W. Vollmer, <sup>¶</sup>Jean-Pierre Simorre

<sup>¶</sup> Institut de Biologie Structurale Grenoble, France.

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<sup>#</sup> Centre de Recherche des Cordeliers, Paris, France

<sup>§</sup> University of Newcastle, Newcastle, U.K.

The cell wall is essential for the survival of bacteria. It gives the bacterial cell its shape and protects it against osmotic pressure, while allowing cell growth and division. It is made up of peptidoglycan (PG), a biopolymer forming a multi-gigadalton bag-like structure, and additionally in Gram-positive bacteria, of covalently linked anionic polymers called wall teichoic acids (WTA).

The machinery involved in the synthesis of this envelop is crucial and is one of the main antibiotic target. Different protein as transpeptidase, transpeptidase activator or hydrolase are recruited to maintain the morphogenesis of the peptidoglycan during the bacterial cell cycle. Based on few examples involved in the machinery of synthesis of the peptidoglycan, we will demonstrates that a combination of liquid and solid-state NMR can be a powerful tool to screen for cell-wall interacting proteins *in vitro* and *on cell*.

In particular, structure of the L,D-transpeptidases that results in *b*-lactam resistance in *M. tuberculosis*, has been studied in presence of the bacterial cell wall and in presence of antibiotic. The NMR study reveals new insights into the inhibition mechanism.

In parallel, we have investigated the potential of Dynamic Nuclear Polarization (DNP) to investigate cell surface directly in intact cells. Our results show that increase in sensitivity can be obtained together with the possibility of enhancing specifically cell-wall signals. It opens new avenues for the use of DNP-enhanced solid-state NMR as an on-cell investigation tool.

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Egan, A.J., Jean, N.L., Koumoutsi, A., Bougault, C.M., Biboy, J., Sassine, J., Solovyova, A.S., Breukink, E., Typas, A., Vollmer, W. and **Simorre, J.-P.** (2014). Outer-membrane lipoprotein LpoB spans the periplasm to stimulate the peptidoglycan synthase PBP1B. **PNAS** *111*, 8197-8202.

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Lecoq, L., Dubée, V., Triboulet, S., Bougault, C., Hugonnet, J.E., Arthur, M., and **Simorre, J.P.** (2013). Structure of Enterococcus faecium l,d-transpeptidase acylated by ertapenem provides insight into the inactivation mechanism. **ACS Chem Biol** *8*, 1140-1146.

Takahashi H, Ayala I, Bardet M, De Paëpe G, Simorre JP, Hediger S. Solid-State NMR on Bacterial Cells: Selective Cell Wall Signal Enhancement and Resolution Improvement using Dynamic Nuclear Polarization. **J Am Chem Soc** 2013.

Kern, T., Giffard, M., Hediger, S., Amoroso, A., Giustini, C., Bui, N.K., Joris, B., Bougault, C., Vollmer, W., and **Simorre, J.P.** (2010). Dynamics characterization of fully hydrated bacterial cell walls by solid-state NMR: evidence for cooperative binding of metal ions. **J Am Chem Soc** *132*, 10911-10919.

# Simultaneous Tuning of Activation and Repression in Intrinsic Disorder-Mediated Allostery

Vincent J. Hilser

*Johns Hopkins University, Baltimore, United States*

Intrinsically disordered proteins (IDPs) present a functional paradox because they lack stable tertiary structure, but nonetheless play a central role in signaling. Like their structured protein counterparts, IDPs can transmit the effects of binding an effector ligand at one site to another functional site, a process known as allostery. Because allostery in structured proteins has historically been interpreted in terms of propagated structural changes that are induced by effector binding, it is not clear how IDPs, lacking such well-defined structures, can allosterically affect function.

Here we show mechanistically how IDPs allosterically transmit signals through a probabilistic process that originates from the simultaneous tuning of both activating and repressing ensembles of the protein, using human glucocorticoid receptor as a model. Moreover, GR modulates this signaling by producing translational isoforms with variable disordered regions. We expect this ensemble model of allostery will be important in explaining signaling in other IDPs.

# **Proximal methods: tools for solving inverse problems in a large scale. Application to biophysics measurements processing.**

Emilie Chouzenoux

*Université Paris Est, Marne-la-Vallée, Laboratoire d'Informatique Gaspard Monge*

In a wide range of recent biophysic instrumental techniques (3D Nuclear Magnetic Resonance Spectroscopy, High Resolution Mass Spectrometry, etc), very large scale optimization problems need to be solved to retrieve the quantities of interest from the acquired measurements.

The goal of this talk is to provide a comprehensive view of a class of optimization algorithms which have been very successful in these areas over the last years: proximal splitting algorithms. These tools allow us to provide a unifying answer to a number of classical dilemma arising when solving optimization problems: constrained vs unconstrained approaches, smooth vs nonsmooth formulations, parallel vs sequential programming,...

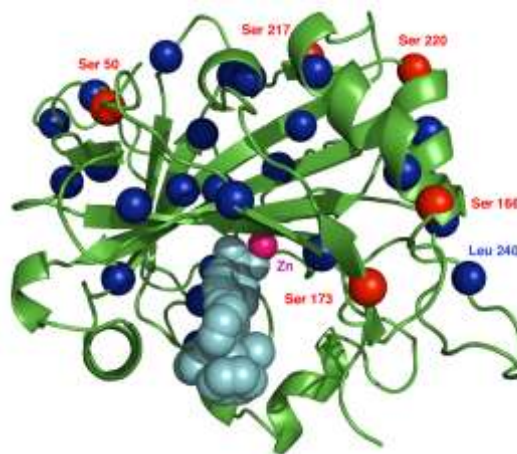
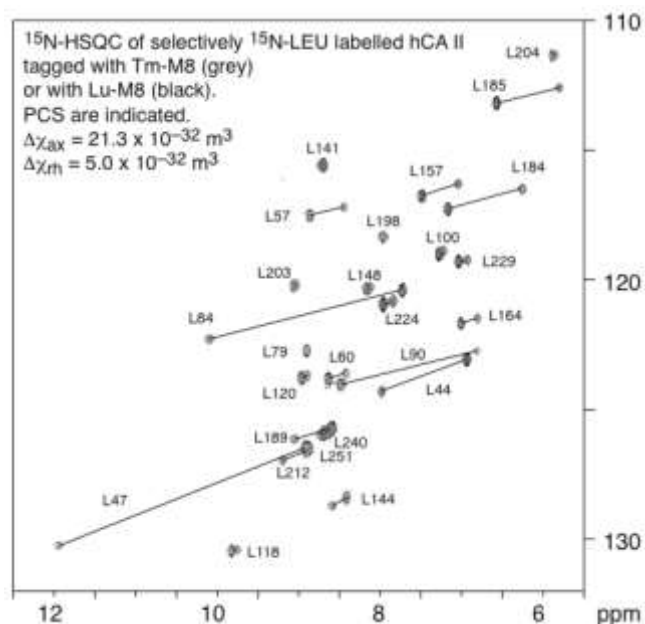
The presentation will be mainly focused on new developments in this area aiming at increasing the convergence speed of these algorithms and making them easily implementable on multicore or distributed computing architectures. We will see that the Majorization-Minimization principle is at the core of many recent advances. Illustrations of these methods on signal reconstruction examples in the context of biophysics will be provided.

# Monitoring Protein–Ligand Interactions in human Carbonic Anhydrase by Long–Range Pseudo Contact Shift NMR.

Kaspar Zimmermann, Heiko Gsellinger and Daniel Häussinger\*

Department of Chemistry, University of Basel, Basel, Switzerland; [daniel.haessinger@unibas.ch](mailto:daniel.haessinger@unibas.ch).

The NMR characterization of protein – protein complexes and protein – ligand complexes by classical, NOE-based methods is a difficult and laborious task due to the short range of the NOE effect. In contrast, synthetic lanthanide chelating tags (lct) that are site-specifically attached via a cysteine thiol to a protein induce pseudo-contact shifts (pcs) in the nuclei of the protein over a distance of up to 70 Å. This long-range effect can be used to determine structure and dynamics of proteins and their complexes by simple and sensitive one- and two-dimensional NMR experiments [1]. We have recently developed [2] an unusually rigid, high affinity lct, DOTA-M8. Here we present applications of this tag to the monomeric 261 residue protein human carbonic anhydrase type II (hCA II). Host guest transition metal complexes bound to hCA II have successfully been applied as synthetic metalloenzymes in homogeneous catalysis [3]. Five different serine to cysteine mutants of hCA II have been designed and the DOTA-M8 tag, loaded with diamagnetic lutetium or with paramagnetic thulium was conjugated to the hCA II. We have thus obtained five linear independent susceptibility tensors that allow the exact positioning in space of any nucleus with an observed pcs in a complex with hCA II. The PCS NMR experiments presented demonstrate the structural characterization of a complex between hCA II and a fluor-containing inhibitor by simple one-dimensional  $^{19}\text{F}$ -NMR experiments. The methodology can in principle be applied to even much larger protein complexes as long as meaningful (TROSY)-HSQC ( $^{15}\text{N}$  or  $^{13}\text{C}$ ) of the protein can be obtained.



1. Hass, M.A.S., Ubbink, M., *Curr Opin. Struct. Biol.* 24, 45-53, (2014).
2. Häussinger, D., Huang, J.-R., Grzesiek, S., *J. Am. Chem. Soc.*, 131, 14761-14767, (2009).
3. Monnard, F.W., Heinisch, T., Nogueira, E.S., Schirmer, T., Ward, T.S., *Chem. Commun.*, 47, 8238-8240, (2011).

## 2D FT-ICR MS: from a laboratory curiosity to an analytical tool

Fabrice Bray, Lionel Chiron, Marc-André Delsuc, Christian Rolando

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2D FT-ICR MS has received little attention relative to 2D NMR since its introduction in 1987. The main drawbacks of the original version of the 2D FT-ICR MS were: a loss of resolution caused by in ICR-cell CID, difficulty of processing data at full resolution FT-ICR MS and the intense noise due to ion fluctuation. In recent years, we have revisited 2D FT-ICR and presented solutions to these problems using methods of fragmentation without gas such as IRMPD and ECD, developing software that can handle files of several gigabytes of data and introducing an innovative algorithm based on mathematical theory sparsity for noise reduction. Acquisition takes about an hour in the order of a LC run. Here we present a procedure for speeding the acquisition.

We recorded the FT-ICR mass spectra with optimized 2D pulse sequence we previously designed. In a first time non-uniform spectra of the sample were obtained by extraction of random values from the spectra uniformly sampled. Reconstruction by a new approach has been done with the SPIKE (Spectrometry Processing Innovative Kernel) which was adapted to the size of the FT-ICR data by implementing a 64-bit version.

So far, we acquired 2D spectra with quadrupole resolution in the first dimension and resolution FT-ICR in the second dimension. In the second dimension, the full resolution costs only a little additional acquisition time due to longer FID. In the first dimension ions can be used only once because they are irreversible fragmented to the opposite of NMR in which the nuclei can be observed again. The first resolution size is very time consuming as the quadrupole resolution requires the recording of two thousand spectra leading to an acquisition period of two hours. 2D and nD NMR used regularly non-uniform sampling and Maximum Entropy reconstruction in particular for the analysis of proteins. This gain can be used to reduce the acquisition time or to increase the resolution at constant acquisition time. Unfortunately, the higher size of FT-ICR spectra compared to NMR one's precludes the use of Maximum Entropy reconstruction. We will present here the gain in resolution obtain with different sampling strategies (randomly, linearly or exponentially spaced) with new algorithms (urQRd, Recital) developed for FT-ICR data.

The power of 2D FT-ICR MS will be illustrated by the analysis of triacylglycerol neutral lipids (TAG) from human plasma. From the 2D spectrum, TAGs with a total carbon of 54 to 64 saturated or with insaturation numbers from 14 to 4 may be identified and their individual fatty acid composition determined. We also found oxidized TAGs that are difficult to elute by chromatography. Families of TAGS are easily distinguished on the 2D mass spectra thanks to neutral loss lines. In one 2D mass spectrum obtained from 25 µL of human plasma over 50 TAGs were identified.



# How scarce sequence elements control the function of single $\beta$ -thymosin/WH2 intrinsically disordered domains in actin assembly

Célia Deville<sup>1</sup>, Francois-Xavier Cantrelle<sup>1</sup>, Dominique Didry<sup>2</sup>, Clotilde Husson<sup>2</sup>, Pierre Roblin<sup>3</sup>, Javier Perez<sup>3</sup>, Eric Guittet<sup>1</sup>, Marie-France Carlier<sup>2</sup>, Louis Renault<sup>2</sup> and Carine van Heijenoort<sup>1</sup>

<sup>1</sup> *Laboratoire de Chimie et Biologie Structurales, ICSN-CNRS, 91198 Gif-sur-Yvette, France*

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<sup>3</sup> *Synchrotron SOLEIL, BP 48 Saint Aubin, 91192 Gif-sur-Yvette, France*

Intrinsically Disordered Domains/Regions (IDRs) appear more and more widespread in eukaryotic proteins, especially those implied in regulatory and signaling processes [1]. We will here focus on the case of actin-binding proteins (ABPs), which often exhibit complex multi-domain architectures integrating and coordinating multiple signals and interactions with the dynamic remodeling of actin cytoskeleton [2]. In most modular ABPs, IDRs relay labile interactions with multiple partners and act as interaction hubs in inter-domain and protein–protein interfaces. They thereby control multiple conformational transitions between inactive and active states and play an important role to coordinate the high turnover of interactions in actin self-assembly dynamics. Understanding the functional versatility of IDRs, here in actin assembly, requires deciphering their conformational plasticity and dynamics by multiple structural and functional approaches.

$\beta$ -thymosin ( $\beta$ T) and WH2 domains are archetypal intrinsically disordered domains that fold upon binding actin. They display significant sequence variability associated to versatile regulations of actin assembly in motile processes [3]. Here we reveal the structural basis by which, in their basic 1:1 stoichiometric complexes with actin, they either inhibit assembly by sequestering actin monomers (Thymosin- $\beta$ 4), or enhance motility by directing polarized filament assembly (Ciboulot  $\beta$ T or WASP/WAVE WH2 domains). Combined mutational, functional, and structural analysis by X-ray crystallography, SAXS and NMR on Thymosin- $\beta$ 4, Ciboulot and the WH2 domain of WASP-interacting protein (WIP) allowed us to show that functionally different  $\beta$ T/WH2 domains do not target alternative actin binding sites but rather differ by alternative dynamics of their C-terminal half interactions with G-actin pointed face. We decipher how this interaction dynamics can be controlled by various subtle variations along the whole sequence of these small intrinsically disordered domains. In particular, it depends in specific cases on the presence of a unique ionic interaction in the central part of the sequence [4]. The results open perspectives for elucidating the functions of  $\beta$ T/WH2 domains in other modular proteins and enlighten how intrinsic structural disorder can lead to a novel mode of functional versatility.

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# Study of hetero-association between tetracycline and caffeine by means of diffusion-ordered NMR spectroscopy

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The ability of molecules to form non-covalent aggregates is one of the keystones of molecular and chemical physics and related fields of science. The most interesting is quantitation of intermolecular aggregation, for example, evaluation of association constants, enthalpy, entropy of complexation, etc.

One of the most well-studied interactions are those between aromatic compounds [1]. In turn, a big part of them was studied by means of NMR spectroscopy which has become a widely used method for analysis of such interactions. The common approach is to carry out a dilution titration (or variable temperature) experiment and subsequent analysis of obtained concentration (or temperature) dependences with the aid of statistical thermodynamic models [2]. However, in this case the error of determination of thermodynamic quantities of complexation is very high.

We propose a new approach to study the association of aromatic as well as non-aromatic (for instance, minor groove binders) molecules. This approach is based on the use of diffusion ordered NMR spectroscopy (DOSY NMR) data. In fact, one experiment results in only one curve (concentration or temperature dependence of self-diffusion coefficients) which, however, contains information about the whole molecular complex. We have proposed a similar statistical thermodynamic model previously [3] for study of self-association of aromatic molecules and minor groove binders. Now we suggest further improvement of this model, particularly, for study of hetero-association of aromatic molecules and minor groove binders. The model is slightly more complicated than the previously proposed one [3] and consists of both theoretical and computational parts.

In order to verify the model proposed we carried out titration and variable temperature DOSY NMR experiments to study hetero-association of well-known antibiotic tetracycline and caffeine. The choice of these compounds is due to huge amount of data available in literature, so one can easily compare our results with published to date.

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# Prion quasi-species and molecular basis of auto-perpetuation of Prion structural information.

Davy Martin<sup>1</sup>, Joan Torrent i Mas<sup>1</sup>, Stéphanie Prigent<sup>1</sup>, Vincent Béringue<sup>1</sup> and Human Rezaei<sup>1\*</sup>

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The prion phenomenon is based on autonomous structural information propagation towards single or multiple protein conformational changes.

Since this last decade the prion concept referring to the transmission of structural information has been extended to several regulation systems and pathologies including Alzheimer and Parkinson's diseases. The unified theory in Prion replication implies structural information transference (SIT) from the prion to a non-prion conformer through a mechanism also called improperly, with regards to biophysical considerations "seeding" phenomenon.

Therefore considering prion replication as a structural information transduction from a donor (*i.e.* template) to an acceptor (*i.e.* substrate) through a transduction interface a new questioning arises: what are molecular mechanisms of the autoperpetuation of the Prion structural information and its faithfulness? Considering the Prion propagation as more or less faithful perpetuation of structural information, in the present work, we explored the concept of prion quasi-species (*i.e.* existence of prion heterogeneous assemblies) and highlighted the existence of prion network, which has an autopoietic behaviour (auto-replicative).

Our observations strongly suggest that specific criteria in term of protein structure, delay-process and thermo-kinetics should be collated before a system become dissipative and autopoietic.

## Dr. Ehrlich's magic bullet: is perfect anticancer drug possible?

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In 1906, the great Paul Ehrlich (1854–1915) has predicted that chemists will soon be able to synthesize substances that bind to and deactivate specific disease-causing agents, while being nontoxic for normal cells. He called these substances “magic bullets,” and the approach to use them - chemotherapy. A century of innumerable attempts to find a magic bullet against cancer has had a limited success. Cancer is now becoming the #1 killer in the industrialized world, while only a fraction of patients positively respond to chemotherapy. National Cancer Institute (NCI) has already tested ≈200,000 small-molecule compounds on the panel of 60 human cell lines representing major types of cancer. Ca. 70,000 molecules are found to have anticancer effect. Yet none of these molecules seems to be the “magic bullet”.

But maybe the bullet has in fact been found, just overlooked? In a new Silver Bullet project, we are testing this hypothesis. Since genetic heterogeneity of tumors and their high mutation rate are the major obstacles in anticancer therapy, the bullet should be lethal at a low concentration (be efficient) for a broad range of tumors (be nonspecific), while nontoxic (be safe) for normal cells. By analyzing the NCI dataset, we identified molecules that combine efficacy E with unspecificity U. We then used the *in silico* prediction tool PASS to assess the *in vivo* safety S of these molecules. By maximizing E, U and S, we obtained a group of “seed” molecules. These provide a training dataset for building a model M predicting E, U & S for related structures. Thousands of such new structures will be generated *in silico* and tested by M. The optimal candidates will then be synthesized and tested experimentally on the NCI-60 panel. Proteomics analysis will predict their drug targets and the mechanism of action. The final assessment will reveal whether Dr. Ehrlich's magic bullet against cancer exists.

# Identifying the Conditions Needed for Stability and Complexity in Living Systems

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The origin of life and the evolutionary process that progressed subsequently have led to the amazing complexity of the Earth biosphere that is expressed at different integration levels. It is found in cellular organization as shown for instance by the interconnected pathways characterizing the metabolism. Multi-cellularity, representing a higher integration level, requires the addition of differentiation into cells achieving different functions and of cellular communications. Moreover, the interdependence between the metabolisms of different species and the predatory or symbiotic modes of subsistence of many species also introduce connections that eventually give the biosphere the appearance of an intricate network in which very few species can be considered as truly autonomous. This high degree of complexity has been analyzed as a feature of living systems that is a direct consequence of the evolution towards increased dynamic kinetic stability (DKS)<sup>1</sup>, the form of stability that is specifically associated with entities capable of multiplying themselves. The drift towards increased complexity was then present from the very beginning of the evolutionary process, as soon as the replicative function and the possibility of transferring improvements to subsequent generations were present<sup>2</sup>. Subsequently, the introduction of any kind of function resulting in an increase in DKS would be selected. It is worth noting that there is *no limitation as regard to the nature of the function working as a feedback process* that can increase DKS so that the evolution of the system becomes unpredictable and there is *no limitation to the degree of complexity*<sup>2-4</sup>. Evolution is intrinsically a divergent process<sup>1</sup>, which makes the issues of function, stability and complexity inseparable. The presentation will focus on the fact that the manifestation of DKS requires far-from-equilibrium conditions that can actually be semi-quantitatively assessed by a kinetic and thermodynamic analysis<sup>3,5,6</sup>. Defining such conditions may render the identification of conditions favourable to the emergence of artificial chemical systems presenting some of the distinctive features of life easier to achieve.

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# 1-methyladenosine in transfer RNA

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RNA modification is a post-transcriptional process by which certain nucleotides are altered after their initial incorporation into an RNA chain. Transfer RNA is the most heavily modified class of RNA molecules. These modifications expand the chemical and functional diversity of tRNA, and enhance its structural stability.

We focussed our research on m<sup>1</sup>A tRNA modification. m<sup>1</sup>A nucleotide is found at position 9, 14, 22, 57 and 58 in tRNA. We solved the X-ray structures of the *T. thermophilus* m<sup>1</sup>A<sub>58</sub> (TrmI) and of the *B. subtilis* m<sup>1</sup>A<sub>22</sub> tRNA (TrmK) methyltransferases.

We then conducted biophysical studies using mass spectrometry, NMR, site-directed mutagenesis and molecular docking to obtain data on the tRNA recognition mode specific to each methyltransferase and on the reaction mechanism.

We showed that TrmI has to maintain its tetrameric organization in order to achieve its enzyme activity whereas TrmK modifies the tRNA as a monomer. TrmI presents two grooves that are large enough and electrostatically compatible to accommodate one tRNA per face of TrmI tetramer. TrmK presents two domains: the 'Rossmann-fold' domain responsible for the methyltransferase activity and a domain that presents two helices that formed a coiled-coil, probably necessary to bind to tRNA. These two domains form a concave surface of positive electrostatic potentials favorable to the tRNA binding just below the catalytic pocket.

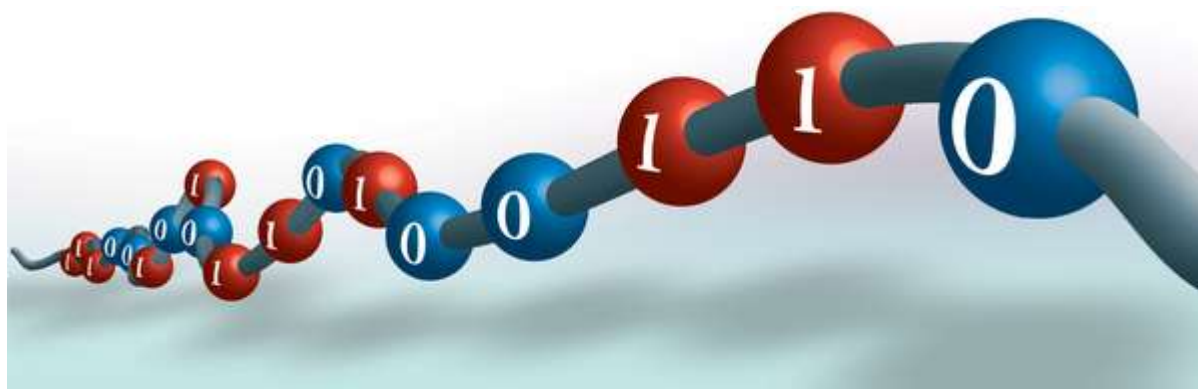
Recent results regarding the specific recognition with tRNA substrates and the reaction mechanisms of these two enzymes will be presented.

# Unnatural Information-containing Macromolecules

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Information-containing macromolecules are polymers that contain a message encrypted in their comonomer sequences. The archetypal example of such a polymer is DNA, which is used in biology to store genetic information.<sup>[1]</sup> However, DNA is certainly not the only polymer that can contain molecular information.<sup>[2]</sup> In principle, a string of information can be created in any copolymer using two comonomers defined intentionally as 0-bit and 1-bit (Figure 1). However, such polymers have to be monodisperse and perfectly sequence-defined. In addition, the message encoded in their chains should be easily read.



**Figure 1.** Schematic representation of an information-containing macromolecule containing a monomer-encoded binary code. Reprinted from reference 2. © Nature Publishing Group 2014.

In this lecture, I will present recent achievements obtained in my laboratory for the synthesis of information-containing macromolecules. Recent progress in the field of sequence-controlled polymers allows synthesis of non-natural macromolecules with precisely controlled primary and secondary structures.<sup>[3-6]</sup> For instance, monodisperse sequence-defined polymers were prepared using chemoselective iterative strategies. Furthermore, the readability of these polymers will be presented. For instance the sequencing of non-natural sequence-defined copolymers by tandem mass spectrometry will be discussed.

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# Chemical cross-linking and mass spectrometry to determine the interaction network of protein complexes

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Understanding the way how proteins interact with each other to form transient or stable protein complexes is a key point in structural biology [1]. Traditional structural methods have complementary attributes, and combining respective information would definitely help in exploring the architecture, dynamics and function of macromolecular complexes. In this context, mass spectrometry was shown as a high potential technique for characterizing intact multiprotein assemblies, especially for determining the complex stoichiometry or monitoring dynamic changes [2].

In this project, we combined chemical cross-linking with MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry) and ESI-MS (electrospray ionization mass spectrometry) to determine the binding stoichiometry and map the protein-protein interaction network of a human HAT-SAGA complex. Glutaraldehyde was first used to determine the stoichiometry of specific stabilized complex; but in order to get more information on the interacting area, cross-linking with isotopically labelled bisulfosuccinimidyl suberate (BS3) d0- d4 was performed. After incubation, the cross-linking reaction was quenched and submitted to tryptic proteolysis, digested peptides were then analysed by nanoLC-MS/MS using a high resolution Orbitrap mass spectrometer. Intra- and intercross-linked peptides were matched by two search engines dedicated to identification of cross-linked peptides: pLink [3] and XQuest [4].

The heterotetrameric stoichiometry was unambiguously resolved, and several interacting areas were definitively revealed. These restraint data were combined to molecular modeling and electron microscopy experiment in order to propose a low resolution interacting model for the complex, illustrating well the potential of an integrative strategy for addressing the structural elucidation of multiprotein complexes.

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# Sensing of Analytes Using Fluorescent Amphiphiles

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Fluorescent amphiphiles are molecules that have a long alkyl chain as a tail and a highly charged fluorescent head group (e.g. dye in scheme). These kind of molecules can be used for sensing in aqueous solutions with many advantages over classical optical chemosensors. Upon addition of an analyte to the amphiphile containing buffer solution, analyte-induced aggregation occurs, resulting in pronounced fluorescence quenching. Fluorescence spectroscopy can be used to identify and quantify the analyte. The amphiphile can be designed according to desired analyte and detection limit. The head group interacts with the analyte and the tail length determines the concentration for aggregation. We have done some studies using this kind of fluorescent amphiphile. In one study, we described a chemosensor for spermine.<sup>1</sup> The sensitivity and selectivity of the sensor is significantly better than what has been described previously for optical spermine chemosensors. We are able to detect spermine down to the low nanomolar concentration range. In an other study, we used a mixture of two amphiphiles with fluorescent head as a sensing ensemble for the pattern-based analysis of aminoglycoside antibiotics.<sup>2</sup> In this study, we were able to differentiate the the seven aminoglycosides amikacin, apramycin, paromomycin, kanamycin A, kanamycin B, neomycin and gentamicin.

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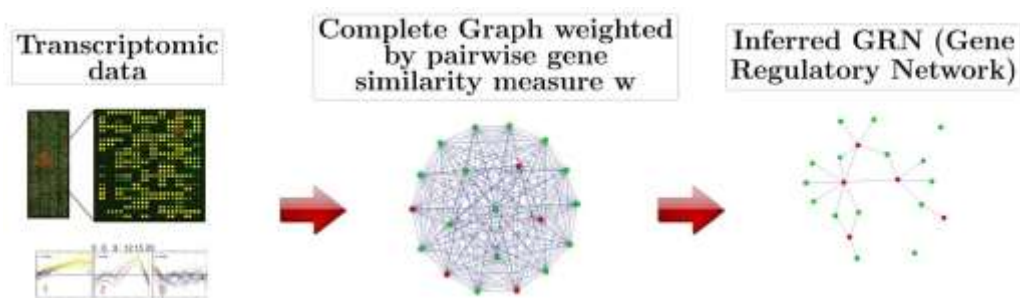
These works were supported by funding from the Swiss National Science Foundation and the Marie Curie Initial Training Network ReAD.

# Gene Regulatory Network Inference using Structural Biological a Priori

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The ability to extract plausible gene regulatory relationships is of paramount importance for improving the knowledge of living organism mechanisms. It benefits medical applications (identification of genes involved in diseases such as cancer) as well as biotechnologies (study of micro-organisms involved in biofuel production), among others. For this purpose, biologists acquire high-throughput gene expression data, from which Gene Regulatory Networks (GRNs) may be inferred. Given all pairwise gene similarity information, we formulate the GRN inference as an energy minimization problem to determine the presence of edges in the final graph. Taking into account expected patterns in the graph structure, biological a priori, such as knowledge about the connectivity of particular genes, are incorporated into the variational formulation. Different priors lead to different mathematical properties of the cost function, for which various optimization strategies can be applied. Experimental results show a performance improvement (in terms of Area Under the Precision-Recall curve) and/or computation time compared with state-of-the-art methods.



## I. INTRODUCTION

A GRN is a graph containing gene regulatory relationships between genes of a given living organism. It may be obtained from gene expression signals: for each gene, the signal corresponds to the gene expression level in different conditions (physico-chemical or temporal conditions, culture medium or mutated strains). Then, inferring a GRN aims at selecting, among all plausible links, a subset of regulatory links reflecting actual regulatory relationships between genes. Unfortunately, recovering useful information from this collection of signals remains a difficult task due to the small number of observations (number of conditions) compared with the number of genes.

## II. MODEL

The graph construction problem may be formulated by defining a cost function to minimize where the variables  $x_{i,j}$  correspond to edge labels for such that  $x_{i,j} = 1$  if the edge  $e_{i,j}$  is in the final graph and 0 otherwise. Weighting all possible pairwise gene relationships by the similarity  $w_{i,j}$  between gene expression profiles for gene  $i$  and  $j$  and assuming that a reliable list of putative regulator genes is available, we define some biological and structural a priori which may be incorporated into our cost function via the parameter  $\lambda$  and function  $\Phi$ :

$$\sum_{(i,j) \in \mathcal{E}} w_{i,j}(1 - x_{i,j}) + \sum_{(i,j) \in \mathcal{E}} \lambda_{i,j} x_{i,j} + \Phi((x_{i',j'})_{(i',j') \in N_{i,j}}),$$

where for every  $(i,j) \in \mathcal{E}$ ,  $N_{i,j}$  denotes some local neighborhood of edge  $e_{i,j}$ .

Depending on the choice of  $\Phi$ , we may constrain the degree of regulated genes to be close to a constant number, or enforce a co-regulation property (i.e. favoring a similar label for  $x_{i',j'}$  when genes  $j$  and  $j'$  are likely to act together). The different choices lead us to employ recent accelerated convex optimization methods or alternatively discrete optimization via maximum flow computations to compute the optimal solution.



# Solid-State NMR as an emerging technique to determine supramolecular interactions in biological and chemical complexes.

Antoine Loquet

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Self-assembly of multiple copies of protein subunits into large supramolecular complexes plays a key role in many cellular processes, notably in bacterial virulence, neurodegenerative disease-related mechanisms, and in biomolecular machines. 3D structures of these supramolecular assemblies are still rare as they are usually noncrystalline, making them often recalcitrant to X-ray diffraction techniques.

I will present recent developments in Solid-State NMR [1-3] to experimentally determine supramolecular interactions at the subunit interfaces, ultimately aiming at atomic resolution structures of large biological supramolecular assemblies [4].

I will discuss the combination of our SSNMR methods with density maps obtained by cryoelectron microscopy and advanced modeling methods in an integrative multitechnique approach [5].

Our approach paves the way to experimentally determine the network of weak interactions at the intermolecular interfaces of biological and chemical supramolecular complexes.

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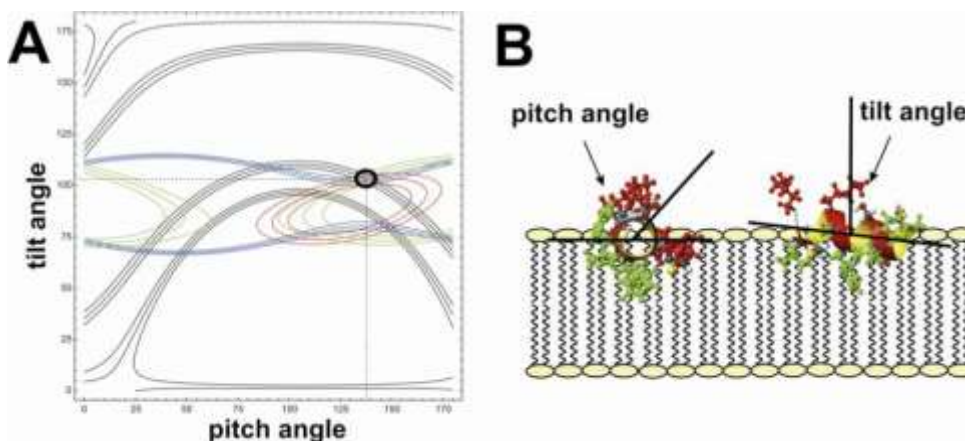
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# Membrane structure and interactions of the amino-terminus of huntingtin and its regulatory role in poly-glutamine aggregation

Arnaud Marquette, Nicole Harmouche, Evgeniy Salnikov, Matthias Michalek, Christopher Aisenbrey, Burkhard Bechinger.

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The amino-terminal domain of huntingtin (Htt17), located immediately upstream of the decisive polyglutamine tract, strongly influences important properties of this large protein and thereby the development of Huntington's disease. Htt17 markedly increases polyglutamine aggregation rates and huntingtin's interactions with biological membranes. An ensemble of low-energy conformations of the protein domain was identified by solution NMR in interfacial environments, and the structure was further refined using solid-state NMR spectroscopy of five selected labelling positions on oriented phospholipid bilayers. Tryptophan-fluorescence provides additional information of the membrane penetration depth. The thus obtained experimental parameters were used to validate a large range of MD calculations. The pronounced structural transitions of Htt17 upon membrane-association result in an in-plane aligned stable  $\alpha$ -helical conformation from K6 to F17. The first five residues appear more flexible and probably interact more weakly with the membrane. The membrane binding of Htt17 and the resulting permeability were quantitatively analyzed and are strongly dependent on lipid composition, whereas the helical tilt angle ( $\sim 77$  degrees) is nearly constant in all membranes investigated. When Htt17-polyQ polypeptides are investigated the presence of membranes has indeed a pronounced effect on polypeptide-lipid association and the polyglutamine aggregation. Therefore structure and lipid interactions of Htt17 have pivotal implications for membrane-anchoring and functional properties of huntingtin, its aggregation kinetics and concomitantly the development of the disease.



The Figure shows the solid-state NMR orientational restraints from three  $^{15}\text{N}$  and one  $^2\text{H}$  labelled sites (A) and the resulting alignment of the solution NMR structure in the lipid bilayer (B).

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Membrane Interactions of the Amino-terminal Domain of Huntingtin: Refined analysis by Tryptophan Fluorescence Investigations, *European Biophysics Journal* 43, 347-60 (2014)

Côté, S., Binette, V., Salnikov, E.S., Bechinger, B., Mousseau, N., Probing the Dynamics and Structure of Huntingtin 1-17 Membrane Anchor on a Phospholipid Bilayer using Molecular Dynamics Simulation, *Biophys J.* (under revision)

# Kinetic mechanism of RNA-mediated genetic regulation

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Riboswitches are structured parts in the 5'-UTR of mRNAs in bacteria and, less often, in the 3'-UTR of mRNAs in plants. Such RNA devices appear in mRNAs coding for proteins involved in the biosynthesis or the import of important metabolites. Their role is of sensing the intracellular level of this metabolite and of 'deciding' whether or not the mRNA has to be used. Remarkably, the decision can affect either translation by preventing ribosome binding, or transcription by detaching RNA-polymerase prematurely, or even alternative splicing leading to mRNA degradation. It is well established that some riboswitches function kinetically [1], and not thermodynamically according to mass action law. We have studied the functioning of three thiamine pyrophosphate (TPP) riboswitches, *thiC* and *thiM*, in *E. coli* regulating, respectively, premature transcription and translation inhibition, and *thiC* in *A. thaliana* regulating alternative splicing. In all cases, we ascertained an 'induced fit' mechanism involving two kinetic steps: initial loose binding of TPP subsequently transformed into tight binding after complete RNA folding.

To address the kinetic aspect we used our newly developed *kinITC* technique [2], which allowed us to obtain all thermodynamic and kinetic parameters for the two steps. These results showed that the two *E. coli* riboswitches, but not the plant riboswitch, function kinetically. A theoretical analysis allowed us to link the experimental parameters obtained by *kinITC* to the probability of these riboswitches to be in the ON-state. This correctly predicted an ON/OFF switch at a TPP concentration in the micromolar range. Remarkably, the regulation efficiency is the same for all kinetically-regulated riboswitches and it is higher than that of thermodynamically-regulated riboswitches. In other words, a kinetic regulation is more abrupt than a thermodynamic regulation around the critical ligand concentration of the ON/OFF switch.

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## ***Posters abstracts***

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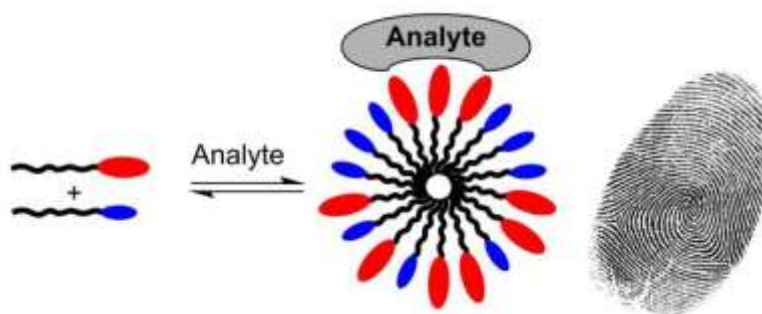
## Pattern-Based Sensing of Aminoglycosides

Kay Severin, Ziya Köstereli

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Aminoglycosides are antibacterial therapeutic agents that inhibit protein synthesis and contain an amino-modified glycoside (sugar) as a portion of the molecule.

Here, we describe a conceptually new 'one-cuvette' sensing system for the pattern-based analysis of aminoglycosides.<sup>1</sup> A mixture of two amphiphiles with fluorescent head groups can be used as a sensing ensemble for the pattern-based analysis of aminoglycoside antibiotics. In buffered aqueous solution, the amphiphiles form a dynamic mixture of micellar aggregates. In the presence of aminoglycosides, the relative amount and the composition of the micelles are modified. The re-equilibration of the system is analyte-specific, and characteristic fluorescence spectra are obtained for different aminoglycosides. Accurate differentiation in the low micromolar concentration range can be achieved by a principal component analysis of the spectral data.



<sup>1</sup>. Z. Köstereli, R. Scopelliti, K. Severin *Chem. Sci.*, **2014**, 5, 2456.

These works were supported by funding from the Swiss National Science Foundation and the Marie Curie Initial Training Network ReAD.

## Raman and Infrared Electrochemical studies of interaction between Adrenodoxin, Adrenodoxin reductase proteins and their complexes

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Metalloproteins that contain iron-sulfur clusters such as (Adx) are included in crucial cellular process as electron transfer. Indeed, protein-protein interactions are involved in the formation of the macromolecular structures and in the regulation of different metabolic pathways. In the present study, we examined interaction between the metalloprotein Adx and the flavoprotein Adrenodoxin reductase (AdR), which is related in the electron transfer mechanism.

Raman and infrared spectroscopy combined to electrochemistry have been used to investigate Adx/AdR proteins interactions. Upon complex Adx/AdR formation, we observed strong shifts of bridging and terminal iron-sulfur vibrations (Fe-Sb/t) in the 420-250 cm<sup>-1</sup> region as compared in Raman spectra to the same vibrations of Adx alone. We observed that the deletion of the C-terminal region of Adx and his mutation close to iron-sulfur cluster had a pronounced effect in the same spectral region. We suggest that hydrogen bonding affects the geometry of the cluster and promote resonance enhancement of some Fe-Sb/t vibrational modes upon complex formation. Furthermore, oxidized minus reduced FTIR difference spectra have been obtained. A significant change was observed for Adx mutant where the C-terminal region was deleted as well as for other mutants beside the primary interaction site.

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## Structural characterisation of highly specific membrane protein-lipid interactions involved in trafficking. A novel regulatory mechanism in membrane dynamics?

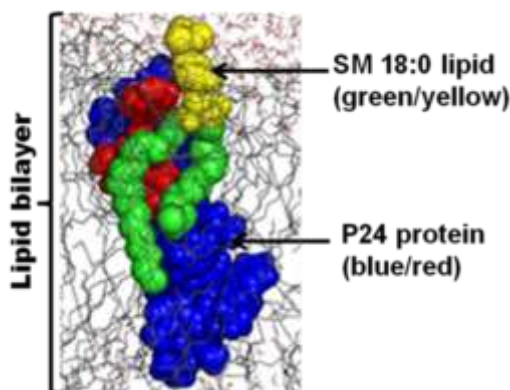
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Cells membranes are complex systems composed of variety of lipids that interacts with proteins to trigger cellular function. The delivery of these lipids to the right compartment is crucial for cells to work efficiently. The coat protein (COP) complex that coats vesicles is involved in transport of lipids and proteins in the early stages of the secretory pathway.

Recently, a highly specific interaction has been found between p24 protein-an abundant transmembrane protein of COPI vesicles- and a sphingomyelin specie (C18:0) [1]. Such highly specific interactions have been reported for protein-protein and protein-nucleic acid interactions to be involved in regulation of cell functions. Therefore, we decided to focus on the study of this proteinlipid interaction.

To characterize this specific interaction, the transmembrane domain of this p24 protein (p24TMD) was synthesized chemically or expressed in bacterial system, and investigated by solid state NMR in presence of sphingomyelin. Based on structural data, we will ultimately propose a model of this p24-sphingomyelin interaction in order to get insights into the function.



The proposed computer model of the p24TMD-SM C18:0 interaction with the binding pocket in red [1]

[1] Contreras and Ernst et al, Nature, 481, 525–529; 10742, (2012)

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ANR Prolipin

Collaborators: Britta Brügger, Felix Wieland, Andreas Max Ernst, François-Xavier Contreras

Group members: Jesus Raya, Delphine Hatey, Elise Glattard for discussion

## Transient contacts in the Intrinsically Disordered Unique domain of c-Src

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The human c-Src is a membrane bound tyrosine kinase involved in several signaling pathways. Anomalous regulation of its activity has been related to a number of tumoral processes. c-Src is the leading member of a group of related proteins, the Src Family Kinases (SFKs), which share a common domain architecture (catalytic SH1 domain, protein-binding SH2 and SH3 domains) but differ in the N-terminal region, the so called Unique domain.

The Unique Domain of c-Src (USrc) is an Intrinsically Disordered Region that binds membranes by means of an N-terminal myristoyl moiety, a positively charged region called SH4 domain and the Unique Lipid Binding Region (ULBR) – recently discovered by our group. Besides membrane binding, protein-protein intramolecular interactions with the SH3 domain have been observed. These interactions are allosterically modulated by a polyproline peptide mimicking the linker region between SH2 and SH1 domains.

We propose that these protein-lipid and protein-protein interactions may be part of a regulatory mechanism for fine-tuning of c-Src activity based on the Unique domain.

Our NMR experiments with a myristoylated construct of USrc in the presence of lipid vesicles show that, upon membrane binding, three regions can be identified (figure 1): the SH4 and ULBR interact with lipids, flanking a third unbound region, comprising amino acids 27 to 59, which interestingly contains the residues that were observed to interact with the SH3 domain.

In order to study the effect of the conformational restrictions on USrc imposed by membrane binding, we prepared a cyclic USrc construct in which the 27-59 region is forced to adopt loop-like conformations. Our NMR results show that the interaction with free SH3 is enhanced in the cyclic form in comparison with the wild type USrc (figure 2).

Finally, the effect of structural bias from the random coil model, which had been previously reported, has also been studied. Using Paramagnetic Relaxation Enhancement (PRE), we have observed that long-range transient contacts between distant regions of USrc take place in solution. Furthermore, we have identified some conserved aromatic residues that are key for these interactions. PRE profiles of mutants lacking only one of these residues show that most long-range contacts are abolished. These observations suggest an intrinsical tendency of USrc towards partially restricted conformations.

These preliminary results support a model in which myristoylated USrc membrane binding greatly enhances the native structural bias, promoting the interaction with the SH3 domain. Given that in nature USrc lipid binding is modulated (by phosphorylation, for example), this may be the basis of a regulatory mechanism in which the Unique domain is not a mere linker but an active player.

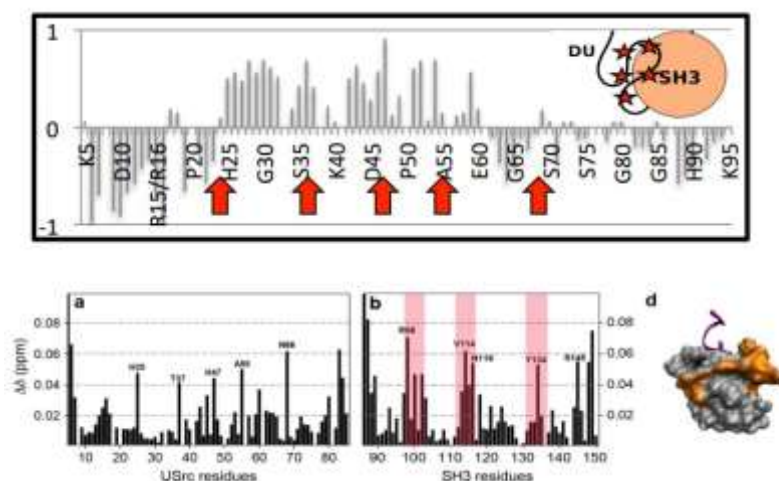


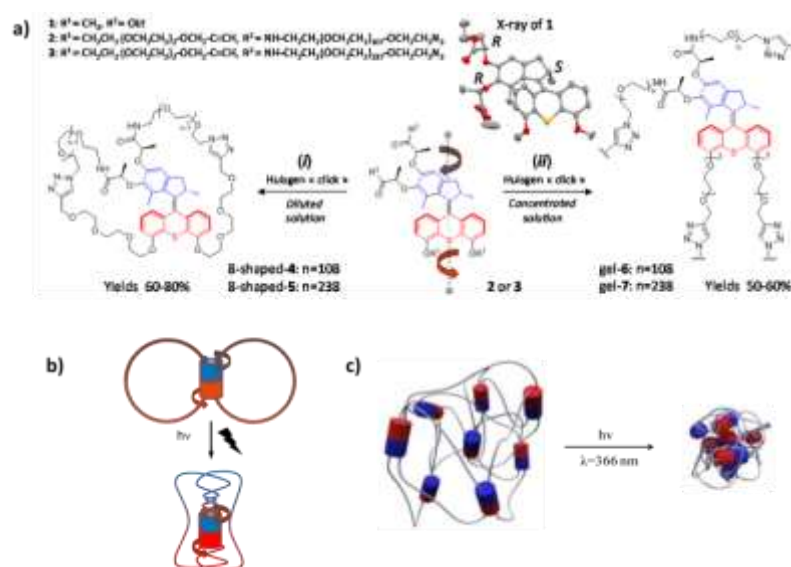
Fig. 1. (Top) NMR intensities show lipid binding regions (negative) and restricted loop regions (positive). Arrows mark SH3 bindingsites (Bottom). Identification of the binding sites in the Unique (a) and SH3 (b) domains. (d) mapping of the Unique interaction site in the SH3 domain opposite to the polyproline site.

## Macroscopic contraction of a gel induced by the integrated motion of light-driven molecular motors

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Natural molecular motors such as ATP synthase, myosin, kinesin and dynein can convert conformational changes, due to chemical energy input, into directed motion for catalysis and transport. Preparing artificial molecular motors and making them work at different scales (from nano to macroscopic scale) have been long-term challenges. Herein we designed and synthesized a light driven rotary molecular motor in highly enantiopure form and in gram scale. This motor is featured by two orthogonal functionalities on its upper and lower part, allowing its further integration into polymeric materials. By performing click reaction under different concentration conditions, either an eight shaped motor-polymer conjugate or a gel containing motors as reticulation units could be obtained. Upon UV irradiation, the polymer chains could be entangled due to the rotation of this motor. For eight shaped polymer, the dimension was changed towards smaller dimension, and the morphology was changed from cycle to collapsed coils (spherical or more elongated). For the gel, due to the twisting of polymer chains induced by the rotation of the motor, it could be contracted significantly (80 %) compared with its original volume. The integration of machines which display motions out of equilibrium at nanoscale to movement in the macroscopic world which is extensively used in natural systems will open very interesting prospects in nanotechnology for further developments.



**Figure 1**(a) Synthesis of 8-shaped motor-polymer conjugate and motor-containing gel; b) Schematic description for the 8-shaped polymer entanglement; c) Schematic description for the gel contraction.

1. Quan Li, Gad Fuks, Emilie Moulin, Mounir Maaloum, Michel Rawiso, Igor Kulic, Justin T. Foy, Nicolas Giuseppone. Macroscopic contraction of a gel induced by the integrated motion of light-driven molecular motors. *Nature Nanotechnology*. 2015, Accepted for publication.

## CASC4DE : Customized solutions for efficient Proteomics data handling

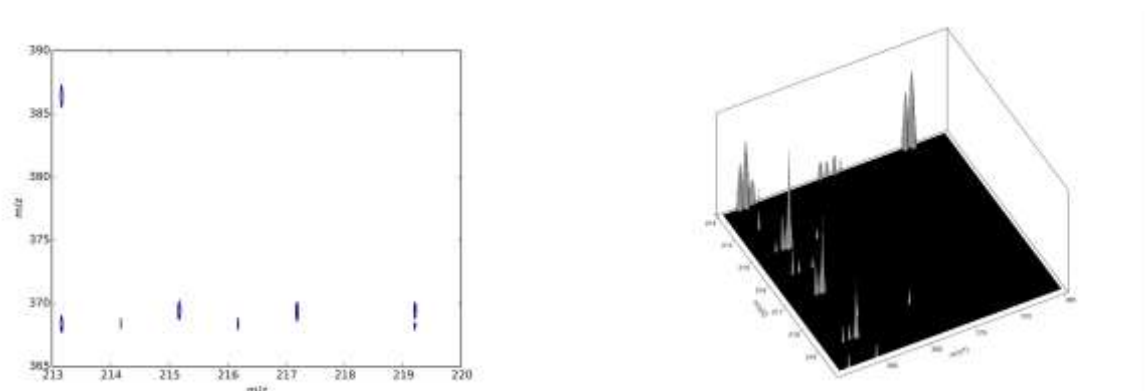
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In Proteomics, biologists generally use directly the data produced by the native processing chain of the spectrometers. But quite often those data are not fully exploited and some relevant information is lost.

Secondly, the speed of the acquisition is commonly seen as only hardware dependent and postprocessing algorithms are often neglected. Finally, visualization softwares provided with the spectrometers are often limited to simple direct views of the data and do not permit very flexible and customized representations of the raw or processed data.

CASC4DE was created for answering to all those limitations and more generally for proposing solutions in the domain of scientific Big data. In the case of Proteomics, CASC4DE develops and provides for example specific tools in order to enhance the resolution by data postprocessing (SPIKE suite), filling the gap between raw data acquisition and standard tools for peptides identification such as MASCOT or OMSSA. CASC4DE also completes the offer with cutting edge visualization tools for improving the analysis and the data representation. We present here few examples of CASC4DE capabilities in data processing and visualization.



*FTICR2D cholesterol dataset processed with SPIKE and spectrum visualization (left : 2D view, right : same view in 3D with fluid WebGL technology).*

# Sequence Adaptive Peptide Materials by Selective Biopolymer Templating

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Biological systems are exceptionally able to respond to new situations. This is achieved through dynamically interacting molecules that assemble, compete and selectively decompose, driven by biological catalysis. We are interested in developing synthetic mimics of these systems for use as adaptive biomaterials. Specifically, we have used a dynamic combinatorial library<sup>1,2</sup> approach to develop peptide based materials that continuously exchange amino acid sequence<sup>3</sup> through enzymatic catalysis. Depending on the presence of different biomacromolecules the peptide which is oppositely charged to this additive starts to predominate, through templating, which in turn results in assembly of nanostructures with different properties.

Initially, we studied the biocatalytic condensation reaction and self-assembly of fluorenylmethoxycarbonyl (Fmoc) capped dipeptides; known for their ability to self-assemble through the combination of  $\pi$ -stacking and hydrogen bonding interactions. We chose charged amino acids in the first position (+ve: Fmoc-Lys and -ve: Fmoc-Cysteic Acid) and Phe-NH<sub>2</sub> on the second position (Figure 1a). This led to form dipeptide derivatives with low yields ( $\leq 10\%$ ). In the presence of oppositely charged biopolymers (+ve: Chitosan and -ve: Heparin) co-assembly stabilized by electrostatic interactions and templating allowed to guide the *in situ* condensation reaction resulting in high yields of peptide products ( $>70\%$ ) with opposite charge. When mixed together in one pot, Fmoc-Lys, Fmoc-Cysteic Acid, F-NH<sub>2</sub> resulted in formation of dipeptide derivatives in low yields. When Chitosan was added to the mixture, Fmoc-Cysteic Acid-Phe-NH<sub>2</sub> was formed as the main product, while Fmoc-Lys-Phe-NH<sub>2</sub> was mainly formed when Heparin was added (Figure 1b). Depending on the selected system (peptide derivative and the biopolymer), different nanostructures and reconfiguration (from spherical aggregates to nanotubes or nanosheets as determined by TEM) were obtained. These results demonstrate for the first time the use of sequence adaptive libraries that respond and adapt to the presence of biopolymers. The approach may have applications for future smart biomaterials.

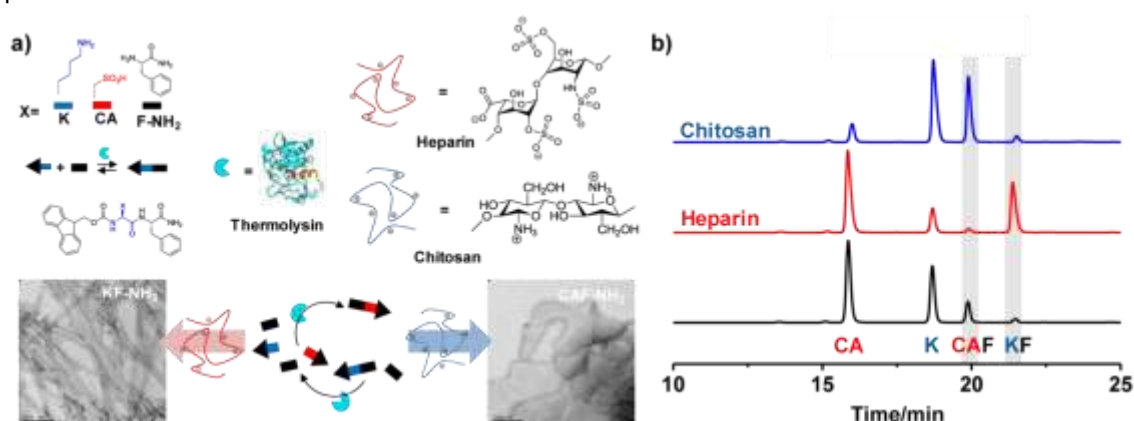


Figure 1. (a) Continuous exchange of amino acid sequence through enzymatic catalysis in the presence and absence of a template (biopolymer) forming different nanostructures; (b) Reversed-phase normalized HPLC traces in the one-pot system.

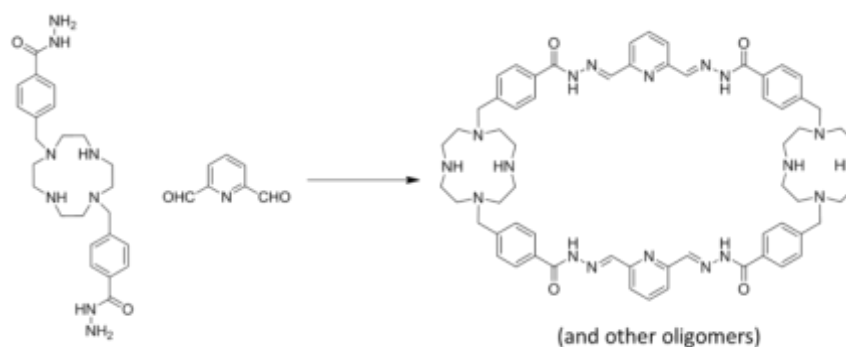
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## Acyl hydrazone based dynamic combinatorial libraries responsive to UV irradiation

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Photoswitchable receptors<sup>1</sup> present an efficient way to control binding and release of guest molecules, both with respect to energy and materials needed. Dynamic combinatorial chemistry, on the other hand, efficiently leads to discovery of new receptors.<sup>2</sup> The two approaches above can be combined by including photoswitchable functionalities into building blocks for dynamic combinatorial libraries, so to obtain receptors that can be switched on or off.<sup>3</sup> In this research a slightly different approach is used, with the photoswitchable functionality being the same as the one used for covalent exchange. Acyl hydrazone chemistry is selected as the means of connecting the building blocks, since it provides reversible covalent bonds, stability in water and, finally, easily photoswitchable double bonds.<sup>4</sup> As the guest binding unit, a cyclen moiety is added, due to its ability to coordinate metal cations and host various anionic guests. The pyridine dialdehyde building block further increases the number of metal coordination sites, but also serves to stabilize the Z-form of the hydrazone C=N bonds. Combined, these two building blocks may present a simple way to obtain dynamic combinatorial libraries of photoswitchable molecules.



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## Fibril self-assembly of a peptide triggered by an oxidation process

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Self-assembly is a spontaneous process by which molecules organize into large ordered structures. One structure commonly presented by peptides and proteins is the formation of large fibril structures known as amyloid fibrils. In many different degenerative pathologies, these fibrils may spontaneously aggregate and produce depositions in tissues. We herein present the evidences of a peptide with the capacity of amyloid formation, triggered by an oxidative event.

This peptide contains a cysteine residue and appears perfectly soluble and unstructured in its native state. Upon the oxidation of the thiol moiety to a disulfide by dimethylsulfoxide, a covalent dimer is produced through the formation of a cystine group. This dimer readily self-assembles in amyloid fibers with a diameter of approximately 6 nm. The kinetic analysis of this phenomenon was followed by NMR together with monomer peptide characterization and the presence of the dimer form by DOSY.

A complementary biophysical study by CD, Fluorescence, EM and SAXS is presented. The fibrils present all the characteristics of amyloid fibers: thioflavin fluorescence,  $\beta$ -sheet secondary structure and consistent diameter. However, unlike most fibrillating peptide that display a rapid aggregation after a nucleation delay, the kinetics followed by NMR shows a pseudo second-order dependence in peptide concentration. Additionally, the free peptide can be reversibly restored from the fibril by the addition of a reducing agent, this property opens-up new possibilities in terms of supramolecular organization and self-assembly, and can certainly be generalized to other systems.

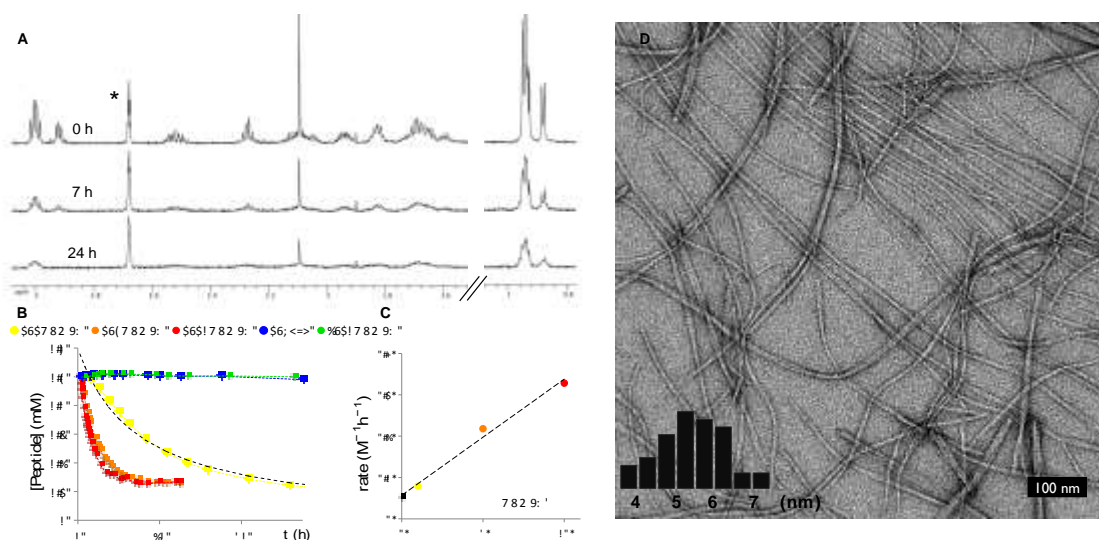


Figure 1. A-C) kinetics of peptide aggregation. D) EM picture of formed fibrils.

Asencio-Hernández et al. 2014 *ChemBioChem*, 15, 2370–2373.

## Molecular packing of amphipathic peptides on the surface of lipid membranes

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When polypeptides bind to the membrane surface they become confined to a restricted quasi two - dimensional space where peptide - peptide interactions become highly relevant and the concept of a crowded medium is appropriate. Within this crowded environment interesting effects like clustering, separation of phases, cooperative alignment and common movements occur.

Here we investigated such effects by measuring distances between fluorophore – labeled peptides in the range  $\leq 1$  nm by fluorescence self - quenching. For helical peptides with dimensions of approximately 1 x 3 nm such a small ‘ruler’ is sensitive to the packing of the labeled peptides and thereby to their molecular arrangement. A novel approach to characterize peptide - peptide interactions within membranes is presented using the designer peptide LAH4. This sequence changes membrane topology in a controlled manner being transmembrane at neutral conditions but oriented parallel to the surface at low pH. Experimental measurements of the fluorescence self - quenching of close - by chromophores, and the changes that occur upon dilution with unlabeled peptides are used to analyze the peptide distribution within the membrane surface. The data shows a strong effect of electrostatic interactions and under some experimental conditions clustering of the peptides. Furthermore the results suggest that at pH 4 the peptides arrange along the membrane surface in an ordered mesophase – like arrangement.

## From carboxyl–carboxyl(ate) supramolecular motifs to complex biomolecular systems

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Carboxyl and carboxylate groups can interact between themselves to form cyclic dimers and can associate in many different ways through a single interlinking hydrogen bond to form specific supramolecular motifs. Further, they can form catemers that are polymeric-like chains formed by hydrogen bonded carboxylic groups in crystals. Through an exhaustive exploration of the Cambridge Structural Database (CSD), the apparently infinite number of single hydrogen bond arrangements involving these groups could be reduced to 17 isolated carboxyl–carboxyl (13) and carboxyl–carboxylate (4) motifs. In addition, it was found that only eight distinct catemer motifs involving repetitive combinations of syn and anti carboxyl groups could be formed. Statistical data related to the occurrence and conformational preferences of these motifs are presented along with data related to the strength of the hydrogen bonds they can form. Indeed, the carboxylic donor group form much stronger hydrogen bonds than the carboxyl(ate) acceptor groups. Such strong hydrogen bonds are found in proteins where Asp/Glu amino acids form recurrent carboxyl–carboxylate motifs that are part of complex interaction networks playing a role in structure and folding. We consequently present data emphasizing how the exploration of small molecules can help understanding larger and more complex biomolecular systems.

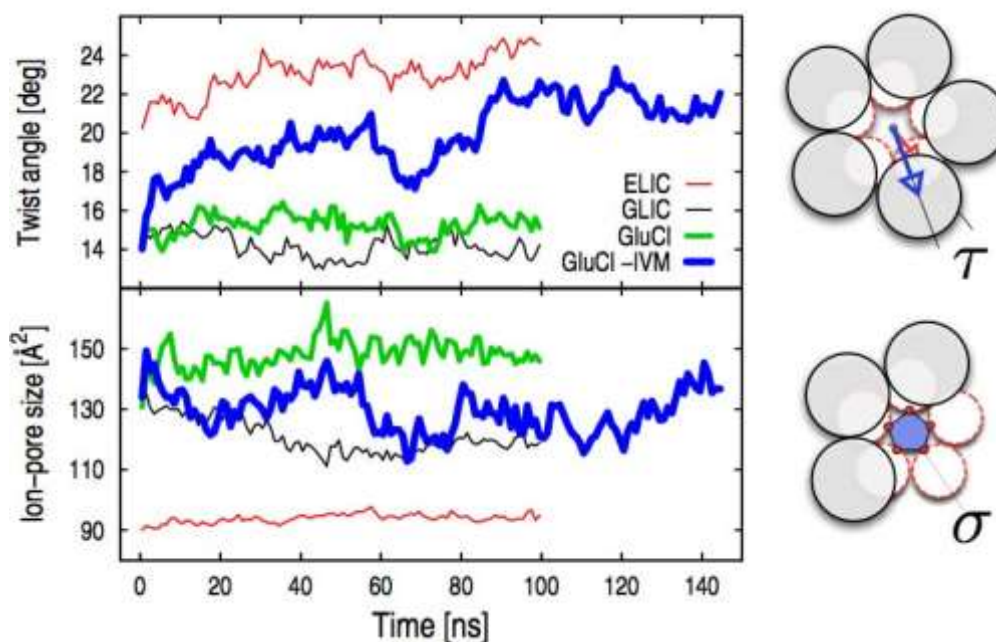
## On the Gating Mechanism of pLGICs

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Pentameric ligand-gated ion channels (pLGICs) mediate intercellular communication in the brain by converting a chemical signal into an ion flux through the postsynaptic membrane.

Despite the recent advances in their structural characterization, the mechanism of gating ions has remained elusive. Here, we present atomistic MD simulations of a glutamate-gated chloride channel (GluCl) simulated with and without the allosteric agonist ivermectin. The relaxation of GluCl induced by the removal of ivermectin captures a sequence of structural events, which elucidate the link between agonist unbinding and ion-channel closing. Based on these results, a mechanism of activation/deactivation in pLGICs is proposed.



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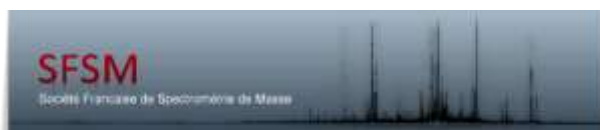
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