International School of Biological Magnetic Resonance

9th Course: Biophysics and Structure

School Directors: J. Puglisi and O. Jardetzky

View from the San Domenico Institute, Ettore Majorana Centre for Scientific Culture

Co-organizers of the Course:
Jody Puglisi and Elisabetta Viani

Program

Erice-Sicily: 22 June - 2 July 2009
URL: http://smrl.stanford.edu/erice2009/

Sponsored by: Stanford University

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SCIENTIFIC PROGRAM

Monday June 22: **Arrivals 7:30pm get together wine (Marsala Room)**

Tuesday June 23

morning

9:00-9:15	**J. Puglisi**	Opening remarks and Perspectives.
9:30-11:00	**J. Puglisi**	Principles of spectroscopy. (1)
11:00-11:30	Coffee break	
11:30-1:00	**A. McPherson**	The nature of crystals and the nature of waves.
12:30-15:30	Lunch	

afternoon

15:00-16:00	**T. Marlovits**	Cryo Electron Microscopy: Principles and Methods.
16:00-16:30	Coffee break	
16:30-17:30	**J. Puglisi**	Principles of Spectroscopy. (2)

Wednesday June 24

morning

9:00-10:00	**A. McPherson**	Diffraction by crystals - The molecules and the lattice.
10:00-11:00	**M. Levitt**	Applications of Molecular Dynamics to Small and Large Systems: C60 and RNA Polymerase.
11:00-11:30	Coffee break	
11:30-12:30	**P. Lukavsky**	NMR structure determination of large RNAs.
12:30-1:30	**T. Marlovits**	The type III secretion system in microbial pathogenesis.

afternoon Free

Thursday June 25

morning

9:00-10:00	**A. McPherson**	The phase problem and how it is solved
10:00-11:00	**M. Levitt**	The Protein Universe: A Current Snapshot
11:00-11:30	Coffee break	
11:30-12:30	**J. Puglisi**	Principles of Spectroscopy. (3)
12:30-15:00	Lunch	

afternoon

15:00-16:00	**M. Levitt**	Mesoscale Modeling of Macro-Molecular Complexes
16:00-16:30	Coffee break	
16:30-17:30	**P. Lukavsky**	Structural insights into RNA transport

Friday June 26 Break day - excursion

Saturday June 27

morning

9:00-10:00	**A. McPherson**	A case study in evaluating crystallographic results – An intact monoclonal antibody
10:00-11:00	**A. Yonath**	Hibernating polar bears and the ribosome structure
11:00-11:30	Coffee break	
11:30-12:30	**C. Aitken**	Dynamics of translation.
12:30-15:00	Lunch	

afternoon Free

SCIENTIFIC PROGRAM

Sunday June 28

morning

9:00-10:00	**H. Oshkinat**	Structure determination of proteins by solid-state NMR.
10:00-11:00	**J. Williamson**	Fourier Transforms in Biophysics
11:00-11:30	Coffee break	
11:30-12:30	**A. Gronenborn**	Structure of a viral capsid.
12:30-15:00	Lunch	

afternoon

15:00-16:00	**R. Efremov**	Biomembranes as pharmacological targets: insights from computer simulations.
16:00-17:00	**M. Delepierre**	Molecular interactions as viewed by NMR.
16:00-16:30	Coffee break	
17:30-18:30	**H. Oshkinat**	Solid-state NMR of membrane proteins.

Monday June 29

morning

9:00-10:00	**A. Yonath**	The stunning ribosomal architecture and dynamics facilitates cell signaling and hints at its evolution.
10:00-11:00	**J. Williamson**	Ribosome assembly in vitro and in cells.
11:00-11:30	Coffee break	
11:30-12:30	**H. Oshkinat**	Dynamic chaperone systems involved in protein homeostasis.
12:30-15:00	Lunch	

afternoon

15:00-16:00	**R. Efremov**	Molecular lipophilicity in protein modeling and drug design.
16:00-16:30	Coffee break	
16:30-17:30	**A. Gronenborn**	Awesome power of RDCs.

Tuesday June 30

morning

9:00-10:00	**J. Puglisi**	Tackling complex disease.
10:00-11:00	**Student Talks (20 minutes each)**	
11:00-11:30	Coffee break	
11:00-12:30	**(continue student talks)**	
12:30-15:00	Lunch	

afternoon **(continue student talks)**

evening **Banquet**

Wednesday July 1

morning

9:00-10:00	**A. Gronenborn**	Lectin-sugar interactions**.**
10:00-11:00	**A. Yonath**	David and Goliath: how do the tiny antibiotics paralyze the giant ribosome? Or: Can structures lead to improved drugs?
11:00-11:30	Coffee break	
11:30-12:30	**M. Delepierre**	The heme acquisition system in Serratia marcescens
12:30-15:00	Lunch	

afternoon

17:00-18:30	**Faculty-Roundtable and tutorial on difficult problems in biophysics**

Thursday July 2 *Departure from Conference Centre.*

ABSTRACT (lecture)

Ribosome Dynamics and Translation

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Translation, the process of protein synthesis, is highly complex and dynamic. To decipher the genetic code and synthesize protein, the ribosome works in concert with mRNA, tRNA, and protein molecules. Understanding the specific molecular events underlying the mechanism of translation is challenging, particularly as a result of ensemble and temporal averaging. Single-molecule fluorescence techniques eliminate these averaging effects and permit direct, time-resolved observation of rare or transient species. We have developed a robust single-molecule fluorescence resonance energy transfer (FRET) system to probe events throughout translation. In particular, we are focused on the role of ribosomal particle dynamics in the mechanism of translation. Using ribosomes labeled specifically on the large (50S) and small (30S) subunits with fluorescent dyes, we have used intersubunit FRET to observe ribosomal conformational changes during translation. We observe two global conformations of the ribosome, consistent with those observed in high-resolution structures. The transition between these two conformations is triggered by discrete chemical events, and is used to drive ribosome function during both initiation and elongation. The methods developed here allow the observation of translation dynamics in real time.

ABSTRACT (poster and oral presentation)

Biophysical characterization of Desmosomal Proteins

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Desmosomes are intercellular complexes that allow strong cell-cell adhesion in epithelial and cardiac tissue. Desmoplakin (DP), a member of the plakin family, is an intracellular protein of the desmosomal complex that is required for anchoring cadherins to the intermediate filament cytoskeleton. Mutations in the N-Terminal region of DP cause Arrythmogenic Right Ventricular Cardiomyopathy (ARVC) – a complex myocardial disease that can lead to sudden cardiac death. These mutations (at amino acids 30 and 90) occur in a region that is required for desmoplakin localization to the desmosome and binding to other desmosomal proteins such as Plakoglobin and Plakophilin2. It was hypothesized that a more detailed biophysical analysis of this region would give insight into the role of DP in normal desmosomal adhesion. To address these questions a variety of constructs including residues 1-179, 10-160, 10-160V30M and 10-160Q90R mutants were expressed in bacteria, purified and structurally

analysed. Our results including Circular Dichroism (CD), 1D-Nuclear Magnetic Resonance (1D-NMR) and Size Exclusion chromatography indicate that the region is intrinsically unstructured. The intrinsic plasticity of the N-Terminal domain of DP could allow it to associate with multiple binding partners and we are currently testing this hypothesis.

ABSTRACT (poster presentation)

Structural studies of new hemicellulases from the filamentus fungus *Fusarium Oxysporum*

Maria Dimarogona[1,2], Maria Moukouli[2], Evangelos Topakas[2], Paul Christakopoulos[2], Evangelia D. Chrysina[1]
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Hemicellulases are key enzymes in the saccharification of plant biomass. Their role is to degrade hemicellulose, a heterogeneous group of branched and linear polysaccharides bound via hydrogen bonds to the cellulose fibrils in the plant cell wall. The catalytic modules of hemicellulases are either glycoside hydrolases (GHs) that hydrolyze glycosidic bonds or carbohydrate esterases (CEs) which hydrolyze ester linkages of acetate or ferulic acid side groups. Here we report on the results obtained from our structural studies on two novel hemicellulases: (i) an alkaline ***endo*-beta-1,4-xylanase (FoXyn10a)** and (ii) **a type C feruloyl esterase (FoFaeC)** from the filamentous fungus *Fusarium oxysporum* F3. In specific, (i) *endo*-β-1,4-xylanases hydrolyze the β-1,4-glycosidic bond between xylose residues in the xylan backbone, yielding short xylooligomers. We have been successful in growing single *Fo*Xyn10a crystals that diffract to 2.03 Å resolution. Preliminary characterization showed that the crystals belong to space group $P42_12$ with unit cell dimensions a=b=123.4 Å, c=117.3 Å, and two molecules in the asymmetric unit. Structure determination of FoXyn10a is currently in progress using the isomorphous molecular replacement method employing the three dimensional structures of previously determined F/10 homologues as starting model. Cocrystallisation efforts are also underway to obtain diffracting crystals of FoXyn10a in complex with linear or branched oligosaccharides. (ii) Feruloyl esterases hydrolyse the ester bond between the hydroxycinnamic acids and sugars present in the hemicellulose side chains facilitating the access of hydrolases to the backbone polymers. They are classified into four types (A-D) according to their amino acid sequence identity and substrate specificity. Our efforts are focused in the structure elucidation of a type C ferulolyl esterase (FoFaeC) which has no close homologue with known crystal structure until present. We have already obtained diffracting crystals of FoFaeC using the sitting drop vapor diffusion method. Our ultimate aim is to solve the structure of FoFaeC in the native form and in complex with various substrates in order to elucidate its mechanism of action.

ABSTRACT (poster and oral presentation)

Substrate-driven conformational changes in ClC-ec1 observed by fluorine NMR

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The CLC "Cl^- channel" family consists of both Cl^-/H^+ antiporters and Cl^- channels. Although gating in CLC channels is known to involve large, cooperative conformational changes between protein subunits, it has been hypothesized that conformational changes in the antiporters may be confined to small movements localized near the Cl^- permeation pathway. However, to date few studies have directly addressed this issue, and therefore little is known about the molecular movements that underlie CLC-mediated antiport. The crystal structure of the *E. coli* antiporter ClC-ec1 provides an invaluable molecular framework, but this static picture alone cannot depict the protein movements that must occur during ion transport. In the present study we employ fluorine NMR to monitor substrate-induced conformational changes in ClC-ec1. Using mutational analysis, we show that substrate-dependent ^{19}F spectral changes reflect functionally relevant protein movement occurring at the ClC-ec1 dimer interface. Our results demonstrate that conformational change in CLC antiporters is not restricted to the Cl^- permeation pathway, and demonstrate the usefulness of ^{19}F NMR for studying conformational changes in membrane proteins of known structure.

ABSTRACT (oral presentation)

NMR characterization of the ligand binding properties of the model siderocalin Q83

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Lipocalins are small secreted proteins able to bind a variety of different hydrophobic molecules (such as lipids, retinol, some hormones and other small hydrophobic molecules) [1]. Despite a limited sequence homology, lipocalins share a conserved eight-stranded b-barrel fold, called "calyx", forming a hydrophobic cavity to which hydrophobic ligands specifically bind.
Siderocalins, which form a particular class of lipocalins, bind small iron-binding molecules called siderophores with a very high affinity (< 0,5 nM). So far, only 2 siderocalins are known. The human

neutrophil gelatinase associated lipocalin (NGAL) [2] and 24p3, the mouse homologue of NGAL [3]. The exact role of siderocalins is still unclear, but they seem to be involved in an iron delivery pathway particularly active during cell growth and differentiation. Furthermore, emerging evidence suggests that siderocalins are involved in cell proliferation and cancer progression. Correspondingly, the quail lipocalin Q83 has been found to be highly overexpressed in quail embryonic fibroblasts transformed by oncogenic v-myc [4]. It has recently been shown that Q83 binds a bacterial siderophore called enterobactin with a picomolar affinity, therefore Q83 belongs to the siderocalin class. Enterobactin consists of a triscatechol derivative of a cyclic triserine lactone, which coordinates ferric iron. To get further insight into the binding-mode of enterobactin to Q83, we investigate with the help of different NMR approaches the interaction of Q83 with vanillic acid, which mimics the catechol moiety of enterobactin. Elucidating the interaction and the binding dynamics of Q83 with vanillic acid will provide insight into the binding contribution of each of the catechol moieties of enterobactin with Q83. The fact that siderocalins are involved in fundamental processes regulating cell growth and cell proliferation, highlights the importance of a detailed structural and dynamic description of the ligand binding properties of siderocalins, as wells as to address their potentiality as new therapeutic targets.

1. Flower DR, North AC & Sansom CE (2000) The lipocalin protein family: structural and sequence overview. *Biochim Biophys Acta* **1482**, 9-24.
2. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN & Strong RK (2002) The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* **10**, 1033-1043.
3. Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, Du T, Erdjument-Bromage H, Tempst P, Strong R & Barasch J (2002) An iron delivery pathway mediated by a lipocalin. *Mol Cell* **10**, 1045-1056.
4. Hartl M, Matt T, Schuler W, Siemeister G, Kontaxis G, Kloiber K, Konrat R & Bister K (2003) Cell transformation by the v-myc oncogene abrogates c-Myc/Max-mediated suppression of a C/EBP beta-dependent lipocalin gene. *J Mol Biol* **333**, 33-46.

ABSTRACT (poster and oral presentation)

Comparison of *In vitro* and *In vivo* 1H NMR Spectroscopy of Rat Brain: Technical Considerations and Effects of Brain Regions and Post-weaning Isolation

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INTRODUCTION. Post-weaning social isolation of rodents leads to some behavioural characteristics similar to those observed in psychiatric disorders, such as schizophrenia. Neurochemical effects of isolation rearing are reported but need further investigation (Fone and Porkess, 2008). *In vivo* magnetic resonance spectroscopy (MRS) is valued as a non-invasive tool providing information about the metabolic state of living tissue. To overcome technical issues, complementary data can be obtained from *in vitro* nuclear magnetic resonance (NMR) spectroscopic analyses of tissue extracts. Previously, *in vivo* and *in vitro* concentrations of choline- and creatine-containing compounds showed good agreement, but *in vitro* N-Acetyl-aspartate (NAA) levels seemed to be lower (Barker et al., 1994; Petroff et al., 1995; Tracey et al., 1996). However, reports on comparisons between MRS and NMR measurements are rare.

AIM. We therefore compared results obtained from NMR spectroscopy and MRS to study inter-regional variations and the effects of social isolation on metabolite levels. We hypothesised that the improved signal-to-noise ratio (SNR) in NMR spectroscopy would produce more precise results, especially for those metabolites that give rise to multiplet resonances.

METHODS. *In vitro* NMR spectra were recorded from methanol extracts (Wu et al., 2008) of the frontal cortex and the hippocampus from 19 isolation-reared Lister-Hooded rats and 27 group housed controls (NOESY pulse sequence, 400 MHz, Bruker). Resonances corresponding to myo-inositol (µ-Ino), taurine (Tau), choline-containing compounds ((G)PChol), aspartate (Asp)*, glutamine (Gln), glutamate (Glu), NAA, γ-aminobutyric acid (GABA), acetate (Acet)*, glycine (Gly)*, alanine (Ala)* and lactate (Lact)* were integrated and related to the resonances of creatine- containing compounds, and group comparisons were made using the Mann-Whitney-U-test. Spectra were also binned in 0.04 p.p.m. wide buckets and analysed using multivariate statistical analysis. *In vivo* spectra from the frontal cortex and the hippocampus of Lister-Hooded rats reared in isolation or groups (8 each) were acquired from a 3x2x2 mm3 voxel using ^{1}H Point-Resolved Spin echo sequence with a 7-T scanner (Biospec, Bruker System) and analysed (jMRUI 3.0, LCModel). The same metabolites (except those indicated by an asterix*) were quantified relative to creatine, and data groups were also compared with the Mann-Whitney-U test.

RESULTS. Results obtained from *in vivo* and *in vitro* data both suggested that post-weaning social isolation did not alter central metabolism in the frontal cortex or hippocampus. Both platforms indicated that the two brain areas exhibited different metabolite patterns, with Glu levels being higher in the frontal cortex (11.51% *in vivo* and 28.56% *in vitro* concentration difference between frontal cortex and hippocampus). Metabolic differences between the two brain areas were more pronounced in spectra obtained from *in vitro* tissue, indicating that levels of Tau (12.62%), NAA (45.60%) and Gln (24.60%) were also higher and those of µ-Ino (21.13%), Ala (7.18%), Acet (62.41%) and Asp (20.87%) were lower in the frontal cortex. The two analytical methods differed greatly with respect to the metabolite concentration estimations relative to creatine, with *in vitro* NMR spectroscopy generally showing more precise results: In the frontal cortex *in vitro* CVs were lower for µ-Ino (5.78% *vs.* 19.29% *in vivo*), Tau (4.80% *vs.* 19.51%), Gln (7.57% *vs.* 13.18%) and Glu (4.44% *vs.* 10.33%) but higher for NAA (15.34% *vs.* 10.82%). Similarly, in the hipppocampus *in vitro* CVs were lower for

µ-ins (4.95% *vs.* 11.57% *in vivo*), Tau (4.39% *vs.* 16.21%), Gln (7.01% *vs.* 16.71%) and Glu (3.80% *vs.* 5.61%) but higher for NAA (26.50% *vs.* 7.65%). Effects of conditions the methanol extracts were subjected to (room temperature over a time span of 44 hours prior spectroscopic analysis) were obvious in results obtained from multivariate analysis ($r^2x(cum)=83.15\%$, $r^2y(cum)=83.36\%$, $R_{prediction}=0.749$): A strong decrease of NAA and simultaneous increase of Acet and Asp were observed. Univariate analysis confirmed these results partly (NAA concentration correlated with this time span only moderately and only in the hippocampus: Spearman correlation coefficient r=0.4826, p«0.001).

CONCLUSION. Results obtained from *in vivo* and *in vitro* NMR spectroscopy were distinctively different in many aspects, but agreed in biological research questions. Post-mortem effects might have occurred in methanol extracts of brain tissue thereby causing unwanted metabolite concentration alterations. For future studies, care must be taken when *in vitro* data is tried to be translated into living organisms.

ABSTRACT (poster and oral presentation)

Structural and functional characterization of PRD-I in GlcT from *Bacillus subtilis*

Sebastian Himmel[1], Sebastian Hübner[2], He-Hsuan Hsiao[3], Gerhard Wolf[1], Henning Urlaub[3], Stefan Becker[1], Donghan Lee[1], Jörg Stülke[2], and Christian Griesinger[1]
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Histidine phosphorylation is a vital post-translational modification in prokaryotes. A well known example of the histidine phosphorylation is histidine-mediated phosphate transfer between proteins such as the bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS). Recently, it has also been shown that a number of target proteins containing PTS regulatory domains (PRDs) are involved in basic cellular processes. In particular, transcriptional antiterminator proteins (LicT, SacT, and GlcT) containing these PRDs are essential for bacteria to adapt to environmental changes in nutrient conditions by regulating the expression of PTS gene products for sugar uptake. Despites the importance of histidine phosphorylation in this regulation, it is still unclear how many histidines and which ones are phosphorylated in GlcT from *Bacillus subtilis*.[(1)] Here, we address these questions using NMR spectroscopy and mass spectrometry. The results will be discussed on the poster.

(1) Schmalisch, M.H., Bachem, S., and Stülke, J. (2003) *J. Biol. Chem.* **278**, 51108-51115.

ABSTRACT (poster presentation)

Cell-free expression of integral membrane proteins

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Integral membrane proteins (MPs) are a large and diverse class of proteins important for many cellular functions, making them popular as drug targets. Overproduction of MPs for biophysical studies can be a challenging task due to cytotoxic effects on *in vivo* systems, mistargeting, their tendency to aggregate or the possibility of being digested by proteases. *In vitro* expression systems, however, potentially overcome these difficulties and enable high-level production. 40 different membrane proteins from different protein classes (GPCRs, UCPs, histidine kinases, GLUTs, AQPs, bacterial rhodopsin and transhydrogenases) were set up for expression trials in this master´s project. The different genes were cloned into the vector pEXP5-NT/TOPO and an S12 extract prepared from *Escherichia coli* was used as a basis for cell-free protein synthesis. Suitable detergents were added to the expression reaction in order to produce cotranslationally solubilized MPs. The choice of detergents depends on the nature of the protein and detergent screens were therefore made for some of the MPs. 37 (92.5%) of the protein targets were produced with the optimized in-house cell-free production system. 12 of these (32%) gave yields higher than the reference protein GFPcyc3 (>0.7 mg/ml). The achieved results support the notion that cell-free expression complements conventional *in vivo* expression of MPs. Obtaining milligram quantities, which is required for detailed biophysical characterization and structural determination, can be an enormous challenge for MPs. In this project, expression levels suitable for biophysical and structural studies for all tested constructs were obtained.

ABSTRACT (oral presentation)

Novel Scaffolds for Protein Design

James T. MacDonald, W. R. Taylor
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There has been significant progress in computational protein design in the past few years. This has predominantly involved redesigning known backbone structures to incorporate new functionality or increase stability. We present a method to systematically construct novel backbone scaffolds for the purpose of exploring fold-space and to provide a vast range of new scaffolds for protein design.

ABSTRACT (poster and oral presentation)

Algorithms for conformational optimization along natural dimensions

Peter Minary and Michael Levitt

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We introduce new algorithms that facilitate macromolecular optimization and sampling along arbitrary degrees of freedom including but not limited to the ones that are naturally observed in experimental X-ray structures. After comparing our method to alternative protocols that have been used for both nucleic acids and proteins we discuss some present and propose various future applications.

ABSTRACT (poster presentation)

Towards a Structure of the HPV Oncoprotein E6

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The focus of this study is the oncoprotein E6 of Human Papilloma Viruses (HPVs). More than 100 HPV types have been described and about 40 of them infect basal keratinocytes of the genital tract. These genital HPVs are classified into high-risk or low-risk types, whether or not they cause cervical cancer. At least 99 % of cervical carcinomas contain high-risk HPV DNA. The HPV oncoproteins E6 and E7 are necessary and sufficient for the transformation and immortalisation of keratinocytes. E6 interacts with numerous cellular proteins. The probably best characterized interaction is the formation of a ternary complex between E6, p53 and E6AP. E6AP alone does not bind to p53. Through formation of the ternary complex E6AP binds to p53 and ubiquitinylates it. This leads to p53 degradation via the proteasome. Degradation or blocking the activity of p53 is a key event in carcinogenesis. So far only a structure of a four-fold mutated C-terminal half of HPV 16 E6 is available. Further structures, optimally full length E6 without mutations, of other HPV types are needed to compare E6 structural features to elucidate what causes the riskiness of a given HPV type. Structural characterization of E6 proteins is hindered by their tendency to aggregate. In the ongoing study several E6 proteins of different HPV types were screened, expression and purification conditions were optimized till one E6 protein was identified which C-terminal half is suited for NMR. At present experiments for structure determination via NMR are recorded.

ABSTRACT

Molecular mechanisms of food allergy

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Ber e 1 is the major allergen present in brazil nut. It belongs to the 2S albumin family, a class of proteins frequently represented in food allergy. However, recent findings show that the protein alone does not describe the molecular origins of the allergenic properties; animal experiments have shown that the presence of endogenous lipids is required for an allergic response. Using fluorescence titrations experiments we have shown that the exposed hydrophobic surface area of Ber e 1 differs significantly compared to SFA8, a homologous protein from sunflower seeds which far less allergenic, yet structurally very homologous. The three-dimensional structure in absence of lipids has been determined by NMR. Ber e 1 forms a relatively ill-defined four-helix bundle stabilized by four highly conserved disulphide bridges. Although the structure is comparable with other 2S albumins, small structural differences can be seen. We have also measured ^{15}N spin relaxation to assess the dynamic properties of Ber e 1. This is the first study of backbone dynamics of any 2S albumin, and can explain some of the structural differences of Ber e 1 compared with other 2S albumins. We have also found that Ber e 1 binds copper(II), making it the first known metal chelating 2S albumin. The binding site has been mapped using paramagnetic relaxation enhancement. By defining the differences between the free and lipid bound state of Ber e 1, as well as differences between a strong and weak allergen, we may be able to describe the requirements for a protein to become allergenic in general.

ABSTRACT (poster presentation)

The binding site of a scorpion alpha-toxin to a sodium channel peptide – insights from NMR

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**Department of Plant Sciences, Tel-Aviv University, Ramat-Aviv, Tel-Aviv 69978, Israel

Voltage-gated sodium channels (NaChs) are large transmembrane proteins that initiate action potential in electrically excitable cells. This central role has placed them as a primary target for a large number of neurotoxins. Scorpion alpha-neurotoxins bind to these sodium channels with high affinity and slow their inactivation, causing a prolonged action potential. Despite the similarity in their mode of action and three-dimensional structure, these toxins exhibit great variations in selectivity toward insect and mammalian NaChs, suggesting differences in the face of interaction of the toxins and the channels. The scorpion alpha-toxin binding site, termed neurotoxin receptor site-3, has been shown to involve

the extra-cellular S3-S4 loop in domain four of the alpha-subunit of voltage-gated sodium channels (D4/S3-S4).

In this study, the binding site for peptides corresponding to the D4/S3-S4 loop of the insect NaCh was mapped on the highly insecticidal alpha-neurotoxin, Lqh(alpha)IT, from the scorpion *Leiurus quinquestriatus hebraeus*, using high resolution 2D NMR spectroscopy.

The intermolecular interactions revealed in this study could further our understanding of the mechanism by which the channel function is modulated by scorpion toxins, contribute to our understanding of channel activation and inactivation and may be used for design of selective drugs and insecticides.

ABSTRACT (oral presentation)

The Conformational Dynamics of HIV-1 Reverse Transcriptase Revealed by Hydrogen/Deuterium Exchange and Mass Spectrometry

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HIV-1 reverse transcriptase is an essential enzyme in the HIV lifecycle and a popular target for drug design. While there are numerous crystal structures of reverse transcriptase (RT) bound to various ligands and inhibitors, there is little experimental information on the conformational dynamics of RT in solution, even though dynamics are believed to play an important role in function. We studied the dynamics of RT using hydrogen/deuterium exchange and mass spectrometry. RT consists of a 66 kD subunit (p66) and a 51 kD subunit (p51), each of which is composed of multiple sub-domains. While various crystal structures indicate that the fingers and thumb sub-domains and RNase H domain of the p66 subunit can assume different orientations, H/D exchange indicates that the secondary structure within these sub-domains is stable. An important exception is the small beta-sheet K in p66 that lies at the base of the thumb domain. This beta sheet is highly flexible and marginally stable. Several residues in this region form contacts with non-nucleoside inhibitors bound to RT. Thumb domain motions are thought to play an important role in the RT replication cycle, and we suggest that the high degree of flexibility seen in beta sheet K facilitates thumb domain mobility. Despite the fact that the p51 subunit has a more compact tertiary structure than the p66 subunit, it shows similar rates of H/D exchange. Additionally, we find that several regions of the RNAse H domain are highly flexible despite containing significant secondary structure, which is consistent with an earlier NMR study of the isolated RNAse H domain.

ABSTRACT (oral presentation)

Longin Domain-SNARE motif interaction: analysis of the SNARE autoinhibition mechanism in Longins

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SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins are considered the engines of all intracellular membrane fusion reactions. SNARE proteins that are localized in opposing membranes form a four helix bundle whose release of free energy contributes to membrane fusion. Each helix comprises a characteristic SNARE motif, a stretch of 60-70 amino acids defining all SNAREs.

Sequence specificity of SNARE motifs residing in different compartments contributes to fusion specificity, but a major role in modulating SNARE function is provided by other protein domains. In particular, the SNARE families of longins and syntaxins possess ~120 amino acid-long N-terminal extensions called Longin domain (LD) and Habc, respectively. LD and Habc can fold back on the SNARE motif and prevent it from pairing to other SNARE motifs. A major role of this "locked", closed conformations is to provide exocytosis with a conformational switch that can specifically trigger membrane fusion.

A number of studies have debated the structural and dynamic features of the closed configuration of syntaxins, whereas this information is still largely missing for Longins. In this study we adopt Nuclear Magnetic Resonance (NMR) to characterize the LD-SNARE motif interaction in the prototypical longin protein VAMP7. In particular, we combine backone resonance assignment, chemical shift perturbations analysis and water/deuterion exchange experiments to demonstrate that VAMP7 is a dynamic, dominantly closed conformation in solution with intriguing structural properties. Our data complement well with the autoinhibitory mechanism of sintaxins and contributes to a better understanding of membrane fusion regulation.

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Ettore Majorana

Ettore Majorana was born a century ago (in August 1906) in Catania in an era when nuclear physics, still in its historical infancy, was gradually being recognized as a distinct science. In earlier times, the greatest physicists, including Sicily's Stanislao Cannizzaro, were first chemists, and this changed only in the twentieth century.

Ettore came from an intellectual and scientific family. His father, Fabio (1875-1934), was an engineer, and later administrator, in the telegraph and telephone industries. Ettore's uncle, Quirino Majorana (1871-1957), was an experimental physicist at the University of Bologna.

Ettore Majorana was something of a prodigy. His first original work, published in 1928, dealt with atomic spectroscopy. Other early work the structure and behavior of atoms, Majorana studied with Werner Heisenberg, and some of his most important work expanded on that of the latter. He was one of the first physicists to publish theories regarding neutrino masses, work considered to be nearly a century before its time. His most recent published paper (much has been published after his death), in 1937, detailed a symmetrical theory on the relationship and corresponding mass of protons and neutrons, and energies that gave stability to atoms of electrons and positrons. This was particularly important in early research into nuclear fission and chain reactions -- principles governing the function of nuclear power reactors and also the first atomic bombs.

Majorana's name is often associated with two distinguished elder Italian colleagues, Enrico Fermi and Emilio Segré (both of whom eventually became Nobel laureates). Majorana served a professorship at the University of Naples. He later was appointed to a physics post at the University of Palermo, but died before actually assuming it. Had he lived longer, Majorana may have become a decisive force in the field of particle physics. His accomplishments were praised by Fermi in glowing terms: "There are many categories of scientists, people of second and third rank, who do their best, but do not go very far. There are also people of first-class rank, who make great discoveries, fundamental to the development of science. But then there are the geniuses, like Galileo and Newton. Well, Ettore Majorana was one of them."

THE "ETTORE MAJORANA" CENTRE

The "Ettore Majorana" International Centre for Scientific Culture takes its inspiration from the outstanding Italian physicist, Ettore Majorana, after whom the Centre was named. Embracing 118 Schools, covering all branches of Science, the Centre is situated in the old pre-mediaeval city of Erice where three restored monasteries provide an appropriate setting for high intellectual endeavour. These monasteries are now named after great Scientists and strong supporters of the "Ettore Majorana" Centre.

- The San Francesco Monastery is now the Eugene P. Wigner Institute where there is the "Enrico Fermi" Lecture Hall.
- The San Domenico Monastery is now the Patrick M.S. Blackett Institute where there is the "Paul A.M. Dirac" Lecture Hall.
- The San Rocco Monastery is now the Isidor I. Rabi Institute where there is the "Richard P. Feynman" Lecture Hall, the Directorate and the main Secretariat of the Centre.
- There are living quarters in all three Institutes for people attending the Courses of the Centre.

ERICE

Located on high ground overlooking the northern coast of western Sicily, ancient Eryce was a prosperous Elimi city, Eryx, famous for its temple to a fertility goddess, Astarte, later identified with Venus and worshipped by the Romans. The city owes its name to Eryx, mythical ruler of the Elimi. Hercules and Aeneas are also associated with ancient Erice. The Phoenicians, Carthaginians and Romans, in turn, conquered the city, which never developed a particularly strong Hellinic culture except for that of the medieval Byzantines of the Eastern Roman Empire. To the Saracens, Erice was an important foothold known as Gebel Hamed, which the Normans christened Monte San Giuliano, a name by which it was known until 1934, when it was given its older Latin nomenclature. With its delightful medieval ambience and splendid location, Erice is an interesting town and popular with travelers. There are ancient Elimi and Phoenician walls around the northeastern side of the city, and two castles, Pepoli Castle, with foundations dating from Saracen times, and Venus Castle, dating from the Norman era but built on ruins of the ancient Temple of Venus. Surrounded by a lush park, the hilltop castles alone are worth a stop in Erice, which offers charming old stone streets and medieval churches. Pepoli Castle was at first a feudal stronghold, though Erice was eventually ceded to the Crown as a demesnial city.

VIEW FROM PEPOLI CASTLE

The view from the castle towers is stupendous. Though both castles have been modified somewhat over the centuries, they still have that distinctively medieval character one expects of such fortresses.

THE MOTHER CHURCH

The beautiful Mother Church, on Via Carvini, is essentially a 14th century Gothic structure whose style reflects certain Romanesque influences. An older tower stands at the entrance, and the church itself was built upon a much older structure. The medieval Church of Saint John the Baptist was modified in recent centuries but still retains something of its original style, especially its exterior. It was built as an Orthodox chapel. The fifteenth century Church of Saint Ursula, which also retains some medieval Gothic elements, is worth a visit. The trek to Erice from nearby Trapani will take you several miles up winding roads, but it's well worth the trip. The Cordici Civic Museum in Piazza Umberto I houses some interesting finds from the area. Its Carthaginian ("Punic") collection is remarkable, but the Greek and Roman pieces are also interesting.

In Erice you can admire the Castle of Venus, the Cyclopeans Walls (~800 B.C.) and the Gothic Cathedral (~1300 A.D.). Erice is at present a mixture of ancient and mediaeval architecture. Other masterpieces of ancient civilization are to be found in the neighborhood: at Motya (Phoenician), Segesta (Elymian), and Selinunte (Greek). On the Aegadian Islands – theatre of the decisive naval battle of the first Punic War (264-241 B.C.) – suggestive neolithic and paleolithic vestiges are still visible: the grottoes of Favignana, the carvings and murals of Levanzo. Splendid beaches are to be found at San Vito Lo Capo, Scopello, and Cornino, and a wild and rocky coast around Monte Cofano: all at less than one hour's drive from Erice.

VIEW TOWARDS ERICE

VIEW FROM ERICE

STROLLING THROUGH ERICE

CITY OF ERICE

MAP OF ERICE

LEGENDA

1 Azienda autonoma soggiorno e turismo
2 Stazione funivia
3 «Ermione» Hotel 1ª categoria
4 Vigili Urbani e parcheggio
5 Pensione «Edelweiss»
6 Dancing «Boccaccio»
7 Dancing-Ristorante «Al Ciclope»
8 Ristorante «Erice»
9 Banco di Sicilia
10 Discoteca «Blu Notte»
11 Ristorante «La Pentolaccia»
12 Ristorante «Nuovo»
13 Ristorante «Re Aceste»
14 Ristorante-Pizzeria «Ulisse»
15 Campo sportivo San Nicola e campo ippico
16 Tennis Club - Campo di bocce
17 Campo di pallacanestro
18 Pizzeria «Criscenti»
19 Hotel «Moderno» 2ª categoria
20 Centro Cultura Scientifica «E. Maiorana»
21 Ufficio Postale
22 Carabinieri
23 Telefoni pubblici
24 Self service «Fork and Knife»
25 Trattoria-Pizzeria «La Vetta» da Mario
26 Tavola calda «Naif»
27 Albergo della Gioventù C.S.I.
28 Istituto Professionale Alberghiero

A Duomo e campanile
B Castello normanno (tempio di Venere)
C Villa Comunale-Balio
D Torri medioevali
E Torretta Pepoli
F Biblioteca-Museo Comunale
G Palazzo Chiaramonte
H Quartiere spagnolo
I Porta Carmine
L Porta Trapani
M Porta Spada
N Casa Militari e Chiesa Carmine
O Chiesa S. Giuliano
P Chiesa S. Carlo
Q Chiesa S. Pietro
R Chiesa S. Martino
S Chiesa S. Francesco
T Chiesa S. Giovanni
U Chiesa S. Orsola
V Chiesa S. Cataldo
Y Chiesa S. Teresa
W Ruderi Porta Castellammare
Z Chiesa S. Domenico

POETIC TOUCH

According to legend, Erice, son of Venus and Neptune, founded a small town on top of a mountain (750 metres above sea level) more than three thousand years ago. The founder of modern history – i.e. the recording of events in a methodic and chronological sequence as they really happened without reference to mythical causes – the great Thucydides (~500 B.C.), writing about events connected with the conquest of Troy (1183 B.C.) said: "After the fall of Troy some Trojans on their escape from the Achaei arrived in Sicily by boat and as they settled near the border with the Sicananians all together they were named Elymi: their towns were Segesta and Erice". This inspired Virgil to describe the arrival of the Trojan royal family in Erice and the burial of Anchise, by his son Enea, on the coast below Erice. Homer (~1000 B.C.), Theocritus (~300 B.C.), Polybius (~200 B.C.), Virgil (~50 B.C.), Horace (~20 B.C.), and others have celebrated this magnificent spot in Sicily in their poems. During seven centuries (XIII-XIX) the town of Erice was under the leadership of a local oligarchy, whose wisdom assured a long period of cultural development and economic prosperity which in turn gave rise to the many churches, monasteries and private palaces which you see today.