交叉学科论坛
Symposium for Advanced Studies

第二十七期：病毒离子通道蛋白的结构与功能研讨会
Symposium on Viral Membrane Proteins

主办单位：中国科学院上海交叉学科研究中心
承办单位：上海巴斯德研究所

Symposium on Viral Membrane Proteins

Shanghai Institute for Advanced Studies, CAS
Institut Pasteur of Shanghai,CAS

30.11. – 2.12 2011

Shanghai, China

Schedule:

Wednesday, 30th of November 2011

Morning Arrival

Thursday, 1st of December 2011

8:00 Arrival

9:00 Welcome — Bing Sun, Co-Director, Pasteur Institute Shanghai

9: 10 – 9:35 — Bing Sun, Pasteur Institute Shanghai
Ion channel study and drug target fuction research of coronavirus 3a like protein.

9:35 – 10:00 — Tim Cross, Tallahassee, USA
The proton conducting mechanism and structure of M2 proton channel in lipid bilayers.

10:00 – 10:25 — Shy Arkin, Jerusalem, IL
A backbone structure of SARS Coronavirus E protein based on Isotope edited FTIR, X-ray reflectivity and biochemical analysis.

10:20 – 10:45 Coffee Break

10:45 – 11:10 — Rainer Fink, Heidelberg, DE
Elektromechanical coupling in muscle: a viral target?

11:10 – 11:35 — Yechiel Shai, Rehovot, IL
The interplay between HIV1 fusion peptide, the transmembrane domain and the T-cell receptor in immunosuppression.

11:35 – 12:00 — Christoph Cremer, Mainz and Heidelberg University, DE
Super-resolution Fluorescence imaging of cellular and viral nanostructures.

12:00 – 13:30 Lunch Break

13:30 – 13:55 Jung-Hsin Lin, National Taiwan University
Robust Scoring Functions for Protein-Ligand Interactions with Quantum Chemical Charge Models.

13:55 – 14:20 Martin Ulmschneider, Irvine, USA
Towards in-silico assembly of viral channels: the trials and tribulations of Influenza M2 tetramerization.

14:20 – 14:45 Jakob Ulmschneider, Shanghai
In-silico peptide partitioning into lipid bilayers and the application to viral peptides.

14:45 – 15:10 Wolfgang Fischer, National Yang-Ming University
Structural modeling of viral channel proteins for drug development.

15:10 – 15:20 Coffe Break

15:20 – 15:44 Melanie Schwarten, Grenoble, FR
NMR characterization of the non-structural protein 5A of the hepatitis C virus.

15:45 – 16:10 Stephen Griffin, Leeds, UK
Structure-guided development of novel hepatitis C virus p7 inhibitors.

16:10 – 16:35 José Villalaín Alicante, ES
Paradigm of a viral membrane protein. HCV protein NS4B.

18:00 – 19:30 Dinner

Friday, 2nd of December 2011

8:45 – 9:00	Bing Sun, Co-Director, Pasteur Institute Shanghai
9:00 – 9: 45	James Chou, Harvard Medical School, USA *Influenza proton channels: from structure to mechanism of drug resistance.*

Departures

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Abstracts:

A backbone structure of SARS Coronavirus E protein based on Isotope edited FTIR, X-ray reflectivity and biochemical analysis.

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SARS Coronavirus E protein is a small membrane protein responsible for viral budding. We have previously shown that a peptide that encompasses its abnormally long hydrophobic segment is capable of dramatically distorting lipid vesicles. As such, in this study we describe a comprehensive structural analysis of the protein based on orientational constraints from isotope edited FTIR, depth restrains from X-ray electron-density profiles and biochemical cross-linking experiments. Together we have arrived at a self-consistent helical hairpin structure of the protein that may explain its ability to deform lipid bilayers.

Influenza proton channels: from structure to mechanism of drug resistance.

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The M2 proton channel ofinfluenza A is an important target of the adamantane-family antiviral drugsthat have been used globally for treating influenza infections. The drugsinhibit the proton transport activity of M2, but mutations in M2 that conferdrug resistance are widespread, greatly compromising the effectiveness of thesecompounds. We have, in the past three years, determined the structures of the M2 channels from both flu A and B viruses, the structures of the drug-bound and drug-free channels, as well as the structures of several resistance mutants. These structural data, together with extensive functional mutagenesis, now enable us to gain a rather clear understanding of the mechanism of proton transport, drug binding and drug resistance. These knowledge will undoubtedly aid new efforts to develop the next generation of anti-flu therapeutics.

Super-resolution Fluorescence Imaging of Cellular and Viral Nanostructures.

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Fluorescence imaging based methods for lightoptical analysis at enhanced resolution beyond the possibilities of conventional epifluorescence microscopy (optical resolution about 200 nm laterally, 600 nm axially) have opened a new avenue towards a highly improved functional understanding of biological nanostructures. The presently established applications range from bacteria to individual protein and chromatin complexes in single 3D preserved mammalian cells. Using Spectral Precision Distance/Position Determination Microscopy with Physically Modifiable Fluorophores ($SPDM_{Phymod}$, in the following abbreviated as SPDM), a special mode of Spectrally Assigned Localization Microscopy (SALM) allowing the application of standard fluorescent proteins and fluorochromes, the spatial distribution of single membrane proteins in intact human cells was determined with a lateral optical resolution down to the 10 - 20 nm range; the intracellular 3D distribution of proteins was measured with a resolution corresponding to about 40 nm in 3D, allowing the simultaneous nanoimaging of two protein types.

As an example for the use of SPDM in the imaging of viral nanostructures, an analysis of *Tobacco mosaic virus* (TMV) particles is presented (collaboration with Max Planck Institute for Metals Research & Institute of Biology, Stuttgart, Germany). Because of its precisely known nanostructure, this virus is especially suited to test the performance of the SPDM method. A mean 2D-localization accuracy around 7-8 nm of Atto488 molecules bound to TMV-coat proteins was achieved. From this, a mean optical resolution around 15 nm in the object plane was estimated, corresponding to about 1/33 of the exciting wavelength used. The best localization accuracy values obtained in these experiments were around 2 nm, corresponding to an optical resolution power in the range of 5 nm. Along the viral axis, up to about 30,000 molecule signals/μm^2 were detected, with a mean next neighbour 2D distance around 6 nm. From these SPDM measurements, the diameter of the TMV particle was estimated correctly with an error in the 2 nm range. Applications to use such methods to study virus-membrane interactions in human cells shall be discussed.

The Proton Conducting Mechanism and Structure of M2 Proton Channel in Lipid Bilayers.

Sharma, M., Yi, M., Dong, H., Qin, H., Can, T., Miao, Y., Luca, S., Peterson, E., Busath, D.D., Zhou, H.-X. & Cross, T.A.

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A combination of oriented sample and magic angle spinning solid state NMR data is used to refine the M2 conductance domain structure in lipid bilayer preparations. In particular, the focus is on the dimer of dimer structure associated with the critical HxxxW sequence. It will be shown that the structure of the histidine tetrad in the conductance domain is the same in purified and reconstituted full length protein and in the full length protein in the plasma membranes of *E. coli.* This later sample has never been exposed to the influence of detergents. In light of this structure the conductance mechanism will be discussed in detail.

Electromechanical coupling in muscle: a viral target?

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Contraction of striated as well as smooth muscle cells is regulated by changes in the myoplasmic free calcium concentration. The initial step of the excitation-contraction coupling involves different cellular mechanisms to achieve the necessary calcium concentration changes. A depolarisation of the membrane potential of the muscle cells can -depending on the type of muscle- either directly stimulate calcium inflow into the myoplasm and/or lead to a release of calcium ions from intracellular stores, in particular the sarcoplasmic or endoplasmic reticulum. Furthermore, it is now widely accepted that calcium regulation can be profoundly disturbed by viral attacks of cells. However, there is still very little known about possible viral-induced changes in excitation-coupling, in particular of cardiac and skeletal muscle.

Structural modeling of viral channel forming proteins for drug development.

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Many biological processes such as the mode of action of viral membrane proteins during genome ejection and channel formation are usually outside the time and size frames manageable by computational methods. A solution to this is to fragment the biological event into 'frames'. Two examples of 'fragment' computing will be reported: VP1 from Polio virus and its role in genome ejection and the assembly of mono and polytopic viral channel forming proteins (VCPs).

The role of VP1 during genome ejection is still under debate. It is suggested that the protein either supports ejection by channel or pore formation, or just lies on the membrane surface. Computational simulations shed light on the putative mechanism. Ideas about routes of assembly of VCPs are outlined and shown on examples of bitopic p7 of HCV and tritopic 3a of SARS-CoV. Computational data are evaluated against relevant data from experiments. The assembly protocol proposed comprises a robust tool to generate relevant protein models which can be used further in drug development.

Structure-guided development of novel hepatitis C virus p7 inhibitors

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Chronic hepatitis C virus (HCV) infection causes severe liver disease and is a major indicator for hepatocellular carcinoma. Of ~200 million infected individuals, many remain undiagnosed and yet more are unable to access current antiviral therapy. Current standard of care comprises PEG-interferon alpha (IFN) combined with ribavirin (Rib), yet this achieves sustained responses in less than 50% of those treated and significant toxicity, high costs and invasive administration lead to a poor rate of patient compliance.

Recently, two direct-acting antivirals (DAA) targeting HCV protease were approved for the treatment of HCV. This reflects a drive to develop IFN-free regimens of DAA to replace current therapy. However, currently new DAA are necessarily given alongside IFN/Rib and, although they significantly improve treatment in many cases, response remains entirely dependent on IFN. More DAA are in clinical development with exciting possibilities for future drug combinations, yet it is highly likely that the long term management of chronic HCV infection will require an ongoing programme of drug development targeting multiple viral proteins.

The HCV p7 proton channel is essential for the secretion of infectious HCV, protecting nascent virions from reduced pH during egress. Several prototype inhibitors with distinct chemotypes block both p7 function and HCV secretion in a strain-dependent fashion, indicative of specific protein-drug interactions. p7 therefore presents as a favourable candidate for DAA development, yet scepticism concerning the clinical use of p7-targeted compounds arose following trials involving a weak prototype inhibitor, amantadine.

We have sought to develop p7 inhibitors as viable HCV therapeutics using a combined molecular modelling/structure-based rational approach. Through these methods we have defined the modes of action for prototypic p7 inhibitors by the identification of specific resistance and characterised their binding sites within the channel complex. Incorporation of a recently solved solution NMR structure for monomeric p7 within channel models has led to the generation of novel inhibitory compounds with nanomolar activities in HCV culture. This represents a critical stage in hit-to-lead development for novel DAA targeting this virus ion channel.

Robust Scoring Functions for Protein-Ligand Interactions with Quantum Chemical Charge Models

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Ordinary least square (OLS) regression has been used widely for constructing the free scoring functions for protein-ligand interaction. However, OLS is very sensitive to the existence of outliers, and models constructed using it are easily affected by the outliers or even the choice of the dataset. On the other hand, determination of atomic charges is regarded as of central importance, because the electrostatic interaction is known to be a key contributing factor for biomolecular association. In the development of the AutoDock4 scoring function, only OLS was conducted, and the simple Gasteiger method was adopted. It is therefore of considerable interest to see whether more rigorous charge models could improve the statistical performance of the AutoDock4 scoring function. In this study, we have employed two well-established quantum chemical approaches, namely the restrained electrostatic potential (RESP) and the Austin-Model 1-Bond Charge Correction (AM1-BCC) methods, to obtain atomic partial charges, and we have compared how different charge models affect the performance of AutoDock4 scoring functions. In combination with robust regression analysis and outlier exclusion, our new protein-ligand free energy regression model with AM1-BCC charges for ligands and Amber99SB charges for proteins achieve lowest root-mean squared error of 1.637 kcal/mol for the training set of 147 complexes and 2.176 kcal/mol for the external test set of 1427 complexes. The assessment for binding pose prediction with the 100 external decoy sets indicates very high success rate of 87% with the criteria of predicted RMSD less than 2 Å. The success rates and statistical performance of our robust scoring functions are only weakly class-dependent (hydrophobic, hydrophilic, or mixed).

Reference

Jui-Chih Wang, Jung-Hsin Lin*, Chung-Ming Chen, Alex L. Perryman, Arthur J. Olson, "Robust scoring functions for protein-ligand interactions with quantum chemical charge models**"**, *J. Chem. Inf. Model.* (2011, in press).

NMR characterization of the non-structural protein 5A of the hepatitis C virus

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The Hepatitis C virus (HCV) is a major human pathogen causing diseases such as chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Its non-structural protein 5A (NS5A) is implicated in a variety of cellular pathways, although their relevance for the viral pathogenesis still has to be elucidated. NS5A is organized into three domains, whereof the first domain is highly conserved among different HCV genotypes and forms a well defined globular structure. It also contains a N-terminal amphipathic helix, which is used to anchor NS5A to ER derived membranes.[1] The domains 2 (D2) and 3 (D3) are less conserved and are intrinsically disordered.[2,3] The low complexity sequence (LCS) connecting D2 and D3 contains two directly neighbored class II PxxP-motifs, which are important for interactions with Src-homology 3 (SH3) domains.

Using NMR spectroscopy, we investigated a NS5A fragment containing amino acid residues 191-369, which comprises the entire D2 and its adjacent LCS regions, as well as a NS5A fragment comprising the complete unstructured region (191-447). NMR spectroscopy is an unique tool to study intrinsically disordered proteins (IDPs) at atomic resolution. However, due to their low chemical shift dispersion, they remain challenging. To obtain nearly complete backbone resonance assignments of the two fragments we used a set of sensitivity and resolution optimized 3D correlation experiments, as well as amino-acid-type editing in ^{1}H-^{15}N correlation spectra.[4,5,6]

Based on our NMR data, we were able to identify four regions with an elevated propensity to adopt α-helical structures. We also have evidence for transient long-range interactions within this NS5A fragment that contributes to the stabilization of the secondary structural elements. Finally, we identified the binding modes of NS5A with several SH3 domains. Most interestingly, 2 out of the 4 transiently populated helices are involved in low affinity SH3 binding.[7,8]

References:

[1]Tellinghuisen TL et al. (2004) *J Biol Chem* 47, 48574-48587
[2]Hanoulle X et al. (2010) *Protein Pept Lett* 17, 1012-1018
[3]Hanoulle X et al. (2009) *Biochem Bioph Res Co* 381, 634-638
[4]Favier A & Brutscher B (2011) *J Biomol NMR* 49, 9-15
[5]Marion D (2010) *J Magn Reson* 206, 81-87
[6]Lescop E et al. (2008) *J Am Chem Soc* 130, 5014-5015
[7]Feuerstein S et al. (2011) *Biomol NMR Assign* 5, 241-243
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The Interplay Between HIV1 Fusion Peptide, the Transmembrane Domain and the T-Cell Receptor in Immunosuppression

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Human immunodeficiency virus (HIV) infection confounds the immune response. Untreated HIV infection usually leads to a state of general immunosuppression, the acquired immune deficiency syndrome (AIDS), and susceptibility to otherwise innocuous opportunistic infections. However, to establish a successful infection and replicate, the virus has to evade immune control, a task that HIV accomplishes by using a broad array of mechanisms. It is known that the fusion peptide (FP) in the N-terminus of the HIV gp41 molecule functions together with other gp41 domains to fuse the virion with host cells. We found that in addition, FP inactivates T-cells. Mode of action studies using various biophysical and biochemical approaches revealed that FP binds specifically to the transmembrane domain (TMD) of T-cell receptor (TCR) and interferes with its functional assembly. Remarkably, by utilizing *in-silico* testing of a TMD sequence library derived from virus protein sequences, we have pin-pointed a nine amino-acid motif shared by the TMD of the α-subunit of the T-cell receptor and the TMD of gp41. A synthetic peptide resembling gp41 TMD co-localizes with CD3 within the TCR complex and inhibits T cell proliferation *in vitro*. This mechanism differs from that of the FP. Finally, these immunosuppressive activities of gp41 FP and TMD can be exploited in the future for the design of new therapies for autoimmune diseases.

Ion channel study and drug target function research of coronavirus 3a like protein.

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Severe acute respiratory syndrome (SARS), also called infectious atypical pneumonia, is an emerging infectious disease caused by a novel variant of coronavirus (SARS associated coronavirus, SARS-CoV), which has high infectivity and mortality. The ORF3 gene of SARS-CoV encodes a viral structural protein 3a, which can forms an ion channel and modulates virus release. Our project is aimed to study the ion channel characteristics of 3a by electrophysiology method, such as voltage clamp and patch clamp, and find the detail mechanism of viral release, combined with techniques in molecular biology and virology. Virus ion channel as a new antiviral drug target, thus demonstration of 3a protein function will guide the design and selection of potential antiviral drugs, and help to control SARS coronavirus infection. On the other hand, this project and research platform could be used for other coronavirus study, and will promote other viral ion channels research, such as HIV and HCV.

Towards in-silico assembly of viral channels: the trials and tribulations of Influenza M2 tetramerization.

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The M2 protein from influenza A forms homotetrameric ion channels in the viral envelope. These channels are vital for acidification of the virion interior, which is a necessary prerequisite for viral un-coating after endosomal uptake by the host cell. Adamantane based antivirals inhibit viral replication by blocking the channel. However, a handful of single-mutants in M2 have recently resulted in near 100% resistance to adamantane based therapies. It would therefore be of great interested if the structural impact of single mutations could be predicted *ab-inito*. Towards this goal we have attempted to fold and assemble an M2 proton channel using unbiased multi-microsecond molecular dynamics simulations, with no other input than the sequence information. We have previously demonstrated the feasibility of folding the trans-membrane segment of M2 monomers in a simplified implicit membrane representation, which resulted in structures with ~2.5 Å root mean square deviation from the experimental NMR structures in bilayers. Here we extend this work to fully explicit bilayers. This has the advantage of more accurately capturing the complex bilayer interfaces. We demonstrate that M2 monomers can be efficiently folded using all-atom molecular dynamics simulations in explicit lipid bilayers. In addition, we attempt channel assembly by allowing previously folded M2 helices to freely oligomerize.

PARADIGM OF A VIRAL MEMBRANE PROTEIN.
HCV PROTEIN NS4B

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The hepatitis C virus (HCV) is the major cause of liver-related diseases, including cirrhosis and hepatocellular carcinoma, infecting more than 180 million people worldwide. So far there is no vaccine available and current treatments are very limited, because of their low effectiveness as well as their major side effects. HCV is an enveloped virus, contains a single strand of positive RNA encoding a polyprotein which after processing gives rise to several structural (core, E1, E2 and p7) and non-structural (NS2, NS3, NS4A, NS4B , NS5A and NS5B) proteins [1]. While proteins E1 and E2 are related to membrane fusion, NS proteins are associated with replication, protein-protein and membrane-protein interactions. Virus entry into the host cell is achieved by endocytosis, where the viral and endosome membranes fuse so that the viral genome can be replicated within the cell. Both replication and morphogenesis are related to a replication complex (RC) resulting from the modification of membranes of the endoplasmic reticulum (ER) [2]. The non-structural NS4B protein, an essential protein in the HCV cycle, is the less known because of its high hydrophobicity. NS4B is a highly hydrophobic protein, is palmitoylated and it is associated with the membranes of the RC, inducing the disruption of ER membranes and appearing to be a multifunctional protein [3]. One of the most important functions of NS4B could therefore be the formation of the framework that gives rise to the RC. NS4B is predicted to have at least four transmembrane domains, and possibly more than one conformation. The C-terminal region of NS4B exhibits many similarities with other proteins of other viruses of the same family, presenting two α-helical regions, been able to interact with membranes and possibly with other proteins [4, 5]. The N-terminal region also has two α-helical regions, some of them capable of crossing the ER membrane, and has been suggested the existence of a leucine zipper domain. Both the N-terminal and the C-terminal could be potential targets to find inhibitors of HCV replication. Our research group, in order to understand the mechanism of interaction with membranes and the replication of HCV, has studied this and other HCV proteins. In this presentation we present our recent studies on HCV NS4B protein, the great unknown among all HCV proteins, its topology, its interaction with membrane model systems as well as our efforts to look for HCV replication inhibitors using NS4B as a molecular target.

[1] D. Moradpour, F. Penin, C.M. Rice (2007) Nat Rev Microbiol, 5, 453-463.
[2] N. El-Hage, G. Luo (2003) J Gen Virol, 84 (2003) 2761-2769.
[3] M. Poenisch, R. Bartenschlager (2010) Semin Liver Dis, 30, 333-347.
[4] J. Guillen, A. Gonzalez-Álvarez, J. Villalaín (2010) Biochim Biophys Acta, 1798, 327-337.
[5] M.F. Palomares-Jerez, J. Villalaín (2011) Biochim Biophys Acta, 1808, 1219-1229.