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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105.

Dear Prof. Rob de Ruyter van Steveninck:

I am writing in response to your notice on naturejobs Ref:(NW45910)R of the position as Assistant Professor in your department. I am currently a research associate in Department of Chemistry at the University of Pennsylvania.

For the past five and a half years, I have studied structures of HIV-1 Vpu protein and its truncated fragments mixed with phospholipid monolayers at the air/water interface and on solid supports via x-ray scattering techniques. Vpu has two different biological activities, namely the enhancement of the release of virus from the infected cell presumably through oligomerization of the transmembrane domain, and the degradation of CD4 molecule in the endoplasmic reticulum (ER). I have also been involving design and structural studies of artificial proteins vectorially-oriented at air/water interfaces. The artificial proteins were designed to possess a variety of functionalities such as generating novel electron transfer with the four-helix bundle motifs and providing model system to understand the mechanism of anesthetic binding. I have been extremely fortunate carrying out x-ray scattering experiments at the CMC CAT at Advanced Photon Source (APS), Argonne National Laboratory and X22B at National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. The phase problem for x-ray and neutron reflectivity from liquid surfaces and from thin films on liquid surfaces has been a difficult issue. As a result, most experimental data has been analyzing by using model-dependent "slab-model" refinement approach with an assumption based on the physical-chemical knowledge of the system investigated. I have been involving the development of data analysis methodology for x-ray and neutron reflectivity from the liquid surfaces namely model-independent "box-refinement" with no prior assumptions to solve the phase problem. The box-refinement is particular effective in determining the structures of complex systems where it is difficult or impossible to assume the unbiased initial structures.

My Ph.D training in Japan applied synchrotron-based x-ray reflectivity in thin films via x-ray fluorescence (XRF) and x-ray excited conversion electron yield (CEY). I was the key person who developed the combination of XRF and CEY techniques together for studying near-surface information of a thick sample at gas environment. The combined technique has been included in "Encyclopedia of Analytical Chemistry" (John Wiley & Sons Ltd, Chichester, UK

2000). I have also applied x-ray absorption fine structure (XAFS) spectra in the soft x-ray region in measuring burned coal fly ash to discover the chemical structures of the surface and core of the ash simultaneously. During the early stages of my scientific career, I also benefited from the highly collaborative environment at the Photon Factory, National Laboratory for High Energy Physics (KEK), Tsukuba, Japan.

I have considerable teaching experience as an adjunct lecturer in a community college of the company that I worked for after I graduated from university and as a teaching assistant in my graduate school. As a community college lecturer, I was chosen to teach analytical methodology and instrumentations. This course included commonly used laboratory techniques, data treatment and instrumental analysis. As a teaching assistant, I chose the courses that were most related to my research interests as you can see from my CV.

In addition to my strong technical skills and varied experience, I would also bring my total commitment and engagement to your department. If any additional information is required, please do not hesitate to contact me. Thank you very much for your consideration. I look forward to hearing from you.

Sincerely,

Songyan Zheng

Songyan Zheng

Statement of research plans

Songyan Zheng

Many specific functions of biological membranes are due to the operation of membrane proteins and that the phospholipids act essentially as a solvent for these proteins. HIV-1 Vpu protein is a 81-residue typical small natural integral membrane phosphoprotein constituted with an N-terminal extremely hydrophobic α-helix transmembrane domain containing 27 residues and a C-terminal cytoplasmic domain containing 51 residues consisting of two amphipathic α-helices connected by a 9-residue loop. Vpu has two different biological activities, namely the enhancement of the release of virus from the infected cell presumably through oligomerization of the transmembrane domain, and the degradation of CD4 molecule in the endoplasmic reticulum (ER). The degradation of CD4 is dependent on the phosphorylation of Vpu at its two phosphoacceptor sites S52 and S56 which are involving the interaction with the cytoplasmic domain of CD4.

Vpu's simplicity together with a completed composition as a nature integral membrane protein provides an attractive system to study functional structures associated with other chemical components and their interactions with surrounding membrane environment. It is particularly meaningful to utilize small membrane proteins in studies of membrane protein-phospholipids interaction because the influence of small membrane protein-phospholipids interactions to their biological functions is much greater than that of larger membrane protein-phospholipids interactions in living cells. Tm and TmCy are sub-molecular fragments of Vpu containing 36 residues and 51 residues truncated from C-terminal side of the cytoplasmic domain. Thus both fragments possess the identical hydrophobic transmembrane α-helix and either with a portion or the entire first amphipathic helix of cytoplasmic domain, respectively. Simultaneous studies of these three proteins allow us to investigate the possible effects of the phosphorylation of the cytoplasmic domain of Vpu to the oligomeric state of the transmembrane domain such as its tilt angle with respect to the normal of the membrane surface and to determine the tertiary structure of Vpu associated with the phosphorylation and its interaction with the cytoplasmic domain of CD4. X-ray crystallography and NMR can provide molecular structures, but not structures in membrane environment and interactions with the membrane environment. For example, in the crystallization process of membrane proteins, the transmembrane domain is not unidirectionally inserted into the hydrophobic environment provided by the detergents unlike the real biological membrane which allows the unidirectional arrangement of the transmembrane domains to form ion channels. In addition, the crystal packing does not provide an unrestricted aqueous environment for the cytoplasmic domain, thus it seems particularly difficult to investigate the phosphorylated Vpu and its interaction with the cytoplasmic domain of CD4. In general, NMR structures of small membrane proteins can be obtained from non-biological environments such as organic solvents and buffer solutions, even if micelles and multibilayers. However, micelle does not provide hydrophobic environment which allows the transmembrane domains to unidirectionally insert as a bundle for ion channel formation and multibilayers do not provide intracellular environment, namely big enough aqueous space between layers for the cytoplasmic domains.

We have employed X-ray scattering techniques in seeking the structures of Vpu proteins, namely Vpu, Tm and TmCy in a membrane-like environment such as proteins/phospholipids monolayers at air/water interface. The advantages of studying membrane protein structure within monolayers are that the water subphase is effectively infinite in extent beneath the phospholipid headgroups and the protein is unidirectionally incorporated into the monolayer thereby providing a good approximation to the surface of an intracellular membrane, especially for the Vpu's cytoplasmic

domains. In addition, the use of Langmuir monolayer technique enables to control directly the temperature and the surface pressure of the monolayer constituted mainly by the host phospholipids to ensure the monolayer to be in physiological conditions. Our initial results have determined general localizations of Vpu proteins within the host phospholipid monolayers. The transmembrane α-helix is localized in the hydrocarbon chain layer of the host phospholipid monolayer and amphipathic α-helices of cytoplasmic domains lie on the surface of the phospholipid headgroups in the water subphase at physiological surface pressures.
I will extend the structural studies of Vpu proteins in more disordered fluid phase and investigate the interactions between Vpu proteins and phospholipids through a pH dependent study. Those studies will not be continued in Dr. J Kent Blasie' research group in University of Pennsylvania because they represent my own research interests. Furthermore, I will develop and apply neutron scattering from selectively deuturated Vpu proteins mixed with phospholipid monolayer at the air/water interface to determine its structure at a single-residue resolution. I will create a research program in phosphorylation of Vpu and its interaction with the cytoplasmic domain of CD4. Structures of phosphorylated Vpu and the complex of phosphorylated Vpu-cytolasmic domain of CD4 within host phospholipids monolayers at air/water interface will be studied via X-ray scattering and neutron scattering. I will also study conformational changes associated with Vpu mutants within the host phospholipids monolayers at air/water interfaces to investigate whether structural elements of Vpu contribute to its conformational changes. Those studies mentioned above will not only be necessary and important in understanding fundamental physiological issues that functional structures of membrane proteins associated with membrane environment but also be specifically meaningful in understanding the mechanism of HIV infection and provide a firm and effective basis for drug discovery in AISD treatment.
X-ray scattering and neutron scattering will be carried out exclusively at national laboratories such as Brookhaven National Laboratory, Argonne National Laboratory and National Institute of Standards and Technology.

1. Structural studies of Vpu proteins in disordered fluid phase

It is known that the mobility of membrane proteins is necessary for efficient operation in a real biological system. The strategy to further investigate a variety of effects of Vpu proteins within more membrane-like environment will be to introduce unsaturated phospholipids into monolayers instead of using only saturated phospholipids. Phospholipids with two asymmetric hydrocarbon chains, one fully saturated and the other mono-cis-unsaturated with a double bond or mono-trans-unsaturated with a double bond can be used as host lipids. Another way to introduce the double bond to the monolayers is to mix saturated phospholipids at certain ratios with unsaturated phospholipids possessing with one or two double bonds. The introduction of the double bond in the acyl chain increases the lateral lipid-lipid spacing in the monolayer and makes the host monolayers to be more fluid like biological membrane environment. In this study, X-ray reflectivity technique will apply in the monolayers of mixed Vpu proteins/unsaturated phospholipids at air/water interface, the electron density profiles derived from experimental data via Box-Refinement with no prior assumption will establish the shape of Vpu proteins with respect to the surface normal within more flexible environment due to the alkyl chains with the double bond introducing more space into the monolayers.

2. Investigate Vpu proteins-phospholipids interaction

Our initial studies have demonstrated that cytoplasmic domains of Vpu proteins closely lie on the surface of the headgroups rather than extended into the infinite bulk subphase. One can think that there are two adhesive interactions between the host phospholipids and the Vpu proteins:

one is that the nonpolar hydrophobic residues of cytoplasmic domains have a tendency to approach the hydrophobic carbon chain region. The detailed localization of the nonpolar residues of cytoplasmic domains will be studied by neutron reflectivity by measuring mixed monolayers of phospholipids and selectively deuturated Vpu proteins synthesized by solid phase synthesis (detailed approach will describe in plan 3). Physical-chemical properties of the phospholipids 2-Dilignoceroyl-sn-Glycero-3-Phosphocholine (abbreviated herein as DLgPC) used in this study are that a headgroup of the phospholipid DLgPC molecule contains groups that are positively and negatively charged, whereas its net electrostatic charge is zero. Note that DLgPC contains phosphocholine the generally predominant headgroup of phospholipids in biological membranes. Negatively charged group is the non-ester phosphate oxygen atoms. Positively charged group is the choline. The cytoplasmic domain of Vpu contains net 11 negatively charged residues with pKa values of 4~5. Negatively charged residues of the cytoplasmic domains of the Vpu may interact with positively charged group of the phosopholipids headgroups within the membrane-like environment. Negatively charged residues of the cytoplasmic domains of the Vpu protein can be neutralized by using the solution pH below 4, in which the cytoplasmic domain is solubilized. X-ray reflectivity studies from Langmuir monolayers of mixed Vpu/phospholipids as a function of subphase's pH will be an effective approach to investigate electrostatic force involved in the protein-phospholipid interaction.

3. Structural studies of Vpu proteins at a single-residue resolution

Electron density profiles derived from the X-ray reflectivity data provide the overall shape of the Vpu proteins with respect to the surface normal. Neutron reflectivity can be employed in studying more detailed structure of Vpu proteins at single-residue resolution for both the transmembrane domain and the cytoplasmic domains.

A. Detailed structural study of the transmenbrane domain of Vpu

The approach will be to selectively deuterate the hydrogen rich residues of the transembrane domain located near at both ends of the helix by using solid phase synthetic method which permits the selective deuteration of particular residues. Neutron reflectivity will be applied to monolayers formed from both selectively deuderated and hydrogenated Vpu proteins at the air/water interface. Experimental data will be analyzed by model-independent Box-Refinement method. The difference between absolute neutron scattering-length density profiles for both specially deuterated Vpu proteins and the fully-hydrogenated Vpu proteins will indicate the localization of the deuterated residues in the monolayer profile structure. As a result, distance measuring between these two deuterated residues via neutron reflectivity will indicate the tilt angle of the transmembrane domain vectorally oriented within the phospholipids monolayers and identify alpha helicity of the transmembrane domain.

B. Detailed structural study of the cytoplasmic domains of Vpu proteins

Localization of nonpolar residues of the amphipathic α-helices of the cytoplasmic domains of Vpu proteins with respect to the polar headgroups and hydrocarbon chains can be studied via neutron reflectivity as a function of surface pressure in the physiological range over a range of protein/phospholipid mole ratios in Langmuir monolayers. Neutron reflectivity will be applied in selectively deuterated non-polar residues of cytoplasmic domains of Vpu proteins and their hydrogenated form monolayers at air/water interface. The difference between absolute neutron scattering-length density profiles for both selectively deuterated Vpu proteins and fully-hydrogenated Vpu proteins will indicate the localization of particularly deuterated nonpolar

residues of the amphipathic helices within the host phospholipid monolayer at single residue level and their role in determining the tertiary structure of the cytoplasmic domains in this membrane-like environment as surface pressure changes.

4. Structural studies of phosphorylated Vpu and complex of phosphorylated Vpu-cytoplasmic domain of CD4

In vitro phosphorylation studies demonstrated that Vpu is phosphorylated by endogenous casein kinase-2 (CK-2) in HIV-1 infected cells. The phosphorylation sites for CK-2 within the Vpu sequences are two serines at positions of S52 and S56. CD4 degradation is mediated through interaction between the phosphorylated sites of Vpu and the cytoplasmic domain of CD4. In this study, I will investigate the structures of phosphorylated Vpu and the complex of phosphorylated Vpu-cytoplasmic domain of CD4 within the host phospholipids monolayers at the air/water interface via X-ray scattering and neutron scattering. Furthermore I will investigate conformational changes of the transmembrane domain of Vpu associated with interaction between phosphorylated Vpu and the cytoplasmic domain of CD4, in which the transmembrane domain has a critical role in regulating virus secretion, presumably through the formation of an ion channel.

Functional studies show that mutations in the transmembrane domain of Vpu partially lost its ability to enhance viral particle release while mutations in the cytoplasmic domain show that Vpu lost its ability to bind the CD4 molecules. To gain further insight into the structure-mutation correlation, I will study mutational structures of phosphorylated Vpu. The hydrophobic transmembrane domain of Vpu will be mutated from the N-terminal, middle to end of the transmembrane domain. Meanwhile the C-terminal of the cytoplasmic domain will be mutated at C-terminal, middle to the end of the cytoplasmic domain. All mutated Vpu will be investigated within in host phospholipids monolayers at the air/water interface. Structural studies of Vpu mutants will be particular meaningful for the drugs development in AIDS treatment.

Statement of teaching plans

Songyan Zheng

I am primarily interested in teaching courses of experimental chemistry, analytical chemistry and biophysical chemistry at the undergraduate level and courses of x-ray scattering techniques in structural biology and instrumental methods of surface analysis at the graduate level. I am also excited by the prospect of developing new courses for advanced undergraduate and graduate students that explore and advance the frontiers of my research area. All courses I teach will provide an opportunity for students to get an intensive and hands-on introduction to the principles and techniques in different levels as well as discussions of the kinds of experiments made possible by these techniques. Meanwhile I involve students in my own research whenever possible.

My teaching philosophy is to help students to develop their own scientific and intellectual abilities by giving them a dynamic opportunity to learn how to be scientists. An emphasis on the interconnectivity of science will be essential. During the courses of my own education and the courses I taught, I have accumulated several teaching strategies which are particularly effective and hope to be able to implement these strategies in the courses that I will teach.

- Cover a broad range of topics with well-organized and well-prepared materials. Finding interesting examples can help students gain a better understanding of principles and motivate students to spend more time with the materials. To be prepared to answer questions that go beyond the lecture materials.

- Promote discussion and critical thinking during a lecture. Encourage involvement and the individual student's constructive suggestions. Respect students and their capabilities, pay attention to their comprehension and interests, and maintain high expectations from them.

- Encourage active learning with modern communication technologies such as searching for information and using software available on the web. Use the e-mail to assign topics for class discussion, provide materials and clarify homework and lab reports. Keeping a regular office hour is not only necessary to directly help individuals with particular questions but also to get feedback from students to strengthen the quality of my teaching. Encourage both independent and collaborative work according to the course materials and issues.

- Teach students the methodology and technical skills in resolving the problems rather than only focus on examples given in class. Help students recognize the issues not only interested by scientific society but also come from daily life.

- In the relative smaller class, I format each class around a discussion in which all students actively participate. Each student will have opportunities to lead an entire class and to take responsibilities to answer questions from other students.