Department of Chemistry
University of Illinois at Chicago
845 W. Taylor St., Room 4500 (M/C 111)
Chicago, IL 60607
Ph: 312-969-8158
Lab: 312-996-8158
rstahe1@uic.edu

October 9, 2005

Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E 3rd Street
Bloomington, IN 47405-7005

Dear Dr. Brun:

I was excited to see your recent advertisement on sciencecareers.org, in search of a potential faculty member. I would like to apply for the position of assistant professor. My expertise in membrane-protein interactions and structure-function studies of proteins involved in signal transduction and membrane trafficking would be a nice addition to your department's ongoing research. I am elated to have this opportunity to apply to a growing department as my expertise with protein-lipid interactions uses biophysical approaches to unravel protein-membrane interactions in signal transduction, which can be useful to the hot new area of lipidomics. Furthermore, this work can spawn collaboration. I am currently a postdoctoral research associate in the laboratory of Dr. Wonhwa Cho at the University of Illinois at Chicago.

Over the years, I have developed innovative *in vitro* and *in vivo* methods to address the mechanism of interaction of proteins with cellular membranes. These proteins, which transiently interact with cellular lipids are termed peripheral proteins and play a crucial role in signal transduction and membrane trafficking. My work has lead to a plethora of information on the forces governing membrane-protein interactions *in vitro* and *in vivo* as well as illustrative mechanistic details of the activation of peripheral proteins such as protein kinases C, sphingosine kinase, and cytosolic phospholipase A_2. Furthermore, my work has lead the field in understanding the mechanism of membrane binding of various conserved membrane-targeting modules. Already, my work has lead to more than 20 peer-reviewed publications and a few invited review articles as well as being a foundation of NIH funding. I have the experience, energy, foresight, and collaborative ties to be a leader in this field for many years.

My research training is broad and encompasses biochemistry, biophysics, chemistry, cell biology, and molecular biology. This combination of knowledge allows me to approach

cutting-edge questions of membrane-protein interactions in signal transduction and membrane trafficking with the ability to illustrate mechanistic details both *in vitro* and *in vivo*. The research project I would intend to pursue involves the investigation of membrane curvature changes induced by peripheral proteins, a process instrumental to the initiation of endocytosis and other membrane trafficking events. The second project entails the role of phosphoinositides and sphingolipids in the membrane recruitment and activation of peripheral proteins harboring C2 domains (A conserved membrane-targeting domain first identified in the protein kinase C family). These projects, I feel will inspire graduate students and future postdocs, while allowing me to continue to produce the highest quality extramurally funded work in my field.

As my curriculum vitae shows, I have had the excellent opportunity to design and teach a biochemistry laboratory course. When I designed this course, I thoroughly researched chemical education journals and utilized the suggestions of a chemical education mentor (see curriculum vitae). Studies have shown that learning for entering the culture and practice of science took place in three program-embedded areas: laboratory-centered, program-centered, and peer-centered. I employed these references to implement a laboratory course that focused on learning the skills of molecular biology and biochemistry then utilized these skills in designing and performing the procedures for a research-like project. The course has seen five successful years of over 250 admiring graduate and undergraduate students. Additionally, the development and assessment of the course was published.

I would bring to this position preparation, experience, enthusiasm, and integrity, not only in research but also in teaching and mentoring. Furthermore, I would bring collaboration experience, which could spawn both inter- and intradepartmental collaboration increasing opportunities for extramural funding. I would also be ecstatic about new course development/revitalization albeit as an independent project or in cooperation with other faculty members. I am confident my research, teaching and mentoring experience along with my positive attitude make me a nice fit for your expanding and energetic department.

I have included my curriculum vitae, research and teaching interests, and I have arranged for four letters or recommendation to be sent to you. I would be happy to provide you with more information such as publication reprints or more detailed research plans. I can be reached at either of the phone numbers listed. I thank you for your consideration and look forward to hearing from you.

Sincerely,

Robert V. Stahelin, PhD

Enclosure: curriculum vitae; research accomplishments, research and teaching interests

Research Description and Accomplishments

Many cellular processes including cell signaling and membrane trafficking involve complex arrays of protein-protein and lipid-protein interactions. Research in the past decade has revealed that a large number of cellular proteins reversibly translocate to specific subcellular locations to form these macromolecular interactions (1). In particular, it has been recognized that many cytoplasmic proteins are recruited to different cellular membranes during cell signaling and membrane trafficking. These proteins are collectively known as peripheral proteins (as opposed to integral membrane proteins) or amphitrophic proteins. Peripheral proteins use different strategies for reversible membrane interactions. A large number of peripheral proteins contain one or more modular domains specialized in lipid binding. These lipid-binding structural modules, also known as membrane-targeting domains, include protein kinase C (PKC) conserved 1 (C1) (2), PKC conserved 2 (C2) (2), pleckstrin homology (PH) (3), Fab1, YOTB, Vac1, and EEA1 (FYVE) (4), phox (PX) (5), band 4.1, ezrin, radixin, moesin (FERM) (6), epsin amino-terminal homology (ENTH) (7) and AP180 amino-terminal homology (ANTH) (7), Tubby (8), and plant homology (PHD) domains (9). Also, there are peripheral proteins that do not have separate membrane-targeting domains but utilize a part of their molecular surface or an amphipathic secondary structure to interact with the membrane. Another class of peripheral proteins has covalently attached lipid anchors that embed in the lipid bilayer.

My research has significantly progressed our understanding of the mechanisms by which reversible binding of the non-lipidated peripheral proteins to different cell membranes is mediated and regulated, with an emphasis on how kinetics and energetics of their membrane-protein interactions are modulated during their membrane targeting and activation. I have done a considerable amount of biophysical studies on membrane targeting domains and their host proteins using model membranes and live cell studies which have revealed how membrane-protein interactions are regulated by different factors, such as amino acid residues (10), Ca^{2+} (11), diacylglycerol (12-14), phosphoinositides (PIs) (15-18) or protein phosphorylation (19), and cellular translocation studies with fluorescence protein-tagged domains which provide new insight into the correlation between the *in vitro* and cellular membrane targeting (20). Additionally, many of the *in vitro* and *in vivo* experimental methods, I have developed myself. My work has spawned multidisciplinary collaborations with computational biologists, cell biologists and biophysicists that have provided a unifying view on how peripheral proteins interact with membranes and how differences in their membrane-binding mechanisms lead to distinct functions. With the availability of whole-genome sequence information for many different organisms, an increasing number of membrane-targeting domains and peripheral proteins will be identified in the near future. Elucidation of the kinetics and thermodynamics of membrane-protein interactions for all these peripheral proteins under physiological conditions is a challenging task that requires multidisciplinary experimental and theoretical approaches. My work has established the groundwork to delineate these interactions in a timely and proficient manner. Specific research accomplishments are highlighted below.

Development of kinetic assay for protein-membrane interactions

Surface plasmon resonance (SPR) (21) allows for the real-time monitoring of macromolecular interactions. Until recently, a robust method for reliably measuring protein-membrane interactions using this system was difficult. I was able to overcome numerous obstacles to successfully and reproducibly monitor protein-membrane interactions for many peripheral proteins in a timely manner. This assay not only has served as a foundation of much of my research, but also has been successfully utilized by countless people in the Cho laboratory as well as at other institutions.

Phosphoinositide induced membrane penetration

PIs play a crucial role in both the spatial and temporal localization of numerous peripheral proteins. My research has lead to a better understanding of how proteins harboring FYVE, PX, or ENTH domains are recruited to the biological membrane. In particular, my work was the first to demonstrate that PIs induce the membrane penetration of these proteins. This is significant, as membrane penetration serves to anchor these proteins to the biological membrane, causes conformational change and subsequent protein activation, or in the instance of the ENTH domain, induces membrane curvature changes necessary for endocytosis (18). I also unraveled the membrane recruitment mechanisms of $p40^{phox}$ and $p47^{phox}$ PX domains, which are essential to NADPH oxidase activation playing an instrumental role in the host defense mechanism of neutrophils and macrophages (17). My work in this field, for the first time, has also quantified the membrane affinity necessary for cellular localization of these proteins.

PKC isoform activation mechanisms

PKCs are a family of serine/threonine kinases that mediate a wide variety of cellular processes (22). More than 10 members of the PKC family have been identified. These isoforms differ in their cellular and tissue distribution, while sharing very similar regulatory and catalytic domains. My work has eloquently elucidated the membrane targeting and activation mechanisms of PKCα (14),–γ (23),-δ (12), and –ε (13) both *in vitro* and *in vivo*. This has had a tremendous impact on understanding how different PKC isoforms are localized to specific cellular compartments in the cell, and how their membrane binding serves in the subsequent activation and phosphorylation of downstream targets.

In vitro versus cellular membrane binding

The development of my SPR assay allowed for monitoring *in vitro* kinetics of peripheral proteins while cellular studies with fluorescently tagged proteins has elucidated the cellular localization of these same proteins. Utilizing these two robust methodologies, my work demonstrated that the subcellular localization of C2 domains is determined primarily by their phospholipids headgroup selectivity and can be tailored by altering their phospholipids headgroup specificity through point mutations (20). This was one of the first studies to show the importance of lipid-protein interactions in the specific cellular localization of peripheral proteins. Furthermore, when kinetics of association and dissociation for *in vitro* vesicle binding for these C2 domains and their host proteins were compared with their cellular membrane translocation rates and activities, excellent quantitative correlation was observed. A threshold affinity necessary for cellular localization was determined, while the dissociation rate constant must also be smaller than a threshold value to exhibit detectable cellular localization and activity.

Identification of sphingosine kinase 1 as a membrane-binding protein

Sphingosine kinase 1 (SK1) catalyzes the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P) (24), which acts as a first and second messenger to activate cellular processes such as differentiation, migration, cytoskeletal changes and protection from apoptosis (25). My work has identified SK1 as a high affinity membrane binding protein that has specificity for the plasma membrane (19). This work also delineates residues involved in the plasma membrane binding and that phosphorylation enhances the membrane affinity and localization to the plasma membrane where the enzyme is active.

Multidisciplinary collaborations

My research has fostered numerous fruitful collaborations with different types of scientists. For instance, I have collaborated with Dr. Diana Murray at Cornell University. Her laboratories expertise in computational biology has been a great asset to my research and has helped give insight into a large amount of our experimental data. Collaborations with Dr. Roger Williams and Dr. Harvey McMahon have lead to elegant studies on the membrane-binding mechanisms of PX (16) and ENTH domains (18), respectively. X-ray reflectivity measurements done in collaboration with Dr. Mark Schlossman have opened up a new avenue to elucidate the membrane penetration depth and angle of orientation for peripheral proteins at the membrane (26). More recently, I have begun collaborations with Dr. David Cafiso to determine membrane-targeting domain penetration depth and orientation at the membrane interface, and with Dr. Hui Lu to create algorithms based upon structure and amino acid sequence to predict proteins that will bind the membrane. These ties have been instrumental in gaining a better view of the forces governing membrane-protein interactions.

References

1. Tereul, M.N. and Meyer T. 2000. Translocation and reversible localization of signaling proteins: a dynamic future for signal transduction. *Cell* 103: 181-84
2. Cho, W. 2001. Membrane targeting by C1 and C2 domains. *J. Biol. Chem.* 276:32407-10
3. Ferguson, K.M., Kavran, J.M., Sankaran, V.G., Fournier, E., Islakoff S.J., et. al. 2000. Structural basis for discrimination of 3-phosphoinositides by pleckstrin homology domains. *Mol. Cell.* 6:373-84
4. Stenmark, H., Aasland, R., Driscoll, P.C. 2002. The phosphatidylinositol 3-phosphate-binding FYVE finger. *FEBS Lett.* 513:77-84
5. Xu, Y., Seet, L.F., Hanson, B., Hong, W. 2001. The Phox homology (PX) domain, a new player in phosphoinositide signaling. *Biochem. J.* 360:513-30
6. Bretscher, A., Edwards, K., Fehon, R.G. 2002. ERM proteins and merlin: integrators at the cell cortex. *Nat. Rev. Mol. Cell. Biol.* 3:586-99
7. De Camilli, P., Chen, H., Hyman, J., Panepucci, E., Bateman, A., Brunger, A.T. 2002. The ENTH domain. *FEBS Lett.* 513:11-18
8. Carroll, K., Gomez, C., Shapiro, L. 2004. Tubby proteins: the plot thickens. *Nat. Rev. Mol. Cell. Biol.* 5:55-63
9. Gozani, O., Karuman, P., Jones, D.R., Ivanov, D., Cha, J. et. al. 2003. The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. *Cell* 114:99-111
10. **Stahelin, R.V.** and Cho, W. 2001. Differential roles of ionic, aliphatic, and aromatic residues in membrane-protein interactions: a surface plasmon resonance study on phospholipases A_2. *Biochemistry* 40:4672-78
11. **Stahelin, R.V.** and Cho, W. 2001. Roles of calcium in the membrane binding of C2 domains. *Biochem. J.* 359:679-85
12. **Stahelin, R.V.**, Digman, M.A., Medkova, M., Ananthanrayanan, B., Rafter, J.D., et. al. 2004. Mechanism of diacylglycerol-induced membrane targeting and activation of protein kinase C delta. *J. Biol. Chem.* 279:29501-12
13. **Stahelin, R.V.**, Digman, M.A., Medkova, M., Anathanrayanan, B., Melowic, H., et. al. 2005. Diacylglycerol-induced membrane targeting and activation of protein kinase cε: mechanistic differences between PKCδ and ε. *J. Biol. Chem.* 280-19784-93
14. **Stahelin, R.V.**, Wang, J., Blatner, N.R., Rafter, J.D., Murray, D., and Cho, W. 2005. The origin of C1 and C2 domain interactions of PKCα in vitro and in vivo. *J. Biol. Chem.* In press
15. **Stahelin, R.V.**, Long, F., Diraviyam, K., Bruzik, K.S., Murray, D., and Cho, W. 2005. Phosphatidylinositol 3-phosphate induces the membrane penetration of the FYVE domains of Vps27p and Hrs. *J. Biol. Chem.* 277:26379-88
16. Karathanassis, D., **Stahelin, R.V.**, Bravo, J., Perisic, O., Pacold, C.M., et. al. 2002. Binding of the PX domain of p47phox to phosphatidylinositol 3,4-bisphosphate and phosphatidic acid is masked by an intramolecular interaction. *EMBO J.* 21:5057-68

17. **Stahelin, R.V.**, Burian, A., Murray, D., Bruzik, K.S., and Cho, W. 2003. Membrane binding mechanisms of the NADPH oxidase PX domains. *J. Biol. Chem.* 278:14469-79
18. **Stahelin, R.V.**, Long, F., Peter, B.J., Murray, D., De Camilli, P., et. al. 2003. Contrasting membrane interaction mechanisms of AP180 N-terminal homology and epsin N-terminal homology domains. *J. Biol. Chem.* 278:28993-99
19. **Stahelin, R.V.**, Hwang, J., Kim, J.H., Park, Z.Y., Johnson, K.R., et. al. 2005. The mechanism of membrane targeting of human sphingosine kinase 1. *J. Biol. Chem.* accepted with revision
20. **Stahelin, R.V.**, Rafter, J.D., Das, S., and Cho, W. 2003. A molecular basis for differential subcellular localization of C2 domains of protein kinase cα and cytosolic phospholipase A_2. *J. Biol. Chem.* 278:12452-60
21. Myszka, D.G. 1997. Kinetic anlaysis of macromolecular interactions using surface plasmon resonance biosensors. *Curr. Opin. Biotechnol.* 8:50-57
22. Newton, A.C. 2001. Protein kinase c: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chem. Rev.* 101:2353-64
23. Ananthanarayanan, B., **Stahelin, R. V.**, Digman, M. A., and Cho, W. 2003. Activation mechanisms of conventional protein kinase c isoforms are determined by the ligand affinity and conformational flexibility of their C1 domains. *J. Biol. Chem.* 278:46886-94
24. Spiegel, S. and Milstein, S. 2003. Sphingosine-1-phosphate: an enigmatic signaling lipid. *Nat. Rev. Mol. Cell. Biol.* 4:397-407
25. Mattie, M., Brooker, G., and Spiegel, S. 1994. Sphingosine-1-phosphate, a putative second messenger, mobilizes calcium from internal stores via an inositol trisphosphate-independent pathway. *J. Biol. Chem.* 269:3181-88
26. Malkova, S., Long, F., **Stahelin, R.V.**, Pingali, S.V., Murray, D., et. al. 2005. X-ray reflectivity studies of cPLA$_2$α-C2 domains absorbed onto langmuir monolayers of SOPC. *Biophys. J.* 89:1861-73

Current Research Interests

Many peripheral proteins involved in cell signaling and vesicle trafficking are specifically targeted to different cell membranes in response to various stimuli, including calcium and lipid mediators (1,2). Growing evidence indicates that 1,2-diacyl-*sn*-glycerol and phosphorylated derivatives of phosphatidylinositol (PtdIns), collectively known as phosphoinositides (PIs), serve as site-specific membrane signals to recruit and activate a variety of peripheral proteins (3,4). However, the molecular mechanisms by which DAG and different PIs modulate the membrane targeting and activation of these proteins is only beginning to unravel as the structural information about the membrane targeting domains becomes available. Over the years, I have studied the mechanisms by which membrane targeting domains, such as C1 (protein kinase C (PKC) conserved 1), C2 (PKC conserved 2), FYVE (Fab1p, YOTB, Vac1p, and EEA1), and PX (Phox) domains, and peripheral proteins harboring these domains interact with model and cell membranes (5-8). These studies have provided some of the first detailed mechanistic information on how DAGs and PIs mediate the membrane recruitment and penetration of these proteins (5-8). A primary objective of my current research is to investigate how DAG and PI turnover effect the subcellular localization and activation of PKCs and PI-binding proteins. This will provide us with important clues to the mechanisms by which DAG and PI divergently regulate the subcellular localization and activation of peripheral proteins, and will thereby establish a direct link between the complex subcellular localization of these proteins during cellular signaling and their actual cellular actions (and biological effects). A long-term objective is to apply the principles learned from these studies to the development of therapeutic agents that can specifically modulate the membrane targeting and activation of different PKC isoforms and PI-binding proteins.

Monitoring Cellular DAG

DAG acts as a key lipid second messenger that mediates a wide variety of cellular processes, including cell proliferation, differentiation, cell cycle progression, and malignant transformation (9). Thus, DAG signaling pathways offer multiple targets for anticancer therapy. Recently, the PKC C1 domain has been found to be a high affinity receptor for DAG. Although much is known about the biology of DAG signaling, much less is known about the spatiotemporal dynamics of the cellular DAG signal in quantitative terms. The paucity of quantitative information makes it difficult to predict and explain the cellular behaviors of multiple DAG receptors in response to fluctuation of the cellular DAG level. Recently, we have investigated how differently C1 domains of PKCs and other proteins interact with soluble and membrane-incorporated DAG and phorbol esters by means of various biophysical tools. Subsequently, we utilized a C1 domain with the highest membrane affinity to construct a DAG sensor. First, we engineered the domain to have increased affinity through site-directed mutagenesis, while also introducing a free cysteine residue near the membrane-binding interface so an environmentally sensitive fluorescent probe could be chemically introduced to this site. This probe exhibits a large increase in fluorescence emission at 450 nm when transferred

from an aqueous to non-polar environment. Thus far, the probe has been successfully utilized *in vitro* and in live cells to monitor DAG generation and concentrations.

Real-Time Monitoring of Cellular PKC Activities

Generally, multiple PKC isoforms are found in a given cell type where in some instances, PKC isoforms lack distinct substrate specificity. On the other hand, the subcellular localization, cellular expression level, and cofactor dependencies can vary among PKC isoforms. Despite the wealth of information on PKC subcellular localization in response to various stimuli, a question still remains as to whether or not PKCs exert their catalytic actions at all these different subcellular locales. Additionally, a quantitative assessment of PKC activity levels at these different sites as well as how distinct PKC isoforms differ in cellular output remains elusive. A basic premise in our PKC activity model is that PKC isoforms can localize to different subcellular membranes in response to DAG/phorbol ester, which is in line with their *in vitro* phospholipids specificity. Thus, the question arises whether or not these PKC isoforms activities follows their subcellular localization in response to DAG or not.

Our data on PKCα activity strongly suggests that PKC isoform activity can be monitored in both a spatiotemporal and quantitative fashion. We have engineered a fluorescently labeled peptide, which can be microinjected or transported with a lipid carrier into live cells. When PKCα was targeted to the cytoplasmic leaflet of the PM, its activity was observed only at the PM. However, when PKCα was targeted to both the PM and intracellular membranes activity was observed in both locales. To address the question of *in vivo* PKC activity at different cellular membranes we have synthesized PKC activity reporters with different amino acid sequences to gain PKC isoform specificity. We are determining the effects of subcellular localization of PKC isoforms on cellular activity. Additionally, the aforementioned C1 domain DAG sensor serves as a control to monitor the spatiotemporal dynamics of PKC in conjunction with cellular activity.

Protein Kinase Cθ Membrane Targeting and Activation Mechanism

T lymphocyte activation requires sustained interaction of T cell receptor (TCR) with MHC-bound peptide antigen of the antigen-presenting cell at the cell-cell contact region, the so-called immunological synapse. Generation of DAG by PLCγ1 isoform in the plasma membrane (PM) in response to TCR triggering is a key in the initiation of T cell activation, which culminates in the cell proliferation and the execution of T cell effectors functions (10). T cells express many PKC isoforms (α, β_I, δ, ε, η, θ, and ζ), but only PKCθ, which is expressed selectively in T cells and muscles, rapidly and stably translocates to the immunological synapse where TCR molecules are clustered upon TCR activation (11) whereas other PKCs translocate to other regions of the PM. Analysis of PKCθ knockout mice showed the importance of this isoform in regulating TCR-derived signals and demonstrated the requirement for PKC in activating the downstream elements AP-1, NF-κB, and IL-2 in T cells (12). My current work is aiming at elucidating the mechanism underlying the selective targeting of PKCθ in T cells. We have shown that

PKCθ has unique DAG binding properties among PKC isoforms and its C2 domain is actually inhibitory for its translocation and membrane binding in contrast to other PKC isoforms. Perhaps, most importantly, PKCθ binds $PI(4,5)P_2$, which may be responsible for its unique TCR localization in T cells. My current research will unravel this obscurity.

Design of an Aptamer to Monitor Native Protein Translocation in Live Cells

The advent of fluorescent antibodies as well as the green fluorescent protein has provided a wonderful means of monitoring protein localization in the cell. However, with these methods, cellular proteins either have to be overexpressed with a fusion tag or cannot be monitored in real-time. Thus, the time is ripe for a method to monitor protein localization in the cell in both real-time and under native conditions Aptamers are oligonucleotides (DNA or RNA molecules) that can bind with high affinity and specificity to a wide range of target molecules, such as proteins. Aptamers have been selected to disrupt the function of their targets and to inhibit or modify the metabolism associated with that target. The remarkable selection between two molecules of very similar structure has suggested that aptamers have the potential as diagnostic reagents. In collaboration with Dr. Tan at the University of Florida, we have synthesized a highly selective non-inhibitory aptamer for PKCδ. The aptamer is fluorescently labeled and allows us to detect native expression of PKCδ in a variety of cell lines. We are currently monitoring the translocation of native PKCδ in conjunction with DAG generation following cellular stimulation. For the first time, results will provide a view of how PKCδ responds to DAG signaling under normal expression levels.

Structural Bioinformatics Prediction of Membrane-Binding Proteins

With the availability of whole genome sequence information for many different organisms, it is expected that an increasing number of membrane targeting domains and peripheral proteins will be identified in the near future. In vitro membrane binding studies and cellular membrane translocation studies have played a major role in identifying new peripheral proteins. Also, structural biology has deciphered the structural basis of specific lipid binding and membrane interactions of membrane targeting domains and peripheral proteins. However, it would be prohibitively time-consuming and expensive to search and identify new peripheral proteins on a genomic-scale by these experimental approaches. Therefore, a fast and accurate bioinformatics-based annotation scheme for peripheral proteins would greatly supplement the effort to identify membrane-binding peripheral proteins on a genomic scale.

Through collaboration, an automated prediction protocol for identifying membrane-binding peripheral proteins was built using a machine-learning algorithm, the support vector machine (SVM) (13). When trained with an ensemble of characteristics of lipid-binding proteins, the SVM learned to distinguish membrane-binding proteins from the non-binding ones with high accuracy. The SVM was also able to single out a membrane-binding C2 domain among highly homologous C2 domains, which was experimentally verified by membrane-binding measurements by surface plasmon resonance (SPR) analysis. This protocol can be used to predict the membrane binding

properties of a large number of modular domains with unknown properties and can be developed into a more general method for genome-wide prediction of membrane-binding peripheral proteins.

Role of Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) in the Membrane Binding and Activation of Class II Phosphatidylinositol-3-Kinase-C2α (PI3K-C2α)

Recently, a growing interest has arisen in members of the class II PI3K family, as several lines of evidence suggest a potential role for these enzymes in agonist-mediated regulation of cellular functions (14). Activation has been shown to be induced by insulin (15), monocyte chemotactic peptide-1 (16), and interactions with clathrin (17). Additionally, PI3K-C2α associates with polypeptide growth factor receptors (18). Despite this evidence of known activators, insight into the activation mechanism of PI3K-C2α is lacking. PI3K-C2α harbors a PX domain, which has been shown to bind $PI(4,5)P_2$ (19). Our collaborator, Roger L. Williams of the Medical Research Council, has solved the X-ray crystal structure of this PX domain. Subsequently, I have mapped out the origin of $PI(4,5)P_2$ specificity of this unique PX domain as well its membrane binding and penetration mechanism. In order to assess the role of the PX domain in the membrane binding and activation of full-length PI3K-C2α, I have expressed and purified the full-length protein in insect cells. Studies with the full-length protein will elucidate how $PI(4,5)P_2$ is involved in the cellular signaling of PI3K-C2α.

Monitoring Spatiotemporal Dynamics of Phosphoinositide (PI) Generation

PIs are now known to regulate many biological processes, including cell proliferation, cell survival, differentiation, signal transduction, cytoskeleton organization and membrane trafficking (20). The regulation of their activities and the mechanisms of their actions have therefore been the subject of much investigation. Signaling roles for PIs that involve their regulated hydrolysis to generate second messengers have been well characterized. For instance, phospholipase C-mediated hydrolysis of $PI(4,5)P_2$ generates the intracellular signals inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and DAG for regulating calcium mobilization and PKC activities. In addition to these roles, the major underlying mechanism by which PIs regulate cellular processes via their ability to serve as site-specific membrane signals to affect the intracellular localization and/or biological properties of effector proteins (or protein complexes). The PH domain was the first discovered effector domain for PI (). This domain of 100-120 amino acids was first identified in pleckstrin and subsequently found in a wide range of signaling proteins, including protein kinases, phospholipases, and regulators of small G proteins. The PH domain shows a broad range of specificity for $PI(4,5)P_2$, $PI(3,4)P_2$, and $PI(3,4,5)P_3$.

Thus far, fluorescent protein fusion constructs of PH domains have been successfully utilized to monitor the generation and localization of PIs (21). However, this method does not allow for a quantitative assessment of PI content. The lack of quantitative information makes it difficult to predict and explain the cellular behaviors of multiple PI receptors in response to fluctuation of the cellular PI levels. Recently, we have investigated how differently PI-binding domains interact with and membrane-incorporated PIs by means of various biophysical tools. Subsequently, we utilized PI

binding-domains with the highest membrane affinity to construct PI(4)P, PI(3,4)P_2, and PI(3,4,5)P_3 sensors. First, we engineered the domain to have increased affinity through site-directed mutagenesis, while also introducing a free cysteine residue near the membrane-binding interface so an environmentally sensitive fluorescent probe could be chemically introduced to this site. This probe, exhibits a large increase in fluorescence emission at 450 nm when transferred from an aqueous to non-polar environment. Thus far, these probes have been successfully utilized *in vitro* and in live cells to monitor PI generation and concentrations.

Concluding Remarks

Over the years, I have made significant progress toward understanding of the mechanisms by which membrane targeting proteins and their host proteins are targeted to different cellular membranes. This contribution to this rapidly developing area of research has been well recognized, as evidenced by a recent invitation to write a comprehensive review article on membrane targeting domains in *Annual Review of Biophysics and Biomolecular Structure.* I have also been successful in developing and adapting new technologies, most notably surface plasmon resonance and microscope analyses, and applying them to various new research projects. This approach has greatly expanded my horizon and allowed me to attack some of the most challenging problems. On the basis of my recent work, I am now poised to address critical and timely questions about DAG and PI signaling, which play a pivotal role in many cellular processes. My current studies, will not only answer the specific questions of how the spatial and temporal DAG and PI signals lead to protein localization and activation, but also lead to a better understanding of how peripheral proteins respond to different quantities of DAG and PI signals.

References

1. Cho, W. (2001) Membrane targeting by c1 and c2 domains *J Biol Chem* **276,** 32407-10.
2. Hurley, J.H. and Meyer, T. (2001) Subcellular targeting by membrane lipids *Curr Opin Cell Biol* **13,** 146-52.
3. Cullen, P.J., Cozier, G.E., Banting, G., and Mellor, H. (2001) Modular phosphoinositide-binding domains—their role in signaling and membrane trafficking *Curr Biol* **11,** 882-93.
4. Itoh, T. and Takenawa, T. (2002) Phosphoinositide-binding domains. Functional units for temporal and spatial regulation of intracellular signaling *Cell Signal* **14,** 733-43.
5. **Stahelin, R.V.,** Long, F., Diraviyam, K., Bruzik, K.S., Murray, D., and Cho, W. (2002) Phosphatidylinositol-3-phosphate induces the membrane penetration of the FYVE domains of Vps27p and Hrs *J Biol Chem* **277,** 13167-13174.

6. **Stahelin, R.V.,** Burian, A., Bruzik, K.S., Murray, D., Cho, W. (2003) Membrane binding mechanisms of the PX domains of NADPH oxidase $p40^{phox}$ and $p47^{phox}$ *J Biol Chem* **278,** 14469-14479.
7. **Stahelin, R.V.,** Digman, M.A., Medkova, M., Ananthanarayanan, B., Melowic, H.R., Rafter, J.D., and Cho, W. (2005) Diacylglycerol-induced membrane targeting and activation of PKCε *J Biol Chem* **280,** 19784-19793.
8. **Stahelin, R.V.,** Rafter, J.D., Das, S., and Cho, W. (2003) The molecular basis of differential subcellular localization of C2 domains of protein kinase C-α and group IVa cytosolic phospholipase A_2 *J Biol Chem* **278,** 12452-12460.
9. Wakelam, M.J. (1998) Diaclyglycerol—when is it an intracellular messenger? *Biochim Biophys Acta* **1436,** 117-126.
10. Downward J., Graves, J.D., Warne, P.H., Rayter, S., and Cantrell, D.A. (1990) Stimulation of p21ras upon T-cell activation *Nature* **346,** 719-723.
11. Diaz-Flores, E., Siliceo, M., Marinez-A, C., and Merida, I. (2003) Membrane translocation of protein kinase Cθ during T lymphocyte activation requires phospholipase C-gamma generated diacylglycerol *J Biol Chem* **278,** 29208-29215.
12. Sedwick, C.E. and Altman, A. (2004) Perspectives on PKCθ in T cell activation *Mol Immunol* **41,** 675-686.
13. Vapnik, V. and Cortes, C. (1995) *Machine Learning* **20,** 273-293.
14. Foster, F.M., Traer, C.J., Abraham, S.M., Fry, M.J. (2003) The phosphoinositide (PI) 3-kinase family *J Cell Sci* **116,** 3037-3040.
15. Brown, R.A. and Shepherd, P.R. (2001) Growth factor regulation of the novel class II phosphinositide 3-kinases *Biochem Soc Trans* **29,** 535-537.
16. Turner, S.J., Domin, J., Waterfield, M.D., Ward, S.G., and Westwick, J. (1998) The CC chemokine monocyte chemotactic peptide-1 activates both the class I p85/p110 phosphatidylinositol 3-kinase and the class II PI3K-C2alpha *J Biol Chem* **273,** 25987-25995.
17. Gaidarov, I., Smith, M.E., Domin, J., and Keen, J.H. (2001) The class II phosphoinositide 3-kinase C2alpha is activated by clathrin and regulates clathrin-mediated membrane trafficking *Mol Cell* **7,** 443-449.
18. Arcaro, A., Zvelebil, M.J., Wallasch, C., Ullrich, A., Waterfield, M.D., and Domin, J. (2000) Class II phosphoinositide 3-kinases are downstream targets of activated polypeptide growth factor receptors *Mol Cell Biol* **20,** 3817-3830.
19. Ago, T., Takeya, R., Hiroaki, H., Kuribayashi, F., Ito, T., Kohda, D., Sumimoto, H. (2001) The PX domain as a novel phosphoinositide-binding module *Biochem Biophys Res Commun* **287,** 733-738.
20. Martin, T.F. (1998) Phosphoinositide lipids as signaling molecules: common themes for signal transduction, cytoskeletal regulation, and membrane trafficking *Annu Rev Cell Dev Biol* **14,** 231-264.

Statement of Teaching Interests

Goals

My unique background in teaching a heterogeneous population of students in biochemistry and chemistry as well as my diverse background in research has prepared me to teach a number of courses. I would do an excellent job teaching general courses such as General Chemistry, Biochemistry, Biochemistry Laboratory, Biophysical Chemistry, or Cell and Molecular Biology. In addition, I would be happy to participate in team-teaching portions of these courses or related ones. I enjoy bringing my broad research experience to the classroom and I would love the opportunity to teach specialized courses such as: Protein Folding, Macromolecular Interactions, Protein Structure and Function, Signal Transduction, or Biophysical Methods. Biochemistry and biotechnology are constantly evolving fields; I would be excited to participate in new course innovation or course revitalization to address these developments. Most of all, I look forward to training and mentoring graduate students as a professor.

Experience

Following my first year as a graduate student, I was given the unique opportunity to design a biochemistry laboratory course for undergraduate and graduate students. I fervently accepted the challenge. I was the lead graduate student on a peer-reviewed curriculum development and research project. I took this opportunity very seriously and thoroughly researched chemical education journals to aid in the development of the course. First, I implemented the course to have a skill-building segment, within which students would learn the common techniques of biochemistry and molecular biology. The second part of the course is innovative pedagogically as it gives students the opportunity to apply the skills learned to designing and performing a research-like project on the pharmacologically important enzyme β-lactamase. This unique and valuable research experience greatly enhances the students understanding of scientific reasoning and the research process. An evaluation of the course indicated successful linkage of skill-building and student-directed activities. We published the curriculum and course evaluation "Development of a Biochemistry Laboratory Course with a Project-Oriented Goal" in Biochemistry and Molecular Biology Education in 2003 (1). I have taught this course for five years.

Prior to my lead in the biochemistry course development, I was a teaching assistant for General Chemistry. Many of my students enjoyed the course and my teaching style. Following this first semester of teaching, I was honored with a teaching merit award based upon students' reviews. The following semester, I was a teaching assistant for a biochemistry laboratory course. This allowed me to generate confidence in my teaching abilities, as I had been well trained in biochemistry and laboratory techniques. It was during this semester that I began to develop a teaching philosophy.

In all these courses I worked with undergraduate and graduate students who, as is true across UIC, are ethnically and academically diverse. I was proud to be able to work with students from throughout northeastern Illinois. In addition, my teaching in both

general chemistry and biochemistry was responsive to their many different career and college plans. I've acquired skills in leading classroom discussions, creating and evaluating exam and essay questions, providing an avenue in the formulation of scientific manuscripts, and relating course materials to students' interests and real world problems. This course has taught me how to prepare and deliver effective lectures. The most rewarding teaching experiences, however, have come from mentoring students one-on-one in the fundamentals of experimental research. As a graduate student and postdoc at UIC, I have participated in training ten undergraduate research assistants and more than 15 graduate students in a wide variety of biochemical and molecular biological techniques. The time devoted training students in laboratory techniques, planning, problem solving, troubleshooting, managing concurrent projects, exploring the literature, and keeping a lab notebook is rewarding, as students become scientists excited about formulating and testing their own hypotheses.

Philosophy

As a teaching assistant and later as a course developer and instructor, I developed a philosophy of teaching. I believe it is important to challenge students with difficult, yet doable problems and assignments. This encourages them to learn about the subject matter and gives them a sense of accomplishment. I also feel it is important to capture their interest by including many real-world problems of applied nature. Problem-based learning uses complex real world problems to captivate student curiosity; motivating them to recognize and research the abstract concepts and principles they need to know to progress through the problems. After motivating the concepts and/or techniques, they can be applied to designing and performing a research-like project whether on the theoretical or experimental level.

An effective mentor fosters critical thinking and heartens students to give their best effort and to accomplish more than they thought they could. Introducing students to the intellectual and experimental approaches that have given rise to the ideas and concepts that classify the field gives them tools with which to grapple new problems and to critically evaluate research results and conclusions. Challenging students to consider biology, chemistry, and physics in the context of larger social and political issues and raising questions regarding values and ethics makes the course material relevant to students' lives. I would also emphasize both oral presentations and writing as I have in the past, since I have observed many biochemistry students need improvement in these areas. It is vital for a teacher to create an atmosphere of mutual respect and intellectual excitement where new thoughts and perspectives are encouraged and ignorant or misguided ideas are gently scrutinized.

References

1. Stahelin, R.V., Forslund, R.E., Wink, D.J., and Cho, W. (2003) Development of a biochemistry laboratory course with a project-oriented goal, *Biochem Mol. Bio. Educ.*, 31, 106-112.