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DEPARTMENT OF BIOLOGICAL CHEMISTRY
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Professor Yves Brun
Systems Biology/Microbiology Faculty Search
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September 21, 2005

Dear Professor Brun,

I am writing to apply for the position of Assistant Professor in the Department of Biology and the Biocomplexity Institute at the Indiana University beginning in fall 2006. I obtained my Ph.D. in bioorganic chemistry in 2003 from the University of California at Berkeley under the guidance of Prof. Peter G. Schultz. In Prof. Schultz's lab at UC Berkeley and later at The Scripps Research Institute, I solved a total of eight structures of catalytic antibodies by X-ray crystallography and carried out detailed mechanistic studies on antibody catalysis and immunological evolution. I also developed a phage display method for the directed evolution of catalytic antibodies and increased the peroxidase activity of a heme binding antibody by ten fold.

I am currently a postdoctoral fellow working with Prof. Christopher T. Walsh at Harvard Medical School. In the Walsh lab I developed an efficient method for labeling proteins with small molecules by Sfp phosphopantetheinyl transferase. I applied this method to high throughput printing of protein microarrays, encoding small molecule libraries with DNA barcodes and live cell imaging of transferrin receptor trafficking dynamics by fluorescence resonance energy transfer (FRET). I also identified an eleven residue peptide tag as the substrate of Sfp by phage display, further improving the versatility of the Sfp-catalyzed protein labeling method.

The main goal of my future research is the elucidation of signal transduction mechanisms inside living cells both at a global level – mapping the signal transduction networks, and at a molecular level – understanding the biochemical mechanism of cell signaling. Posttranslational modification of p53 and histone, and ligand induced dimerization and endocytosis of ErbB receptors will be the focus of my research. Another goal of my research is enzyme-directed evolution for the combinatorial biosynthesis of "unnatural" natural products with desired biological activities. Specifically, I intend to engineer the substrate specificity of erythromycin polyketide synthase by directed evolution for the biosynthesis of new erythromycin analogs. A wide variety of biochemical and biophysical methods will be used in my research, including DNA library construction, phage display, organic synthesis, cell culturing, chemical genetics, high throughput proteomics, enzyme kinetics and molecular imaging. Besides my strong interest in research, I am also very much dedicated to teaching students at both undergraduate and graduate levels.

The Department of Biology at the Indiana University has an outstanding history of academic success at the frontiers of biological sciences. I believe my academic career will thrive in your department and I will make significant contributions to your department both in teaching and in basic research. I have enclosed a CV, a statement of my teaching interests, a summary of my past research accomplishments, my future research interests, three research proposals and five of my recent publications. I have also arranged to have Prof. Christopher T. Walsh, Prof. Peter G. Schultz, Prof. David R. Liu and Prof. David E. Golan provide recommendation letters on my behalf. Thank you for your time and consideration.

Sincerely yours,

Jun Yin

Research Accomplishments

Postdoctoral research (08/2003 – present) with Prof. Christopher T. Walsh at Harvard Medical School.

High throughput cloning of biosynthetic gene clusters by phage display.

A shotgun genomic library of *Bacillus Subtilis* was displayed on the surface of M13 phages and the acyl carrier protein (ACP) and peptidyl carrier protein (PCP) domains involved in polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) were selected by Sfp phosphopantetheinyl transferase catalyzed biotin – coenzyme A (CoA) modification of the phage displayed proteins. In this way, the DNA sequence of ACP and PCP gene can be quickly identified by sequencing the selected phage clones and the full gene cluster of PKS and NRPS quickly assembled for the combinatorial biosynthesis of pharmaceutically important natural products. Currently we are trying to use this method to clone biosynthetic gene clusters from other bacteria such as *Streptomyces* or cyanobacteria.

Labeling proteins with small molecules by Sfp phosphopantetheinyl transferase.

A general strategy for site specific protein labeling with small molecules was developed based on Sfp catalyzed posttranslational modification of 80 residue PCP domains by small molecule – CoA conjugates. The target proteins are expressed as fusions to PCP and Sfp catalyzes the covalent attachment of the small molecules of diverse structures to a specific serine residue on PCP. The labeling reaction proceeds with high specificity and efficiency and CoA conjugated small molecule probes of diverse structures ranging from biotin to fluorescent dyes can all be covalently attached to PCP by Sfp. With this method, PCP fused target proteins in the cell lysates were specifically labeled with biotin and spotted on streptavidin coated glass slides for high throughput printing of protein microarrays. Transferrin receptor 1 (TfR1) was also fused to PCP and the TfR1-PCP fusion protein on the cell surface of live cells was specifically labeled with fluorophore by Sfp. The trafficking of transferrin – TfR1-PCP complex during the process of transferrin-mediated iron uptake was imaged by fluorescence resonance energy transfer (FRET) between the fluorescently labeled transferrin ligand and TfR1 receptor, demonstrating Sfp catalyzed protein labeling as a useful tool for live cell molecular imaging.

PCP was also displayed on the surface of phage and small molecules were attached to the phage surface by Sfp for one-phage-one-compound display of small molecule libraries on phage. A unique 20bp DNA sequence was inserted into the phagemid DNA so that each small molecule attached to phage surface was encoded by a unique DNA barcode sequence encapsulated inside the phage particle. Small molecule libraries were selected for binding to the target proteins in a high throughput way and the selected small molecules were quickly identified by a DNA decoding array.

To reduce the size of PCP as a tag for protein labeling, an eleven residue peptide was identified by phage display as a PCP mimic for Sfp catalyzed protein modification. The peptide tag was labeled with various small molecules by Sfp when it was fused to the N- or C- termini of target proteins or inserted in a flexible loop in the middle of a target protein.

Related publications:

1. **Yin, J.**, Straight, P. D., McLoughlin, S. M., Zhou, Z., Lin, A. J., Golan, D. E., Kelleher, N. L., Kolter, R. & Walsh, C. T. (2005). Genetically encoded short peptide tag for versatile protein labeling by Sfp phosphopantetheinyl transferase. *Proc Natl Acad Sci U S A*, in press.

2. **Yin, J.**, Lin, A. J., Buckett, P. D., Wessling-Resnick, M., Golan, D. E. & Walsh, C. T. (2005). Single-cell FRET imaging of transferrin receptor trafficking dynamics by Sfp catalyzed site specific protein labeling. *Chem Biol*, in press.
3. McLoughlin, S. M., Mazur, M. T., Miller, L. M., **Yin, J.**, Liu, F., Walsh, C. T. & Kelleher, N. L. (2005). Chemoenzymatic approaches for streamlined detection of active site modifications on thiotemplate assembly lines using mass spectrometry. *Biochemistry*, in press.
4. Vaillancourt, F. H., **Yin, J.** & Walsh, C. T. (2005). SyrB2 in syringomycin E biosynthesis is a nonheme FeII α-ketoglutarate- and O2-dependent halogenase. *Proc Natl Acad Sci* U S A 102, 10111-6.
5. **Yin, J.**, Liu, F., Schinke, M., Daly, C. & Walsh, C. T. (2004). Phagemid encoded small molecules for high throughput screening of chemical libraries. *J Am Chem Soc* 126, 13570-1.
6. **Yin, J.**, Liu, F., Li, X. & Walsh, C. T. (2004). Labeling proteins with small molecules by site-specific posttranslational modification. *J Am Chem Soc* 126, 7754-5. This paper was featured by *Chem & Eng News* (2004) volume 82, page 31.

Graduate research (09/1997 – 07/2003) with Prof. Peter G. Schultz at the University of California at Berkley and The Scripps Research Institute.

Directed evolution of catalytic antibodies.

To improve the peroxidase activity of antibody 7G12 - mesoheme complex, a catalytic turnover based selection strategy was developed to select 7G12 mutants with enhanced peroxidase activity from a phage displayed antibody Fab library. In the selection, biotin tyramine conjugate was used to covalently crosslink biotin with catalytic active Fabs displayed on the phage surface upon Fab catalyzed oxidation of tyramine. In this way, phages displaying 7G12 mutants with enhanced peroxidase activity were labeled with biotin and selected by streptavidin binding. As a result, mutant Fabs with more than a ten fold increase in peroxidase activity were acquired.

Related publication:

7. **Yin, J.**, Mills, J. H. & Schultz, P. G. (2004). A catalysis-based selection for peroxidase antibodies with increased activity. *J Am Chem Soc* 126, 3006-7.

Immunological evolution of catalytic antibodies.

The generation of antibodies by the immune system through V-(D)-J recombination and somatic hypermutation is similar in many aspects to the combinatorial and mutational processes that occur during the evolution of enzymes in nature. As a prototypical example of enzyme evolution, the affinity-maturation processes of catalytic antibodies 7G12 and 28B4 were studied by solving the x-ray crystal structures of the unliganded Fab fragments of the germline antibodies, the germline Fab-hapten complexes and the unliganded Fab of the affinity-matured antibodies. A comparison of these structures with the previously solved crystal structures of the affinity-matured Fab-hapten complexes of antibodies 7G12 and 28B4 reveals that the germline antibody combining sites undergo significant conformational changes upon hapten binding to achieve structural complementarity with their haptens. In contrast, the affinity-matured antibodies show minimal changes upon the binding of haptens, consistent with a "lock and key" type of binding. Thus a greater degree of conformational flexibility exists in the active sites of the germline antibodies, which may play a major role in expanding the binding potential of the germline repertoire. However during affinity maturation, somatic mutations remove the structural flexibility in the germline antibodies and create rigid, preorganized antibody combining sites well adapted for hapten binding. The dynamics of antibody – hapten interaction in the germline and affinity-matured antibodies was also probed with resonance Raman

spectroscopy and photon echo shift spectroscopy. Structural studies on antibody 7G12 also reveal the mechanism by which the immune system evolves binding energy to catalyze the porphyrin metallation reaction.

Related publications, Research papers:

8. Venkateshrao, S., **Yin, J.**, Jarzecki, A. A., Schultz, P. G. & Spiro, T. G. (2004). Porphyrin distortion during affinity maturation of a ferrochelatase antibody, monitored by Resonance Raman spectroscopy. *J Am Chem Soc* 126, 16361-7.
9. Jimenez, R., Salazar, G., **Yin, J.**, Joo, T. & Romesberg, F. E. (2004). Protein dynamics and the immunological evolution of molecular recognition. *Proc Natl Acad Sci U S A* 101, 3803-8.
10. **Yin, J.**, Beuscher, A. E. t., Andryski, S. E., Stevens, R. C. & Schultz, P. G. (2003). Structural plasticity and the evolution of antibody affinity and specificity. *J Mol Biol* 330, 651-6. This paper was featured on the cover of the issue.
11. **Yin, J.**, Andryski, S. E., Beuscher, A. E. t., Stevens, R. C. & Schultz, P. G. (2003). Structural evidence for substrate strain in antibody catalysis. *Proc Natl Acad Sci U S A* 100, 856-61.
12. **Yin, J.**, Mundorff, E. C., Yang, P. L., Wendt, K. U., Hanway, D., Stevens, R. C. & Schultz, P. G. (2001). A comparative analysis of the immunological evolution of antibody 28B4. *Biochemistry* 40, 10764-73.
13. Romesberg, F. E., Santarsiero, B. D., Spiller, B., **Yin, J.**, Barnes, D., Schultz, P. G. & Stevens, R. C. (1998). Structural and kinetic evidence for strain in biological catalysis. *Biochemistry* 37, 14404-9.

Reviews:

14. **Yin J.** & Schultz P. G. (2004) Immunological evolution of antibody catalysis. In "*Catalytic Antibodies*" (Keinan, E. ed), Wiley-VCH, Weinheim, pp. 1-29.
15. Schultz, P. G., **Yin, J.** & Lerner, R. A. (2002). The chemistry of the antibody molecule. *Angew Chem Int Ed Engl* 41, 4427-37.

Graduate research (09/1995 – 08/1997) with Prof. Stephen Anderson at the Rutgers University, New Brunswick.

t-PA stimulation by amyloid β peptide fibrils.

The fibrillar form of amyloid β (Aβ) peptides found in the patients with Alzheimer's disease has a marked stimulatory effect upon plasminogen activation by tissue-type plasminogen activator (t-PA). The t-PA stimulation effect of Aβ peptide fibrils was followed in a time course during the process of Aβ peptide aggregation. Two distinct morphologies of Aβ peptide fibrils were found to appear in sequential order during the aggregation of Aβ peptides and have different stimulatory effect on t-PA.

Undergraduate research (09/1994 – 08/1995) with Prof. Baohuai Wang at the Peking University.

Enthalpies of dissolution of fullerenes in organic solvents.

Enthalpies of dissolution of fullerenes C_{60} and C_{70} in *o*-xylene, toluene, and CS_2 were measured by microcalorimetry at temperatures from 25°C to 40°C. It was observed that the dissolution of C_{60} in the three organic solvents was exothermic while the dissolution of C_{70} in the same solvents was endothermic.

Related publications:

16. **Yin J.**, Wang, B. H., Li, Z. F., Zhang Y. M., Zhou, X. H. & Gu, Z. N. (1996) Enthalpies of dissolution of C_{60} and C_{70} in o-xylene, toluene, and carbon disufide at temperatures from 293.15 K to 313.15 K. *J Chem Thermodynamics* 28, 1145-1151.

Teaching Philosophy and Interests

The primary goal of my teaching is to provide a fun and stimulating learning environment to the students. Based on my experience as a teaching assistant for two introductory organic chemistry courses at the chemistry department of the University of California at Berkeley, I found a lot of students lost confidence at the start of the course not due to lack of commitment but due to lack of interest. I believe teaching and learning should be a fun and mutually inspiring experience and the most important role that I should play as a teacher is not to have students memorize all the materials covered in the classroom and do well in the exams, but to cultivate and engage their interests in the subject so that they would enjoy learning, exploring and being challenged by hard concepts. In order to achieve this, I will employ a variety of active learning techniques including in-class demonstrations, small group discussions, hands-on practices and visual presentations. I will be very accessible to my students so that they would not feel it is their own battle to learn something new. I believe once the students are curious about and interested in a subject, they will be self-motivated to learn more and to excel. Thus my teaching is to help the students to discover and appreciate the fun and the beauty inside the subject of learning.

At the graduate level, I will try to be a mentor that plays a supportive role to the students' pursuit of scientific achievements at the early stages of their careers. I will create a lab environment that is friendly to discussion and questions, where the graduate students are encouraged to think freely and creatively and to take the control of their own projects. I will be a source of experience and advice for them and my lab will be a place where they can learn from experimentation, their own creativity, their own mistakes, and interaction and collaboration with others. By doing so, I will have the chance to learn from them as well and continue to improve my teaching practices.

My teaching interests at the Indiana University include general chemistry, biological chemistry, organic chemistry, bioorganic chemistry, chemical biology and enzymology.