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Children's Hospital Oakland Research Institute

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

Re: FACULTY Application

Oct. 19th, 2005

Professor Brun/Systems Biology-Microbiology Faculty Search Committee:

I am writing to apply for an assistant professor position in the Department of Biology and Biocomplexity Institute, Indiana University. I am enclosing relevant materials for your review.

I received Ph.D degree in Biochemistry in Iowa State University under the direction of Professor Richard Honzatko. In 2001, I joined Molecular BioIron group as a postdoctoral fellow of Professor Elizabeth Theil's in Children's hospital Oakland Research Institute (CHORI), to investigate the mechanisms of the multifunctional protein-ferritin. Currently, I am an assistant staff scientist in CHORI.

My postdoctoral research on ferritin has been fruitous. This is reflected in a number of first-authored research publications and reviews, including two PNAS papers (one cover article) and one in Accounts of Chemical Research (cover article). The main contributions are: 1, discovery of structural basis and mechanism of iron pore gating of ferritin iron release through kinetic and spectroscopic approaches; 2, functional assignment of ferroxidase site of ferritin through kinetic studies with chimeric ferroxidase sites on inactive L-type ferritins through protein engineering; 3, screening and characterization of peptides that alter ferritin iron pore gating in an effort to manage iron overloading in sickle cell and thallassemia patients. In addition, I have been involved in collaborative studies, such as mechanistic studies of ferritin iron oxidation (Professor Vincent Huynh Group, Emory University); nutritional studies with soy ferritin as source of bioiron (Professor Bo Lonnerdalin Group, Univ. California, Davis), and CD/mCD study of ferritin iron binding (Professor Edward Solomon, Stanford University).

Recently, I became interested in studying the mechanisms of bacterial pathogenesis and environmental adaptation by investigating the molecular and cellular mechanisms of bacterial Dps (DNA protection during starvation) proteins. Focus has been on the study on the molecular mechanisms of DNA binding/protection, Fe^{2+} oxidation/storage, O_2/H_2O_2 detoxification, and iron release. A set of cellular experiments are under development to investigate molecular mechanisms of pathogenic *Bacilli* Dps functions and cellular roles of Dps in iron homeostasis and peroxide resistance, two important factors in host pathogen interaction; In addition, investigations on roles of Dps in gene regulation and virulence are planned. Further developments include bacterial chromosome dynamics and global gene regulation with *B. anthracis* Dlp (Dps like proteins) as tools. Main research interests and detailed plans can be found in the Research Plan section.

I am currently supported by Cooley's Anemia Foundation research fellowship (2004-2006). In addition, with the preliminary results on *B. anthracis* Dps study (manuscript currently under revision), an NIH-R03 proposal has been submitted in which I am the sole PI (PA 03-108, IC: NIAID/DMID & NIEHS/COSPB, Study section: IDM/BACP).

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My teaching and mentoring experience are listed in resume. I am serving in two institutional committees that help me gain administrative skills.

I believe my expertise in biochemistry, biophysics, molecular and cell biology, and protein engineering can strengthen the programs research. Projects under development may synergize the existing department research through collaborations.

I am submitting my Curriculum Vitae, a statement of research accomplishments with detailed information on past and current research activities, a research plan, reprints of three important papers, and a statement of teaching interest for your review. Reference letters will be/have been sent to your department directly from referees.

I am available for research discussions in the coming scientific events. I am attending 2005 ASCB annual meeting held in San Francisco, and I plan to attend the coming FASEB meeting next April.

Please let me know if you have questions. I can be reached with the contact information below. I appreciate your consideration and look forward to hearing from you.

Sincerely,

Xiaofeng Liu, Ph.D
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Statement of Research Accomplishments

Past research effort has been focused on the elucidation of the molecular basis of protein-ligand, protein-protein, and protein-DNA interactions through chemical, physical, molecular and cellular biology approaches to gain insight into the molecular basis of important components in cellular processes, targeting disease treatment and prevention.

I. Postdoctoral Research: Molecular mechanisms of ferritin activities: iron in and iron out

Ferritin like compounds, including ferritins, bacterioferritins, bacterial ferritins, and bacterial Dps (DNA protection during starvation) proteins, are cage-like proteins. They concentrate iron in their large canocavities (5–8 nm) for cofactor synthesis. In animals, ferritin proteins are assembled by two different subunits (H and L) with different molar ratios, in a cell and tissue specific manner to which the mechanism remains unknown. Ferritin manages cellular iron and oxygen by coupling ferrous ions and dioxygen. Oxidation products, such as diferric oxo/hydroxo species, eventually form mineral in the ferritin nanocavity. The biological importance of ferritin in regulating cellular iron and oxidative stress is exemplified by the lethality of ferritin gene deletion in mammals and oxidant sensitivity after deletions of the multiple ferritins in bacteria.

a) Molecular requirement of diferric peroxo intermediate formation in ferritin

Ferritin oxidizes iron through ferroxidase sites, residing in the center of four-helix bundle of each subunit. Ferroxidase site has been proposed based on crystal structures of ferritins co-crystalized with poor metal analogs such as Mg^{2+} and Ca^{2+}, which could not account for di-iron coupling/oxidation. To investigate molecular requirement of ferritin iron oxidation/intermediate formation, we constructed a series of ferroxidase ligands in ferroxidase inactive L ferritin, based on crystal structure models and sequence conservation, and studied chimeric proteins functionally. The results elucidated that diferric peroxo intermediate formation in ferritin depends on coupling iron atoms in two iron substrate sites, E, ExxH for site A and E, QxxD for site B, which contrasts with di-iron cofactor sites where A and B are both E, ExxH and iron remains bound to the protein. The difference in site B and the similarity in site A between di-iron cofactor and di-iron substrate sites among members of the di-iron carboxylate family, and the weaker ligand set in site B of the ferritin iron substrate site indicate that the B site determines whether the metal–protein interaction is very stable (di-iron cofactor) or transient (di-iron substrate). Furthermore, results indicated the importance of second shell residues in intermediate decay/product translocation.

b) Localized reversible unfolding of ferritin iron pores: a control mechanism of iron efflux

Global ferritin structure is very stable, resisting 6 M urea and heat (85°C) at neutral pH. Eight pores, each formed by 3 adjacent subunits, restrict mineral access to reductant, protons, and/or chelators. Ferritin pores are "gated", based on crystal structures and functional iron chelation assays. Ferritin iron pore structure and pore gate residues are highly conserved, and pore gating can be altered by mutation, temperature and low concentrations of urea, using iron chelation assay. The existence of low-melting ferritin subdomains recorded by CD spectroscopy (melting midpoint 53°C), accounting for 10% of ferritin α-helices, is unprecedented. The low-melting ferritin subdomains are pores, based on percentage helix and destabilization by either low concentration of urea (1 mM) or Leu/Pro substitution, which both increased Fe^{2+} chelation. Biological molecules may have evolved to control gating of ferritin pores in response to cell iron need and, if mimicked by designer drugs, could impact chelation therapies in iron-overload diseases.

c) Ongoing projects

1. Identification and characterization of ferritin pore-specific binding peptides from a combinatorial library, their functional effects, and sequence relationships to known proteins.
2. Investigation of ferrous binding/oxygen activation/diferric peroxo intermediate decay by aromatic CD/MCD approach (collaboration with Professor Edward Solomon lab, Dept. of Chemistry, Stanford University)

II Research in Ph.D training: Human brain hexokinase: multiple mechanisms of G6P inhibition

Human brain hexokinase is one of the four isoforms of mammalian hexokinases. It catalyzes the first step in glycolysis pathway. In brain tissue, it anchors itself to mitochondria outer membrane through its N-terminal hydrophobic tail. It presumably regulates cell apoptosis through direct interaction with mitochondrial permeability transition pore (PTP).

Brain hexokinase (HKI) is inhibited potently by its product glucose 6-phosphate (G6P). However, the mechanism of inhibition is unsettled. Crystal structure of hexokinase revealed two G6P binding sites: one is in N-terminal half of HKI as allosteric site; the other is in active site in C-half. Single mutations within G6P binding pockets, as suggested by crystal structures, at either the N- or C-terminal half of HKI, have no significant effect on G6P inhibition, whereas double mutations, both at the active and at allosteric sites, completely abolished product inhibition in HKI. Evidently, potent inhibition of HKI by G6P can occur from both active and allosteric sites. Furthermore, kinetic data, in conjunction with equilibrium binding data, are consistent with inhibitory sites of comparable affinity linked by a mechanism of negative cooperativity.

With the crystal structure of hexokinase/ADP complex, a series of ATP site mutants have been studied kinetically. Kinetic results show that hexokinase's specificity towards nucleotide substrate is in catalytic specificity, but not substrate specificity. In addition, a few mutations in ATP binding site abolishes G6P inhibition completely in full-length hexokinase. The loss of G6P sensitivity in these mutants is assigned to the structural disturbance for an allosteric inhibition. Combined with structural information, the molecular basis of allosteric inhibition is elucidated.

Other projects:

Characterization of glucoamylase from *Aspergillus awamori* mutants suitable for industrial use

The goal was to engineer a thermo stable glucoamylase through random mutantion (directed evolution). The thermostable glucoamylase mutant was characterized by kinetic, chemical and physical approaches. The results showed that the Tm value for the mutant is 5.4 degrees higher than that of wild-type protein. The saccharification and condensation experiments, which mimic industrial condition in the use of glucoamylase, showed that thermostable glucoamylase mutant had a better specificity toward glucose production and a lower specificity to isomaltose, a side product in fructose syrup.

Research Plan

Dps proteins are a family of recently discovered proteins with multiple functions. Dps protein was first discovered in *E. coli*, and later studies revealed DNA binding activity in *E. coli* cells. In stationary phase, *E. coli* cells expressed as many as 200,000 Dps to bind chromosomal DNA, and the Dps-DNA complex forms defined a biocrystalline structure called "bacterial chromatin". Dps-bound DNA is resistant to multiple physical and chemical challenges. Further characterization of Dps revealed ferritin like function, including iron oxidation/storage, and iron mobilization. Cellular and *in vitro* studies discovered peroxide detoxification with coupled Fe^{2+}. A broad range of bacterial cytoprotection is associated with Dps multiple biochemical functions. Molecular study of biochemical and cellular functions of Dps proteins is necessary for new anti-bacterial strategy and drug development.

Bacterial Dps (DNA protection during starvation) proteins are a family of recently discovered proteins universally expressed to protect bacterial chromosomal DNA integrity during oxidative stress or stationery growth phase. In stationery phase, Dps and chromosomal DNA form crystalline, and the Dps-DNA complex, so called "bacterial chromatin" is resistant to multiple physical and chemical challenges. In human pathogens, involvement of Dps in virulence is indicated by the proteins' biochemical functions. Biochemical functions of Dps proteins include DNA binding and Fe^{2+} oxidation with H_2O_2 and O_2, Dps storage and iron mobilization. Molecular mechanisms of Dps functions were not established, nor were functional coordination on Dps or between Dps isoforms. Little detail is known about cellular roles of Dps. In pathogenic *bacilli,* Dps isoforms presumably have distinct biological functions, exemplified by *Bacillus anthracis* (anthrax) Dlp-1 and Dlp-2. The two Dps isoforms have separate and complementary functions in DNA binding/protection and Fe^{2+}/H_2O_2 reaction, which indicate that cytoprotection by *Bacilli* Dps is achieved through selective presentation of biochemical functions, by functional site rearrangements/conformational change, by controlling ratios of Dps isoform expression. Recombinant *B. anthracis* Dlp-1 and Dlp-2, already purified and are currently under biochemical and biophysical studies, will be used in the investigation, because they have separate functions in DNA binding/protection and Fe^{2+}/H_2O_2, which facilitate functional site assignment. Specifically, I am interested in: 1, investigating molecular basis of Dps functions, to deduce structural basis of functional coordination/cooperation between Dps isoforms, and assigning functional sites of multiple functions with *B. anthracis* Dlp-1 and Dlp-2, based on preliminary biochemical characterization the two proteins; 2, studying cellular roles of Dpl-1, Dlp-2, Dlp-1 + Dlp-2 in iron homeostasis, peroxide resistance, and gene regulation; regulation of Bacilli chromosome dynamics and cell cycle, and global gene regulation in response to environment signals.

I. Molecular studies of *Bacillus* Dps-1/DNA binding; Structural requirements for Fe^{2+} mineralization with H_2O_2 in Dps-2; Mechanism of Fe release.

Characterization of sites in Dlp-1 and Dlp-2 related to iron and peroxide consumption, and DNA binding/protection will clarify the role of Dps proteins in bacterial resistance to oxidative challenges and provide potential antibacterial drugs targets to Dps proteins.

a') Fe^{2+} oxidation by O_2 and H_2O_2 in Dlp-1 & Dlp-2, structure/function relationships of multiple biological functions. (Currently under investigation)

Dps proteins possess iron oxidation activities under *in vitro* and *in vivo* conditions. However, the general mechanism proposed for di-iron cofactor coupled Fe^{2+} oxidation by O_2 & H_2O_2 in Dps proteins is inconsistent with several current experimental observations. Assignment of active site residues through direct studies of oxidase function is required for a thorough understanding of Dps functions. Dlp-1 and Dlp-2 are particularly useful models because of the differences in reactivity coupled with high conservation of structure.

Assignment of active site will use proposed Dps active site models as guide and functional consequences of putative active site residues evaluated kinetically; *Catalytic activities* of mutant and wild type proteins will be characterized by measuring Fe oxidation with stopped flow kinetics under aerobic and anaerobic conditions, *various oxidants* and *pH effect* will be studied kinetically; *Fe^{2+} binding stoichiometry* will be studied with fluorescence quenching and isotherm titration (ITC) anaerobically. Relationship among Dps isoforms; *relationships* of structure and function between Dps and ferritin will be explored with functional assessment of chimeric ferritin active sites with structural based protein design/engineering.

b') DNA binding by Dlp-1

Dlp proteins offer DNA protection through two aspects: 1, DNA protection against hydroxyl radicals (•OH) is achieved by oxidizing Fe^{2+} thus scavenges cellular source for radical formation; 2, Dps further protects DNA by around DNA and forms DNA-Dps complex, shielding DNA from nuclease attack. A set of experiments are designed to investigate molecular basis of DAN binding and protection with Dlp-1 and Dlp-2, and cooperative DNA protections *in vivo*.

DNA binding site assignment will be explored on the current working model built on structural variations between Dlp-1 and Dlp-2, and between Dlp and other Dps proteins. Selected candidates will be altered and their contributions to DNA binding will be investigated in vitro with EMSA and/or filter binding assays; *Effects of Dps oligomerization on DNA binding and protection* will be quantified on wild type and mutant proteins by DNA mobility shift assays and/or filter binding Assays with fluorescent and isotope probes; Mutants with altered iron oxidation/release activities will be subject to DNA interaction to study *functional coordinations*. *Stoichiometry* between DNA and Dlp-I (wild-type and mutants) [DNA] will be examined by a combination of gel retardation assay, size exclusion chromatography, and sedimentation velocity (analytical ultracentrifugation) with variation of protein concentrations to deduce modes of DNA/Dlp-1 interaction.

c') Fe Exit pores in Dlp-1 and Dlp-2

Dps iron pores, formed at the three subunit junctions, are structurally highly conserved. Up to 500 Fe atoms in Dlp-1 and Dlp-2 can be mobilized. Dps iron pores share significant structural features with ferritins. Solution state studies on Dps proteins revealed existence of flexible iron pore structure, which reversibly unfolds to gate iron flux, a mechanism akin to ferritin pore gating. We will investigate structural elements for these interactions in Dlp-1 and Dlp-2, and find ways to manipulate pore stability to control Dps iron mobilization for drug development.

Assignment of conserved interactions to stabilize Dps pores will be studied with mutants that putatively alter hydrophobic interaction, salt bridges, hydrogen bonding, and secondary structures on the dynamics of iron pores. Individual mutation will be characterized kinetically, and effects on pore dynamics will be investigated by equilibrium fluorescence and circular dichroism spectroscopy; *Manipulation of Dps pore dynamics* will investigated with **1** environmental factors (salt, pH, chaotropes and detergents), **2** bacterial siderophores, **3** commercial combinatorial small compound libraries and **4** peptides from commercial phage displayed peptide library (cell based screening and *in silico* target validation with colormetric chelation assays).

d') Functions of Dlp hetoropolymer with different Dlp-1/Dlp-2 subunits assembled *in vivo* and *in vitro*

Presence of two *dps* genes in *Bacilli* presents an evolutionary advantage than other bacteria which contain only one *dps* gene: through differential expression of two *dps* genes whose products have separate but complementary biochemical functions, *Bacilli* have better adaptability to changing environmental challenges. Dps-1 and Dps-2 subunits inside *Bacilli* may spontaneously coassemble similar to mammalian ferritin H/L heteropolymer assembling in response to different environmental challenge/cellular needs. Explorations of co-assembled Dlp heteropolymers with different ratios between Dlp-1 and Dlp-2 subunits in Fe^{2+}/O_2 and Fe^{2+}/H_2O_2 reactions, Fe mobilization, DNA binding and protection are planed.

Fe^{2+}/O_2 and Fe^{2+}/H_2O_2 by Dlp heteropolymers will be assessed for their ability and molecular details on the relationship between DNA binding/ protection and Fe^{2+} oxidation with a combination of spectroscopic methods; DNA binding and protection by Dlp heteropolymer variants for EMSA, Fe^{2+}/H_2O_2 challenge and filter binding assays.

II Cellular roles of Dlp in iron homeostasis, gene regulation, and genome protection

Dlp-1 and Dlp-2 regulate cellular iron status and defend iron and peroxide related oxidative challenge, redirect cellular iron forms, regulate bacterial iron and oxidative elements through various iron/peroxide responsive transcription factors.

Bacillus subtilis will be used as model bacteria in the study, since *B. subtilis* Dps-1 and Dps-2 share high conservation with *B. anthracis* Dlp-1 and Dlp-2, and *B. subtilis* is the most studied gram-positive soil bacteria, interpretation of results will be facilitated. To investigate cellular roles of Dlp-1 and Dlp-2 in *iron homeostasis*, which we hypothesize to be complimentary or cooperative, we propose to examine effects of recombinant Dlp-1 and Dlp-1 wild type and mutant proteins on *B. subtilis* with Dps deletions under iron and peroxide stress:

a') Fe distribution during iron overloading, iron limitation, and iron repletion

B. subtilis mutants with single *dps* gene knockout or double knockouts will be complemented with *B. anthracis* Dlp-1 and/or Dlp-2. To test Fe oxidation/storage, 5 mM $^{55}FeSO_4$ is included in cell culture and 30 minutes after incubation, *B. subtilis* cells are harvested and fractionated. Cell supernatant will be separated by native gel electrophoresis, and Fe content in Dlp proteins will be evaluated. To test *Fe mobilization*, cells were treated by above method will be treated with 5 times 10 minute buffer exchange, followed by immediate addition of exogenous reduced glutathinone (GSH) and chelator. Amount of ^{55}Fe will be read 30 minutes and 1 hour after induced Fe mobilization, to check possible cellular re-distribution of ^{55}Fe before and after GSH/chelator treatment. Levels of reactive oxygen species will be compared in live cells.
Wild-type and *dlp* mutant cells will be grown under iron-rich and iron-depleted growth conditions. *B. subtilis* were grown overnight in minimal media containing 0·1 mM $FeCl_3$. Cultures were diluted into identical media to an OD600 of 0.025. Growth was continued for 4 h before 2', 2'-dipyridyl was added to one culture at a final concentration of 0·5 mM.
1"). Generation of B. subtilis mutants with single and double dps gene deletions
DNA fragments carrying Tetracyclin resistant gene (tet^R) and kanamycin resistant gene (kan^R) will be inserted into integration vector pCP115 (Bacillus genetic stock center in OSU), and transformation of two plasmids generates two single *dps* deletion strains with tet^R replacing *dpsA*, kan^R replacing *mrgA,* two *dps* isoform genes in *B. subtilis.* Deletion of single or double *dps* genes damaged cell's ability to resist oxidative stress, but did not kill the bacterium. 3 rounds of antibiotic selections will be performed to select for homozygous mutant, followed by a second round *dps* deletion. Again double deletion is selected with incubating *B. subtilis* with both antibiotics. PCR products of specific loci, where genes were deleted, will be sequenced to confirm gene deletion.
2"). Complementation of Dlp-1 and Dlp-2 in double knockout B. subtilis mutant
dlp-1 and dlp-2 genes will be introduced in shuttle vector pDG148-Stu which allows IPTG induction for protein expression. Constructed pDG148-stu vectors carrying *dlp* genes will be transformed in *B. subtilis,* alterations in iron homeostasis components and elements responding to particular stress to Dlp protein expression will be examined to study Dlp's role feedback regulation of particular regulatory network. 3 hours after addition of IPTG cells were washed 3 times to stop protein production.

b') Cellular roles of Dlp in gene regulation through iron mobilization

This study targets regulation by different forms of cellular iron. 1") *B. subtilis* mutant cells will be supplemented with Dlp-1, Dlp-2, and Dlp-1 + Dlp-2 with methods mentioned above. Iron pore mutations from Aim 1 will also be applied to examine altered pore dynamics on cellular iron homeostasis. Cells will be grown under iron limited, iron overloading, and iron repletion conditions with $^{55}FeSO_4$ or ^{55}Fe labeled ferric ammonium citrate (FAC) media. Iron mobilization is triggered with exogenous GSH and bipyridyl. 2") Total RNA is extracted from samples taken with 30 minute intervals, and known genes under fur regulation will be quantified by quantitative PCR and Northern analysis with ^{32}P labeled probes, to assess individual *fur* and Per controlled gene transcription; mRNA levels of individual genes will be compared under different treatment to assess different cellular iron forms on gene induction; 3) activities of induced gene products will be analyzed.

Long term goal: Dps proteins in chromosome dynamics, cell cycle and global gene regulation

Long term future plan of studying Dlp bound chromosomal DNA (bacterial chromatin) dynamics is projected: 1) regulation *of B. subtilis* cell cycle by a DNA-Dps "bacterial chromatin" complex with Dlp complementation in *B. subtilis* mutant; 2) roles of Dlp-1+Dlp-2 in bacterial chromosome spatial and plastid regulation will be examined under confocal microscopy with fixed cells (through collaboration); 3) physical alteration of chromosomal DNA due to Dlp binding by atomic microscopy (collaboration); 4) global gene expression regulation with microarray chip analysis and data analyzed by statistical analysis (collaboration).

Statement of Teaching Interest

I believe that scientific education, and only education, has enabled and will ensure the leading role of this country in science and technology. With that in mind, the ultimate goal of college teaching is not how much students are expected to learn, but is how much students can learn.

I think leaning involves not only memorizing the knowledge of particular topics, but also the ability to learn and application of the knowledge to solve real problems. Therefore, goals of teaching include:

1. Teach students how to grasp essence of basic biological concepts and develop students' creative and critical thinking.
2. Help student to develop learning strategy and organizational skills.
3. Train students to develop problem solving ability.
4. Facilitate students to develop effective communication skills in oral and written forms.

Achieving the above goals is not easy, especially when knowledge accumulating with an accelerating speed. Instructors need to be aware of latest developments in many new fields. This requires constant learning from textbooks, research papers and scientific meetings. In addition, as cross disciplinary studies are increasing in an unprecedented speed, it requires instructors to learn more before they can pass to students. However, teaching occurs not with traditional lecturing with heavier load to students, which can be daunting for students to learn; new approaches need to be explored, since students need to learn effectively within limited time and resources. Active participation of students is one of the most successful ways of achieving the goal. Today's biological not only know the latest concepts and developments of biological science, they are required to have know-how and hands-on experiences to fully understand the sets of concepts and developments. Instructors should integrate the latest knowledge effectively and properly with clear conceptual structures; in addition, new techniques should be incorporated into lab course and prepare students adjust better in research and professional careers. Most importantly, instructors need to clearly state the goals of the course and the expectations for students, and should be aware if the set of teaching methods work effectively by constant feedback from students and close collaborations with graduate student instructors, and make corresponding adjustments to teach more effectively.

Since biological science is still largely an experimental science, individual research labs should be open to students who are interested in pursuing research development or additional research technical trainings.

At graduate level, graduate students should be trained more in effective scientific communications, in ways such as group/individual presentations, oral discussions, et al, or assignments of group projects to train students independent research abilities in organizational skills in project development; At higher level courses, graduate students are required to write research proposals, to train them in hypothesis development and consolidation, and presentation of suitable research activities to test hypotheses. The purpose of these trainings is necessary for graduate students to prepare qualification exams, manuscript writing, fellowship proposal writing, and future proposal writing in independent scientific careers.

Learning does not only occur in class and research labs. Graduate students will be encouraged to attend scientific meetings to present results, and be exposed to bigger scientific communities to learn to communicate effectively.

As graduate student instructor, I had in worked with students in the lab, led review discussions, which helped me to consider goals of teaching; the two lectures I taught in a senior/graduate biochemistry course helped me to develop effective teaching methods; Extensive experiences in supervising/directing student interns, rotation students and junior research associates expanded my expertise in training junior scientists.

The following are the courses I taught:

- General Chemistry, Changchun College of Traditional Medicine (lecturer);
- Organic Chemistry I (lab), Western Michigan University (graduate teaching assistant);
- Organic Chemistry II (lab), Western Michigan University (graduate teaching assistant);
- Advanced Protein Chemistry (lab), Iowa State University (graduate teaching assistant);
- Biochemistry (senior & graduate level), Iowa State University (graduate teaching assistant).