Example GUP2 review for reference

These proposals are for GUP2 (Chameleon access). In this early access phase this would include a Chameleon session to generate up to 4 grids to be shipped to the user. They may use these grids to apply for a GUP1 (Krios access) session, but this category of access is only for specimen preparation.

1) Project ID: NCCAT-GUP2-WT181207
Project Name: Use spotiton (chameleon) to prepare grid for human presequence protease.
Primary user name: Wei Jen Tang
eRA Commons user name: WEI-JEN
Institution: University of Chicago, IL
Submission Date: December 7, 2018 8:18 pm

Averaged URC scores:
(i) scientific impact: **1.0**
(ii) scientific feasibility: **1.7**
(iii) technical feasibility: **2.3**
(iv) resources requested: **1.3**
(v) geographical demographics or need: **1.0**

Raw average score: 1.5

Comments:

Reviewer 1: The proposal requests Chameleon access to improve the quality of the structure of the presequence protease in its open state. Preliminary structures solved from data collected at NYSBC using conventional blotting techniques yielded ~5Å structures and revealed severe orientation bias. Many particles also appeared to be partially denatured, presumably due to their interactions with the hydrophobic air-water interface. The request to use Chameleon to outrun particle interactions with the air-water interface is therefore completely appropriate and a logical way to improve the quality of their reconstruction. Although not explicitly stated in the proposal, the biochemical yield of their particle appears abundant (based on their gel filtration profile in Fig. 3A), but I would encourage NCCAT to confirm that the concentration of the preps is sufficiently high for Chameleon.

Reviewer 2: The goal of this proposal to obtain structures of structure of PreP. Samples have preferred orientation based on the data provided. Use of Chameleon/spotiton should be granted to see if this research group could overcome the orientation bias by outrunning air-interface interactions during blotting.

Reviewer 3: The applicant intends to purchase a commercial version of spot-it-on (Chameleon) and views this activity as not only solving the problem at hand, but establishing best practices in the use of the instrumentation. There is an excellent chance that spot-it-on will solve both the orientation and degradation issues and the resources requested are reasonable.

Project ID: NCCAT-GUP2-WT181207
Primary user name: Wei Jen Tang
eRA Commons user name: WEI-JEN
Project name: Use spotiton (chameleon) to prepare grid for human presequence protease.

Abstract:
Mitochondria require the import of many nucleus-encoded proteins for their function and the mitochondrial targeting presequences are required for such importing process. Upon entering into mitochondria, presequences are cleaved off by mitochondrial processing peptidases. Presequences are rich in hydrophobic and positively charged residues and therefore highly toxic to mitochondria. Presequence protease (PreP) is a ubiquitously expressed M16 metalloprotease that localizes to the mitochondrial matrix and degrades presequence peptides into non-toxic pieces. PreP also degrades amyloid beta, another peptide toxic to mitochondria which has been linked to Alzheimer's disease. Our overarching goal is to gain structural and functional insights to elucidate the molecular basis of how PreP is involved in maintaining mitochondrial proteostasis. Our crystal structure of 117 kDa human PreP in the closed state has shown that PreP contains homologous 55 kDa domains at its N- and C-termini, named PreP-N and PreP-C, respectively. The structure of substrate-free and amyloid beta bound PreP elucidates how PreP forms an enclosed catalytic chamber to entrap and degrade its substrate, amyloid beta (King et al Structure 22:996-10017, 2014). However, many key questions regarding PreP function remains due to the luck of structural information of PreP open state. For example, how does PreP transition between open and closed states to capture its substrate or release its reaction products? The structure of the catalytic chamber in the PreP closed state appears to preclude both of these activities. What is the molecular mechanism which allows PreP to recognize presequence peptides and amyloid beta, which are highly diverse in sequence and length? How does PreP interact with other mitochondrial proteins for its non-catalytic activity? We have started to employ single particle electron microscopy to elucidate the structure of open state PreP and explore conformational dynamics. Using the Titan Krios at NCCAT, we have obtained 5.2 Angstrom PreP open state and 4.5 Angstrom PreP partial open state reconstructions and models. The relatively poor resolution of two PreP conformational states from our 2-day data collection is due, in part, to a severe orientation preference. The problem is compounded with a preferential denaturation of the PreP-C domain so that only ~20% PreP particles have both PreP-N and PreP-C intact. Many conventional strategies described in the supplemental data to prevent the denaturation of PreP-C domain have failed. We will explore the spotiton to "outrun" the denaturation of PreP-C by ~10-time faster sample spotting and grid freezing than vitrobot. Spotiton is instrumental in how the research team led by Clint Potter and Bridget Carragher and our group could obtain the cryoEM structures of open state and insulin bound state of human insulin degrading enzyme (IDE) (Zhang et al eLife 7:e33572, 2018). We anticipate that spotiton can do the same for PreP structure as IDE and PreP belong to the same M16 metalloprotease family and share a similar domain organization. Such studies should provide valuable insights into how to better prepare the grid for PreP in order to investigate the molecular basis of how PreP recognizes the targeted peptides and offer insights for developing PreP-based therapies.

Scientific Impact:
1. Structural basis for the function of PreP: PreP is vital for mitochondrial proteostasis. The gene knockout of PreP is embryonic lethal in mice and missense mutations in human PreP are associated with neuronal disorders such as mental retardation and psychosis (Brunetti et al EMBO Molecular Medicine 8;176, 2016). PreP belongs to the M16C family of metalloproteases. Members of this family are involved in diverse biological processes, such as falcilysin, which is vital for the catabolism of hemoglobin in the malaria parasite. PreP is one of several chamber-containing proteases that selectively degrade amyloid beta, a key contributor for the progression of Alzheimer's disease (Malito et al Cell Mol Life Sci 65:2574, 2008). CryoEM analysis of PreP structures should provide insights in how PreP and its homologs work.
2. Technical challenges in solving PreP cryoEM structures: The protein denaturation at the air-water interface

represent the major hurdle in identifying the suitable condition to vitrify protein sample for cryoEM study. By ingenious design, spotiton eliminates the paper blotting step that removes the excess liquid and thus significantly reduces the time for protein to stay at air-water interface. We found that PreP, a small, 117 kDa, monomeric enzyme with homologous 55 kDa N- and C-domains exhibits preferred denaturation of PreP-C domain. The observed orientation of PreP particles wherein PreP-C is denatured, suggests that Prep-C faces the air-water interface. This is consistent with the notion that PreP-C is preferentially denatured in vitrified ice by exposure to the air-water interface. This makes PreP a compelling test case to investigate how the reduced grid preparation times afforded by spotiton can alter the kinetics of protein denaturation at the air-water interface.

Scientific Feasibility:
Human PreP has ~50 kDa N- and C-terminal domains, PreP-N and PreP-C. The rigid body motion between these two domains allows PreP to adopt at least two distinct conformational states (open and closed). PreP requires large conformational changes for its catalytic activities, which hinder the effort in solving the open state PreP structure by crystallography. From our recent data collected at NCCAT, we have obtained a 5.1 angstrom Coulomb potential density map and open state model as well as a 4.5 angstrom partial open state map that are distinct from the previous solved human PreP closed state structure. The availability of a PreP closed structure (King et al Structure 22:996-10017, 2014) has allowed us to build a preliminary structural model of the PreP open state (see supplemental material). The major challenge that needs to be overcome is preferential denaturation of the PreP-C domain, to the point that only ~20% PreP particles have both PreP-N and PreP-C intact. It is our belief that the drastic reduction in the time for protein sample to stay in the thin film before plunged into liquid ethane provided by spotiton will substantially reduce the time for PreP to be exposed to air-water interface and thus decrease the rate of PreP-C denaturation.

Technical Feasibility:
Our goal is to obtain the near atomic resolution structures of PreP alone and PreP in complex with functionally relevant substrates, e.g., amyloid β and the model sequence peptide for mitochondrial targeting. We have performed 2D and 3D classifications of PreP alone from a dataset collected using a NCCAT 300 kV Titan Krios microscope equipped with a K2 camera (see the attached progress). In this 2-day data collection, we have obtained ~3,300 micrographs and ~650,000 particles. The 3D classification of the dataset revealed two novel PreP structures, a 5.2 Angstrom PreP open state and a 4.5 Angstrom PreP partial open state. The major roadblock preventing higher resolution reconstruction is the preferential denaturation of 50 kDa PreP-C domain. We propose to take the advantage of spotiton to reduce the exposure time of PreP at air-water interface to reduce/eliminate the denaturation issue of PreP-C. Using our current grid-making protocols, we estimate that only ~20% of the particles contain a properly folded PreP-C domain. As mentioned above, PreP particles exhibit an orientation preference such that PreP-C faces the air-water interface. This observation, coupled with the lack of improvement stemming from modifying buffer conditions, lead us to conclude that unfavorable interactions at the air-water interface represent the primary catalyst for PreP-C denaturation. As such, decreasing the amount of time PreP has to spend at the air-water interface is a top priority. Grid preparation via spotiton has been shown to decrease the time protein spends on the grid prior to freezing by a factor of 10. With such a dramatic reduction in time, we anticipate that, by utilizing spotiton to generate PreP grids, it would be feasible to expect greater than 50% of the particles on the resulting grids to possess properly folded PreP-C domains, potentially adding ~200,000 intact particles of a two-day data collection to our analysis.

Resources Requested:
We will travel to NCCAT and work with the scientists at NRAMM to prepare various grids suitable for use by spotiton, if such grids are not commercially available. We will vary the parameters known to allow successful grid preparation using spotiton. We will examine the quality of PreP particle on site. PreP particles which

possess an intact PreP-N and denatured PreP-C display distinct features which are readily identifiable. As such, the quality of particles can be assessed from merely several micrographs. Once suitable conditions have been identified, we will submit a proposal for the collection of a full dataset. We anticipate that we will need three 2 day sessions to complete the search for the optimal and consistent grid making protocol.

Geographic/Demographics:
We consider that spotiton (chameleon) technology will revolutionize the grid-making process. My colleagues and I will apply for an instrument grant to purchase such a machine. To gain familiarity in the use of spotiton, we would like to learn from the scientists from NRAMM how to best apply this technology and transfer it back to Chicago and the Midwest region. This will complement the 300 kV Titan Krios equipped with Gatan K2 camera, which will be installed in the University of Chicago in late 2018 and early 2019.

Progress in CryoEM analysis of human PreP

Recombinant human PreP was expressed in *E. coli* and purified using Ni-NTA, Source Q anion exchange, and S200 columns according to King et al Structure 22:996-10017, 2014. The purified human PreP behaves as a monomer based on size exclusion column, a finding consistent with previous SAXS analysis. Human PreP has 16 cysteine residues, which contributes to its high sensitivity to oxidative inactivation. PreP is a zinc metalloprotease and the presence of EDTA can strip the catalytic zinc ion, rendering PreP inactive. We thus vary the reducing agents (beta-mercaptoethanol, dithiothreitol (DTT), or Tris(2-carboxyethyl)phosphine (TCEP)) and metal chelator (EDTA) in addition to the conventional conditions, i.e., NaCl (50-300 mM), buffers (Tris, Hepes), the type of grid (Au, Cu), and blotting time controlled by vitribot for the grid preparation. By optimizing these variables using the 200 KV Talos at the University of Chicago, we have found conditions that are optimal to image the monomeric PreP particles in the PreP alone condition using a 200 mesh Cu grid.

Previous approval by the review committee allowed us to visit NCCAT for two-day on-site data collection from October 4-6. In this period, we obtained ~3,300 micrographs containing ~650,000 particles. Using Relion 3.0 and Cryosparc, we have obtained good 2D class averages (Figure 1A). To avoid model bias, we used the structure of plant PreP as a model, instead of the human PreP structure. After significant effort in our data analysis, a satisfactory 3D classification emerged to reveal two major classes of PreP structures that are distinct from the previously published, closed state, crystal structure of PreP (Figure 1 and 2). A 5.1 angstrom structure of open state PreP was reconstructed from 45,292 particles while a 4.5 angstrom structure of a partial open state PreP was reconstructed from 34,404 particles.

Figure 1 CryoEM analysis of PreP open state structure. (A) 2D classification. (B) PreP open state model (ribbon) and Coulomb potential density map. (C) Comparison of PreP open state structure with PreP closed state. (D) FSC plot of PreP open state structure, indicating 5.1 angstrom resolution. (E) Angular distribution plot depicting the severe orientation bias. (F) ResLog plot for the resolution estimation when the additional data is added.

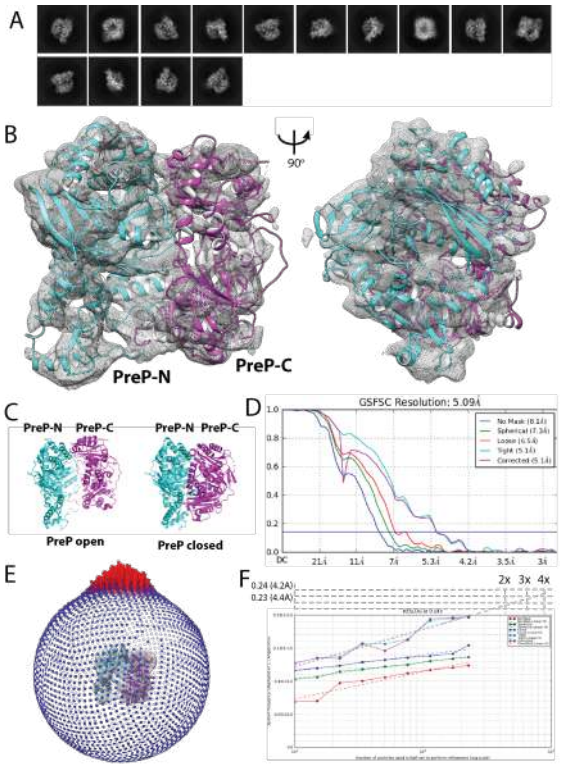
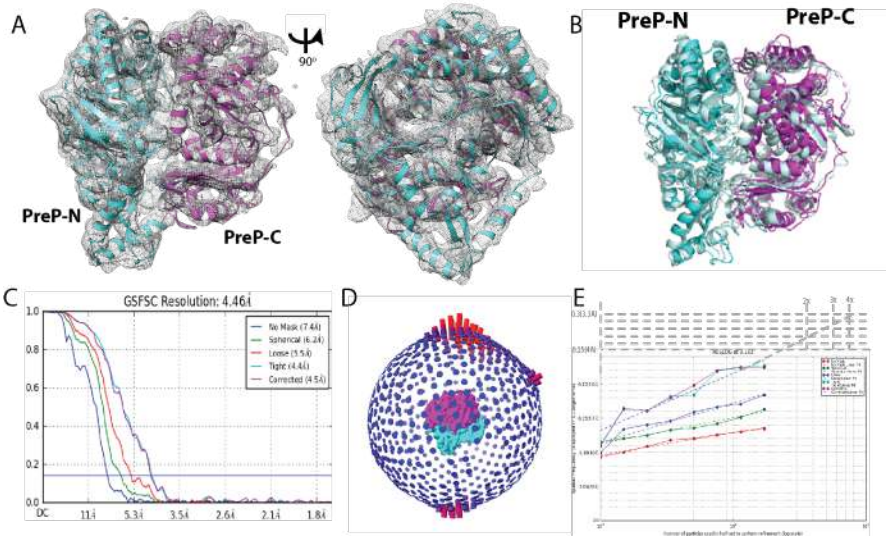


Figure 2 CryoEM analysis of PreP partial open state. (A) PreP partial open state (ribbon) and Coulomb potential density map. (B) Comparison of PreP partial open state (cyan and magenta) with PreP closed state (pale cyan). (C) FSC plot of PreP partial open state structure, indicating 4.5 angstrom resolution. (D) Angular distribution plot. (F) ResLog plot for the estimated resolution improvement with additional data.

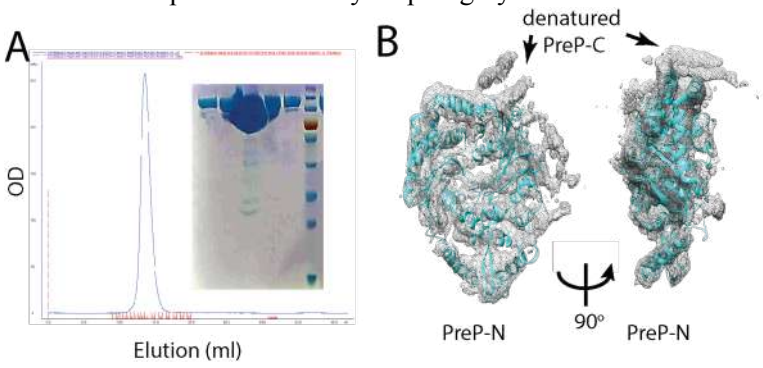


PreP has ~50 kDa N and C domains (PreP-N and PreP-C, respectively) connected by an alpha-helical hair-pin (Figure 1C). Compared with the PreP closed structure, the open-state PreP structure reveals a large-scale pivot motion between PreP-N and PreP-C (Figure 1C, 1D). However, detailed analysis of this molecular motion is hampered by poor map quality stemming from severe orientation bias (Figure 1E). We estimate that 3 times additional data with the tilted series should improve the resolution up to 4.3 angstrom and better density map for the modeling (Figure 1F).

The partial open PreP structure represents a ~7 angstrom opening between PreP-N and PreP-C in comparison with the crystal structure of PreP closed state (Figure 2A-C). In this case, we observed a mild orientation bias. We estimate that 3 times additional data should push the resolution of this structure beyond 3.5 angstrom.

In addition to the expected open state structures of PreP from our 3D classification, we also obtained a PreP structure that has an intact PreP-N and denatured PreP-C despite the fact that purified PreP behaved as a single uniform species in size exclusion column and greater than 95% PreP is intact based on SDS-PAGE (Figure 3). This structure prefers to adopt an orientation such that the presumably denatured PreP-C faces the incoming electron beam while PreP-N is opposite to that. Thus, it is reasonable to assume that this structure is derived from unfavorable interactions between PreP-C and the air-water interface, which is known to cause the denaturation of protein. This also explains the significant difficulty that we had in our 3D classification for generating an interpretable density map and the lower number of usable particles for the two open state PreP structures. We have attempted to use amine-to-amine crosslinker, DSS to rigidify the PreP-C domain so that PreP can withstand the denaturation by the air-water interface. However, such treatment has worsened the denaturation problem. The addition of additives, e.g., detergent or organic solvent has also failed to modify the vitrified ice and/or change the surface properties better favor intact PreP particles. In fact, these efforts have mostly resulted in conditions where PreP is excluded from the grid holes. We will continue to explore the conditions that can favor intact PreP in the vitrified ice, yet is our belief, as detailed above, that the primary cause of PreP-C denaturation is prolonged exposure to the air-water interface during grid preparation. We anticipate that the use of spotiton in grid preparation will vastly decrease the amount of PreP-C denaturation. The commensurate increase in analyzable intact PreP particles should also reduce the amount of data collection necessary to reach our desired resolution, ultimately saving both time and money.

Figure 3 Partially denatured PreP structure. (A) The size exclusion profile of purified PreP and SDS-PAGE and Coomassie blue staining of PreP peak fraction. (B) The partially denatured PreP structure with PreP-N in ribbon representation and Coulomb potential density map in grey.



BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Wei-Jen Tang

eRA COMMONS USER NAME (credential, e.g., agency login): WEI-JEN

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
National Taiwan University	B.S.	10/1978-05/1982	Zoology
University of Texas, Austin	Ph.D.	08/1984-05/1988	Biological Science (Mentor: William R. Folk)
University of Texas, Austin	Postdoctoral fellow	06/1988-08/1988	Microbiology (Mentor: William R. Folk)
University of Texas Southwestern Medical School	Postdoctoral fellow	09/1988-06/1991	Pharmacology (Mentor: Alfred G. Gilman)

A. Personal Statement

My research program involves in elucidating the molecular basis of protein functions relevant to human health and diseases. The research is based on the premise that the better understanding of protein-protein and protein-ligand interaction is key to elucidating the fundamental principles governing cellular signaling network that contributes to normal physiological and pathological settings. I apply structure biological (e.g., X-ray crystallography and single particle EM), proteomics, biochemical, biophysical, cellular and pharmacological tools to address the protein functions and regulations. I am known for the studies on the catalysis and regulation of mammalian adenylyl cyclase, anthrax and pertussis adenylyl cyclase toxins, and human insulin degrading enzyme (IDE). I am also known in the drug discovery for anthrax toxins, edema factor and lethal factor, and human insulin degrading enzyme. I am a strong believer of collaboration, which shows nicely from the collaborative nature of many research projects in my publications. An example is my effort to assemble a team of researchers from academy and industry to show the efficacy of approved antiviral drug, Adefovir in inhibiting anthrax edema factor and anthrax pathogenesis. This allows the repurpose of the existing anti-hepatitis B virus drug for the biodefense against anthrax bacterium, a bioweapon for mass destruction and a proven bioterrorism agent used in 2001. The other example is the involvement of my lab with many scientific teams to develop the small molecule modulators of human IDE. IDE plays the key role in the clearance of insulin and amyloid β thus is vital for the progression of type 2 diabetes and Alzheimer's disease. The small molecule modulators could be further developed for the treatment of these chronic diseases that are continuing in the rise.

a. **Tang, W.-J.** & Gilman, A. G. (1991) Type-specific regulation of adenylyl cyclase by G protein βγ subunits. Science 254:1500-1503.

b. Drum, C.L., Yan, S.-Z., Bard, J., Shen, Y.-Q., Lu, D., Soelaiman, S., Grabarek, Z., Bohm, A., & **Tang, W.-J.** (2002) Structural basis for the activation of anthrax adenylyl cyclase exotoxin by calmodulin. Nature 415:396-402. (Highlighted in N&V Nature 415: 373, 2002; N &V Nature Structure Biology 9:156, 2002; Minireview Cell 108:739, 2002)

c. Shen, Y.-Q., Zhukovskaya, N.L., Zimmer, M.I., Soelaiman, S., Wang, C.R., Gibbs, C.S., & **Tang, W.-J.** (2004) Selective inhibition of anthrax edema factor by adefovir, a drug for chronic hepatitis B virus infection. Proc. Natl. Acad. Sci. U.S.A. 101:3242-3247.

d. Shen, Y., Joachimiak, A., Rosner, M.R., & **Tang, W.-J.** (2006) Structures of human insulin degrading enzyme reveal a new substrate recognition mechanism. Nature 443:870-874. (Highlighted in N&V Nature 443:761, 2006).

B. Positions and Honors

Positions and Employment

1982-1984	Lieutenant, Air Force, Taiwan
1991-1993	Instructor, Dept. of Pharmacology, University of Texas Southwestern Medical School
1993-1994	Assistant Professor, Dept. of Pharmacology, UT Southwestern Medical School
1994-1998	Assistant Professor, Dept. of Pharmacol. & Physiol. Sciences, The University of Chicago
1998-2001	Assistant Professor, Dept. of Neurobiol. Pharmacol. & Physiol., The University of Chicago
2001-2007	Associate Professor, Ben-May Institute for Cancer Research, The University of Chicago
2007-present	Professor, Ben-May Department for Cancer Research, The University of Chicago

Other Experience and Professional Memberships

1992-present	American Society for Biochemistry and Molecular Biology
1986-2013	American Association for the Advancement of Science
1998-present	Ad Hoc NIH and NSF grant reviewing panels
2007-2011	Regular member of NIH MSF-C study section
2009	The advisory Board, Structure Biology Center, APS, Argonne National Lab.
2012-2014	Regular member of American Heart Association Signaling 4 study section.

Honors

1987-1988	University Fellowship, University of Texas, Austin
1999-2002	American Heart Association Established Investigator

C. Contributions to Science

1. ***Regulation and catalysis of mammalian adenylyl cyclases:*** Cyclic AMP is a prototypic intracellular second messenger that controls diverse physiological events in response to the stimulation of a plethora of hormones and neurotransmitters. My early publications establish the molecular basis for the regulation and catalysis of mammalian adenylyl cyclase, which is an enzyme that raises the intracellular cyclic AMP level in response to the extracellular stimuli. Upon the activation by G protein coupled receptors, hormone-regulated heterotrimeric G protein is dissociated into α and βγ subunits. The dogma at the time is that α subunit of G protein, but not βγ subunit is responsible to regulate mAC. After involving in the cloning of first mammalian adenylyl cyclase (type 1), I characterized its regulation biochemically to show surprisingly that βγ can effectively suppress the activity of type 1 adenylyl cyclase. I also subsequently showed that G protein βγ subunit could directly activate the activity of $G_{s\alpha}$-activated type 2 adenylyl cyclase. This finding made the seminal contribution to establish the direct roles of G protein βγ in modulating the activity of downstream effectors. Mammalian membrane-bound adenylyl cyclase consists of two trans-membrane domains, each followed by a conserved cytoplasmic domain. I also have combined protein-engineering and genetic approaches to construct a $G_{s\alpha}$-activated soluble adenylyl cyclase from two conserved cytoplasmic domains of adenylyl cyclase and used it to address the catalysis and regulation of adenylyl cyclase by $G_{s\alpha}$ and forskolin. As the soluble adenylyl cyclase is amenable to structural analyses, such construct played a key role for the structural studies of mAC. Together, the molecular basis of how mammalian adenylyl cyclases are regulated by G proteins, calmodulin, and other pharmacological agents such as forskolin was elucidated. I was initially a postdoctoral fellow and then a junior faculty under the guidance of Dr. Alfred G. Gilman at UT Southwestern Medical School and then became the principal investigator at the University of Chicago for these studies.

   a. **Tang, W.-J.** & Gilman, A. G. (1991) Type-specific regulation of adenylyl cyclase by G protein βγ subunits. Science 254:1500-1503.
   b. **Tang, W.-J.** & Gilman, A.G. (1995) Forskolin and $G_{s\alpha}$ sensitive soluble adenylyl cyclase. Science 268:1769-1772.

c. Yan, S.-Z., Hahn, D., Huang, Z.-H., & **Tang, W.-J.** (1996) Two cytoplasmic domains of mammalian adenylyl cyclase form a $G_{s\alpha}$ and forskolin-activated enzyme in vitro. J. Biol. Chem. 271:10941-10945.
d. Yan, S.-Z., Huang, Z.-H., Rao, V.D., Hurley, J.H., & **Tang, W.-J.** (1997) Three discrete regions of mammalian adenylyl cyclase form a site for $G_{s\alpha}$ activation. J. Biol. Chem. 272:18849-18854.

2. ***Structural and functional analyses of anthrax edema factor:*** I have been studied the molecular basis of how toxins and virulent factors disrupt the cellular signal transduction to benefit the bacterial pathogenesis. I have primarily used *Bacillus anthracis*, bacteria that causes anthrax, as the model system. Anthrax bacteria, a bioweapon for mass destruction and a proven bioterrorism agent used in 2001, secrete three major toxins, edema factor (EF), lethal factor (LF), and protective antigen (PA). EF has the calmodulin (CaM)-activated adenylyl cyclase activity. We have determined the structures of EF and EF-CaM complex to address the structural basis of how CaM binds and activates EF, highlighting the diverse mode of binding and mechanism of action of CaM to modulate their effectors. Furthermore, this work reveals that bacterial adenylyl cyclase toxins and eukaryotic adenylyl cyclases use two-metal mediated catalysis despite they share no structural similarity. Advanced Photon Source at Argonne National Laboratory has highlighted our work for their contribution to the biodefense as the structures of EF are the first anthrax toxin solved by the use of synchrotron facility in US. I have led a team to develop and characterize small molecule inhibitors against EF and LF. One example is our teamwork of researchers from academy and industry to show the efficacy of approved antiviral drug, adefovir in inhibiting the activity of EF and anthrax pathogenesis. This allows the repurpose of the existing anti-hepatitis B virus drug against the anthrax infection. I also have done collaborative work to address the roles of EF in anthrax pathogenesis and develop the experimental models to study EF-induced tissue damages.

   a. Drum, C.L., Yan, S.-Z., Bard, J., Shen, Y.-Q., Lu, D., Soelaiman, S., Grabarek, Z., Bohm, A., & **Tang, W.-J.** (2002) Structural basis for the activation of anthrax adenylyl cyclase exotoxin by calmodulin. Nature 415:396-402. (Highlighted in N&V Nature 415: 373, 2002; N &V Nature Structure Biology 9:156, 2002; Minireview Cell 108:739, 2002)
   b. Shen, Y.-Q., Zhukovskaya, N.L., Zimmer, M.I., Soelaiman, S., Wang, C.R., Gibbs, C.S., & **Tang, W.-J.** (2004) Selective inhibition of anthrax edema factor by adefovir, a drug for chronic hepatitis B virus infection. Proc. Natl. Acad. Sci. U.S.A. 101:3242-3247.
   c. Lee, Y.-S., Bergson, P., He, W.-S., Mrksich, M., & **Tang, W.-J.** (2004) Discovery of a small molecule that inhibits the interaction of anthrax edema factor with its cellular activator, calmodulin. Chem. & Biol. 11:1139-46.
   d. Shen, Y., Zhukovskaya, N.L., Guo, Q., Florián, J., and **Tang, W.-J.** (2005) Calcium-independent calmodulin binding and two-metal-ion catalytic mechanism of anthrax edema factor. EMBO J. 24:929-941.

3. ***Structural and functional analyses and drug discovery of human insulin degrading enzyme (IDE) and presequence protease (PreP):*** Type 2 diabetes mellitus (T2DM) and Alzheimer's disease are human chronic diseases that affect millions of people in US alone. Aberrant levels of insulin and improper responses to insulin and other hormones that control glucose levels are the primary causes of T2DM. Aβ peptide, the primary component in amyloid plaques, plays a central role in the progression of AD. Insulin Degrading Enzyme (IDE) and Presequence Protease (PreP) are structurally related, ~110 kDa M16 Zn^{2+}-metalloproteases that use an enclosed catalytic chamber to recognize and degrade peptide substrates into fragments. IDE is involved in the clearance of peptides diverse in structure and sequence, including three glucose-regulating hormones (insulin, amylin, and glucagon), Aβ, and other bioactive peptides <80 aa. The involvement of IDE in the clearance of insulin and Aβ links IDE to the progression of Type 2 diabetes mellitus and Alzheimer's disease. PreP is localized at mitochondrial matrix, where it degrades presequences cleaved from proteins imported into the organelle. PreP also effectively degrades Aβ *in vitro* and may degrade Aβ imported into mitochondria to prevent Aβ toxicity in mitochondria. The defect in PreP is embryonic lethal in mice and is linked to the neurological disorder such as mental retardation and spinocerebellar ataxia. I have used structural, biochemical, and biophysical analyses to construct a working model to how human IDE and PreP use their catalytic chambers to recognize the <80 aa substrates in a distinct manner. We also decipher the molecular basis of how IDE recognizes amyloidogenic peptides. Furthermore, we have developed potent inhibitors of human IDE and PreP to explore the biological

functions and therapeutic potential of these proteases. Together, our studies pave the way to explore IDE and PreP-based therapies.

a. Shen, Y., Joachimiak, A., Rosner, M.R., & **Tang, W.-J.** (2006) Structures of human insulin degrading enzyme reveal a new substrate recognition mechanism. Nature 443:870-874. (Highlighted in N&V Nature 443:761, 2006)
b. McCord L.A., Liang, W.G., Dowdell, E., Kalas, V., Hoey, R.J., Koide, A., Koide, S., & **Tang, W.-J.** (2013) Conformational states and recognition of amyloidogenic peptides of human insulin-degrading enzyme. Proc. Natl. Acad. Sci. USA 110(34):13827-32.
c. King, J.V., Liang, W.G., Scherpelz, K.P., Schilling, A.B., Meredith, S.C., & **Tang, W.-J.** (2014) Molecular basis of substrate recognition and degradation by human presequence protease. Structure 22:996-1007.
d. Maianti, J.P., McFedries, A., Foda, Z.H., Kleiner, R.E., Du, X.-Q., **Tang, W.-J.**, Charron, M.J., Seeliger, M.A., Saghatellian, A., & Liu, D.R. (2014) Anti-diabetic activity of insulin degrading enzyme inhibitors mediated by multiple hormones. Nature 511:94-98. (Previewed by Cell Metabolism 20:201, 2014.
e. Zheng, Z., Liang, W.G., Bailey, L.J., Tan, Y.Z., Wei, H., Wang, A., Farcasanu, M., Woods, V.A., McCord, L. A., Lee, D., Shang, W., Deprez-Poulain, R., Deprez, B., Liu, D.R., Koide, A., Koide, S., Kossiakoff, A.A., Li, S.*, Carragher*, B., Potter, C.S.*, and Tang, W.-J.*, (2018) Emsemble cryoEM elucidates the mechanism of insulin capture and degradation by human insulin degrading enzyme. ELife In press (*co-corresponding authors).

4. ***Structural and functional analyses of human chemokines*** Chemokines are 8-14 kDa chemotactic cytokines that modulate inflammation and infection, affecting many chronic human diseases and thus potential therapeutic targets. CCL3 (a.k.a. MIP-1α), CCL4 (a.k.a. MIP-1β), CCL5 (a.k.a. RANTES) are proinflammatory chemokine that are linked to many human diseases, e.g., atherosclerosis, AIDS, and cancer. These chemokines readily dimerize and then form high molecular weight, >500 kDa oligomers. Our structural studies reveal how these chemokines form the rod-shaped, double helical oligomers and how oligomerization regulates their functions at the ligand level. Glycosaminoglycans (GAGs) are complex polysaccharides that are either free or attached to proteoglycans that are present at the glycocalyx layer of the cell surface or in the extracellular matrix. The binding of chemokines to extracellular GAG is a key for chemokines' function. Our GAG bound CCL3 and CCL5 structures also provide the structural basis of how GAG binds these chemokines, which allows further exploration how GAG regulates chemokine functions.

a. Ren, M., Guo, Q., Guo, L., Lenz, M., Qian, F., Koenen, R.R., Xu, H., Schilling, A.B., Weber, C., Ye, R.D., Dinner, A.R., and **Tang, W.-J.** (2010) Polymerization of MIP-1 chemokine (CCL-3 and CCL-4) and clearance of MIP-1 by insulin degrading enzyme. EMBO J. 29:3952-3966.
b. Liang, W.G., Ren, M., Zhao, F., and **Tang, W.-J.** (2015) Structures of human CCL18, CCL3, and CCL4 reveal molecular determinants for quaternary structures and sensitivity to insulin degrading enzyme. J. Mol Biol 427:1345-1358.
c. Liang WG, Triandafillou CG, Huang T-Y, Zulueta MML, Banerjee S, Dinner AR, Hung S-C, & **Tang W.-J.** (2016) Structural basis for oligomerization and glycosaminoglycan-binding of CCL5 and CCL3. *Proc Natl Acad Sci USA* 113:5000-5005.

5. ***Structural and functional analyses of bacterial virulent factors:*** In addition to study the virulent factors secreted by anthrax bacteria, I have also done biochemical and structural analyses of CaM-activated adenylyl cyclase toxin secreted by *Bordetella pertussis*, bacteria that cause whooping cough. Our studies led to the surprising finding that the mode of CaM binding by pertussis adenylyl cyclase toxin is completely different from that of EF, highlighting that the diverse means that CaM effectors can evolve to be regulated by CaM. I have the broad interest in the structure and functions of bacterial toxins. Toward this, I have determined the structure of anthrolysin O, an anthrax-secreted, pore-forming toxin and shown that anthrolysin O can disrupt the integrity of gut epithelial monolayer, thus potentially contributing to gastrointestinal anthrax. I have also involved in studying an anthrax-secreted protease, inhA and showed that inhA interferes blood coagulation in the quorum-acting manner.

a. Guo, Q., Shen, Y., Lee, Y.-S., Gibbs, C.S., Mrksich, M., & **Tang, W.-J.** (2005) Structural basis for the interaction of adenylyl cyclase toxin of *Bordetella pertussis* with calmodulin. EMBO J. 24:3190-3201.
b. Bourdeau, R.W., Malito, E., Chenal, A., Bishop, B.L., Musch, M.W., Villereal, M.L., Chang, E.B., Mosser,

E.M., Rest, R.F., & **Tang, W.J.** (2009) Cellular functions and X-ray structure of anthrolysin O, a cholesterol-dependent cytolysin secreted by *Bacillus anthracis*. J. Biol. Chem. 284:14645-56.

c. Bishop, B.L. Lodolce, J.P., Kolodziej, L.E., Boone, D.L., & **Tang, W.J.** (2010) The role of anthrolysin O in gut epithelial barrier disruption during *Bacillus anthracis* infection. Biochem. Biophys. Res. Commun. 394:254-259.
d. Kastrup, C.J., Boedicker, J.Q., Pomerantsev, A.P., Moayeri, M., Bian, Y., Pompano, R.R., Kline, T.R., Sylvestre, P., Shen, F., Leppla, S.H., **Tang, W.-J.,** & Ismagilov, R.F. (2008) Spatial localization of bacteria controls coagulation of human blood by 'quorum acting'. Nature Chem Biol 4:742-750.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1b5sZCJCab_kp/bibliography/44138238/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

Grant-in-Aid 17GRNT33400028 Tang (PI) 01/01/2017-12/31/2018
American Heart Association
Structure and functions of chemokine CCL5-CXCL4 hetero-oligomer
This study is to use biophysical and structural methods to investigate the molecular basis of heteromer formation between CCL5 and CXCL4.

R01 GM 121964 Tang (PI) 09/01/2017-08/31/2021
NIH NIGMS
Structure-function analysis and small molecule modulator discovery of human insulin degrading enzyme
This study is to analyze molecular basis of how human insulin degrading enzyme undergoes the requisite conformational changes for substrate recognition and destruction as well as to develop the small molecule modulators that can either enhance or inhibit the activity of human insulin degrading enzyme.

Completed Research Support (within past three years)

R01 GM81539-08 Tang (PI) 10/01/2011-09/30/2016
NIH NIGMS
Regulation and catalysis of human insulin degrading enzyme
This study is to analyze molecular basis for substrate recognition and regulation of human insulin degrading enzyme.