

Example GUP2review for reference

These proposals are for GUP2 (Chameleonaccess). 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.

Project ID: NCCAT-GUP2-WT190629

Project Name: Grid preparation for the substrate-bound human presequence protease by chameleon

Primary User Name: Wei Jen Tang

eRA Commons User Name: WEI-JEN

Institution: The University of Chicago

Submission Date:

Averaged URC scores:

(i) training goals: 1.5

(ii) training plan: 1.5

(iii) resources requested: 1

(iv) user EM background and history: 1

(v) geographical demographics or need: 1

Raw average score: 1.2

Comments:

Reviewer 1:

The proposal requests assistance to prepare 4-8 grids using Chameleon of the presequence protease (PreP) enzyme in complex with substrates. The PI has demonstrated the success of structure determination of PreP without substrate using Spotiton/Chameleon. Therefore, inclusion of substrate is a logical step forward and likely to achieve successful results. Some clarification should be provided before finalizing the project, including the rationale of using two different substrates (are they expected to bind differently?), biochemical evidence supporting substrate binding (to increase confidence that complex is stable in solution; ideally nM to low uM Kd), and the stability of PreP in the absence of zinc. Overall, enthusiasm for this application is high depending on the stability of the PreP(+EDTA)-substrate complex.

Reviewer 2:

This proposal rests on an impressive foundation of initial data, including proof-of-concept that the Chameleon reduces sample denaturation and results in grids suitable for high-resolution imaging and reconstruction. My only concern is uncertainty that more data will ultimately prove to be helpful. The authors have already, according to Figure 1, collected nearly 2 million particles from Chameleon-prepared grids and reached what may be a plateau in resolution.

Project ID: NCCAT-GUP2-WT190629

Project name: Grid preparation for the substrate-bound human presequence protease by chameleon

Primary username: Wei Jen Tang

eRA Commons username: WEI-JEN

Institution: The Chicago University

Submission date: 6/29/19

Abstract:

Mitochondrial targeting presequences are required for the import of many nucleus-encoded proteins that are essential to mitochondria function. 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 M16 metalloprotease that degrades presequence peptides into non-toxic fragments. 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 insights into PreP function and leverage these insights to elucidate the molecular basis of how PreP is involved in maintaining mitochondrial proteostasis. Human PreP is a 117 kDa, monomeric protein ubiquitously expressed in all tissues which localizes to the mitochondrial matrix. We have solved the crystal structure of a human PreP closed state to elucidate how PreP forms an enclosed catalytic chamber to entrap and degrade amyloid beta (King et al Structure 22:996-10017, 2014). These structures also reveal that PreP likely needs to undergo a large scale open-closed transition to degrade presequences or amyloid beta. However, the structure of the catalytic chamber in the PreP closed state precludes the capture of its substrate or the release of its reaction products, key steps in the PreP catalytic cycle. Furthermore, the molecular mechanism which allows PreP to recognize presequence peptides and amyloid beta remains elusive. To address these questions, we have employed single particle cryo-electron microscopy to elucidate the structure of open state PreP and explore conformational dynamics to provide valuable insights into the molecular basis of how PreP recognizes the targeted peptides and offer insights for developing PreP-based therapies. PreP has N- and C-terminal domains (PreP-N and PreP-C) that form a sizable catalytic chamber to entrap, unfold, and degrade amyloid peptides and mitochondrial targeting sequences. Previous data collection at NCCAT revealed that $\sim 2/3$ of PreP particles had a denatured PreP-C when the vitrobot-prepared grids were used, but the preparation of grids via chameleon completely eliminated the denaturation issue (Figure 1). From this study, we have obtained open state (4.6 angstrom) and partial open state (4.2 angstrom) PreP structures (Figure 2). The partial open PreP structure can be sub-classified into three distinct PreP partial closed states (4.5, 6.2, and 6.4 angstrom), revealing conformational dynamics within the catalytic region which potentially explain the molecular basis of how PreP recognizes amyloid beta and presequences (Figure 2). Further map improvement and structural analysis is ongoing. This proposal is to request the preparation of substrate-bound PreP using chameleon. The substrate-bound structure will provide the critical insight into the substrate recognition of PreP, which will pave the way to design the small molecule modulators to enhance PreP activity to regulate the mitochondrial proteostasis.

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 the 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 substrate-bound PreP structures should provide insights into how PreP and its homologs work.
2. Technical challenges in solving PreP cryoEM structures: PreP is a small, 117 kDa, monomeric enzyme with

homologous 55 kDa N- and C-domains that exhibit quasi symmetry. Furthermore, PreP requires multiple conformations for its catalytic cycle. The relatively small size, pseudo-symmetry, and conformational heterogeneity offer the opportunity to test the efficacy of state-of-art sample preparation technology and apply and refine the use of cryoEM data acquisition and analysis methodologies to obtain the near atomic resolution structures of such proteins.

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 efforts to solve the open state structure of PreP by crystallography. From our recent data collected at NCCAT, we have obtained a 4.6 angstrom Coulomb potential density map and open state model and three distinct partial closed state map (4.5 - 6.4 angstrom) that are distinct from the previous solved human PreP closed state structure (Figure 2). PreP is a zinc metalloprotease. By treating with the chelating agent, EDTA, PreP can be rendered catalytically inactive. PreP will be mixed with amyloid beta or the presequence of citrate synthase in a 1:5 - 1:10 molar ratio to ensure the high occupancy. Such structures will provide insight into the molecular basis for the substrate binding and selectivity of PreP.

Technical Feasibility:

Our goal is to obtain the near atomic resolution structures of PreP in complex with two substrates, amyloid beta and the presequence of citrate synthase. We have performed 2D and 3D classification on cryoEM datasets of PreP alone collected using a NCCAT 300 kV Titan Krios microscope equipped with a K2 camera and chameleon prepared grids. we have obtained a PreP open state (4.6 angstrom) and 4.2 angstrom partial open state PreP structure (Figure 2). The partial open PreP structure is sub-classified into three distinct PreP partial closed states (4.5, 6.2, and 6.4 angstrom). We envision that the substrate-bound PreP will behave similarly to PreP alone particles, if not better, since the presence of substrate could further stabilize PreP structure.

Resources Requested:

We request the preparation of 4-8 grids for both amyloid-beta bound and citrate synthase presequence bound PreP as well as confirmation of the grid quality by screening microscopy.

Geographic/Demographics:

Currently, Chicagoland does not have 300 kV EM machine for single particle cryoEM analysis. The University of Chicago has purchased a titan Krios but it is not yet installed. We also do not have the access to spotiton or chameleon locally. The University of Chicago has placed an order for a 300 kV Titan Krios equipped with Gatan K2 camera. We have traveled to NCCAT for our first two-day data collection on October 4-6, 2018. In addition to their assistance in our successful data collection, the staff at NCCAT was extremely helpful in teaching about facility setup and data collection. We have transmitted what we learned to help in setting up and commissioning the 300 kV Krios system that is scheduled to be installed at The University of Chicago. We will continue to learn the operational aspects of NCCAT and NRAMM and attempt to implement what we learn to improve the efficiency of our facility.

When grids are prepared via Vitrobot, only 1/3 of the PreP particles are intact and the rest is only half of the size of PreP (Figure 1A). 3D classification revealed that the half particles are derived from particles with an intact PreP N domain and a denatured PreP C domain (Figure 1C). However, 2D classification from the particles collected using grid prepared by chameleon completely eliminated the half particles (Figure 1B,D). Currently, the precise reasoning for why the differences in grid preparation by vitrobot vs chameleon affect the denaturation of PreP-C domain remains elusive and we will work with Bridget Carragher and Clint Potter to use cryoET to investigate the localization of denatured PreP particles to further study the mechanism of denaturation.

The 3D classification from this data reveals maps for PreP open and partial open states that have the 4.6 and 4.2 angstrom resolution, respectively (Figure 1D, 2A). These two states are distinct from each other and different from the previously reported closed structure of PreP solved by X-ray crystallography (Figure 2B). We have performed 3D classification of both states. While no new meaningful states can be obtained from PreP open state, the 3D classification of PreP partial open state reveal three distinct states that vary mostly at the catalytic and linker region between PreP-N and PreP-C. Such motions within the catalytic domain of PreP can be interpreted as the key conformational switch necessary for PreP to unfold and select unstable peptides in mitochondria, such as presequences. In this proposal, we will address substrate bound state PreP structure to address the structural basis for the interaction of PreP with its substrates.

Figure 1 Comparison of 2D and 3D classification of PreP particles from grids prepared by vitrobot (A,C) or chameleon (B,D).

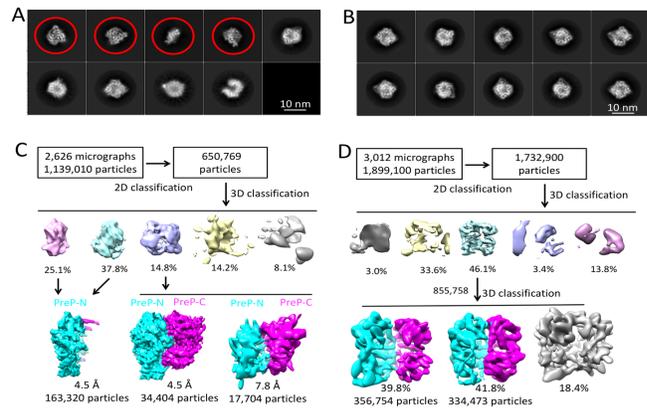
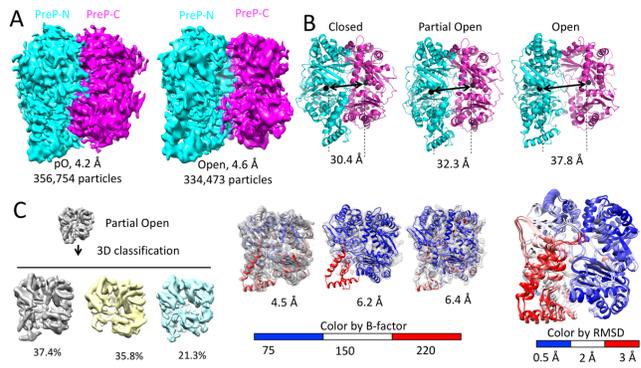


Figure 2 The analysis of PreP open and partial open state structures.



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 $\beta\gamma$ 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 $\beta\gamma$ subunits. The dogma at the time is that α subunit of G protein, but not $\beta\gamma$ 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 $\beta\gamma$ can effectively suppress the activity of type 1 adenylyl cyclase. I also subsequently showed that G protein $\beta\gamma$ 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 $\beta\gamma$ 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 $\beta\gamma$ 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_{sa} 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_{sa} 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. A β 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 Zn²⁺-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), A β , and other bioactive peptides <80 aa. The involvement of IDE in the clearance of insulin and A β 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 A β *in vitro* and may degrade A β imported into mitochondria to prevent A β 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) Ensemble cryoEM elucidates the mechanism of insulin capture and degradation by human insulin degrading enzyme. *ELife* 7:e33572 (*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-1 α), CCL4 (a.k.a. MIP-1 β), 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. Kastrop, 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

R01 GM 121964
NIH NIGMS

Tang (PI)

09/01/2017-08/31/2021

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
NIH NIGMS

Tang (PI)

10/01/2011-09/30/2016

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.

Grant-in-Aid 17GRNT33400028
American Heart Association

Tang (PI)

01/01/2017-12/31/2018

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.