BIOGRAPHICAL SKETCH

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NAME: Wei-Jen Tang

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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.	05/1982	Zoology
University of Texas, Austin	Ph.D.	05/1988	Biological Science
University of Texas, Austin	Postdoctoral fellow	08/1988	Microbiology
University of Texas Southwestern Medical School	Postdoctoral fellow	06/1991	Pharmacology

A. Personal Statement

My research program involves in elucidating the molecular basis of cellular signal transduction. 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. I apply X-ray crystallography, 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 protease family that degrade amyloid peptides, such as human insulin degrading enzyme (IDE), human presequence protease, and *Mycobacterium tuberculosis* Zmp1. 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. In this proposal, I will work with a team of scientists to address the structural basis for conformational dynamics and substrate recognition of amyloid peptide-degrading enzymes including human presequence protease (PreP), human neprilysin (NEP), *Mycobacterium tuberculosis* (*Mtb*) zinc metalloprotease 1 (ZMP1), and human angiotensin-converting enzyme (ACE). The collaborators include Tony Kossiakoff, Minglei Zhao, Tobin Sosnick, and Aaron Dinner who are in the same institute (U. of Chicago), Bridget Carragher and Clint Potter at NRAMM/NCCAT, Sheng Li at UCSD, and Carla Koehler at UCLA. The track record of our collaboration is evident from our past publications and in the proposals.

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. 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).
d. 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 7:e33572 (*co-corresponding authors).

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-present	The advisory Board, Structure Biology Center, APS, Argonne National Lab
2012-2014	Regular member of American Heart Association Signaling 4 study section
2014-2015	Society of Chinese Bioscientists in American Nomination committee
2016	Review panel member for APS XSD chemical and materials science review
2016-present	NIH New Innovator Award
2018-2020	American Heart Association Fellowship Basic Cell - proteins and crystallography review

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 United States of America. 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. 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 7:e33572 (*co-corresponding authors).
e. Liang, W. G., Wijiya, J., Wei, H., Noble, A., Mo, S., Lee, D., Mancl, J. M., King, J. L., Pan, M., Liu, C., Koehler, C., Zhao, M., Potter, C. S., Carragher, B., Li, S., and **Tang, W. J.** (2022) Structural basis for the mechanisms of human presequence protease conformational switch and substrate recognition. *Nature Communications* 13, 1833.

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:*** I have the broad interest how bacterial virulent factors work. In addition to study edema factor secreted by anthrax bacteria, I have also performed biochemical and structural analyses of two other bacterial nucleotidyl cyclase toxins. We have structurally characterized CyaA, the calmodulin-activated adenylyl cyclase toxin secreted by *Bordetella pertussis*, bacterium that cause whooping cough. Our studies led to the surprising finding that the mode of calmodulin binding by pertussis adenylyl cyclase toxin is completely different from that of edema factor, highlighting that

the diverse means that calmodulin effectors can evolve to bind and be regulated by calmodulin. We have also characterized ExoY, a toxin secreted by *Pseudomonas aeruginosa*, bacterium that causes nosocomial infections. We have shown that, in addition to be activated by F-actin, ExoY also effectively bundles F-actin. We also have determined the molecular basis for the bundling activity of ExoY. I have also 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. We have also use cryoEM to determine the open state structures of zinc metalloprotease 1 secreted by bacterium that causes tuberculosis, *Mycobacterium tuberculosis* to provide the structural basis of open-closed conformational change of M13 metalloproteases.

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. Mancl, J.M., Suarez, C., Liang, W.G., Kovar, D.R., **Tang, W.-J.** (2020) *Pseudomonas aeruginosa* exoenzyme Y directly bundles actin filaments. J Biol Chem 295:3506-3517.
d. Liang, W.G., Mancl, J.M., Zhao, M., **Tang, W.-J.** (2021) Structural analysis of *Mycobacterium tuberculosis* M13 metalloprotease Zmp1 open states. Structure 29(7):709-720.

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. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 GM 121964 Tang (PI) 09/01/2021-08/31/2025
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 the substrate recognition and destruction of amyloid peptides as well as to develop the means to enhance the activity of human insulin degrading enzyme.

Completed Research Support (within past three years)

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.