Tsinghua-*Science* Workshops

Computational Structural Biology

Session 8: Computational Structural Biology – Protein Design

Friday, July 16th, 2021 8-10 pm (GMT +08:00, Beijing)

20:00-20:45

Peilong Lu, Westlake University, China

Computational design of transmembrane proteins

20:45-21:00 Q&A

21:00-21:45

Dek Woolfson, University of Bristol, UK

Protein Design: Learning from Nature to Build Completely New Protein Structures and Functions

21:45-22:00 Q&A

Host

Prof. Xueming Li

Xueming Li received his Ph.D in condensed matter physics from Institute of Physics at the Chinese Academy of Sciences in 2009. Then, he joined the laboratory of Yifan Cheng at University of California San Francisco as a postdoctoral scholar, and changed his research direction to electron cryo-microscopy (cryoEM) in structural biology. Xueming Li joined Tsinghua University since 2014. He is currently associate professor of the School of Life Science, and principal investigator of Beijing Advanced Innovation Center for Structural Biology and Tsinghua-Peking Joint Center for Life Sciences.

Xueming Li has been engaged in the research of electron cryo-microscopy (cryoEM) methods and techniques, as well as the applications of cryoEM in structural biology, for more than ten years. He had made remarkable contributions in the development of electron counting detector and beam-

induced motion correction algorithm, which promoted the "Resolution Revolution" of cryoEM. In recent years, Xueming Li aimed to develop high-efficient and high-resolution cryoEM technologies, and introduced new algorithms from other fields into cryoEM, such as deep learning in artificial intelligence and particle filter in electronic engineering. Xueming Li also worked on microcrystalline electron diffraction (MicroED) and greatly enhanced the applicability of this technology. His efforts provide new ideas for the development of image processing technology in cryoEM.

Currently Xueming Li's researches are focusing on the methodological and technological development of electron cryo-tomography (cryoET) with a goal to obtaining in situ structures of cellular organelle and biological macromolecules at 1 nm or even atomic resolution, and exploring the possibility of medical application of cryoET technology.

Speakers

Dr. Peilong Lu

Dr. Lu received his bachelor's degree in Biological Science in 2009, from University of Science and Technology of China (USTC). He got his doctoral degree at Tsinghua University in 2014, trained in Prof. Yigong Shi's lab. In 2015, he joined Prof. David Baker's lab at University of Washington for postdoctoral training. In 2019, he joined School of Life Sciences at Westlake University as a principal investigator. His lab mainly focuses on computationally design of new generations of functional multi-pass transmembrane proteins, and design of protein therapeutics.

Computational design of transmembrane proteins

The computational design of transmembrane proteins with more than one membrane-spanning region remained a major challenge. Here, we present the design of transmembrane homodimers and tetramers that adopt the target oligomerization state in detergent solution. Crystal structures of the designed dimer and tetramer—a rocket-shaped structure with a wide cytoplasmic base that funnels into eight transmembrane helices—are very close to the design models. More interestingly, we have

successfully designed two hexameric and octameric transmembrane protein pores formed by two concentric rings of α-helices. Patch clamp electrophysiology experiments show that a hexameric 12-helix transmembrane pore expressed in insect cells allows passage of ions across the membrane with selectivity for potassium over sodium. An octameric 16-helix transmembrane pore, but not the hexameric pore, allows passage of biotinylated Alexa Fluor 488 when incorporated into liposomes using in vitro protein synthesis. A cryo-EM structure of the octameric transmembrane pore fused to helical repeat domains closely matches the design model. The ability to produce structurally well-defined transmembrane channels opens the door to the creation of designer pores and other functional transmembrane proteins for a wide variety of applications.

Prof. Dek Woolfson

Dek Woolfson is Professor of Chemistry and Biochemistry; Principal Investigator of BrisSynBio, a UKRI-funded Synthetic Biology Research Centre; Director of the Bristol BioDesign Institute at the University of Bristol; founding member of the Max Planck-Bristol Centre for Minimal Biology; and Founder of Rosa Biotech.

Dek took his first degree in Chemistry at the University of Oxford, UK in 1987. In 1991, he gained a PhD in Chemistry and Biochemistry at the University of Cambridge. He then did post-doctoral research at University College London (1991 – 92) and the University of California, Berkeley (1992 – 94). He returned to the UK to take up a Lectureship in Biochemistry at the University of Bristol (1994 – 95). From 1996 – 2005 he was Lecturer through to Professor of Biochemistry at the University of Sussex. He moved back Bristol in 2005 to a joint chair in Chemistry and Biochemistry.

Dek's research has always been at the interface between chemistry and biology, applying chemical methods and principles to understand biological phenomena such as protein folding and stability. He has a long-standing interest in the challenge of rational protein design, and how this can be applied in synthetic biology and biotechnology. His particular emphasis is on making completely new protein

structures not known to natural biology using a combination of rational and computational design. The current focuses of his group are in the parametric design of protein structures, assemblies and materials, and porting these into living cells to intervene in and to augment natural biological functions.

In 2011, Dek became the first recipient of the Medimmune Protein and Peptide Science Award of the Royal Society of Chemistry; in 2014, he received a Royal Society Wolfson Research Merit Award, and he gained an ERC Advanced Grant; in 2016 he won the Interdisciplinary Prize of the Royal Society of Chemistry; and in 2020 he received a Humboldt Research Award (also known as the Humboldt Prize).

Protein Design: Learning from Nature to Build Completely New Protein Structures and Functions

Protein design—*i.e.*, the construction of entirely new protein sequences that fold into prescribed structures—has come of age: it is now possible to generate a wide variety stable protein folds from scratch using rational and/or computational approaches. A new challenge for the field is to move past protein structures offered up by nature and to target the so-called 'dark matter of protein space'; that is, protein structures that should be possible in terms of chemistry and physics, but which biology seems to have overlooked or not used prolifically. This talk will illustrate what is currently possible in this nascent field using *de novo* α-helical coiled-coil peptides as building blocks.[1]

Coiled coils are bundles of 2 or more α helices that wrap around each other to form rope-like structures. They are one of the dominant structures that direct natural protein-protein interactions. Our understanding of coiled coils provides a strong basis for building new proteins from first principles. The first part of my talk will survey this understanding,[1] our design methods,[2,3] and our current "toolkit" of *de novo* coiled coils.[4-5]

Next, I will describe how the toolkit can be expanded used to generate some dark-matter protein structures. I'll focus on the rational and computational design of α-helical barrel proteins, which have 5 or more helices surrounding accessible central channels.[6] Finally, I'll discuss how these synthetic barrel proteins can be put to use to make new nanotube materials,[7] rudimentary catalysts,[8] membrane-spanning pores,[9] components of a new types of sensing devices,[10] and proteins that switch conformational state.[11]

1. Coiled-coil design: updated and upgraded.
DN Woolfson. ***Subcellular Biochemistry 82***, 35-61 (2017)
2. CCBuilder: an interactive web-based tool for building, designing and assessing coiled-coil-protein assemblies.
CW Wood *et al.* ***Bioinformatics 30***, 3029-3035 (2014)
3. ISAMBARD: an open-source computational environment for biomolecular analysis, modelling and design
CW Wood *et al.* ***Bioinformatics 33***, 3043–3050 (2017)

4. A basis set of *de novo* coiled-coil peptide oligomers for rational protein design and synthetic biology

JM Fletcher *et al.* ***ACS Synth Biol 1***, 240-250 (2012).

5 A set of *de novo* designed parallel heterodimeric coiled coils with quantified dissociation constants in the micromolar to sub-nanomolar regime

F Thomas *et al.* ***J Am Chem Soc 135***, 5161-5166 (2013).

6. Computational design of water-soluble α–helical barrels.

AR Thomson *et al.*, ***Science 346***, 485-488 (2014)

7. Modular design of self-assembling peptide-based nanotubes.

NC Burgess *et al.*, ***J Am Chem Soc 137,*** 10554-10562 (2015)

8. Installing hydrolytic activity into a completely *de novo* protein framework.

AJ Burton *et al.*, ***Nature Chemistry 8***, 837-844 (2016)

9. A monodisperse transmembrane α-helical peptide barrel.

AJ Scott *et al.*, ***Nature Chemistry 13,*** 643-50 (2021)

10. De novo-designed alpha-helical barrels as receptors for small molecules

F Thomas *et al.*, ***ACS Synth Biol 7***, 1808-16 (2018).

11. Structural resolution of switchable states of a de novo peptide assembly.

WM Dawson WM *et al.* ***Nature Communications 12***, ARTN:1530 (2021).