Introduction

Structural biology: Gaining atomic level insight into the biological function of macromolecules

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From the first X-ray structures of myoglobin and hemoglobin that began to uncover how secondary structural elements organize into functional three-dimensional folds, to the first structure of the classical DNA double-helix, to the more recent determination of complex molecular machines, such as the ribosome and RNA polymerase, the field of structural biology has fundamentally altered how we perceive biological macromolecules. Visualization of proteins at the atomic level has allowed researchers to gain a detailed fundamental understanding of the molecular interactions and enzymatic processes essential for life, and is the foundation for hypothesis-driven structure–function research. Beyond its role in understanding biological processes, structural biology has dramatically impacted the development of new therapeutic modalities to treat human disease, including small molecule drug design and the identification of new biotherapeutics.

Since its modest beginnings by today's standards, significant advances have been made in the field of X-ray crystallography, including production of recombinant proteins, crystallization of macromolecules, innovations in synchrotron radiation, cryo-cooling crystals, detector technology, phasing methodology, and structure refinement. These advances have greatly reduced the barriers for solving macromolecular structures, resulting in a structural biology revolution.[1] This was particularly evident where shortly after the human genome was sequenced, the National Institutes of Health founded the Protein Structure Initiative in 2000, aimed at generating structural information from the newly acquired genetic information.[2] This included identifying the majority of the unique three-dimensional tertiary folds that proteins adopt. These efforts resulted in approximately 7000 depositions in the Protein Data Bank (PDB).[3] Currently, the PDB houses more than 48,000 distinct protein sequences, over 44,000 structures of human proteins, and over 11,000 structures that contain a nucleic acid component.[4] Despite these advances, we are still just scratching the surface with our structural understanding of multiprotein complexes. As such, there is a growing need to gain structural information for larger macromolecular machines that underlie important biological processes, such as transcription, DNA replication and repair, protein trafficking, and signal transduction, many of which are difficult to isolate, crystallize, and determine their structures using conventual methods in X-ray crystallography. Certainly, recent advances in cryo-electron microscopy (cryo-EM) have augmented these efforts and are now producing structures with resolutions that can accurately place the sidechains of amino acids into electron density maps.[5] While still financially limiting, cryo-EM is rapidly being implemented at an exciting pace to solve structures of macromolecular complexes, including transmembrane receptors and large multiprotein complexes, as well as the structure of viral particles.[6]

Given the importance of structural biology to our understanding of biological processes and its close ties to therapeutic discovery, as well as its interdisciplinary nature, the Society's flagship journal, *Experimental Biology and Medicine*, has expanded its focus to include structural biology as a category of interest. The journal is now considering manuscripts that are focused on the structural aspects of macromolecules or have a structural component. To highlight the journal's new focus area, we have assembled a Thematic Review on Structural Biology that spans a broad range of topics from leading experts in their respective areas of research. The first two mini-reviews are from the Blacklow and Kovall laboratories where both have made seminal contributions towards understanding the roles and regulation of Notch signaling at the structural level.[7,8] Seegar and Blacklow highlight recent structural advances in understanding the similarities and differences of ADAM (a disintegrin and metalloproteinase) proteins, which contain both catalytically active proteases and non-catalytic members, which play a critical role in Notch receptor activation.[7] Hall and Kovall survey the latest structure–function studies of transcription complexes that regulate Notch signaling, focusing on our understanding of coregulator complexes with CSL and how these affect transcriptional repression and activation.[8] Two additional mini-reviews from the Thompson and Hinck laboratories highlight the

TGFβ signaling pathway.[9,10] Goebel *et al.* provide a summary of our current structural understanding of TGFβ ligand–receptor interactions, including a recent structure of the ternary receptor complex from an activin class ligand.[10] In addition, the review highlights recent advances in our understanding of mechanisms whereby ligands are regulated through interactions with its own prodomain and extracellular antagonists. Sun *et al.* focus their review on recent insights into the co-receptors betaglycan and endoglin, describing how the co-receptors have a similar domain architecture, but functionally they appear to have distinct functional mechanisms for augmenting TGFβ ligand signaling.

Additional mini-reviews were provided by the Horn and Luca laboratories, which focused on the use of protein engineering strategies to augment structural studies.[11,12] Certainly, a major bottleneck in obtaining structural information is a soluble, well-behaved protein sample that does not aggregate. Over the last 20 years, accessory-binding molecules have played an instrumental role in resolving intractable structural projects. At the forefront has been the implementation of nanobodies, or single-chain antibodies, that bind and stabilize the macromolecule of interest. Hoey *et al.* summarize recent advances in nanobody development and their utilization not only in structural studies but also in their broader use as therapeutics and diagnostics.[11] Ming *et al.* discuss the broader application of protein engineering and strategies for capturing low-affinity macromolecular complexes.[12] While these methods are certainly more involved and time-consuming, they can be essential for trapping and visualizing low affinity protein–protein interactions.

The last three mini-reviews cover a broad range of topics. West and Deng discuss the structural advances in understanding the immune regulator receptors of the B7 and CD28 families.[13] The authors describe the structural diversity of the receptors, highlighting the complexities of targeting them during immunotherapy. Following this review, Matlahov and van der Wel discuss the structural basis of Huntington Disease and the Huntington protein.[14] This review discusses the challenges associated with understanding the pathogenic versions of this protein, which can form misfolded aggregates. Certainly, understanding the structural basis for a disease that is associated with pathogenic aggregation is complex, requiring multiple complimentary techniques, including X-ray crystallography, cryo-EM, nuclear magnetic resonance spectroscopy (NMR), and other biophysical approaches. The authors highlight the recent advances and implementation of solid-state NMR to study the large polyglutamine aggregates which are intractable by solution-based NMR methods. Finally, the review by Shurina and Page discusses how structural biology can help with rational small molecule drug design.[15] In this review, the authors discuss how up to 20 structures have been determined of different small molecules binding to carbapenemase-2, an enzyme that can inactivate β-lactam antibiotics. Collectively, these structures provide a foundation for future development of small molecule inhibitors that target β-lactamases, allowing the β-lactam drugs to remain effective.

In summary, this Thematic Review provides a small snapshot of the various disciplines that structural biology extends into and enlightens, as well as to encourage future submissions to *Experimental Biology and Medicine* that incorporate structural biology approaches and experimental results into their articles.

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REFERENCES

1. Dauter Z. Current state and prospects of macromolecular crystallography. *Acta Crystallogr D Biol Crystallogr* 2006;**62**:1–11
2. Terwilliger TC, Waldo G, Peat TS, Newman JM, Chu K, Berendzen J. Class-directed structure determination: foundation for a protein structure initiative. *Protein Sci* 1998;**7**:1851–6
3. Montelione GT. The protein structure initiative: achievements and visions for the future. *F1000 Biol Rep* 2012;**4**:2005–10
4. Burley SK, Berman HM, Bhikadiya C, Bi C, Chen L, Di Costanzo L, Christie C, Dalenberg K, Duarte JM, Dutta S, Feng Z, Ghosh S, Goodsell DS, Green RK, Guranović V, Guzenko D, Hudson BP, Kalro T, Liang Y, Lowe R, Namkoong H, Peisach E, Periskova I, Prlić A, Randle C, Rose A, Rose P, Sala R, Sekharan M, Shao C, Tan L, Tao YP, Valasatava Y, Voigt M, Westbrook J, Woo J, Yang H, Young J, Zhuravleva M, Zardecki C. RCSB protein data bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. *Nucleic Acids Res* 2019;**47**:D464–74
5. Herzik MA, Wu M, Lander GC. High-resolution structure determination of sub-100 kDa complexes using conventional cryo-EM. *Nat Commun* 2019;**10**:1–9
6. Yifan C. Single particle cryo-EM – how did it get here and where will it go. *Science* 2018;**361**:876–80
7. Seegar TC, Blacklow SC. Domain integration of ADAM family proteins: emerging themes from structural studies. *Exp Biol Med* 2019; DOI:10.1177/1535370219865901
8. Hall DP, Kovall RA. Minireview structurally conserved binding motifs of transcriptional regulators to notch nuclear effector CSL. *Exp Biol Med* 2019;DOI:10.1177/1535370219877818
9. Kim SK, Henen MA, Hinck AP. Minireview structural biology of betaglycan and endoglin, membrane-bound co-receptors of the TGF-beta family. *Exp Biol Med* 2019;DOI:10.1177/1535370219881160
10. Goebel EJ, Hart KN, Mccoy JC, Thompson TB. Minireview structural biology of the TGF b family. *Exp Biol Med* 2019;DOI:10.1177/1535370219880894

11. Hoey RJ, Eom H, Horn JR. Minireview structure and development of single domain antibodies as modules for therapeutics and diagnostics. *Exp Biol Med* 2019;DOI:10.1177/1535370219881129
12. Ming Q, Gonzalez-Perez D, Luca VC. Molecular engineering strategies for visualizing low-affinity protein complexes. *Exp Biol Med* 2019; DOI:10.1177/1535370219855401
13. West SM, Deng XA. Considering B7-CD28 as a family through sequence and structure. *Exp Biol Med* 2019; DOI:10.1177/1535370219855970
14. Matlahov I, van der Wel PCA. Conformational studies of pathogenic expanded polyglutamine protein deposits from Huntington's disease. *Exp Biol Med* 2019;DOI:10.1177/1535370219856620
15. Shurina BA, Page RC. Influence of substrates and inhibitors on the structure of Klebsiella pneumoniae carbapenemase-2. *Exp Biol Med* 2019;DOI:10.1177/1535370219854322