Published in final edited form as:

Methods Mol Biol. 2015 ; 1329: 279–280. doi:10.1007/978-1-4939-2871-2_22.

Summary and Future Directions

Nicholas Noinaj¹ and Susan K. Buchanan^{2,*}

¹Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, Indiana, 47907

²National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, 20892

Summary

β -barrel outer membrane proteins (OMPs) are found in the outer membranes (OMs) of all Gram-negative bacteria, yet exactly how they are folded and inserted remains unknown. The last decade has provided a wealth of discovery including the identification of the BAM complex, a multi-component complex responsible for the biogenesis of all OMPs into the OM. It is anticipated that the next decade will further advance our knowledge of how the BAM complex is able to perform its unique and interesting function.

Keywords

BamA; OMP; Outer membrane protein; β -barrel membrane protein; BAM complex; Protein folding; Lateral opening

The BAM complex serves an essential role in Gram-negative bacteria by orchestrating the biogenesis of all β -barrel membrane proteins found within the outer membrane. The last decade has witnessed not only the discovery of the BAM complex itself, but an experimental tour de force in dissecting the individual components and how those components come together to function as a molecular machine. Functional assays, both *in vitro* and *in vivo*, along with clever genetics experiments have given clues to how each Bam component interacts with the others, the role each may play within the full complex, and how each may be regulated with the cell. The structures of all the Bam components have also contributed significantly and together have provided a molecular blueprint to serve as a guide in steering all on-going and future experiments aimed at understanding exactly how the BAM complex folds and inserts new β -barrel membrane proteins into the outer membrane.

Significant work has been done, yet significant work remains to be done in order to fully understand the mechanism for how the BAM complex functions. The last decade saw a flurry of exciting findings that have provided the foundation for what we anticipate will be an even more enlightening next decade of discovery. At the forefront is the importance of determining structures of the fully assembled complex, as well as, structures of subcomplexes, both in apo form and with substrate. The power of structural biology in

*Corresponding author: ; Email: skbuchan@helix.nih.gov; Tel. 1-301-594-9222

providing molecular insight and guiding functional experiments remains unparalleled and therefore knowing the structure of the fully assembled complex would be invaluable in unraveling how it works. Further, an improved *in vitro* functional assay that can yield information about how the BAM complex sorts and handles the various sizes and properties of new β -barrel membrane proteins would be necessary to verify and validate all current and future mechanistic models. And lastly, the therapeutic potential of the BAM complex and its components both as vaccine targets and as targets for antibiotic development, particularly against multidrug resistant Gram-negative bacteria, should be explored in more depth towards efforts to discover new drugs that are more effective and sustainable. Multi-drug resistant bacteria are an immediate threat to the world that requires an immediate response from the research community.

This volume presents twenty chapters of experimental protocols based on methods that have been used to study the BAM complex. It was these methods that provided us with the wealth of knowledge we now know about this system and while new technology will bring new methods that will further assist in studying the BAM complex, it will be these methods and protocols presented here that will continue to be used in making novel discoveries about this fascinating system.