SPECIFIC AIMS

This project proposes continued development of advanced interactive visualization and analysis software for experimental data, including and emphasizing data from state-of-the-art (<3Å resolution) cryo-electron microscopy (cryo-EM), with the ultimate goal of understanding how cells and their molecular machinery function. The enormous growth in recent years in both the size and complexity of biological data sets, and especially structural data at various scales in length and time has created new and significant challenges for biomedical researchers. New and innovative software tools are vital to the successful outcomes of the myriad NIH-funded experimental research projects. There is no indication that the growth in data size and complexity will abate, and thus approaches to integrating, interpreting, and otherwise making the best use of the data require continuing, focused attention. We propose to address this challenge via the following specific aims:

Aim 1: Continue the design, implementation, documentation and dissemination of ChimeraX for the interactive visualization and analysis of atomic and cryo-electron microscopy data sets. In our vision for a multi-scale modeling environment, ChimeraX is the molecular visualization and analysis component that provides integrated interactive tools for analyzing a variety of data types that vary in scale from atomic structures to cryo-EM density maps to large molecular complexes such as the nuclear pore.

- Aim 1A: Further strengthen ChimeraX's ability to efficiently read, display and manipulate very large homogeneous and heterogeneous data sets.
- Aim 1B: Integrate two-dimensional diagrams with the three-dimensional graphics for linking non-spatial data with molecular visualization.
- Aim 1C: Implement novel analysis and modeling functionality and user interfaces, integrate existing web resources, and migrate selected Chimera tools to ChimeraX.
- Aim 1D: Create an export mechanism to facilitate disseminating results and hypotheses generated in ChimeraX via a WebGL-based viewer.

Aim 2: **Within the ChimeraX framework, develop tools for atomic-resolution modeling from cryo-electron microscopy data sets.** Develop software for visualization, modeling, validation and analysis of molecular assemblies imaged with state-of-the-art cryo-EM, especially at atomic resolutions (2-4Å).

- Aim 2A: Develop analyses of map quality for model building. Calculate and show spatial variations in resolution, find domain motions, identify and quantify approximate symmetries, analyze difference maps, perform segmentation.
- Aim 2B: Create interactive model building and measurement tools. Locate template structures from within the PDB, calculate domain and secondary structure motions for optimal fits, utilize *de novo* backbone tracing algorithms. Compare related models with real-time animation of conformational changes. Measure motions, distances, variation in contact areas, and create schematic 2D and 3D illustrations of measured quantities.
- Aim 2C: Devise new model validation tools, including atomic model validation, comparing models to maps, detecting atomic clashes and unfavorable geometries, manually correcting problems, and launching automated refinement methods. Highlight conformational and sequence differences in models derived from different experimental conditions or mutants.

Aim 3: Design, implement and document software interfaces to facilitate community development of new algorithms and methods by building upon the ChimeraX platform. Community development has proven to be a very successful paradigm for our older Chimera application. The goal of this aim is to ensure that ChimeraX will also provide a highly attractive visualization and analysis infrastructure for the development of novel algorithms and tools by others.

- Aim 3A: Create and document semantically versioned application programming interfaces (API) for ChimeraX libraries.
- Aim 3B: Design and implement a mechanism for interactive installation of new tools and updates to existing tools in ChimeraX from an RBVI-provided repository accessible via the Web.
- Aim 3C: Create a Web interface for external developers to deposit and distribute their tools using the RBVI repository.