# Chimera Extensions for Modeling, Evaluation and In-silico Mutagenesis of 3D Protein Structures

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## Background

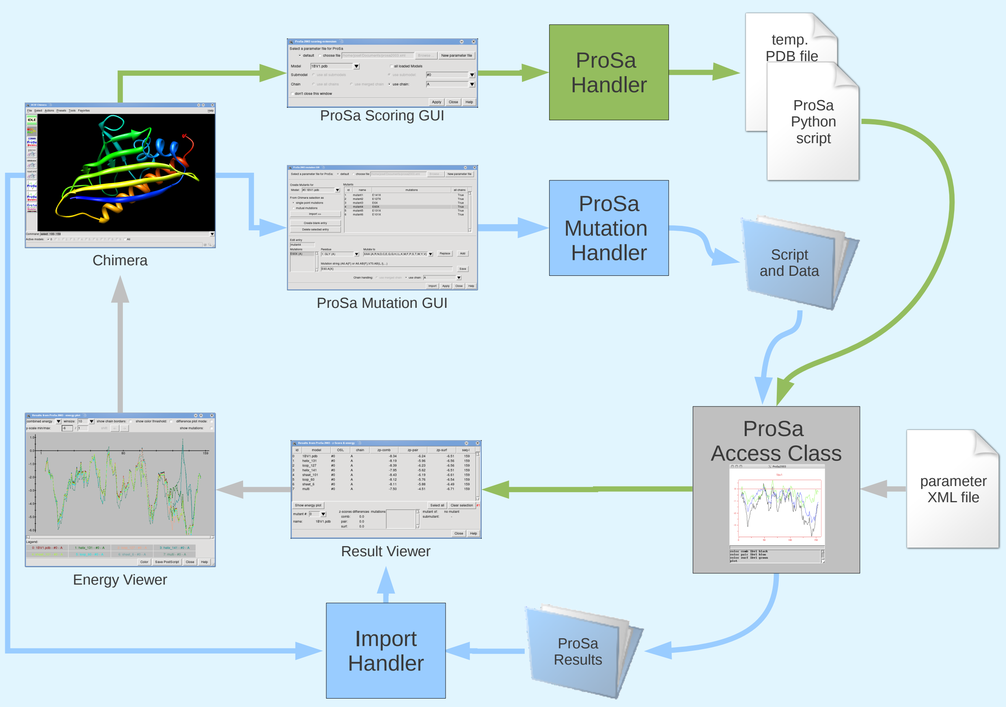
Knowledge of the 3D structure of a protein is essential for the understanding of its biological function. Moreover, it supports the focused planning of wet lab experiments for many purposes. Experimental structure determination is time consuming and associated with practical limitations. Therefore computational methods have been devised for the prediction of a 3D structure from its sequence, the prediction structural changes upon mutations and the evaluation of structural models.

The corresponding computational tools are commonly very powerful but often difficult to use for unexperienced users and cumbersome to combine in an efficient manner. We developed an extension bundle for the visualization software UCSF Chimera[1], which integrates several state of the art methods into a single workspace, which allows the user an easy access to the methods without any particular knowledge in their operation.

## Implementation

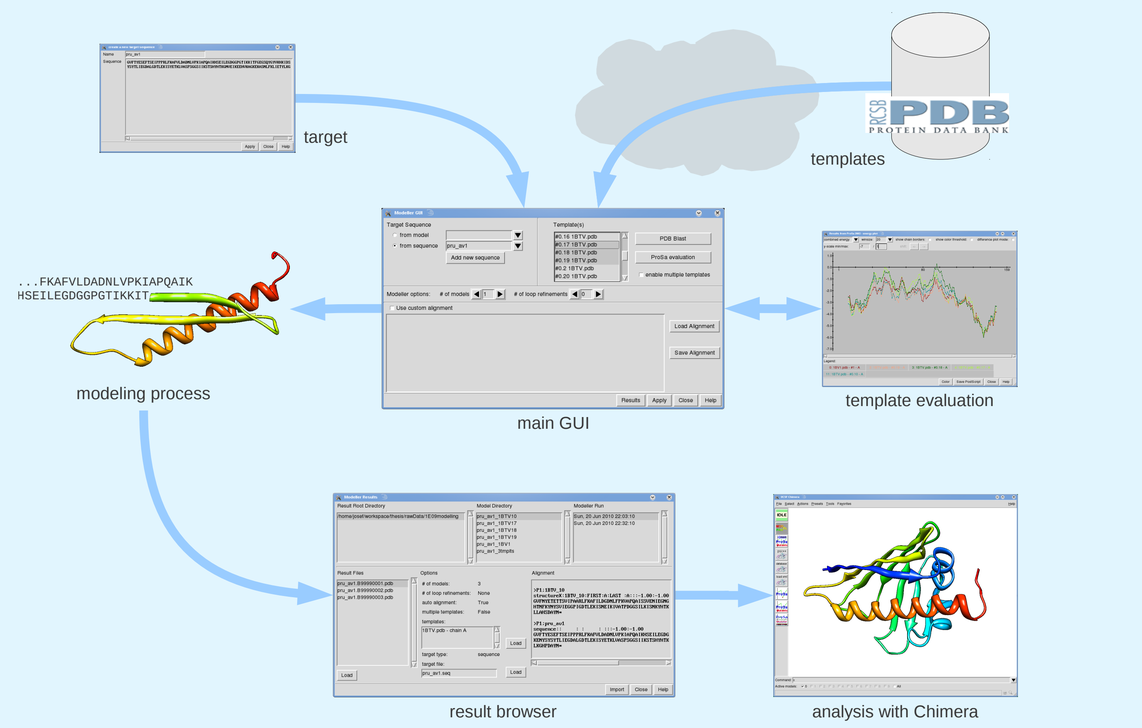
Our extensions are based on third-party software, namely **ProSa 2003**[2], a software tool for the validation of 3D protein structures by knowledge-based potentials, and **MODELLER**[3], a software tool for the prediction of 3D protein structures based on homology modeling techniques.

### Evaluation & In-silico Mutagenesis




The evaluation of protein structures and mutations is based on ProSa 2003. A configurable handler gives easy access to ProSa functionality and prepares the results for the Chimera extensions. Results are presented in tables and graphs. Numeric results can be mapped to the 3D structure drawings.

### Comparative Modeling



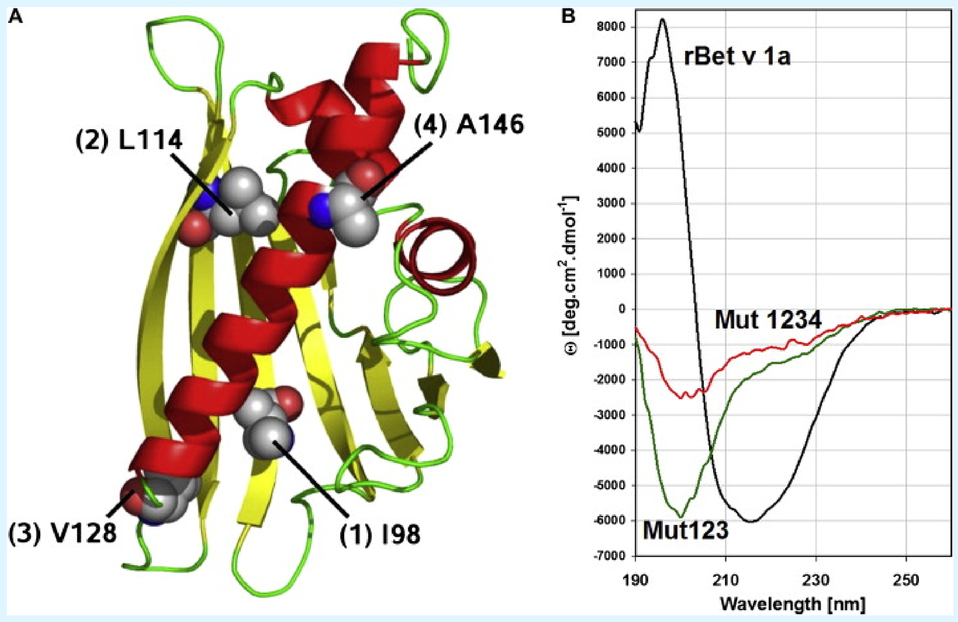

The comparative modeling extension is based on Sali's MODELLER. MODELLER is configured by Python scripts which are created and called based on the given settings. The results are shown in a browser for a comfortable file handling. The extension 'Blast Protein' can be used for template selection.

## Approach for Hypoallergen Design

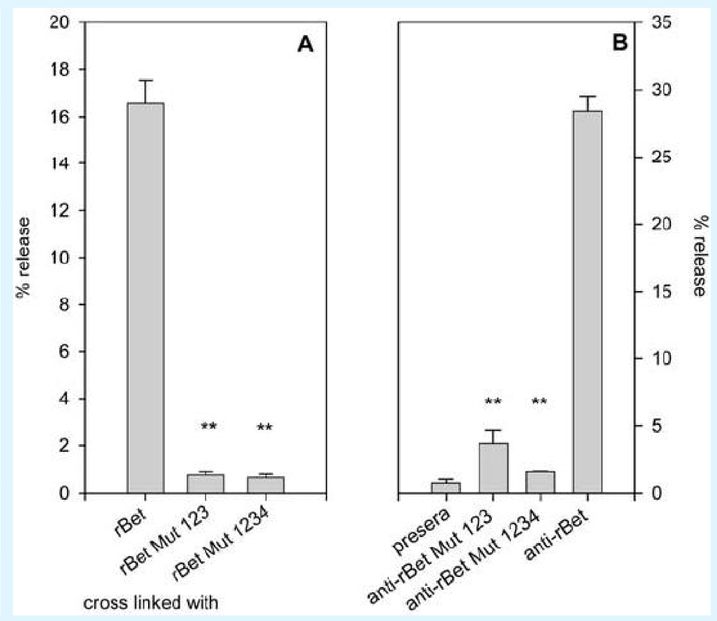
Our *in silico* mutagenesis extension provides a mode for the search for the most destabilizing or stabilizing mutations for a given 3D structure. The approach is based on the assumption that an increased ProSa z-score indicates a destabilization of the structure, where a decrease indicates a stabilization of the structure.
For the design of a hypoallergenic variant of a protein, the most destabilizing mutations are determined by an iteratively search:

```
best       <- wildtype
iterations <- 4
for i <- 1 to iterations:
  foreach residue in sequence:
    foreach possible mutation:
      m <- create mutant
      if z-score(m) > z-score(best):
        best <- m
fix mutations of best
```

Thalhamer and coworkers[4] successfully used this approach for the design of hypoallergenic variants of Bet v 1a, the major allergen of the european white birch.




The four mutation points, determined by the *in silico* mutagenesis approach, within the 3D structure (A) and the corresponding CD spectra analysis (B), which indicates a structural change[4].*




The mutants show a significantly reduced IgE cross-linking capacity after an immunization with wild-type Bet v 1a (A). An immunization with the mutants leads to a negligible level of Bet v 1a cross-linking IgE (B)[4].*

## Conclusion

We implemented extensions for the 3D-visualization software UCSF Chimera, which provide functionality for the evaluation and *in silico* mutagenesis of 3D protein structures, based on ProSa 2003, as well as for comparative modeling, based on MODELLER. The extensions are easy to use without any knowledge about operating the underlying software and results are presented within Chimera. All components are freely available for academic research.

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## References

[1] **UCSF Chimera–a visualization system for exploratory research and analysis.** Pettersen EF *et al.* J Comput Chem. Oct;25(13):1605-12, 2004. http://plato.cgl.ucsf.edu/chimera/

[2] **Recognition of errors in three-dimensional structures of proteins.** Sippl MJ Proteins Vol. 17, pp. 355-362, 1993. http://www.came.sbg.ac.at/

[3] **Comparative protein modelling by satisfaction of spatial restraints.** Sali A *et al.* J. Mol. Biol. 234, 779-815, 1993. http://salilab.org/modeller/

[4] **Designing hypoallergenic derivatives for allergy treatment by means of in silico mutation and screening.** Thalhamer T *et al.* J Allergy Clin Immunol., 125(4):926-934.e10, 2010.
*Figure taken from [4]