Homology modeling of Subtilisin mutated with a photocaged serine

Homology modeling is a computational tool to predict the 3D structure of a protein using the existing structure of a related protein, called template. Homology modeling is a powerful method which can yield a credible indication of the general fold of a protein. Using molecular modeling software, we aimed to predict the tertiary structure of the Subtilisin E protease, with 221 serine replaced by a non-natural amino acid, the photocaged serine. This summary is contribution for the Aachen University iGEM project.

Most existing modeling software based their methodology on calculating internal constraints of the template 3D structure such as internal distances, packaging etc. and collect and use data almost exclusively from natural amino acids. To our knowledge, only the SwissSidechain database (http://www.swisssidechain.ch) is able to model a limited number of non-natural amino acids. Since photocaged serine is not included we cannot use this automatic method. In order to obtain a credible prediction of the 3D structure of a protein containing a variant of an amino acid completely different in volume and shape, the computational prediction needs to include several steps: 1) Build an initial guess of the 3D structure of the non-natural amino acid. 2) Make an initial model of the protein with the natural amino acid 3) Parametrize the modified serine into one of the molecular dynamics software (e.g. Amber or Gromacs) 4) Replace the natural amino acid with the modified one 5) Run minimization and molecular dynamics (MD) simulation with Amber or Gromacs to obtain a realistic physico-chemical model with the modified amino acid. Only using MD, the 3D structure with a new chemical entity maybe able to relax and provide credible rotamer information, fold conformational changes and secondary structure predictions following this change. Since the amino acid sidechains are highly flexible, several of conformations can be obtained. But MD is a highly consuming computational method which will require high skills and large ammounts of computational time to reach conformational equilibria.

Unfortunately, due to a limited time framework we are not able to complete the structural modeling of the Subtilisin E protease. Nevertheless, using the Chimera

software, we did come to a few conclusions regarding the mutated Subtilisin E, while changing the 221 serine into a photocaged serine.

Figure 1 shows the structure of natural Subtilisin (PDB 1SCJ), it is evident that the serine (colored in green) is mostly buried inside the proteins core. Therefore, every small change in this area will probably impact dramatically the tertiary structure of the protein. Furthermore, the photocaged serine has a much larger number of atoms (37 atoms) than the regular serine (14 atoms) resulting in an immense steric effect that will most probably disrupt the native folding of the protein.

In conclusion, we believe that the protein, modified with photocaged serine, will fold differently than the natural Subtilisin, at least in its local environment amino acids. Therefore we believe it is necessary to have the suitable conditions, so once the photocaged serine will be exposed to light and the cleavage will occur, the protein will fold properly and the Subtilisin E protease will be activated.

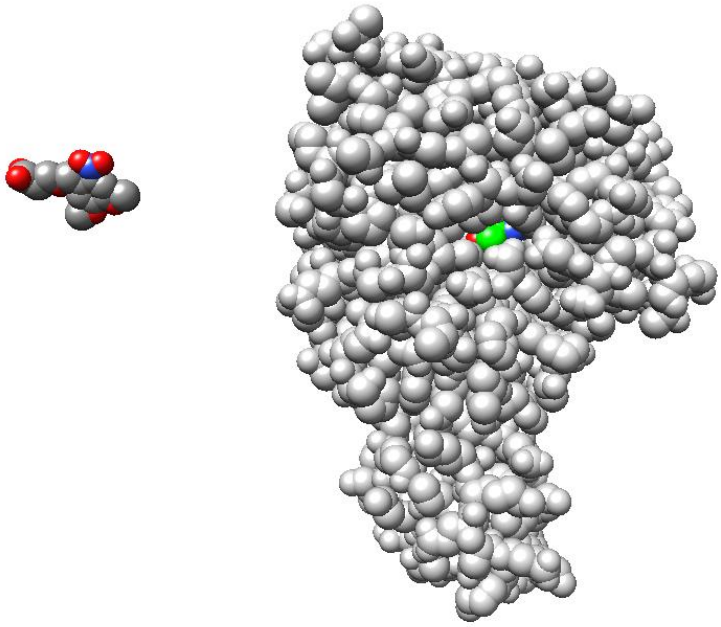

Figure 1: Molecular structure of the Subtilisin E (right PDB 1SCJ) compared to the structure of the photocaged serine (left). Natural serine inside the protein is colored (green C, red O, blue N).

Acknowledgments

We would like to express our gratitude to Dr. Fabian Glaser for sharing his knowledge with us.

Reference

Gfeller, D., Michielin, O., and Zoete, V. (2012). **Expanding molecular modeling and design tools to non-natural sidechains**. J Comput Chem *33*, 1525–1535.