PROTEIN ENGINEERING FOR PRACTICAL BIOTECHNOLOGY

Pietro Gatti-Lafranconi, Stefania Brocca, Gaetano Invernizzi and Marina Lotti
Dept. of Biotechnologies and Biosciences, Univ. of Milano-Bicocca,
Piazza della Scienza 2, 20126, Milan, Italy
pietro.gattilafranconi@unimib.it

The complex structure and behaviour of proteins have been studied for decades and addressed with many different approaches to elucidate their basics. The more we get to know, however, the more we have to admit nature is such a good protein engineer it will still take some time to reach the point were ex-novo molecular design will be a common reality.
Protein engineering has been, so far, a great tool to explore protein properties and develop new methodologies or functions. A number of *in vitro* studies demonstrated the possibility to isolate protein domains and their functions, to transfer properties from one structure to another, to modulate catalysis or binding according to special need and to add new function to an existing protein.

In our group, we study several proteins, starting from model systems to well established biocatalysts, regulatory proteins and unusual enzymes. The main focus of our research is the understanding of basic mechanisms of protein activity, binding, *in* vitro stability and *in vivo* solubility. To this aim, we used several techniques (enzyme isolation, site-directed and random mutagenesis, creation of chimeric proteins) on biotechnologically relevant enzymes and proteins. The well-known *Candida rugosa* lipase has been addressed with directed mutagenesis to asses its dependence upon glycosilation, a model for protein-sugar conjugates and to test the effect of glycosil residues on protein stability in unusual process conditions [1, 2]. Moreover, substrate preferences have been selectively modified to tune enzyme's specificity on the desired substrate [3]. The protein beta-lactoblobulin from bovine milk, that naturally binds to different molecules and has been proposed as a molecular carrier, was produced in cheap and safe cellular systems, its properties investigated at structural level and later modified for faster and easier practical applications [4, 5]. The study of cold-adapted lipases and of partially unstructured proteins is a new field where structure and function have to be considered independently and protein properties investigated under a new light [6-8]. We use several biochemical and biophysical methods (i.e. FT-IR, CD, Fluorimeter, 2D-PAGE, Confocal Microscope, etc) available in our department according to the model we are studying.

To conclude, the ability to manipulate protein structure and to characterize the effects of mutagenesis in biochemical and structural terms allows for the development and tuning of the biological moiety.

References:

[1] Brocca, S., M. Persson, E. Wehtje, P. Adlercreutz, L. Alberghina, and M. Lotti, *Mutants provide evidence of the importance of glycosydic chains in the activation of lipase 1 from Candida rugosa.* Protein Sci, 2000. **9**(5): p. 985-90.

[2] Brocca, S., C. Schmidt-Dannert, M. Lotti, L. Alberghina, and R.D. Schmid, *Design, total synthesis, and functional overexpression of the Candida rugosa lip1 gene coding for a major industrial lipase.* Protein Sci, 1998. **7**(6): p. 1415-22.

[3] Brocca, S., F. Secundo, M. Ossola, L. Alberghina, G. Carrea, and M. Lotti, *Sequence of the lid affects activity and specificity of Candida rugosa lipase isoenzymes.* Protein Sci, 2003. **12**(10): p. 2312-9.

[4] Invernizzi, G., L. Ragona, S. Brocca, E. Pedrazzoli, H. Molinari, P. Morandini, M. Catalano, and M. Lotti, *Heterologous expression of bovine and porcine beta-lactoglobulins in Pichia pastoris: towards a comparative functional characterisation.* J Biotechnol, 2004. **109**(1-2): p. 169-78.

[5] Invernizzi, G., M. Samalikova, S. Brocca, M. Lotti, H. Molinari, and R. Grandori, *Comparison of bovine and porcine beta-lactoglobulin: a mass spectrometric analysis.* J Mass Spectrom, 2006. **41**(6): p. 717-27.

[6] Alquati, C., L. De Gioia, G. Santarossa, L. Alberghina, P. Fantucci, and M. Lotti, *The cold-active lipase of Pseudomonas fragi. Heterologous expression, biochemical characterization and molecular modeling.* Eur J Biochem, 2002. **269**(13): p. 3321-8.

[7] Gatti-Lafranconi, P., S. Caldarazzo, A. Villa, L. Alberghina, and M. Lotti, *Unscrambling thermal stability and temperature adaptation in evolved variants of a cold-active lipase.* submitted, 2008.

[8] Santarossa, G., P.G. Lafranconi, C. Alquati, L. DeGioia, L. Alberghina, P. Fantucci, and M. Lotti, *Mutations in the "lid" region affect chain length specificity and thermostability of a Pseudomonas fragi lipase.* FEBS Lett, 2005. **579**(11): p. 2383-6.