Proceedings of the 5th International Conference on Nanotechnology: Fundamentals and Applications
Prague, Czech Republic, August 11-13, 2014
Paper No. 316

Protein Engineering for the Design of Protein-polymer Systems

Ulrich Schwaneberg
RWTH Aachen University
Lehrstuhl für Biotechnologie, Worringer Weg 3, 52074 Aachen, Germany
u.schwaneberg@biotec.rwth-aachen.de

Extended Abstract

Directed protein evolution has become in biocatalysis and in chemical/pharmaceutical industries a widely accepted and broadly applied method for tailoring of enzymes to application demands. Advances in mutagenesis technologies and screening systems are fuelling progress and enabling novel reengineering strategies (Ruff et al., 2013) by (I.) focused mutagenesis (selected residues are randomized; e.g. 3.2 million protein variants generated (Denning et al., 2011) and screened (Ruff et al., 2012) in two days), (II.) random mutagenesis (mutations are randomly introduced over the whole gene), and (III.) gene recombination (stretches of genes are mixed to chimeras in a random or rational manner (Marienhagen et al., 2012).

These methods offer in combination with computation methods robust options to shape protein properties to demands in material science. After introducing concepts and limitations of protein engineering technologies, the potential will be outlined on the example of two ß-barrel proteins (FhuA and nitro-bindin). Protein-hybrids comprise examples on triggered release systems (reduction trigger (Onaca et al., 2008) and light trigger (Güven et al., 2011), hybrid catalyst design (Philippart et al., 2013), elongating ß-strands to match thickness of polymer membranes, increase channel diameter of FhuA, and development of a production technology in gram scale.

References

Ruff, A. J., Dennig, A., and Schwaneberg, U. (2013). To get what we aim for: progress in diversity generation methods. FEBS J., 280, 2961-2978.

Dennig, A., Shivange, A.V., Marienhagen, J., and Schwaneberg, U. (2011). OmniChange: The sequence independent method for simultaneous site-saturation of five codons, PLoS One, 6, e26222.

Ruff, A. J., Dennig, A., Wirtz, G., Blanusa, M., and Schwaneberg, U. (2012). Flow cytometer-based high throughput screening system for accelerated directed evolution of P450 monooxygenases. ACS Catalysis, 2012, 2, 2724−2728.

Marienhagen, J., Dennig, A., and Schwaneberg, U. (2012). Phosphorothioate-based DNA Recombination: an enzyme-free method for the combinatorial assembly of multiple DNA fragments. BioTechniques, Rapid Dispatch, 6.

Onaca, O., Sarkar, P., Roccatano, D., Friedrich, T., Hauer, B., Grzelakowski, M., Güven, A., Fioroni, M., Schwaneberg, U. (2008). Functionalized nanocompartments (synthosomes) with a reduction-tiggered release system, Angew Chem Int Ed Eng., 47, 7029-7031.

Güven, A., Dworeck, T., Fioroni, M., and Schwaneberg, U. (2011). „Residue K556-A Light triggerable gatekeeper to sterically control translocation in FhuA", Adv. Eng. Mat., B324-B329.

Philippart, F., Arlt, M., Gotzen, S., Tenne, J., Bocola, M., Chen, H.H., Zhu, L., Schwaneberg, U.*, and Okuda, J.* (2013). A hybrid ring-opening metathesis polymerization catalyst based on engineered ß-barrel protein FhuA. Chemistry-A Eur. J., 19, 13865-13871.