The interplay between protein design and directed evolution

Andreas Plückthun, Dept. Biochemistry, University of Zurich
www.bioc.uzh.ch/plueckthun, plueckthun@bioc.uzh.ch

Two systems will be discussed, which are both of basic science interest and considerable economic importance.

The first is the challenge of designing binding sites around given ligands, to change the paradigm of peptide binding reagents: the current state of conventional reagent antibodies is extremely unsatisfactory, as about half of them do not show the presumed specificity, and none of them is molecularly defined and thus not renewable [1,2]. Even with current recombinant technologies, still, for each protein and each modification, an individual reagent has to be separately generated and validated. We have been working on creating a completely modular detection technology for polypeptides (e.g., unstructured regions, denatured forms or proteolytic digests) bind to a modular counterpart, built from Armadillo repeat proteins (ArmRPs). Combining evolutionary engineering, NMR, X-ray crystallography and structure-based computation, we have now achieved well crystallizing ArmRPs with bound peptides, picomolar affinities, and a well functioning selection and evolution technology, as well as a portfolio of biochemical and biophysical analysis technologies for the engineered ArmRPs [3-10].

The second example is to create ligands for a given protein, which however, is so unstable that its structure determination is a challenge. G protein coupled receptors (GPCRs) have enormous pharmacological relevance but our understanding of GPCR architecture and signaling mechanism has remained limited, as have the design features of agonists and antagonists. We have now developed evolution methods to solve this problem [11-15]. The crystal structure of a stabilized GPCR, NTR1, with agonist bound was determined [16, 17] in short chain detergents directly from protein made in E. coli, not requiring insertion of lysozyme. The stabilized receptors have been used successfully for computational docking, leading to agonists that work on cells.

1. Bradbury, A and Plückthun, A (2015) and 110 co-signatories, Nature 518, 27-29.
2. Bradbury, A and Plückthun, A (2015) Protein Eng. Des. Sel. 28, 303-305.
3. Reichen, C., et al., (2016). Acta Crystallogr. D 72, 168-175.
4. Reichen, C., et al., (2014). Protein Science 23, 1572-1583.
5. Reichen, C., et al., (2014). J. Struct. Biol. 185, 147-162.
6. Watson, R. P. et al., (2014) Structure 27, 985-995.
7. Varadamsetty, G., et al., (2012). J. Mol. Biol. 424, 68-87.
8. Alfarano, P., et al., (2012). Protein Science 21, 1298-1314.
9. Madhurantakam, C., et al., (2012). Protein Science 21, 1015-1028.
10. Parmeggiani, F., et al., (2008). J Mol Biol 376, 1282-1304.
11. Sarkar CA et al., Proc. Natl. Acad. Sci. USA. 2008;105:14808-13.
12. Dodevski I and Plückthun A., J. Mol. Biol. 2011;408:599-615.
13. Schlinkmann KM et al., Proc. Natl. Acad. Sci. USA. 2012;109:9810-5.
14. Schlinkmann KM et al., J. Mol. Biol. 2012;422:414-28.
15. Scott DJ and Plückthun A., J. Mol. Biol. 2013;425:662-77.
16. Egloff P et al., Proc. Natl. Acad. Sci. USA. 2014;111:E655-62.
17. Hillenbrand M et al., Proc. Natl. Acad. Sci. USA. 2015;112:E1181-90.