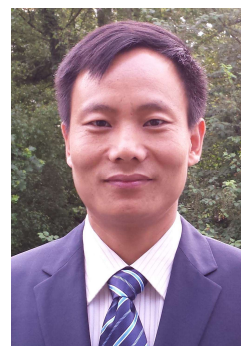# The Crucial Role of Methodology Development in Directed Evolution of Selective Enzymes

Name

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Abstract

Directed evolution of stereo-, regio-, and chemoselective enzymes has enriched the toolbox of synthetic organic chemistry and biotechnology and provides a means to generate biocatalysts for synthetically interesting transformations. In order for this protein engineering technique to be efficient, fast and reliable, methodology development was and still is necessary. Saturation mutagenesis (SM) at sites lining the enzyme's binding pocket has emerged as a particularly viable approach to control selectivity and activity. Traditionally, NNK codon degeneracy encoding all 20 canonical amino acids is used, but as the size of the randomization site increases beyond a single residue, oversampling of transformants needed to ensure ≥95% library coverage rapidly reaches astronomical dimensions, impossible to screen in a practical manner (bottleneck of directed evolution). Therefore, many groups have been content with screening only a small segment of the designed protein sequence space, but this means that the best mutants will be missed. Alternatively, it has been shown that the use of highly reduced amino acid alphabets allows the generation of small and smart libraries requiring less screening. Here we address the question of which approach is more efficient. Statistical analyses clearly show that it is more efficient to opt for rationally designed reduced amino acid alphabets because this approach results in a distinctly higher frequency of active mutants, stereoselectivity also being notably higher.

Brief Biography

Zhoutong Sun obtained his Ph.D in microbiology at Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences in 2012, then he moved to Nanyang Technological University in Singapore as a research fellow. One year later, he moved to the MPI für Kohlenforschung and Marburg University in Germany for a postdoc with Prof. Manfred T. Reetz. In 2016, he became a full professor at Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences via the "CAS Pioneer Hundred Talents Program". His research interests are in the discovery, design and engineering of biocatalysts as well as new enzymatic reactions design and catalytic mechanism analyses. He has published more than 30 peer-reviewed papers in Chem Rev, J Am Chem Soc, Angew Chem Int Ed, ACS Catal, Green Chem, Adv Synth Catal and Metab Eng, and a dozen patents.

Brief CV

Zhoutong Sun, Ph.D.

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Education:

B.S   Bioengineering, Henan University, China, 2005;

Ph.D.   Microbiology, Shanghai Institutes for Biological Sciences, CAS, China, 2012

Professional Career:

2012-2013: Nanyang Technological University, Singapore, Postdoctoral Fellow.

2013-2016: MPI für Kohlenforschung and Marburg University, Germany, Postdoctoral Fellow.

2016-Present: Tianjin Institute of Industrial Biotechnology, CAS, China, Professor.

Research Interests:

1. Genome mining of new types of biocatalyst by bioinformatics analysis;

2. Protein engineering and directed evolution of important industrial enzymes to improve or achieve novel functions;

3. Rational design and *in silico* directed evolution of new biocatalysts;

4. Methodology development in directed evolution;

5. Novel enzymatic reaction design and catalytic mechanism studies.

Selected publications

1. Qu G. et al. ***Angew. Chem. Int. Ed.***, 2019, DOI: 10.1002/anie.201901491.
2. Li A. et al. ***ACS Catal.***, 2019, 9, 7769-7778.
3. Liu B. et al. ***Adv. Synth. Catal.***, 2019, 361, 3182-3190.
4. Qu G. et al. ***Bioresour. Bioprocess.*** 2019, 6:18.
5. Sun Z. et al. ***Chem. Rev.***, 2019, 119, 1626-1665.
6. Qu G. et al. ***J. Chem. Inf. Model.***, 2019, 59, 832-841.
7. Dai Z. et al. ***Metab. Eng.***, 2019, 51, 70-78.
8. Qu G. et al. ***Green Chem.***, 2018, 20, 777-792.
9. Qu G. et al. ***ChemBioChem***, 2018, 19, 239-246.
10. Sun Z. et al. ***J. Am. Chem. Soc.***, 2018, 140, 310-318.
11. Acevedo-Rocha CG. et al. ***ChemBioChem***, 2018, 19, 2542-2544.
12. Yang J. et al. ***Biotechnol. Biofuels***, 2018, 11, 290.