Enzyme evolution by genetic complementation


Evolution of an aspartate aminotransferase

- What are the authors trying to do?
- What is the process by which they conduct their evolution? Explain the Materials and Methods section.
- What method of library generation do they use? Is this a good choice?
- How do they carry out their selection?
- What are the results? Does their explanation of why mutants lead to increased activity make sense?
- Why is this evolution worthwhile? JOC paper
Aminotransferase catalysis

Image of aminotransferase catalytic cycle removed due to copyright restrictions.
Explanation of mutations from crystal data

Image removed due to copyright restrictions. Please see Fig. 3 in Yano, T., S. Oue, and H. Kagamiyama. “Directed evolution of an aspartate aminotransferase with new substrate specificities.” PNAS. 95(1998): 5511-5515.
Evolution of a cephalosporin acylase

• What are the authors trying to do? Why is this significant?
• How do the authors make their library? Is this reasonable?
• What is the selection method in this evolution?
• What are the results?
• What are the problems or pitfalls in this paper?
Cephalosporin synthetic pathway

Adipate feed → Acyltransferase → Expandase → Expandase/Hydroxylase → Acylase

Acyltransferase

Acylase
Positions of mutated residues

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For next week...chemical complementation