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7.344 Directed Evolution: Engineering Biocatalysts □ □
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Session 10 Lecture Notes

1. Why engineer antibodies? The authors discuss this a bit – what do you think? Implications for cancer biology. What are the limitations the authors identify with previous display methods that make their system attractive? Expression bias in e.coli – phage display requires soluble expression of a protein for incorporation as a fusion with a phage coat protein – problem for disulfide-bond containing proteins. Phage display may also select for alternative properties of enzymes including reduced host toxicity or increased phage infectivity. Major advantage for cell-based methods is the total number of surface fusions – as many as thousands and can be screened by FACS – a very fast method. One problem, steric interference of the cell wall glycans may prevent substrate binding.
2. Proteins are made as a fusion to the yeast surface receptor α -agglutinin, which is placed under an inducible GAL1 promoter (production in the presence of galactose-rich media). Fusion proteins are exported and linked to cell surface via two disulfide bonds.
3. Surface expression is verified by incorporation of two tags, HA and c-myc (N and C-terminal respectively), which can be independently investigated using fluorescent Abs. Total number of cell fusions is quantified using an elisa-like approach. Fluorescence is DTT sensitive – must be because disulfide bonds are required to form the complete display complex. When would this be a problem? If mildly reducing conditions are required for protein stability and reactivity the fusions could be lost from the surface of cells, losing genotypic-phenotypic link.
4. Enrichments of about 600-fold are observed using FACS. Pretty comparable to bacterial display systems and better than phage.
5. Mutator strains of e.coli are used to mutagenize the DNA prior to insertion in the yeast display vector. Kinetic selection by competition of FITC-dextran labeled cells with 5-aminofluorescein was used to enrich populations – lowest K_{off} was selected. Three rounds of sorting yielded a 2.2 fold improvement for mutants relative to wild-type. Use of polyvalent antigen aided in enrichment for clones with increased avidity.
6. Benefits/challenges – eukaryotic host (big plus!). Same general limitations as bacterial surface display.
7. What is this strategy being used for now? Engineering horseradish peroxidase with enhanced enantioselectivity – collaboration between Wittrup and Klivanov labs.

8. The authors are trying to engineer a better lipase for the hydrolysis of fatty acid esters in organic solvents for bioindustrial applications. They are trying to improve both hydrolysis and esterification properties. Ultimately they hope to use the yeast displaying the proteins as a bioreagent themselves.
9. The authors are using a self-designed system of surface display based on the alpha-agglutinin surface protein – see figure from Wittrup paper for reference. Error-prone PCR is used for generation of a library of mutant ROLs. Lipases are assayed in liquid culture using PNP-palmitate. Lipases are screened from plated cultures using a halo assay. Methyl palmitate is added to the plated media and active library members are identified by halo formation. Libraries are first screened by halo assay and then examined in aqueous/organic solvents.
10. Displayed enzyme itself is 30 fold higher in aq reactivity and in organic solvents. Why is this? Could be that the enzyme itself is much less soluble in org solvents, but the cells help with that insolubility. This is true for both hydrolysis and esterification. Large fusions of enzymes often lead to stability. Why? Five of the 13 mutants had 3 to 6-fold higher reactivity. Combinations of beneficial mutations could lead to even more active ROLs – use a shuffling technique for library formation!
11. Pros: proteins are stabilized, can be readily produced by standard fermentation – a big plus for industry. Cons: library screening is very low throughput and is based only on assay for hydrolysis. Might be missing some good esterification activity because the assay doesn't exist.