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7.344 Directed Evolution: Engineering Biocatalysts □ □  
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## Session 8 Lecture Notes

1. Debrief on Codon Devices next week since we have 3 papers this week.
2. Remind everyone about the assignment coming up due Session 10. People should have picked out their enzyme by next session and bring choice to class for approval.
3. What has phage display historically been used for? Is this a reasonable application? Briefly get someone to explain the system of superinfection, phage biology etc...
4. The authors are trying to use phage display to evolve a metalloenzyme – specifically a beta lactamase (zinc containing) using catalytic elution. They put forth that this method should be general for any enzyme that requires the presence of a cofactor.
5. The elution works as follows: phage are produced that have a p3 fusion of the metalloenzyme. Any metals are removed from the system by complexation and phage are loaded onto a solid support coated with substrate. Addition of the metal cofactor allows for enzyme turnover and phage are released, since they have more affinity for the substrate than for the product.
6. Phage-bound enzyme is characterized by complete sequencing of the DNA, analysis of the fusion proteins by western blot – get someone to explain figure 1, and by determination of enzymatic activity. The authors find that there is low expression of the fusion enzyme – why is this a problem? Do you value their explanation? Any other possibilities?
7. Authors assess elution by plating on lactams.
8. Model experiments are there to test stringency of the elution process for background. Get someone to explain each.
9. The authors find that their mutagenesis is primarily deleterious for function – why is this? They find 9 mutants with a max of 170% WT activity. None of the mutations are close to the active site, mostly just surface residue changes. How would these contribute to increased activity?
10. Potential pitfalls: what if enzyme doesn't bind well in the absence of the cofactor? What if the affinity for the product is too high? The enzyme activity is very sensitive to the conditions used to prepare the phage. Phage that have only one copy of the p3 fusion don't bind well to the solid-support – could be missing active components of the library based on expression – generally a problem with phage display. Benefits – you don't have any crazy tethers to the phage itself and no superinfecting with a different kind of helper and trying to sort out the total copy number of each type of p3 fusion. Should

work for most metalloenzymes – particularly proteases, but kind of overdone for that – there is an easier way.

11. The authors are looking to evolve an adenylate cyclase – the enzyme that converts ATP into cyclic AMP using phage display. They realize that the substrate must be displayed on the same phage as the enzyme to retain the genotype-phenotype link. They make a substrate that contains a maleimide to react with surface cysteine groups for substrate display – what’s the immediate red flag that goes up here? Crosstalk!!
12. Substrate is designed using structural data to assess which positions can tolerate functionalization. Remember – you get what you select for – if you don’t use the real substrate you may get enzymes that can’t use it! An elegant use of phage display for Scfv selection, however.
13. Classic test of selection capability – all good phage display papers have it, a mock selection. Can anyone explain the treatment of the samples with trypsin? Why does this affect infectivity?
14. Generality of strategy is still a problem – you have to make an antibody to do your selection – very hard for certain classes of molecules. Ask if students know which kinds.
15. Have someone explain how the Schultz strategy works. They spend a lot of time talking about how disulfide bond formation isn’t specific without their method and that covalent bond formation is important – explain findings in the Walker lab. Key point: Enzyme must be maintained in an inactive state during phage attachment and then activated.
16. This paper explains the entire process of producing phage and conducting a selection very elegantly. Make sure everyone understands this process. Do students see why some enzymes aren’t going to survive this with activity?
17. Why is the efficiency of immobilization so poor? (How do they determine the extent of immobilization? Infectivity post washing – what if phage attached to the beads are just not infective anymore?) Poor expression of fusions – it is linked to the srp/pelb expression machinery... Why is there leakage of phage off the solid support? They say release of streptavidin or disulfide exchange. They didn’t do an extensive purification, so what about endogenous proteases?
18. Results, Mock selection shows active enzymes may be rescued. Strategy is viable. Discuss Figure 3.
19. Generality? Who wants to make a 50 AA synthetic peptide every time you want to evolve something? Not practical or cost effective. Introduce Sec strategy.