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7.344 Directed Evolution: Engineering Biocatalysts □ □  
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## Session 6 Lecture Notes

1. The authors are trying to create general strategy for the reporting of enzyme activity. They use small molecule receptors that are bound to a transcription factor and a DNA binding protein to activate transcription of reporter genes (LacZ).
2. The complementation system is engineered by making a strain of yeast that encodes LexA-DHFR and B42-GR fusion proteins under control of the GAL1 promoter. The LacZ reporter plasmid (spectinomycin) is transformed in as is the vector containing the enzyme (kanamycin).
3. The Cornish group uses the cephalosporinase enzyme to test their system. Cleavage of the B-lactam bond in the cephalosporin results in expulsion of the leaving group at the C3 position and breaks the bond between Mtx and Dex. The substrate (Mtx-Cephem-Dex) is added to the growth media in varying concentrations.
4. The result is that enzyme activity can be detected in the presence of M-C-D using blue-white screening. Talk through figure 3, panels b and c. A rescue of active enzyme in a pool of inactive variants is possible from blue-white screening. (Serine to alanine mutation mixed 95 to 5%). Is this a stringent test of the capabilities of this method? Perhaps it would be better to link to the transcription of genes that are essential for survival. – see the next Cornish paper.
5. Transcription change is due to enzyme turnover because Mtx-Dx with no cleavable linker cannot be disrupted by the presence of the enzyme (and transcription is unaffected.)
6. Is this strategy general? There is a lot of chemical synthesis involved in making the substrates. Also, how many enzymes will tolerate these modified substrates? How many substrate classes can easily be uptaken in cells? Is this sort of assay easily translated to bacterial systems?
7. What are the benefits? One can make a system that responds to enzymatic activity of any kind provided they have the skill set necessary to make the substrates.
8. The Doyle method is quite like the Cornish method, but way more confusing. The authors use nuclear receptors fused to DNA binding domains to activate transcription of downstream genes allowing for histidine production. Selection occurs on plates lacking histidine.
9. The authors test their system using mutant enzymes of the RXR ligand binding domain. These mutants are made with the help of structural data

suggesting the importance of specific residues in binding the ligand. They show that some of the mutants behave even better than the wild type in terms of transcriptional activation. Figure 5 and 6.

10. The authors seem to believe that nuclear receptors could be generated for any small molecule using genetic selection – this may be true, but is a hard way to start an enzyme engineering project. Perhaps the riboswitch method is better. (quite similar, actually) This is a very challenging and contrived system. Look at the small molecules used – taxol takes 50 synthetic steps to make!
11. The authors are trying to use their method to engineer a better glycosynthase from a glycosidase based on earlier work that showed that removal of the glutamate residue from the active site makes an enzyme capable of using glycosyl fluorides as donor molecules. Figure 1.
12. The authors make disaccharide-containing Mtx and Dex molecules and select for clones capable of living on leucine deficient media. The reporting genes are the LEU2 gene under the control of 6 tandem LexA operators. How did they figure out where to attached their linkers in their substrates? There was a lot of structural data on the Cel7b glycosynthase.
13. They are able to link glycosynthase activity to growth in a dose dependent fashion. Figure 4 – pretty amazing that those big substrates are utilized or uptaken by the cell. Maybe the cell exports the enzyme? Figure 5 shows that the enzyme must already be mutated to glycosynthase capabilities to use the substrate. The authors do a mock selection.
14. A library of Glu197 saturation mutants was made using cassette mutagenesis and overlap extension PCR. These mutants are then selected in a single assay. Several variants were picked up and then screened further in an assay for pnp expulsion. One mutant was 5 fold better than wt. In vivo expression of enzymes may be a limiting factor.