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7.344 Directed Evolution: Engineering Biocatalysts
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Enzyme evolution by chemical complementation

Baker, K.; Bleczinski, C.; Lin, H.; Salazar-Jimenez, G.; Sengupta, D.; Krane, S.; Cornish, V.W. Chemical complementation: a reaction-independent genetic assay for enzyme catalysis. *Proc. Natl. Acad. Sci. USA* **2002**, *99 (26),* 16537-16542.

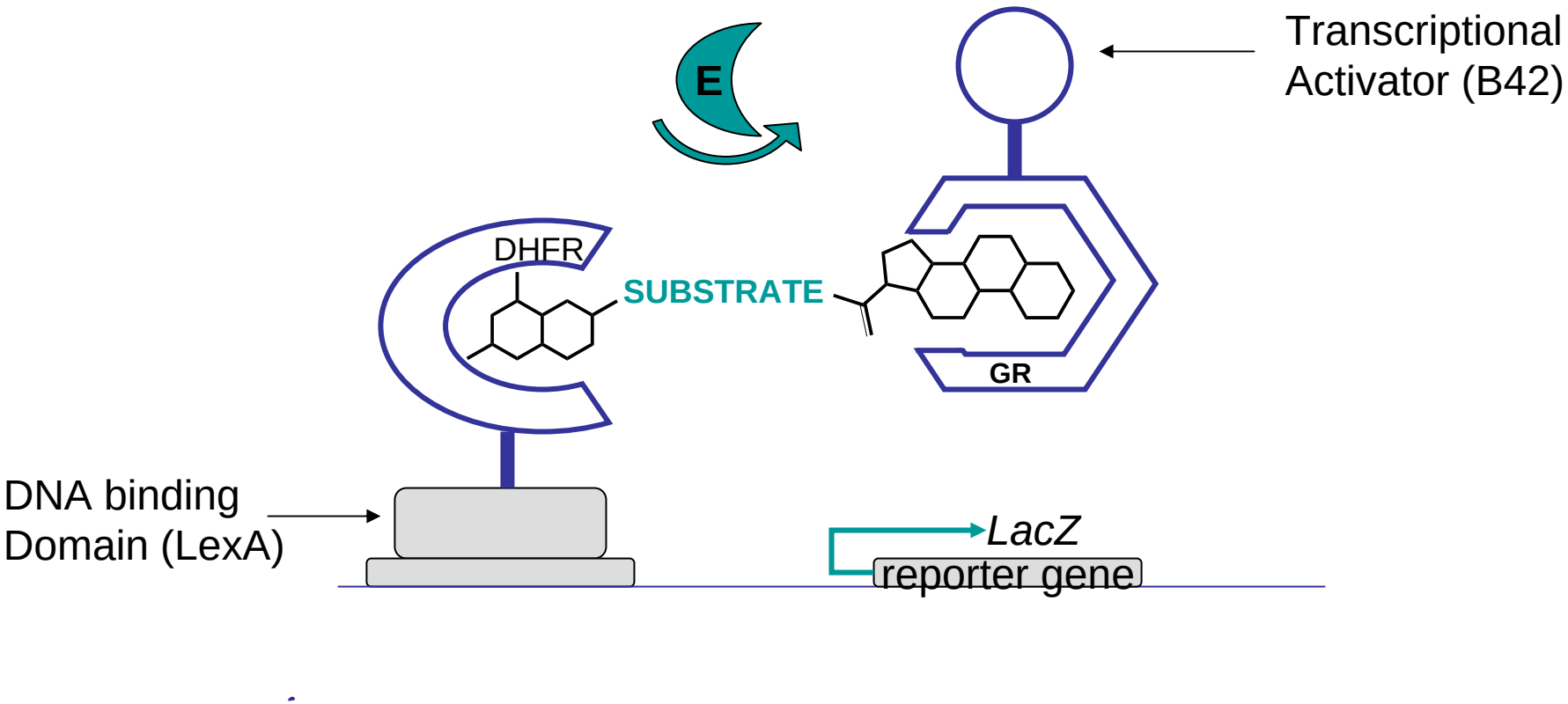
Azizi, B.; Chang, E.I.; Doyle, D.F. Chemical complementation: small-molecule-based genetic selection in yeast. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 774-780.

Lin, H.; Tao, H.; Cornish, V.W. Directed evolution of a glycosynthase via chemical complementation. *J. Am. Chem. Soc.* **2004**, *126*, 15051-15059.

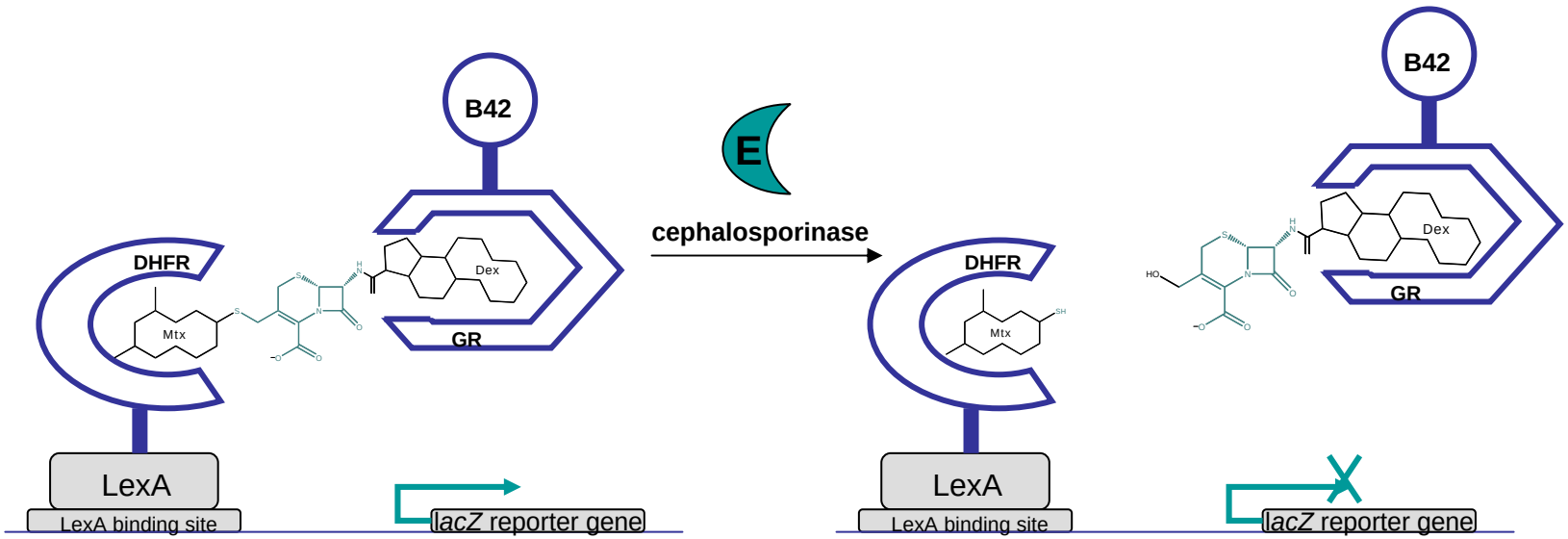
Chemical complementation as developed in the Cornish lab

- What are the authors trying to do?
- How do they go about engineering their system of complementation?
- What enzyme do they use to test their system? How does this work? (Figure 2, panel B)
- What are the results? (Figure 3, panels B and C) How do the authors confirm that the change in transcription is caused by enzyme turnover?
- Is this strategy general? What are the benefits and pitfalls?

The Cornish group strategy

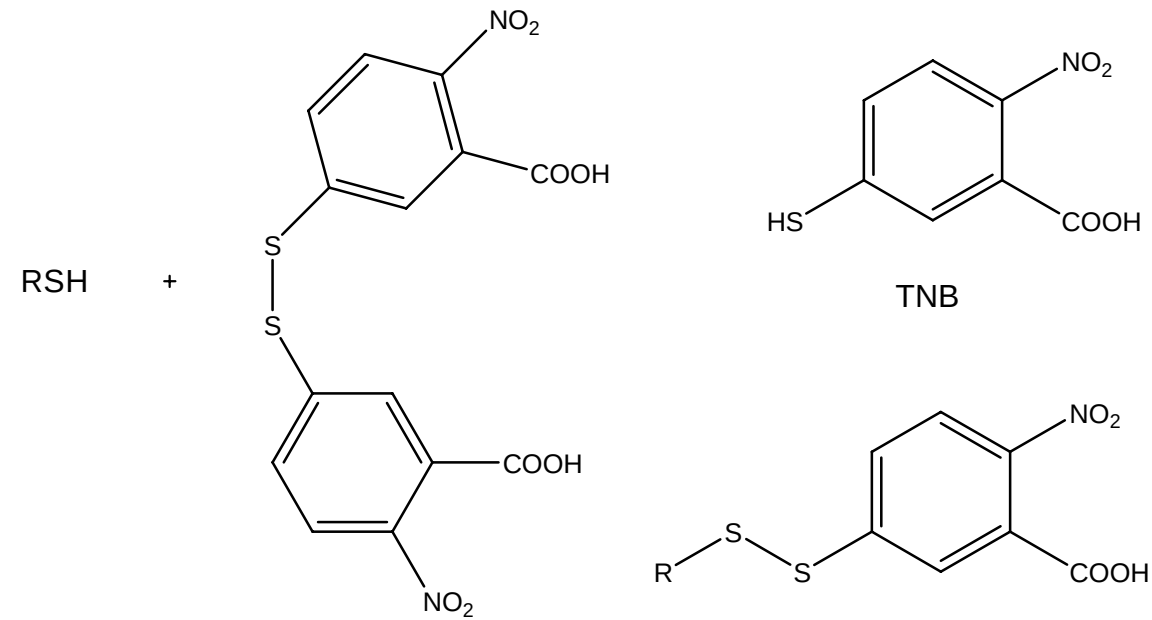


Chemical complementation for detection of cephalosporinase activity



How is enzyme activity reported? How are the enzymes tested with the substrates for activity? What chemical functional group is detected?

Detection of free thiols (RSH) using Ellman's reagent



MCD is a good *in vivo* substrate

Images of plate assay and liquid culture assay using Ellman's reagent removed due to copyright restrictions.

Active enzyme can be isolated from inactive variants

Image removed due to copyright restrictions. Please see Fig. 1 in Baker, K., C. Bleczinski, H. Lin, G. Salazar-Jimenez, D. Sengupta, S. Krane, and V. W. Cornish. “Chemical complementation: a reaction-independent genetic assay for enzyme catalysis.” *PNAS*. 99(2002):16537-16542.

Chemical complementation as developed by the Doyle lab

- What are the authors trying to do?
- How do they go about engineering their system of complementation?
- What enzyme do they use to test their system? How does this work?
- What are the results?
- Is this strategy general? What are the benefits and pitfalls?
- Can you see why this paper got much less attention than the previous one?

Doyle's system of chemical complementation

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Genetic selection using the system

Retinoid receptor

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Pregnane receptor

Image removed due to copyright restrictions. Please see Fig. 4 in Azizi, B, E. I. Chang, and D. F. Doyle. “Chemical complementation: small-molecule-based genetic selection in yeast.” *Biochem. Biophys. Res. Commun.* 306(2003): 774-780.

Mutant LBDs can be better than WT

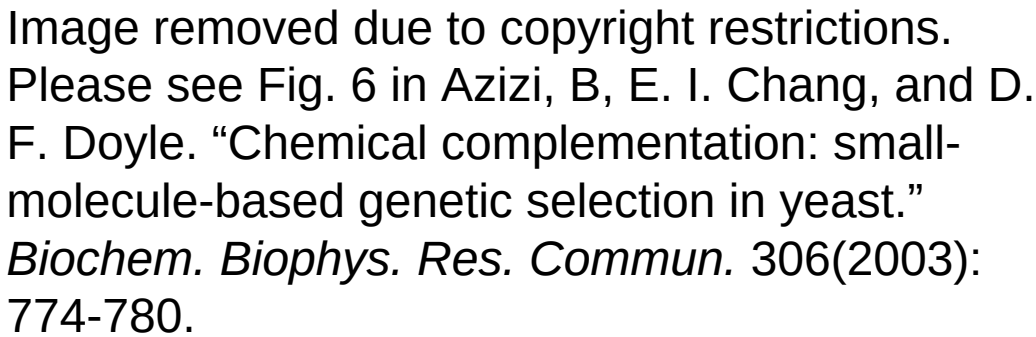
Image removed due to copyright restrictions. Please see Fig. 6 in Azizi, B, E. I. Chang, and D. F. Doyle. “Chemical complementation: small-molecule-based genetic selection in yeast.” *Biochem. Biophys. Res. Commun.* 306(2003): 774-780.

Enzyme evolution by chemical complementation

- What are the authors trying to do?
- How do they go about engineering their system of complementation?
- What enzyme do they use to test their system? How does this work? (Figure 2)
- What are the results? (Figure 5)
- How do they evolve this enzyme? What is the library generation strategy? How do they characterize the mutants?
- Is this strategy general? What are the benefits and pitfalls?

Chemical complementation for complex carbohydrate synthesis

Image removed due to copyright restrictions. Please see Fig. 1 in Lin, H., H. Tao, and V. W. Cornish. “Directed evolution of a glycosynthase via chemical complementation.” *J. Am. Chem. Soc.* 126(2004): 15051-15059.

Making a glycosidase into a glycosynthase

Image removed due to copyright restrictions. Please see Fig. 2 in Lin, H., H. Tao, and V. W. Cornish. “Directed evolution of a glycosynthase via chemical complementation.” *J. Am. Chem. Soc.* 126(2004): 15051-15059.

Chemical complementation links glycosylation activity to LEU2 transcription