7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008

Alternate methods for enzyme design

Hill, C. M.; Li, W.-S.; Thoden, J. B.; Holden, H. M.; Raushel, F. M. Enhanced Degradation of Chemical Warfare Agents through Molecular Engineering of the Phosphotriesterase Active Site *J. Am. Chem. Soc.* **2003**, *125(30)*, 8990-8991.

Williams, G.J.; Woodhall, T.; Nelson, A.; Berry, A. Structure-guided saturation mutagenesis of N-acetylneuraminic acid lyase for the synthesis of sialic acid mimetics. *Prot. Eng. Des. Select.* **2005**, *18(5)*, 239-246.

Reetz, M.T.; Bocola, M.; Carballeira, J.D.; Zha, D.; Vogel, A. Expanding the range of substrate acceptance of enzymes: Combinatorial active-site saturation test. *Angew. Chem. Int. Ed.* **2005**, *44*, 4192-4196.

Molecular engineering of PTE

- What is the PTE and what are its substrates? What is the mechanism for substrate degradation and what residues are important?
- What is the mutagenesis method used? Why do the authors choose this method?
- How are the active mutants recovered?
- What are the results?

Active site structure of WT PTE

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Results: activities of mutants on soman analogue

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Active site of triple mutant

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Saturation mutagenesis of NAL

- What is NAL and what is it used for? Why is this significant?
- How do the authors propose to assay NAL activity? Is this meaningful?
- How is the library generated? Why do the authors use this method?
- What are the results?
- What is a key problem with this paper?

NAL mechanism

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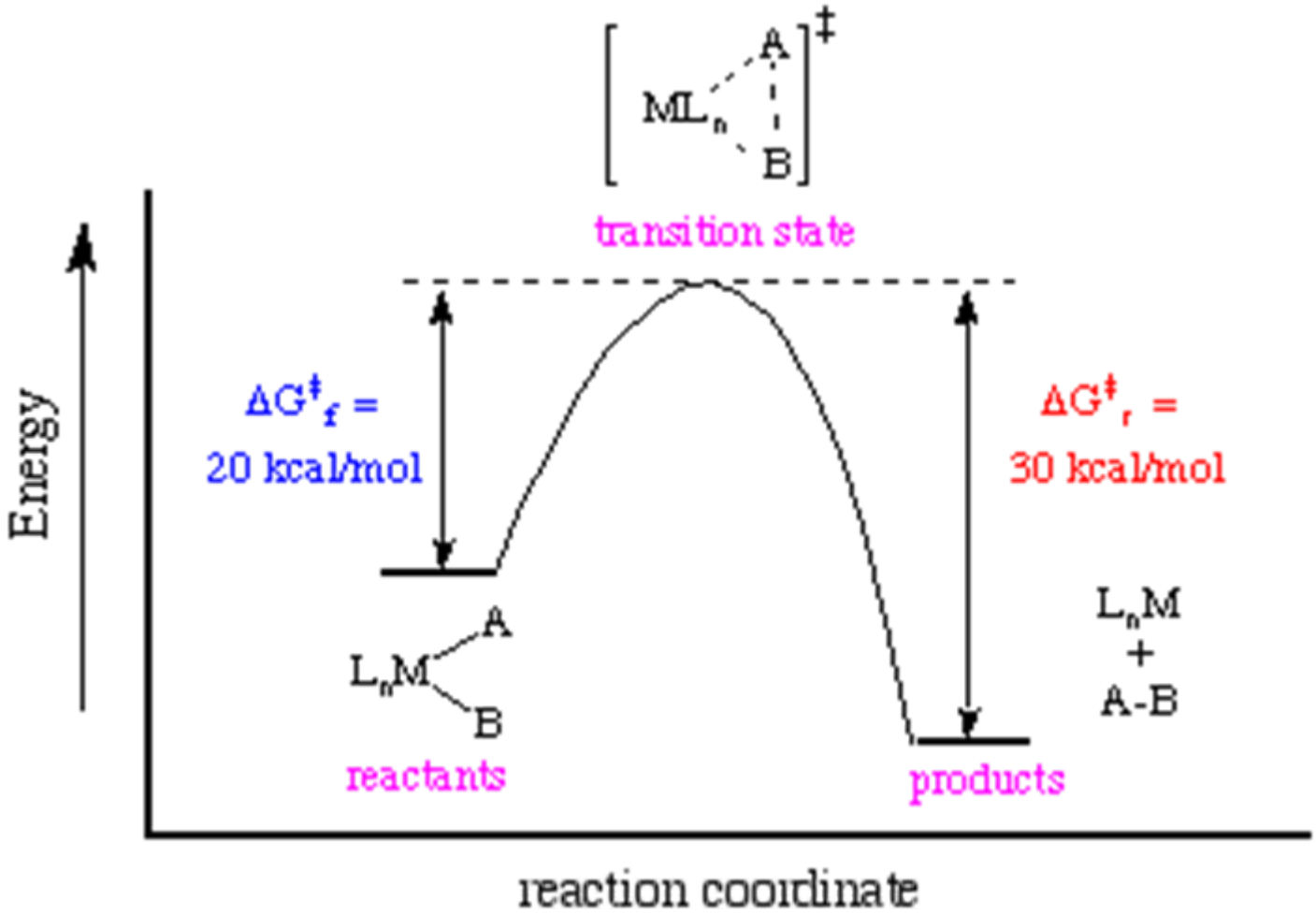
The principle of microscopic reversibility

- If a certain series of steps constitutes the mechanism of a forward reaction, the mechanism of the reverse reaction (under the same conditions) is given by the same steps traversed backwards. (**Note:** The phrase "under the same conditions" means that this applies only to thermal reactions, not photochemical ones.)
- The sequence of transition states and reactive intermediates in the mechanism of a reversible reaction must be the same, but in reverse order, for the backward reaction as for the forward reaction.
- If the mechanism in one direction is known, then the mechanism in the opposite direction is known.
- The lowest-energy pathway in the forward direction will be the lowest-energy pathway in the reverse direction.

The principle of microscopic reversibility



Results: (Figure 4)

Image removed due to copyright restrictions. Please see Fig. 4 in Williams, G. J., T Woodhall, A. Nelson, and A. Berry. “Structure-guided Saturation of *N*-acetylneuraminic acid lyase for the synthesis of sialic acid mimetics.” *Prot. Eng. Des. Select.* 18(2005): 239-246.

CASTing for altered substrate specificity

- What is the method developed by the authors? Is this a reasonable way to create libraries? Does it offer advantages over traditional methods?
- What is the enzyme mutagenized? How do they choose their residues? How do they assay enzyme variants?
- What are the pitfalls of this method?

Structural guides for CASTing

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Please see Fig. 1 in Reetz, M. T., M. Bocola, J. D. Carballeira, D. Zha, and A. Vogel. “Expanding the Range of Substrate Acceptance of Enzymes: Combinatorial Active-Site Saturation Test.” *Angew. Chem. Int. Ed.* 44(2005): 4192-4196.

Library generation for lipase mutants

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Please see Fig. 2 in Reetz, M. T., M. Bocola, J. D. Carballeira, D. Zha, and A. Vogel. “Expanding the Range of Substrate Acceptance of Enzymes: Combinatorial Active-Site Saturation Test.” *Angew. Chem. Int. Ed.* 44(2005): 4192-4196.

Results: Figure 3

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Please see Fig. 3 in Reetz, M. T., M. Bocola, J. D. Carballeira, D. Zha, and A. Vogel. “Expanding the Range of Substrate Acceptance of Enzymes: Combinatorial Active-Site Saturation Test.” *Angew. Chem. Int. Ed.* 44(2005): 4192-4196.