7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008

# Enzyme evolution using phage display

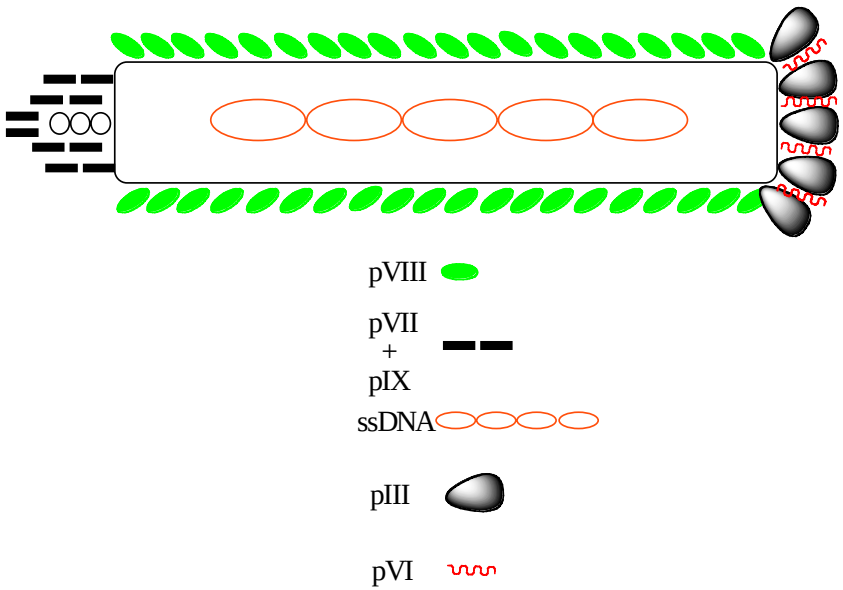
Ponsard, I.; Galleni, M.; Soumillion, P.; Fastrez, J. Selection of metalloenzymes by catalytic activity using phage display and catalytic elution. *Chembiochem* **2001**, *2* 253-259.

Strobel, H.; Ladant, D.; Jestin, J.L. *In vitro* selection for enzymatic activity: a model study using adenylate cyclase. *J. Mol. Biol.* **2003**, *332*, 1-7.

Pedersen, H.; Holder, S.; Sutherlin, D.P.; Schwitter, U.; King, D.S.; Schultz, P.G. A method for the directed evolution and functional cloning of enzymes. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 10523-10528.

# Phage display: the basics

## M13 phage particle



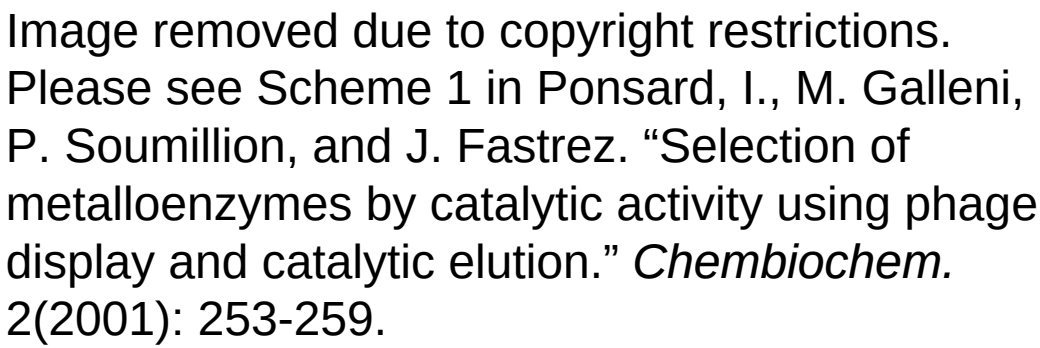

## Phagemid

Image removed due to copyright restrictions. Please see: Fig. 2 in <http://www.biology-medicine.com/Technical-Articles/Molecular-Biology/2008-01-11/90.html>

# Enzyme evolution using phage display and catalytic elution

- What are the authors trying to do?
- How does catalytic elution work?  (explain figure 1)
- How is the phage-bound enzyme characterized?  What do the authors find?
- How is the elution process assessed?  What does the term enrichment factor mean?
- What is the point of the three sets of model experiments?
- What are the results of the evolution?
- Is this strategy general?  What are the benefits and pitfalls?

# Catalytic elution



# Characterization of phage

Image removed due to copyright restrictions. Please see Fig. 1 in Ponsard, I., M. Galleni, P. Soumillion, and J. Fastrez. “Selection of metalloenzymes by catalytic activity using phage display and catalytic elution.” *Chembiochem.* 2(2001): 253-259.

# Adenylate cyclase paper

- What are the authors trying to do?  Explain their method.
- How do the authors design their substrate?
- How are reactive phage identified?
- How do they test of AC activity can be selected?
- What are the results?
- Is this strategy general?  What are the benefits and pitfalls?

# Cartoon of strategy

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Please see Fig. 1 in Strobel, H., D. Ladant, and J. L. Jestin. "*In vitro* selection for enzymatic activity: a model study using adenylate cyclase." *J. Mol. Biol.* 332(2003): 1-7.

# Schultz method for phage display enzyme evolution

- What are the authors trying to do?  Explain their method.
- How do the authors design their substrate?  Why do they do it this way?
- How are reactive phage identified?
- Can you get any information about enzyme kinetics using phage display?
- What are the results?
- Is this strategy general?  What are the benefits and pitfalls?

# Acid/Base peptide for covalent substrate attachment

Image removed due to copyright restrictions. Please see Fig. 2 in Pedersen, H., S. Holder, D. P. Sutherlin, U. Schwitter, D. S. King, and P. G. Schultz. "A method for the directed evolution and functional cloning of enzymes." *PNAS* 95(1998): 10523-10528.

# Results

Image removed due to copyright restrictions. Please see Fig. 3 in Pedersen, H., S. Holder, D. P. Sutherlin, U. Schwitter, D. S. King, and P. G. Schultz. "A method for the directed evolution and functional cloning of enzymes." *PNAS* 95(1998): 10523-10528.

# Method for enzyme/substrate display on phage

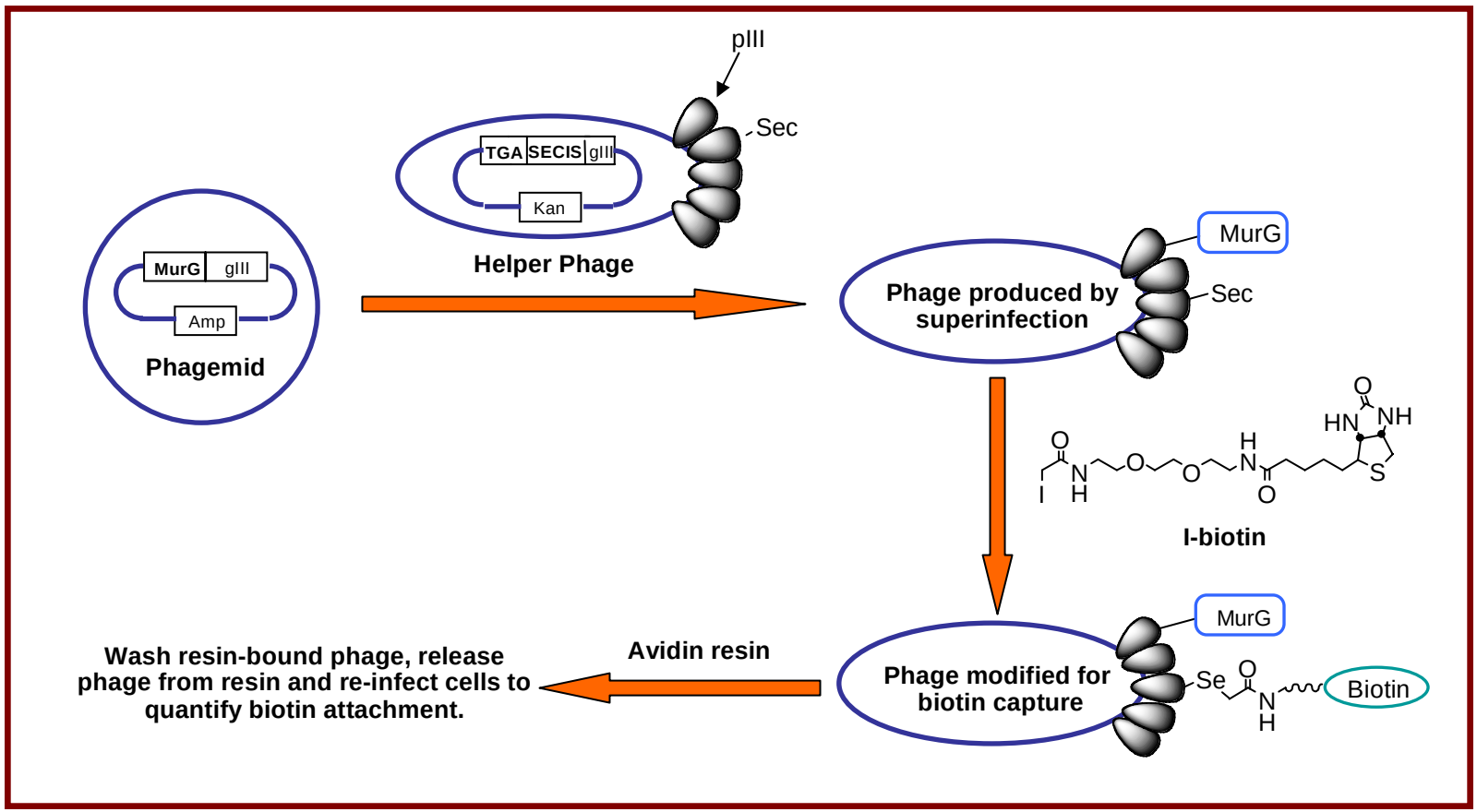


Image adapted from Scheme 1 in Love, K. R., J. G. Swoboda, C. J. Noren, and S. Walker. *ChemBioChem*. 7(2006): 753-756.

# Sec attachment strategy for MurG evolution