iGEM 2015: Evolution through time and SPACE-P

The adaptation and connection of PACE, BACTH and Affibodies to the new project, Structural Phage Assisted Continuous Evolution of Proteins

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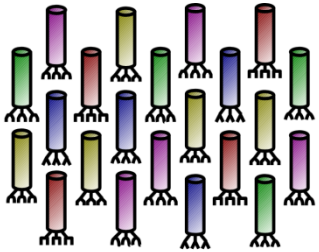
Motivation from Phage Display

Diversity phage display: 10^8-10^9

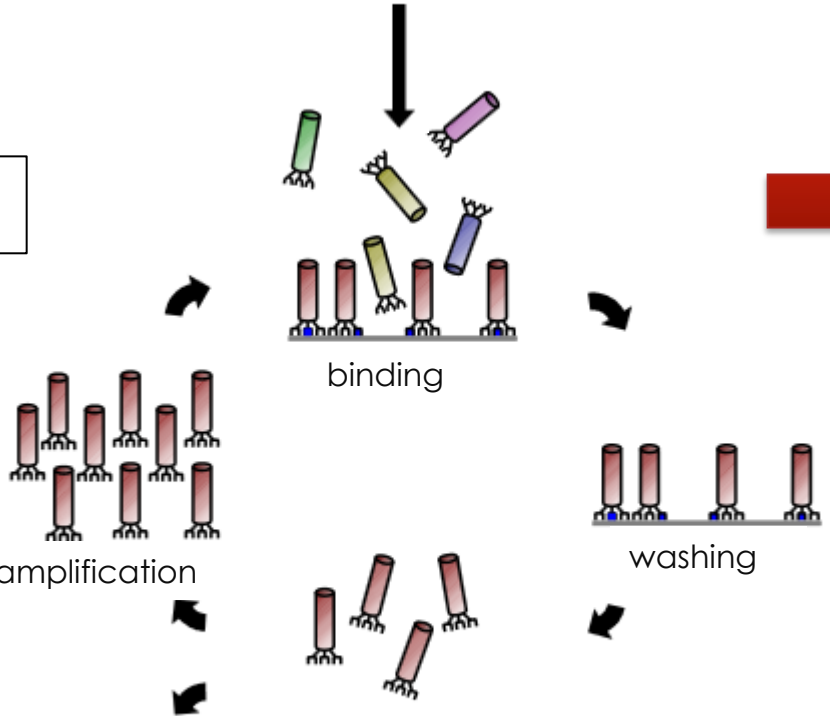
All possibilities for ~ 7 aa

(20^7=1.3×10^9)



Directed

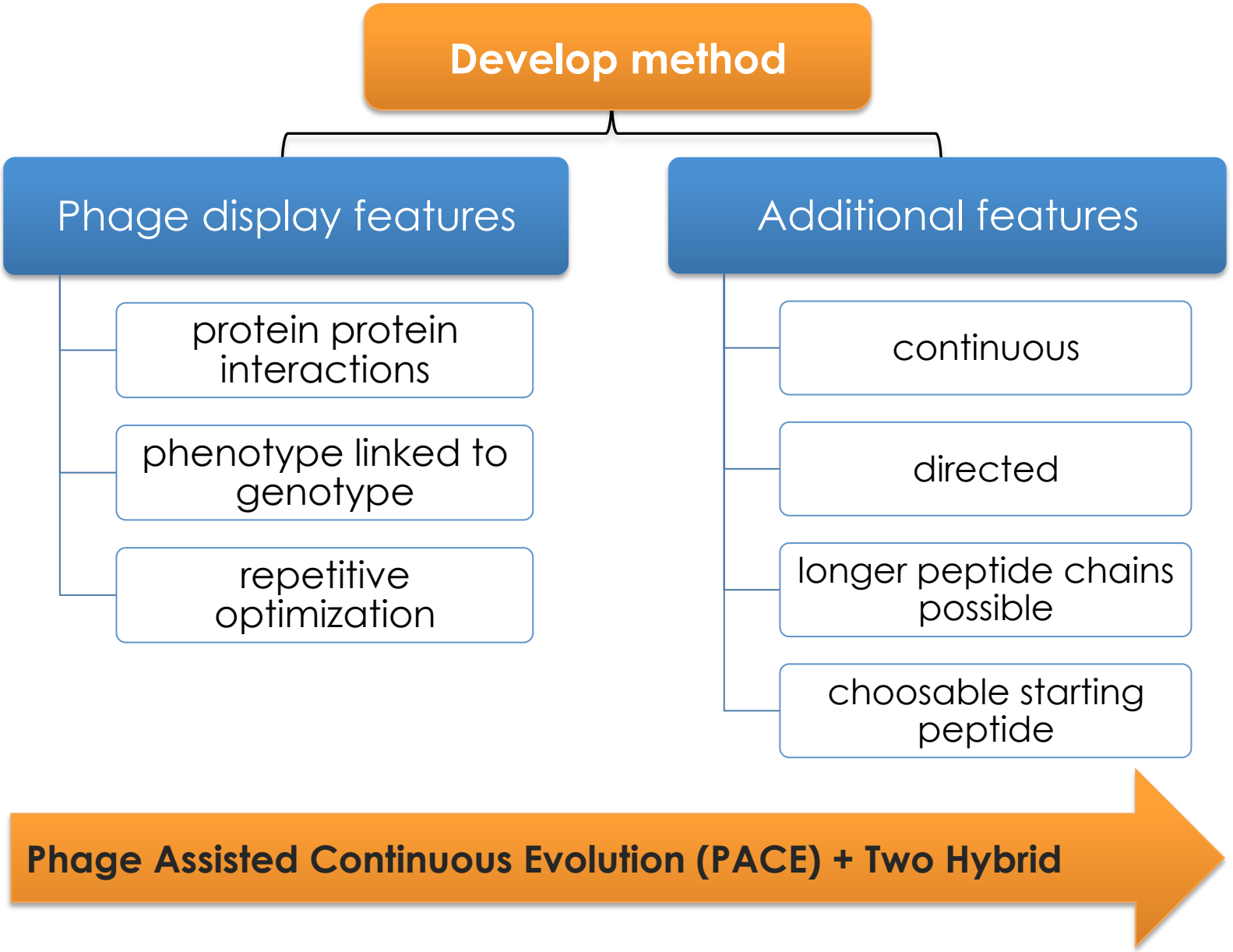
Iterative



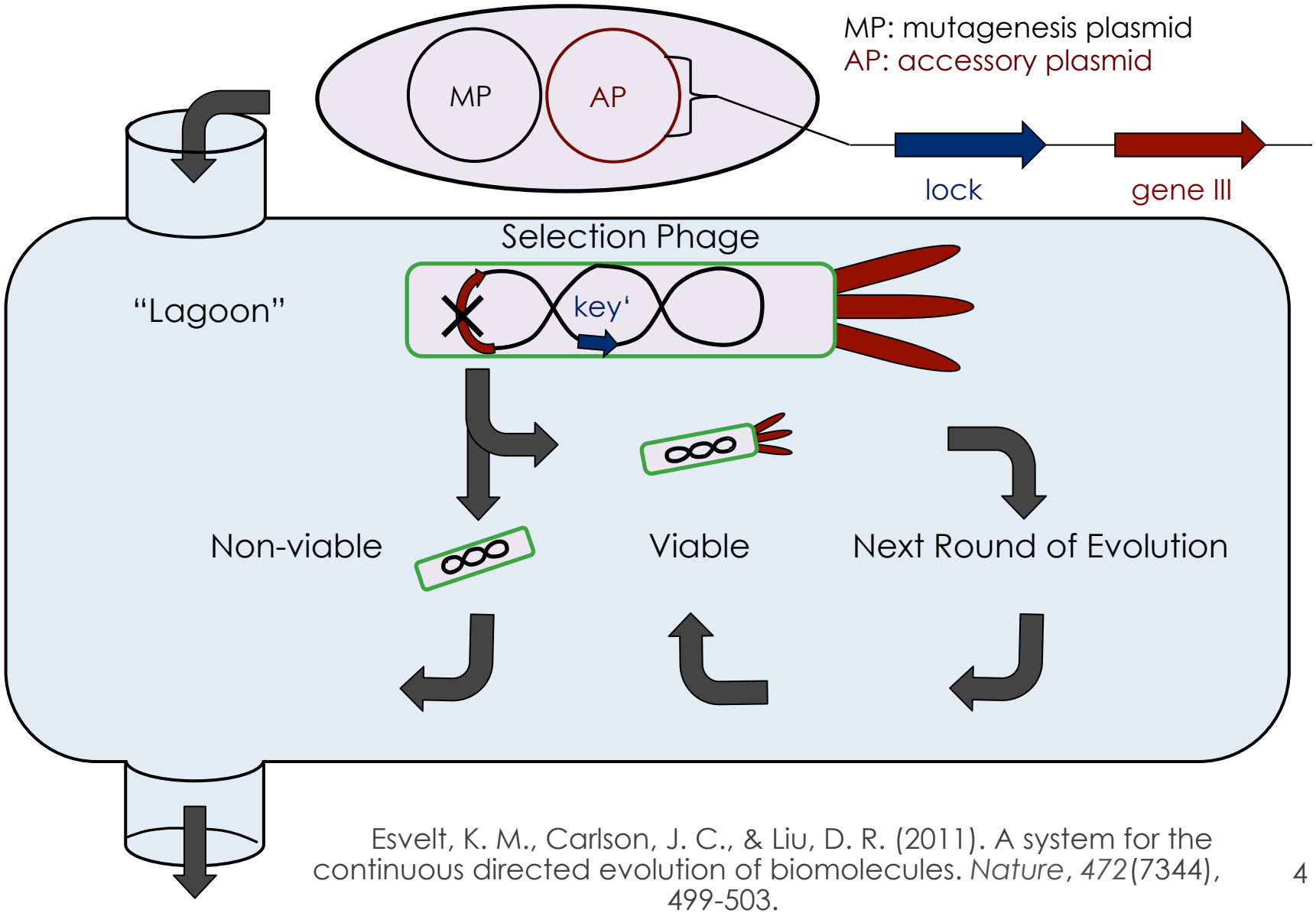
Continuous

www.wikimedia.org

Aim of the Project

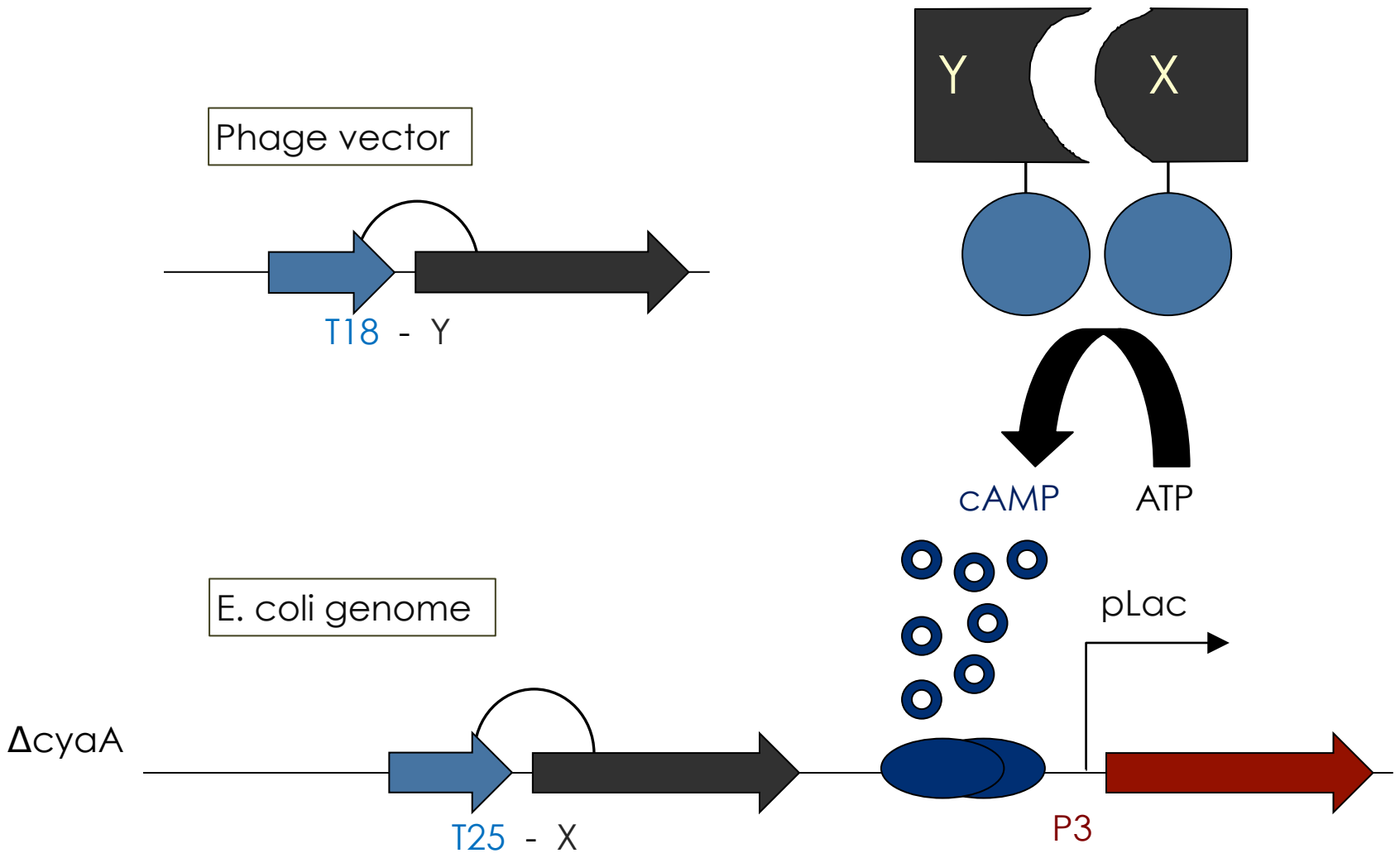


Phage Assisted Continuous Evolution



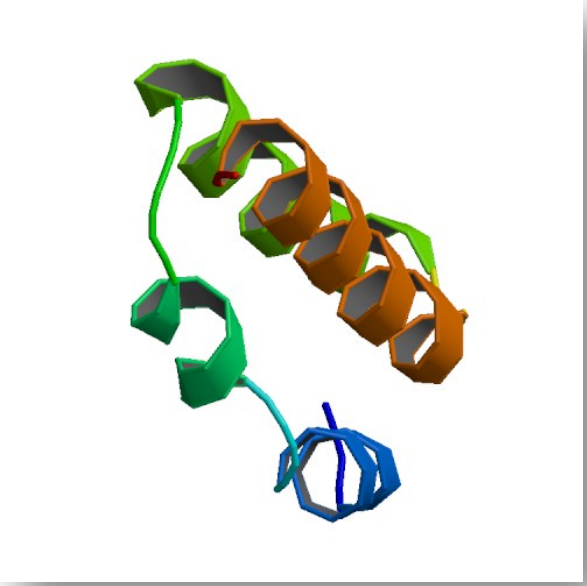
Esvelt, K. M., Carlson, J. C., & Liu, D. R. (2011). A system for the continuous directed evolution of biomolecules. *Nature*, *472*(7344), 499-503.

Combination with BACTH



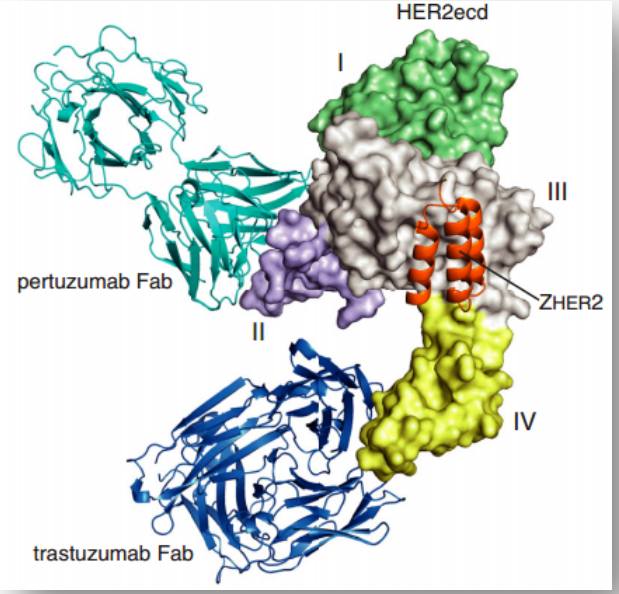
Battesti, A., & Bouveret, E. (2012). The bacterial two-hybrid system based on adenylate cyclase reconstitution in Escherichia coli. Methods, 58(4), 325-334.

X & Y Choice



Y: Scaffold protein – affibody

- highly stable core
- variable regions
- no disulfide bridges



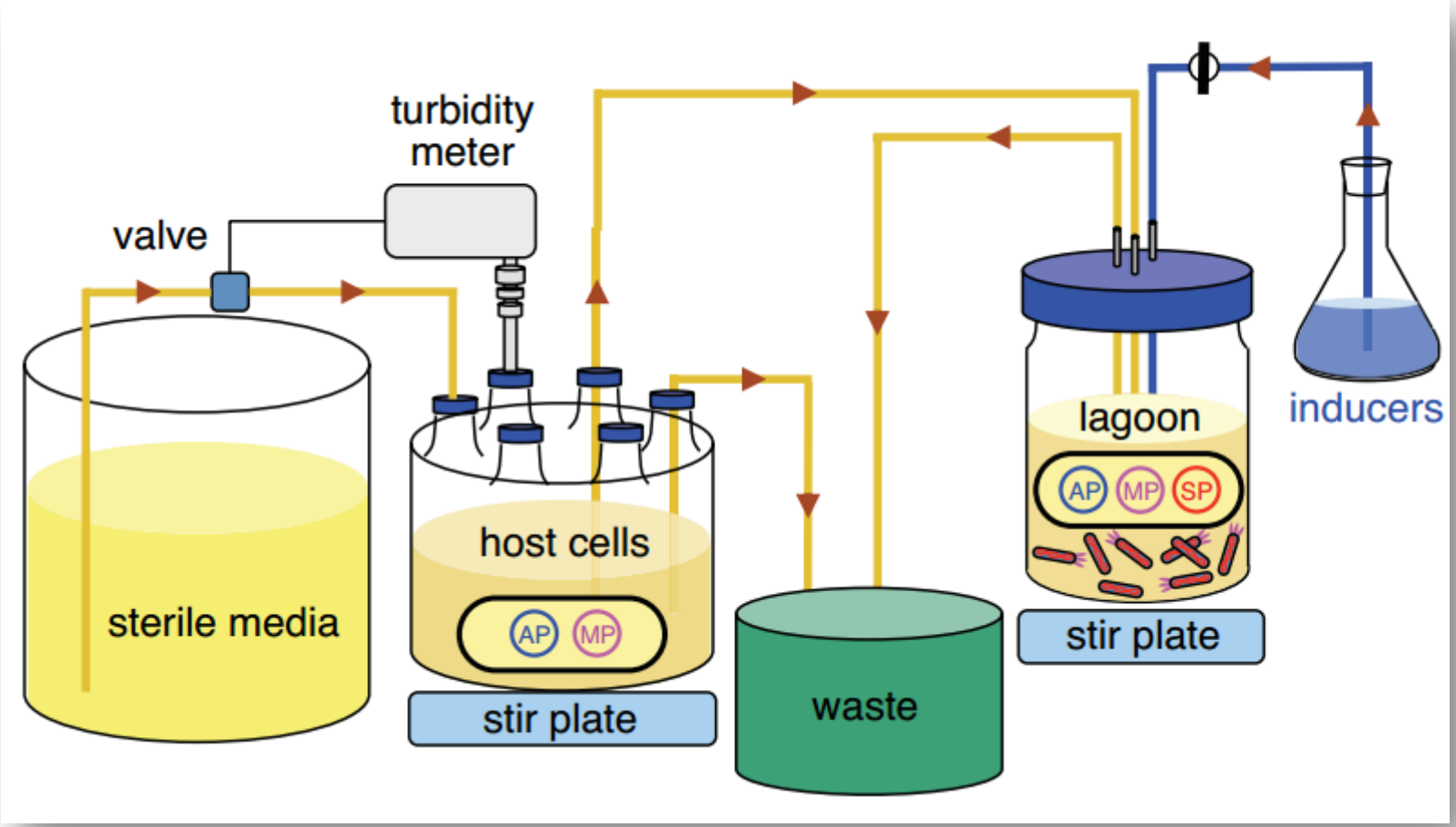
X: human epidermal growth factor receptor (HER2)

- established positive control
- of scientific interest

long-term goal → **replacement of antibodies**

Eigenbrot, C., Ultsch, M., Dubnovitsky, A., Abrahmsén, L., & Härd, T. (2010). Structural basis for high-affinity HER2 receptor binding by an engineered protein. *Proceedings of the National Academy of Sciences, 107*(34), 15039-15044.

Planned Setup



Conclusion

SPACE-P is a simple tool that aims to enhance the development and detection of protein-protein interactions.

The System combines methods from both PACE and BACTH.

This method has promising applications to develop new affibodies for targeting epitopes of interest.

It may be a potential replacement for currently less efficient techniques used in drug discovery and research.

Open Questions

? N- or C-terminal fusion of cyclase to X & Y

? T18/T25 fused to X/Y

? Parameters for setup

? Inducible promoter/riboswitch promoter for MP