



Harvard iGEM

2006

Drug Delivery

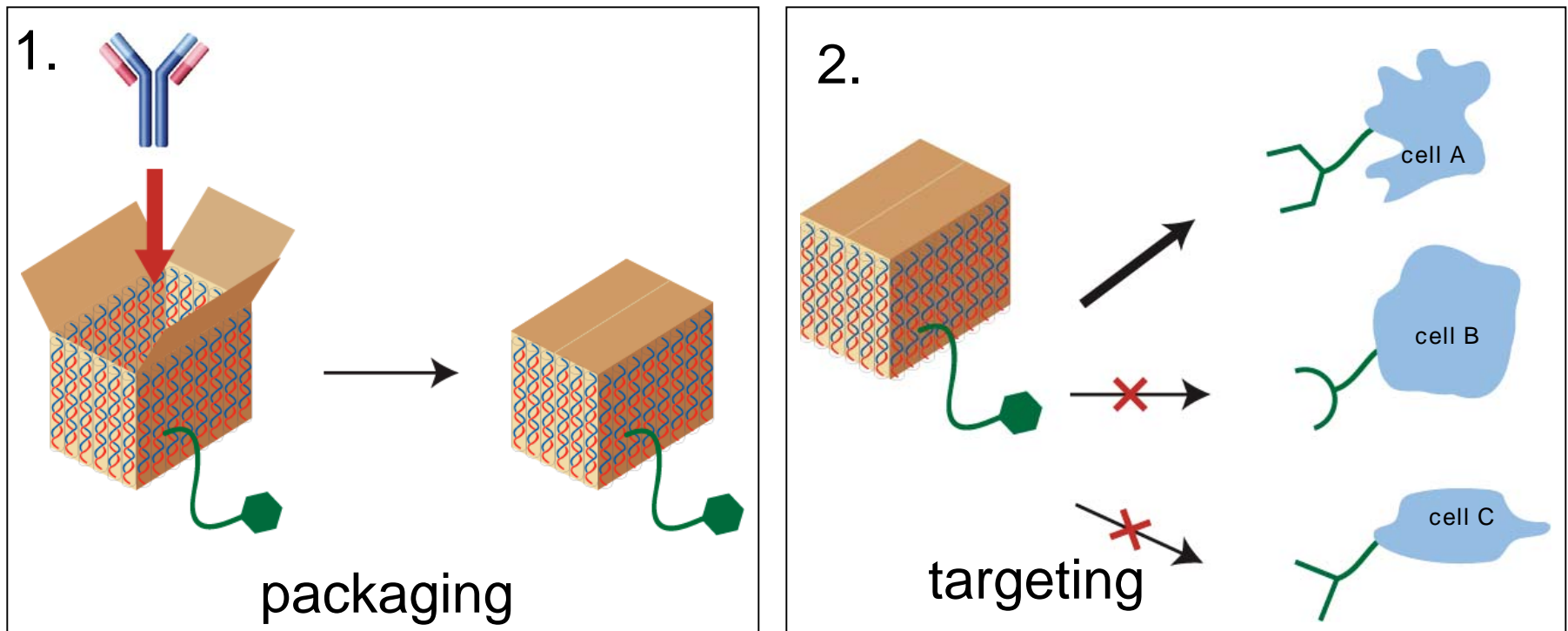
DNA Nanostructures

Cell Targeting

Bio-Oscillator

Cyanobacterial
Oscillator

Our Vision: modular drug delivery



Why DNA?

- Strong Watson-Crick base pairing
- Covalent modifications
- Relatively inexpensive
- Self-assembles
- **Highly programmable**

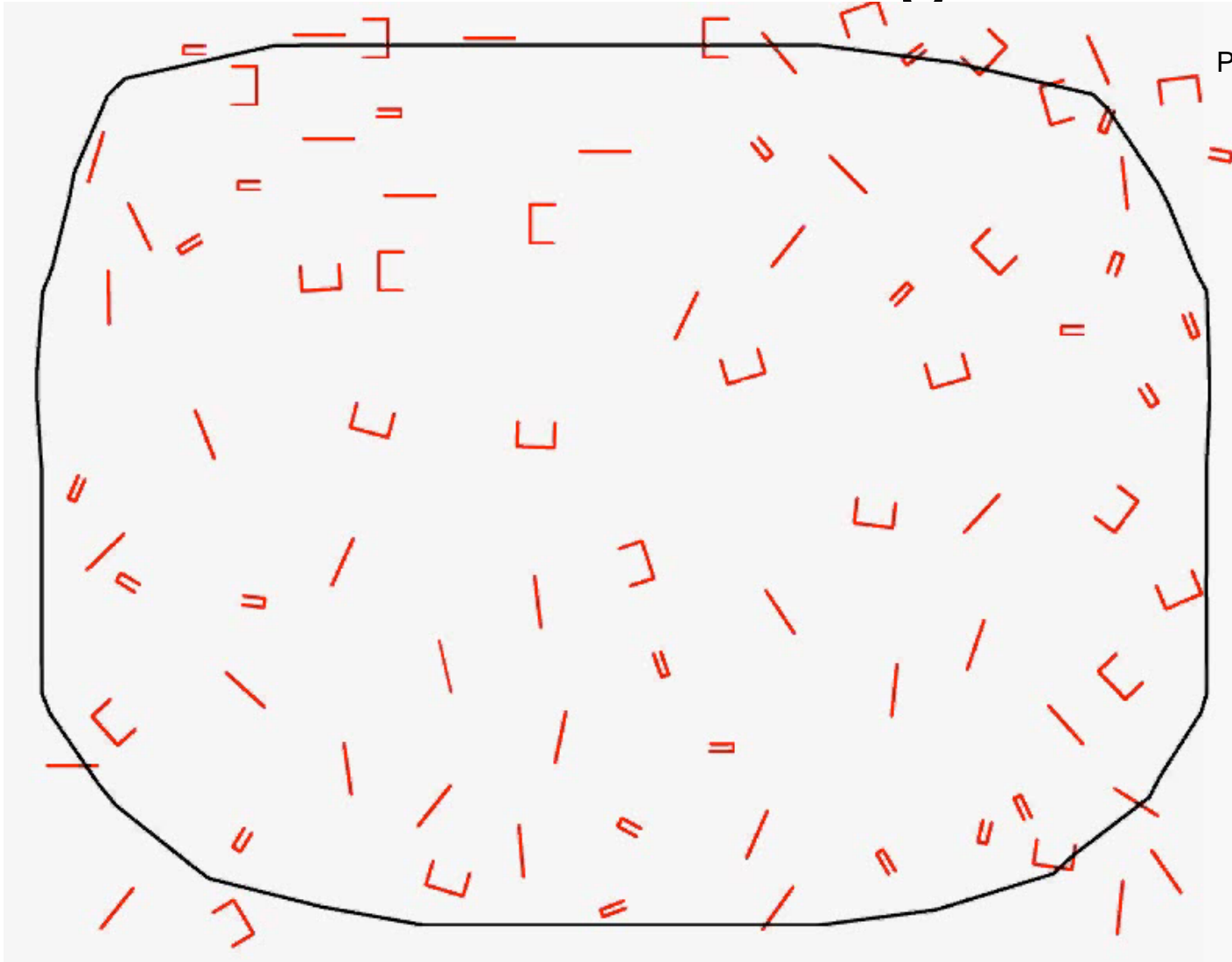


How: engineered crossing over

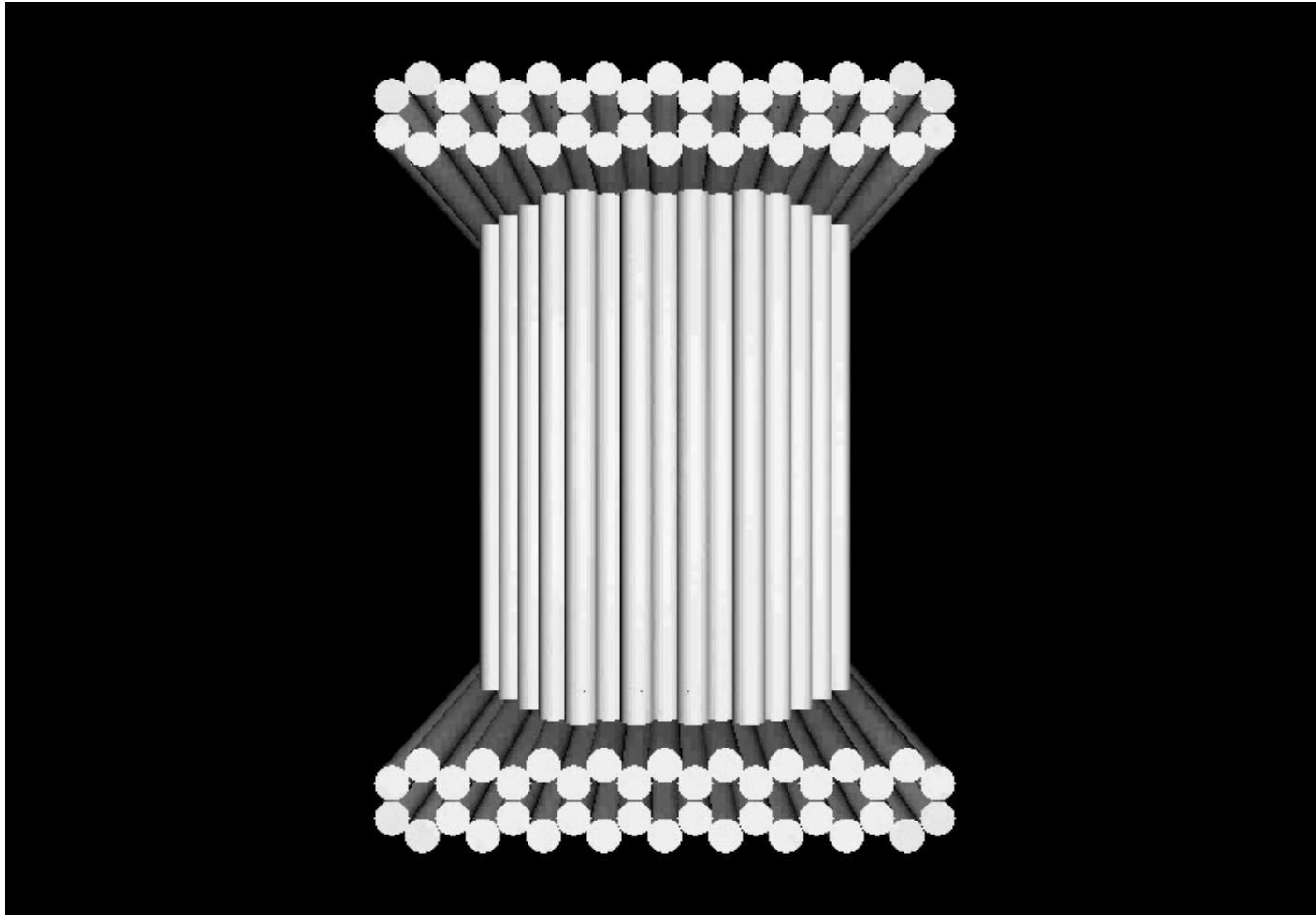


How: scaffolded origami

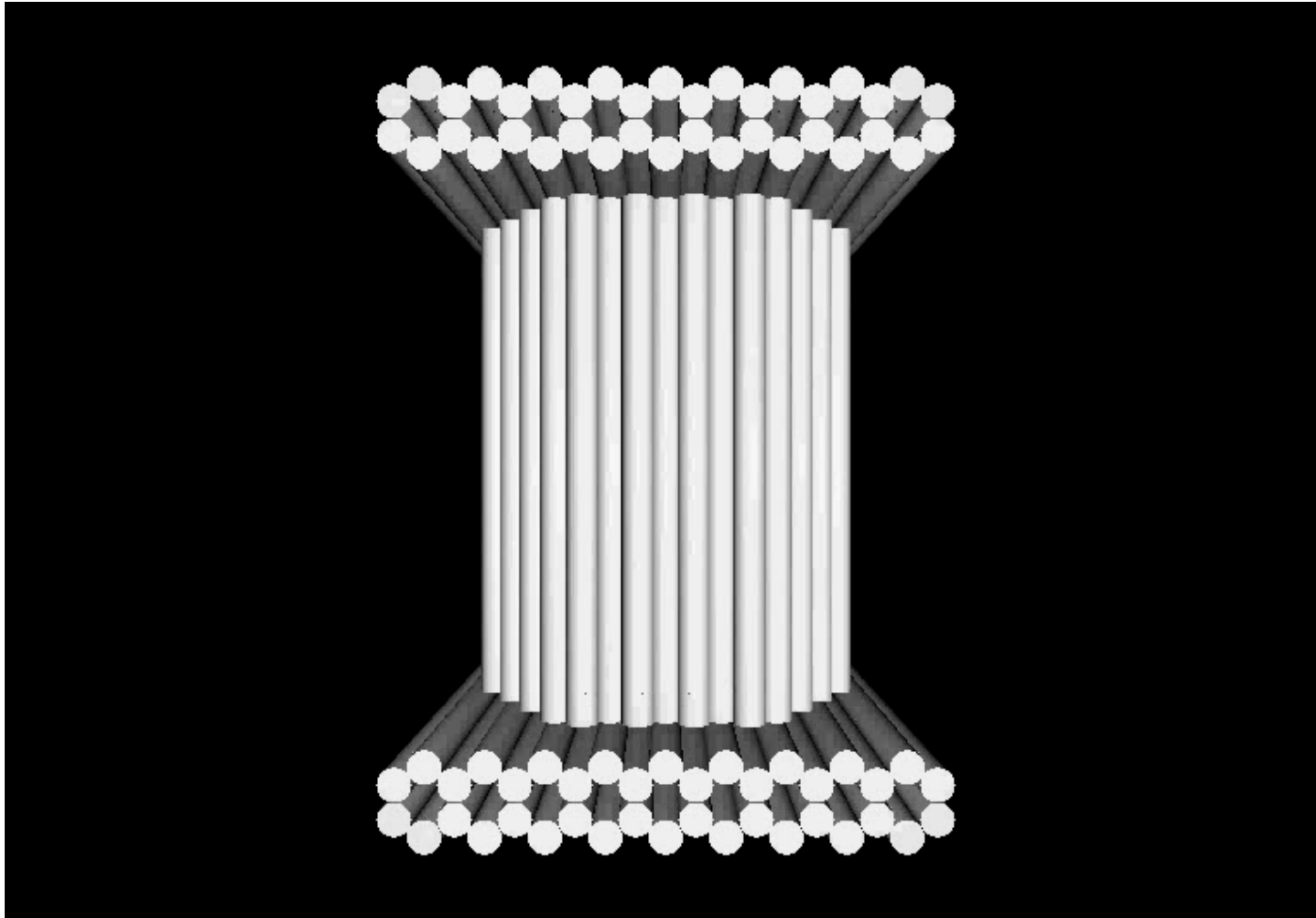
N. Seeman
P. Rothemund
W. Shih
et. al



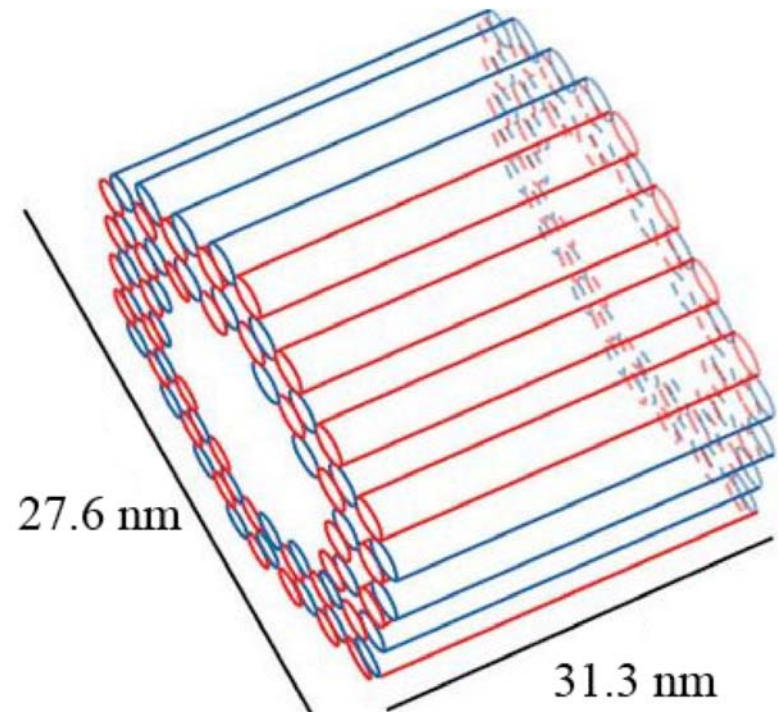
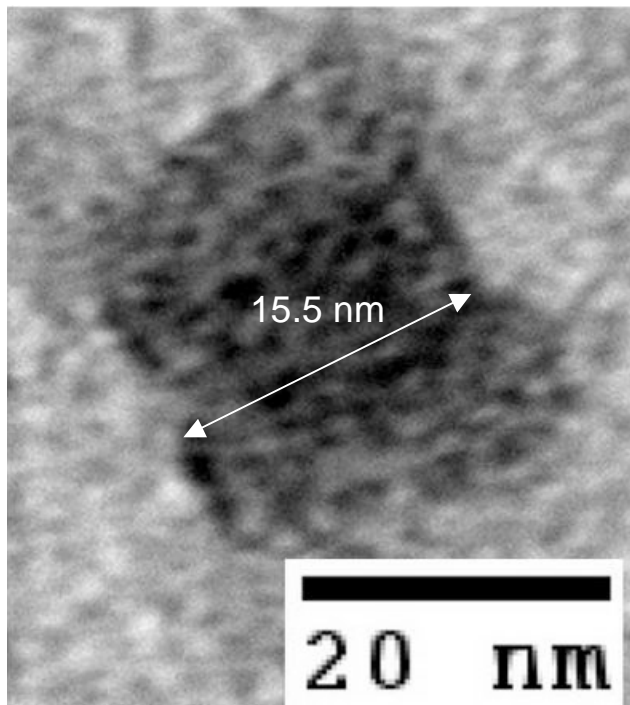
Our Design: a novel structure



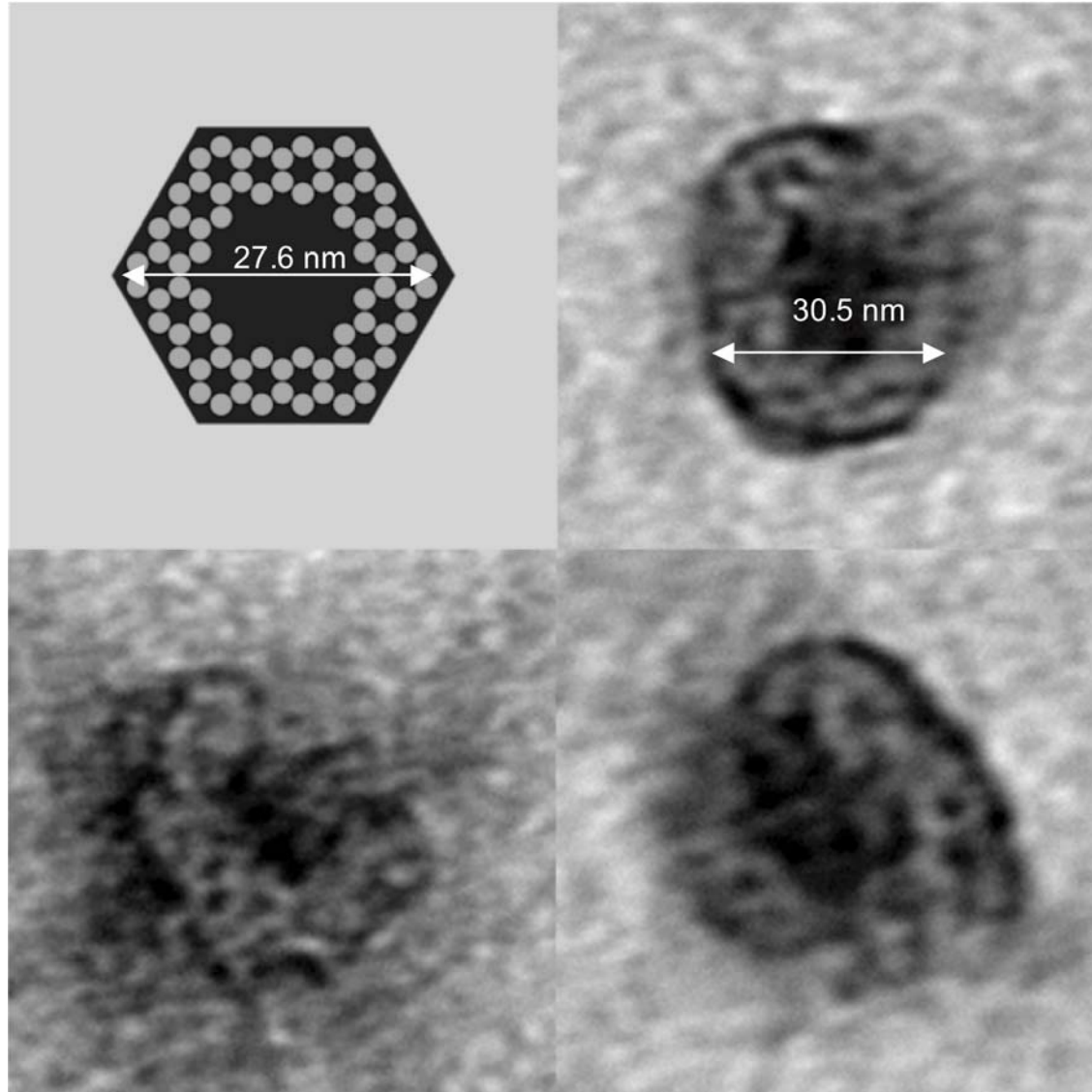
Our Design: a novel structure



Evidence: EM images

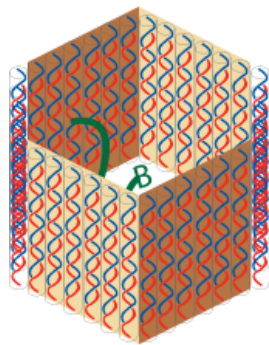


Evidence: EM images



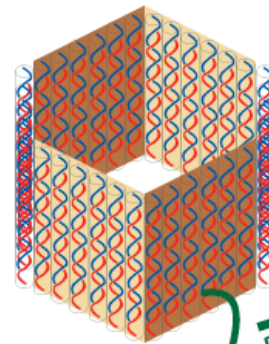
Evidence: protection assay

Protected biotin on the inside
of our container from large
streptavidin beads



Streptavidin bead

Payload protected



Streptavidin bead

Control: biotin bound



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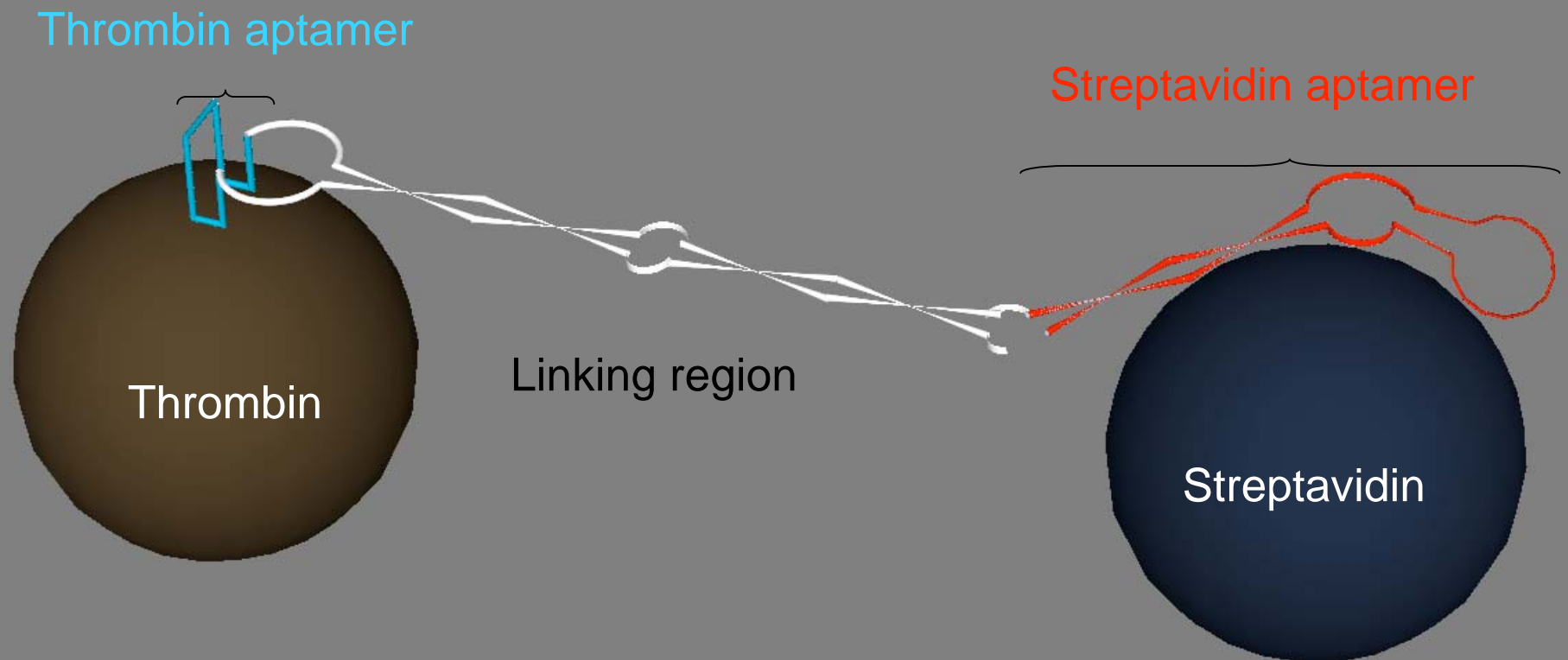
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Adaptamers



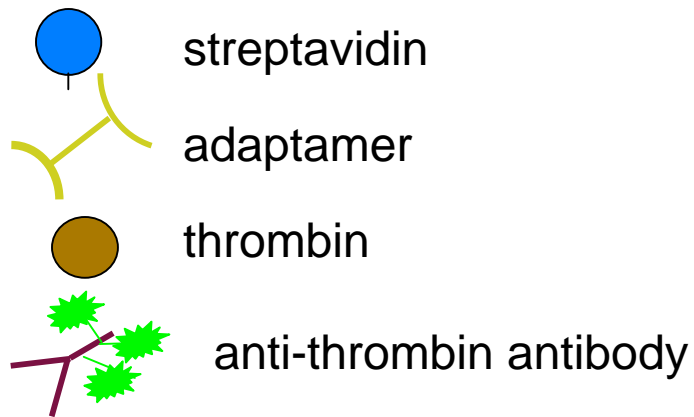
Aptamer: nucleic acid sequence that can bind a substrate with high specificity and affinity.

Why develop adaptamers?

- To offer a new way to **target substrates to cells.**
- To create a tool for studying **cell-cell interactions.**
- To provide a foundation for **engineered catalysts.**

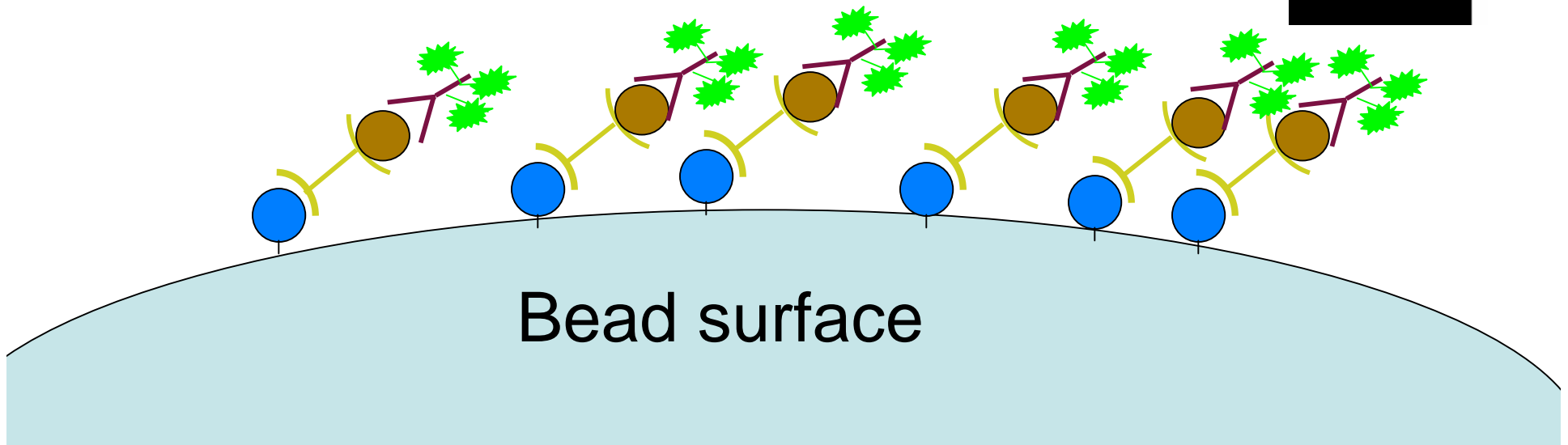
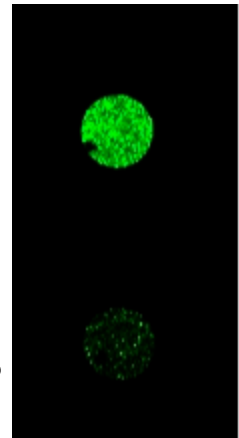


Adaptamer testing

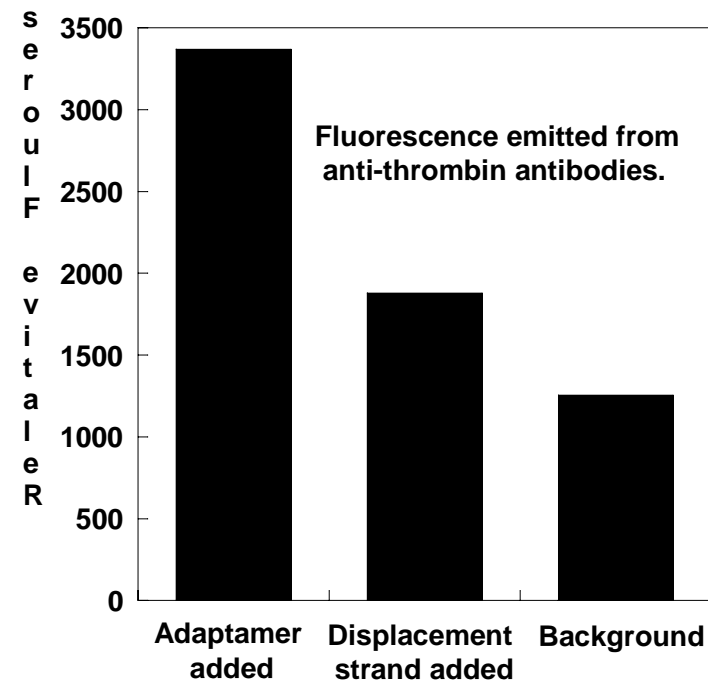
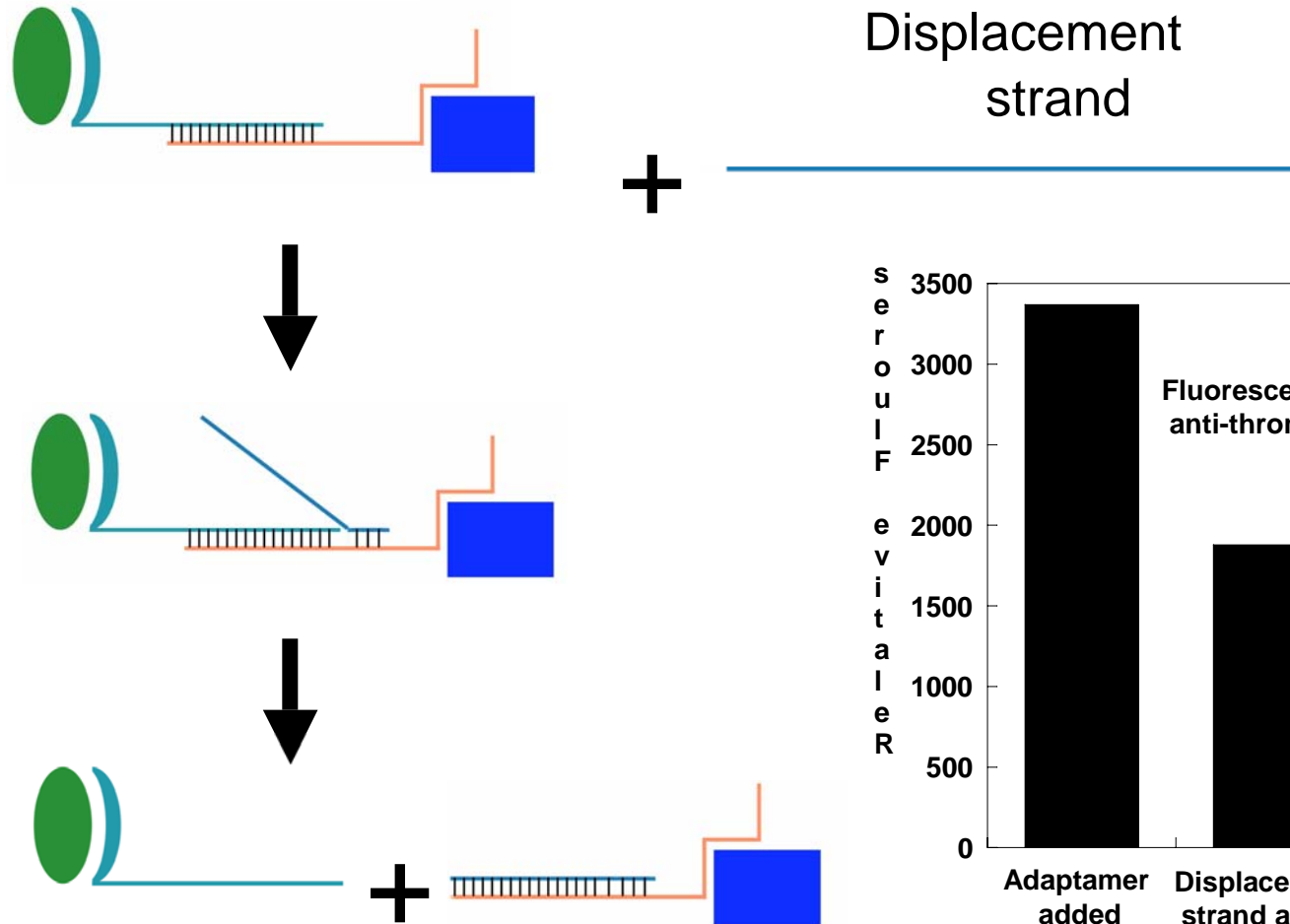


Adaptamer + thrombin

(-) control: naked beads



Adaptamer quenching



Cell Surface Targeting

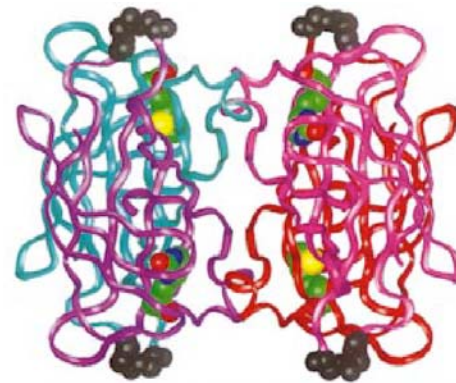
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Streptavidin on the cell surface

Streptavidin

- Binds strongly to biotin molecule
- Used to bind biotinylated nucleic acids or peptides



Big idea:

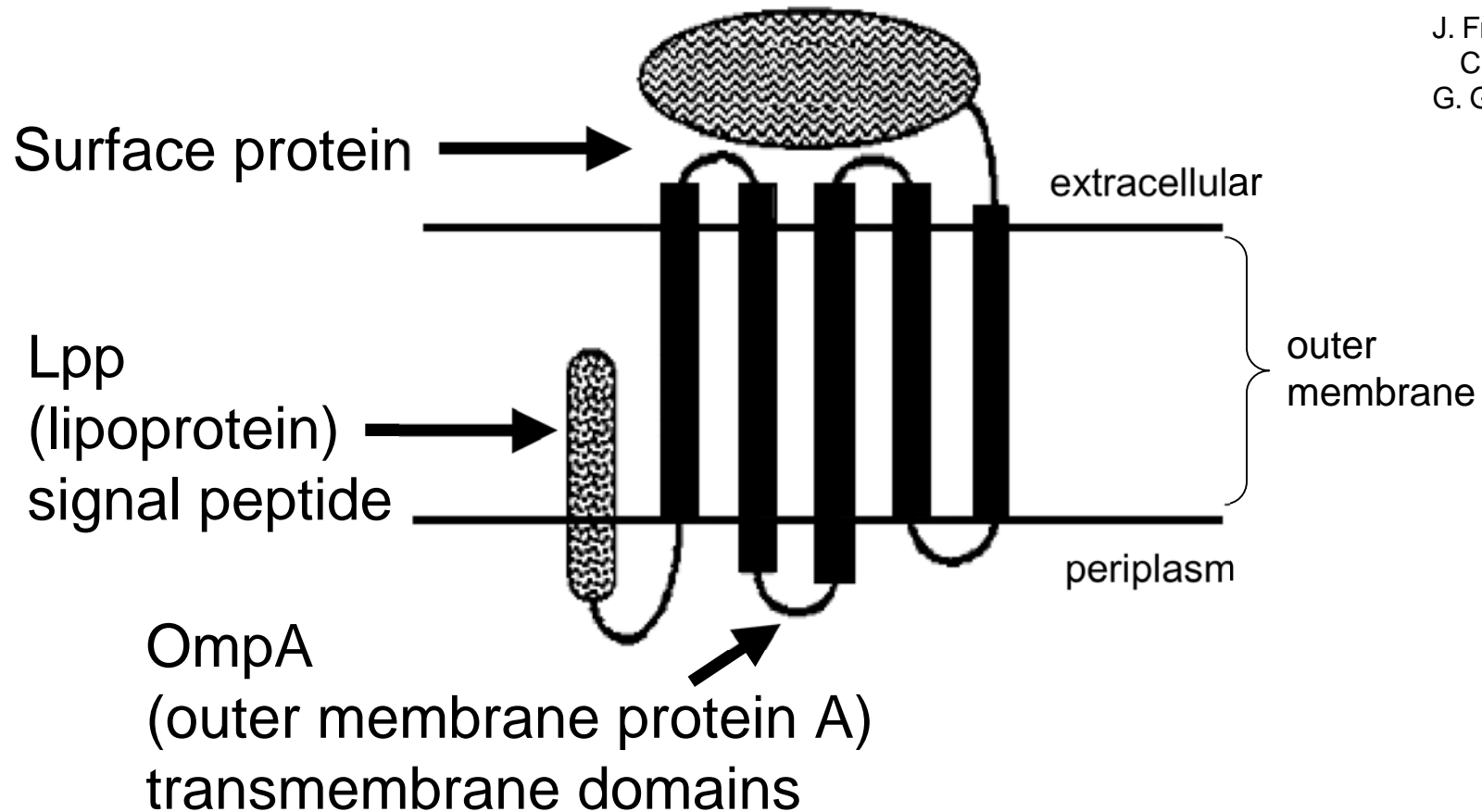
Streptavidin protein expressed on the cell surface can be used to target any biotinylated DNA/protein to the cell surface.

McDevitt, 1999



Lpp-OmpA surface display

J. Francisco
C. Earhart
G. Georgiou
1992



BioBricks assembly of protein domains

I. Phillips
P. Silver
2006

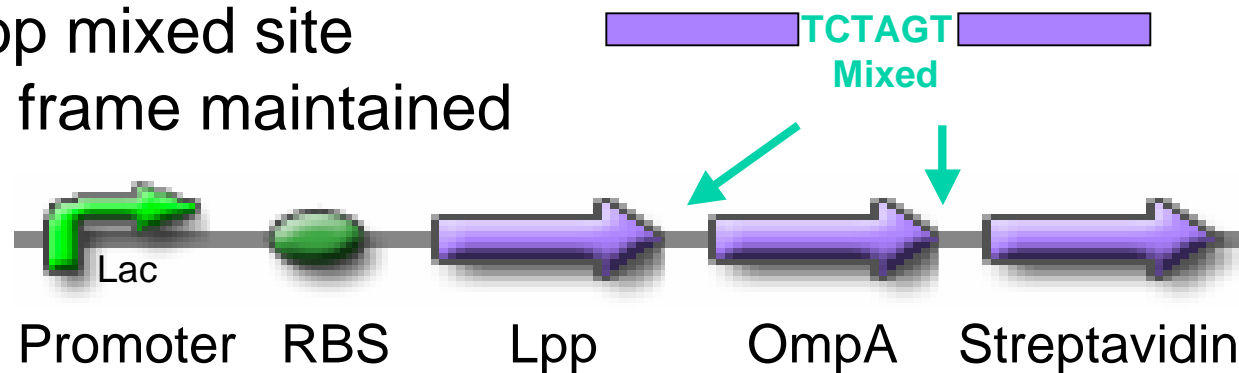
Standard BioBrick

5'...TCTAGAG [] TACTAGT...3'
XbaI SpeI

Protein domain BioBrick

5'...TCTAGA [] ACTAGT...3'
XbaI SpeI

6-bp mixed site
Reading frame maintained

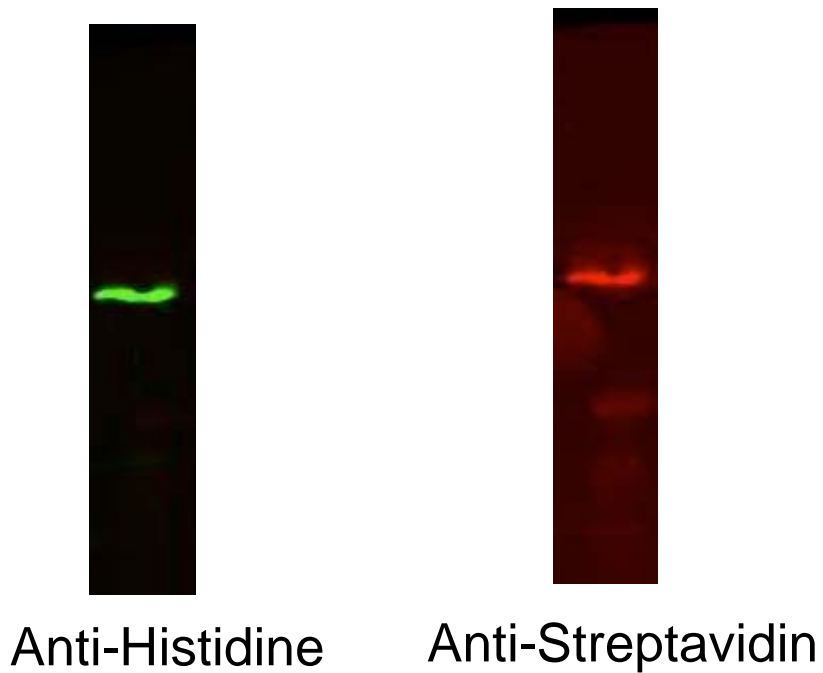


Cell Surface Targeting

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Expression of fusion protein in frame



- Inducible expression
- Histidine tag at end, so construct in frame
- Streptavidin domain recognized by Ab





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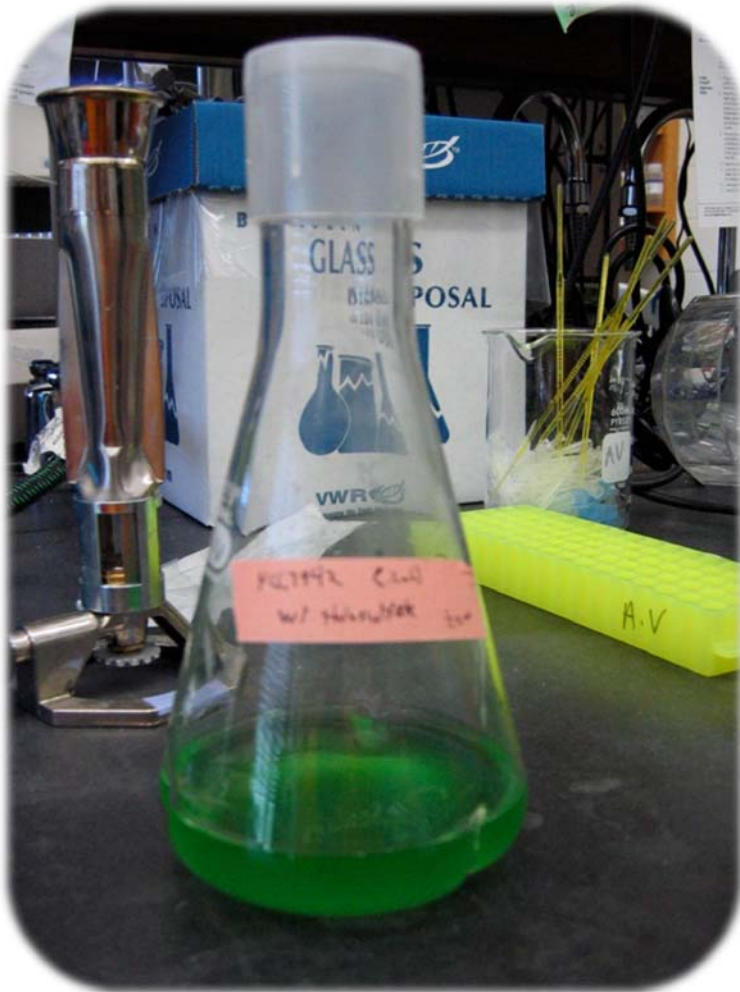
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From cyanobacteria...

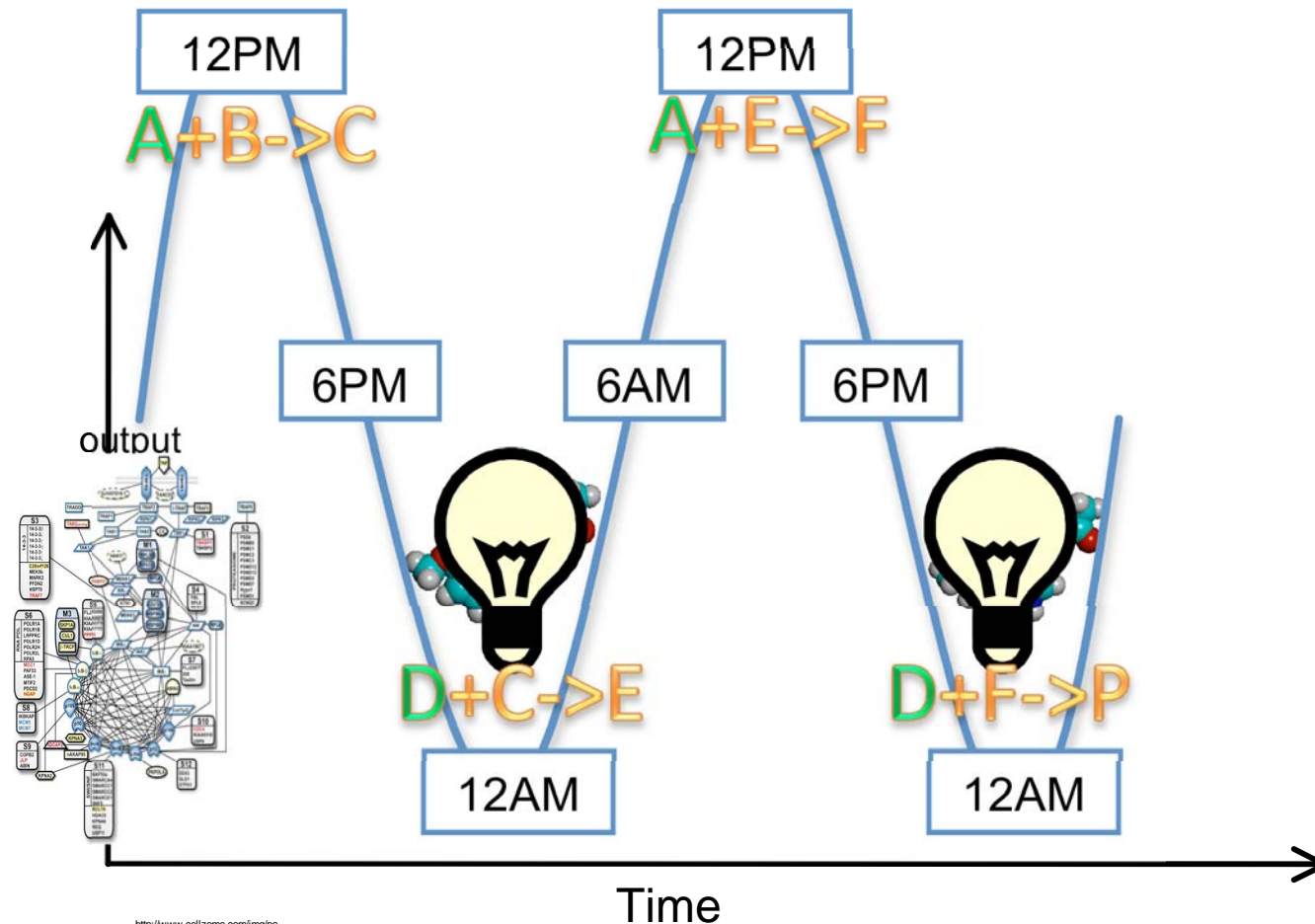
- Photosynthetic
- Circadian rhythm
- Evolved over billions of years

...to *E. coli*

- Model organism for synthetic biology
- BioBrick registry



Applications of a Bio-oscillator



- Clock
- Nightlight
- Timed drug delivery
- Pharmaceutical processes
- Bio-circuitry
- Investigate natural systems

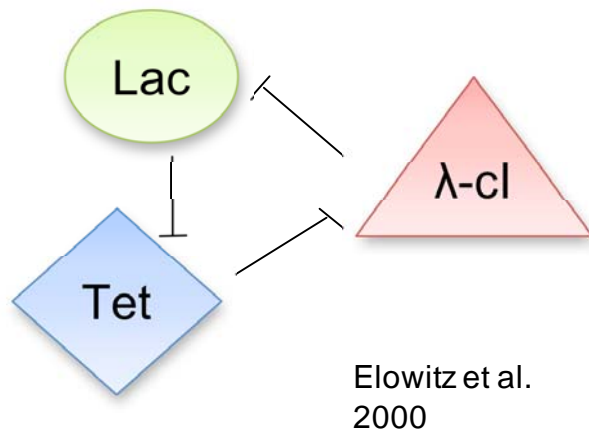
<http://www.cellzome.com/img/paths/ways.jpg>

Cyanobacterial Oscillator

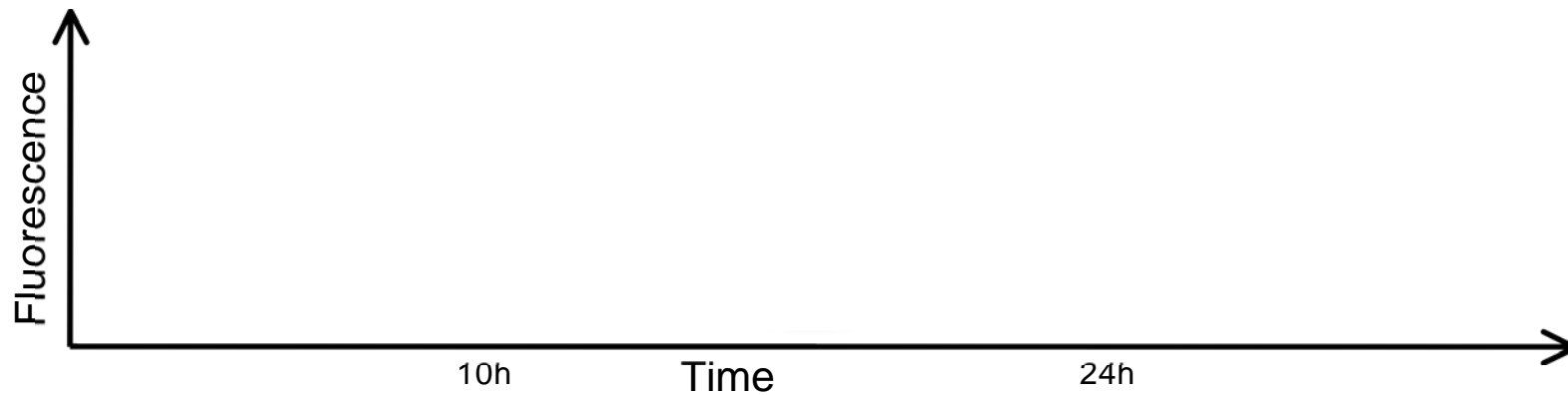
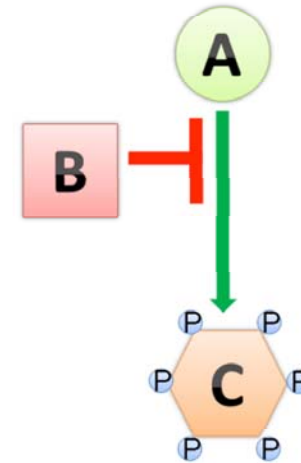
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The Repressilator



Cyanobacteria Bio-oscillator

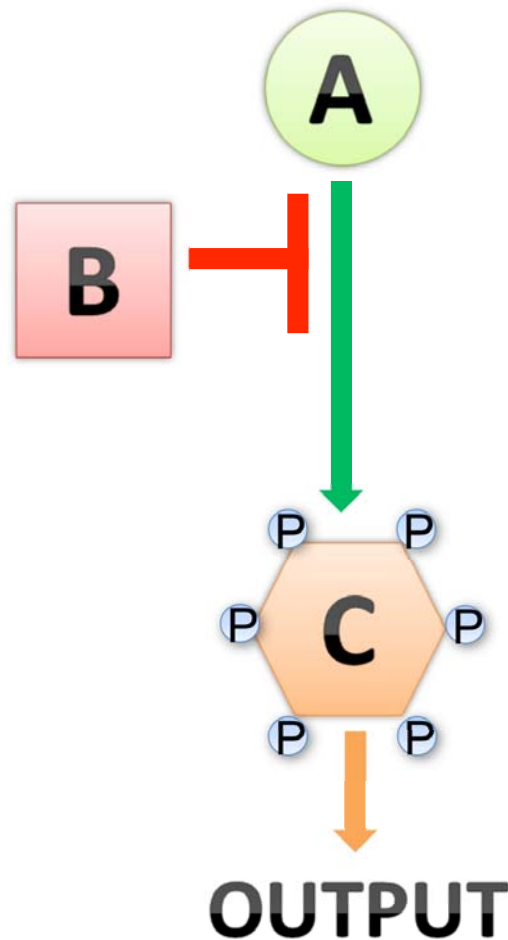


Cyanobacterial Oscillator

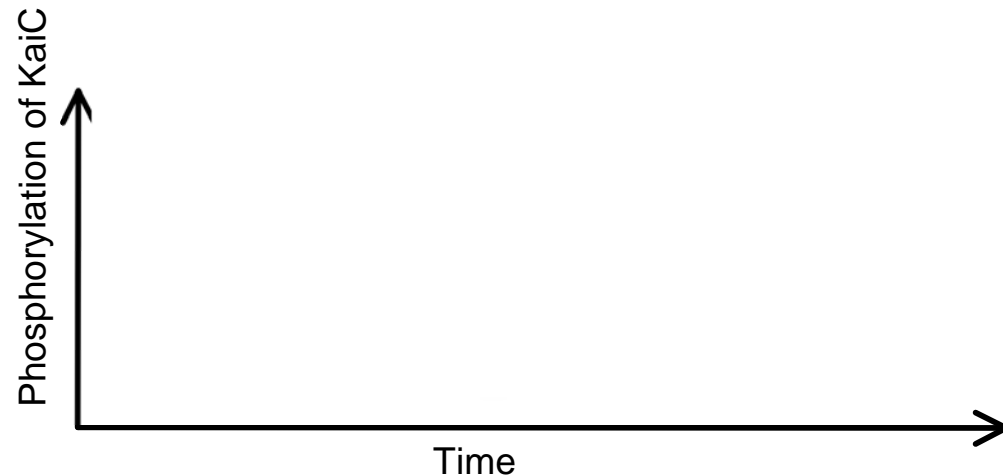
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The Kai Clock in Cyanobacteria



- KaiC autophosphorylates and dephosphorylates
- KaiA promotes phosphorylation
- KaiB inhibits KaiA
- Transcription-translation independent
- Period: 14-60 h



Achievements

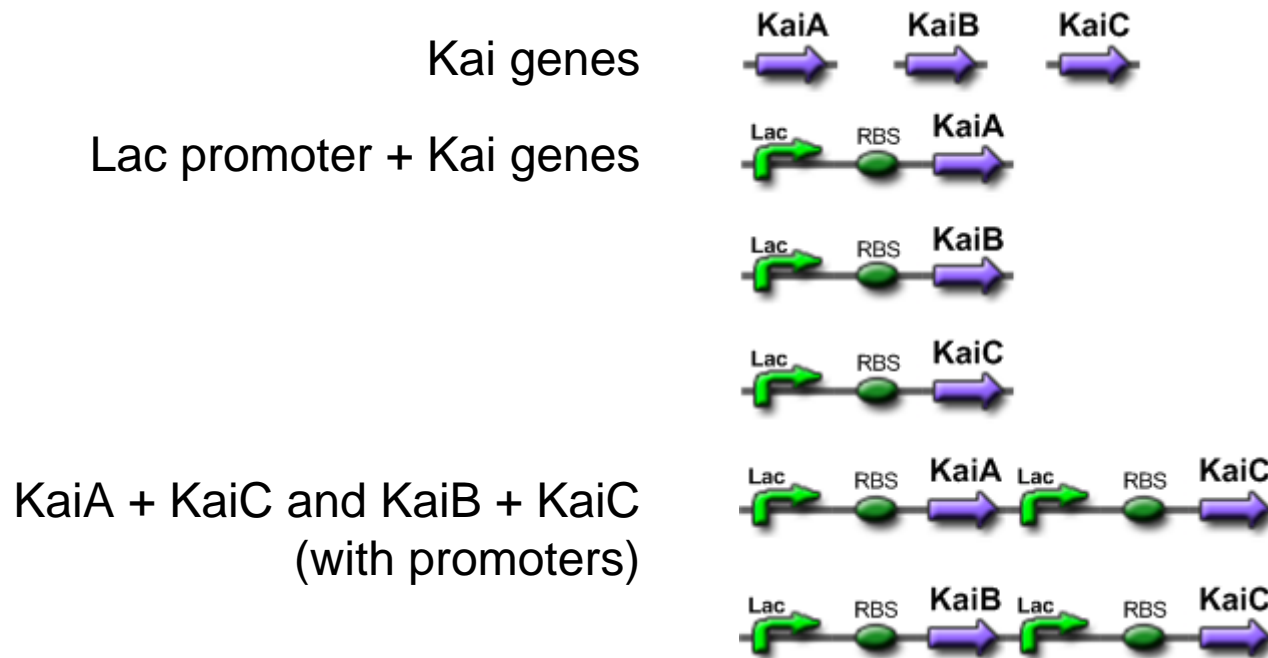
Goal: reconstitute the cyanobacteria Kai oscillator in *E. coli*

1. Created KaiA, KaiB, and KaiC BioBricks.
2. Combined the above with registry parts to form functional BioBricks.
3. Expressed Kai proteins in *E. coli* and verified interaction.

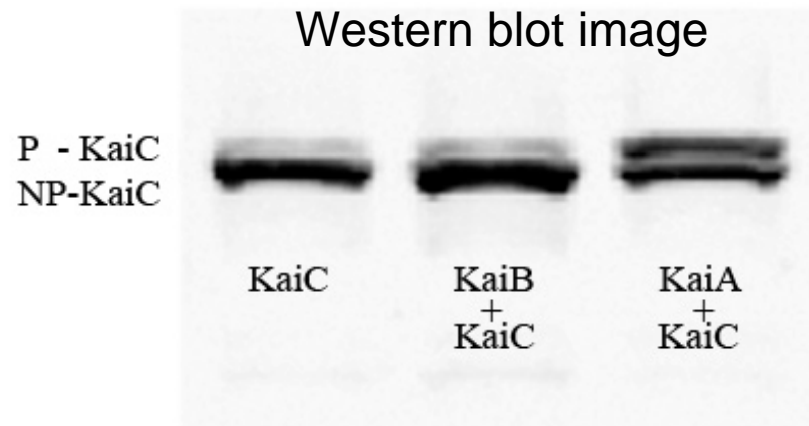


Results: Constructs Created

We've made the following constructs:

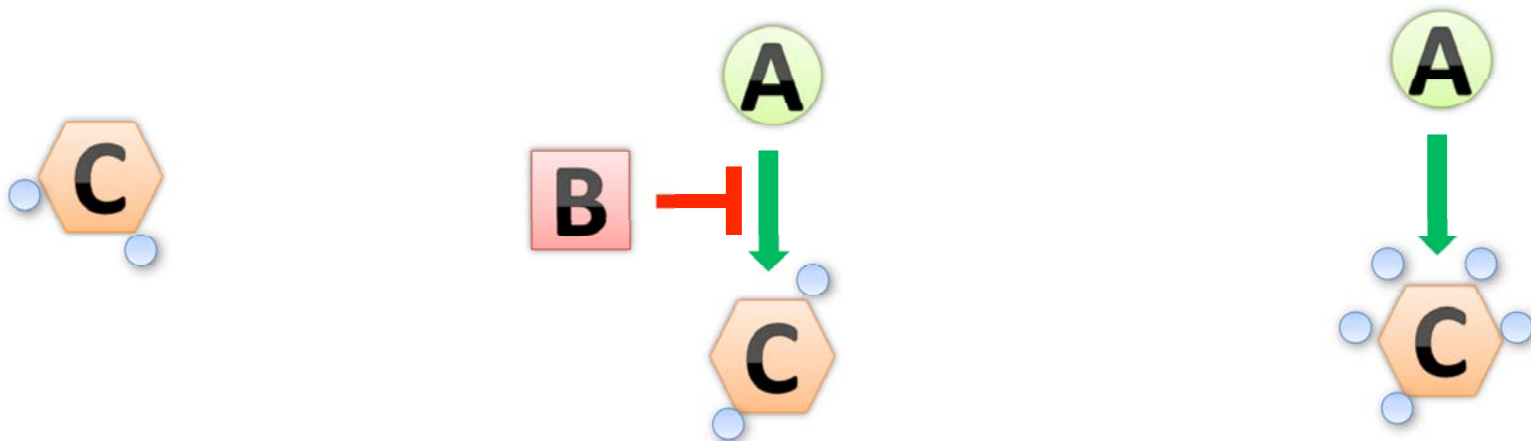
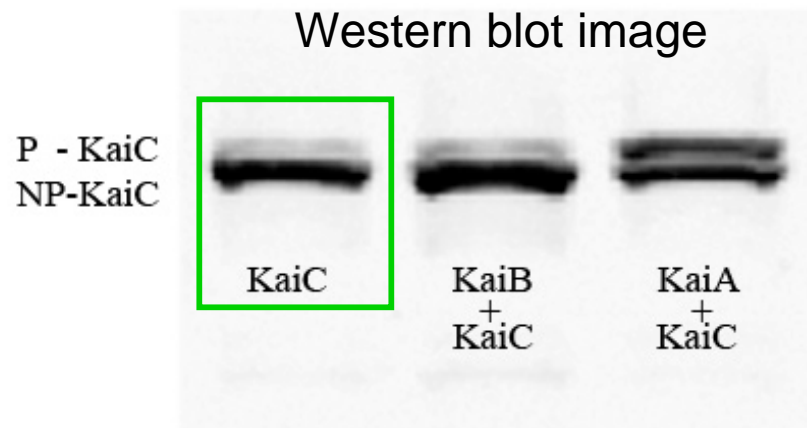


Results: Proteins Interact in *E. coli*

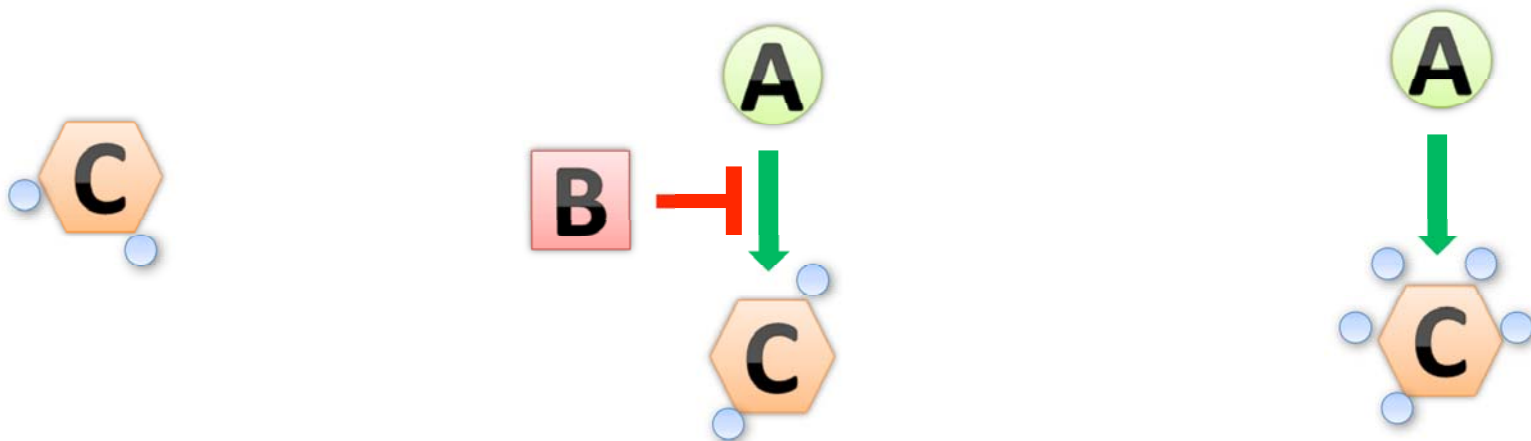
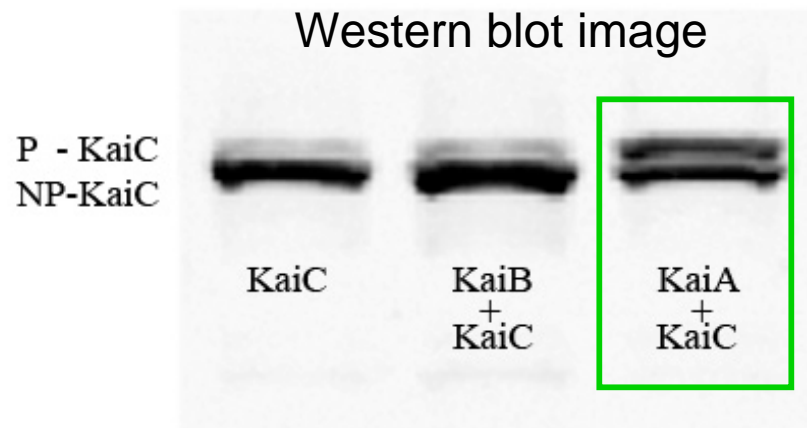


- Constructs transformed in *E. coli*
- Cultures sampled at OD 0.5
- Western blot probed with anti-KaiC antibodies

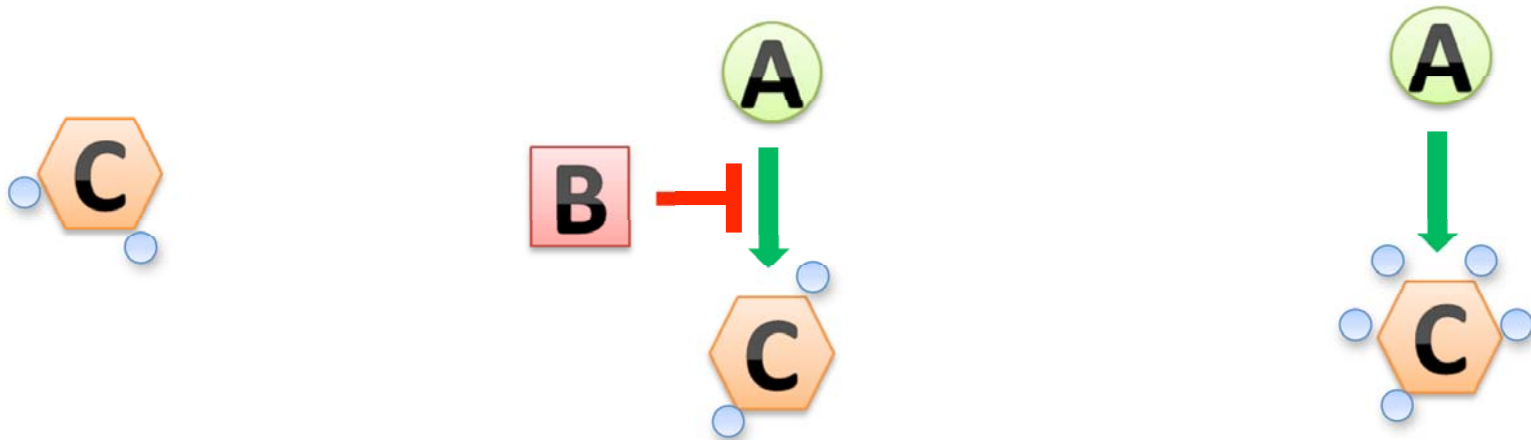
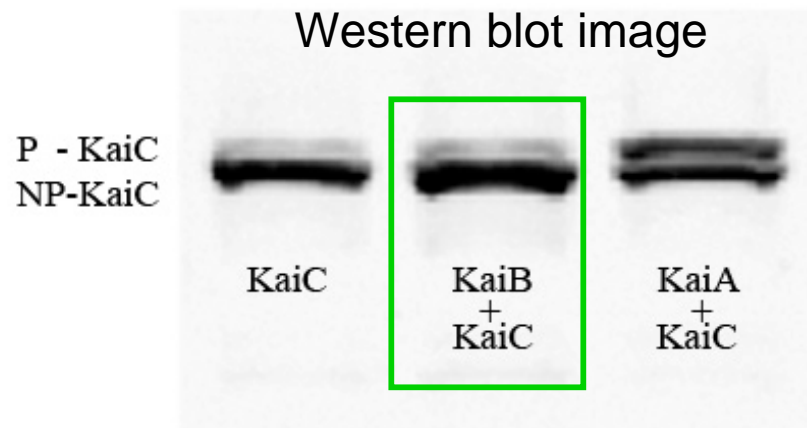
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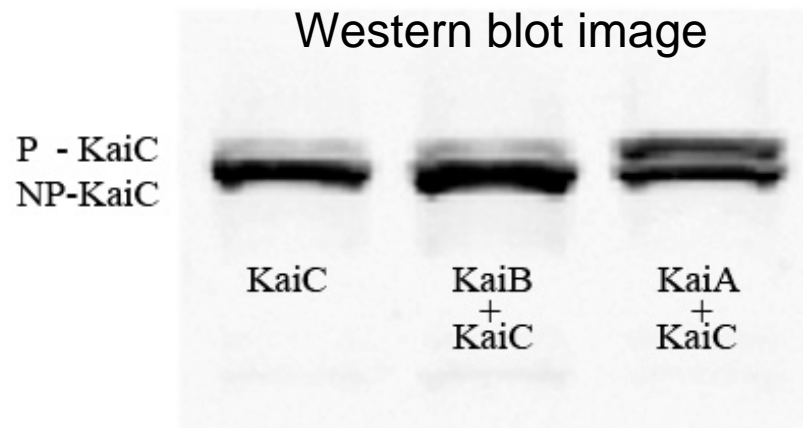
Results: Proteins Interact in *E. coli*



Results: Proteins Interact in *E. coli*



Results: Proteins Interact in *E. coli*



Conclusion:

- KaiA and KaiC are expressed and interacting
- KaiB not verified, but results consistent with predictions

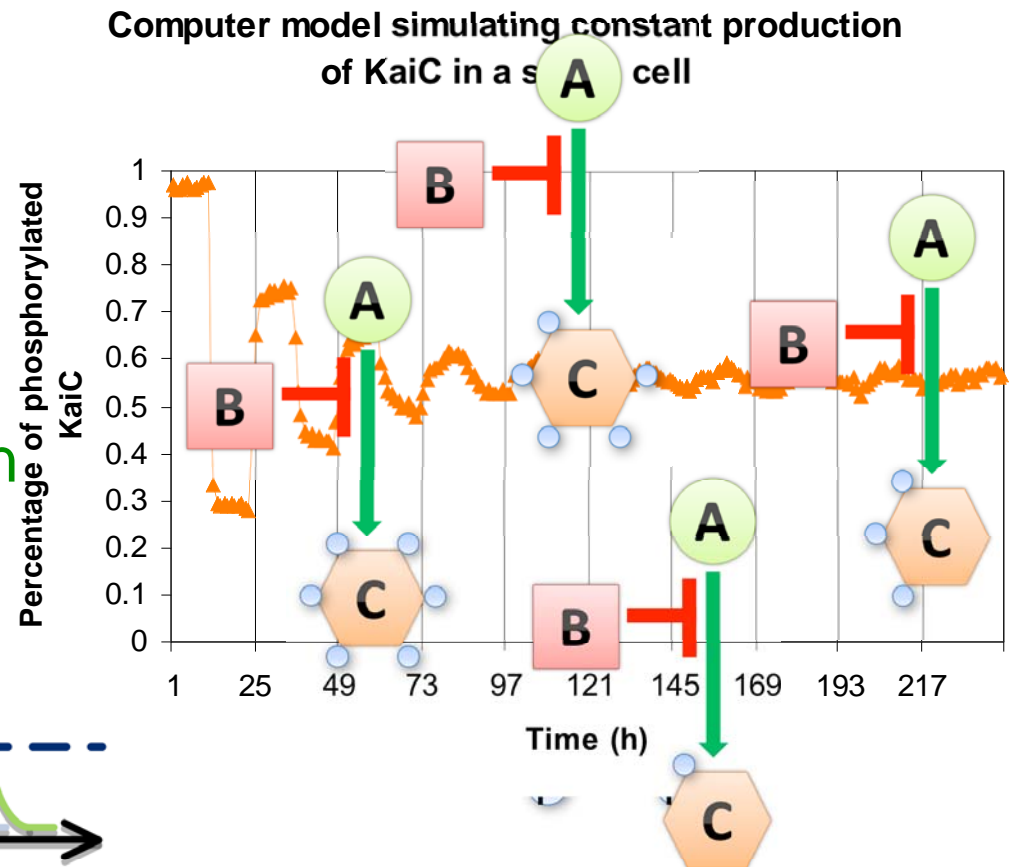
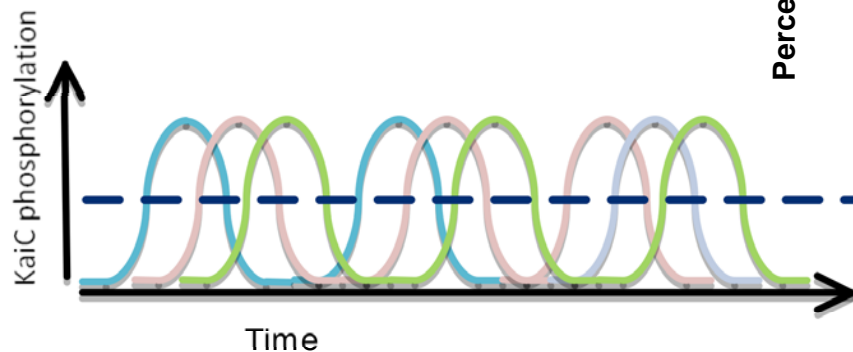
Further challenges

Verify oscillation in *E. coli*.

Synchronization problem

- Between cells
- Within cell

Solution: pulsed expression



Cyanobacterial Oscillator

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