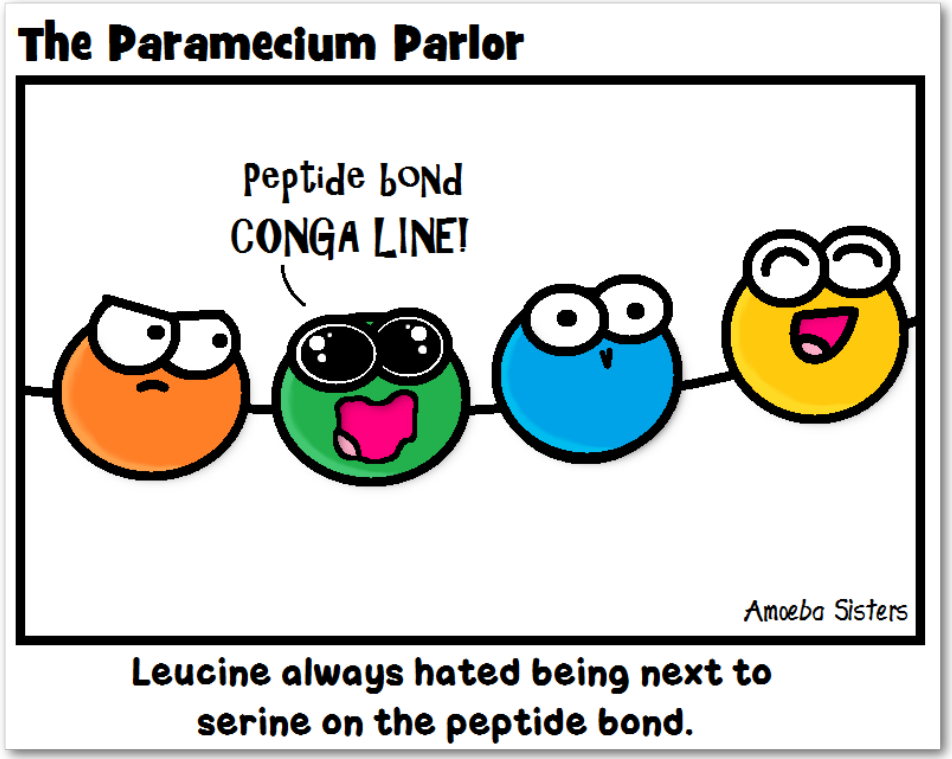
M2D1:
Determine peptide design strategy

1. Prelab discussion
2. Review literature to design display peptide sequence
3. Submit primers for peptide sequence



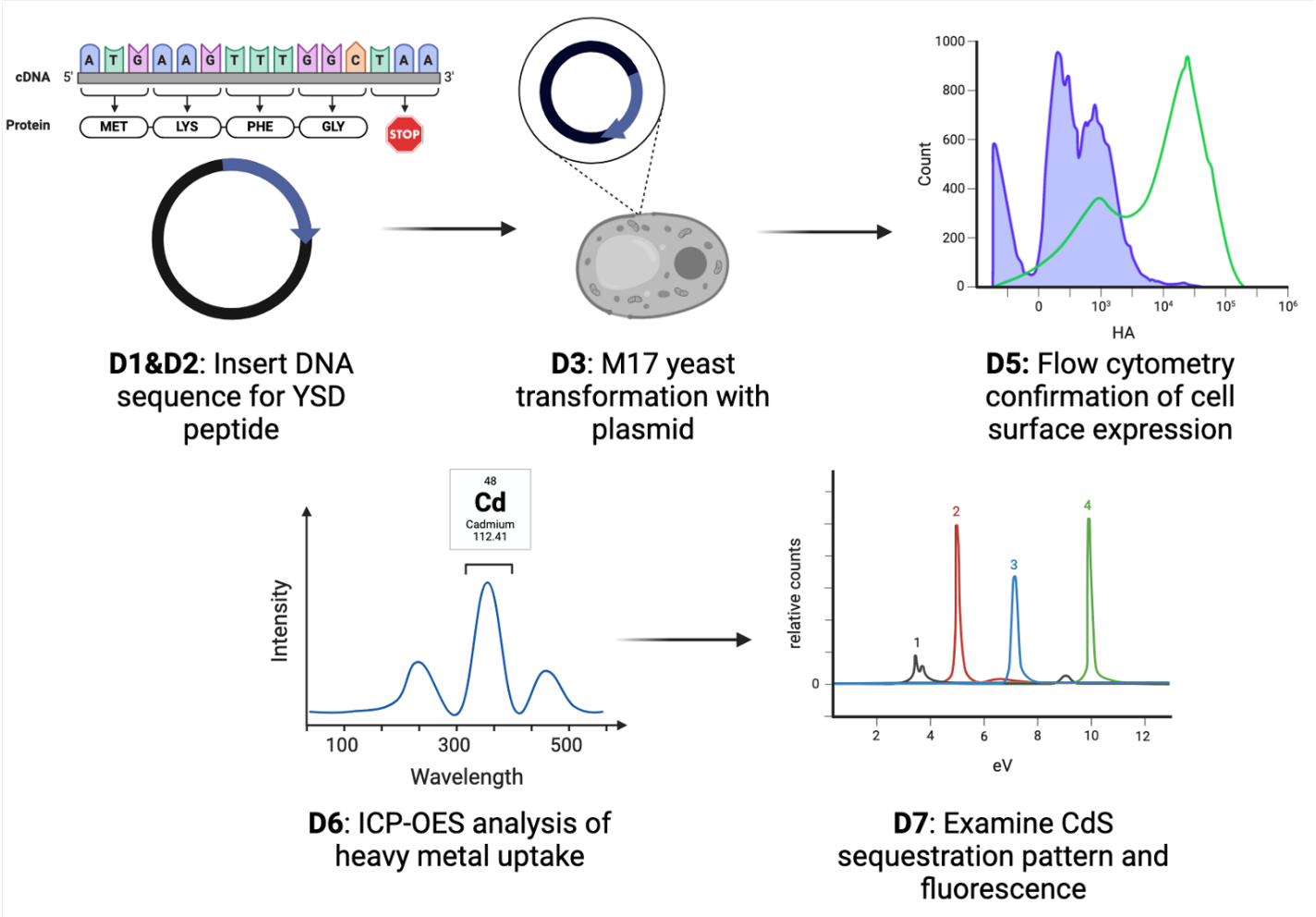
What is your research goal?

- Genetically engineer a cells surface display peptide to capture cadmium in a model of bioremediation

What is your experimental approach?

- Engineer peptide to capture cadmium sulfide from environment
- Test peptide design by assessing metal uptake and composition

Overview of Mod 2 experiments:



D1&D2: Insert DNA sequence for YSD peptide

D3: M17 yeast transformation with plasmid

D5: Flow cytometry confirmation of cell surface expression

D6: ICP-OES analysis of heavy metal uptake

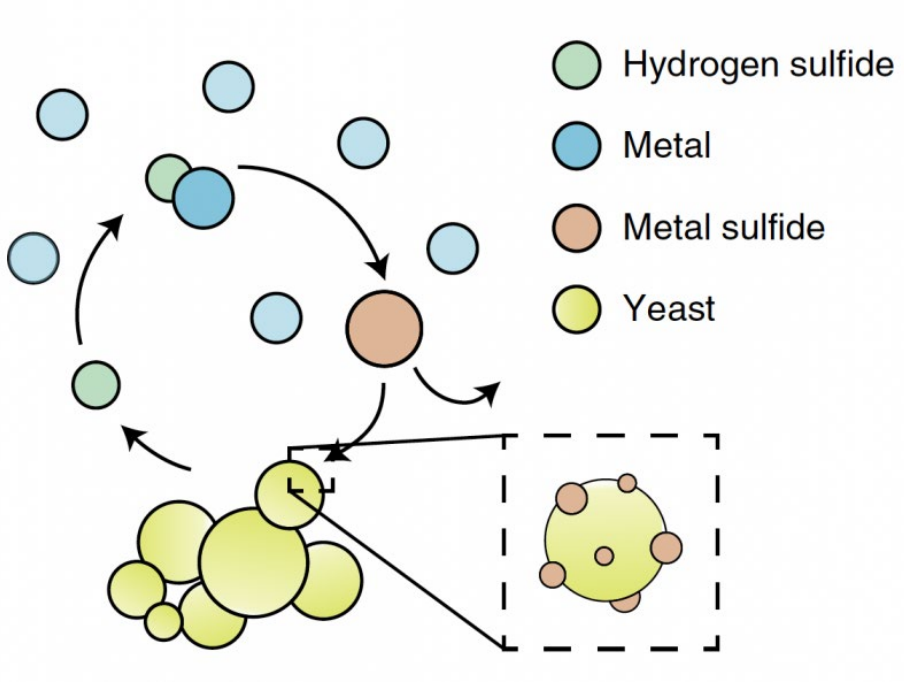
D7: Examine CdS sequestration pattern and fluorescence

Foundational work driving your bioremediation system

Concept: yeast cells produce hydrogen sulfide gas which reacts with metals causing metal sulfide formation

- Yeast cells with ΔMet17 mutation produce excess hydrogen sulfide gas

- Precipitated metals can be captured on the surface of yeast cells using peptides

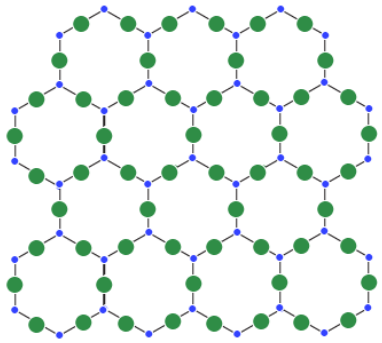


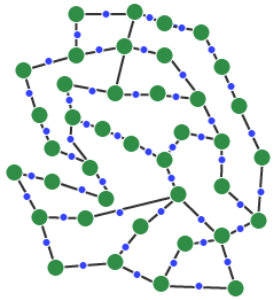
Your research will build on these foundations

- Take advantage of △Met17 yeast cells to increase production of hydrogen sulfide that can precipitate with cadmium in the environment

- **Design peptide that is:**
  1. specific to cadmium sulfide

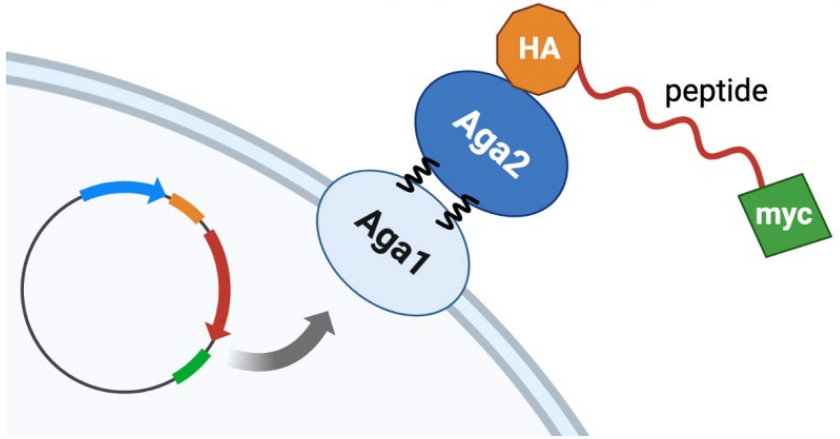
  2. promotes cadmium sulfide to accumulate in crystalline arrangement

crystalline 

amorphous 

How will you display your peptides on the yeast cell surface?

- Yeast surface display (YSD) used to ‘show’ peptides on the membrane of cells
- Genetic fusions used to attach display peptides to cell wall protein
- Sequence for display peptide inserted into expression vector to generate fusion
- Why display peptide on yeast cell surface?



How will you engineer your peptides?

- What amino acid residues have been shown to remove cadmium from an environment?
- What amino acid residues have been shown to stably bind cadmium?
- What amino acid residues have been shown to slow precipitation of cadmium sulfide?

Primers used to insert peptide sequence into expression vector

1. Choose amino acid residues for your display peptide

2. Translate amino acid residues into nucleic acid codons

3. We will include the tags and order!

For today…

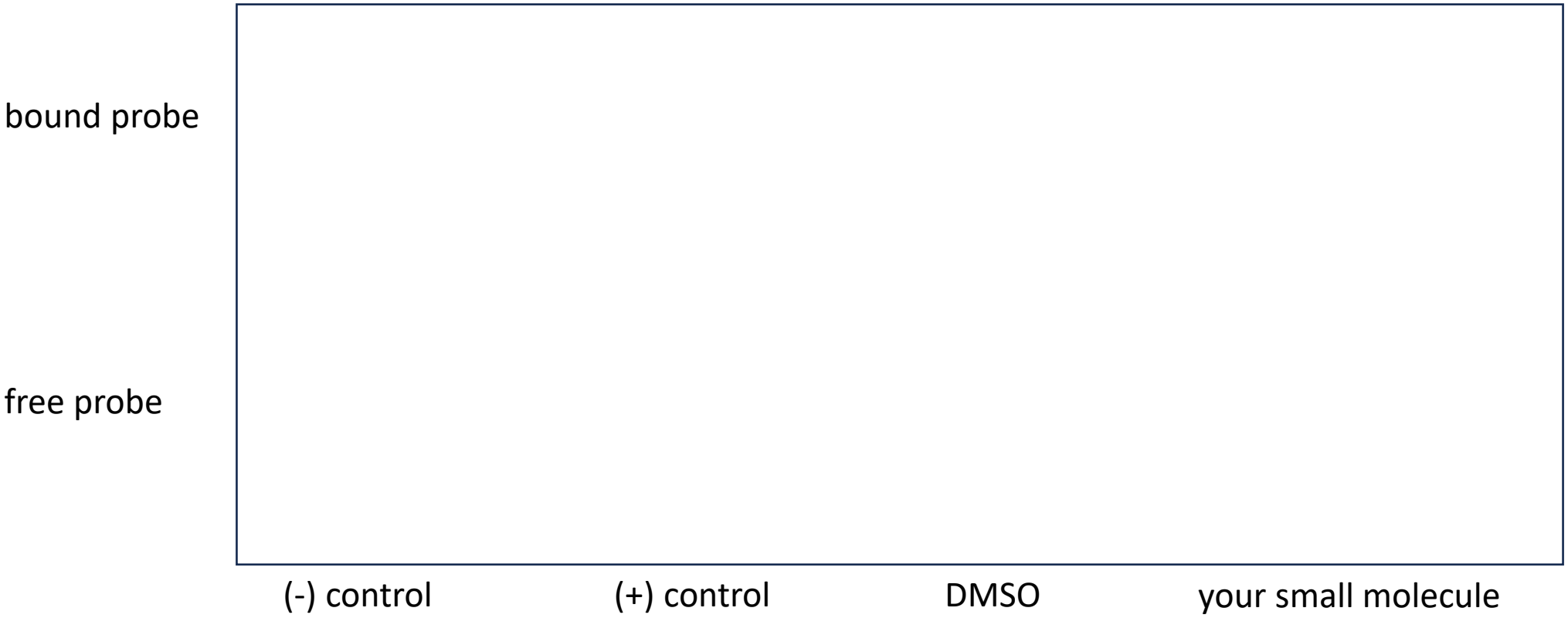
- Primers must be completed / submitted by 5p!

For M2D2…

- Reserve article for Journal article presentation and submit summary that highlights why you think the research is interesting

Notes on your EMSA results…

- What did you expect to see?



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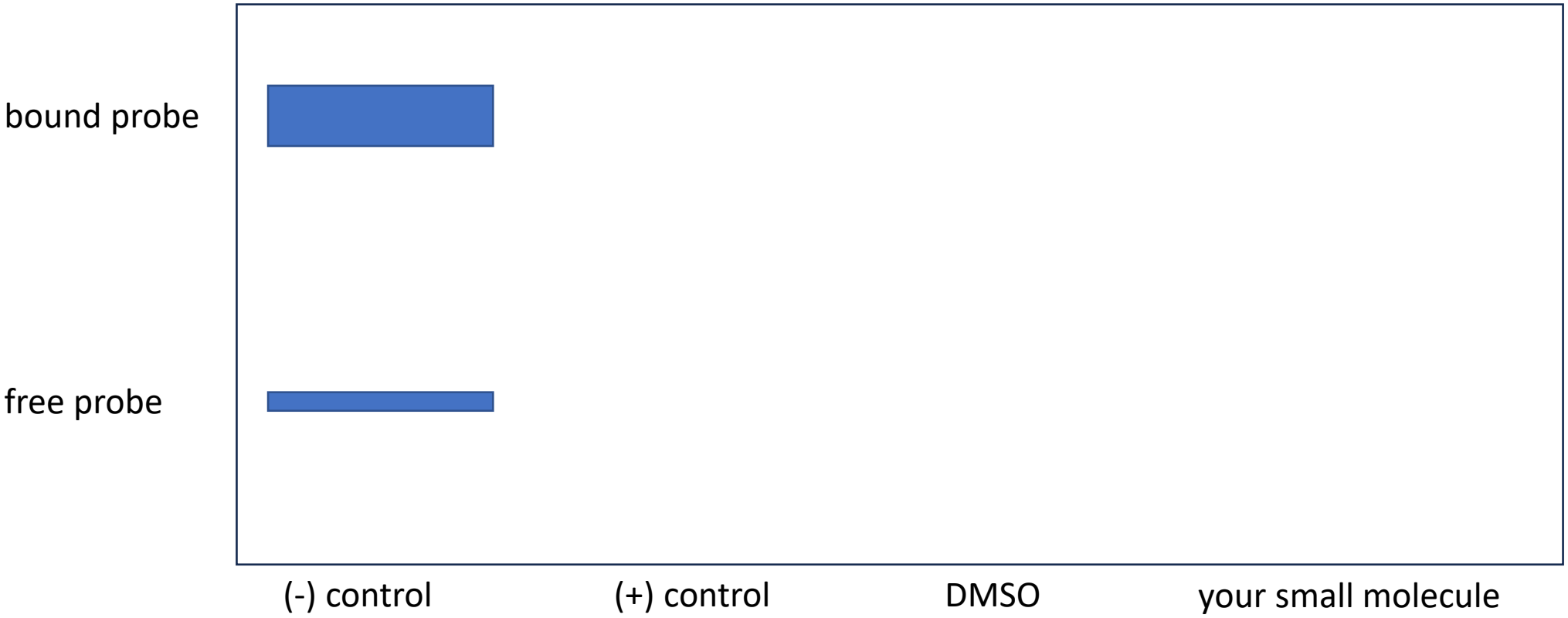

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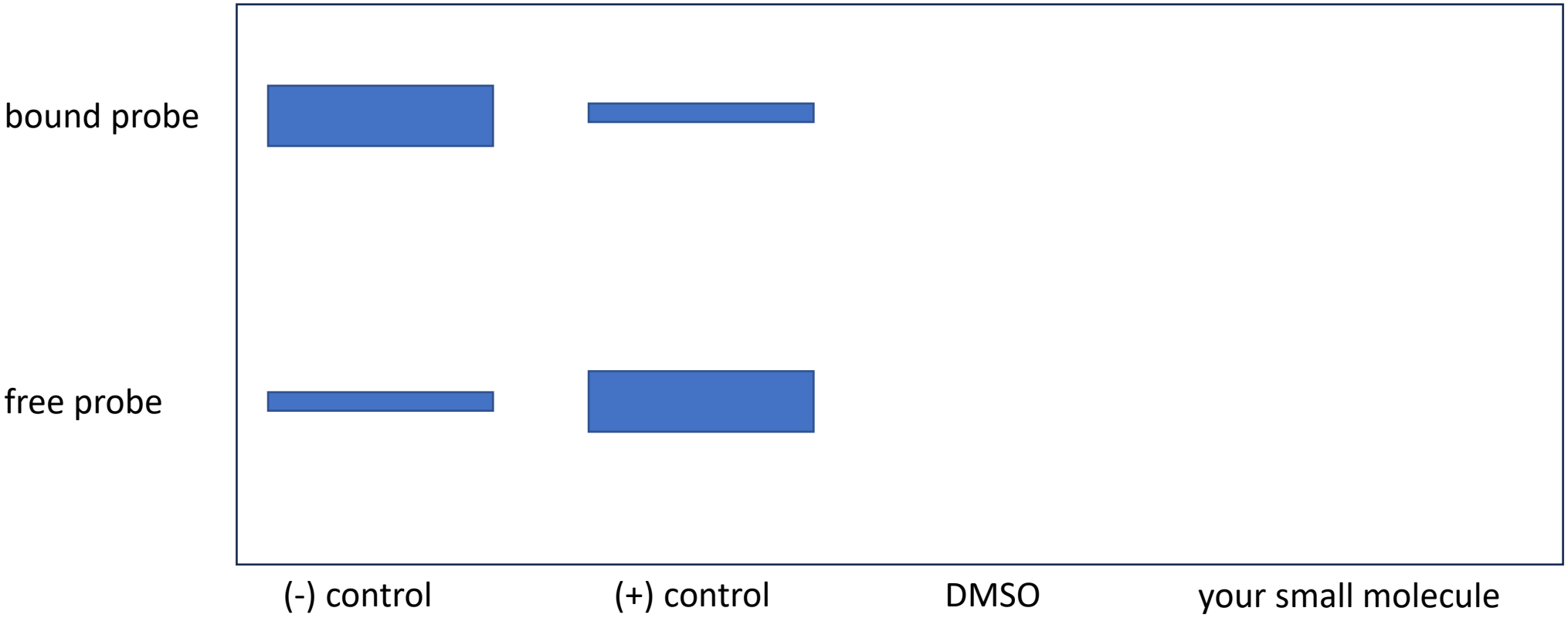

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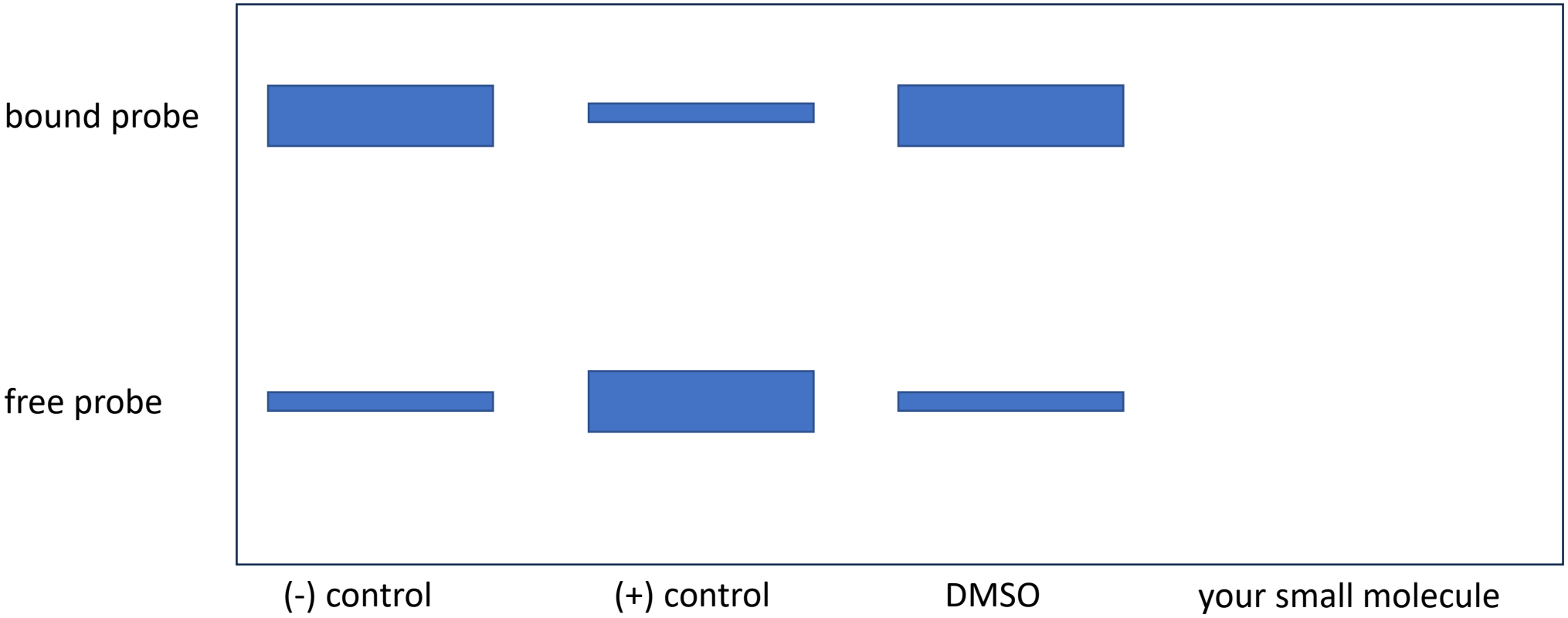

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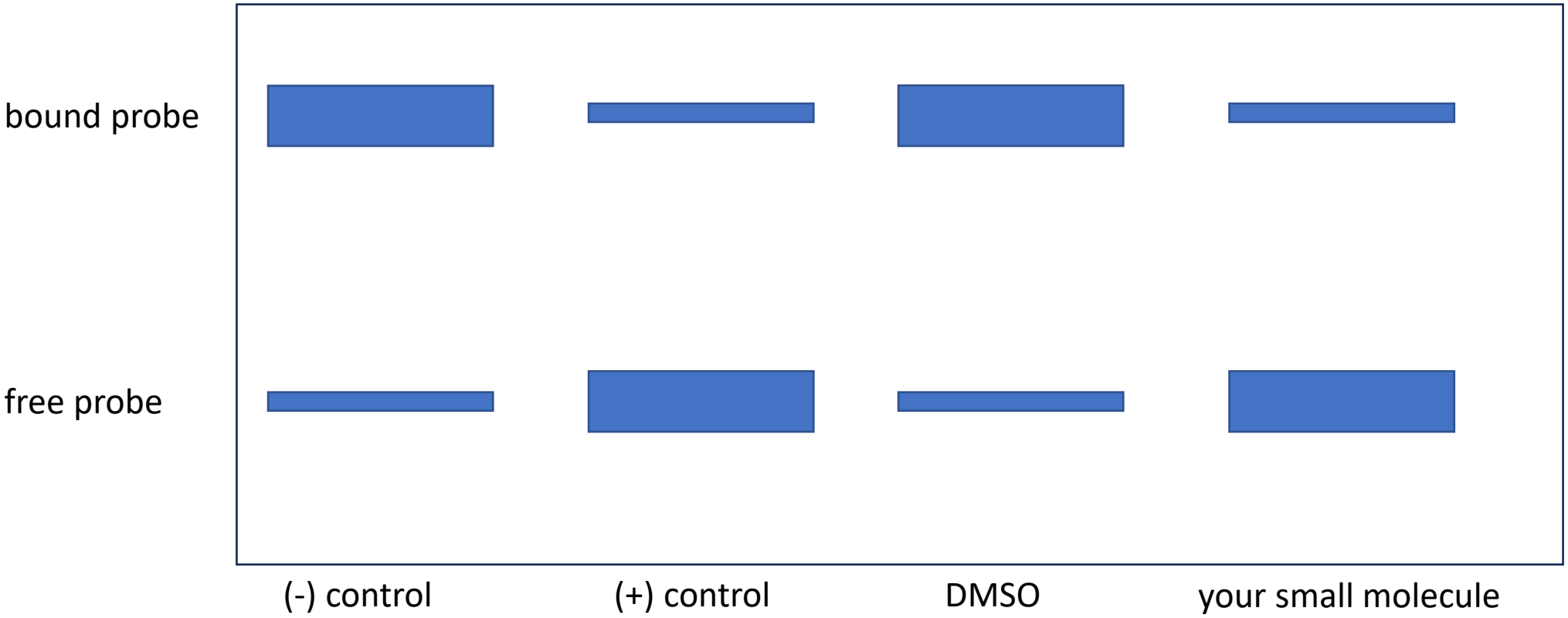
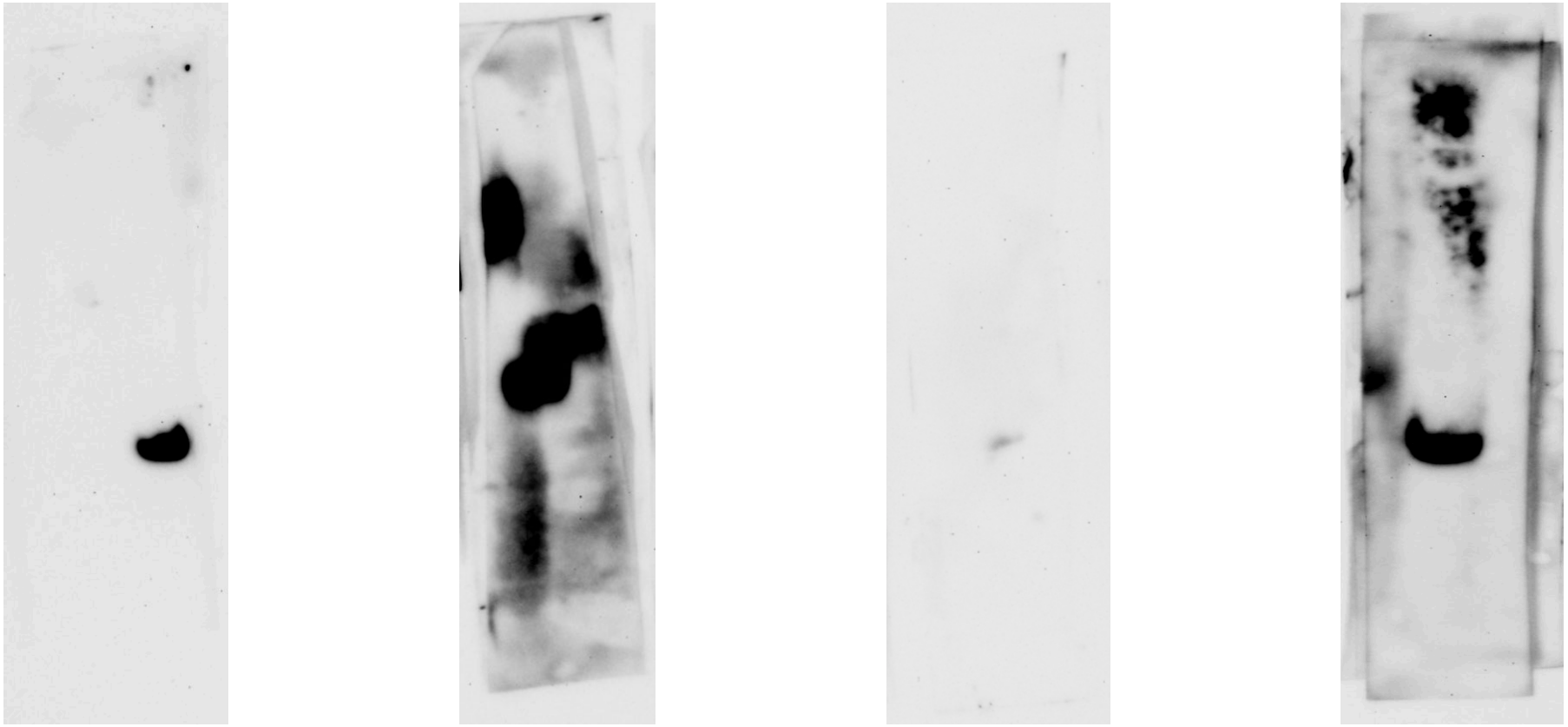


Notes on your EMSA results…

- What do you actually see? #realscience

free probe



DMSO