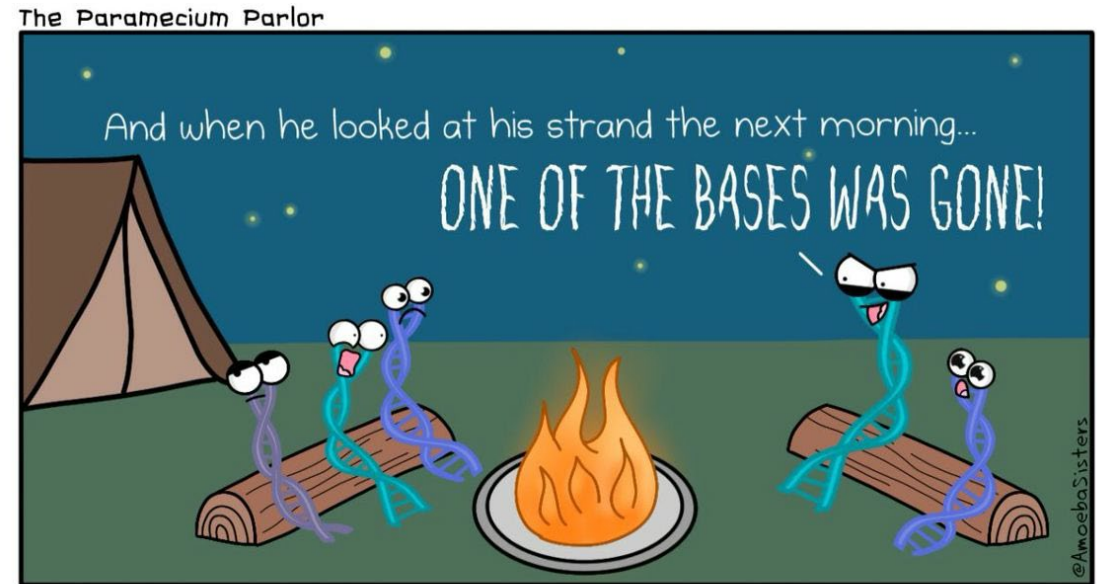


M2D1:

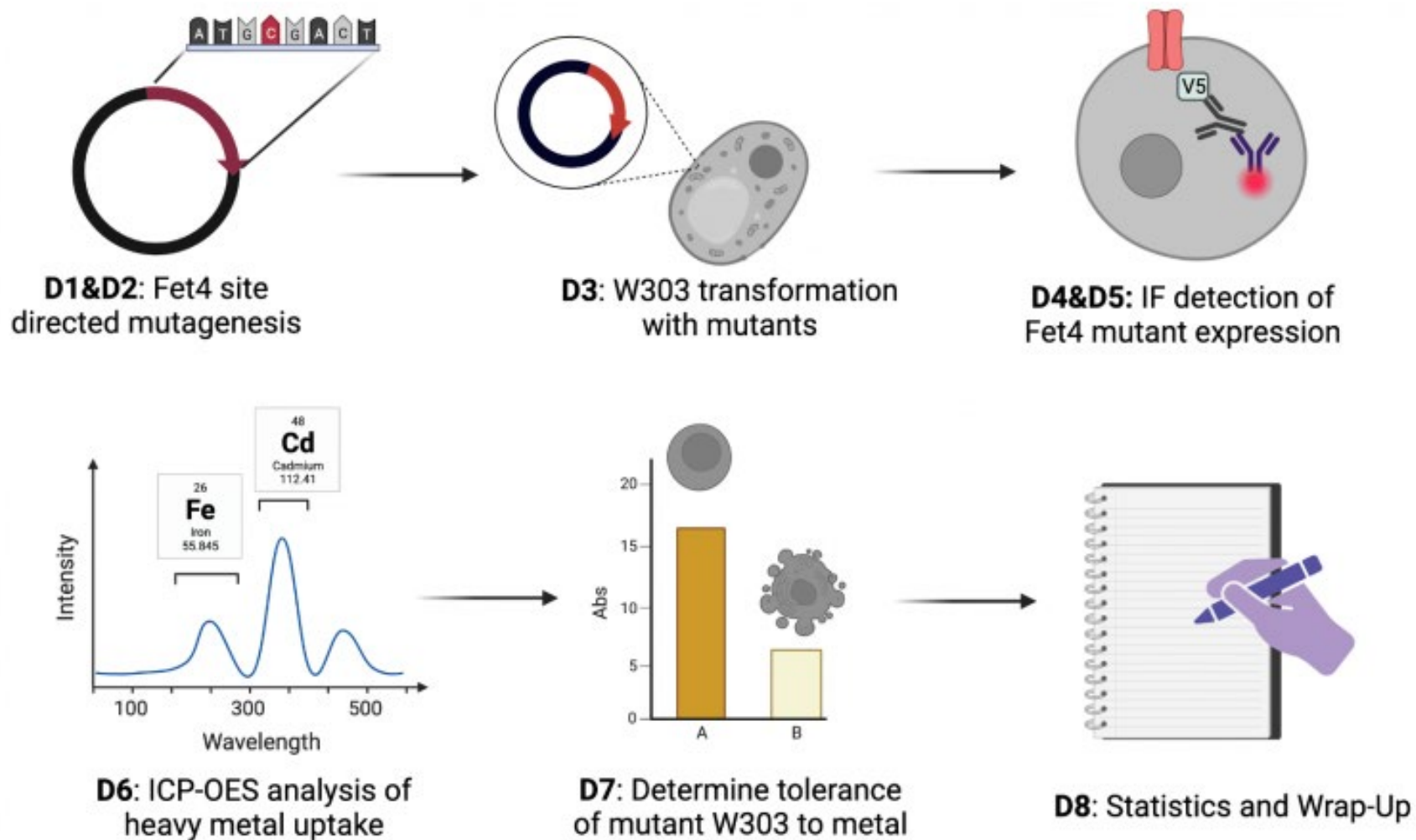
Determine mutagenesis strategy

1. Prelab discussion
2. Review literature and identify point mutation site
3. Design mutagenesis primers



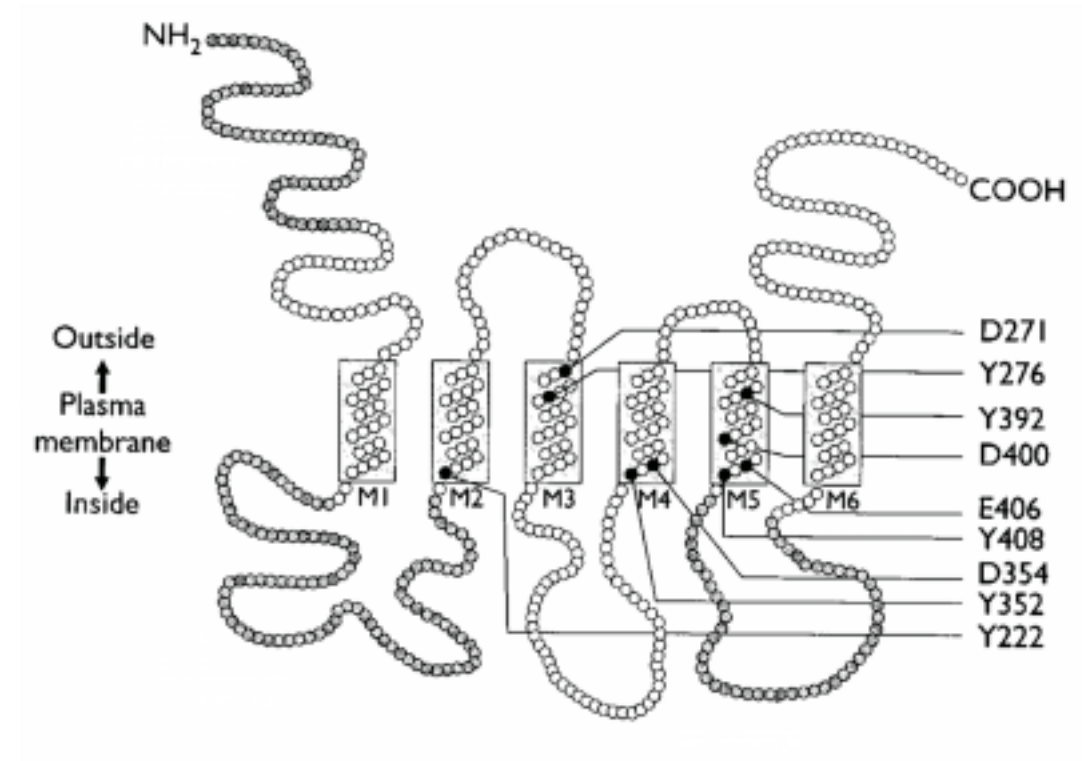
Sharing mutation stories was a DNA camping tradition.

Overview of Mod 2 experiments:



FET4 functions as a Fe^{2+} transporter protein

- Integral membrane protein located in plasma membrane
- Regulated by environmental iron levels
- Residues important in Fe^{2+} binding located in transmembrane domain



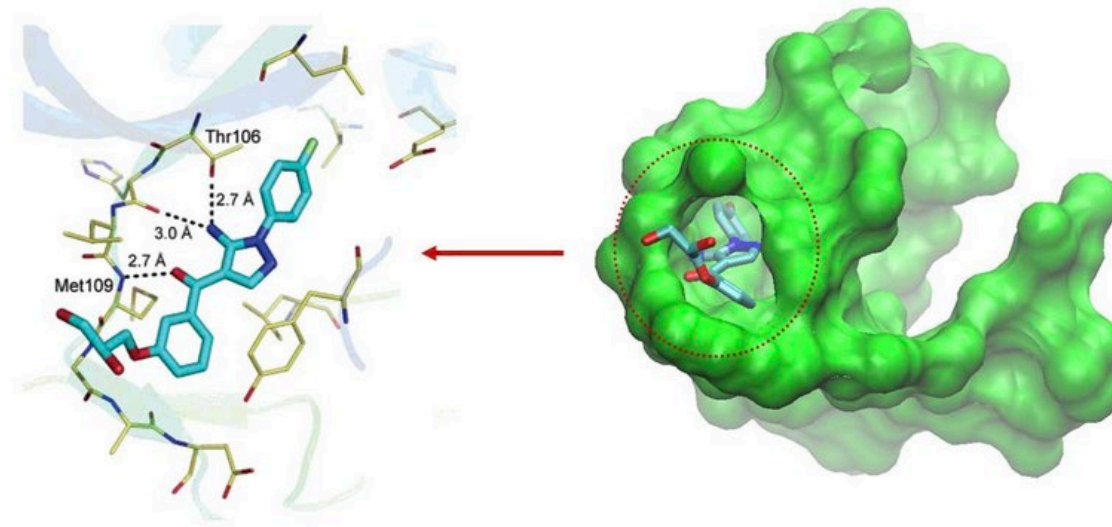
What is your research goal?

- Genetically **modify the FET4 Fe²⁺ iron transporter** to preferentially take-up cadmium

What is your experimental approach?

- Incorporate **point mutations** into FET4
- Analyze **data from class** to assess ~14 unique mutations

How do point mutations alter binding?



Change in single protein residue can:

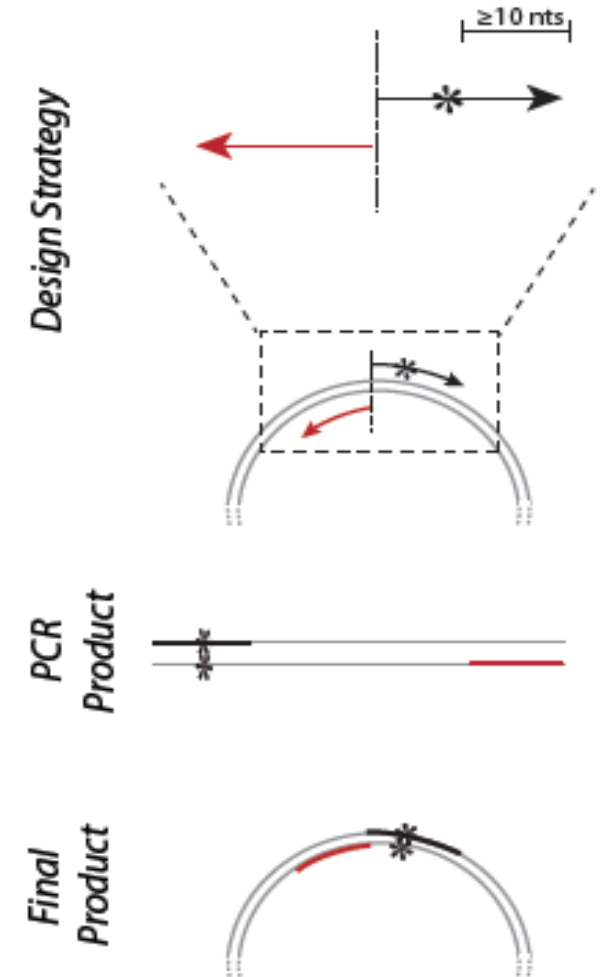
- Alter size / shape of the binding pocket
- Alter charge of the binding pocket
- Collapse the binding pocket
- Eliminate / promote access to binding pocket

How will you generate point mutations?

- Gene sequence for FET4 cloned into expression vector
- Primers used to amplify FET4-containing expression vector
 - Forward primer incorporates sequence that contains wanted point mutation
- Amplification reaction results in linear PCR product that contains point mutation
- Linear PCR product ligated to generate mutated FET4-containing expression vector

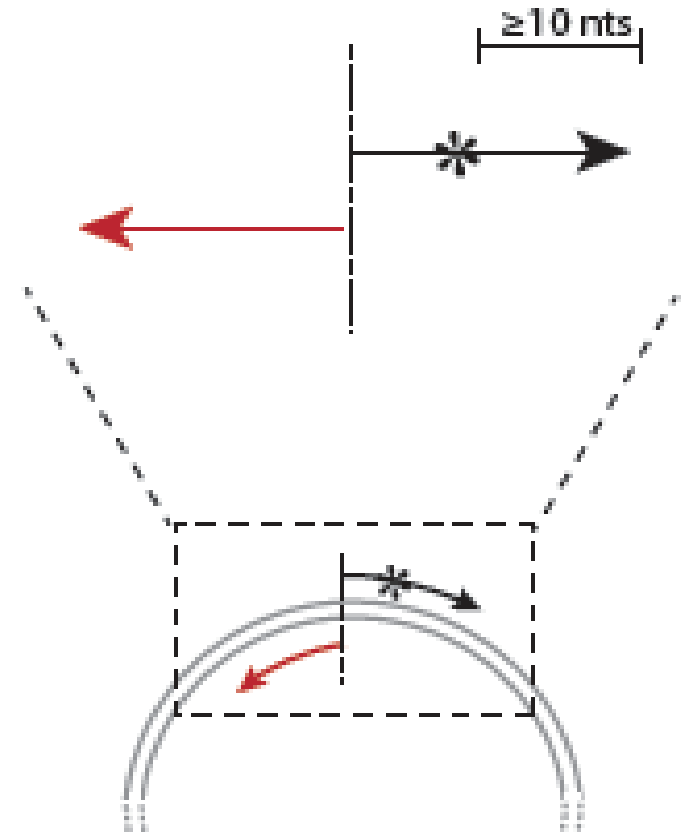
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- Linear PCR product ligated to generate mutated FET4-containing expression vector



How will you design your primers?

- Wanted mutation must be in the center of the forward primer
 - Ensures primer is able to bind sequence located upstream and downstream of mutation site
- Forward and reverse primers must 'face away' in opposite directions
- Primers should: be 25-45 bp, contain 40% G/C bps, have melting temperature <78C, and terminate in G/C



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