Transcriptional Regulation by cAMP Receptor Protein (CRP)

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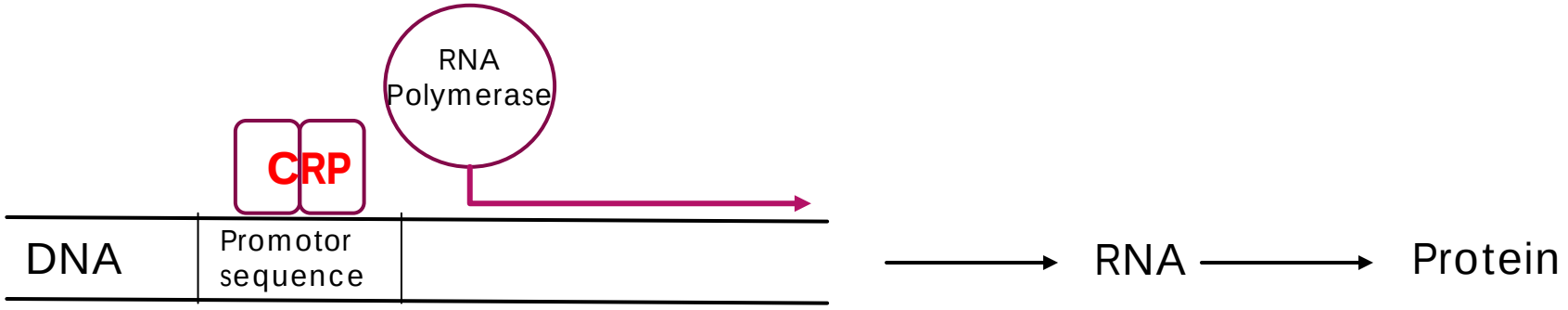
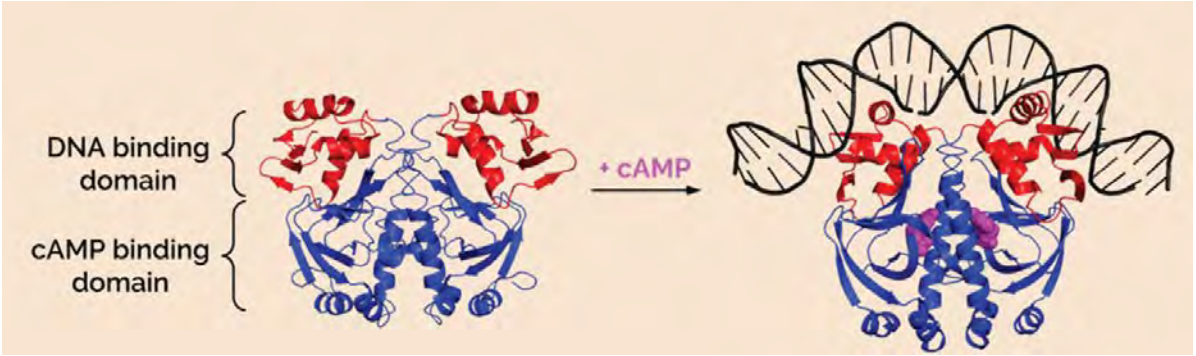
Overview: What is Cyclic AMP Receptor Protein?

- CRPmtb is a homodimer
- CRPmtb is a transcription factor that controls DNA expression
- cAMP is a ligand that activates and deactivates different functions of the protein



Goal of my research

The goal of my research was to study the effect of cyclic AMP (cAMP) on DNA binding to Cyclic AMP Receptor Protein in Mycobacterium tuberculosis (CRPmtb)



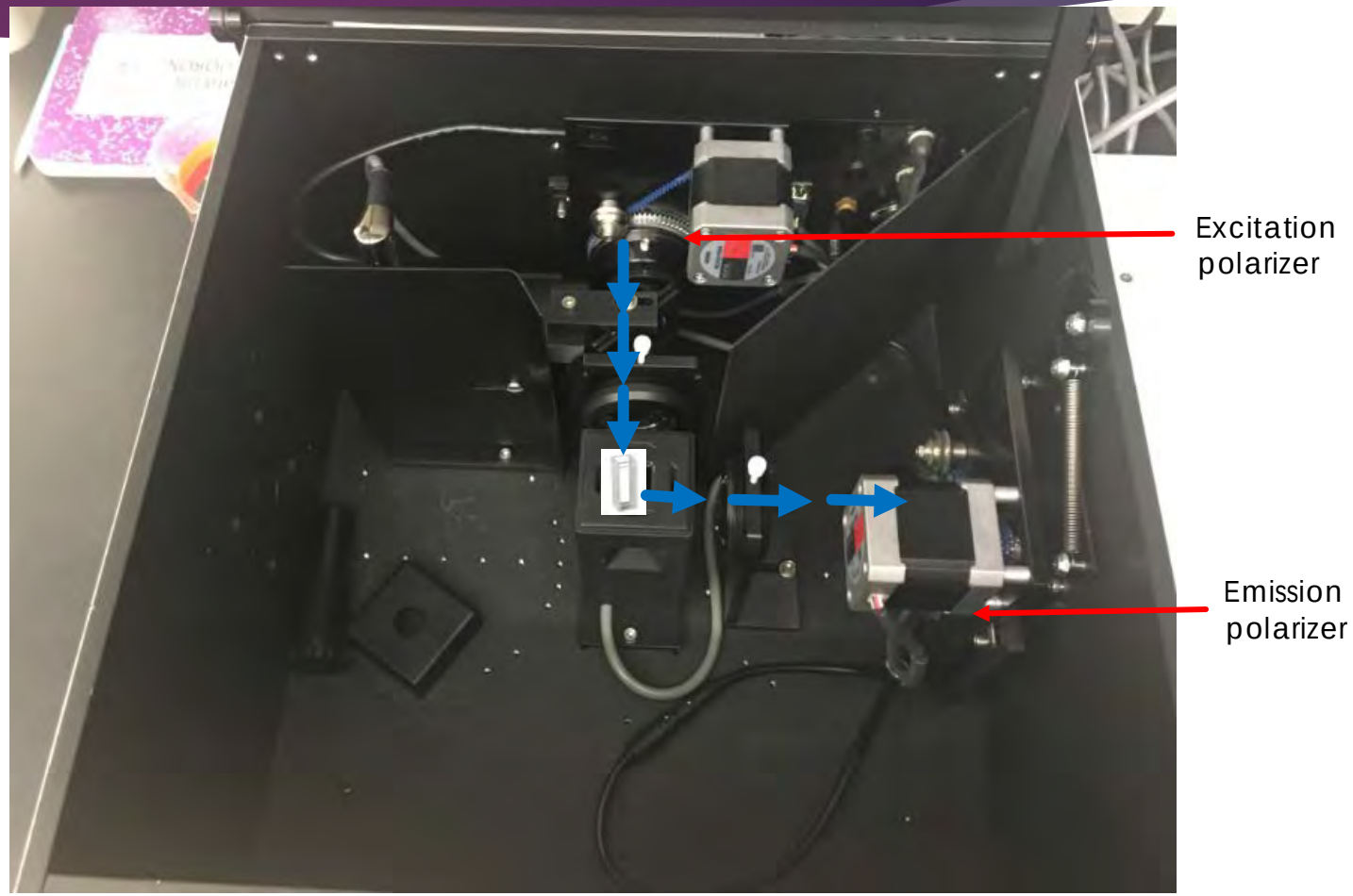
My role in the lab

- Worked July 5th- August 30th
- Performed Anisotropy experiment for the first 5 weeks
- Worked on Protein purification for the rest of my time

Anisotropy

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

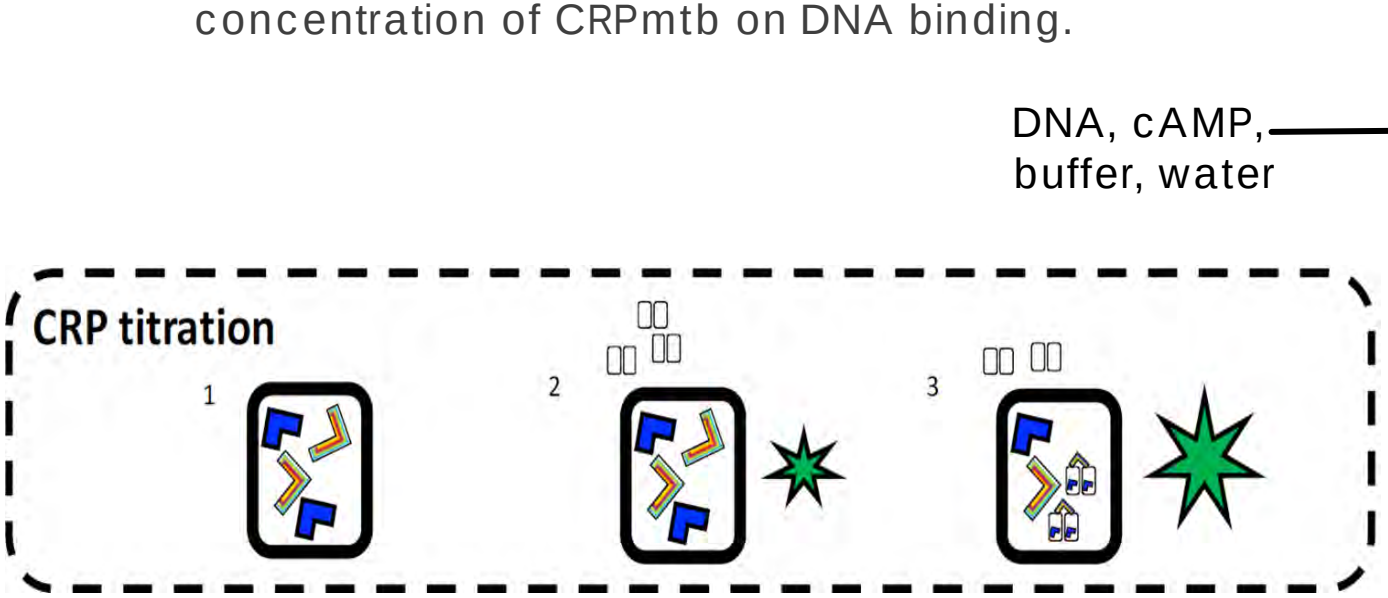
- Anisotropy is a measurement of the degree of polarization/rotation of the sample
- A method of measuring the binding interaction between two molecules
- Polarize light excites the fluorophore, and the fluorophore emits light

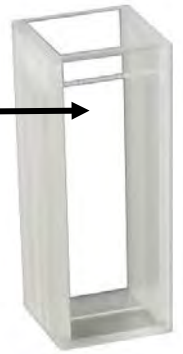


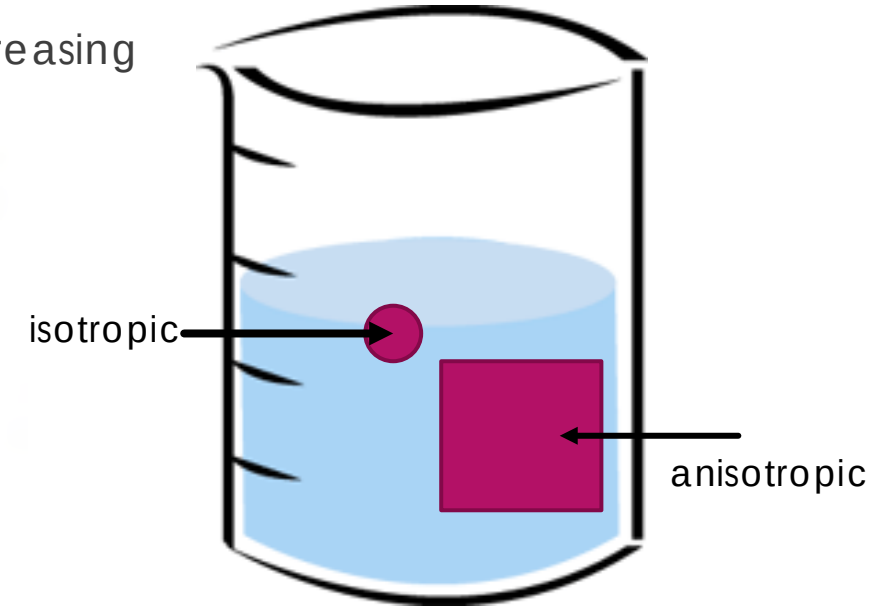
Fluorometer

Anisotropy

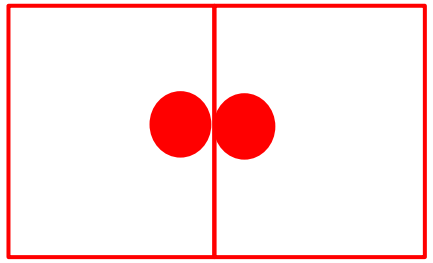
- Studied the effect of cAMP on DNA binding to CRPmtb through the anisotropy experiment.
- DNA binding to CRPmtb can be measured through anisotropy using fluorescently- labeled DNA as the fluorophore
- We titrated DNA and cAMP with CRPmtb and studied the effect of increasing concentration of CRPmtb on DNA binding.



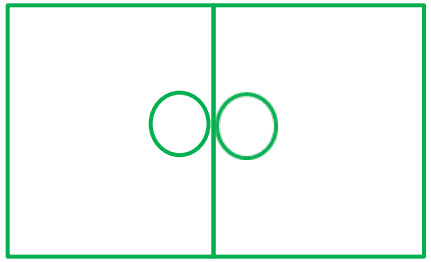




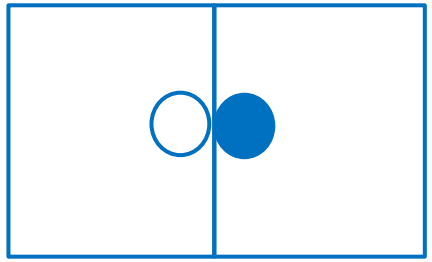
Results of Anisotropy with [DNA]= 3 nM, Slit width= 10 nm



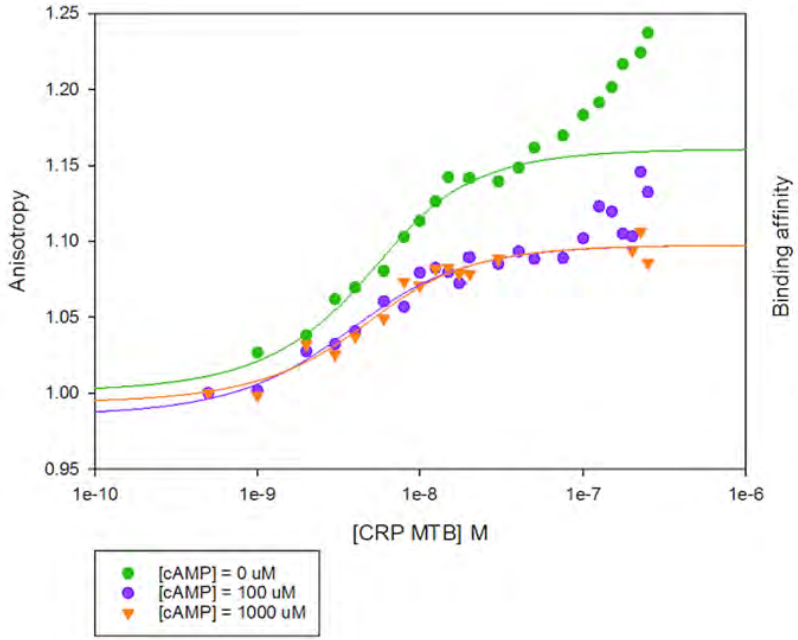
[cAMP]= 1000 µM

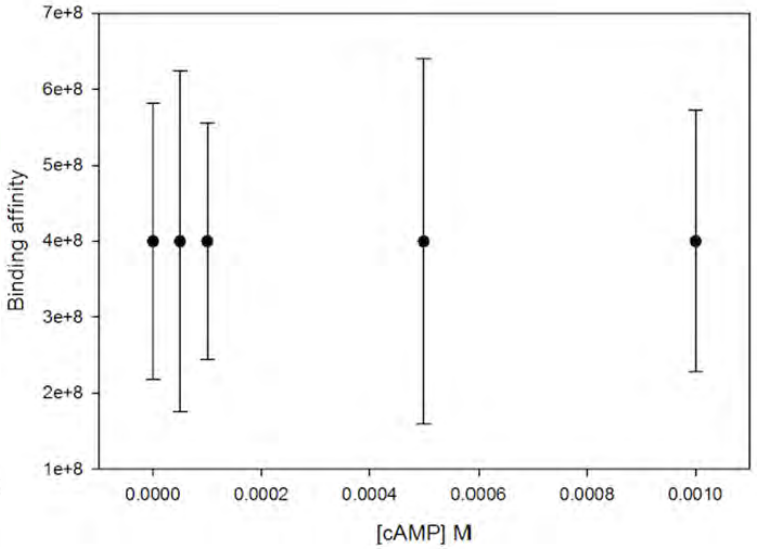


[cAMP]= 0 µM



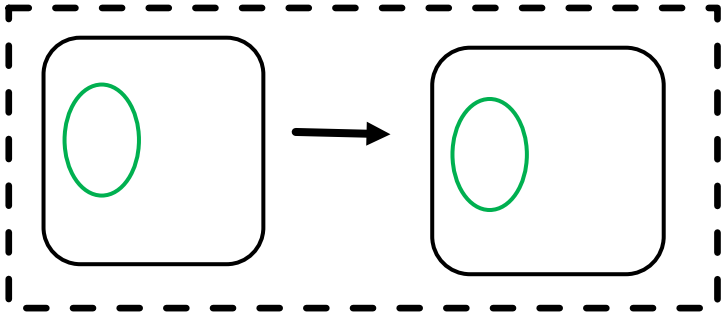
[cAMP]= 100 µM



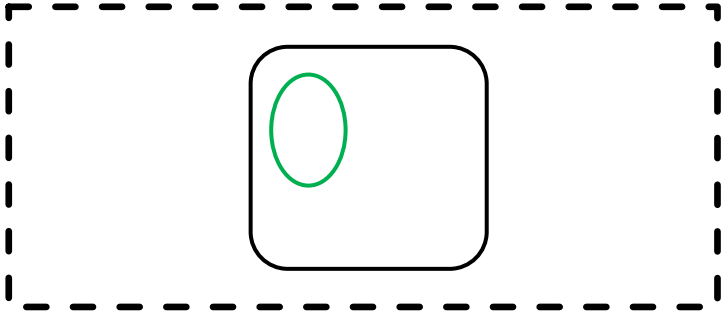


Protein Purification

Step 1. Transformation

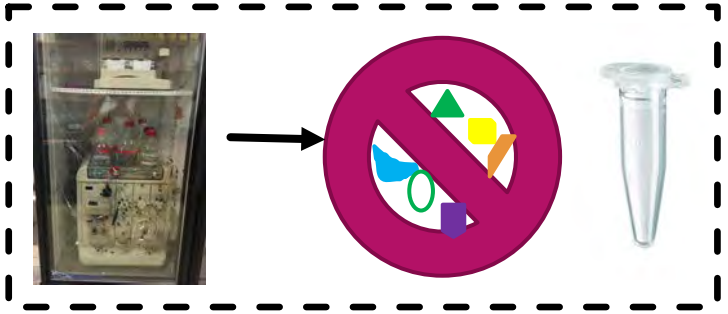


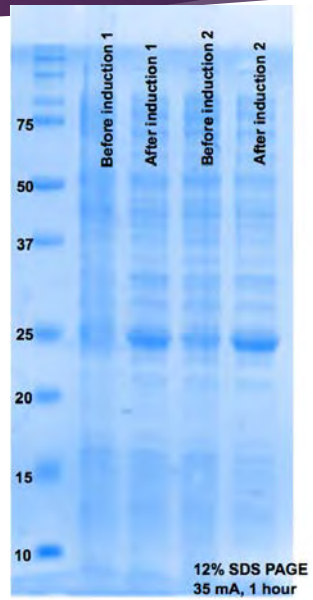
Step 2. Induction

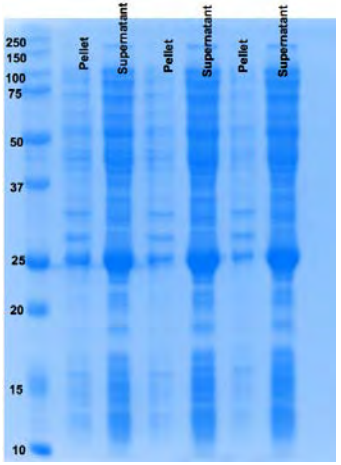


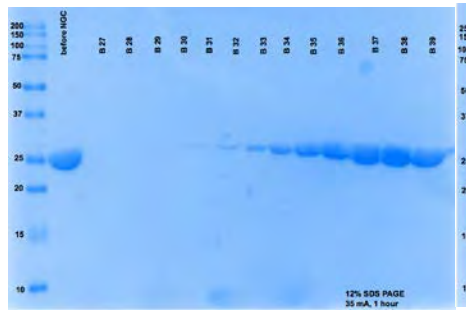
Step 3. Break Cells and centrifuge

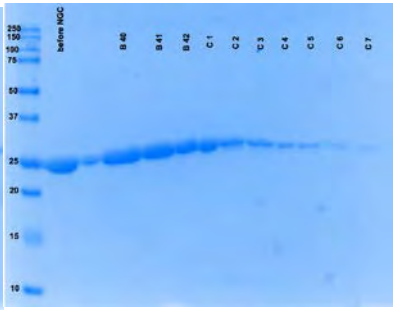
Step 4. Size Exclusion Chromatography









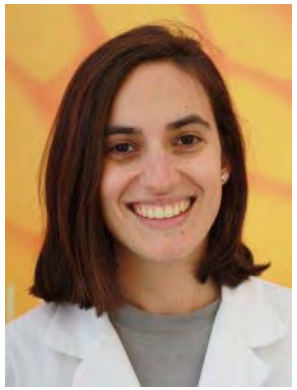


Mistakes Made and Lessons Learned

- Mistakes
  - Never come to the lab in open-toed shoes
  - Spilled cuvettes a few times
- Lessons learned
  - It’s okay to make mistakes!
  - Research takes a long time and a lot of patience
  - Ask a lot of questions

Thank you to:

Rodrigo Maillard: Principal investigator (PI)



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Sahar Foroutannejad PhD student; Chemistry

Virginia Glick Student; Bioc hemistry

Jenny England PhD student; Chemistry

Sources

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