Developing a Ribose Binding Protein FRET Sensor

Henry Sims, SEAS 2026

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Introduction

- Ribose is a key component in the creation of nucleotides within cells
- Not much is known about the metabolism, uptake, and quantity of ribose in cells, so it is important to have a way to measure it
- The quantity of ribose can be measured using Förster Resonance Energy Transfer (FRET). When Ribose Binding Protein (RBP) is combined with two specific chromophores, it gives a fluorescent signal when the protein is bound to ribose
- A ribose sensor has already been developed with cyan and yellow chromophores, but I aimed to do so with green and red ones (mClover3 and mRuby3), forming GR-RBP, because they would theoretically be more sensitive

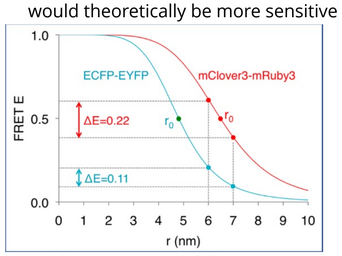

Figure 1. Display of the change in FRET energy as the distance between chromophores changes

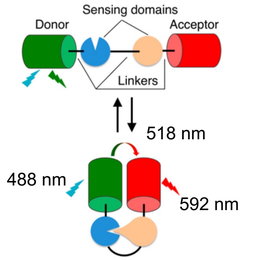

Figure 2. Schematic of GR- RBP as it binds to ribose

Protein Purification

- After the protein was grown and expressed in BL21DE3Gold cells, it was purified with FPLC machine using both a nickel affinity column and size exclusion column

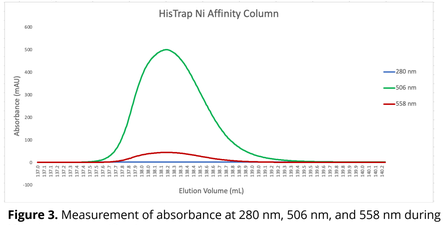

Figure 3. Measurement of absorbance at 280 nm, 506 nm, and 558 nm during Nickel column purification

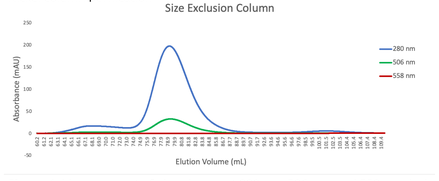

Figure 4. Measurement of absorbance at 280 nm, 506 nm, and 558 nm during size exclusion column purification

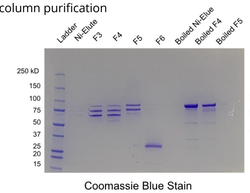

Figure 5. Gel containing FPLC fractions, stained with Coomassie blue

FRET Measurements With Purified Protein

- The purified protein was excited with 488 nm light using a spectrofluorometer and emitted light of the following wavelengths. This was done both in the presence of ribose and without to determine if there is a difference in FRET signal

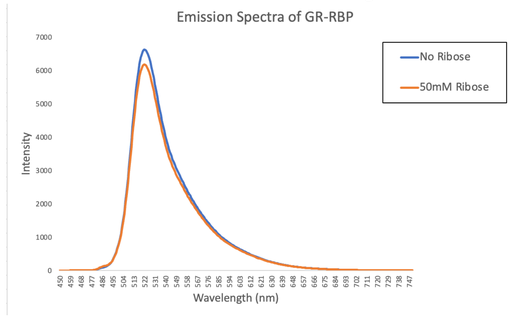

Figure 6. Fluorometry data of GR-RBP, excited with 488 nm

- Unfortunately, there was no FRET signal both with ribose and without it, as there was no emission of red light

FRET Measurements in Living Cells

- After determining it was possible that the protein became denatured during the purification process, we decided to express the protein in HEK293T cells, where the FRET signal could be measured in vivo
- This was done by placing the cells under a confocal microscope, exciting with 488 nm light, and recording the image produced when reading light in the ranges of 506 nm to 546 nm for green and 580 nm to 620 nm for red.
- After these images were taken as a control, ribose was added and the process was repeated
- The images were processed by going through them pixel by pixel, finding the ratio of the red pixel's intensity to the green's, and then calculating the average ratio of the entire image

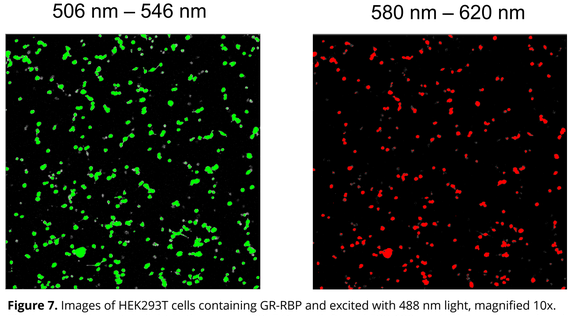

Figure 7. Images of HEK293T cells containing GR-RBP and excited with 488 nm light, magnified 10x. The images only contain light in the ranges 506-546 nm (left) and 580-620 nm (right), and are colored just for display purposes

FRET Measurements in Living Cells cont.

- This process was repeated in 3 different areas of 3 different wells full of cells and the average pixel ratios were as shown below

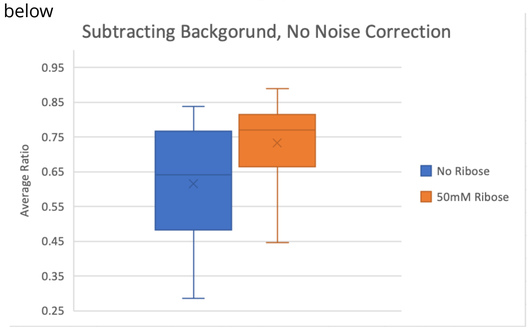

Figure 8. Plot of average pixel ratios of the cell images before and after adding ribose

- While this method allowed us to see a FRET signal, there was almost no difference in the signal when ribose is present, so the GR-RBP is not sensitive to ribose

DNA Sequencing

- To make sure that the protein contains the exact DNA sequence that we are looking for, I transformed the DNA for GR-RBP in a mammalian vector and sent it for DNA sequencing
- This confirmed that the protein I had been working with did in fact have the exact sequence that I assumed it did

Different Protein Expression Conditions

- To see if the conditions the protein was being expressed under were affecting anything, I grew small samples of the protein under different conditions of time, temperature, and IPTG concentration. However, the conditions I had been using were shown to be the best

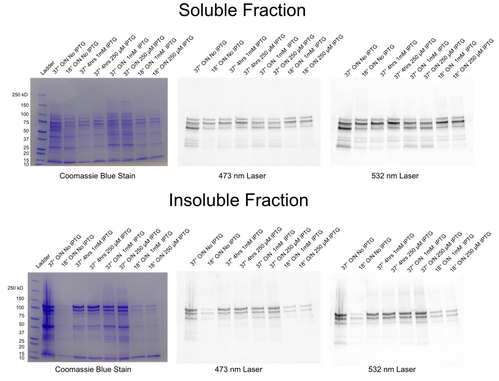

Figure 9. Gel data of GR-RBP expressed in different conditions. After the cells were lysed and separated, gel electrophoresis was performed on both the soluble and insoluble fractions

Green Sensor Only

- To test if the green chromophore, mClover3, was the one causing the problem, I purified a version of the protein that only contained it and not the red
- The fluorescence was tested again to make sure it still worked and the ITC was used to see if the mClover3 was inhibiting RBP's ability to bind to ribose

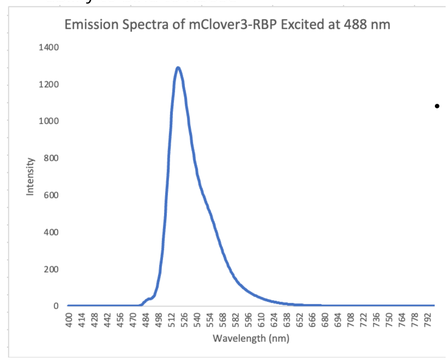

Figure 10. Fluorescence data of mClover3-RBP, excited with 488 nm

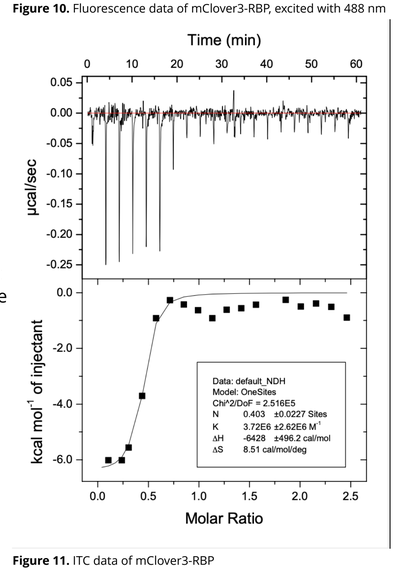

Figure 11. ITC data of mClover3-RBP

- The fluorescence measurement showed that mClover3 was able to absorb and emit light by itself
- The ITC showed that the Kd, or constant of binding, was 2.69E-7
- The N value, which is supposed to be close to 1, was only 0.403, but otherwise the data suggests that mClover3 does not affect RBP's ability to bind to ribose

Acknowledgements

1. Lager, I.; Fehr, M.; Frommer, W. B.; Lalonde, S. Development of a Fluorescent Nanosensor for Ribose. FEBS Letters 2003, 553 (1-2), 85–89.
2. Bajar BT, Wang ES, Zhang S, Lin MZ, Chu J. A Guide to Fluorescent Protein FRET Pairs. *Sensors*. 2016; 16(9):1488.