Early Designs of a Ribose Binding Protein FRET Sensor

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Introduction

- While the uptake and quantity of free ribose in mammalian cells is mostly unknown, ribose is key for nucleotide synthesis[1,2].
- Ribose can be studied with a variety of techniques, including mass spectrometry, magnetic resonance spectroscopy, and Förster resonance energy transfer (FRET)[2].
- Ribose binding protein (RBP) is a periplasmic binding protein (PBP) that undergoes a conformational change upon binding ribose[2].

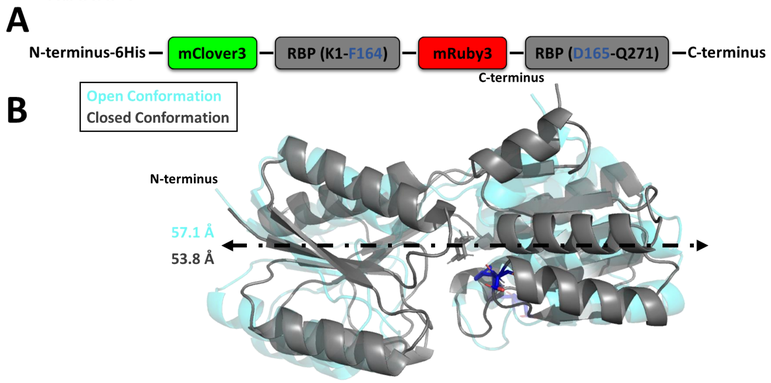

Figure 1. (A) Domain arrangement of GR-RBP sensor. (B) Structural representation of RBP open (PDB 1urp) and close (PDB 2dri) conformation.

Motivation

- Green and red fluorescent proteins (FPs) can be added to RBP to create a FRET sensor (GR-RBP)[1,3].
- Green and red FPs were found to show reduced phototoxicity[3].
- A FRET-based RBP biosensor can be genetically introduced into living cells for the purpose of quantifying free ribose and observing the binding and uptake of ribose[3].
- Literature suggests ribose is utilized for cellular pathways associated with cancer cells[2], raising the need for a ribose-based fluorescent sensor for use in live cells.

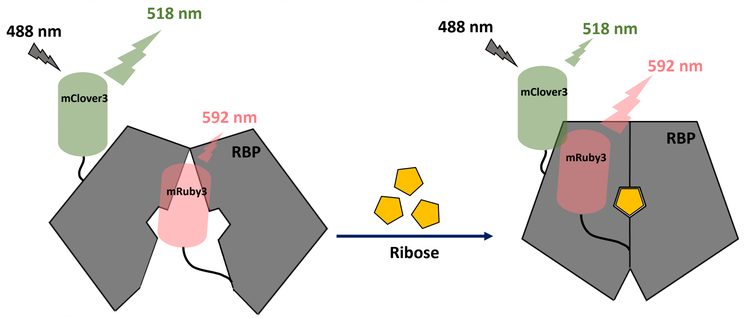

Figure 2. Schematic of GR-RBP sensor behavior upon binding to ribose.

Protein Purification

- The function of a protein is related to structure[4].
- Proteins fold to the most thermodynamically stable structure[4].
- Some proteins fold and refold with the assistance of other proteins, called chaperone proteins[4].
- Chaperones bind to or encapsulate the target protein, allowing it to fold or refold in a protected environment[4].
- Introducing Mg^{2+} during protein expression may assist in releasing chaperones from the target protein after refolding[5].

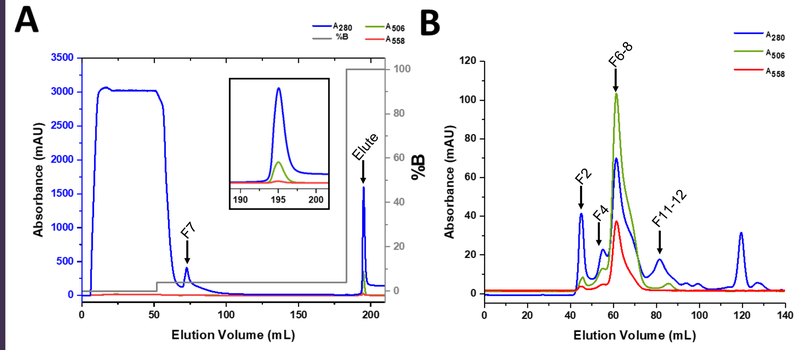

Figure 3. (A) Ni^{2+}-column purification of GR-RBP sensor monitored by absorbances at 280 nm, 506 nm (mClover3), and 558 nm (mRuby3). Protein was eluted at %100 B buffer containing 0.5 M imidazole. (B) SEC column on elution fraction of GR-RBP monitored by absorbances at 280 nm, 506 nm (mClover3), and 558 nm (mRuby3). Multiple peaks are evidence of different folding states of the protein.

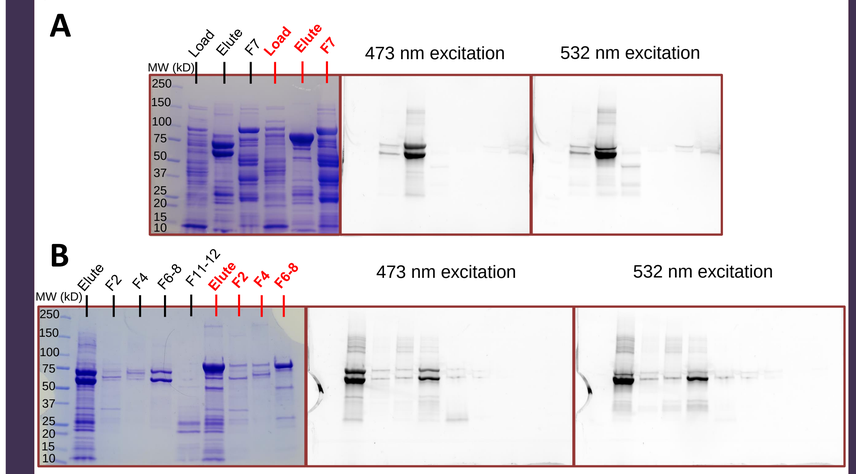

Figure 4. Gel data of FPLC fractions stained with Coomassie blue (left) and excited at 473 nm (center) and 532 nm (right) using PMT 500. Contrast was enhanced on fluorescent images for viewing purposes. Red labeled samples were boiled in water for 5 minutes to denature proteins. BioRad Precision Plus All Blue Standard was used in lane 1. (A) SDS-PAGE gel of Ni^{2+}-column load, elution, and flowthrough samples. (B) SDS-PAGE gel of select concentrated fractions from SEC run on elution fraction.

FRET Measurement

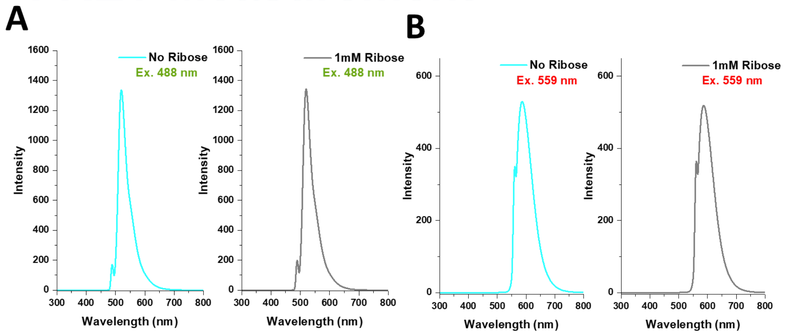

Figure 5. Fluorometry data of 1 μM of GR-RBP in PBS collected from combined SEC fractions 6-8. (A) Fluorescence intensity readings when sample is excited at 488 nm in the absence (left) and presence (right) of 1 mM ribose. Sample excitation at 488 nm is indicative of green FP's presence. A single peak from the donor (green) but no excitation of the acceptor (red) is indicative of no FRET activity from GR-RBP. (B) Fluorescence intensity readings when sample is excited at 559 nm in the absence (left) and presence (right) of 1 mM ribose. Sample excitation at 559 nm is indicative of red FP's presence.

Discussion

- The gel and fluorometry data do not support that Mg^{2+} alone assists in the expression and purification of a functional GR-RBP FRET sensor.

Future Directions

- Redesign protein with a new insertion site for red FP and a linker molecule to allow space for RBP folding.

References

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