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Conformational Changes of Gai1 nucleotide exchange catalyzed by Ric-8A

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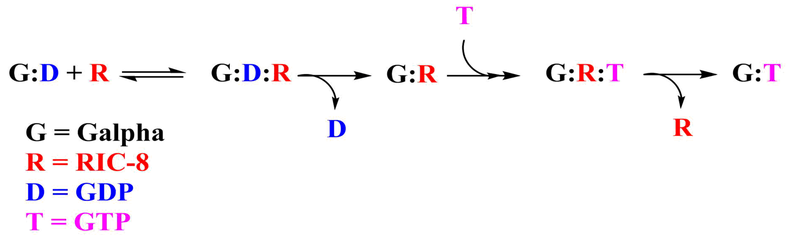
Introduction

- G-Proteins regulate several cellular processes, and when defective, have been associated with several endocrinal disorders.
- Heterotrimeric G-Proteins are regulated by the binding of GDP (inactive state) and GTP (active state) on the α subunit.
- Ric-8A is a ~60-kDA cytosolic protein that acts as a GEF to exchange GDP for GTP as shown in schematic 1 *in vitro*, and a chaperone for the Gα subunit *in vivo.*
- Previous studies with DEER spectroscopy have demonstrated a Ric-8A induced structural change in which, the Ras domain pivots away from the helical domain, exposing the nucleotide binding site and resulting in the release in GDP.
- The aim of this study is to elucidate the kinetics of Ric8A induced conformational changes in $G_{\alpha i1}$ and potentially, any uncharacterized intermediates such as $G_{\alpha\ i1}$:GDP:Ric-8A, using stopped-flow FRET (Förster Resonance Energy Transfer) spectroscopy.
- FRET is a distance dependent physical process where energy is transferred from an excited fluorophore (donor) to another fluorophore (acceptor) by intramolecular long-range dipole-dipole coupling.
- To analyze Ric8A induced $G_{\alpha\ i1}$ conformational changes, cysteines introduced in the Ras-like and Helical domain (figure 3), from a $G_{\alpha\ i1}$ construct with cysteines removed (figure 2), will be labeled with acceptor and donor fluorescent dyes.
- Stopped flow enables the measurement of FRET upon the addition of Ric-8A and allows the FRET signal to be monitored as $G_{\alpha\ i1}$ undergoes conformational changes.

Purification of Hexa I-$G_{\alpha i1}$

E-Coli Protein Expression	BL21 (DE3) – RIPL	P-DEST 15 Vector
Cell culture	TB Media	200 RPM, 37°C, OD 0.6
Affinity column	GST-Beads	Separate Gαi1 from Supernatant
Ion Exchange	Q-Column	Separate Gαi1 based on PI
Size-Exclusion	Superdex-200	Separate based on size pure Gαi1

RIC-8A GEF Mechanism



Schematic 1. Ric-8A acts as a GEF *in vitro* to mediate the release of GDP from GDP:$G_{\alpha i1}$ for GTP, resulting in an active conformation of Gα. This mechanism is poorly understood, and the kinetics have not been well established.

Hexa I-$G_{\alpha i1}$ Constructs

Green- Helical domain
Grey-Ras-like domain
Red- GDP

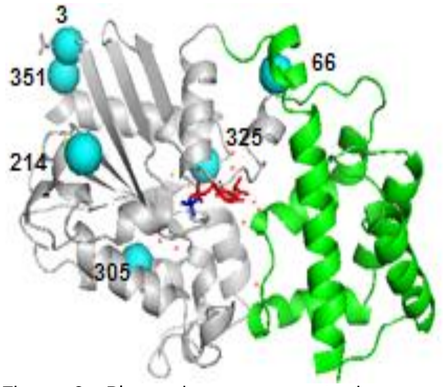


Figure 2. Blue spheres represent six naturally solvent exposed cysteines that were removed. Obtained from protein data bank (1GIT).

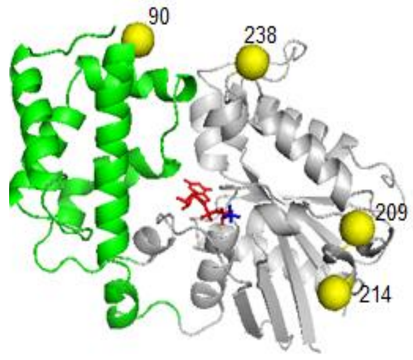


Figure 3. Yellow spheres represent cysteines that were introduced for labeling. Three FRET pairs containing either 90-238, 90-209, and 90-214 will be used for this study. Obtained from protein data bank (1GIT).

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SDS-PAGE of Hexa-I

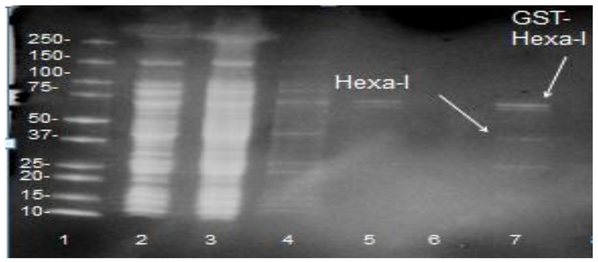

Figure 3. SDS-PAGE during purification of Hexa I-$G_{\alpha i1}$ utilizing a GST column. Lane 1 displays the ladder, Lane 2 lysate, Lane 3 flow-through, Lane 4 wash, Lane 5 GST beads, Lane 7 elution post TEV-digestion.

Acknowledgments

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Literature Cited

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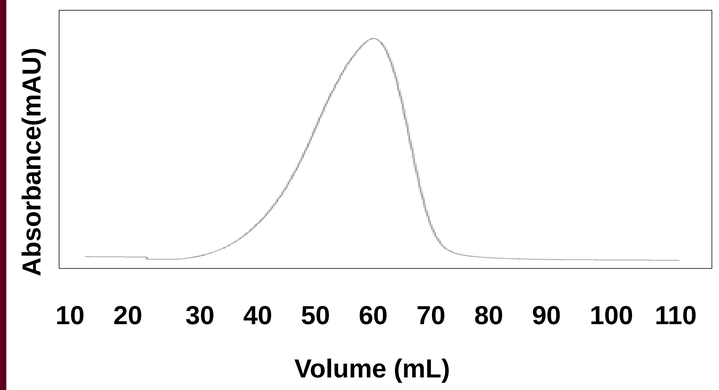
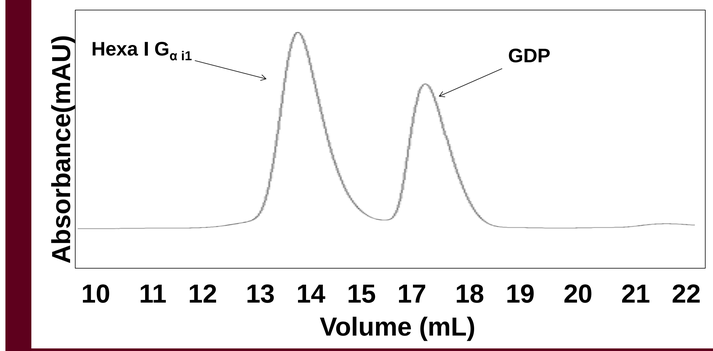
2) Van Eps, N., Thomas, C. J., Hubbell, W. L., & Sprang, S. R. (2015). The guanine nucleotide exchange factor Ric-8A induces domain separation and Ras domain plasticity in Gαi1. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(5), 1404–1409.

Q-Column Hexa I-$G_{\alpha i1}$



Size-Exclusion Hexa I-$G_{\alpha i1}$



Potential Stopped-Flow Results

$$G_{\alpha\ i1} \cdot GDP \overset{k_1}{\rightleftharpoons} G_{\alpha\ i1}{:}GDP{:}Ric\text{-}8A \overset{k_2}{\rightleftharpoons} G_{\alpha\ i1} \cdot Ric\text{-}8A$$

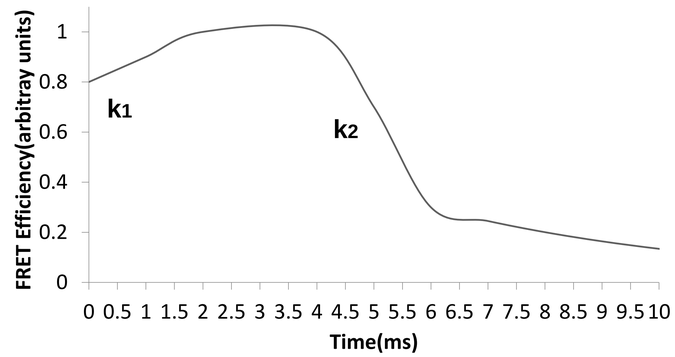


Figure 4. Potential Stopped-flow results from the experiment. The experiment could potentially elucidate a $G_{\alpha\ i1}$:GDP:Ric-8A intermediate along with the rate constants of Ric8A GEF activity. The mechanism shown above the graph corresponds to the position of the graph. The change of FRET per unit of time , will match with conformation al changes of $G_{\alpha i1}$, correlating with Ric-8A GEF activity.

Future Directions

- Label Hexa I-$G_{\alpha i1}$ with fluorescent dyes, and run stopped flow to determine the kinetics of Ric-8A induced conformational changes of $G_{\alpha i.}$