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

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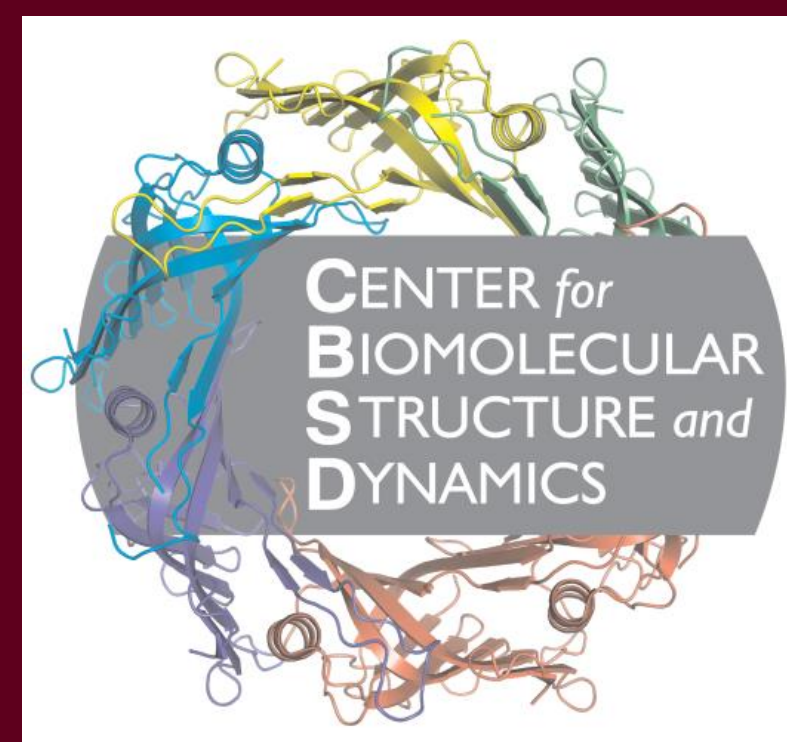
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# Conformational Changes of G $\alpha$ i1 Nucleotide Exchange Catalyzed by Ric-8A

Jake Johnston, Levi McClelland : Stephen Sprang

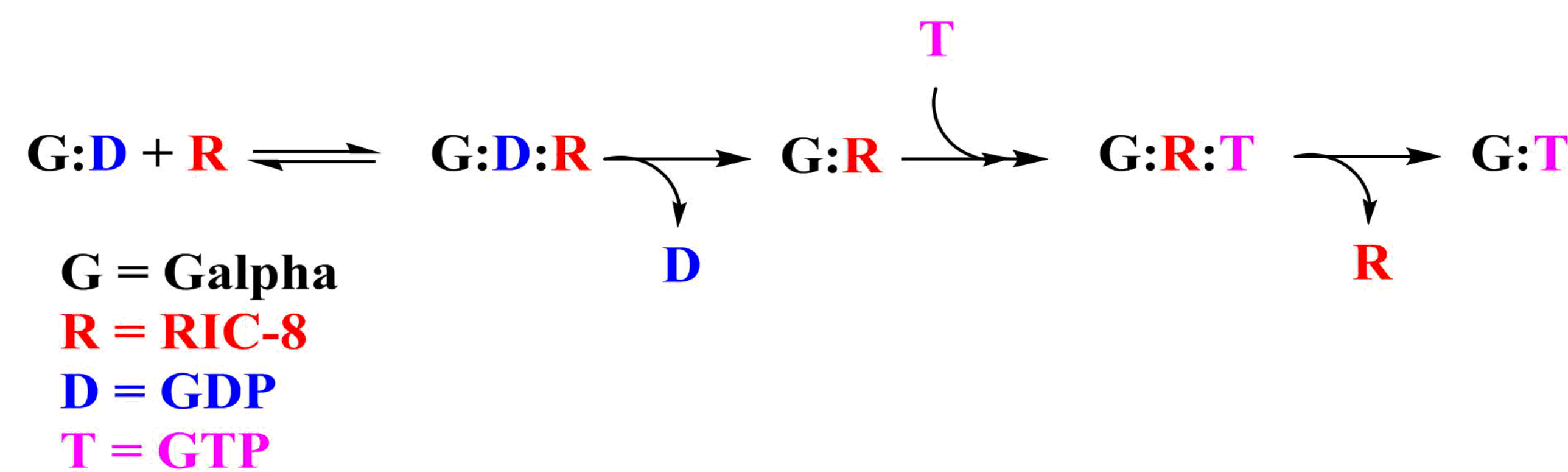
Center for Biomolecular Structure & Dynamics, The University of Montana, Missoula, MT



## Introduction

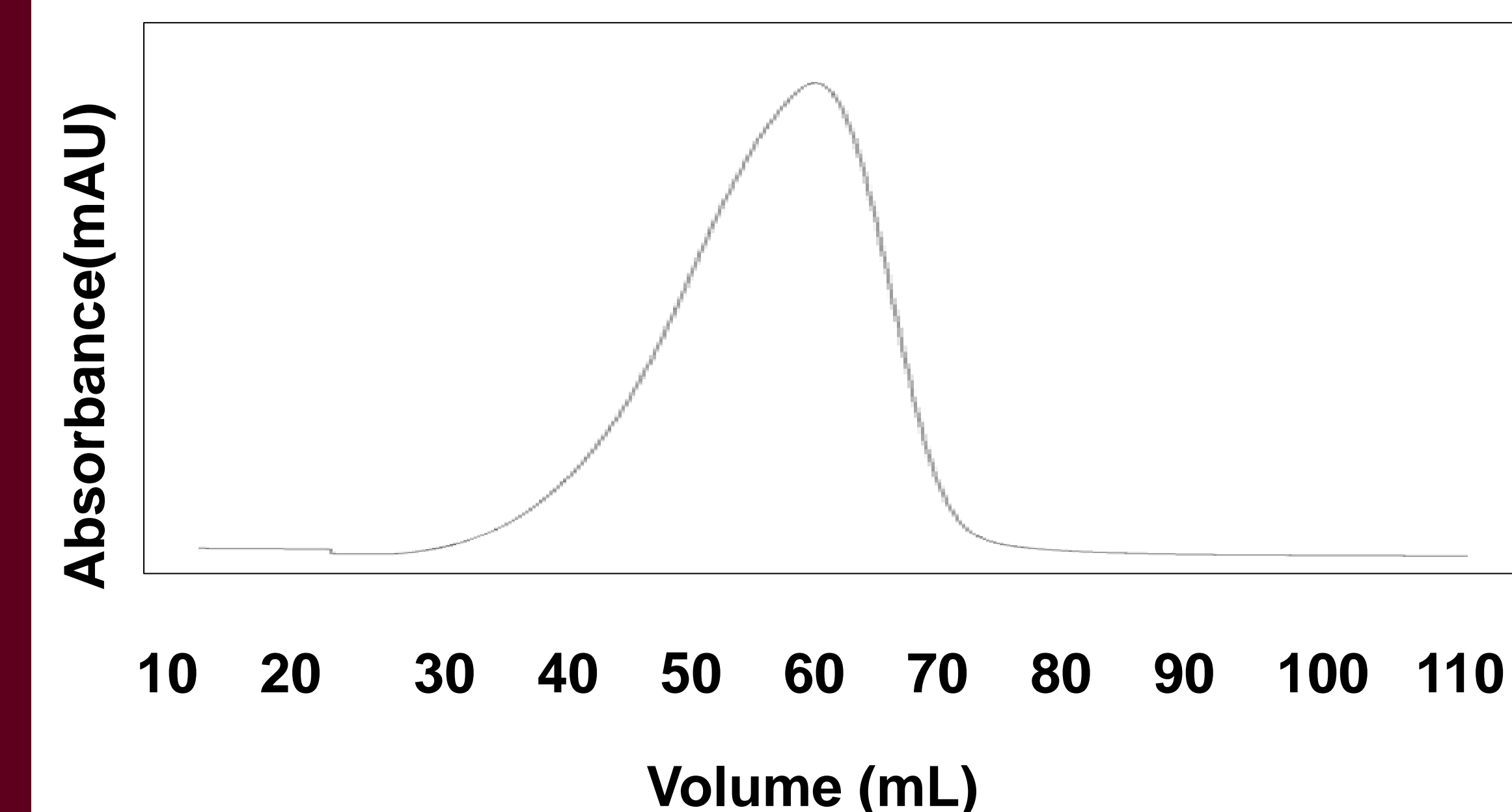
- G-Proteins regulate several cellular processes, and when defective, have been associated with several endocrinal disorders.
- Heterotrimeric G-Proteins are regulated by the binding of GDP (inactive state) and GTP (active state) on the  $\alpha$  subunit.
- Ric-8A is a ~60-kDA cytosolic protein that acts as a GEF to exchange GDP for GTP as shown in schematic 1 *in vitro*, and a chaperone for the G $\alpha$  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.

## 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 G $\alpha$ . This mechanism is poorly understood, and the kinetics have not been well established.

## Q-Column Hexa I-G $\alpha$ i1



## Hexa I-G $\alpha$ i1 Constructs

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

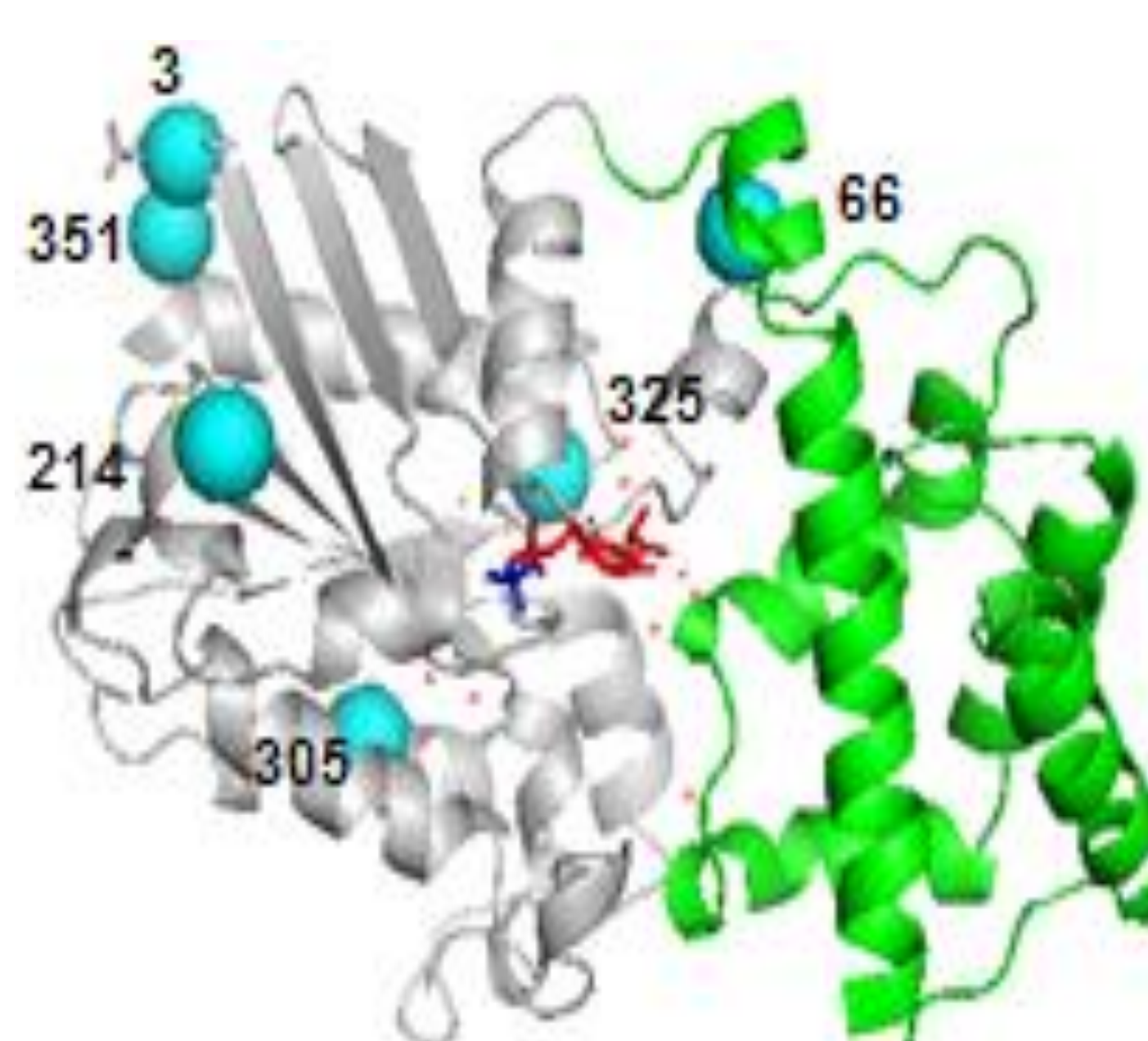


Figure 2. Blue spheres represent six naturally solvent exposed cysteines that were removed. Obtained from protein data bank (1GIT).

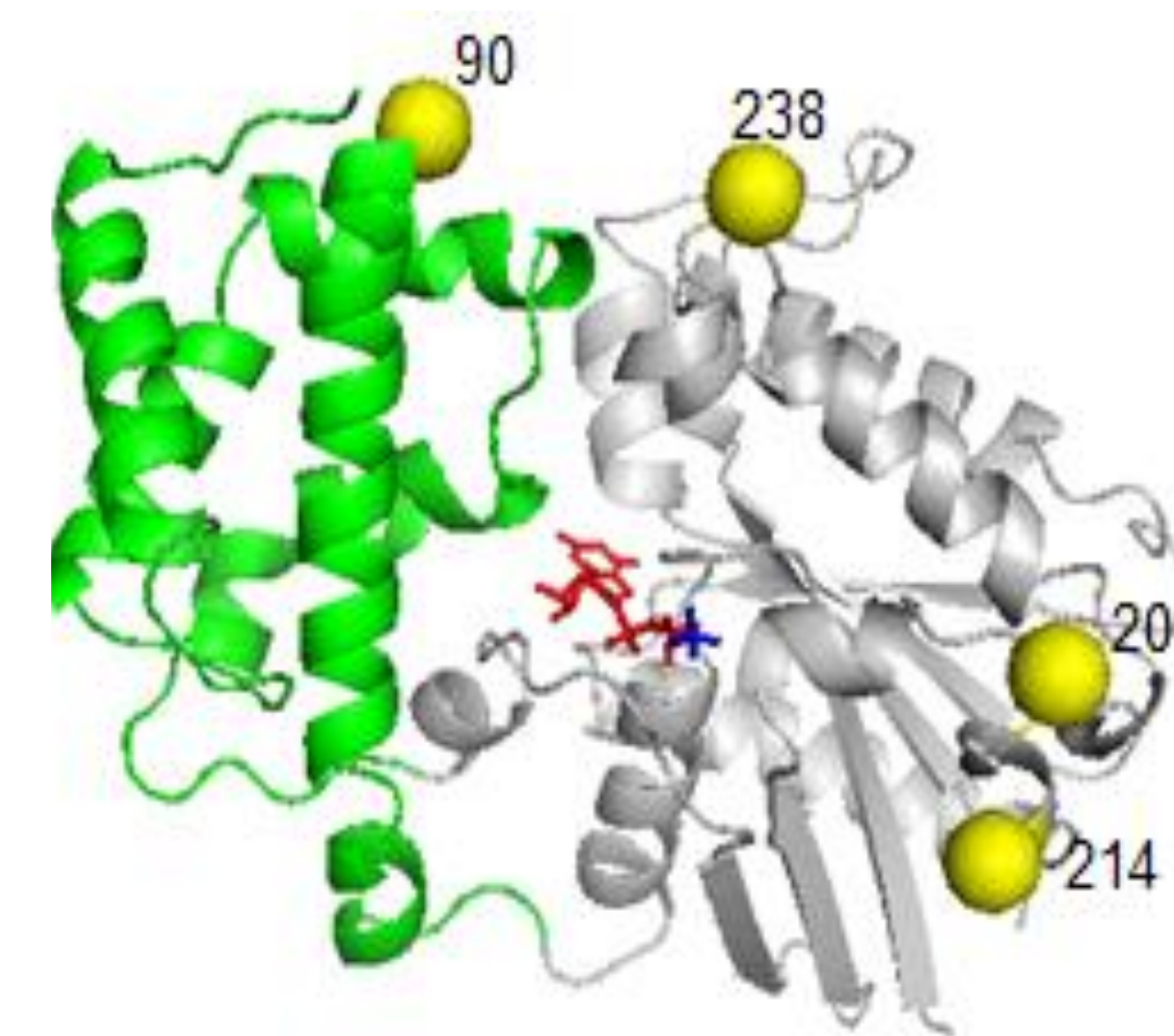
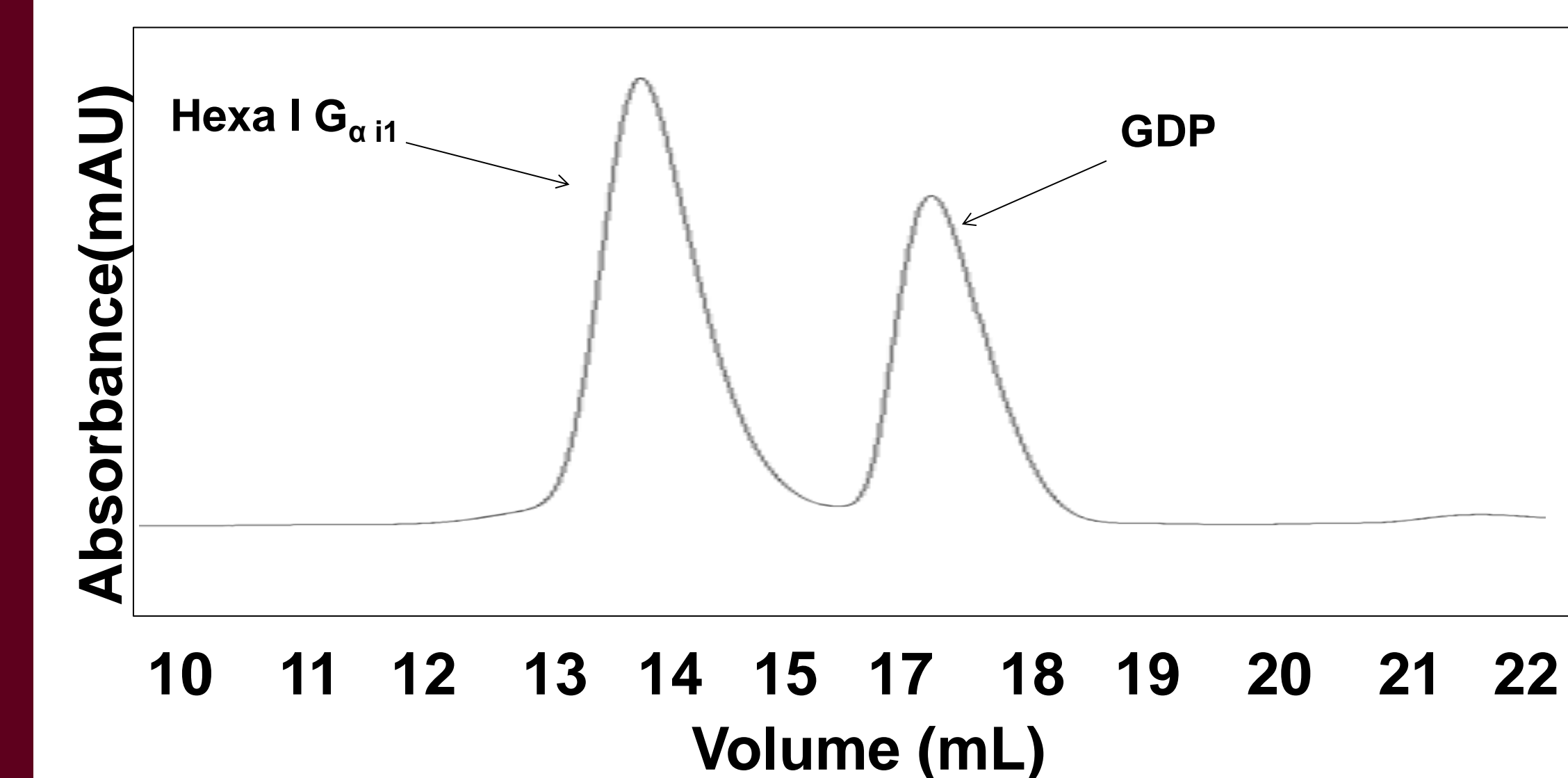


Figure 3. Yellow spheres represent cysteines that were introduced for labeling. Three FRET pairs containing either 90-238, 90-209, and 90-214 will be used for this study. Obtained from protein data bank (1GIT).

## Size-Exclusion Hexa I-G $\alpha$ i1



## Potential Stopped-Flow Results

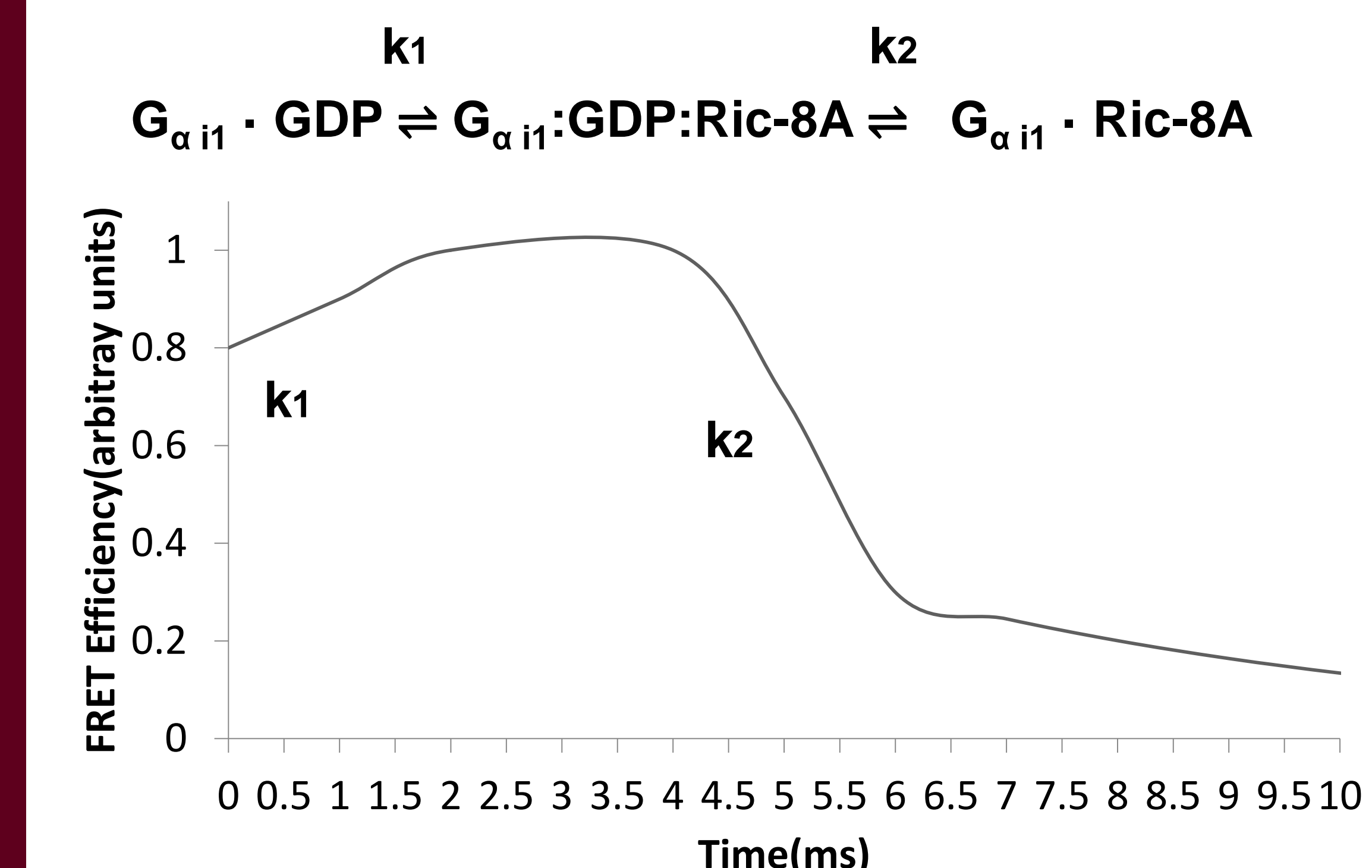
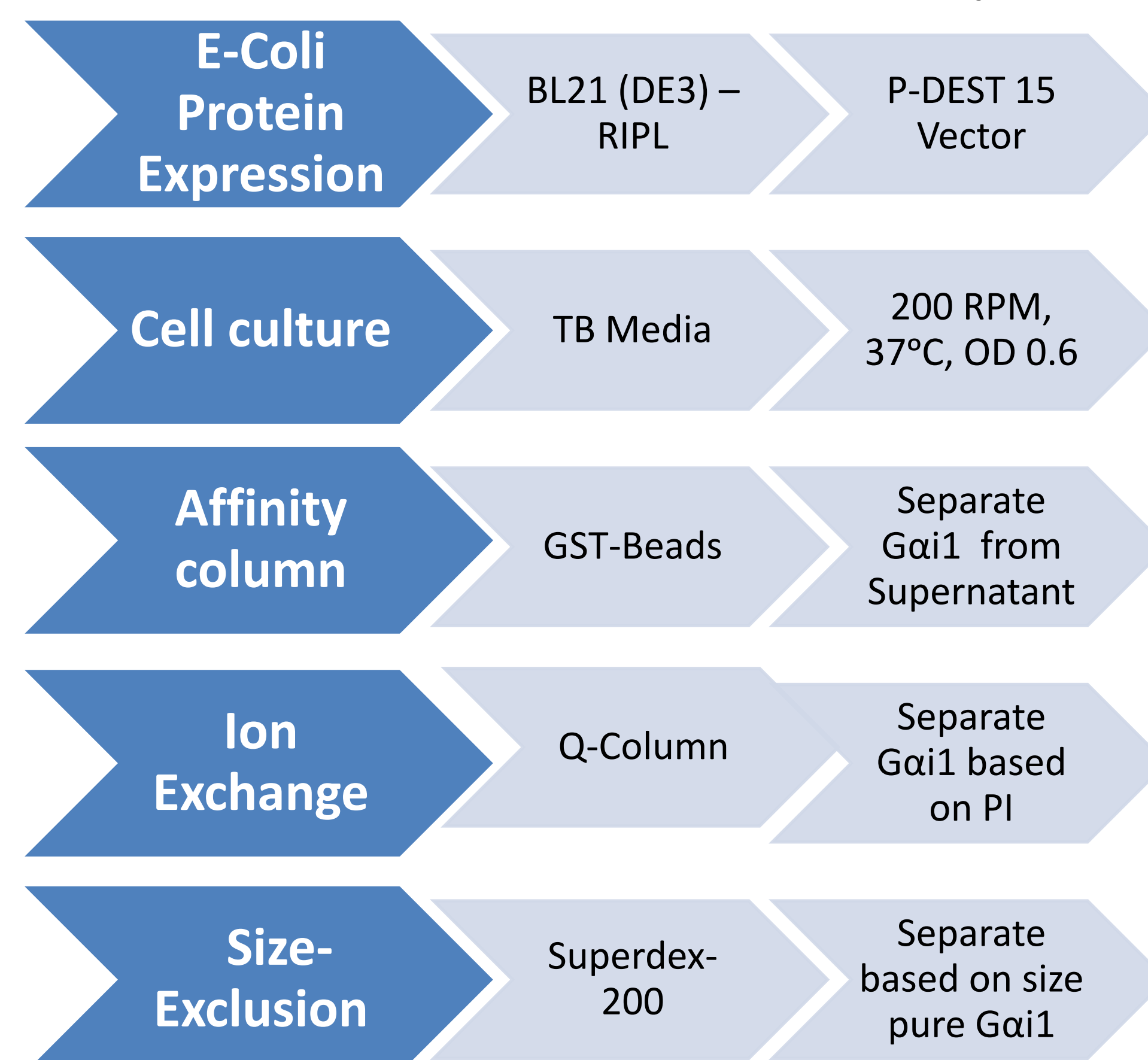


Figure 4. Potential Stopped-flow results from the experiment. The experiment could potentially elucidate a G $\alpha$ i1:GDP:Ric-8A intermediate along with the rate constants of Ric8A GEF activity. The mechanism shown above the graph corresponds to the position of the graph. The change of FRET per unit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

## Purification of Hexa I-G $\alpha$ i1



## 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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- 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 G $\alpha$ i1. *Proceedings of the National Academy of Sciences of the United States of America*, 112(5), 1404-1409.

## 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 $\alpha$ i1.