Identification of protein complexes formed by the major brain G protein $G\alpha_o$

Michael Koelle and Santosh Kumar
Dept. of Molecular Biophysics & Biochemistry
Yale University

Thinking = neurons signaling each other

Signals: ~ 7 neurotransmitters & ~100 neuropeptides
Receptors: ion channels & G protein coupled receptors

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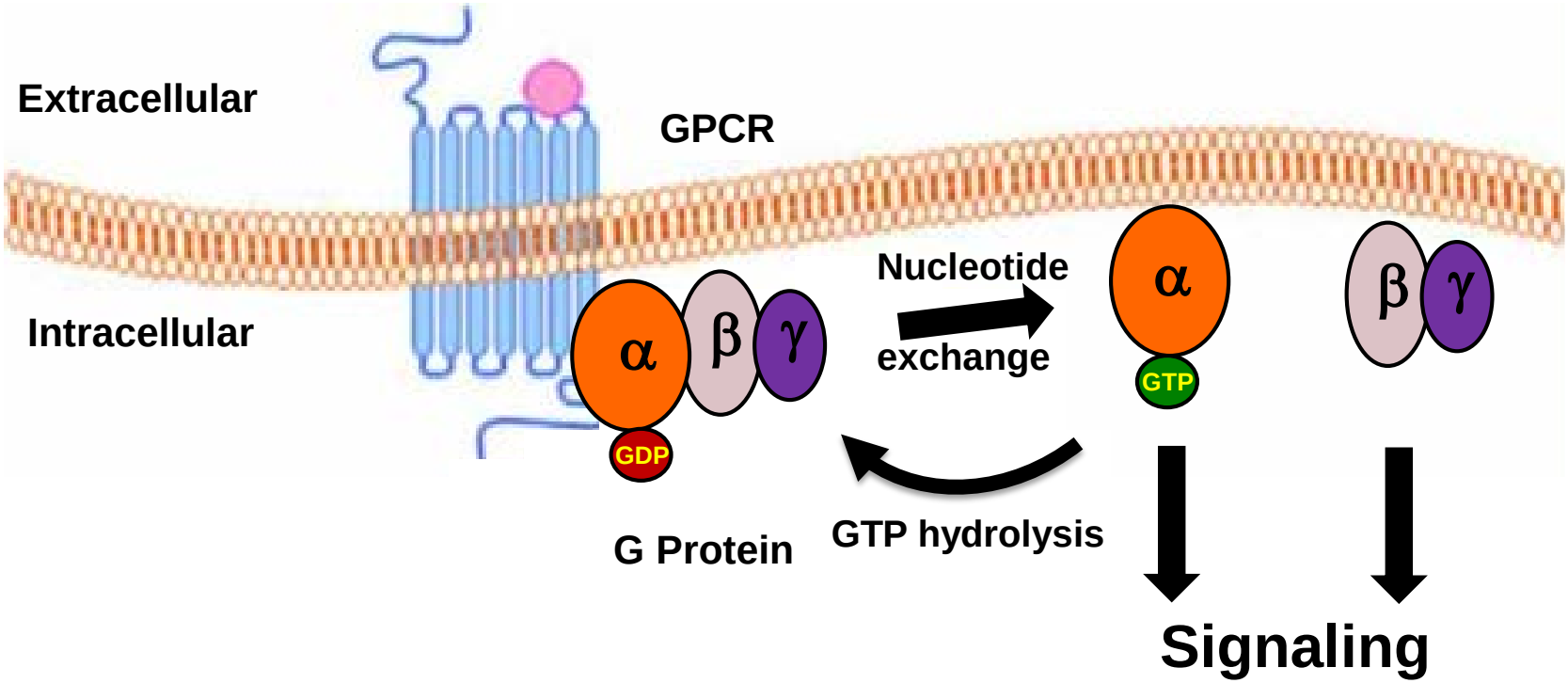
Emotions, memory, pleasure, etc.

Neural GPCR signaling has a menu of 4 outcomes



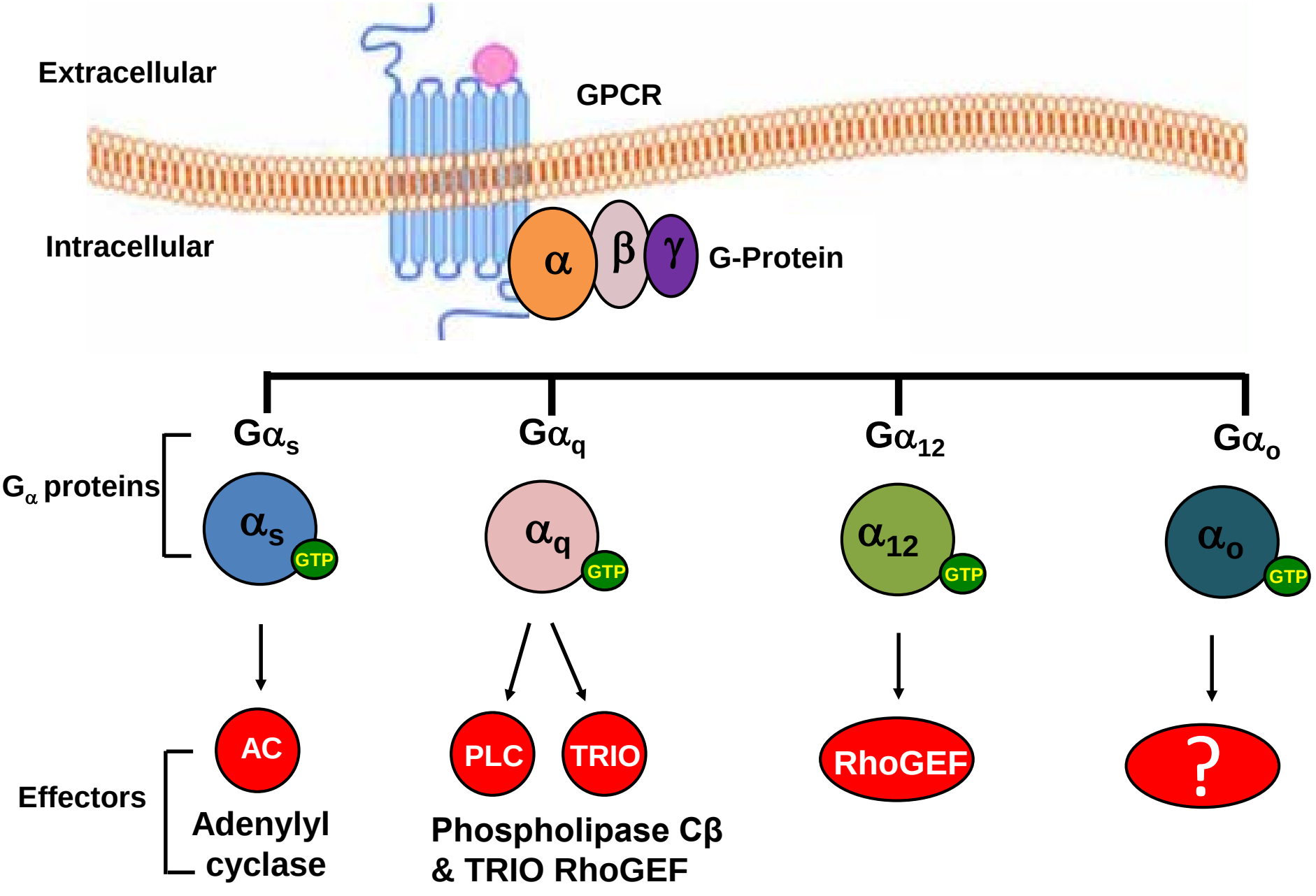
100's of GPCRs

~one type of Gβγ

~4 types of Gα

Each Gα type activates different "effector" proteins

$G\alpha_o$ is the $G\alpha$ protein without a known effector



35 years of failure to identify $G\alpha_o$ effectors

- 2 hybrid and other interaction screens
- Purification of $G\alpha_o$ binding proteins
- *C. elegans* genetic screens

Results: Some binding proteins- no convincing effectors

Do $G\alpha_o$ effectors even exist?

- $G\alpha_o$ could serve only to release $G\beta\gamma$
- But, this idea is refuted by *C. elegans* genetics

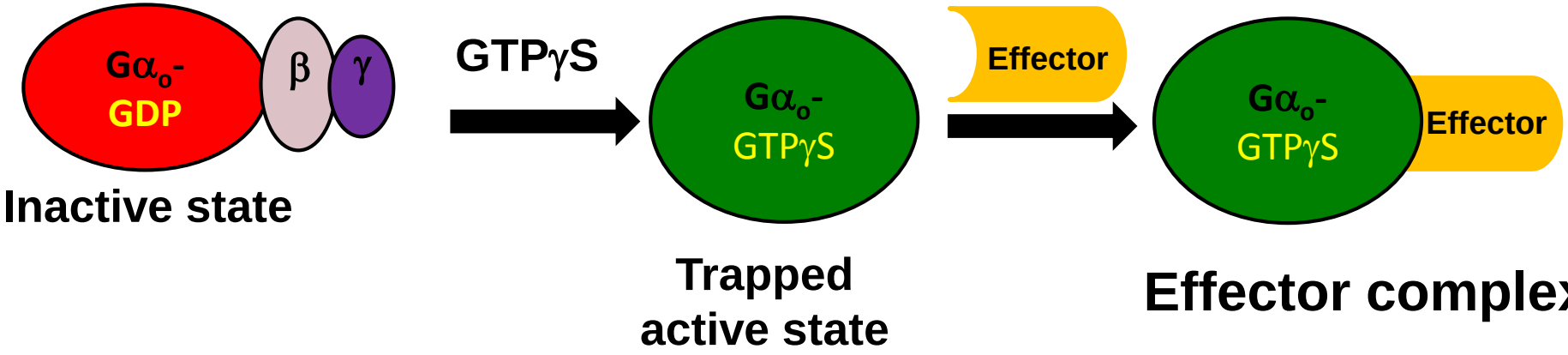
We are trying another approach…

Discover the effector proteins of $G\alpha_o$

Methodology:

1. **Immunopurify $G\alpha_o$-effector protein complexes**
2. **Identify the $G\alpha_o$-binding proteins by mass spectrometry**

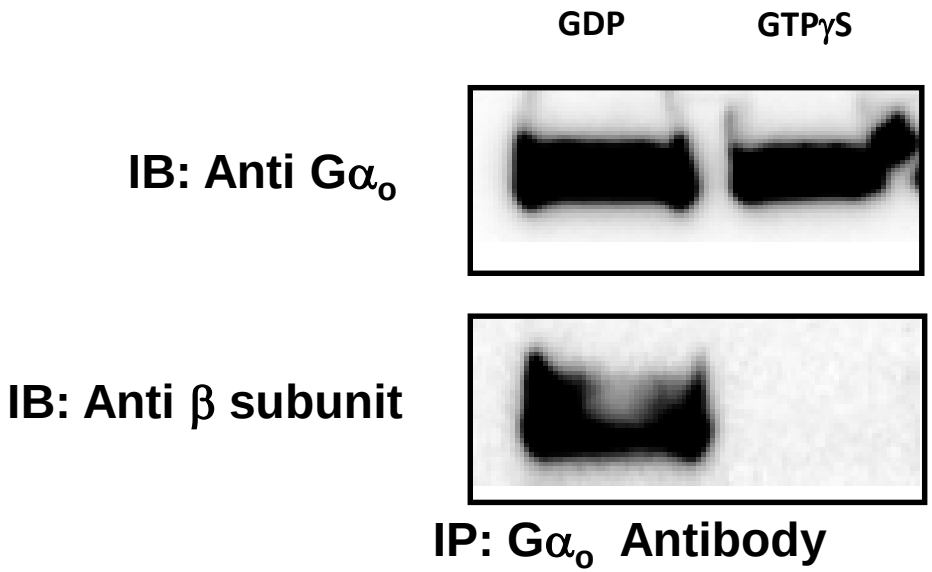
In-vitro activation of $G\alpha_o$ by GTPγS in protein lysates



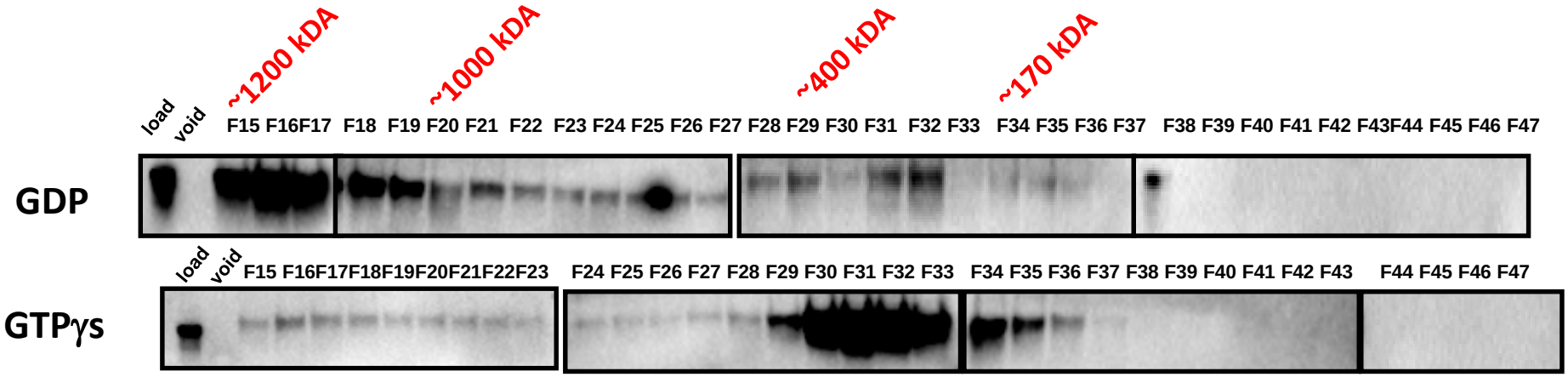
Inactive state

Trapped active state

Effector complex



Fractionating mouse brain lysate proteins by gel filtration shows that $G\alpha_o$ is part of large macromolecular complexes

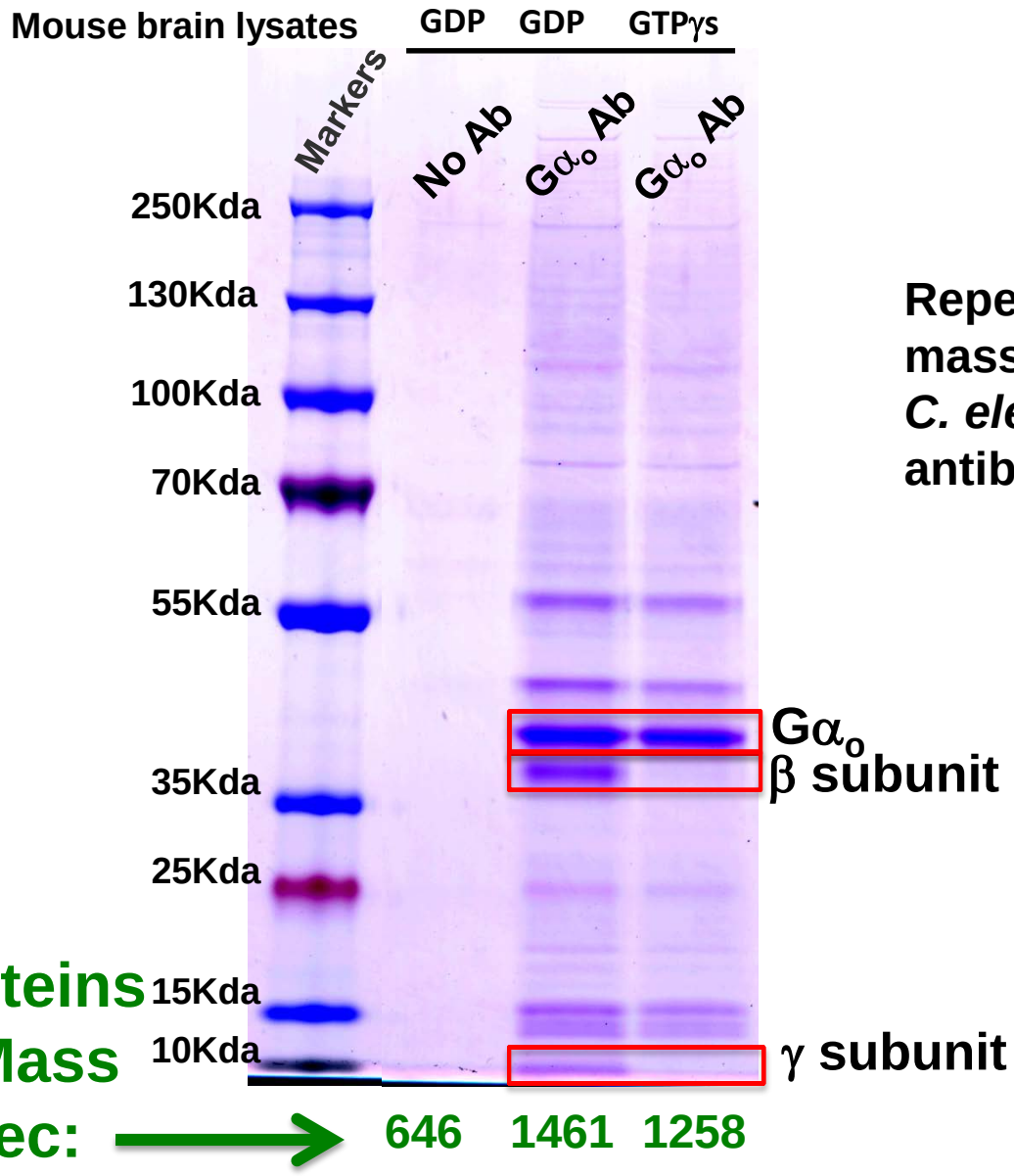


Anti-$G\alpha_o$ Western blots of fractions

$G\alpha_o\beta\gamma$-GDP is expected to be only ~82 kDa

$G\alpha_o$-GTP is expected to be only ~40 kDa

Immunoprecipitation/Mass spectrometry to identify proteins associated with $G\alpha_o$



Repeated the immunopurification / mass spec, using mouse brain and *C. elegans* lysates and different antibodies.