Characterization of a Novel Function of Gαi as an Intracellular pH Sensor

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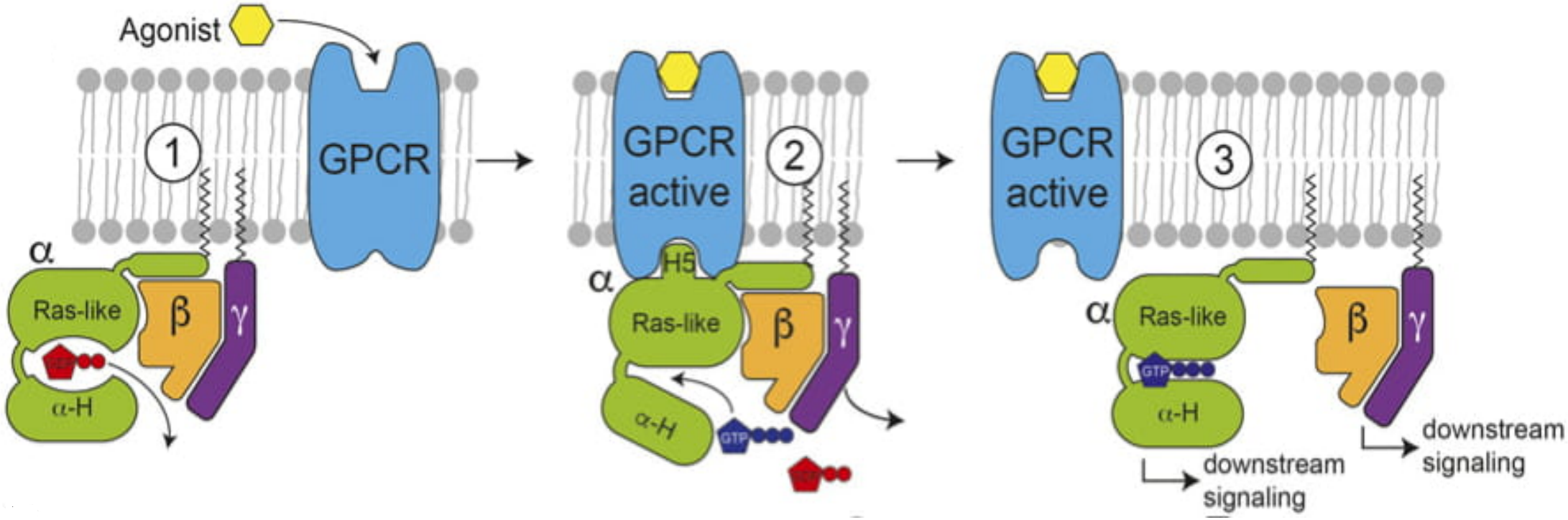
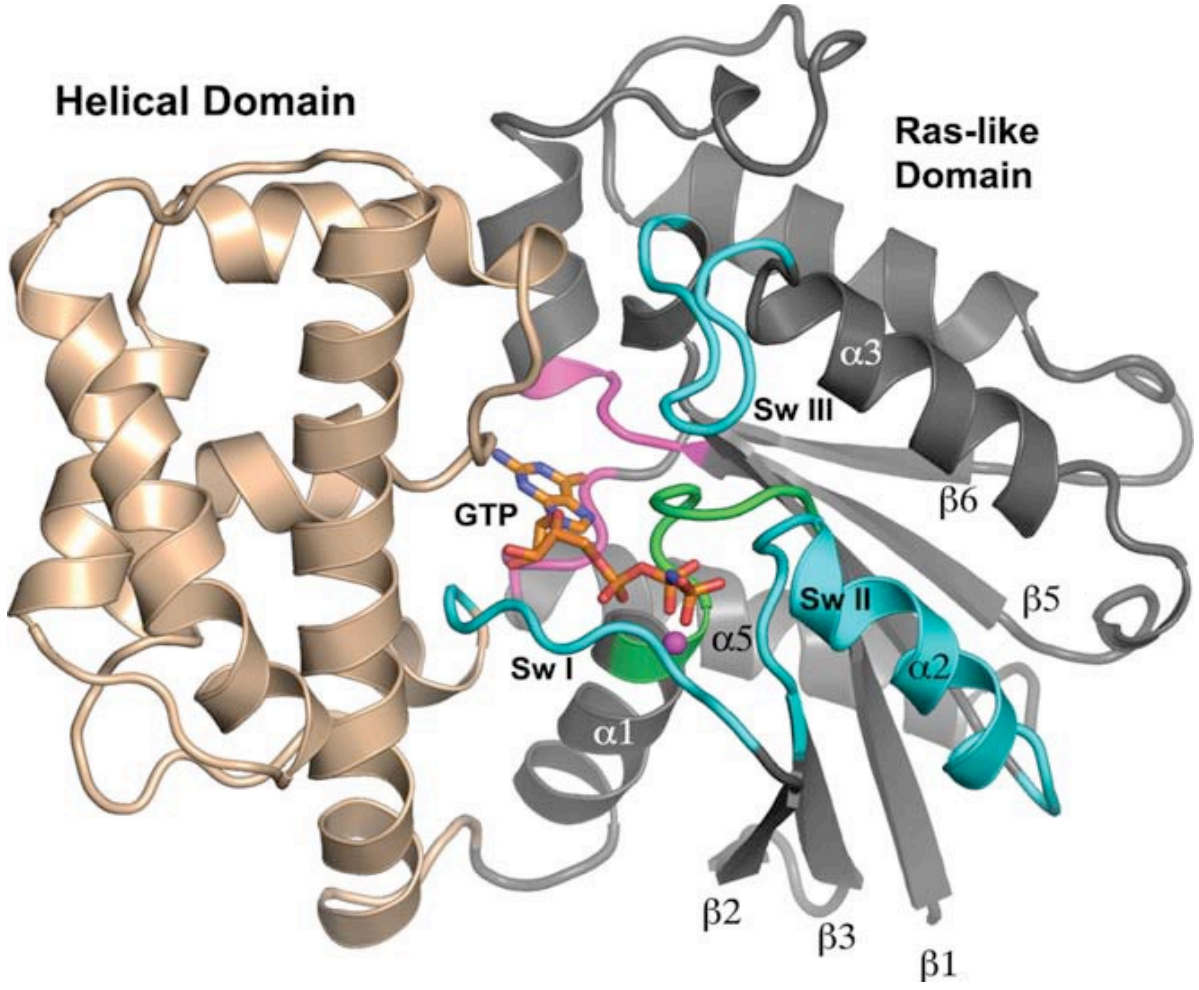
Gα is a downstream component of GPCR

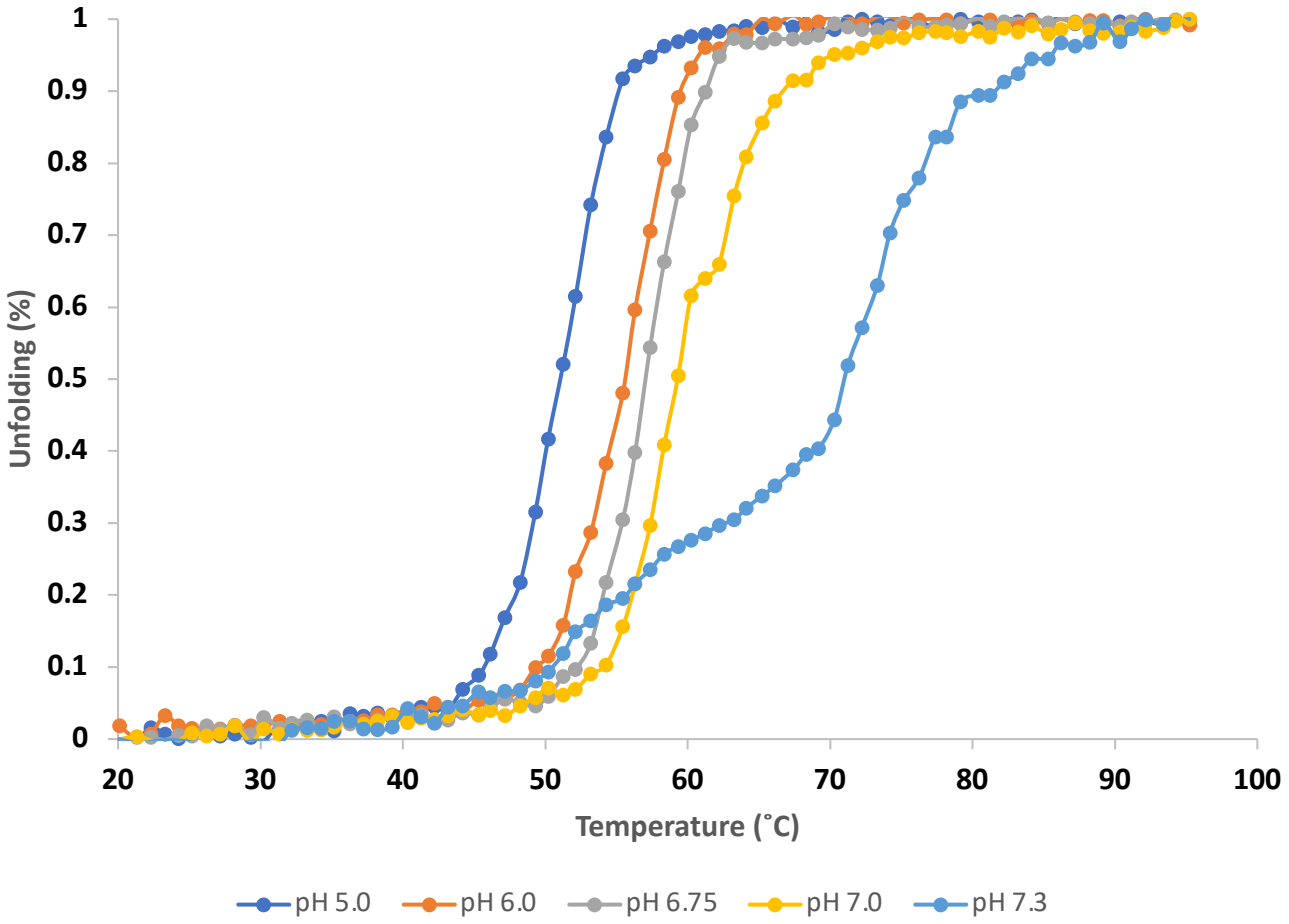


Goricanec D, Stehle R, Egloff P, et al. *Proc Natl Acad Sci U S A*. 2016.

Gα is a molecular switch

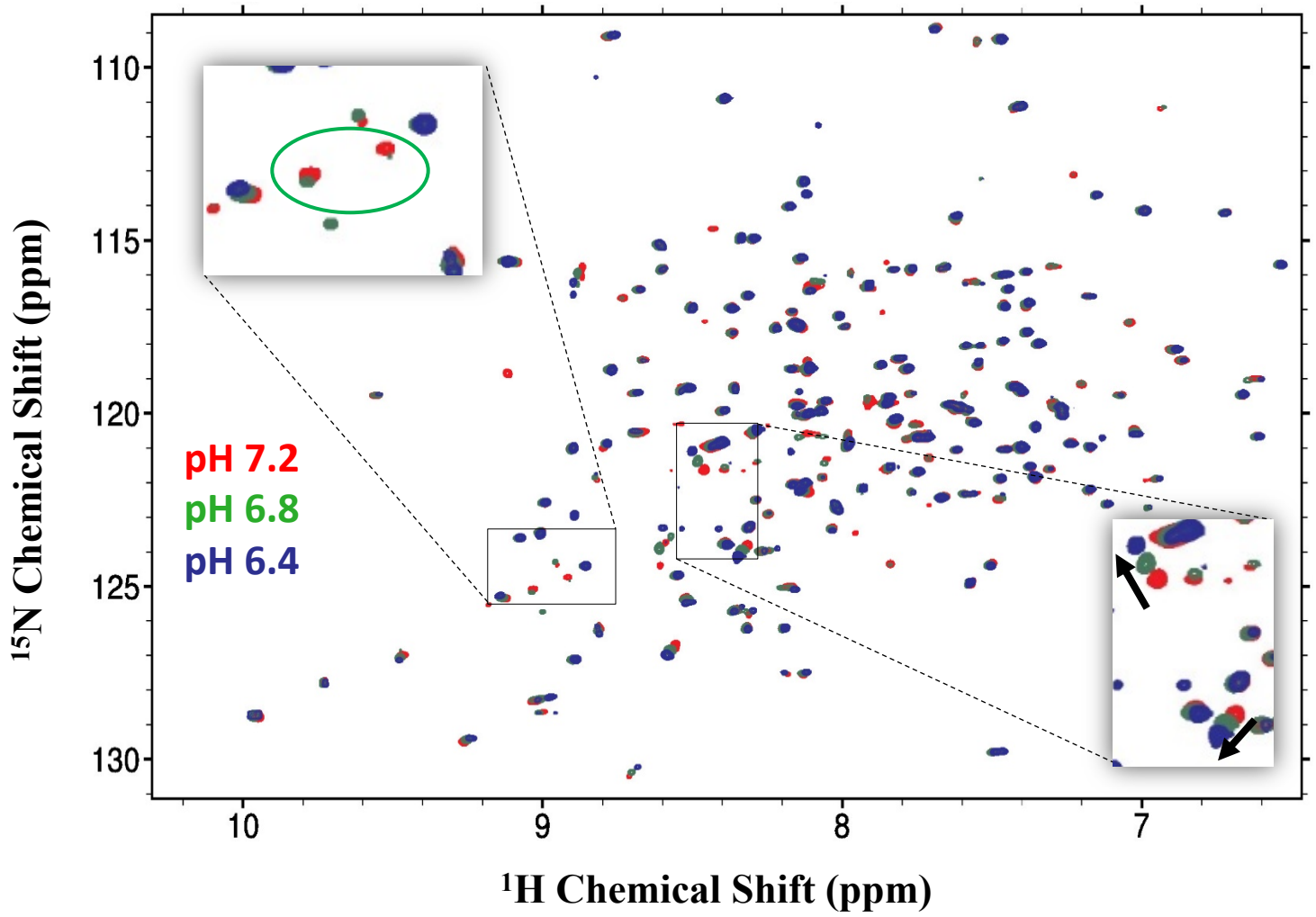


Thermal stability of Gαi-GDP is pH-dependent



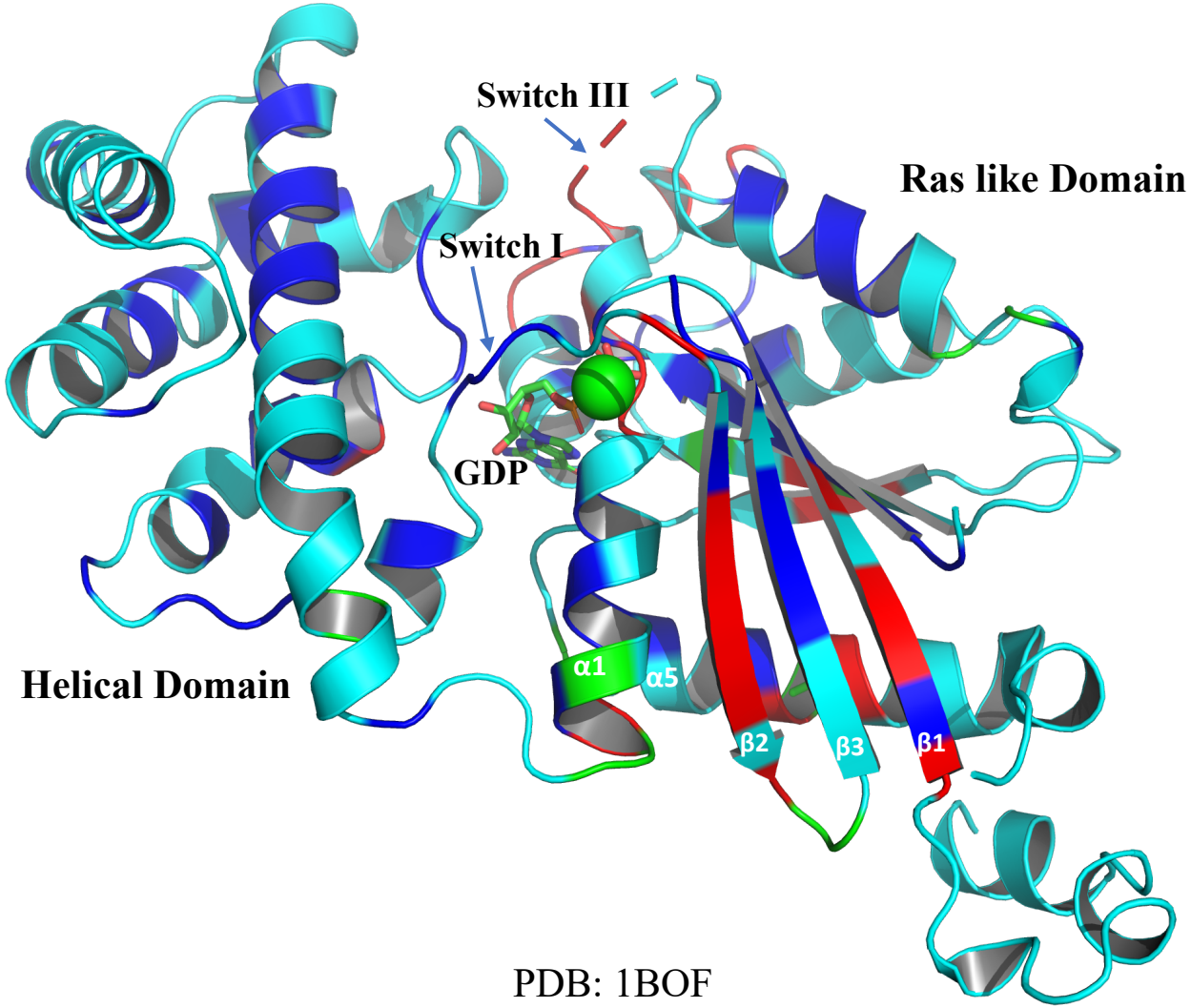
pH	Tm
5.0	50.8
6.0	55.6
6.75	57.2
7.0	58.3, 64
7.3	56.3, 74.2

Chemical shift and broadening of Gαi-GDP is pH-dependent



Juhi Rasquinha. Unpublished Data

Residues in switch regions show NMR chemical shift and broadening for pH 6.4 to 7.2



PDB: 1BOF

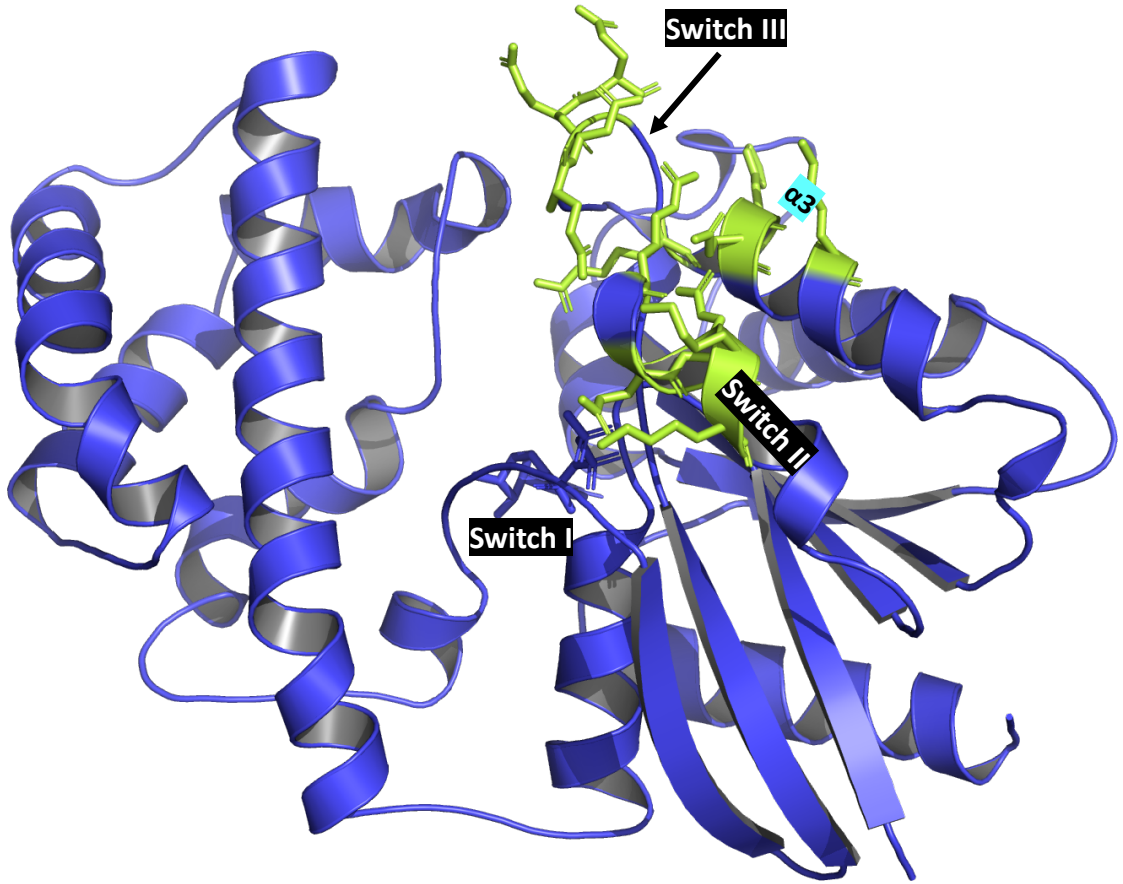
Broadened residues

Shifted residues

Missing/unassigned residues

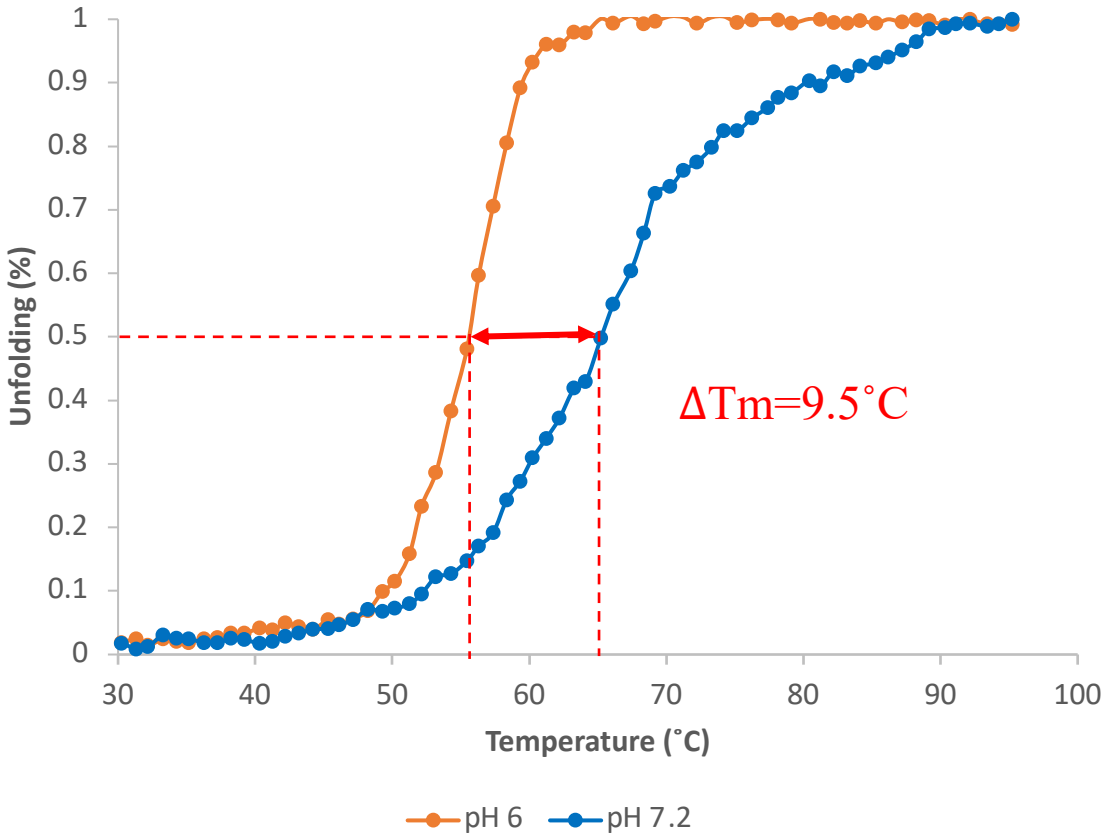
Juhi Rasquinha. Unpublished Data

Switch regions of Gαi contain charged amino acids forming interactive network

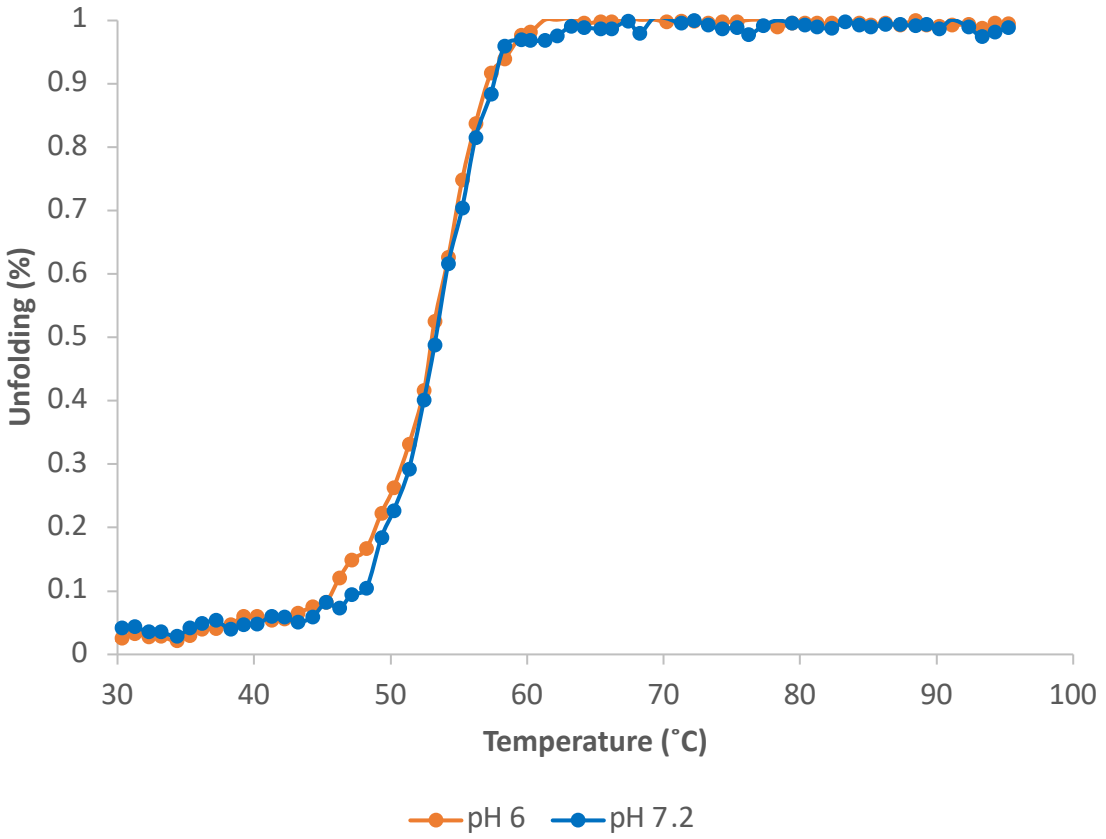


PDB: 1CIP

Gαi-GDP Triple mutant from the switch network abolishes pH-dependent thermostability

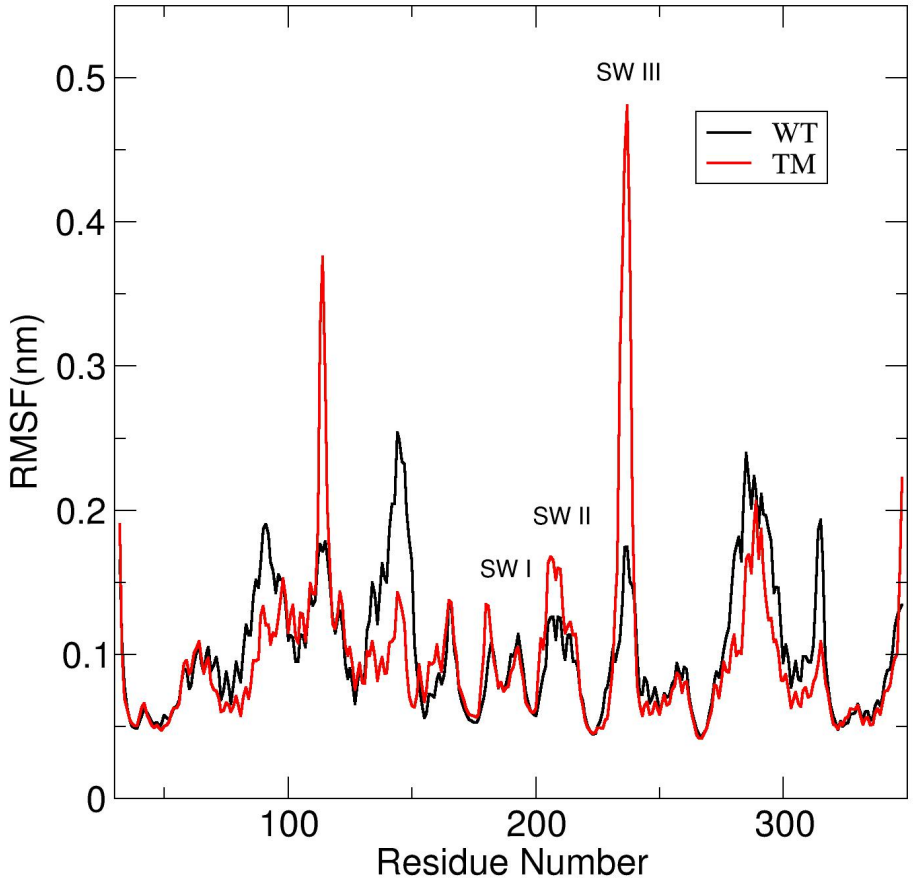


WT Gαi

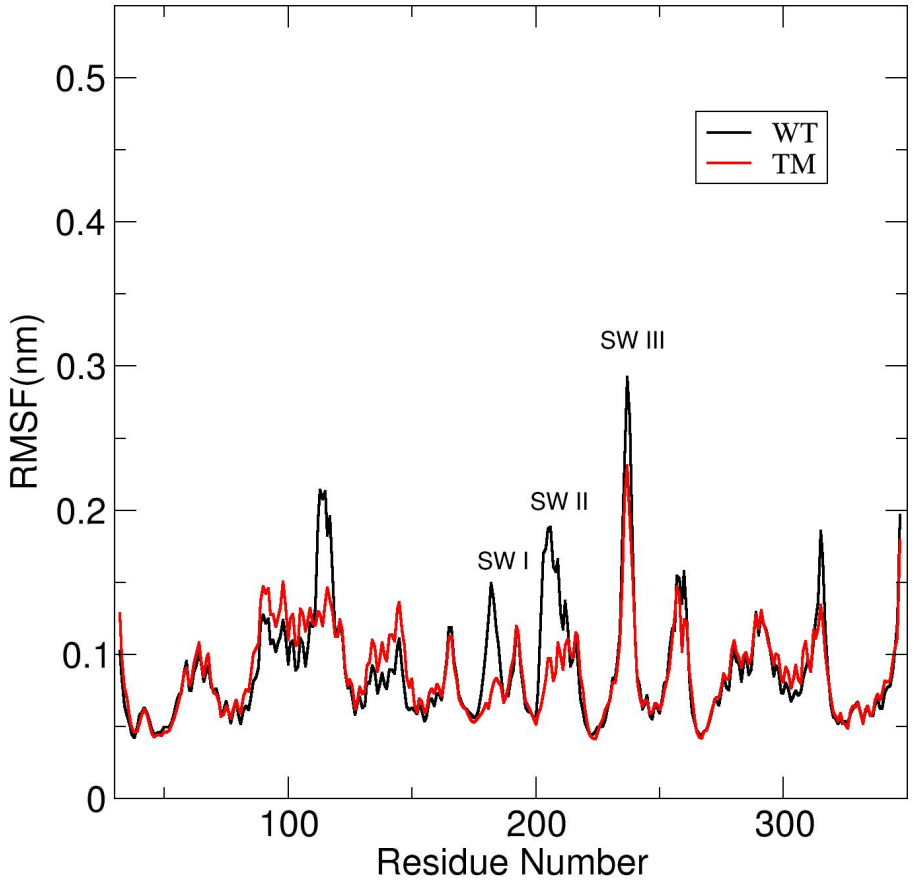


Triple mutant

Triple mutant makes switches more dynamic in Gαi-GDP but not in Gαi-GTP

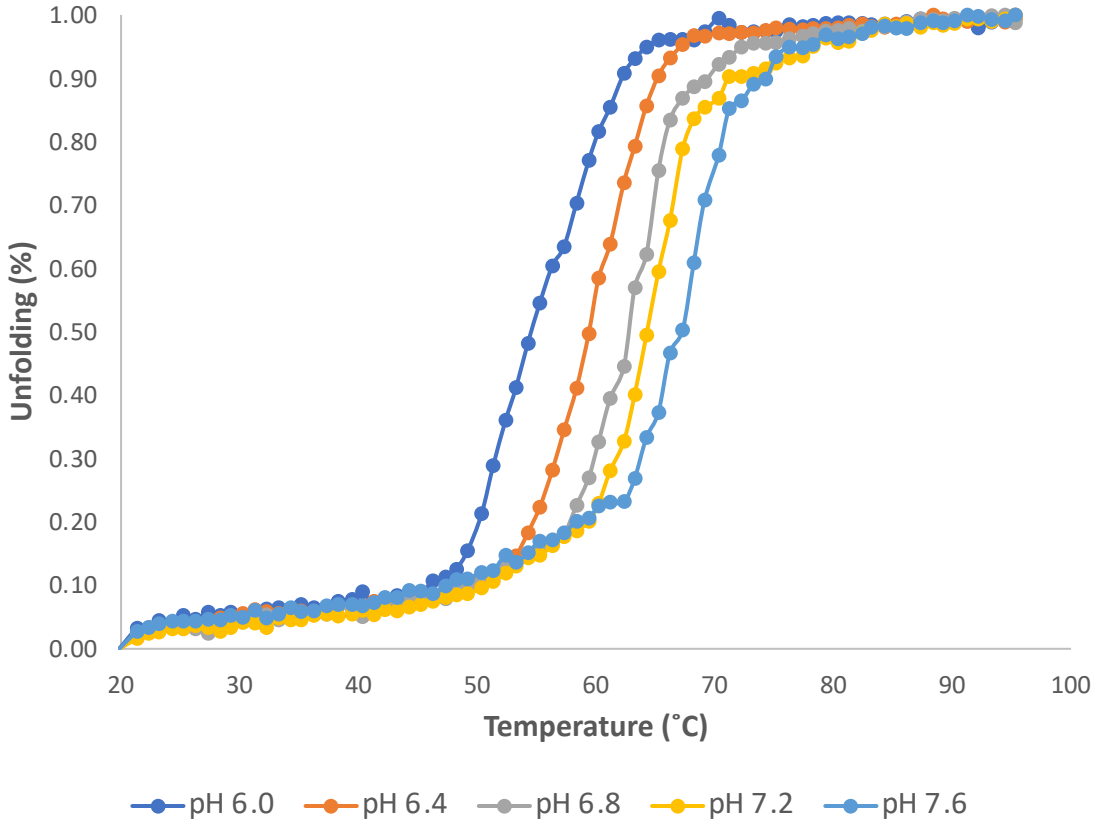


Gai-GDP

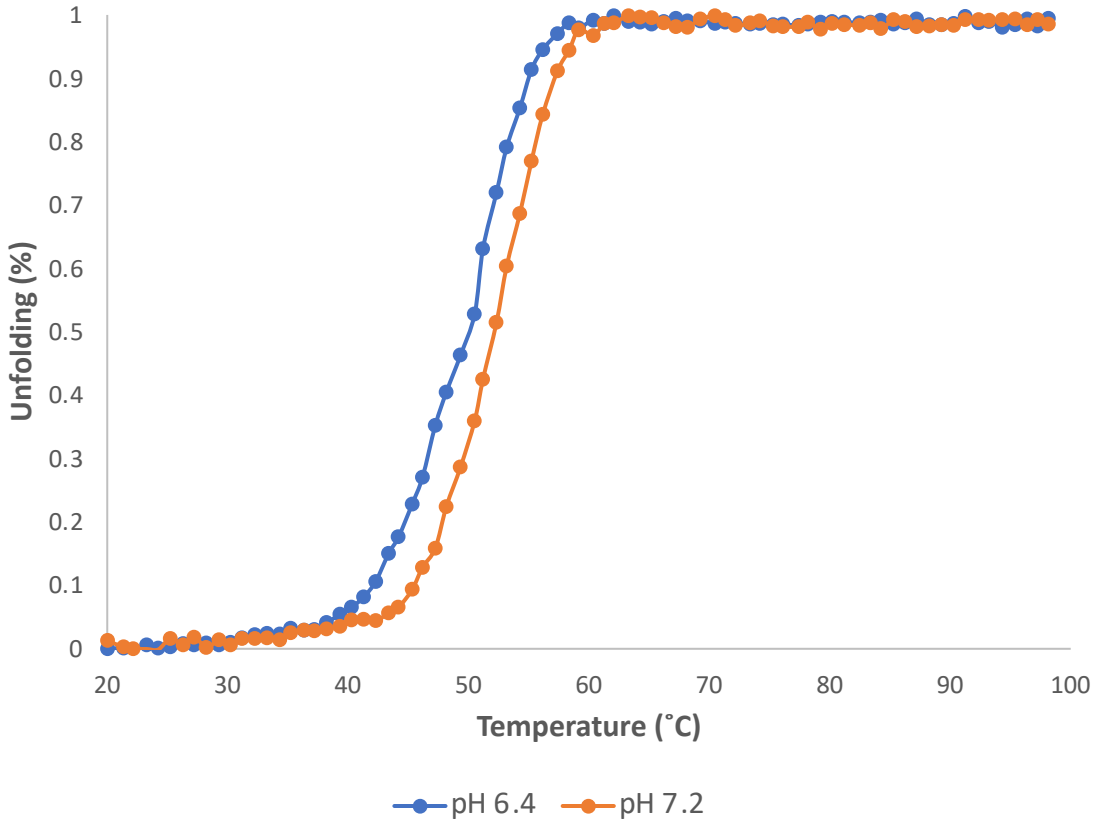


Gai-GTP

Gαi-GMPPCP Triple mutant does not completely abolish pH-dependent thermostability

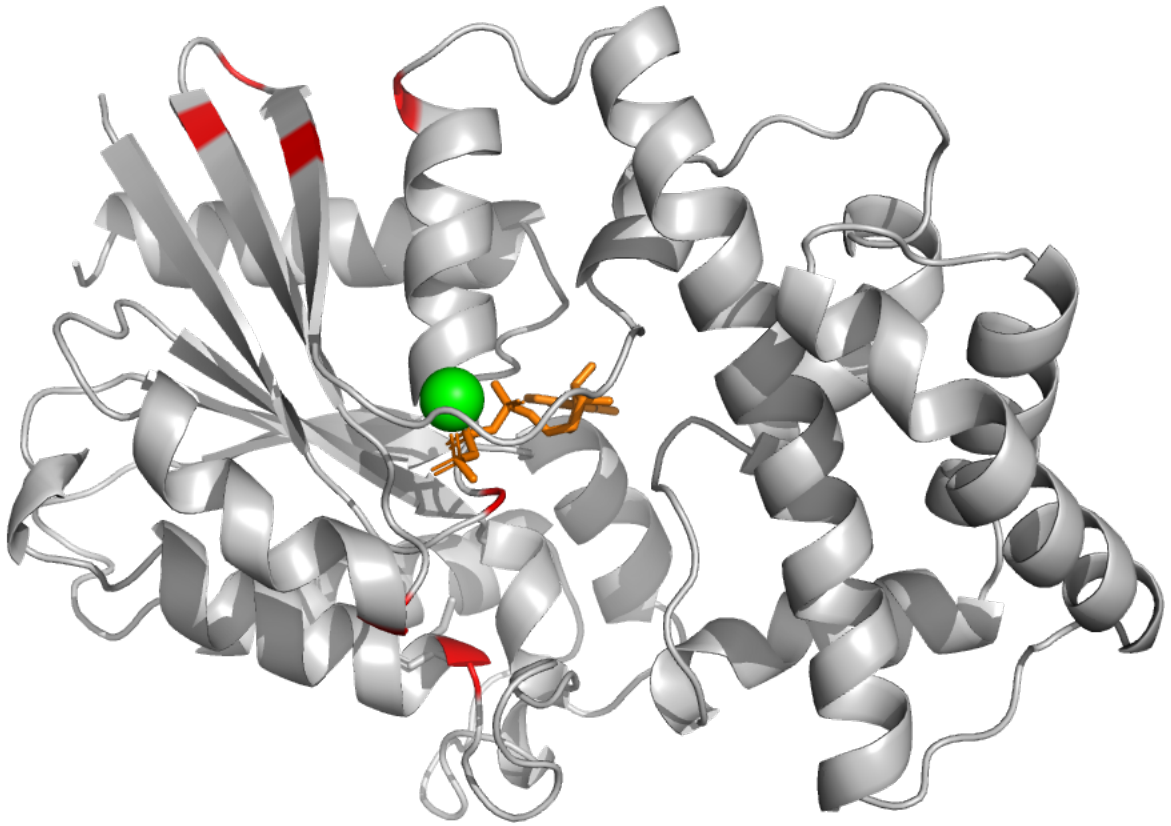


WT Gαi



Triple mutant

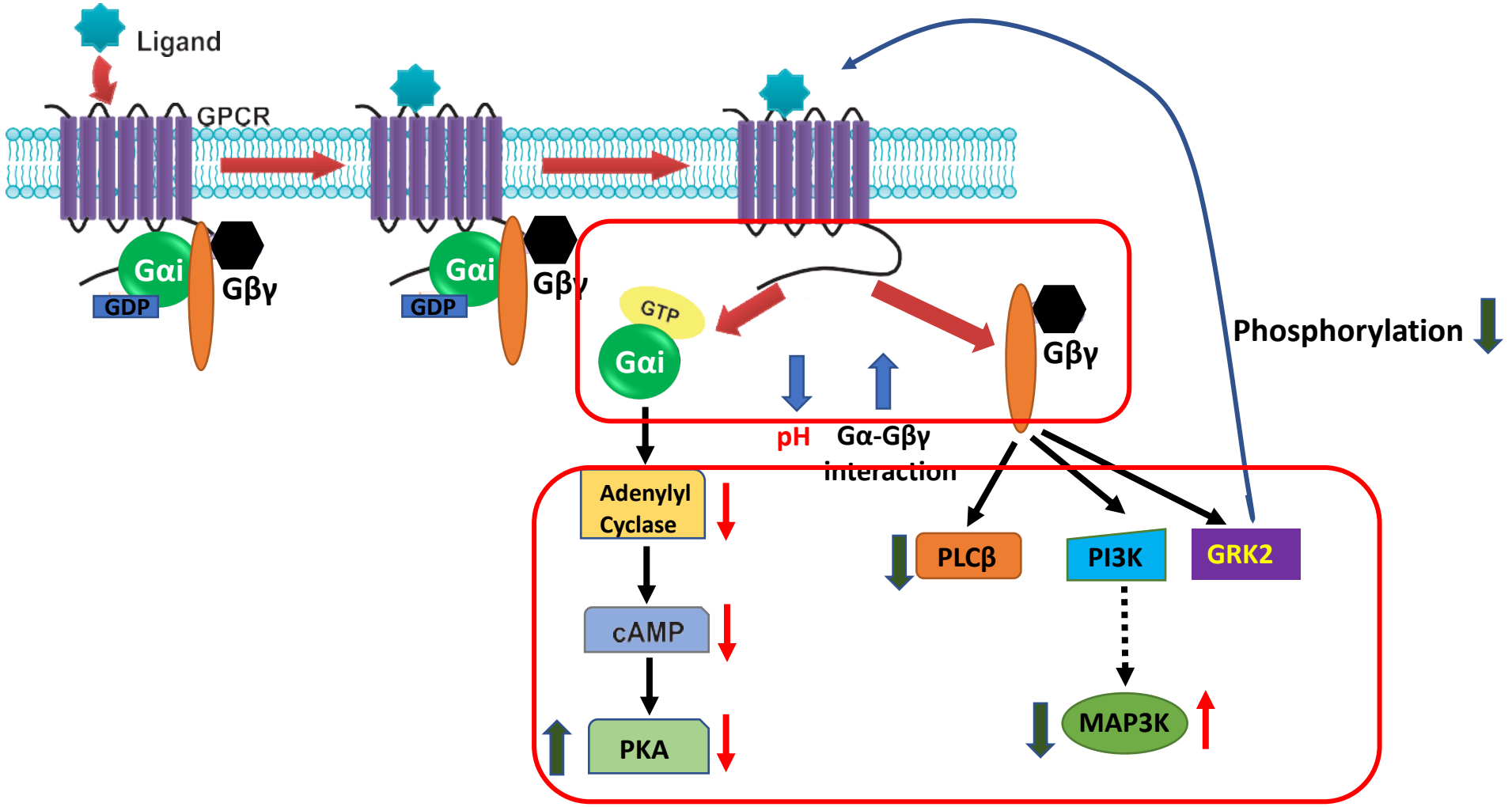
Some residues in the GDP release network may affect pH-dependent thermostability in Gαi-GMPPCP



GDP release network residues

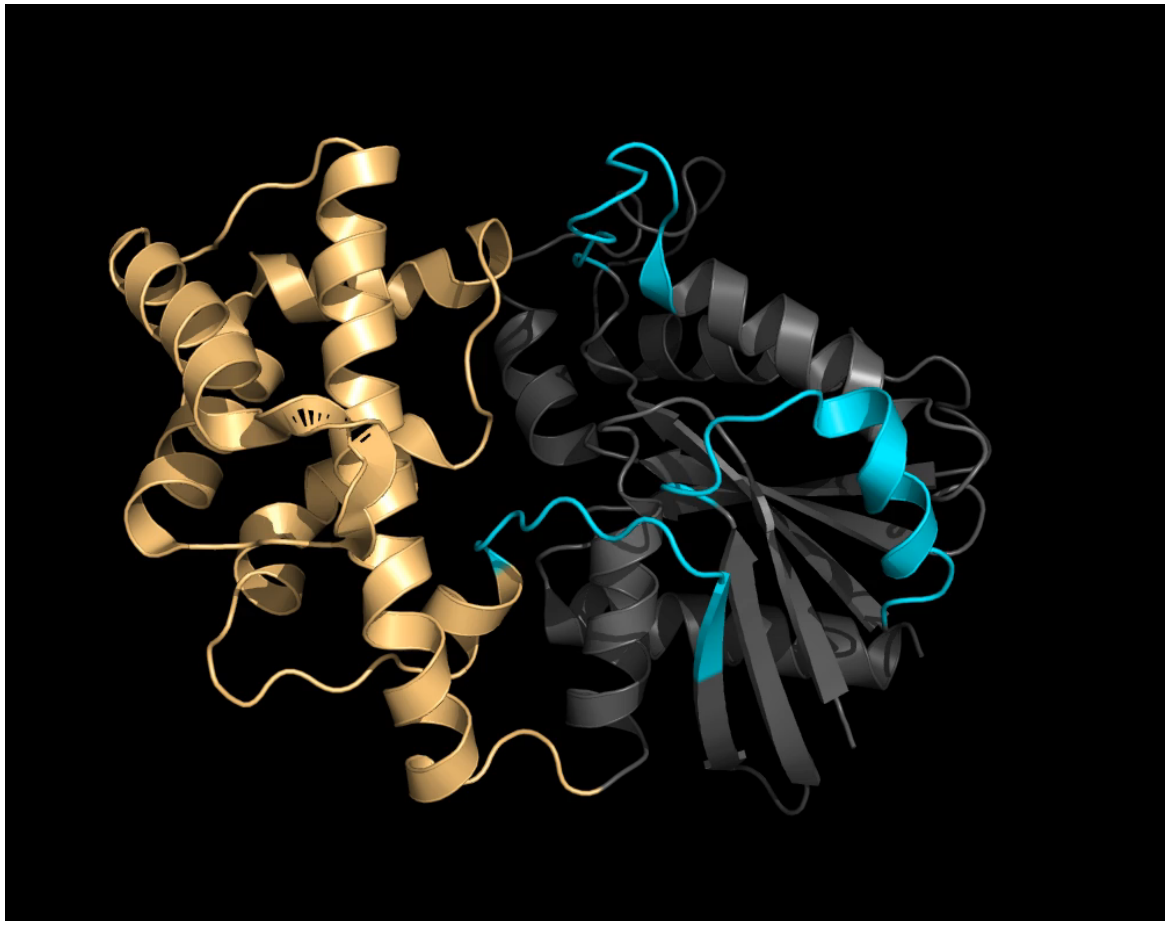
Residue	Tm (pH 6.4)	Tm (pH 7.2)	Δ
WT	59.5	64.5	5
E43	60	64	4
H57	59	64	5
H188	60	66.5	6.5
K192	56	63	7
H195	59	64.5	5.5
R205	54.5	63	8.5
R242	56.5	65.5	9
TM	50	52	2

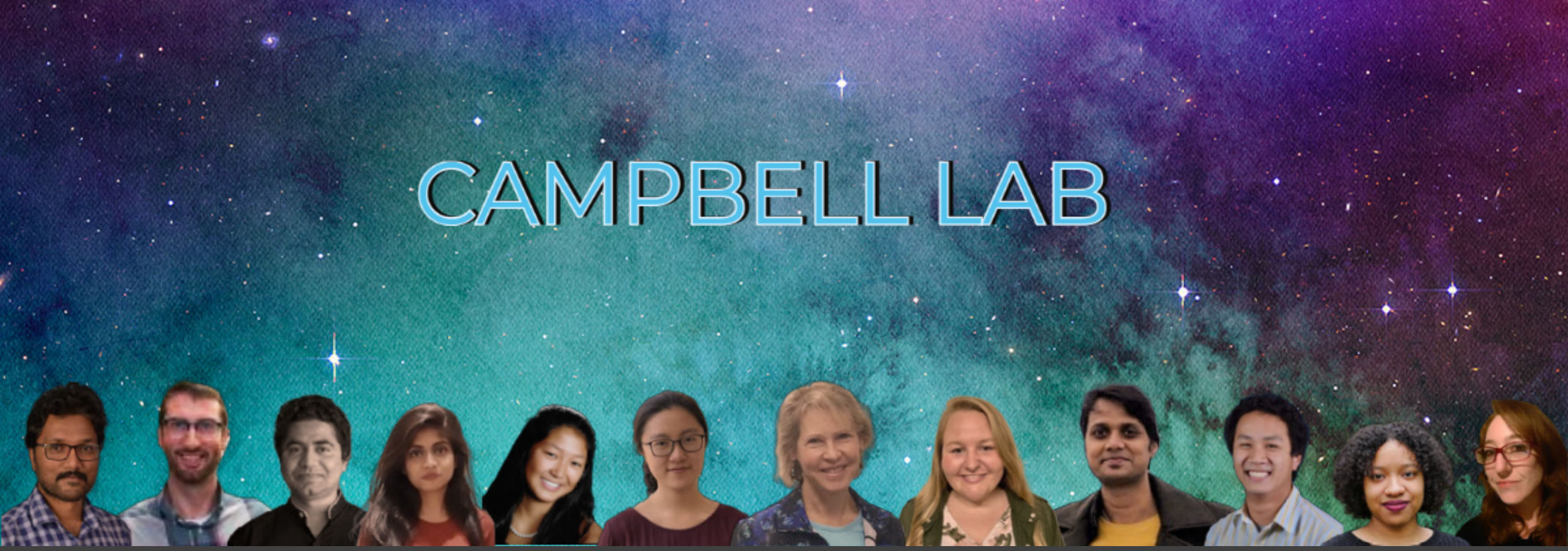
Future Perspective



Conclusion: Gα is an intracellular pH sensor

- Thermal stability of Gαi is pH-dependent in both active and inactive forms
- Low pH alters Gα structure and destabilizes Gα demonstrated by lower overall T_m
- Mutant Gα acts as low-pH mimetic suggesting the identification of key pH-sensing residues
- Active Gα may have additional pH-sensing network





Thank you!