

EFFECTS OF THE A2AR C-TERMINUS ON RECEPTOR STABILITY

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G-protein coupled receptors (GPCRs) are seven-transmembrane domain membrane proteins which are targets for nearly half of all pharmaceuticals on the market. The adenosine A2A receptor (A2AR) is a class A GPCR that is often a drug target due to its involvement in neurodegenerative diseases, diabetes, inflammatory diseases, cancer, and heart disease. At present, the only crystal structures of A2AR are of a truncated variant of the receptor (A2A Δ 316R). However, the full length C-terminus has been shown to be critical in downstream signaling. Here, we use ligand binding to investigate the effects of the C-terminus on A2AR stability by comparing full-length A2AR, A2A Δ 316R, and Rag23, a thermostable A2A Δ 316R variant with 5 point mutations favoring agonist binding (Magnani, Shibata, Serrano-Vega, & Tate, 2008). Receptors were overexpressed using a multicopy vector, pITy, purified in detergent micelles, and incubated with fluorescent agonist FITC-APEC. Fluorescence anisotropy was used to determine FITC-APEC binding after receptors were exposed to various conditions (Swonger & Robinson, 2017). We quantified equilibrium binding, temperature stability, kinetic rates, and competition with agonists and antagonists and find that beyond downstream signaling, the C-terminus contributes strongly to A2AR stability in micelles.

Magnani, F., Shibata, Y., Serrano-Vega, M. J., & Tate, C. G. (2008). Co-evolving stability and conformational homogeneity of the human adenosine A2a receptor. *Proceedings of the National Academy of Sciences*, 105(31), 10744–10749. <https://doi.org/10.1073/pnas.0804396105> Swonger, K. N., & Robinson, A. S. (2017). Using fluorescence anisotropy for ligand binding kinetics of membrane proteins. *Current Protocols*. Submitted