



Ligand Gated Ion Channel Functionality Assays

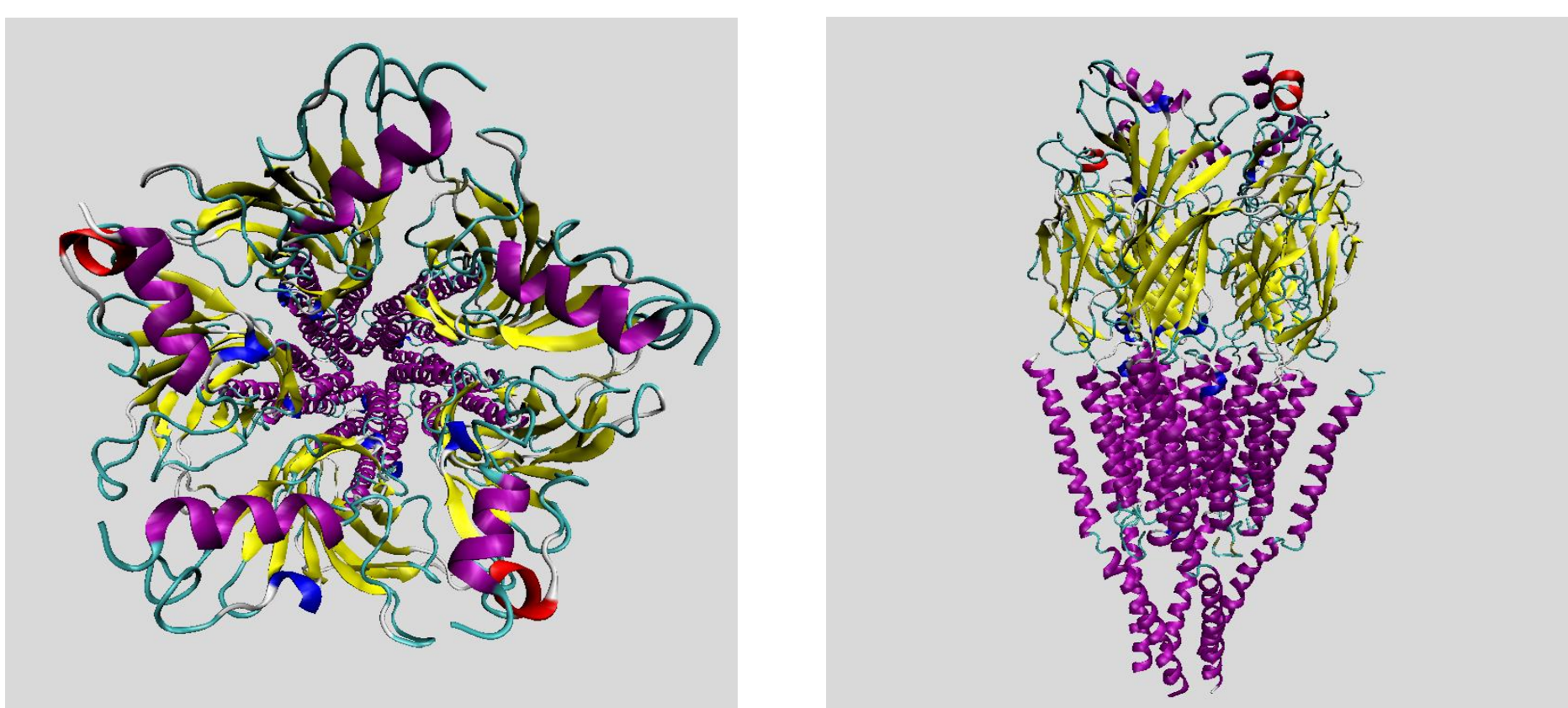
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Human Type 3 Serotonin Receptor (5HT3a)

- Belongs to the cysteine-loop ligand gated ion channel superfamily
- Pentameric Protein
- No crystal structure currently exists for 5HT3a
- nAChR is a suitable model receptor for comparison

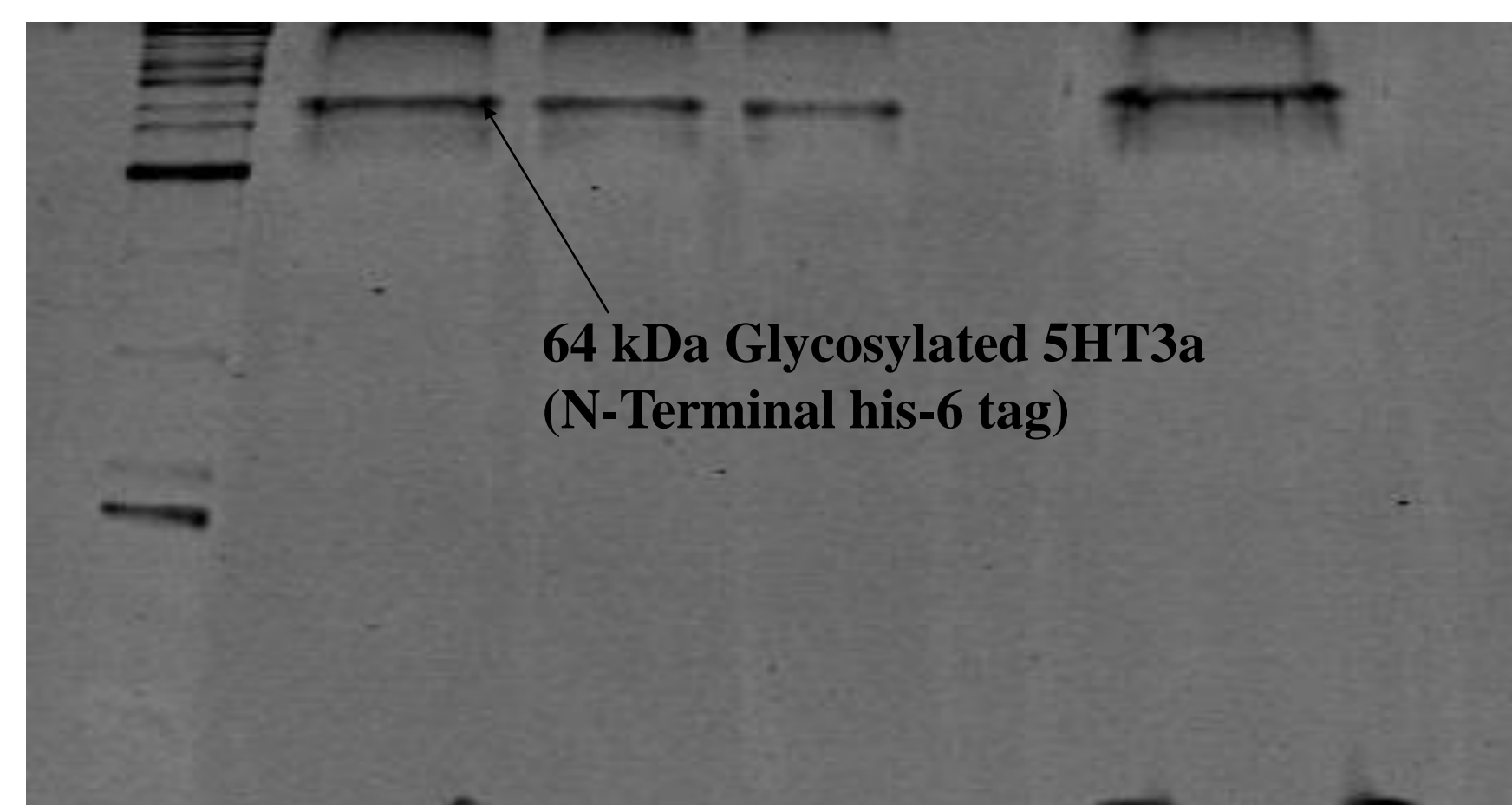


1. nAChR (Top View)

2. nAChR (Long View)

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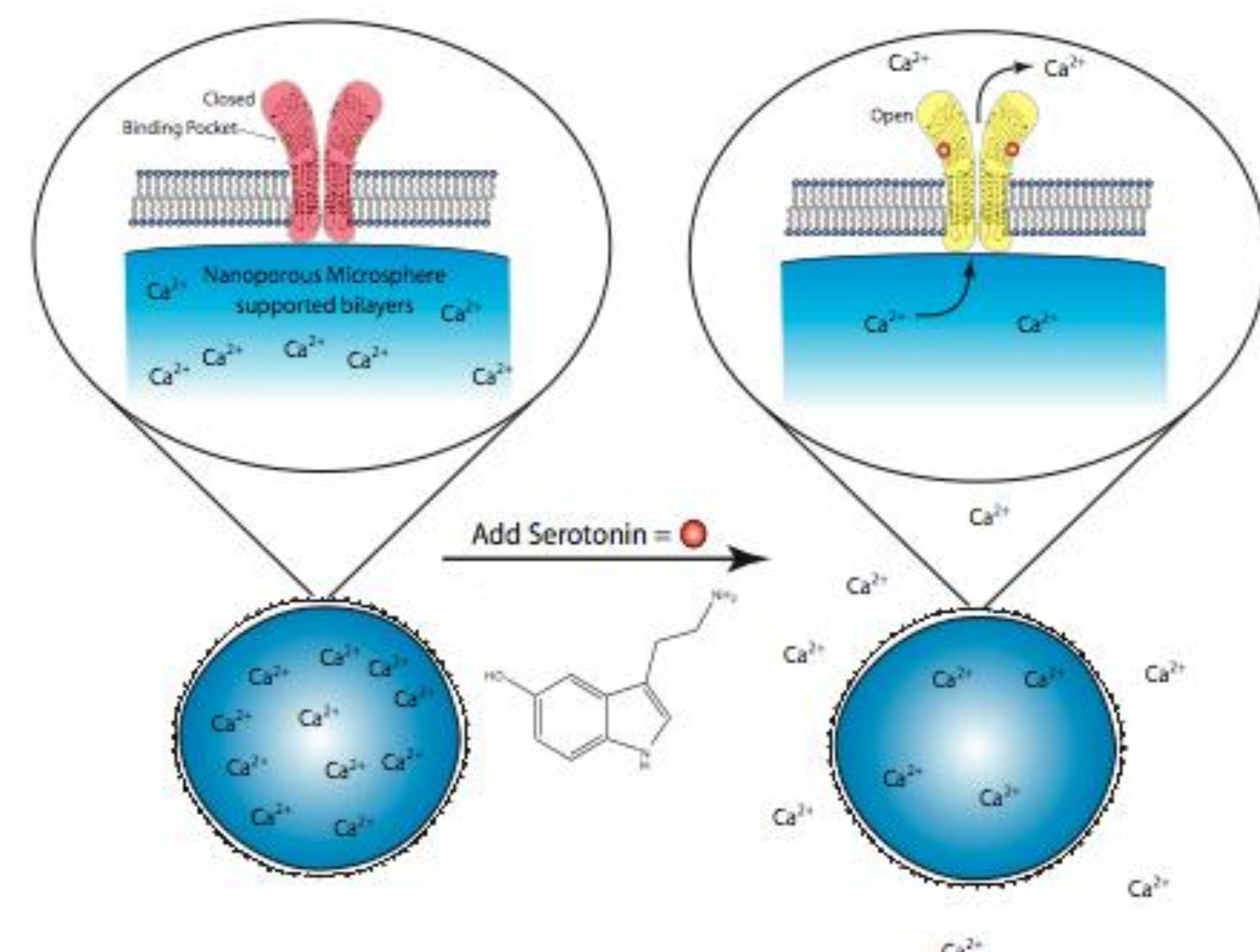
SDS-PAGE Results



10-20% gradient Tris-glycine gel with SYPRO ruby stain
Lane 1 - 0.5 microliters Protein Marker
Lane 2 - 15 microliters of Sample Protein

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Structure of Biomimetic Assembly



Abstract

Ligand-gated ion channels (LGIC) are integral membrane proteins that open when bound by a chemical messenger or neurotransmitter and allow the flow of ions through a cell membrane. In this project, the human type 3 serotonin receptor ion channel (5HT3a) was studied to determine its functionality in a biomimetic assembly. The purpose of this research was to devise a method suitable to test the functionality of the entire family of cysteine-loop ligand gated ion channels.

Serotonin receptors were obtained before this work by method of transfection into human embryonic kidney cells. The presence of the 5HT3a protein in the samples was verified using the standard biochemical method SDS-PAGE. Once it was determined that 5HT3a was present, the protein was tested for functionality.

The biomimetic assembly used in the functionality assay consisted of a nanoporous silica bead coated with a lipid bilayer of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine). The silica bead substrate was infused with calcium ions that were trapped by the POPC bilayer. Once the assembly was formed, a membrane disrupting detergent, c12e9, was used to aid in the insertion of 5HT3a into the lipid bilayer.

Electrochemical measurements were taken using a calcium ion sensitive electrode. The assemblies were interrogated both before and after the injection of serotonin HCl; the ligand responsible for opening the ion channel. The difference in calcium release between blank POPC assemblies and 5HT3a reconstituted POPC assemblies was significant. When 5HT-HCl was introduced to the blank POPC, without ion channels, the resultant calcium release was 1.4 micromoles. The 5HT3a reconstituted sample demonstrated a 92.7 micromolar calcium release. A fluorescently labeled 5HT3a sample was also tested and demonstrated a 26.1 micromolar calcium release. These results suggested that a biomimetic assembly was successfully constructed and protein functionality was maintained.

Conclusions

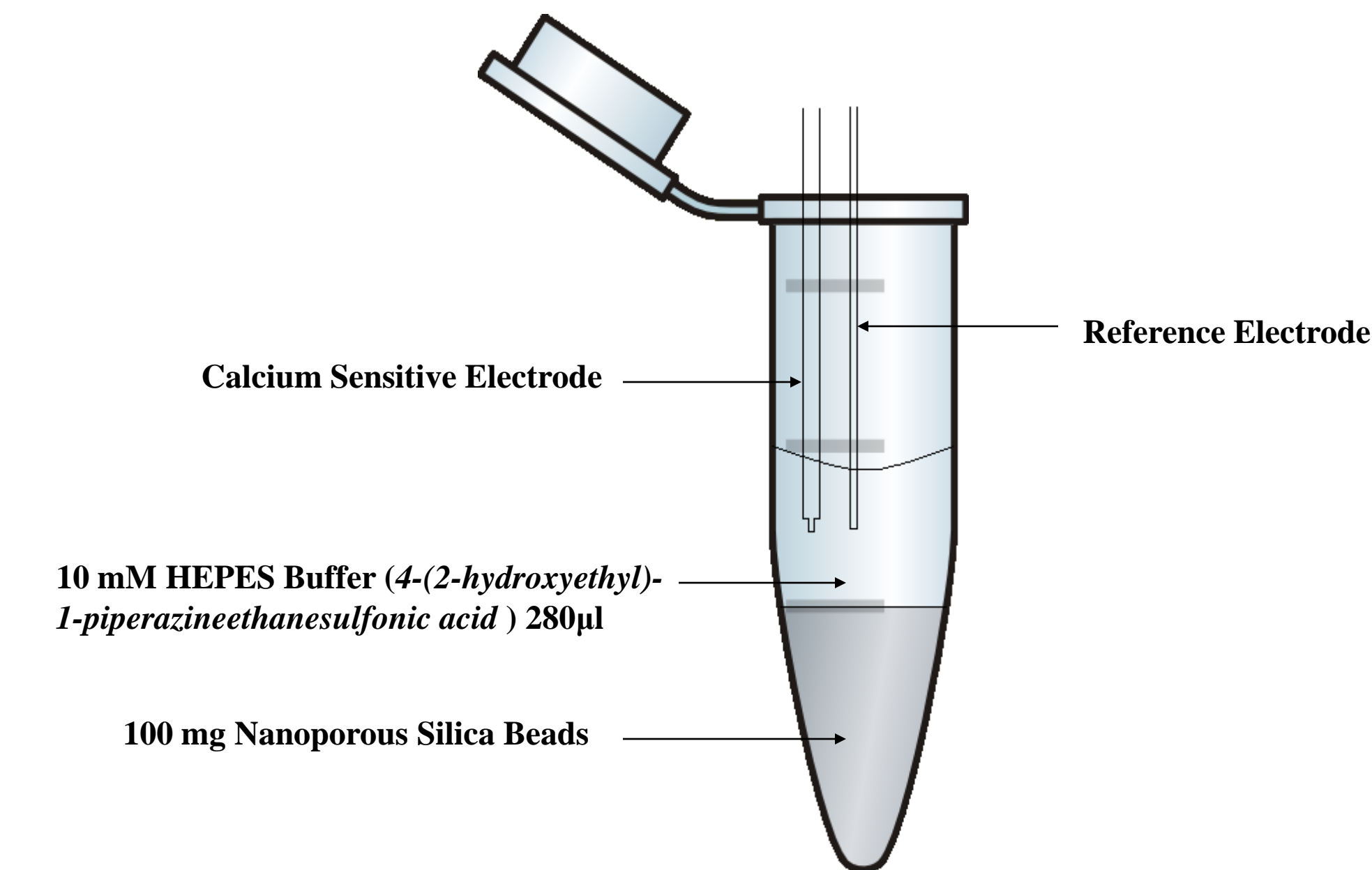
In conclusion, these biomimetic assemblies can be altered in order to test the functionality of many different LGIC's. Further research on this topic will continually utilize this technique to test the functionality of fluorescently labeled serotonin receptors for use in biophysical studies. Various kinetic experiments can be conducted using single molecule fluorescent spectroscopy / microscopy once a series of fluorescently labeled ligands and receptors are developed.

Acknowledgments

The authors would like to acknowledge support for this REU project provided by NSF Grant 0851502.

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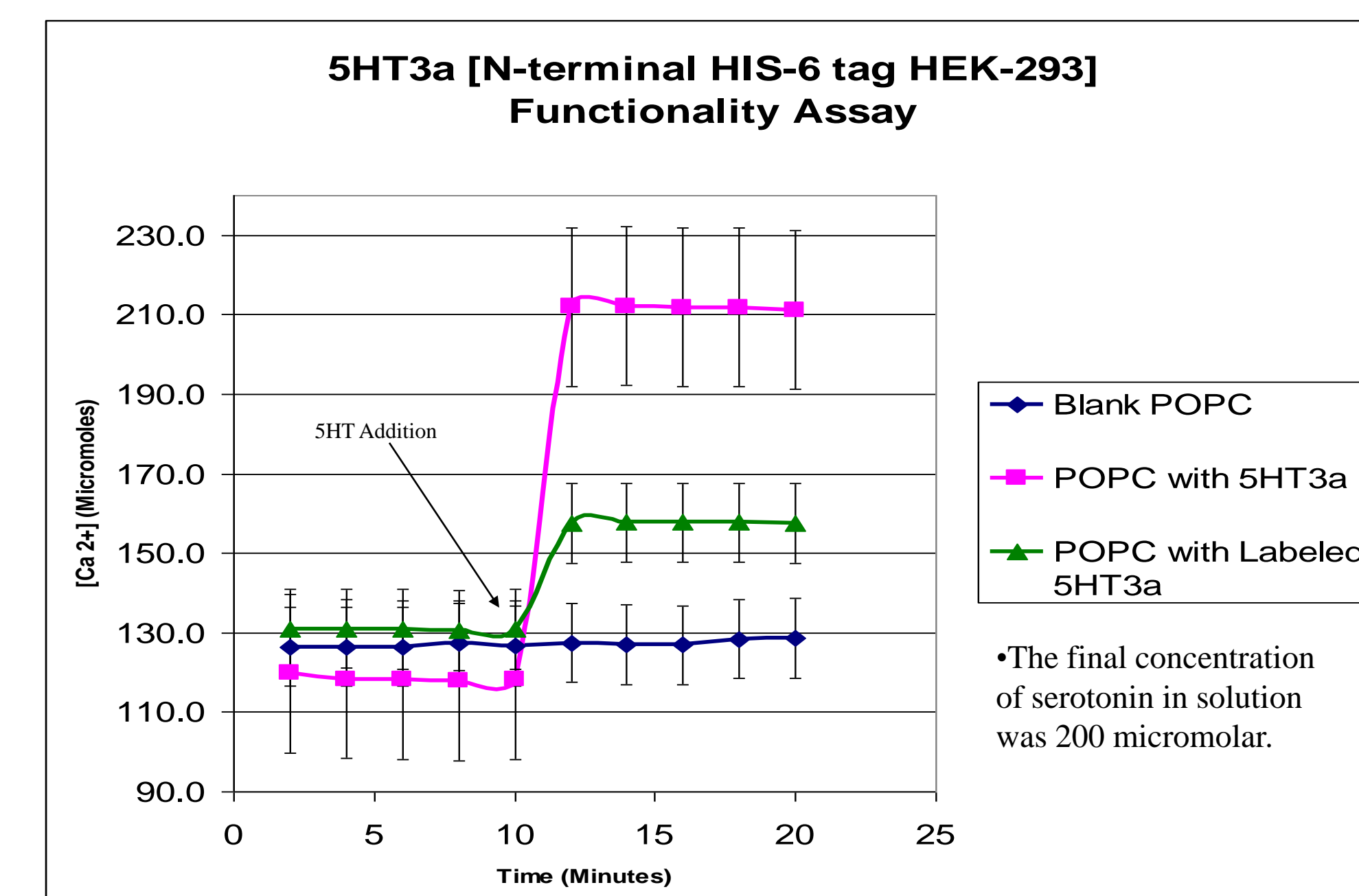
Experimental Methods



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Electrochemical Studies



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Future Work

1. Further electrochemical studies of Alexa 555 fluorescently labeled protein.
2. FRAP (Fluorescence Recovery after Photobleaching)
 - Laser excitation will be utilized to photodestroy the Alexa 555 dye and the differences in image can be used to determine lateral diffusion within the lipid bilayer
3. A variety of other lipids will be investigated.
4. Western Blot Assay to further confirm presence of 5HT3a.