Johns Hopkins Engineering

Methods in Neurobiology

Genetically Encoded Optical Indicators

Small Molecule Probes v. Genetically Encoded Indicators (GEI)

Small molecules PROS	Small molecules CONS
High sensitivity	Subcellular localization cannot be controlled
Rapid kinetic response	Diffuse to all type of cells in a tissue/area
No need for gene transfer	Difficult to load in non vertebrate organism
	Leaking out from cells during experiment

In addition, GEI…

| Genetic Features	Optical Features
Can be constructed from proteins that respond to a variety of neuronal events;	Optical imaging enables thousands of neurons to be simultaneously observed *in vivo*, more than currently feasible with electrodes, and thus enables sophisticated analyses of ensemble neural activity
Allows selective sampling of neuronal subsets, including genetically defined neuronal subtypes; sparse random subsets of cells; or cells with specific patterns of anatomical connectivity, for example, via axonal or trans-synaptic labeling;	Imaging can be less biased than electrodes, as some neuron types resist extracellular electrical recordings owing to unfavorable cell morphology, weak electrical dipoles, or the organization of the extracellular tissue
Can be stably expressed to study how neuronal dynamics evolve over time in individual animals during the course of learning, life experience, brain development, or disease progression;	Voltage and calcium indicators can reveal spatiotemporal activity patterns within neurons such as dendritic integration, voltage propagation, or dendritic spiking
	Minimization of neuronal damage.

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But..

Need to be expressed in cells/tissues/animals first…

- Viral expression;
- Transgene expression;
- In utero electroporation.
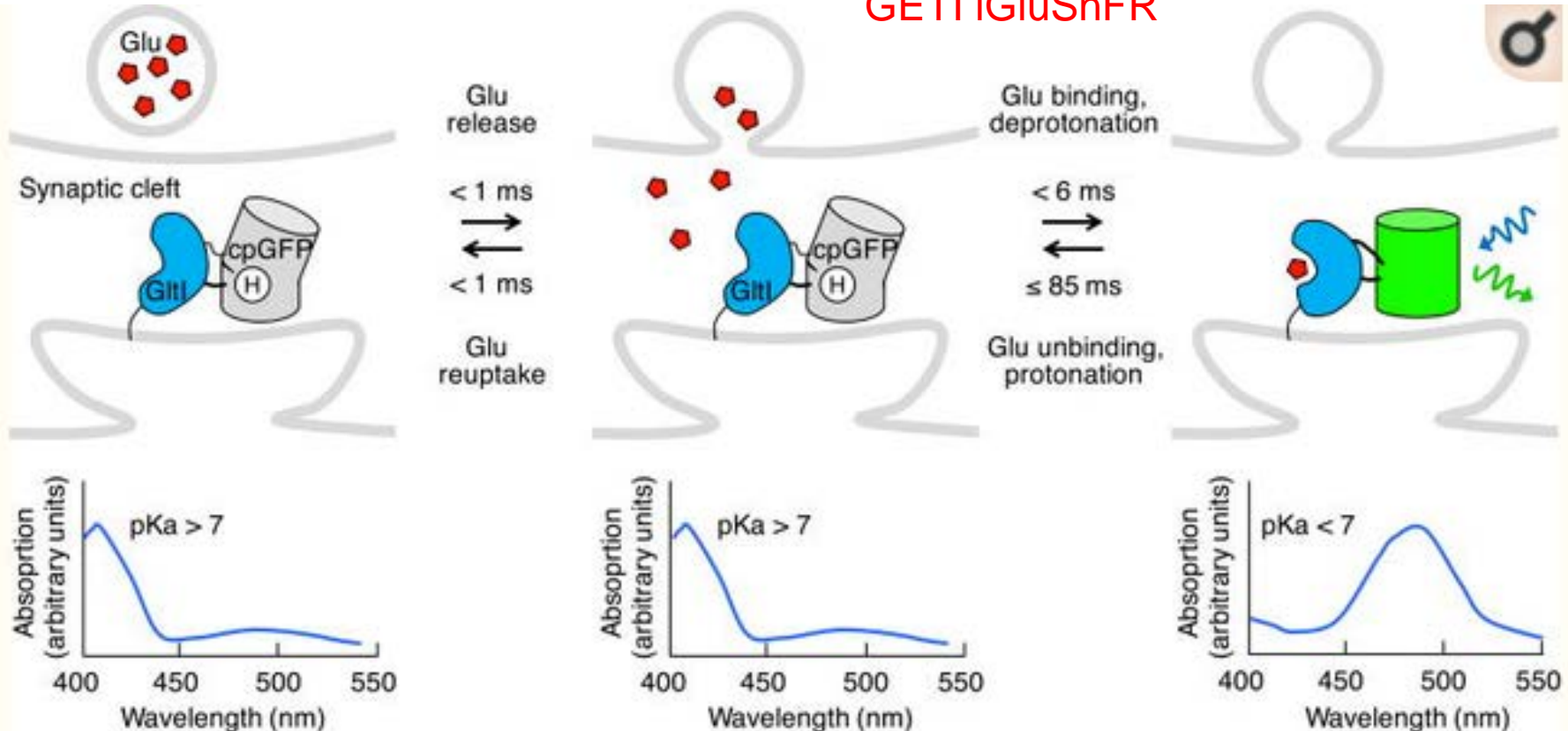
Examples of Genetically Encoded Indicators

| Name	Target	Mechanism	Reference
FLIPE, GluSnFR iGluSnFr	Glutamate	FRET iFRET	Hires et al PNAS 2008 Marvin et al Nat Met 2013
ASAP1, FlicR1, Opsins, Butterfly1.2	Membrane voltage	cpGFP FRET	St-Pierre F et al Nat Neurosci 2014; Abdelfattah AS J Neurosci 2016; Akemann J Neurophys 2012
Ecliptic pHluorin, EGFP, pH Tomato	Vesicular Release (pH)	GFP sensitive to pH	Miesenböck G Nature 1998; Sankaranarayanan Biophysic J 2000
Camgaroo, Pericam and G-CaMP Camaleons (YC2.60, YC3.60, D3cpv, and TnXL)	Ca^{2+}	YFP-CaM FRET	Miyawaki Nature 1997;
KIRIN1, KIRIN1GR, GINKO1	K^+	FRET GFP	Shen et al Commun Biol 2019
HyPer	H_2O_2	cpYFP	Belousov et al Nat Met 2006
Flamindo, Flamindo 2,	cAMP	Citrine (YFP)	Odaka et al PLOS One 2014
FUCCI	Cell cycle and Division	GFP/RFP	Sakaue-Sawano et al Cell 2008

Genetically Encoded pH Indicators (GEPIs)
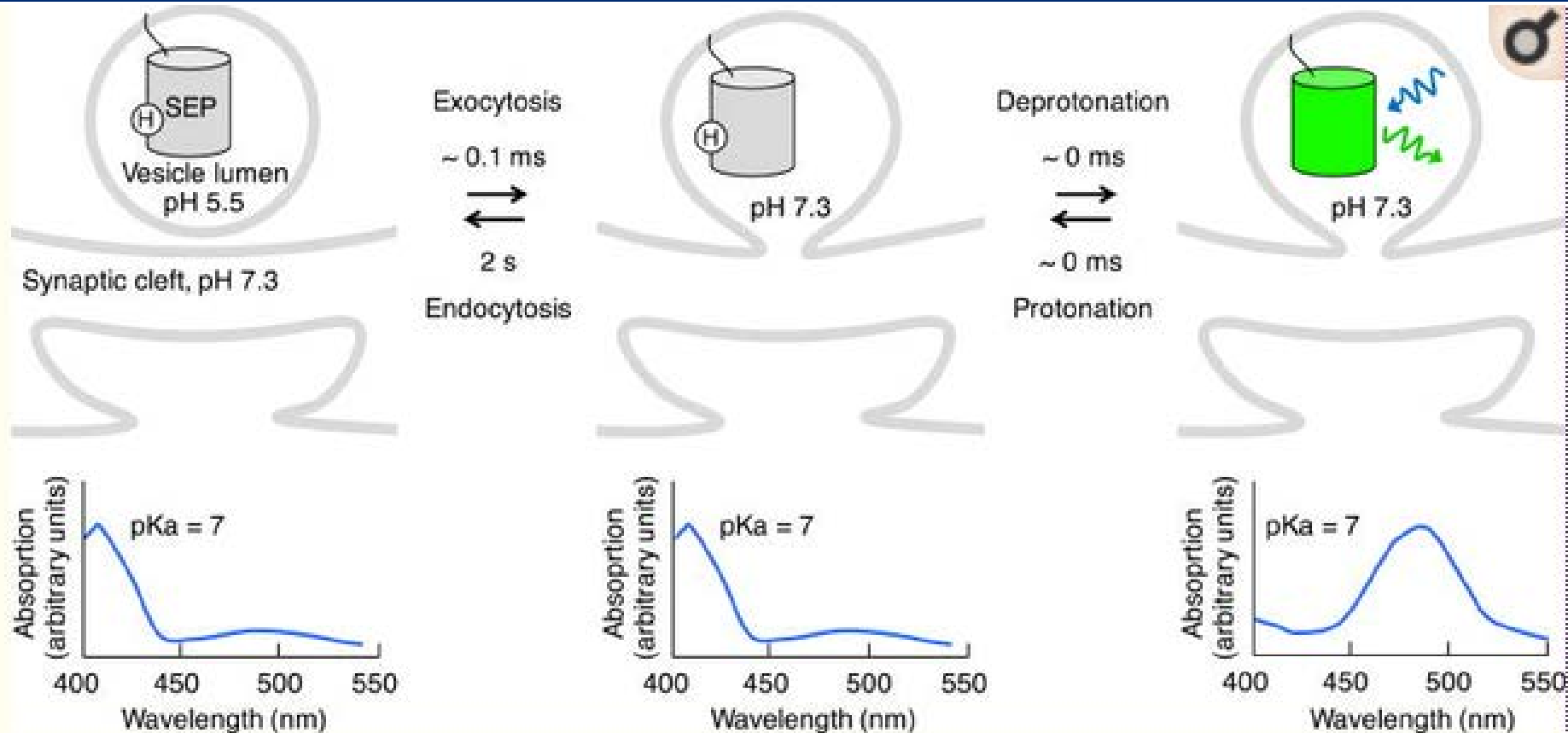
Neurotransmitter Indicators (GETI)

GETI iGluSnFR

Genetically Encoded- Ca²⁺ Fluorescent Indicators (GECI)
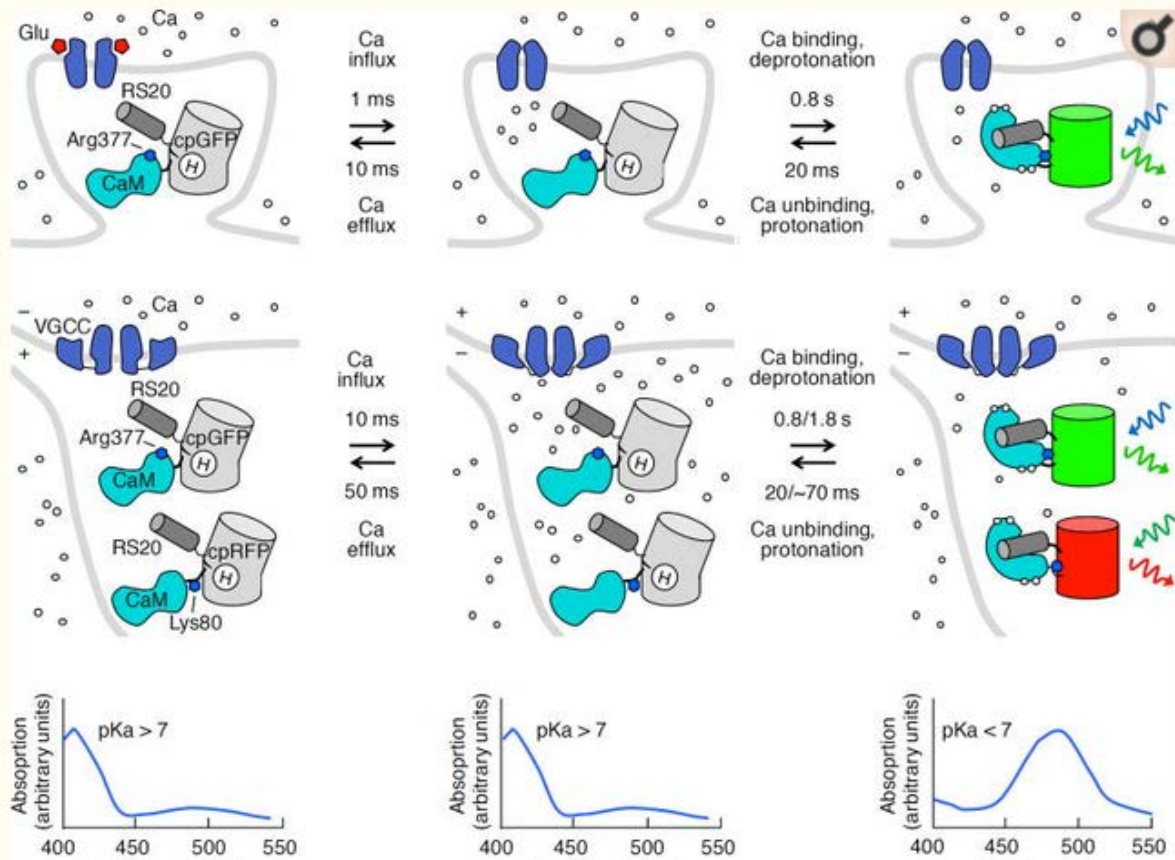
References

Slide	Reference
6,7,8	Zin, M.Z., Schnitzer, M.J.Genetically encoded indicators of neuronal activity. 2016 Nat Neurosci. 19(9):1142-1153. https://www-nature-com.proxy1.library.jhu.edu/articles/nn.4359.pdf