Design of state-dependent labels for ion channel voltage sensors

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Yarov-Yarovoy sphere of influence
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Visualizing nature's transistors with tarantula toxins

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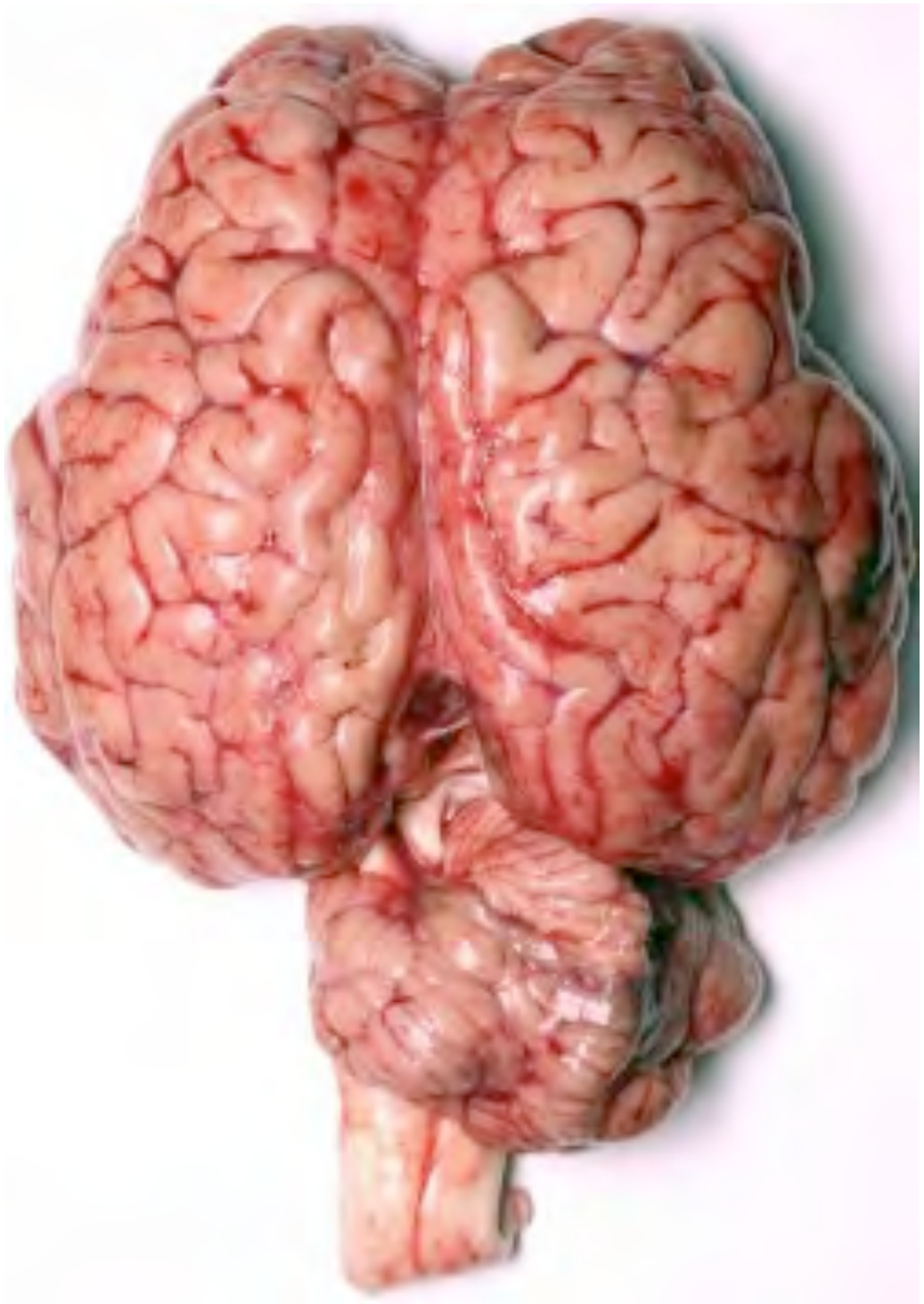
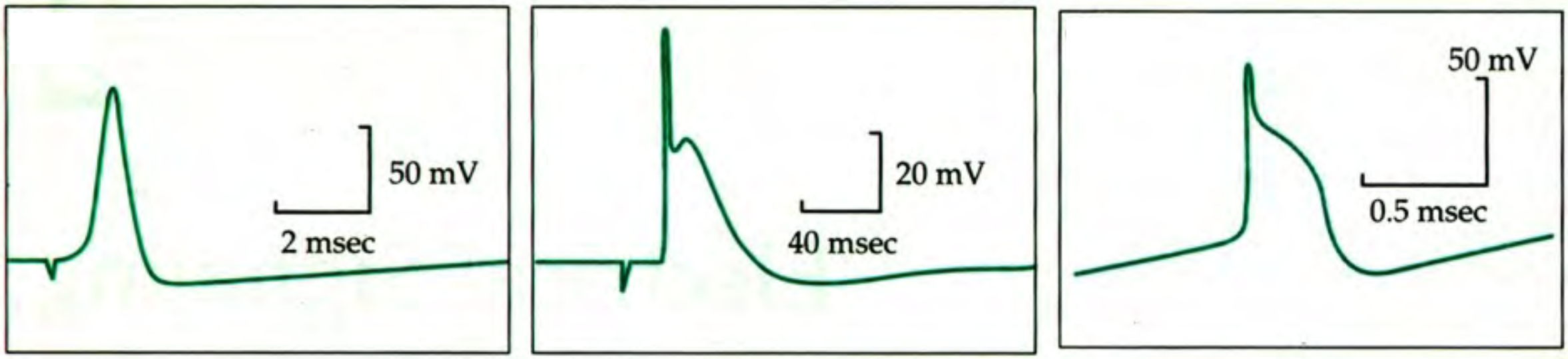
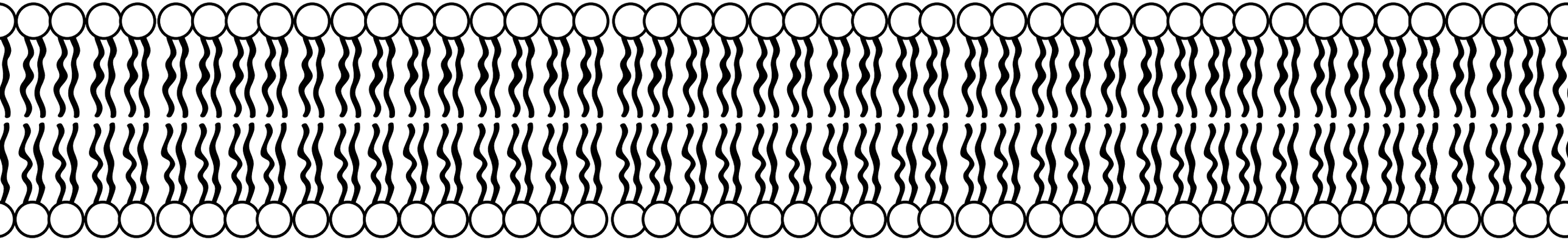


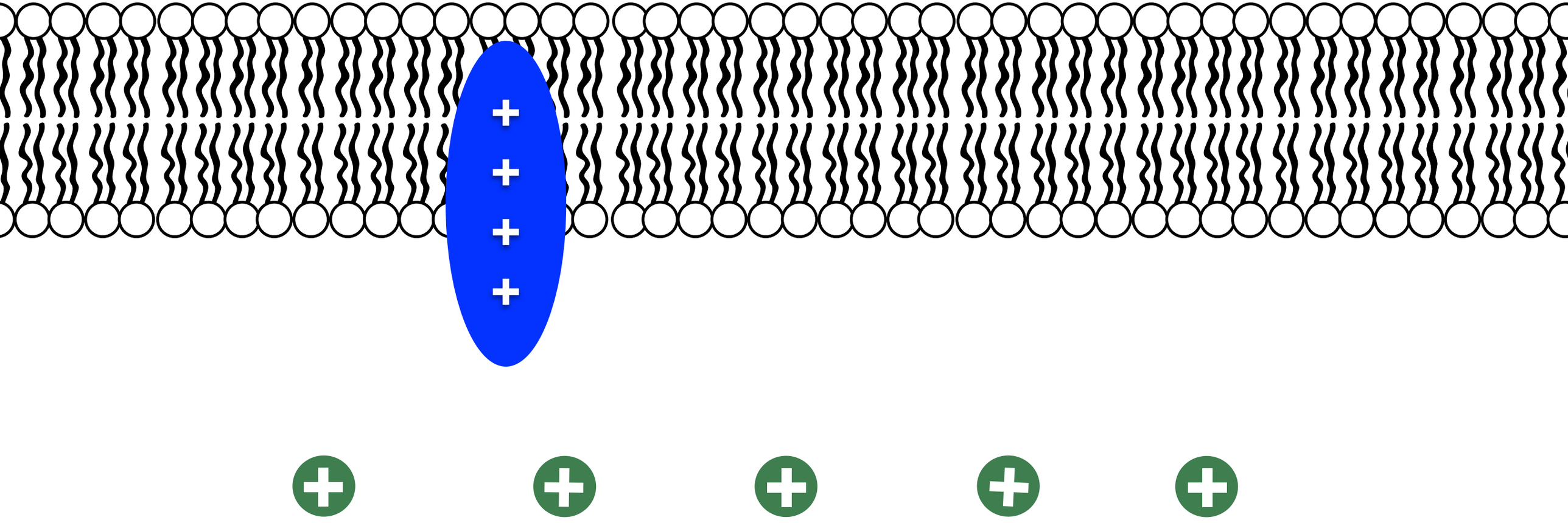
biologic electrical waveforms are diverse



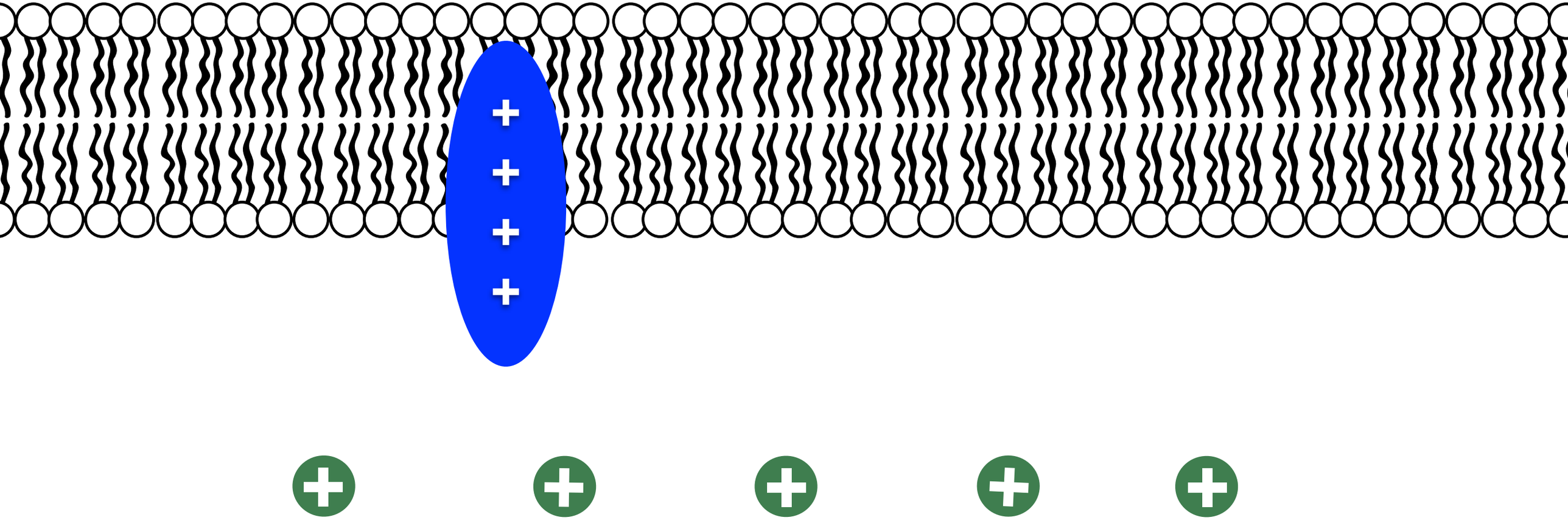
transmembrane voltage is biology's electrical signal



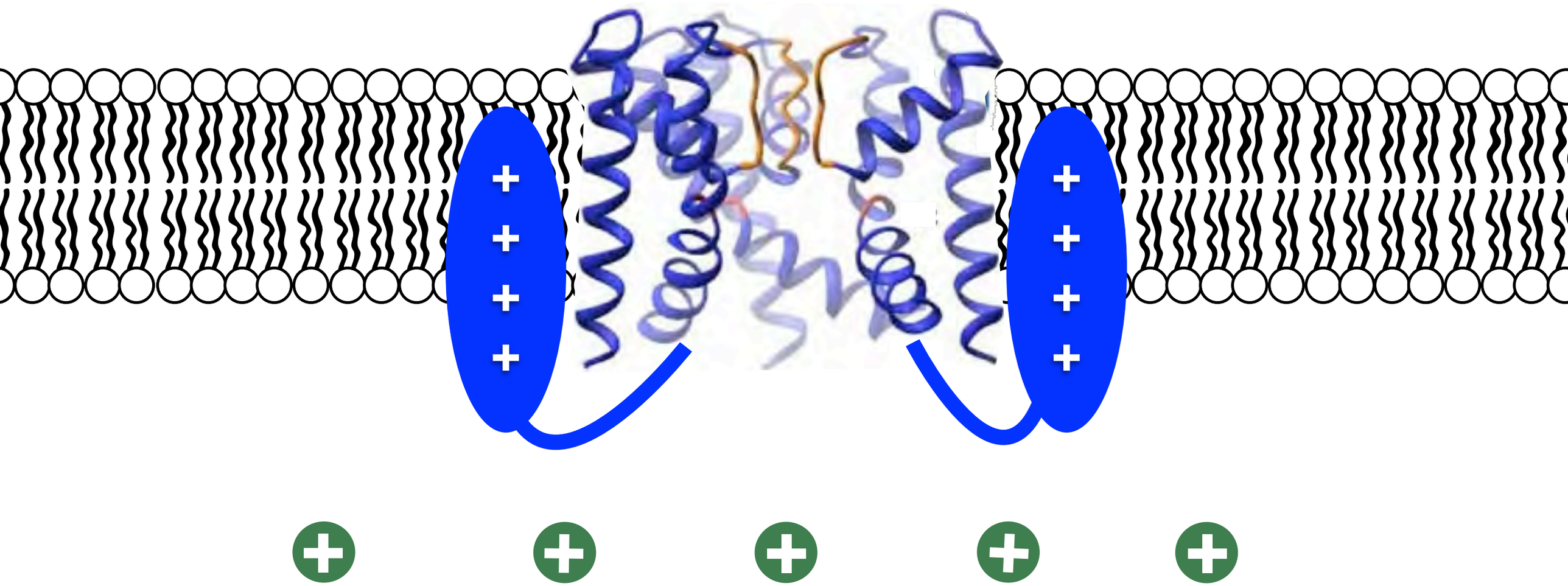
voltage sensors respond to membrane voltage



state dependent voltage sensor ligands can visualize voltage change

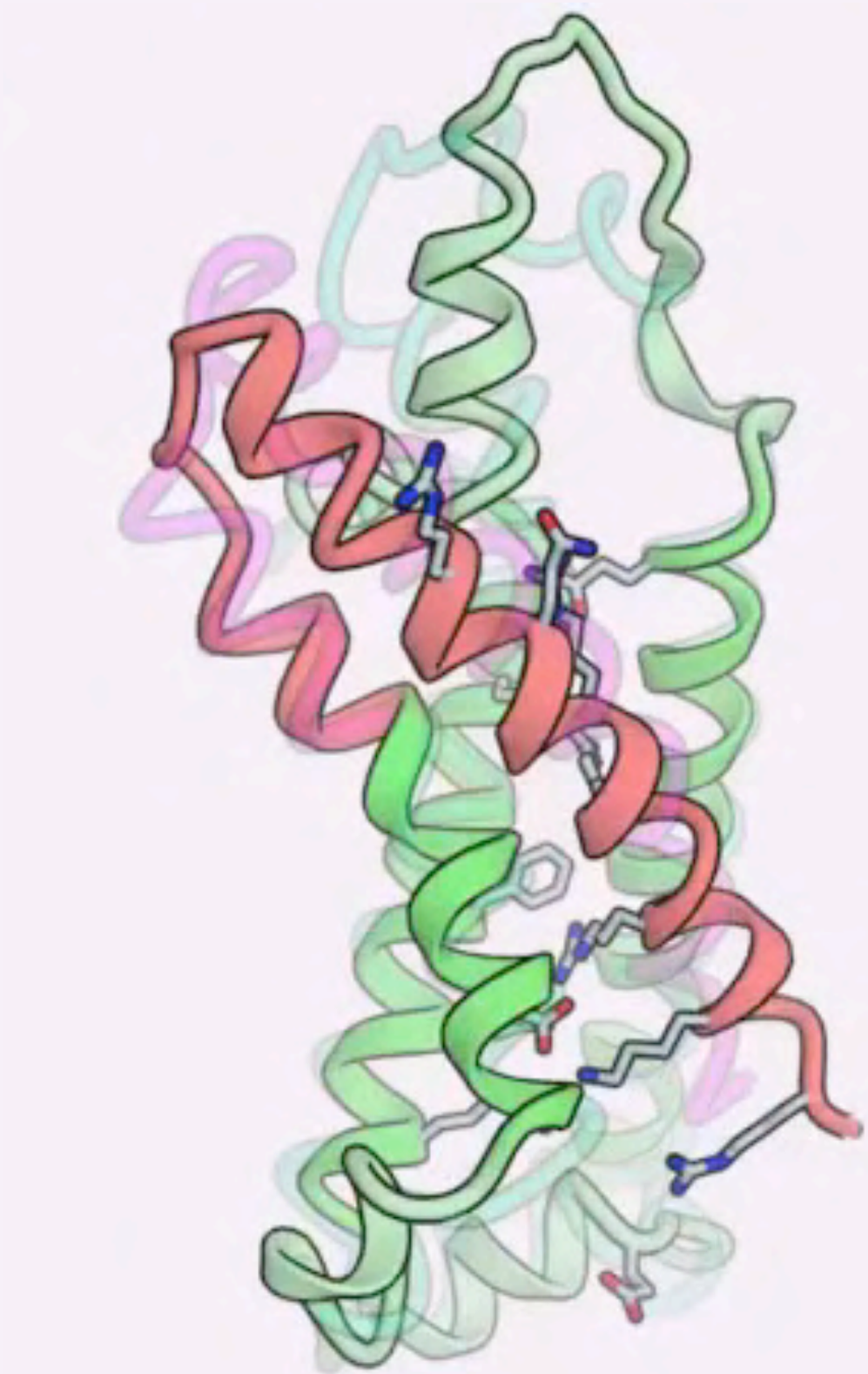


voltage sensors couple to effector domains



voltage sensors are beautiful

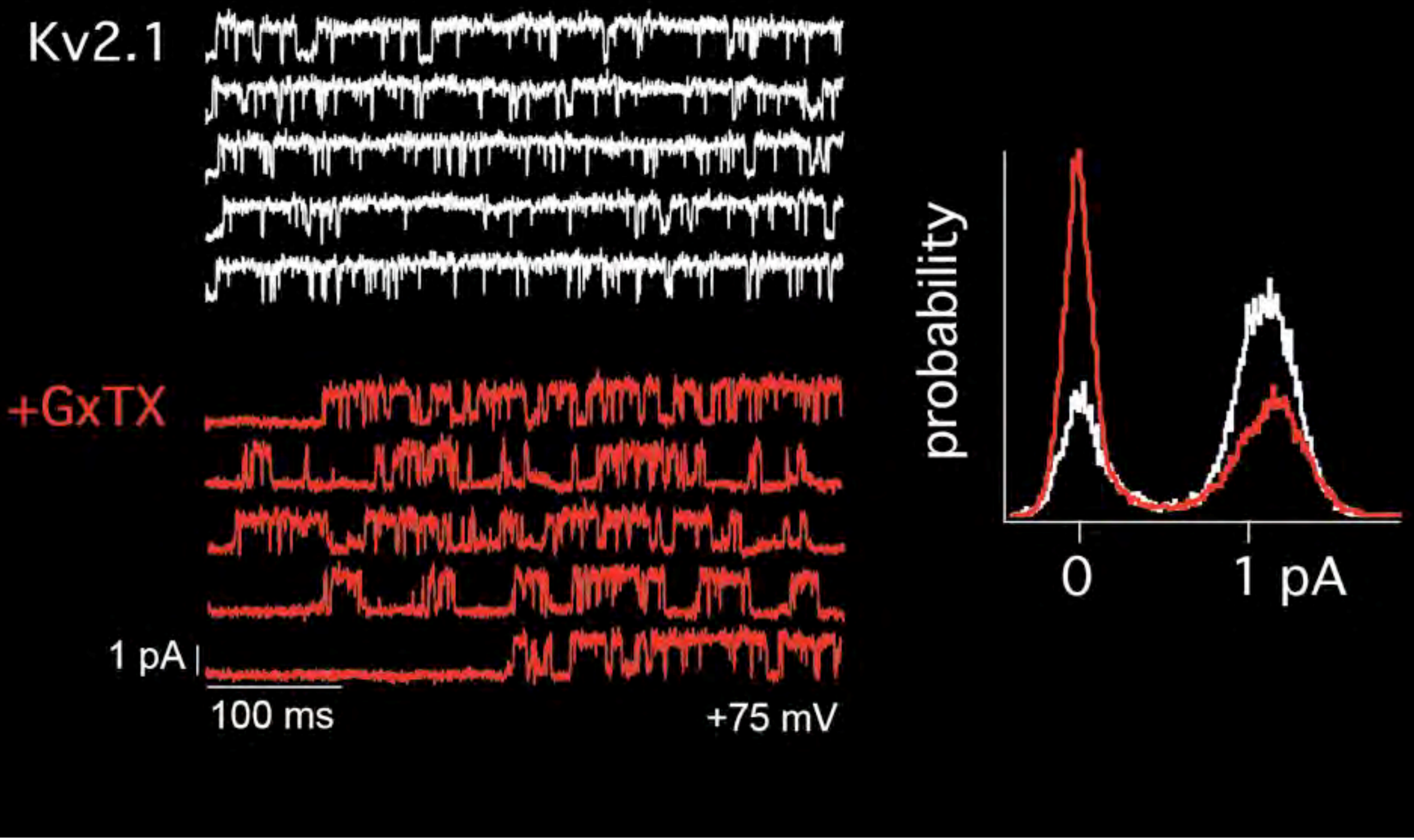
voltage sensors move



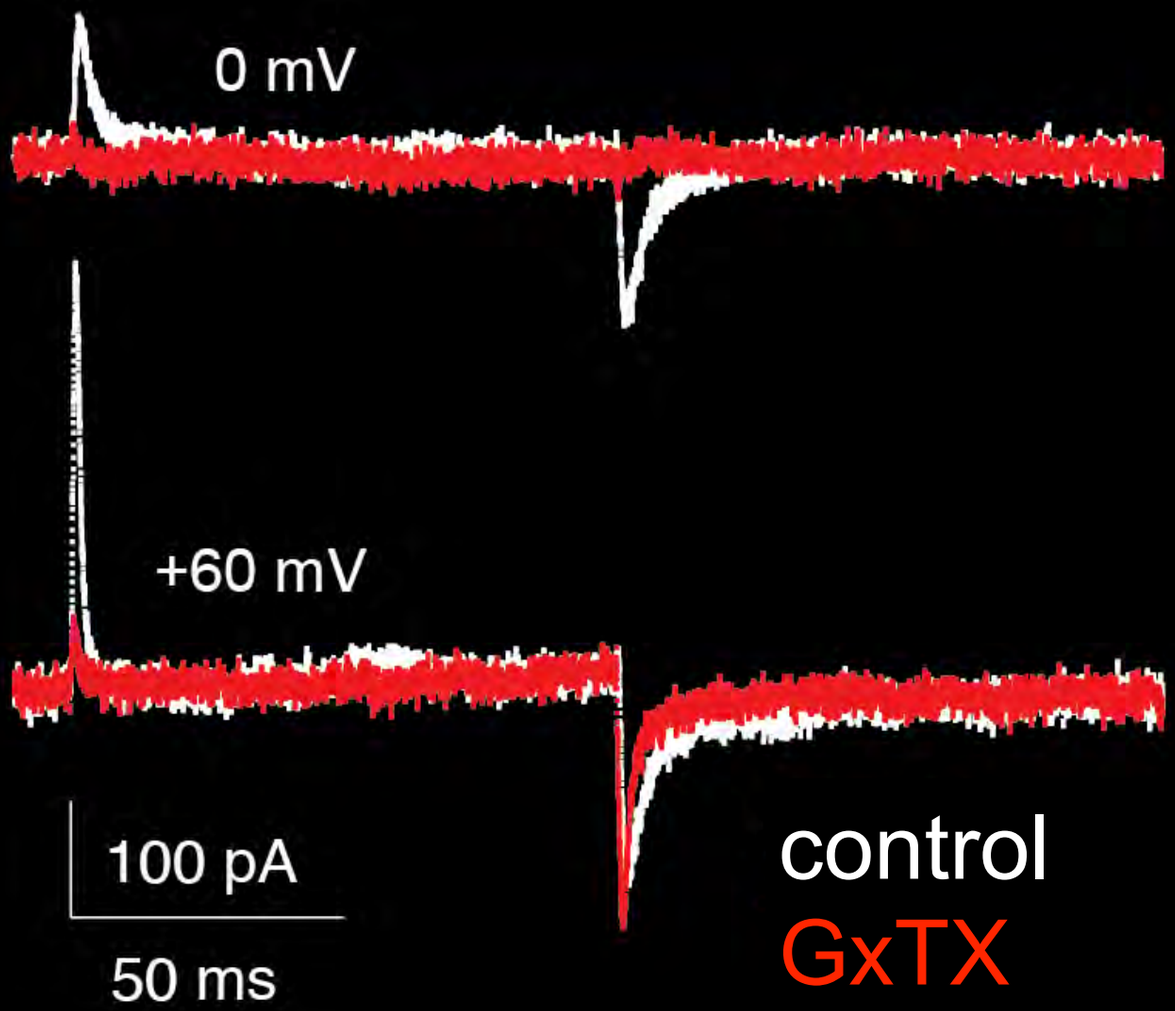
Jensen...Leffler et al. Science 2012

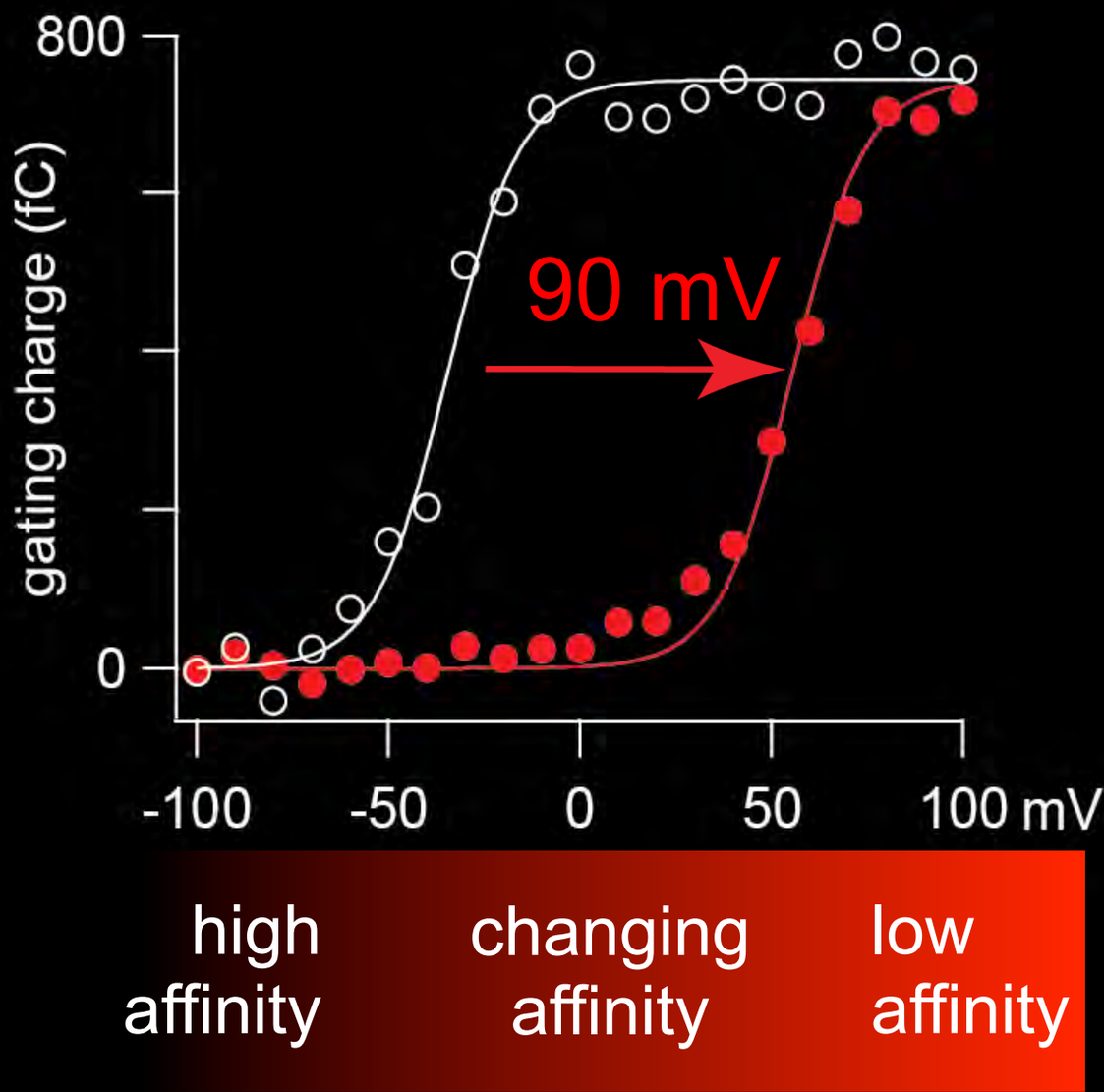
Chilobrachys guangxiensis eats birds

guangxitoxin modulates voltage-sensitive open probability

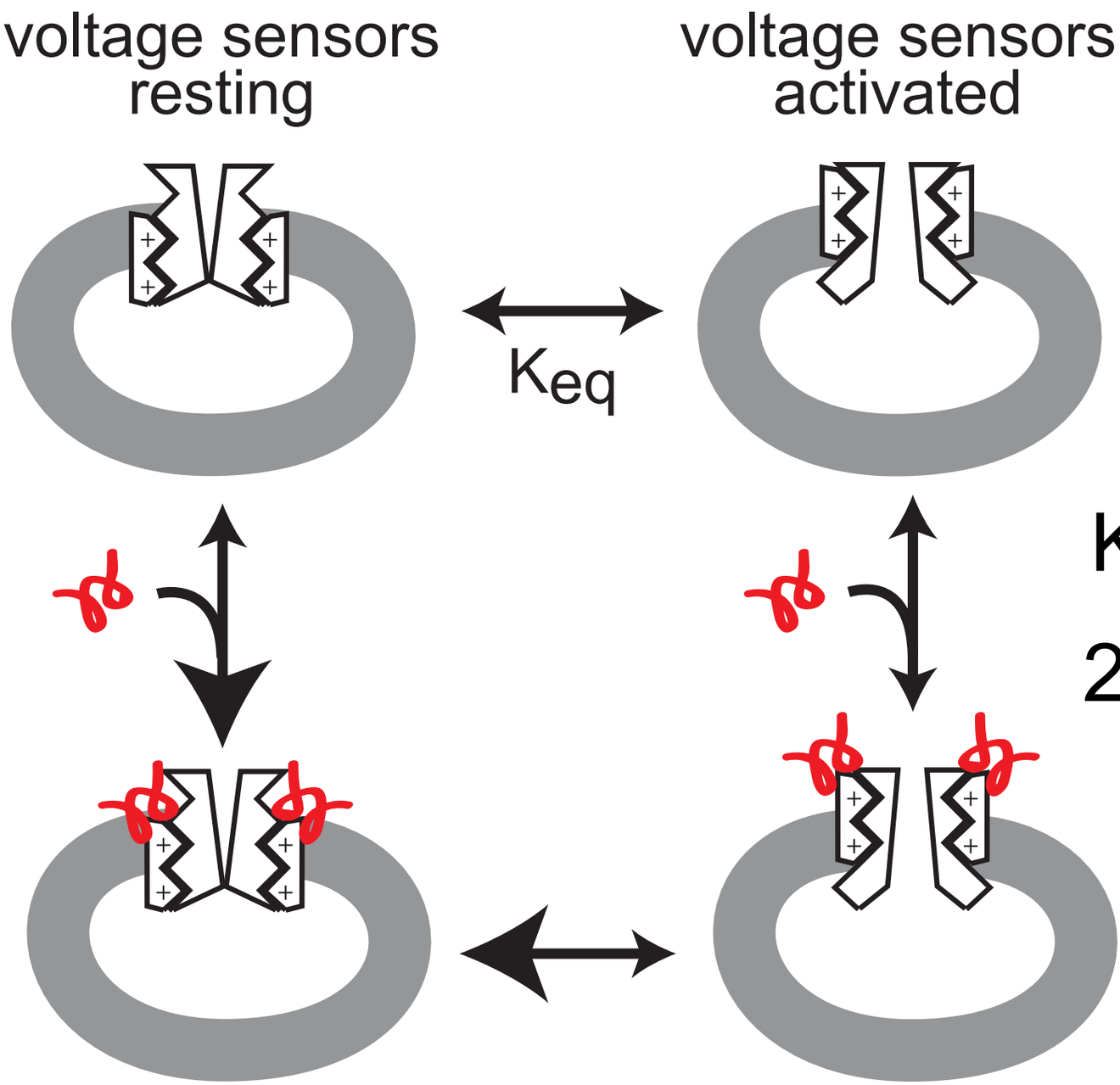


guangxitoxin modulates voltage sensor conformation





guangxitoxin is an allosteric modulator

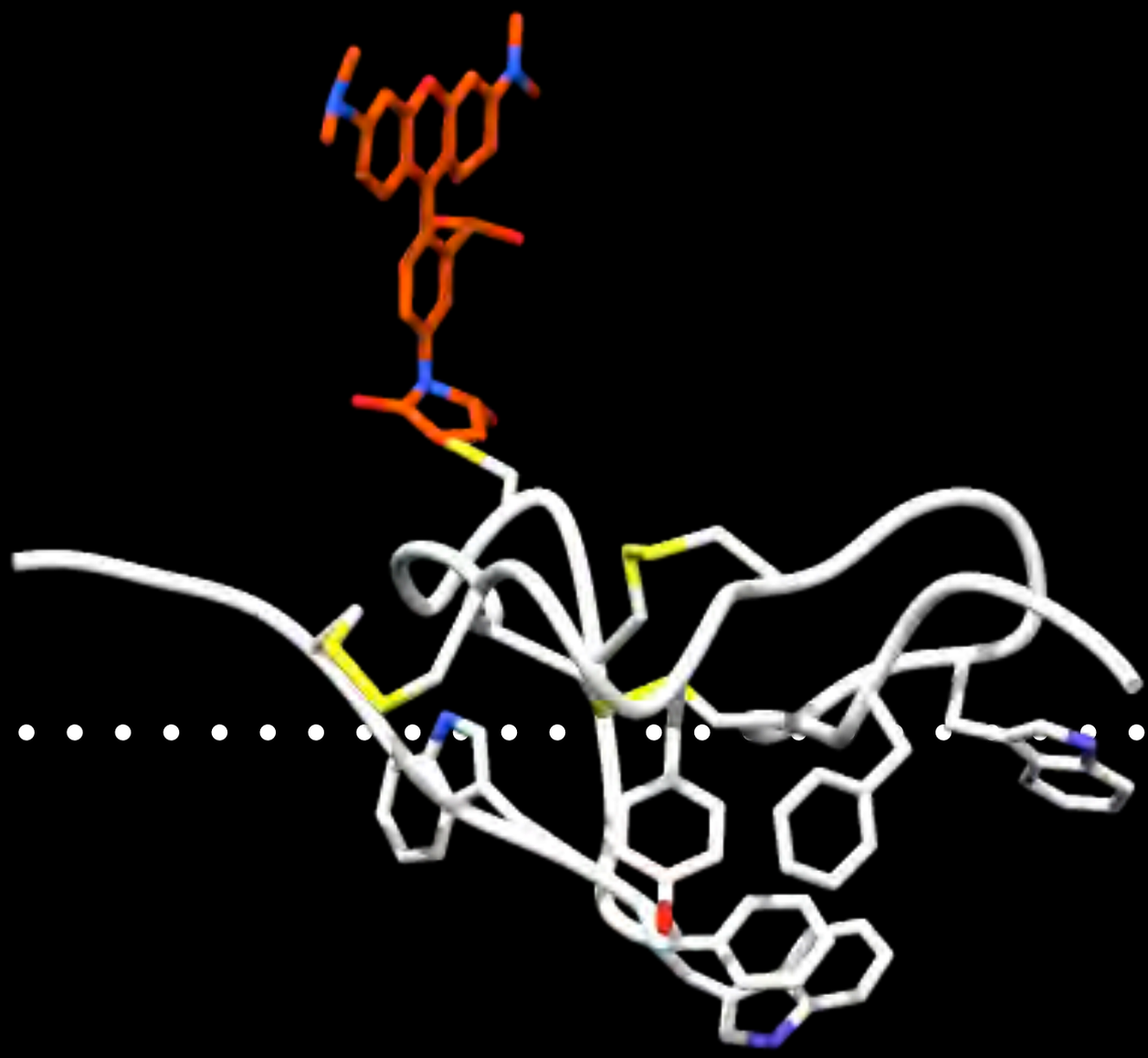


$C = 18{,}000^{-1}$

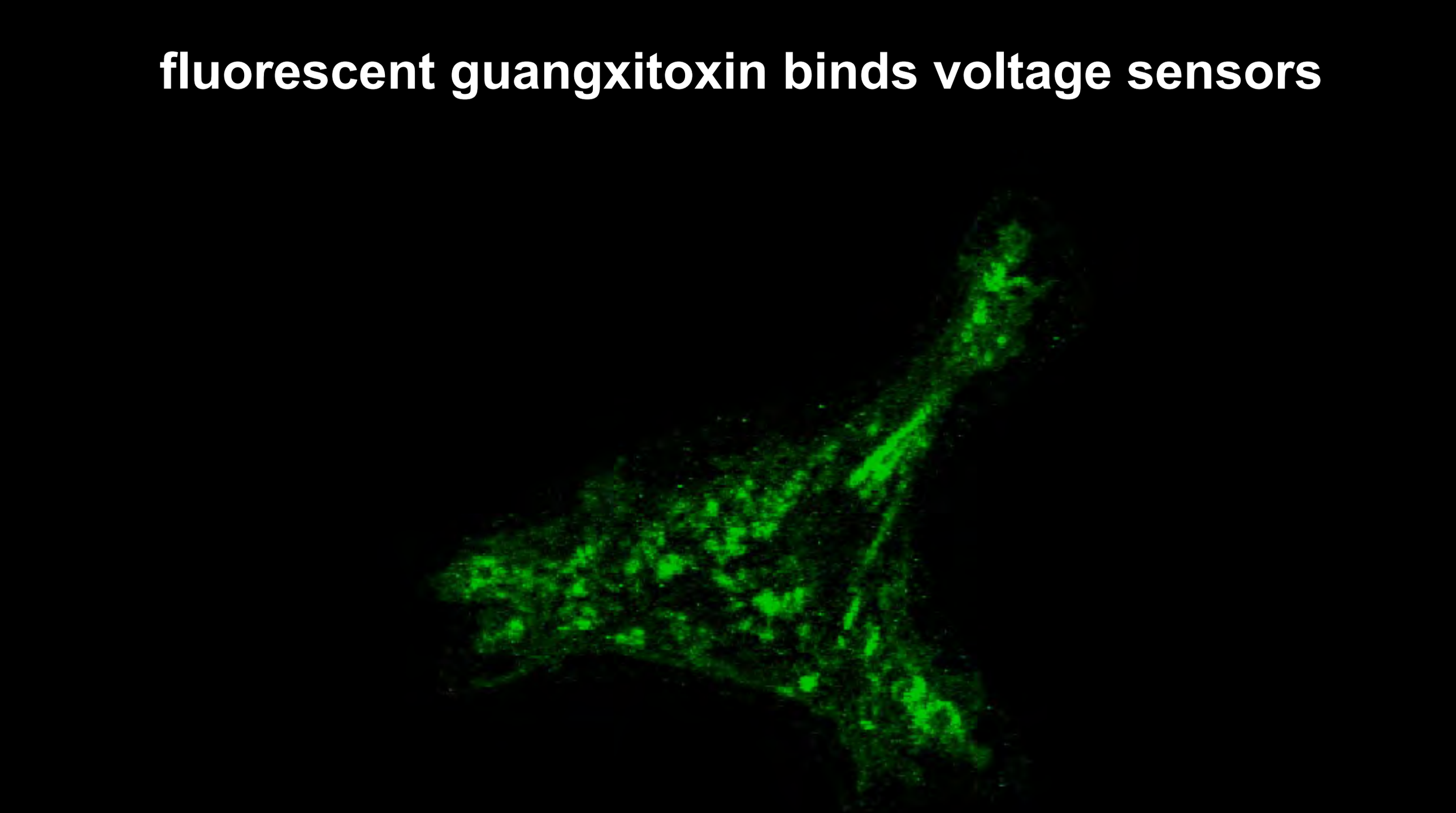
$\Delta G = 10\ kT$

$z\Delta V = 90\ mV$

labeled guangxitoxin peptide retains activity

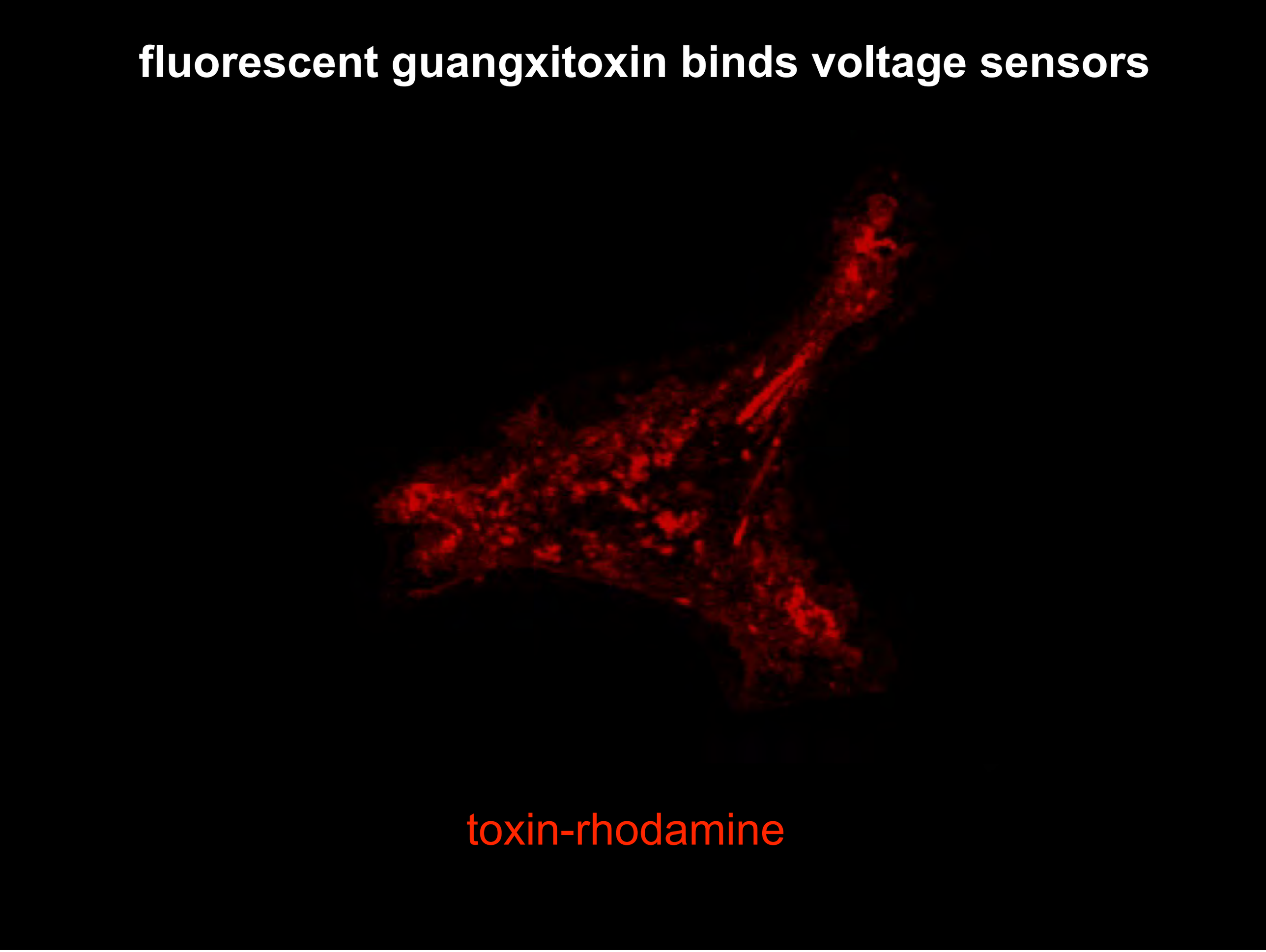


fluorescent guangxitoxin binds voltage sensors

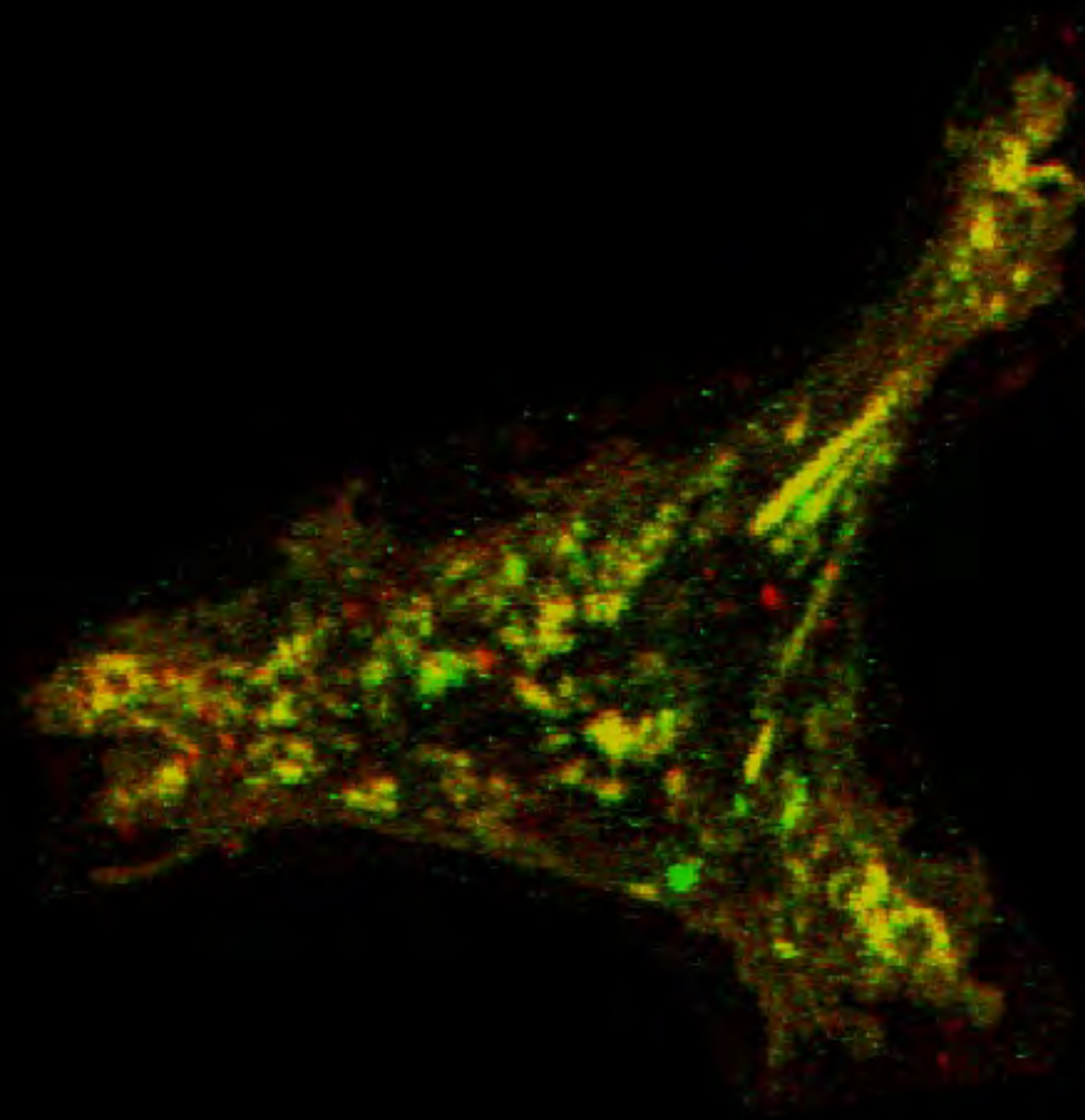


voltage sensor-GFP

fluorescent guangxitoxin binds voltage sensors



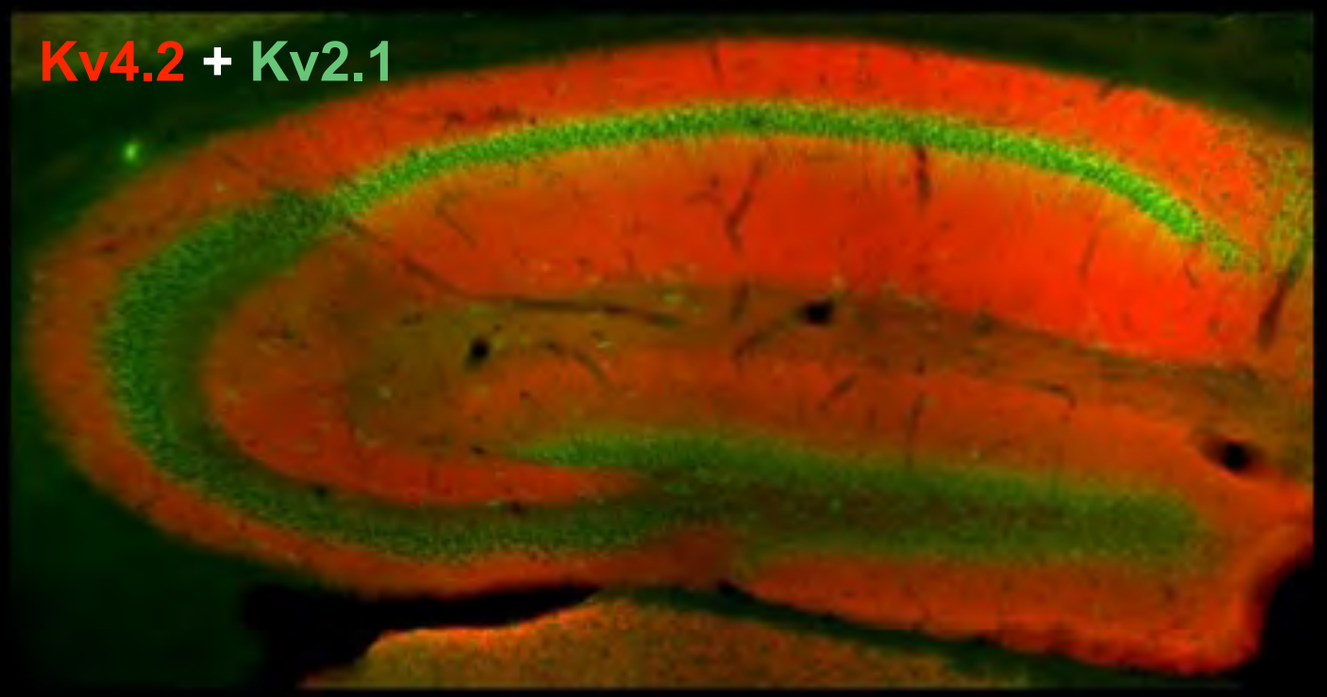
fluorescent guangxitoxin binds voltage sensors

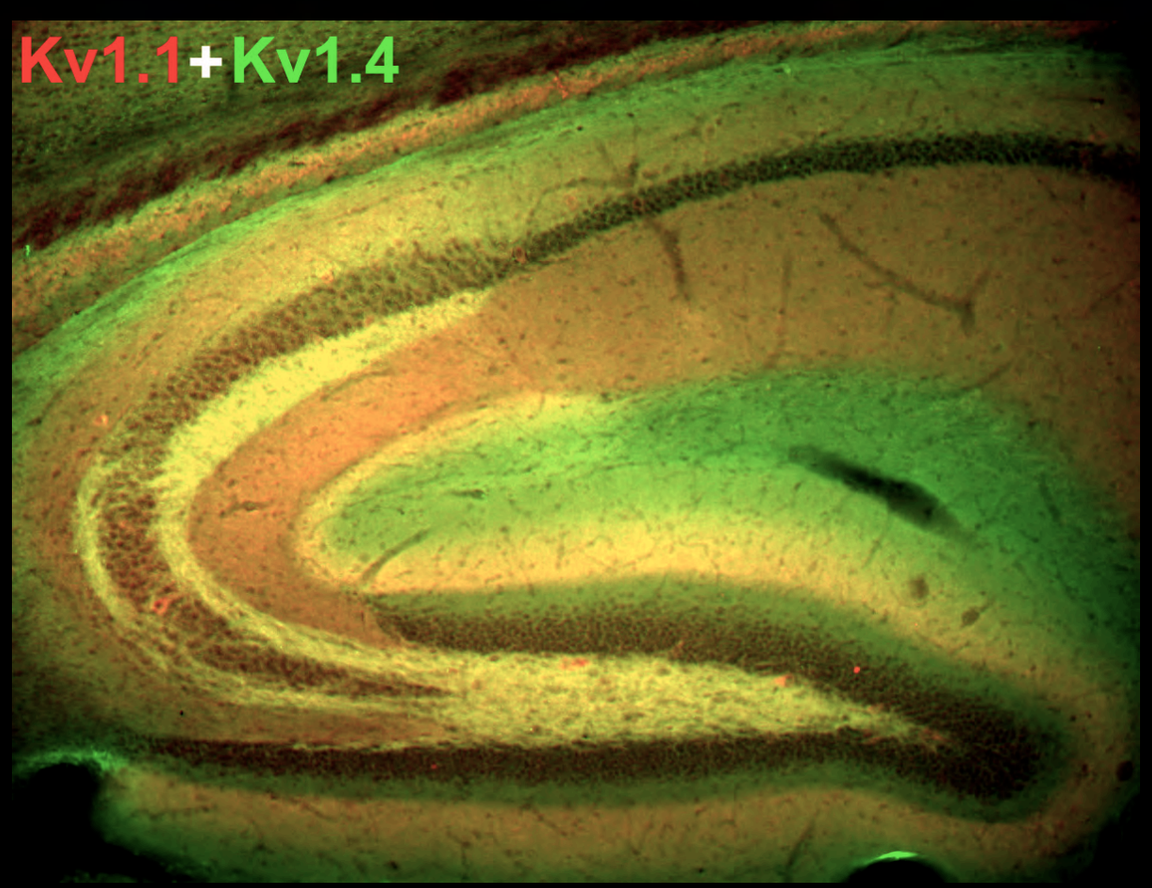


voltage sensor-GFP

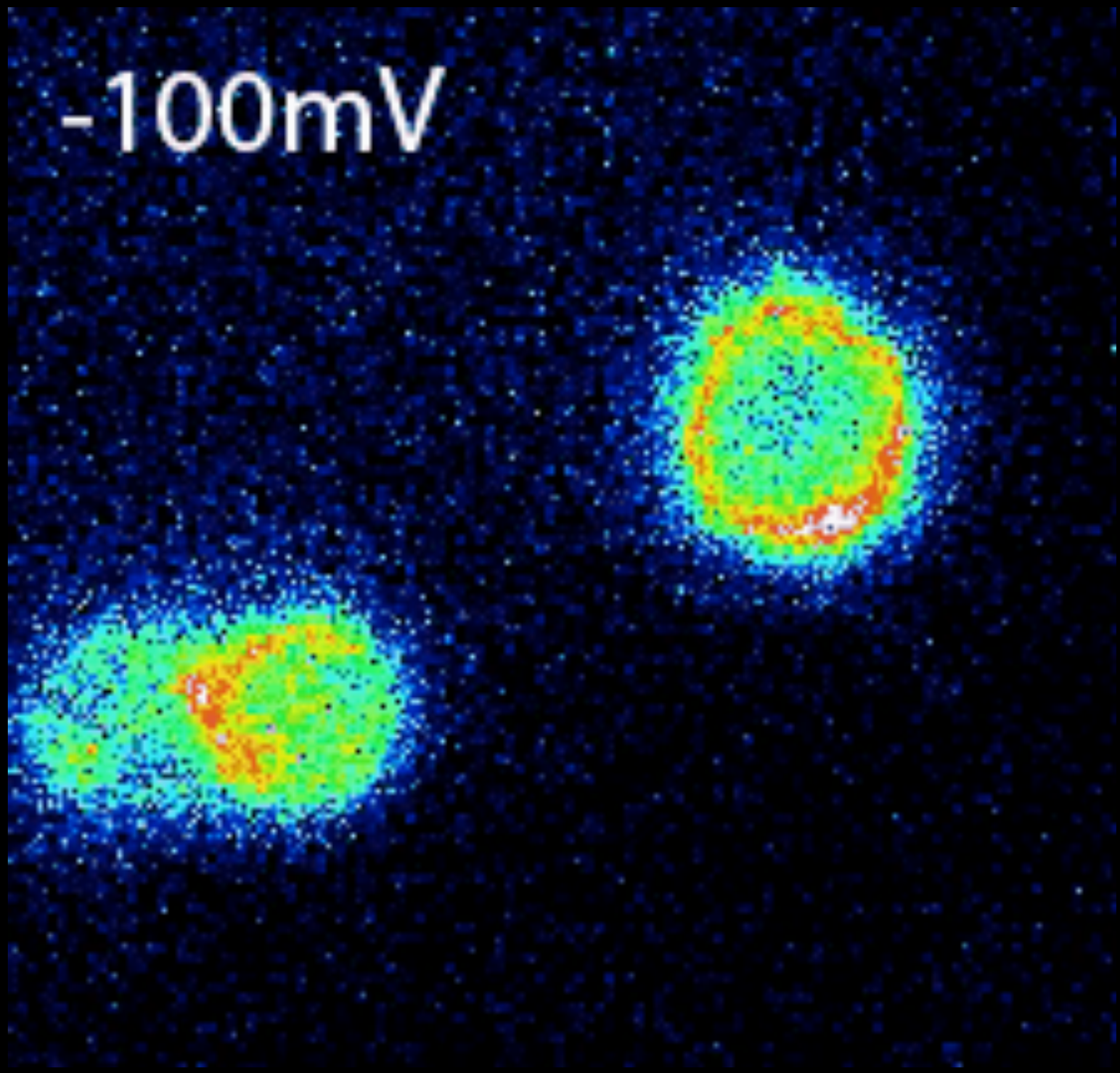
toxin-rhodamine

60+ voltage sensor proteins have different neuronal distributions

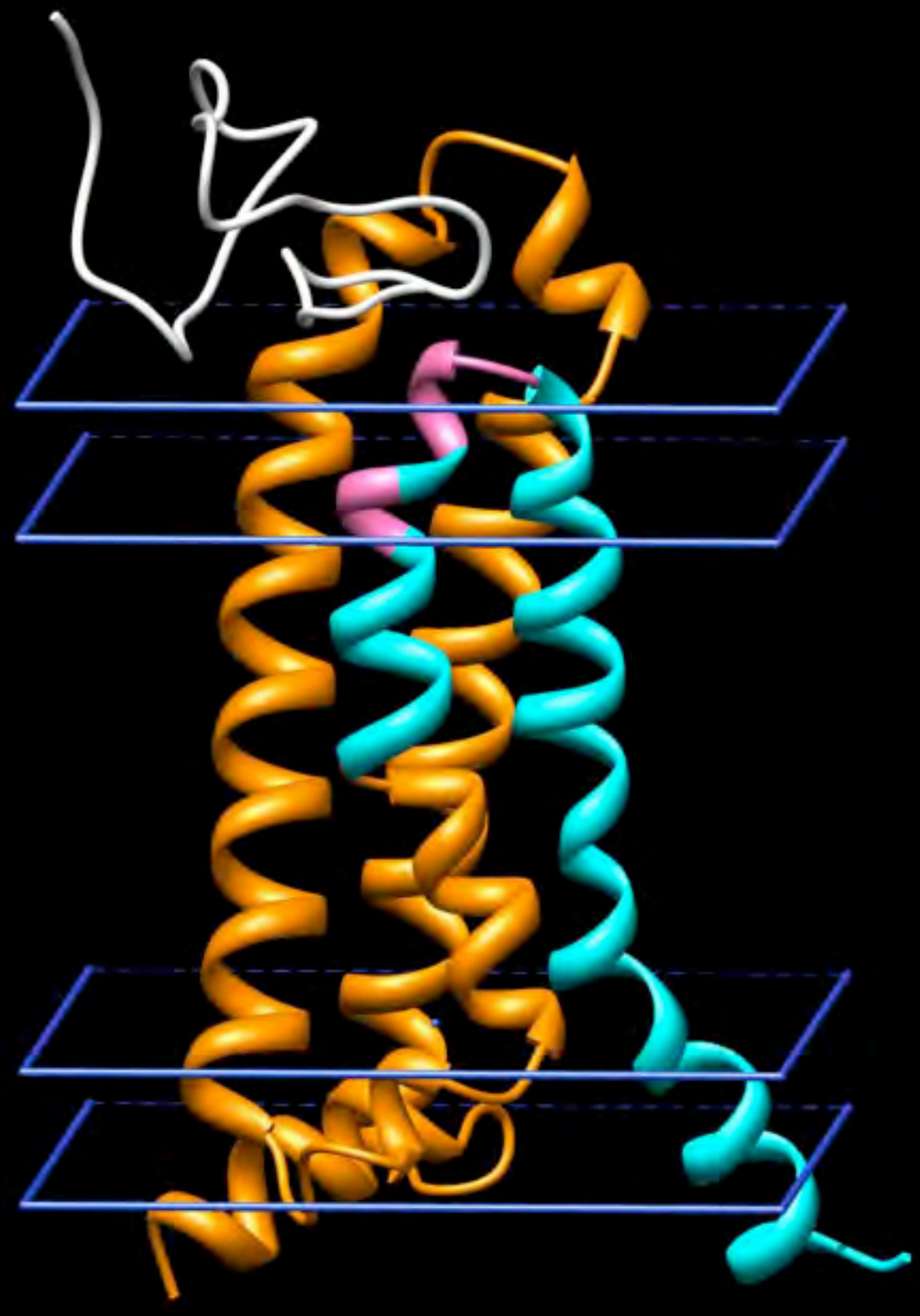




fluorescent tarantula toxin is a voltage sensitive dye



voltage sensors change conformation



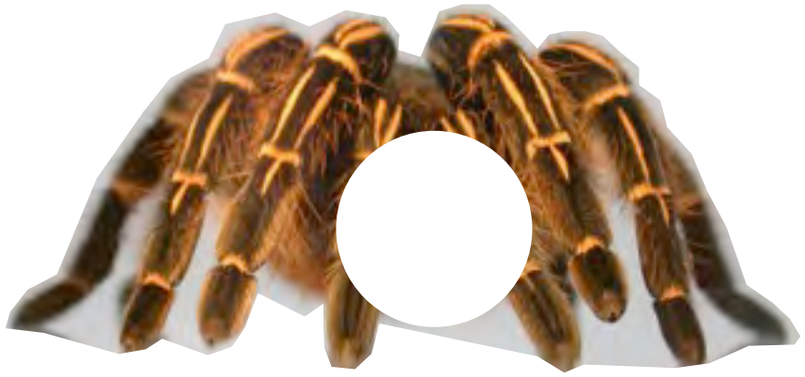
design goals with Rosetta

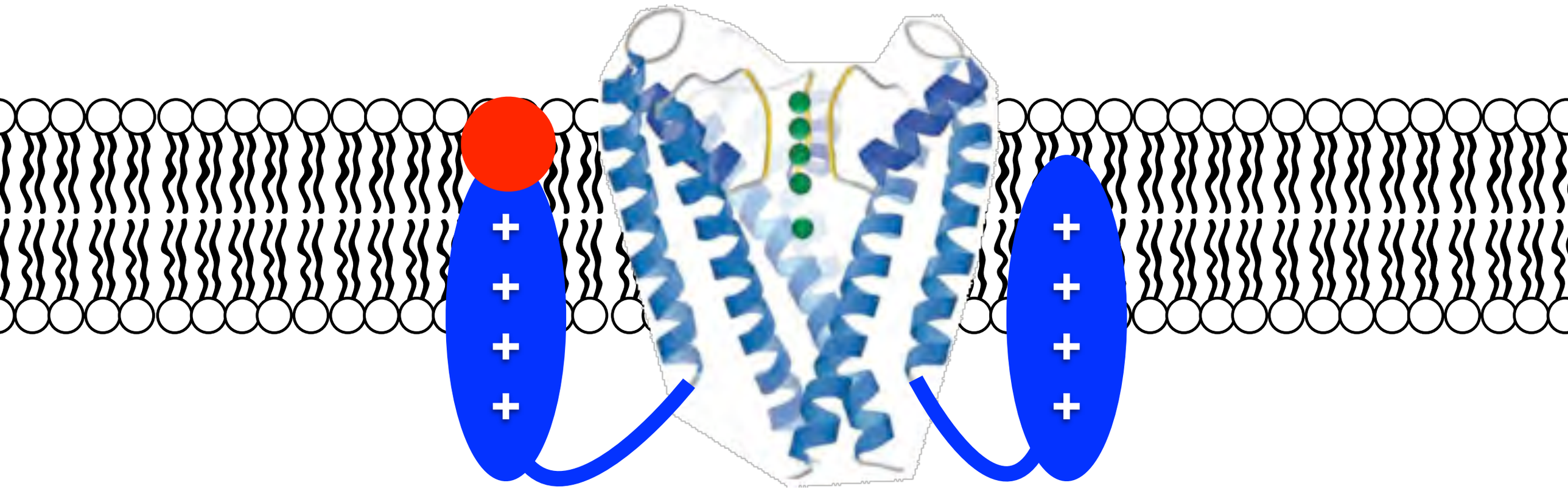
1) model toxin-voltage sensor binding interface

2) redesign interface for chemical genetics

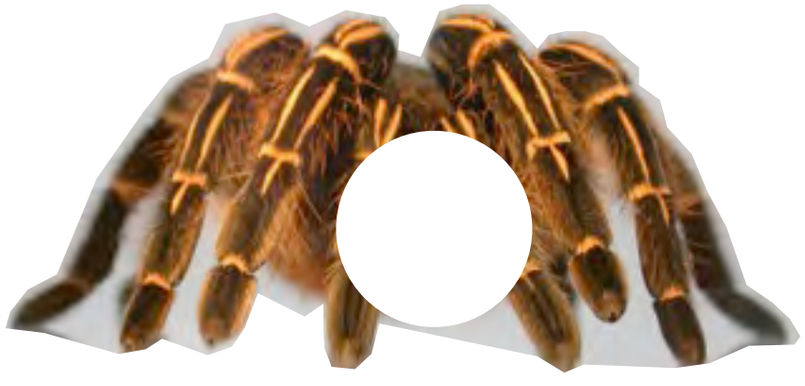
3) alter affinity for + vs – voltage sensor conformations

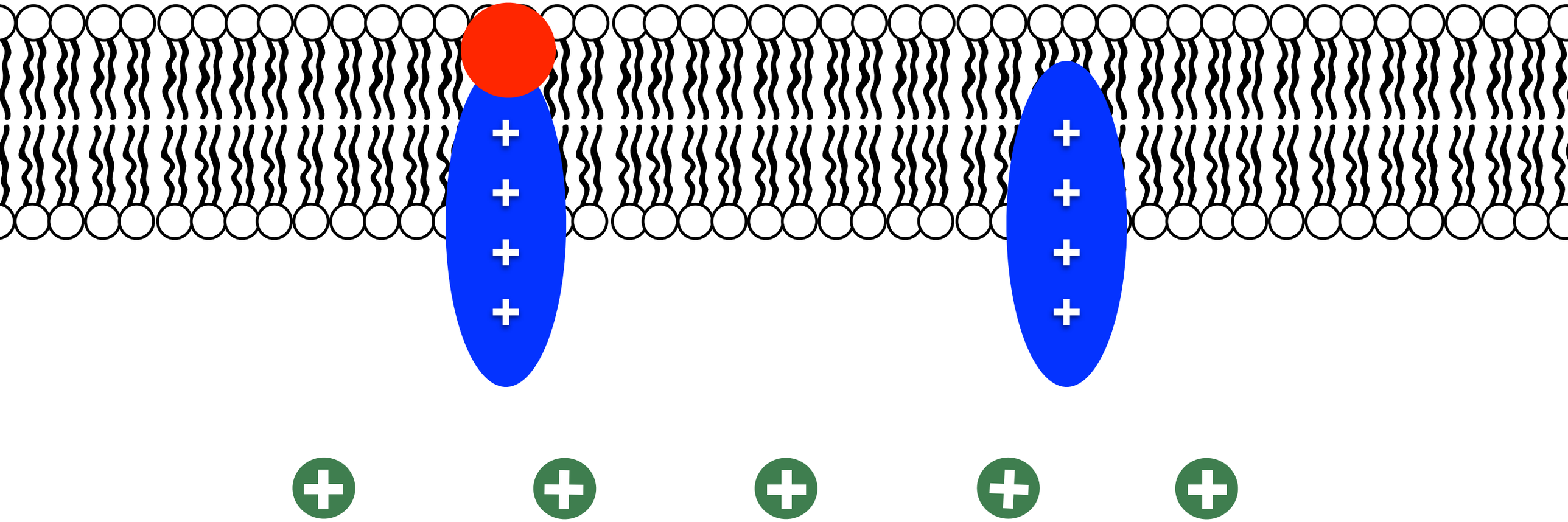
interface redesign for orthogonal binding





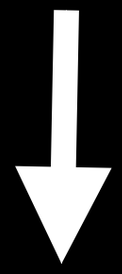
interface redesign for orthogonal binding





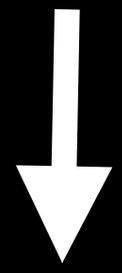
Design strategy

channel crystal + peptide NMR



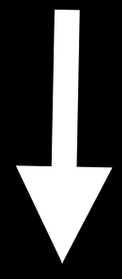
rigid body dock

structural hypotheses



empirical vs. Rosetta alanine scans

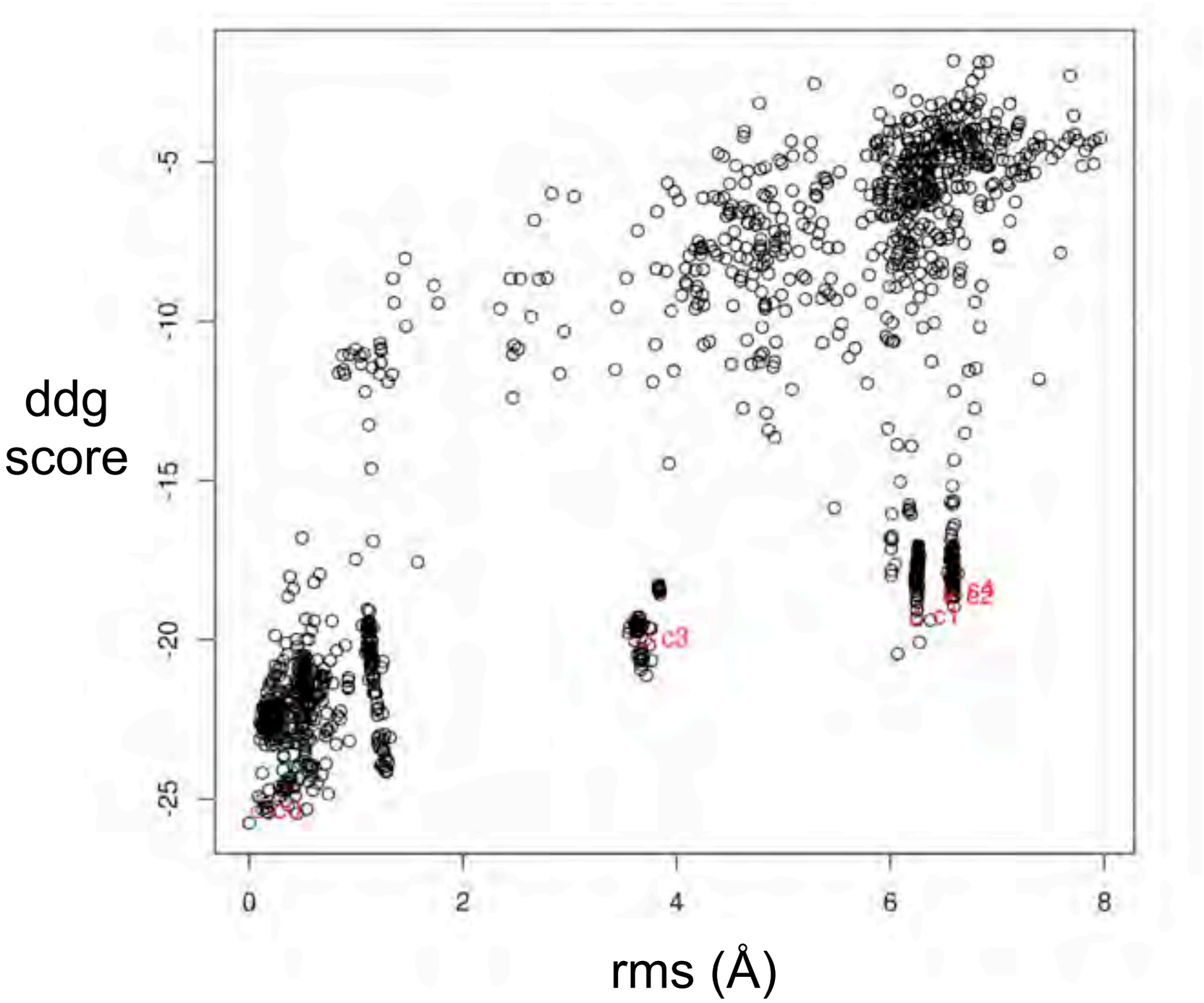
grounded structural hypotheses



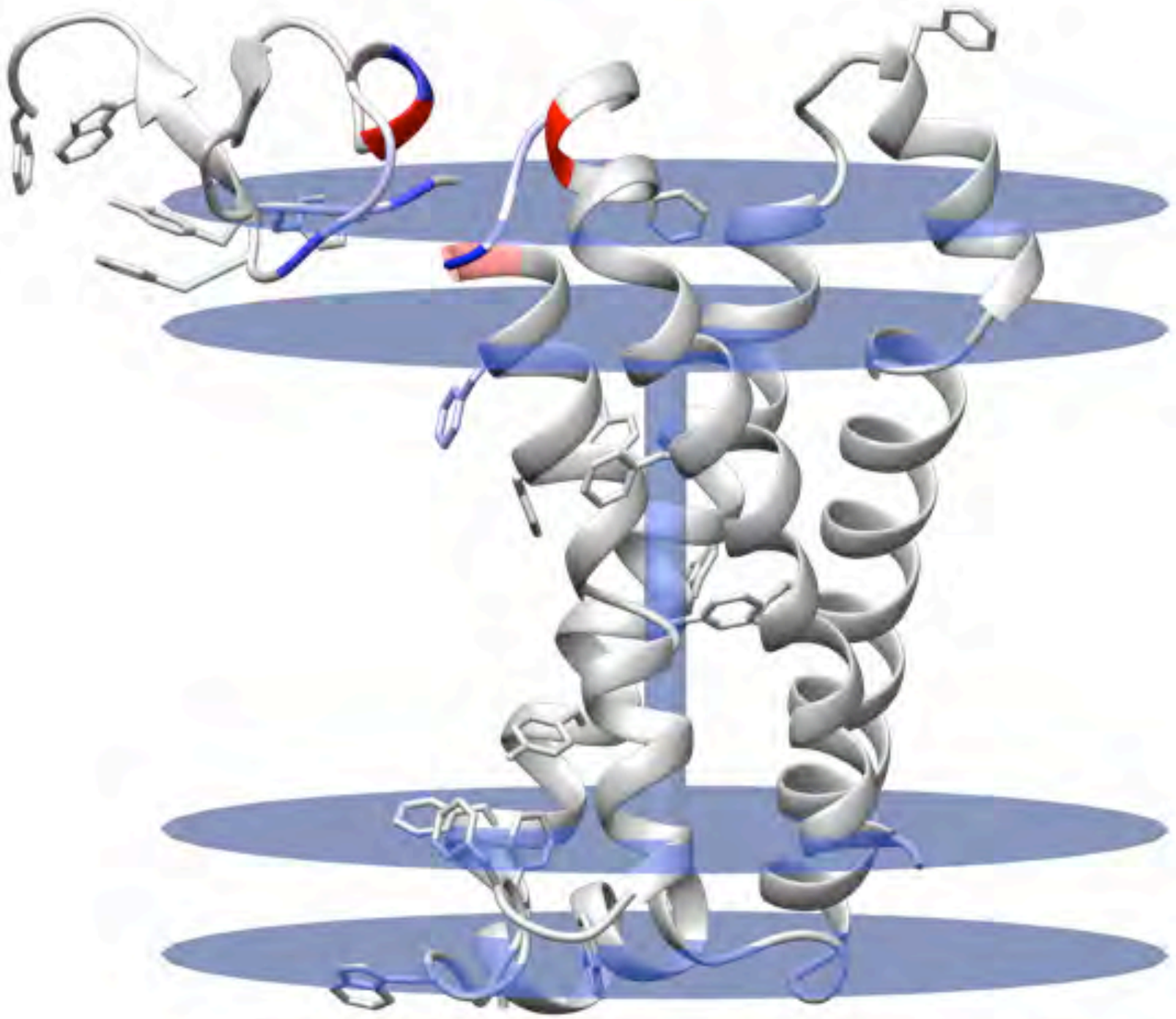
interface redesign, synthesis

orthogonal toxin-voltage sensor pair

tarantula toxin-voltage sensor docks yield clusters



alanine scans permit empirical evaluation



one-bead-one-compound method enables synthetic "display"

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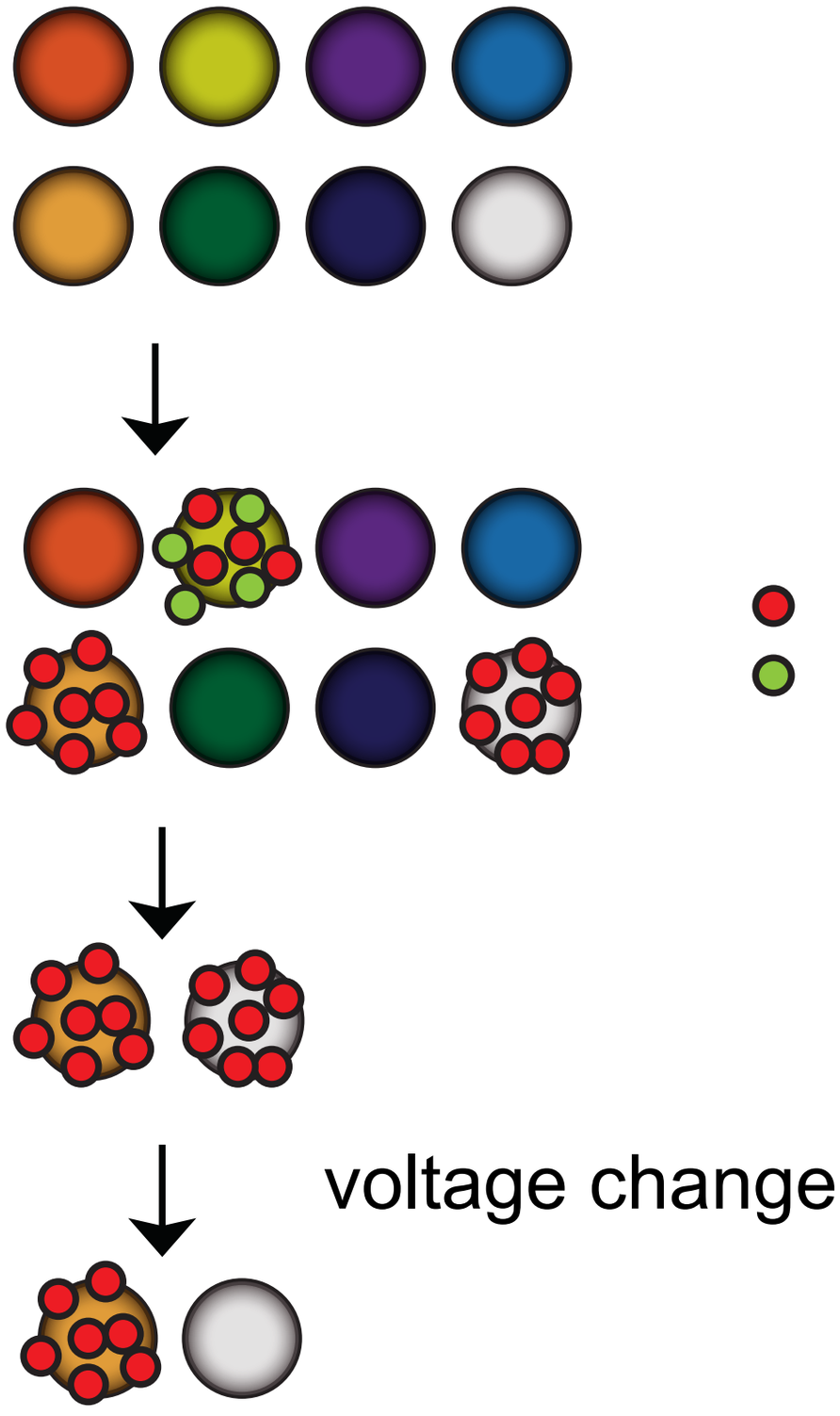
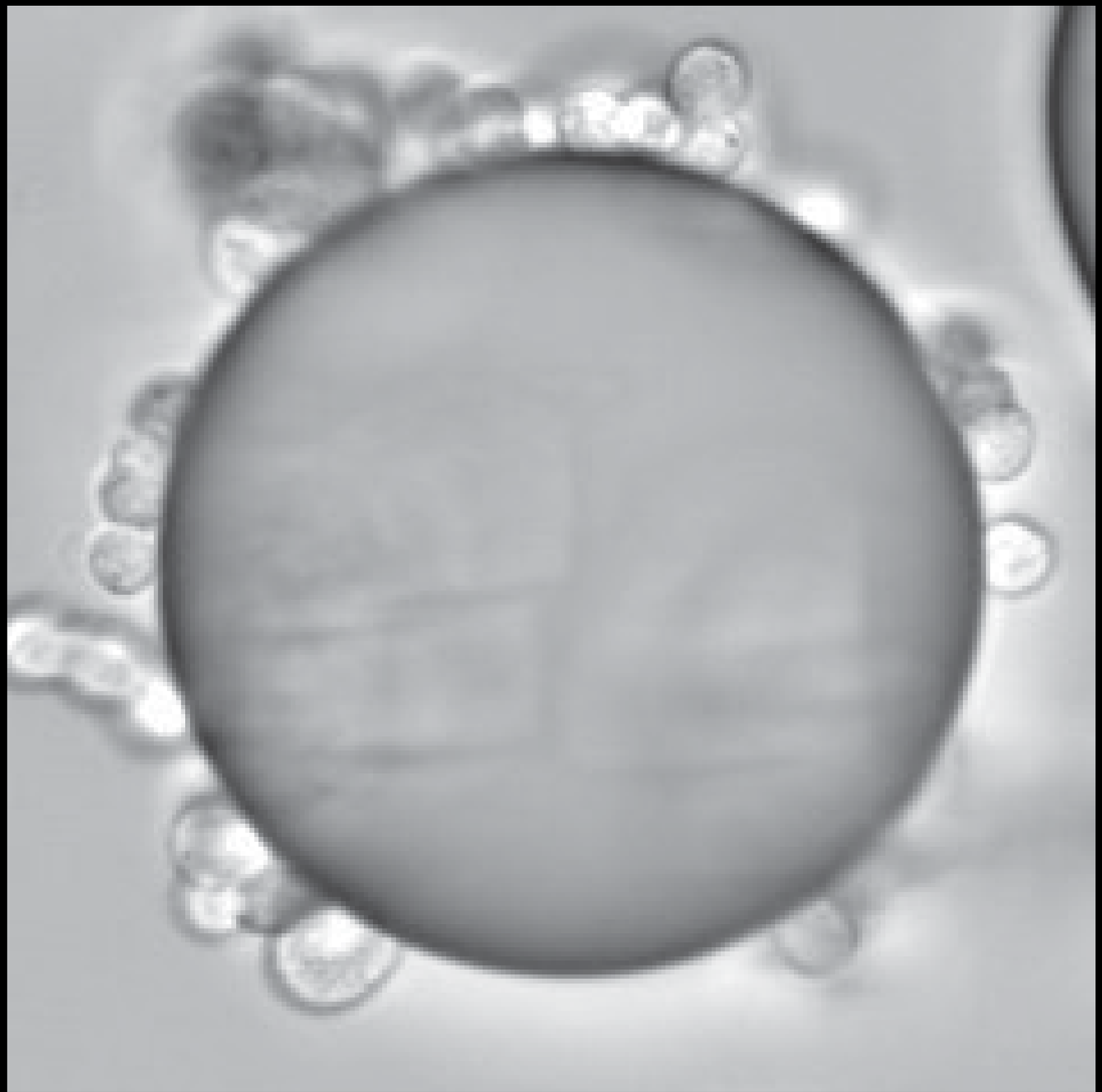


10^5 - 10^7 peptides
each on one resin bead

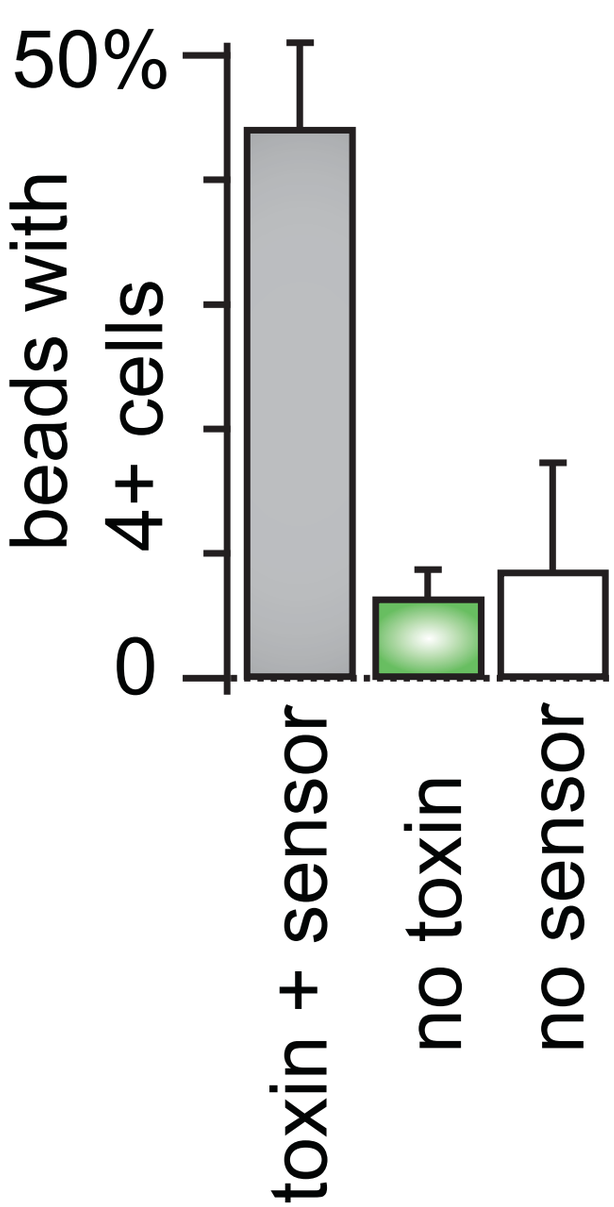
- cell with voltage sensors
- control cell

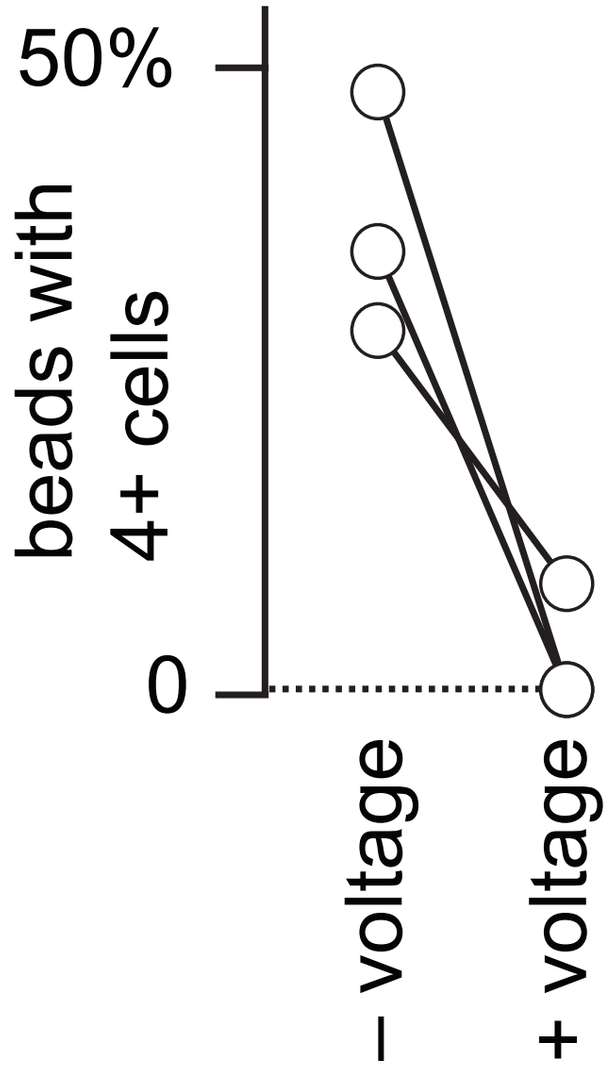
voltage change

Tarantula toxin beads bind voltage sensor cell



toxin-sensor interaction is amenable to display library voltage screening





challenges with Rosetta

1) model toxin-voltage sensor binding interface

protein docking protocols
membrane scoring function

2) redesign interface for chemical genetics

non-canonical amino acids

3) alter affinity for + vs – voltage sensor conformations

low-resolution conformational constraints

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