Spectroscopic Studies of Purified Rat TRPV1

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Abstract

Transient receptor potential vanilloid-1 (TRPV1) ion channels are polymodal signal integrators of noxious stimuli including heat, vanilloids such as capsaicin, peptide toxins, acid, and inflammatory mediators. It is unknown whether activation of TRPV1 by different stimuli is achieved through the same structural mechanism or if different stimuli activate the channel through different structural mechanisms. Clinical trials using TRPV1 antagonists resulted in patients exhibiting hyperthermia, suggesting that TRPV1 plays a role in maintaining body temperature and highlighting the need to ensure that therapeutics targeting the channel do not disrupt thermal homeostasis. Hence, knowledge of different structural mechanisms for channel activation would aid in design of therapeutic agents targeting TRPV1. To address this, we have expressed a series of functional single-cysteine rat TRPV1 channels for spectroscopic analysis, including electron paramagnetic resonance, double electron-electron resonance, and Förster resonance energy transfer spectroscopy. By probing several structural regions within TRPV1 we can determine which regions of the channels move during activation and whether those are the same for different noxious stimuli.

Screening mutants suitable for spectroscopic analysis

Mutagenesis

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FSEC to verify structural integrity

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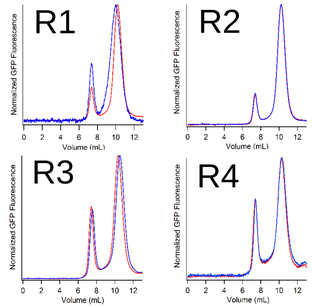
Functional verification

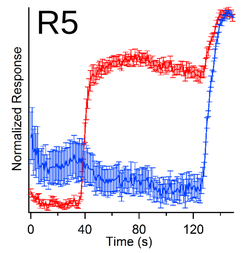
⇩

Large-scale expression for spectroscopic assays

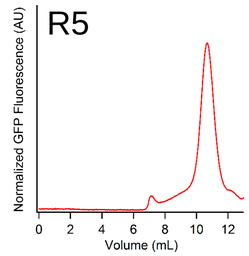
FSEC identifies positive mutants



Calcium imaging to verify response to capsaicin



Virally-expressed protein for purification and labeling



Future Directions

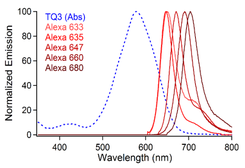
- Optimize large scale purification for each candidate mutant
- Optimize labeling efficiency of FRET pairs for tetrameric channels
- Measure bulk FRET values of purified protein
- Measure single-molecule FRET
- Measure lifetimes using single-photon counting with and without FRET pairs
- Immobilize channels with non-canonical amino acids to increase the range of R0 values

FRET pairs to screen a range of distances

FRET is steeply distance dependent

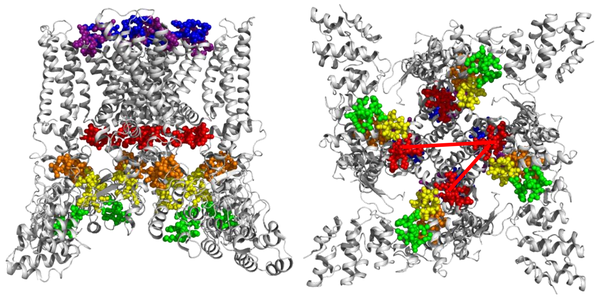
$$E = \frac{1}{1 + (\frac{r}{R_0})^6}$$

Less bulky FRET pairs with shorter R0s are needed to observe small structural changes



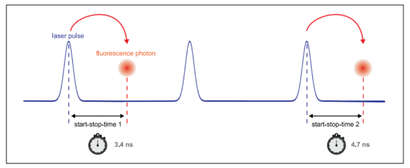
Fluorophore	R0
Alexa-633	46.8
Alexa-635	49.3
Alexa-647	40.6
Alexa-660	37.2
Alexa-680	32.9

Amino acids with a broad range of intersubunit distances have been mutated

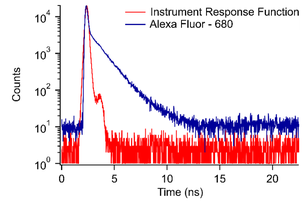


Calculating lifetimes using Time Correlated Single Photon Counting

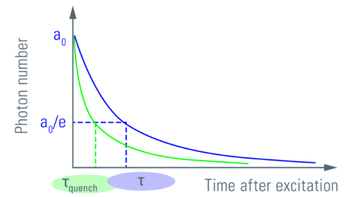
The time between fluorophore excitation and photon detection is measured



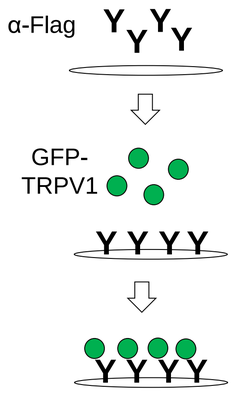
Fluorescence lifetime is calculated by measuring time decay



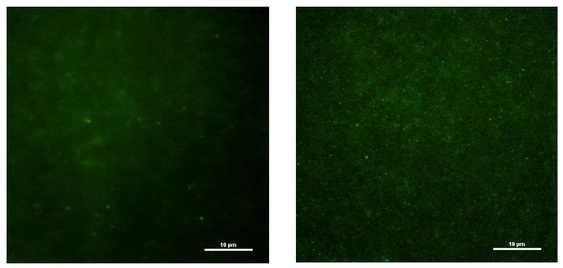
Fluorescence lifetime is reduced in presence of FRET



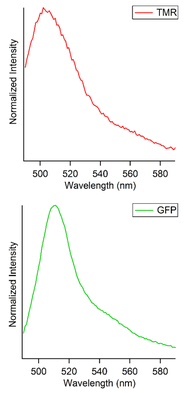
Single Molecule Fluorescence with GFP-TRPV1



Anti-FLAG immobilized TRPV1-GFP



Label single-cysteine TRPV1 with dyes for analysis



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https://www.leica-microsystems.com/science-lab/fret-with-flim/

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