Red-shifted thallium flux assay

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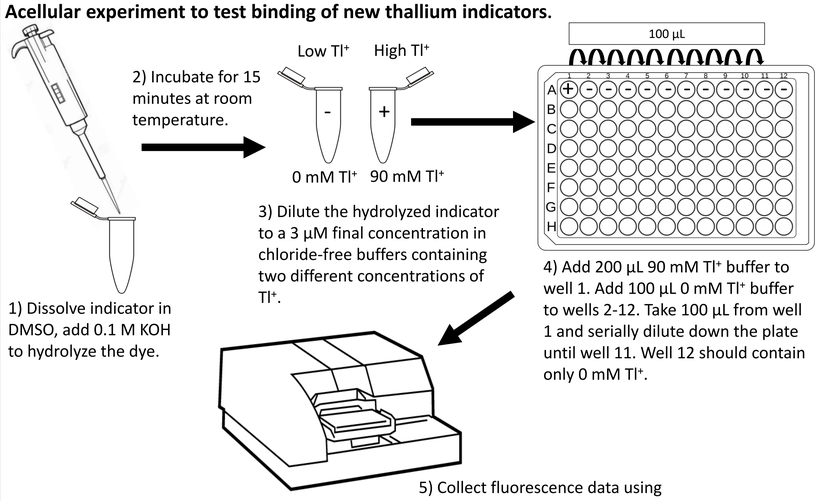
Abstract

Over the past 15 years, fluorescence-based measurements of thallium (Tl^+) flux have empowered the discovery of small-molecule modulators for a host of ion channels, transporters, GPCRs and other targets of interest for both drug discovery and physiological research.[1] The monovalent Tl^+ cation readily passes through sodium (Na^+) and potassium (K^+) channels, thereby acting as a surrogate to Na^+ or K^+. When paired with Thallos, a green-emitting thallium indicator whose fluorescence increases with increasing thallium concentrations, one can measure the flux of Tl^+ ions through membrane bound monovalent cation channels and transporters. The excellent selectivity of Thallos for Tl^+ over other monovalent cations, overcomes the major challenge when screening K^+ activity of detecting small decreases of intracellular potassium concentration with available potassium indicators.

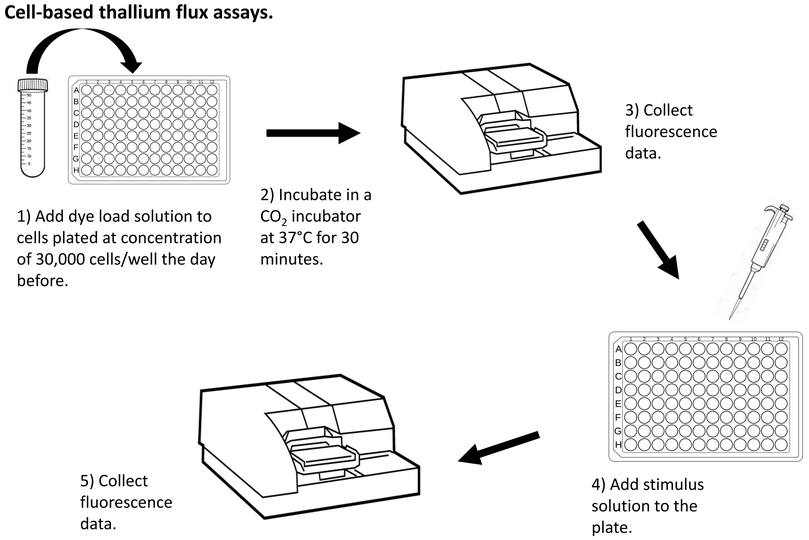
Although Thallos is one of the best thallium sensors on the market, its spectral properties overlap with many common green fluorophores. To monitor protein expression, scientists frequently tag their ion channel of interest with green-fluorescent protein (GFP), which spectrally overlaps with Thallos and thus interferes with ion channel activity measurement. Consequently, there is a need for a Tl^+ sensitive indicator with red-shifted spectral properties. To address this need, four thallium indicators with red-shifted excitation and emission were synthesized and evaluated in vitro. The best performing indicator was used to commercialize two red-shifted thallium flux assays, **Brilliant Thallium *Gold***, and **Brilliant Thallium *Gold* snapshot**, a novel endpoint compatible potassium and sodium channel assay.[2]

Methods

Acellular experiment to test binding of new thallium indicators.



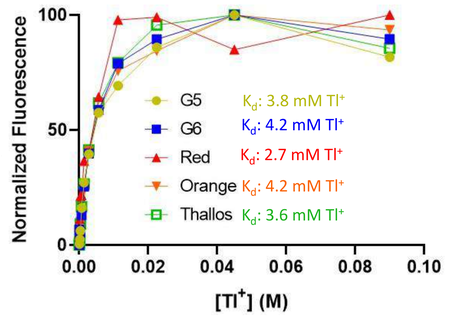
Cell-based thallium flux assays.



Results

Evaluating indicator performance.

Figure 1. Acellular saturation binding curve. Titrations of Thallos Gold 5 (G5), Thallos Gold 6 (G6), Thallos Red (Red), Thallos Orange (Orange), and Thallos were conducted in gluconate buffers.



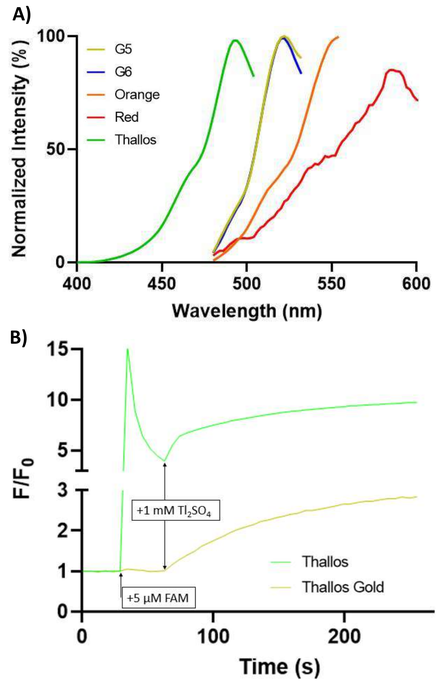


Figure 2. Spectral properties. A) Excitation spectra of the thallium sensors demonstrate their red-shifted properties. **B)** CHO K1 cells were loaded with Thallos and Thallos Gold (G5). After the addition of FAM (a fluorescent compound whose spectral properties coincide with Thallos), the fluorescence detected for Thallos Gold was minimally affected.

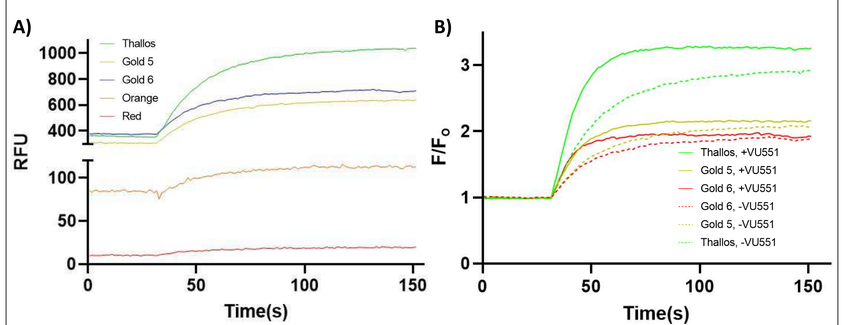


Figure 3. Indicator loaded into CHO cells overexpressing GIRK channels. **A)** Stimulus solution with final concentration of 1 mM Tl^+, was added at time 30 seconds. Data was recorded at ~1 Hz using a Molecular Devices FlexStation®. **B)** VU551 is a potent activator of GIRK1/2, resulting in an increased flux of Tl^+. Gold 5 and Gold 6 measure an increase in Tl^+ flux in the presence of VU551, albeit, not as marked as Thallos.

Results

Developing the Brilliant Thallium *Gold* assays with indicator G5.

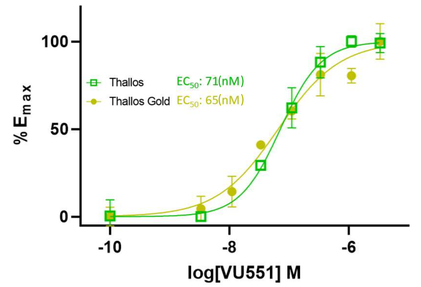


Figure 4. Thallos and Thallos Gold response. Concentration response curve of VU551 in CHO cells overexpressing GIRK channels. Thallos fluorescence (Ex: 485 nm, Em: 525 nm, Cutoff: 515 nm) and G5 fluorescence (Ex: 520 nm, Em: 555 nm, Cutoff: 550 nm) were recorded at ~1 Hz using a Molecular Devices FlexStation®. Error bars represent standard deviation (n=3).

Brilliant Thallium *Gold* snapshot assay

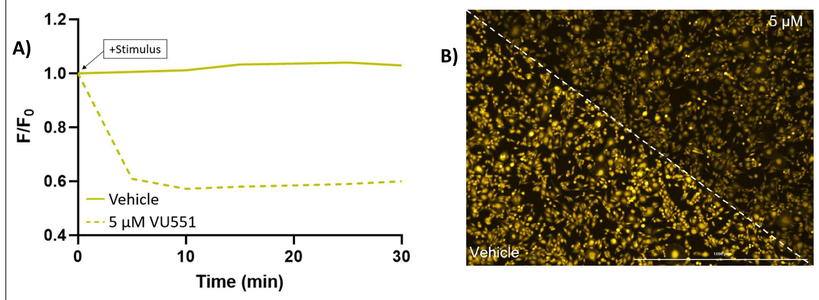


Figure 5. CHO GIRK1/2 response to a GIRK activator after being loaded with Tl^+ along with Thallos Gold. A) After equilibration, signals are sustained for 30 minutes as demonstrated using a standard fluorescence plate reader (Cytation 5), reads were acquired at 5 min intervals. B) Representative fluorescence images of vehicle- (left) and VU551-treated (right) CHO G12 cells. Images acquired 30 min after VU551 addition using propidium iodide filters and a 4X objective. The images were pseudo-colored with a gold lookup table (LUT) after acquisition.

Conclusions

- Out of all indicators tested, G5 (Thallos Gold) offered fluorescence levels comparable to that of Thallos and the greatest intracellular response to stimulus. Thus, it was chosen to commercialize two assays.
- Thallos Gold gave comparable potassium channel assay results to Thallos and displayed minimal assay interference when combined with a green fluorescent compound, FAM.
- Thallos Gold was also an adequate indicator for *snapshot* – our endpoint-compatible potassium channel assay that offers:
  - Sustained signal amplitude
  - Greater convenience
  - Reduced interference from background flux

References

1. Weaver CD. Thallium Flux Assay for Measuring the Activity of Monovalent Cation Channels and Transporters. Methods Mol Biol. 2018;1684:105-114.
2. Dutter, et al. Rhodol-based Thallium Sensors for Cellular Imaging of Potassium Channel Activity. Org Biomol Chem. 2018 Aug 8; 16(31): 5575-5579.