

# A Homogenous Thallium Flux Assay for High Throughput Screen of Potassium Channels

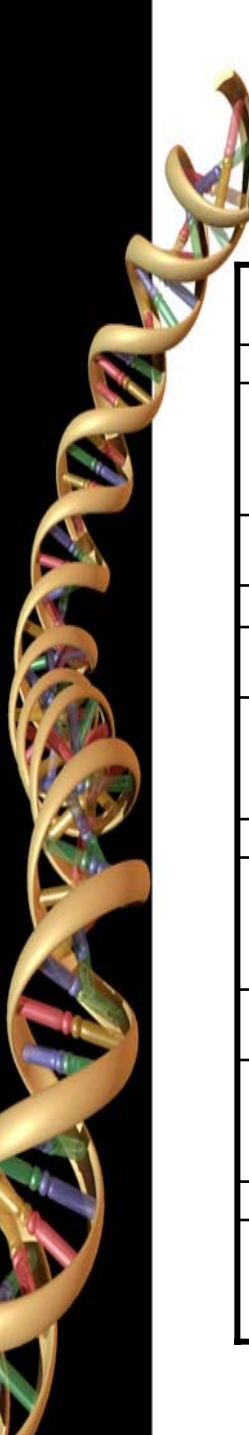
Wei Zheng

NIH Chemical Genomics Center, National Human Genome Research Institute,  
National Institutes of Health

[wzheng@mail.nih.gov](mailto:wzheng@mail.nih.gov)

[www.ncgc.nih.gov](http://www.ncgc.nih.gov)

# Screen Assays for hERG Potassium Channels



Assay	Type	Format	Comments
Binding assay ( <sup>3</sup> H MK-499)	Filtration	96-well	Classical assay (but only one binding site)
	SPA	96/384-well	Homogeneous
Electrophysiology	Patch-clamp	manual	“Gold standard”
	Automated patch-clamp	Chips (16, 96, 384wells)	Increased throughput. Expensive instrument
Ion flux assay	Radioisotopes ( <sup>86</sup> Rb <sup>+</sup> )	96 wells	High radioactivity and low assay window
	Rubidium flux	96 and 384-well	Special instrument
	Thallium flux	96, 384, 1536-well	Fluorescence kinetic reader
Fluorescence dyes	Membrane potential dye	96, 384 and 1536- well	Indirect measurement (effect of other channels)



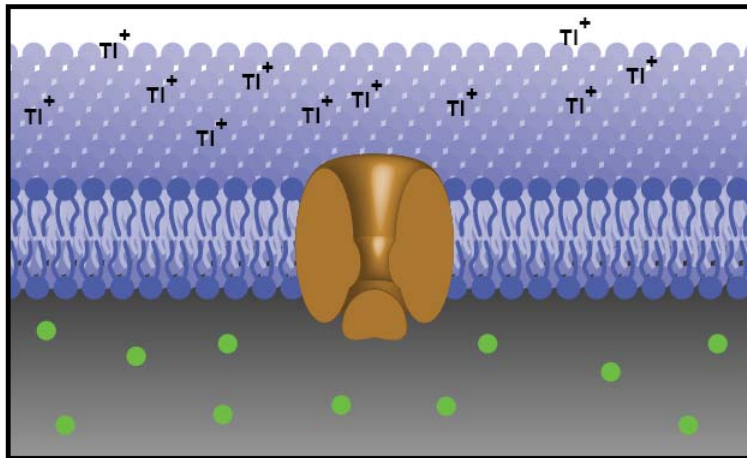
## Thallium Flux Assay – Using $Tl^+$ as a Surrogate Ion for Measurement of Potassium Channel Activity

- **Hille, B.** Potassium channels in myelinated nerve. Selective permeability to small cations. *J Gen Physiol* 61, 669-686 (1973).
- **Weaver, C.D., et al.** A thallium-sensitive, fluorescence-based assay for detecting and characterizing potassium channel modulators in mammalian cells. *J Biomol Screen* 9, 671-677 (2004).
- **Niswender, C.M. et al.** A novel assay of Gi/o-linked G protein-coupled receptor coupling to potassium channels provides new insights into the pharmacology of the group III metabotropic glutamate receptors. *Mol Pharmacol* 73, 1213-1224 (2008).
- **Jorgensen, S et al.** Fluorescence-based  $Tl(+)$ -influx assays as a novel approach for characterization of small-conductance  $Ca(2+)$ -activated K (+) channel modulators. *Methods Mol Biol* 491, 257-266 (2008).

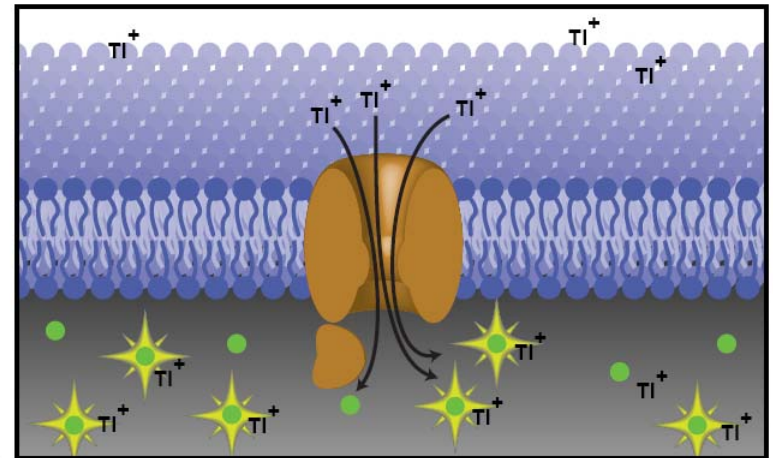
# Principle of Thallium Flux Assay

- ❑ FluxOR, a pro-fluorescent dye, is loaded into cells
- ❑ Thallium ions move down concentration gradient through open potassium channels across cell membrane
- ❑ When thallium ion binds to FluxOR dye, it emits a bright fluorescent emission at 530 nm upon an excitation at 480 nm

(a) Channel closed

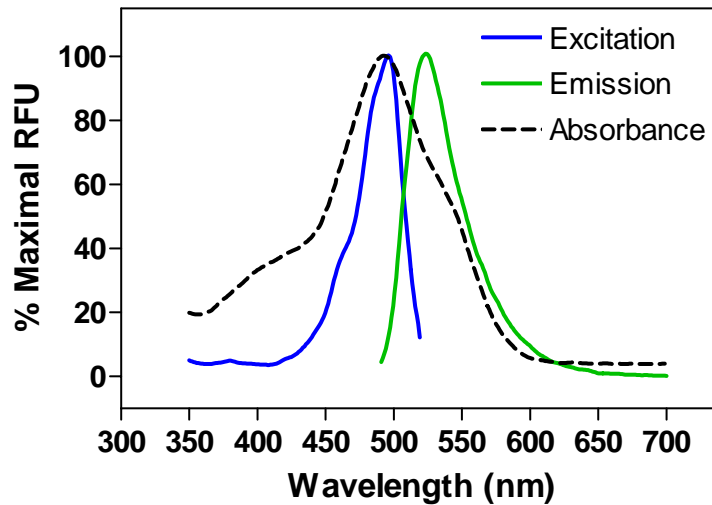


(b) Channel opened

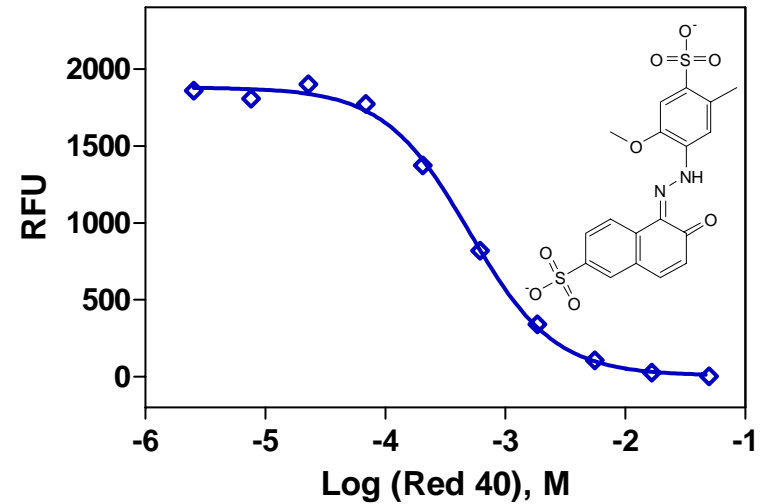


# Fluorescence Thallium Dye and Red-40 Quencher

A. Thullian dye - FluxOR

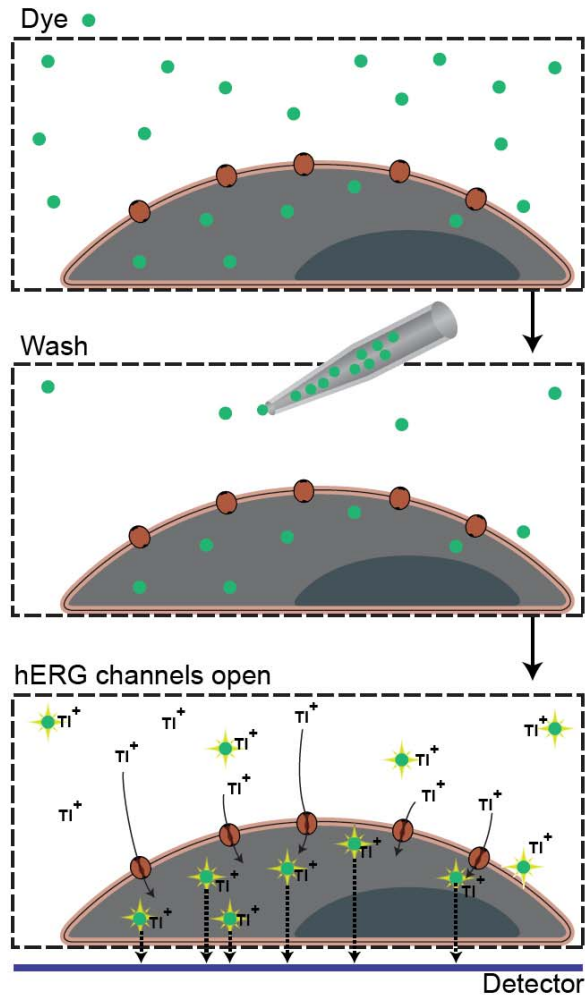


B. Red-40 quenching effect

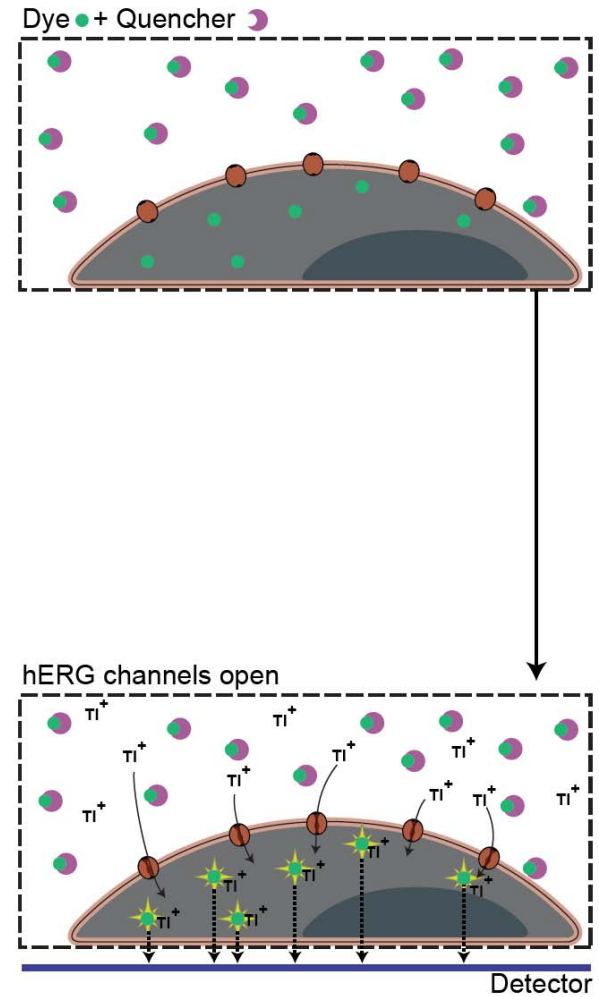




## Original assay requires cell wash



## No-wash assay





## A Homogenous Protocol for Thallium Flux Assay in 1536-well Plates

Step	Parameter	Value	Description
1	Cells	2000 cells (4 $\mu$ l/well)	U2OS cells transduced with hERG channels are loaded with dye and seeded in 1536-well plate
2	Compound	20 nl/well	Compounds in DMSO solution
3	Incubation	10 min	Room temperature
4	Detection	10 reads	Basal signal. FDSS-1000 kinetic plate reader at 1 Hz (Ex= 480 and Ex = 530 nm)
5	Reagent	1 $\mu$ l/well	Stimulation buffer with Tl <sup>+</sup>
6	Detection	180 reads	Total signal. FDSS-1000 kinetic plate reader at 1 Hz (Ex= 480 and Ex = 530 nm)

# Hamamatsu FDSS-7000 Fluorescence Kinetic Plate Reader

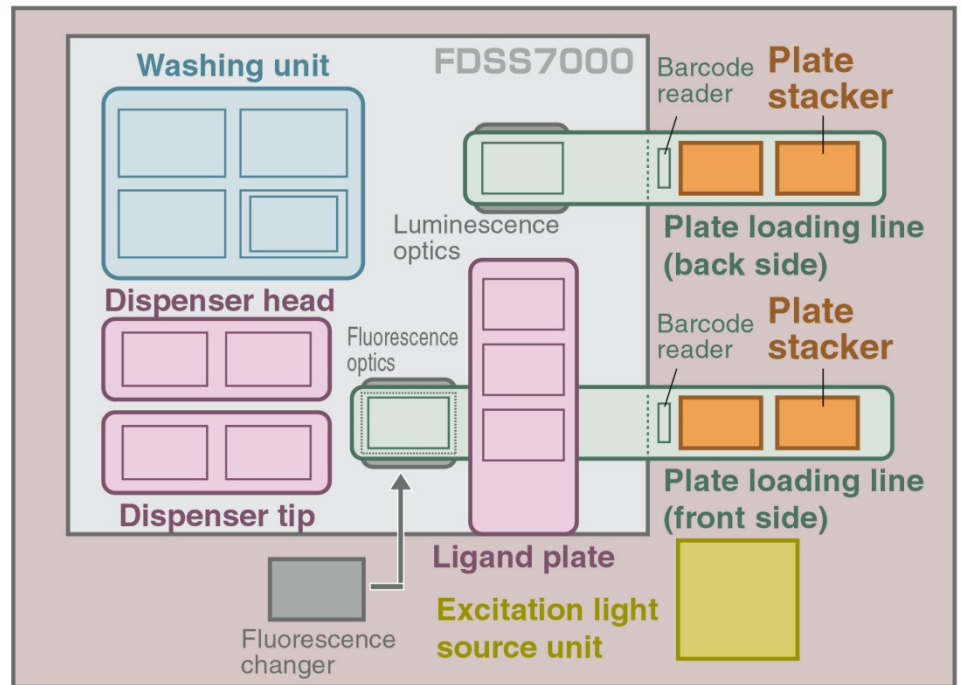
A.



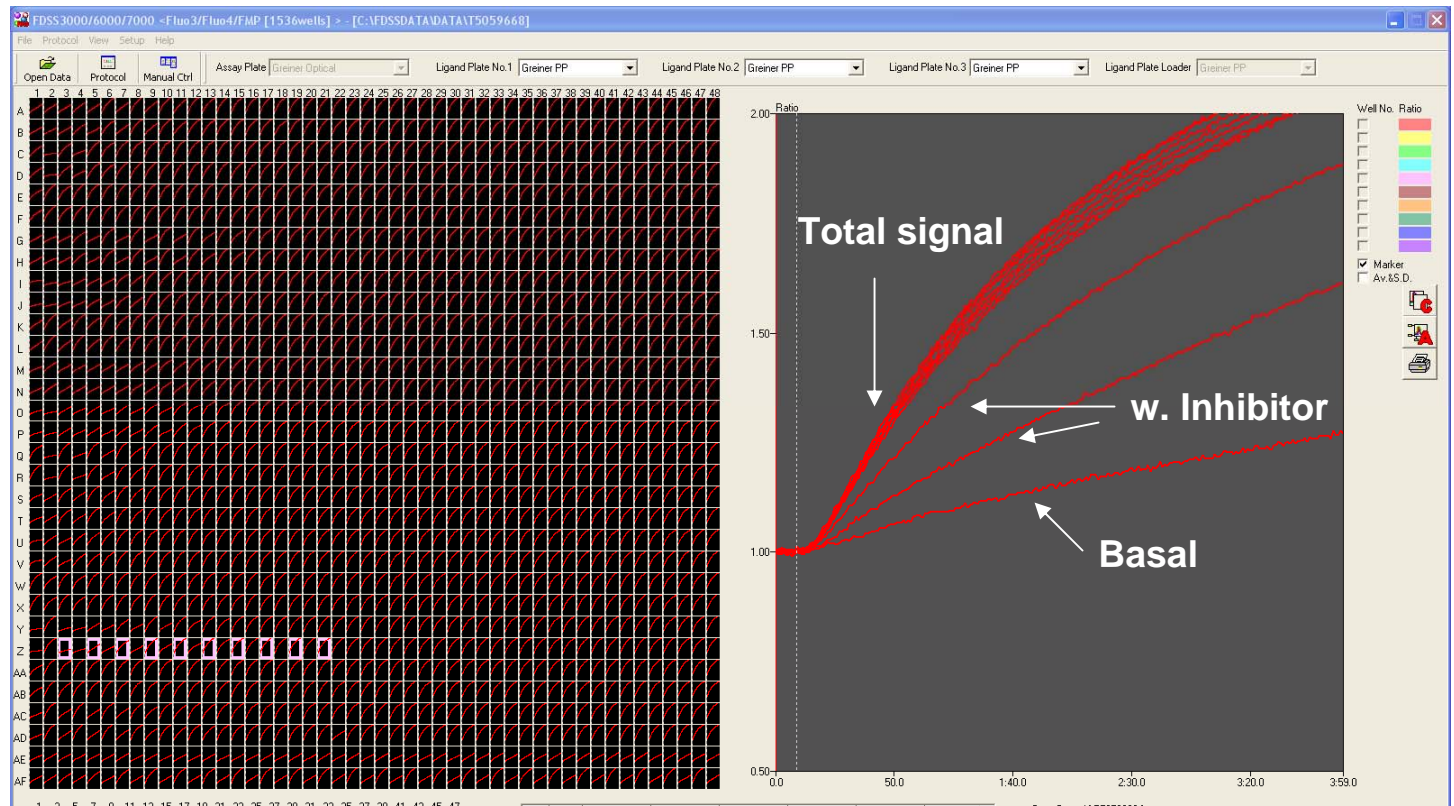
B.



C.

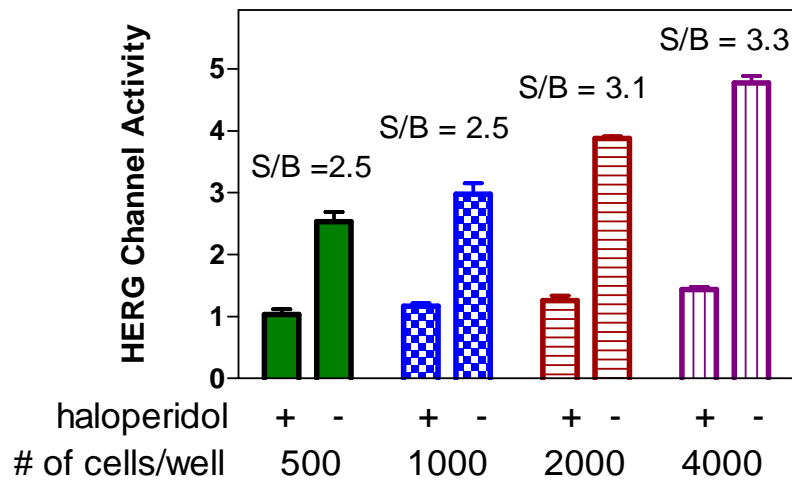


# Result of Thallium Flux Assay is Calculated as the Slope of Fluorescence Intensity Development

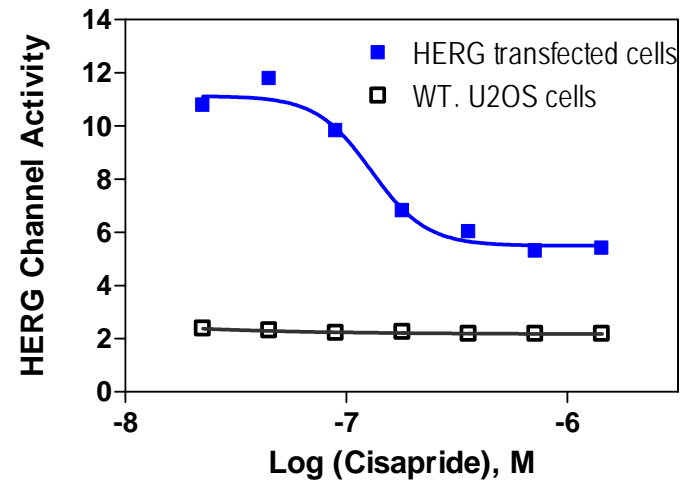


# Thallium Flux Assay Optimization in 1536-well Plates

## a. Cell density titration:

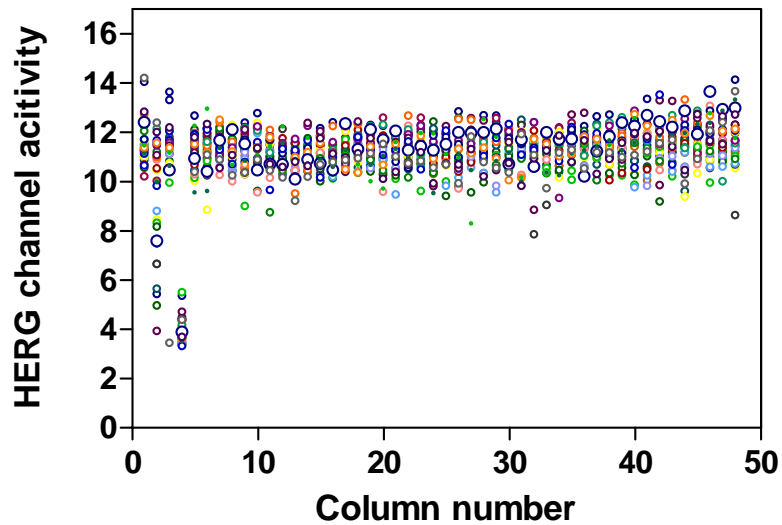


## b. Known inhibitor response:





## DMSO Plate Test in a 1536-well Plate



**DMSO plate test:**

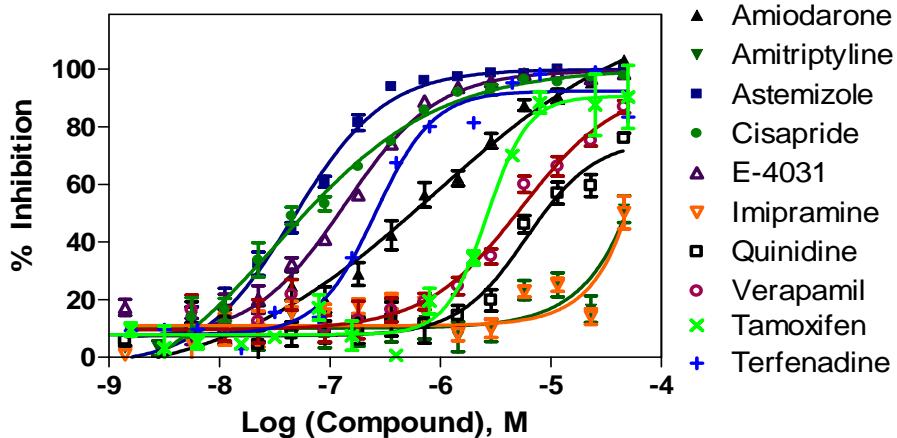
**S/B = 2.9 fold**

**CV = 6 %**

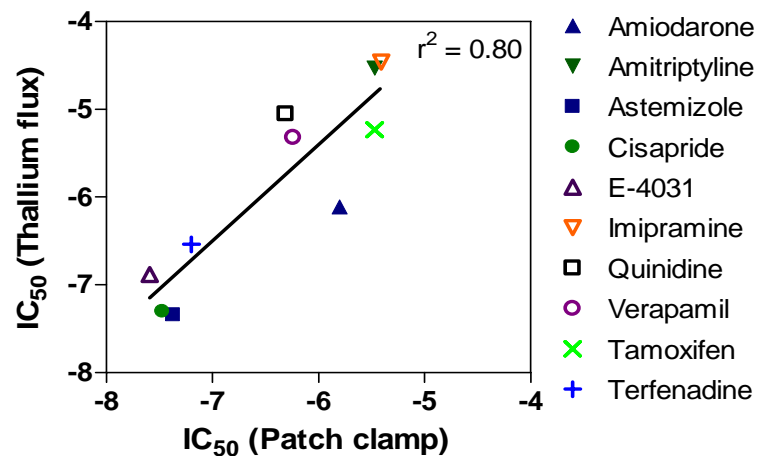
**Z' = 0.52**

# IC50s of 10 Known hERG Inhibitors and Correlation between Thallium Flux and Patch Clamp Assays

A.

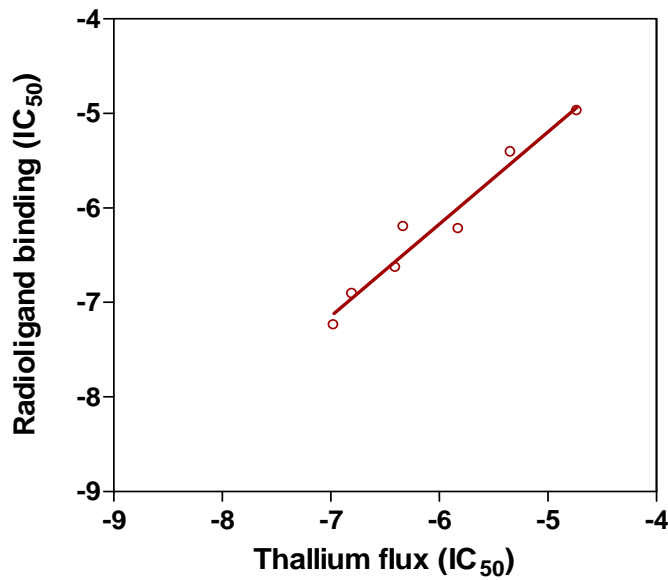


B.

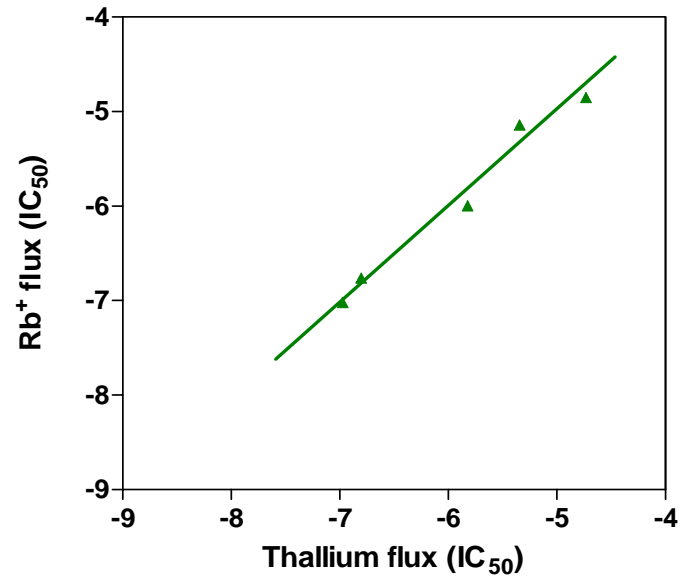


# Correlation of IC50s of Known hERG Inhibitors

With binding assay



With Rb<sup>+</sup> flux assay





# Summary

- **Thallium flux assay has robust signal-to-basal ration and has been optimized in a homogenous assay format**
- **Correlation of compound IC50s has been observed between thallium and patch clamp assays**
- **This thallium flux assay in 1536-well plate format can be used for the primary screen of compound libraries and for the large volume compound profiling for potassium channels**



# NIH Grant Information for HTS Assays

## **(1) RO3 grant for HTS assay submission:**

- \$50,000 for direct cost
- For HTS ready assay
- Obtaining small molecule probes
- PAR-09-129 (Three times per year. Next deadline is 9/3/2009)

## **(2) R21 grant for HTS assay development:**

- \$100,000 for direct cost
- For development of an assay for HTS
- Obtaining small molecule probes (fast tracker)
- Par-08-024 (Two times per year. Next deadline is 11/20/2009)



# Outreach Program for Assistance on RO3 Assay Grant Application

- Initial consulting for assay design and assay format and screen technologies (teleconference or in person)
- Assay miniaturization and HTS validation
- Help on RO3 grant writing and submission
- Contact Wei Zheng at NIH Chemical Genomics Center for help (Tel. 301-217-5720 and [wzheng@mail.nih.gov](mailto:wzheng@mail.nih.gov))



# Acknowledgement

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