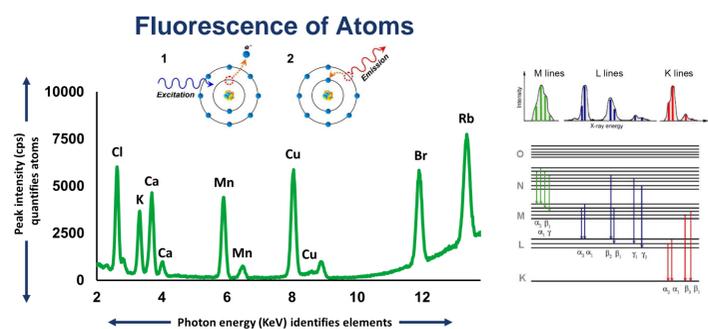


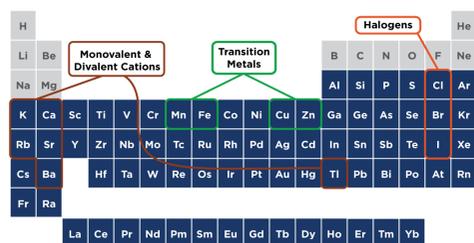
Abstract

XRpro[®] technology leverages the unique capabilities of X-ray fluorescence for label-free activity measurements of transporters and ion channels. Here, we demonstrate XRpro for analysis of a broad range of pharmacologically important targets, including solute carrier (SLC) transporters and TRP ion channels. SLC transporters comprise a diverse set of secondary-active transporters, including families responsible for uptake and efflux of inorganic ions. The ability of XRpro to directly quantify most elements on the periodic table enables measurement of SLC targets including Zn²⁺ (SLC30, SLC39), sodium phosphate (SLC20, SLC34), and nonelectrogenic cation chloride (SLC12) transporters. XRpro analysis of TRP ion channels includes both Rb⁺ and Sr²⁺ flux measurements for TRPA1, TRPV1 and TRPC5 experiments, in buffer and 100% serum. Combined with straight-forward cell biology, XRpro provides a powerful solution for investigating a wide range of otherwise challenging targets.

XRpro[®] Technology

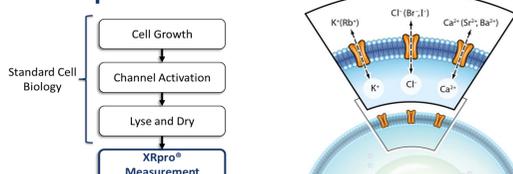


- X-rays excite inner-shell electrons. Fluorescent X-ray photons are emitted when the resulting hole in the electron configuration is filled.
- Each element generates unique X-ray peaks
- Area under the peak quantifies elements



- XRpro directly measures elements shown in blue
- Element content of cells and buffers is measured directly: No dyes, fluorophores or radiolabels
- Simultaneous measurement of multiple elements enables internal quantitative standards
- Detection unaffected by chemical environment, and works with complex and opaque solutions, including 100% serum

XRpro Workflow



- XRpro measures ion influx and efflux for direct measurement of ions or using tracer ions such as Rb⁺ and Sr²⁺
- Ion flux is measured in standard 384-well plates
- Standard cell biology for cell growth and activation provides flexibility in experiment design

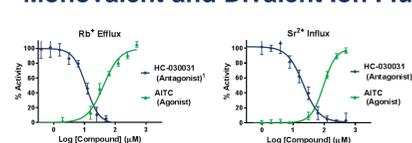
TRP Channels

TRPA1

TRPA1 conducts both monovalent (K⁺) and divalent (Ca²⁺) ions. Like other drug targets, compounds that inhibit TRPA1 may be significantly bound to serum proteins.

Here, we demonstrate the use of XRpro to monitor both monovalent and divalent ion flux and to measure compound IC₅₀ values in buffer and 100% human serum.

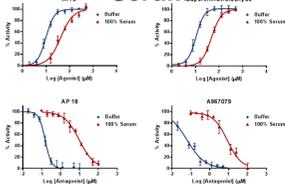
Monovalent and Divalent Ion Flux



Compound	Rb ⁺ Efflux EC ₅₀ (μM)	Sr ²⁺ Influx EC ₅₀ (μM)	Expected EC ₅₀ (μM)
AITC	9.8 ± 0.5		4 ^α
Supercinnamaldehyde	10.5 ± 0.3		16 ^α
TCS5861528 [†]	12 ± 1	12 ± 1	14 ^δ
A967079 [†]	0.06 ± 0.03	0.051 ± 0.006	0.07 ^β
HC-030031 [†]	11 ± 1	22 ± 1	8 ^α
AP 18 [†]	0.15 ± 0.01	0.69 ± 0.04	3.1 ^γ

[†] With 200 μM AITC
[‡] With 100 μM supercinnamaldehyde
^α Eid et al. Mol Pain. 2008, 4:48
^β Chen et al. Pain. 2011, 152(5):1165-72
^γ Petrus et al. Mol Pain. 2007 3:40
^δ http://www.scbt.jp/datasheet-361378.html

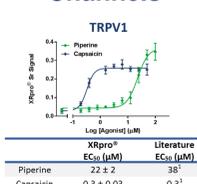
TRPA1 in 100% Serum



Compound	XRpro [®] (Fraction Bound) EC ₅₀ (μM)	Equilibrium Dialysis (Fraction Bound) EC ₅₀ (μM)
TCS 5861528 [†]	0.95 ± 0.03	0.95
HC 030031 [†]	0.90 ± 0.05	0.90
A 967079 [†]	0.993 ± 0.001	0.99
AP 18 [†]	0.99 ± 0.01	0.99

[†] With 200 μM AITC
[‡] With 100 μM supercinnamaldehyde

Additional TRP Channels



Compound	XRpro [®] EC ₅₀ (μM)	Literature EC ₅₀ (μM)
Piperine	22 ± 2	38 ¹
Capsaicin	0.3 ± 0.03	0.3 ¹

Compound	XRpro [®] EC ₅₀ (μM)	Literature EC ₅₀ (μM)
ML 204	6.6 ± 0.5	9 ¹
Rosiglitazone	36 ± 4	30 ¹

Results

- Measurements for monovalent efflux and divalent influx
- Results for TRPA1, TRPV1 and TRPC5 match literature
- Assays in 100% serum for serum shift measurements

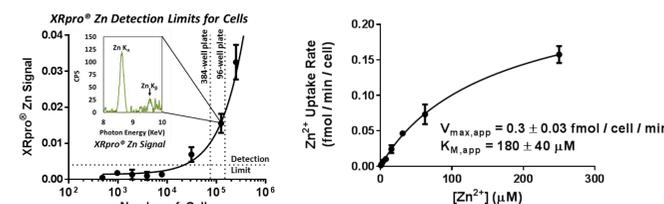
Analysis of SLC Transporters

Zn²⁺ Transporters

Zn²⁺ is the second most common trace metal in the body. Zn²⁺ transporters have been linked to a number of diseases, including diabetes (Znt8), pancreatic cancer progress (Zip4) and Alzheimer's disease (Znt3).

XRpro quantifies total cellular Zn²⁺ to measure activity of Zn²⁺ transporters.

Zn²⁺ Detection and Activity Measurements



XRpro Zn Detection limits showing detection for the number of cells grown in a 96- or 384-well plates, and XRpro Zn signal (inset).

Zn²⁺ uptake measurements for endogenous transporters in HEK-293 cells. Rates were determined from linear fits of Zn²⁺ over time from 20 minutes to 4 hours after activation. Apparent values for V_{max} and K_M likely represent a mixed population of transporters.

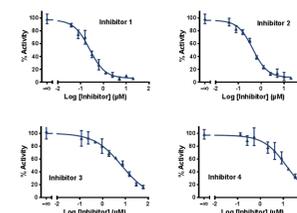
Results

- Direct measurement of cellular Zn²⁺
- Both endogenous and overexpressed transporters can be assayed
- Stopped-kinetic measurements for V_{max} and K_M determinations

Cation Chloride Transporters

Cation Chloride Cotransporters (CCC) regulate intracellular chloride, and have been implicated in a number of neurological conditions.

The ability of XRpro to directly monitor ion flux enables nonradioactive Rb⁺ flux assays for CCC transporters.



IC₅₀ measurements for test compounds, following Rb⁺ uptake through a target CCC transporter. XRpro IC₅₀ values match other techniques.

Inhibitor	IC ₅₀ (μM)	
	XRpro [®]	Known Value
Inhibitor 1	0.5 ± 0.2	0.5
Inhibitor 2	0.3 ± 0.1	1.1
Inhibitor 3	7 ± 1	9
Inhibitor 4	17 ± 1	18

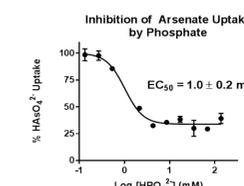
Results

- Validated assay for a challenging system
- Matched known IC₅₀ values
- Reduced 4 wash steps to a single wash
- Z' for entire assay > 0.7.
- Throughput of >30K wells / day / instrument for Rb⁺

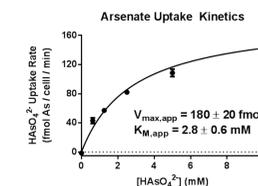
Sodium Phosphate Transporters

Sodium phosphate transporters (SLC20, SLC34), are responsible for renal phosphate reabsorption and maintenance of plasma phosphate concentrations.

XRpro can monitor both target ions and chemical surrogates enables studies of ions, like phosphate, with high background concentrations in cells.



Arsenate uptake is mediated by phosphate transporters. Arsenate uptake rates (Y-axis) are reduced at elevated phosphate concentrations, with a mM EC₅₀, as expected.



Measurement of endogenous phosphate transporter activity in HEK-293 cells following arsenate as a tracer. Rates are based on linear fits to arsenate uptake at times from 20 to 120 minutes.

Results

- Arsenate can be used to measure activity of phosphate transporters
- V_{max} and K_M determinations for endogenous transporters in HEK-293 cells

Conclusions

XRpro provides a direct solution for challenging transporters & ion channels

- Direct measurements with no dyes, fluorophores, or radiolabels
- Flux measurements of transporters & ion channels
 - Analysis of Zn²⁺, phosphate and nonelectrogenic transporters
 - Straight-forward analysis of TRP channels
 - Uptake and efflux measurements
 - Assays in 100% serum and High DMSO

XRpro[®] technology and other ion channel & transporter services provided by Icagen. Find out more at www.icagen.com

Additional Poster:
See our second poster for additional information about Icagen ion channel assays.