

# Measuring Ion Channel and Transporter Function

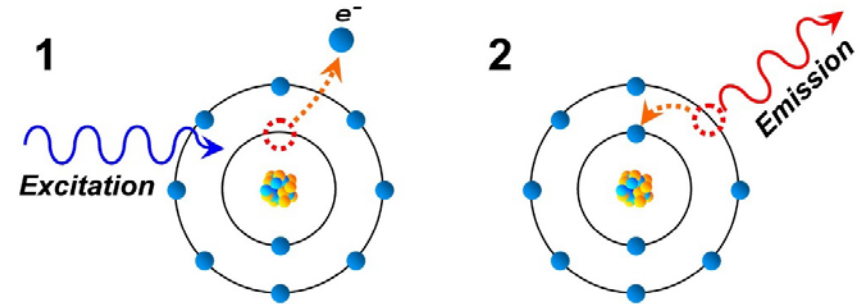
## *A New Way*

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Aurora Ion Channel Retreat  
27 June, 2014

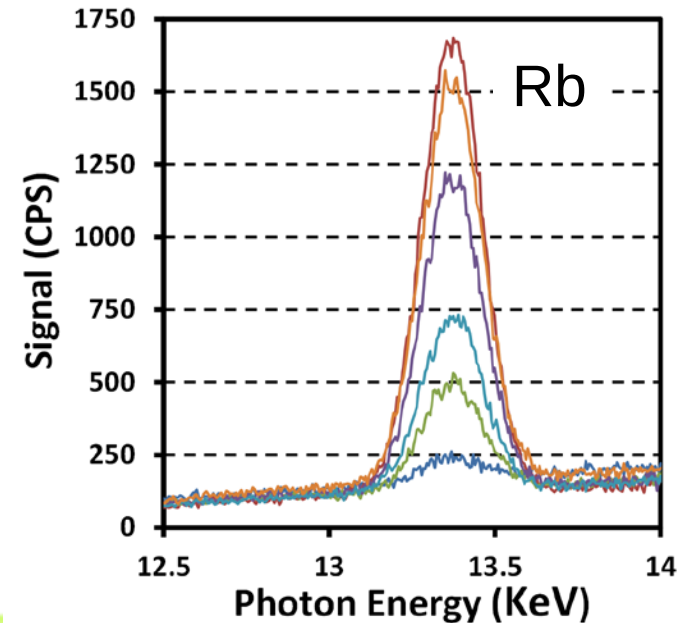
## Fluorescence of Atoms

- Each element generates unique peaks
- Peak intensities quantify atoms
- All elements atomic number  $\geq$  Al



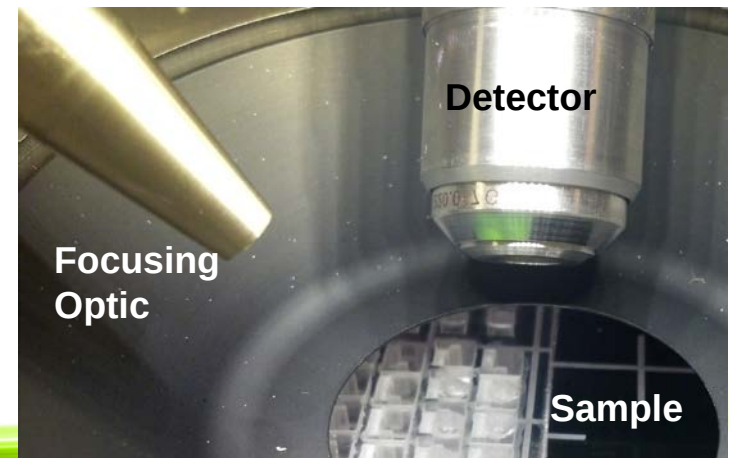
|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |    |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--|----|
| H  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  | He |
| Li | Be |    |    |    |    |    |    |    |    |    | B  | C  | N  | O  | F  | Ne |    |  |    |
| Na | Mg |    |    |    |    |    |    |    |    |    | Al | Si | P  | S  | Cl | Ar |    |  |    |
| K  | Ca | Sc | Ti | V  | Cr | Mn | Fe | Co | Ni | Cu | Zn | Ga | Ge | As | Se | Br | Kr |  |    |
| Rb | Sr | Y  | Zr | Nb | Mo | Tc | Ru | Rh | Pd | Ag | Cd | In | Sn | Sb | Te | I  | Xe |  |    |
| Cs | Ba | Lu | Hf | Ta | W  | Re | Os | Ir | Pt | Au | Hg | Tl | Pb | Bi | Po | At | Rn |  |    |
| Fr | Ra |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |  |    |
|    |    | La | Ce | Pr | Nd | Pm | Sm | Eu | Gd | Tb | Dy | Ho | Er | Tm | Yb |    |    |  |    |

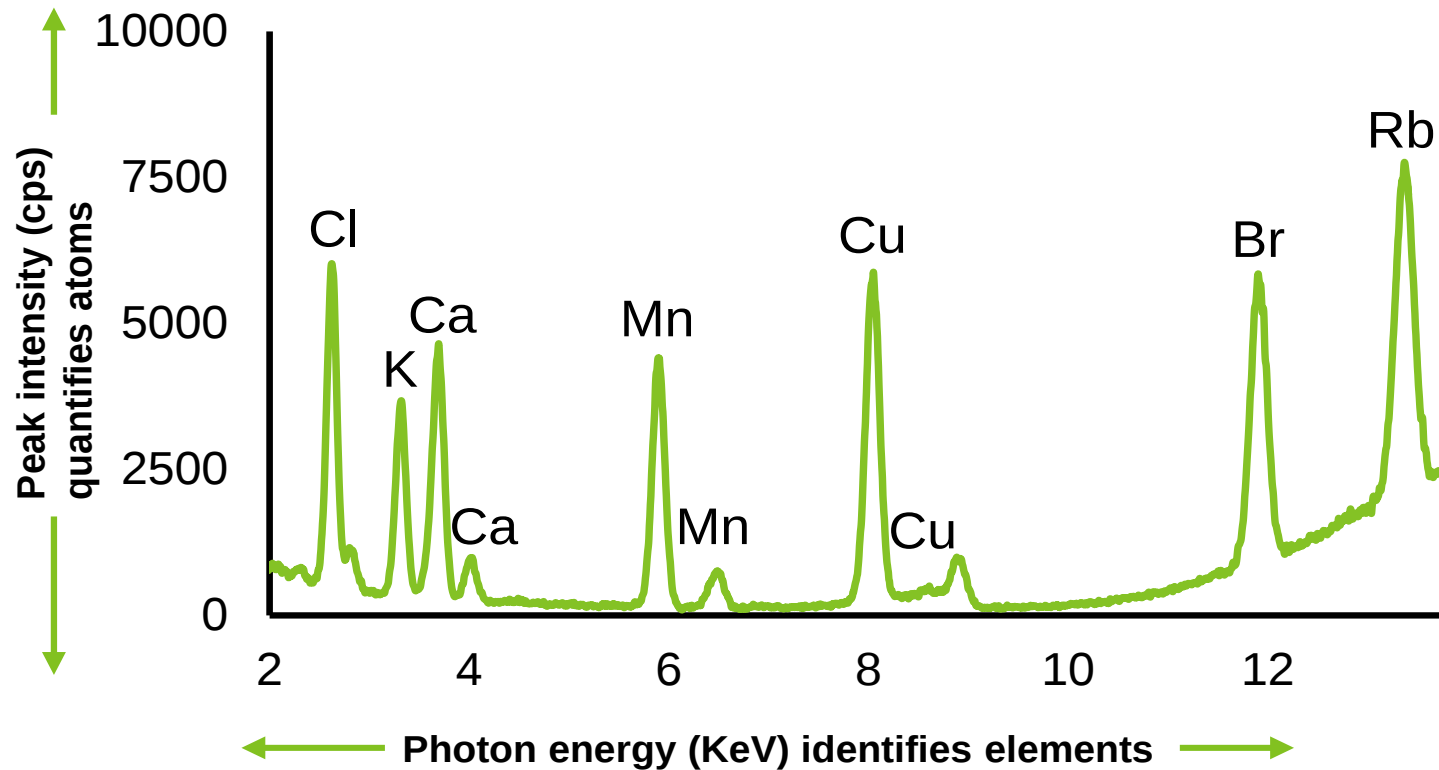
Elements in green are XRpro® fluorophores



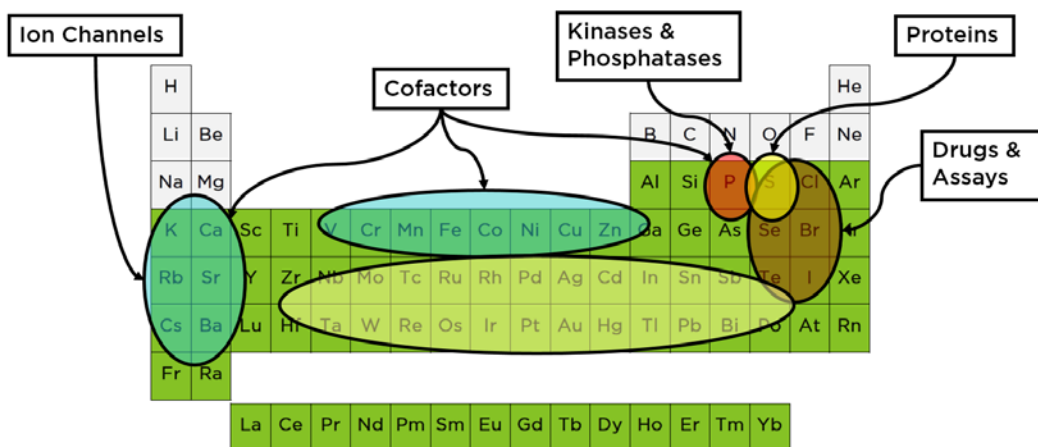
## Instrument Features

- Bench top
- Microfocused X-rays
- Energy resolving detector
  - Counts photons
  - Measures photon energies
- Programmable Stage
  - 96-, 384-, 1536-well plates
  - Custom programs
- Safe
  - Fully shielded and interlocked
- Nondestructive
  - Low X-ray power
  - Works with live cells



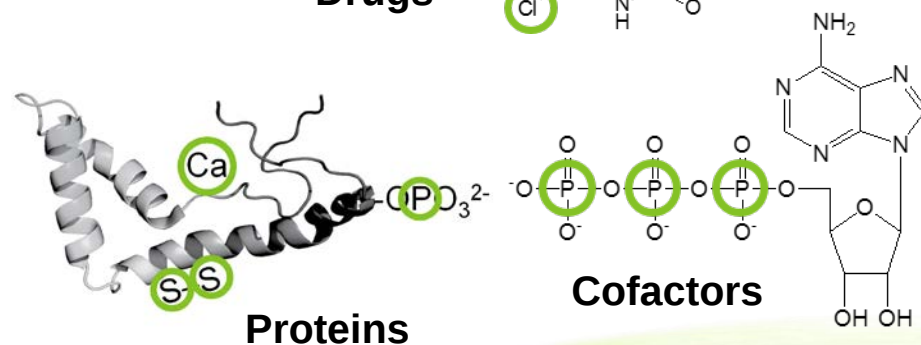
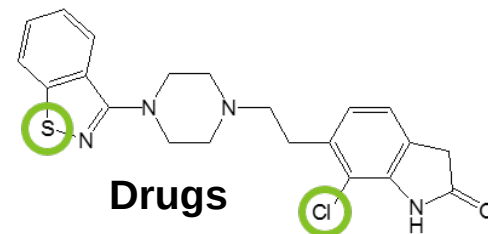
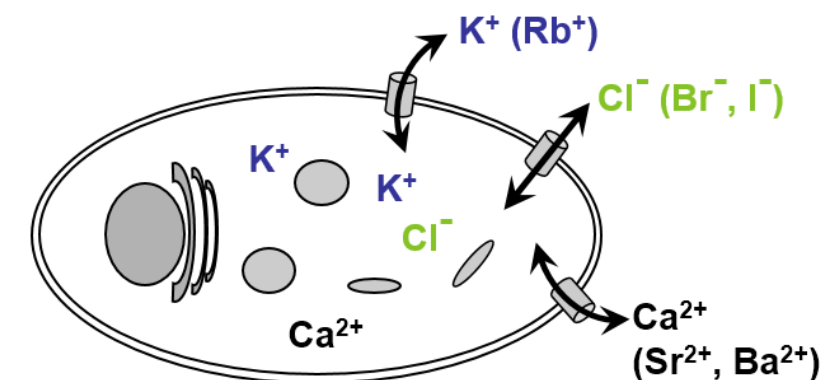


- All elements are measured in a single spectrum.
- Spectra are quantitative: Peak areas quantify elements.



Elements in green are XRpro® fluorophores

- Ion Channels and Transporters
- Cofactors
- Kinases and Phosphatases
- Proteins
- Drugs and Small Molecules



## Transporters & Ion Channels

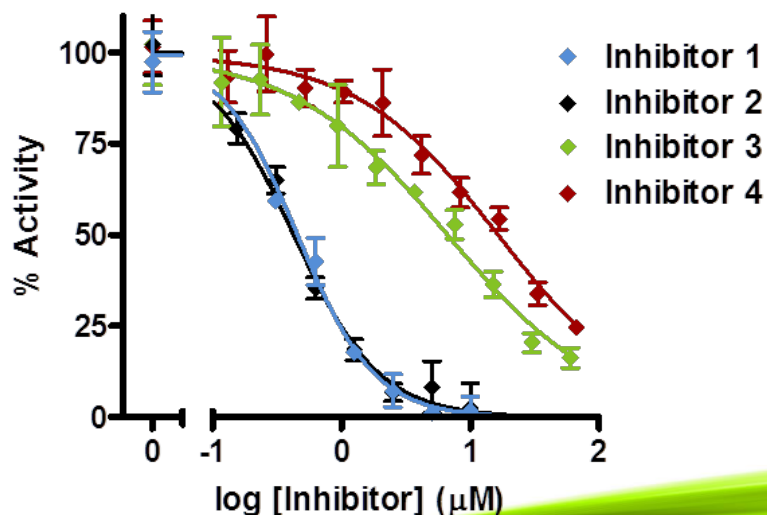
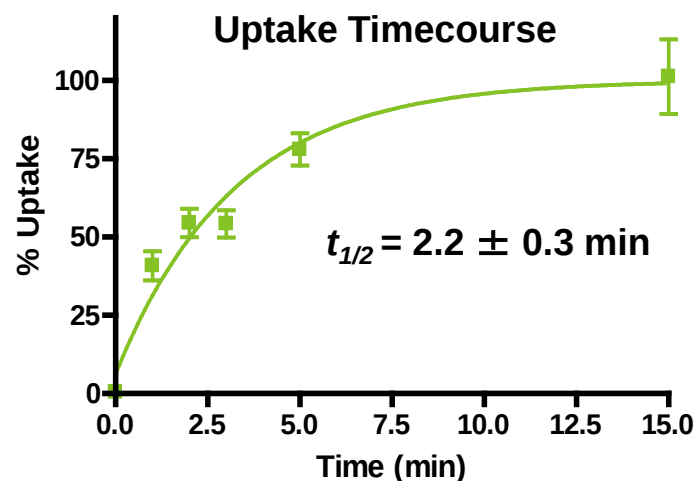
- Flux measurements
- Cell populations

## Advantages

- Direct measurements
  - No dyes, fluorophores, radiolabels
- Multiple elements simultaneously
- Works with up to 100% serum, high DMSO, complex matrices
- Standard well plates, standard cell biology
- Thousands of samples per day

## Rapid Protocol Optimization

- **Non-electrogenic** K<sup>+</sup> transporter
- Uptake with Rb<sup>+</sup> surrogate substrate
- Measure of cell pellets
- Optimizations:
  - Rb<sup>+</sup> concentration
  - Uptake time
  - Minimize sample handling
- Z' = 0.7 to 0.8



## IC<sub>50</sub> Values

|             | IC <sub>50</sub> (μM) |                |
|-------------|-----------------------|----------------|
|             | XRpro®                | Customer 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             |

## Experiment

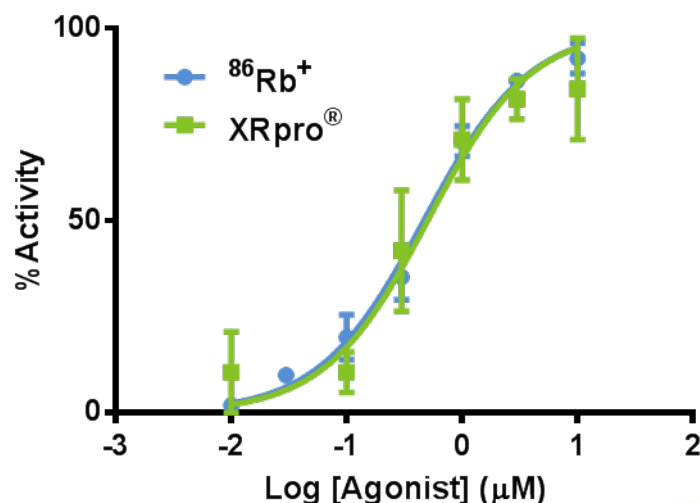
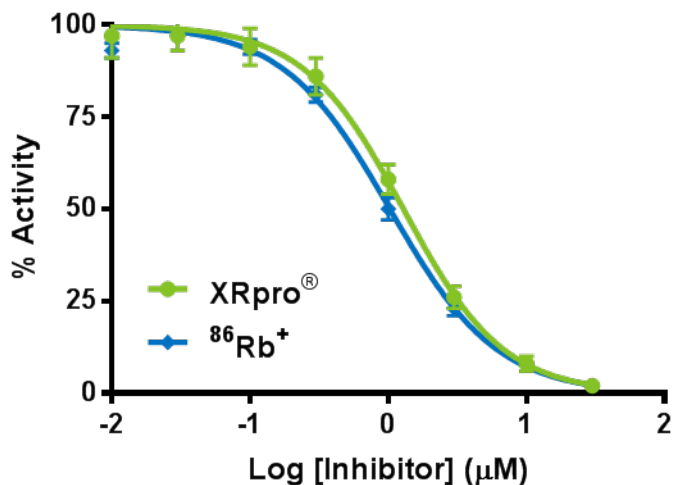
- Voltage gated K<sup>+</sup> channel
- Efflux with Rb<sup>+</sup> surrogate substrate
- **XRpro<sup>®</sup> uses cold Rb<sup>+</sup>**
- Measuring cell pellet and efflux buffer

## Comparison

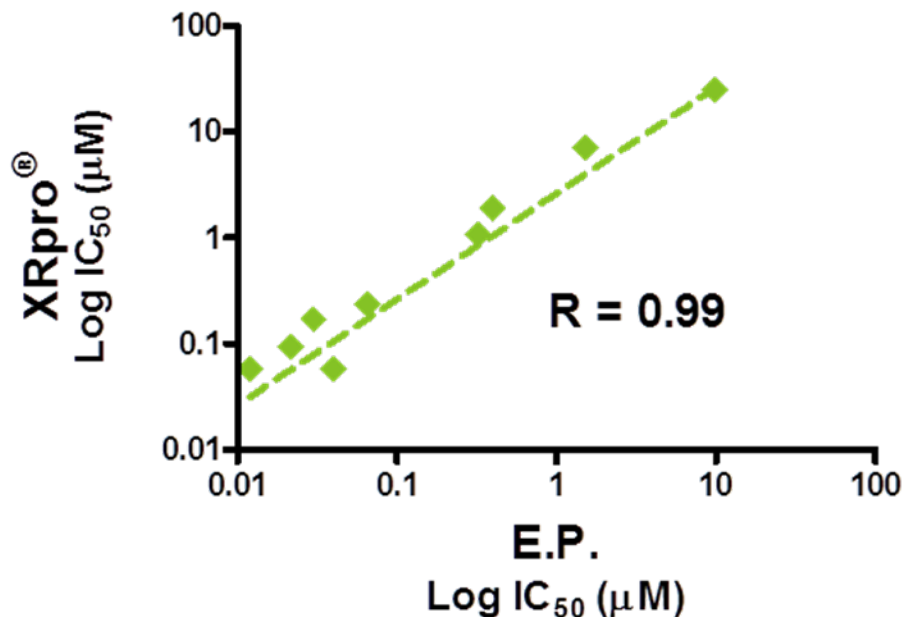
|             | XRpro <sup>®</sup>                       | <sup>86</sup> Rb <sup>+</sup>            |
|-------------|------------------------------------------|------------------------------------------|
|             | IC <sub>50</sub> / EC <sub>50</sub> (μM) | IC <sub>50</sub> / EC <sub>50</sub> (μM) |
| Inhibitor 1 | 1.33 ± 0.03                              | 1.02 ± 0.02                              |
| Agonist 1   | 0.11 ± 0.03                              | 0.33 ± 0.03                              |
| Agonist 2   | 0.29 ± 0.08                              | 0.21 ± 0.02                              |

## Results

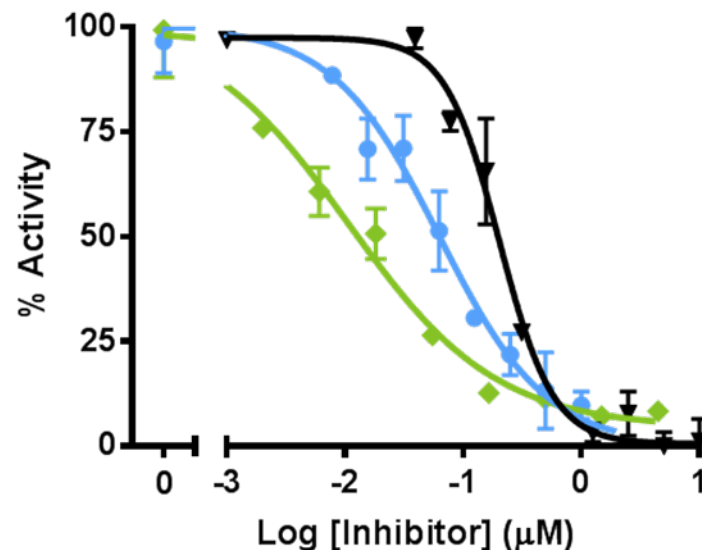
- Z' = 0.7 to 0.85
- XRpro<sup>®</sup> results match <sup>86</sup>Rb<sup>+</sup> analysis



XRpro<sup>®</sup> hERG IC<sub>50</sub> Correlation



hERG Inhibition



|                |                  |
|----------------|------------------|
| ◆ E-4031:      | 0.011 ± 0.002 μM |
| ● Dofetilide:  | 0.06 ± 0.01 μM   |
| ▼ Haloperidol: | 0.20 ± 0.02 μM   |

## Conclusions

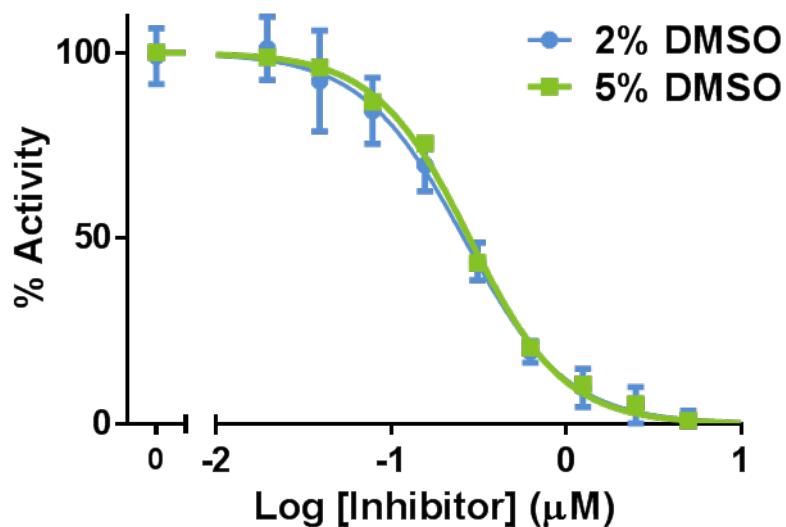
- Good correlation with established values.
- Hundreds of samples per hour.
- $Z' \geq 0.7$

## Experiment

- K<sup>+</sup> transporter
- Uptake with Rb<sup>+</sup> surrogate substrate
- IC<sub>50</sub> with varying [DMSO]

## Comparison

| [DMSO] | IC <sub>50</sub> |
|--------|------------------|
| 2%     | 0.26 ± 0.03      |
| 5%     | 0.11 ± 0.03      |



## Results

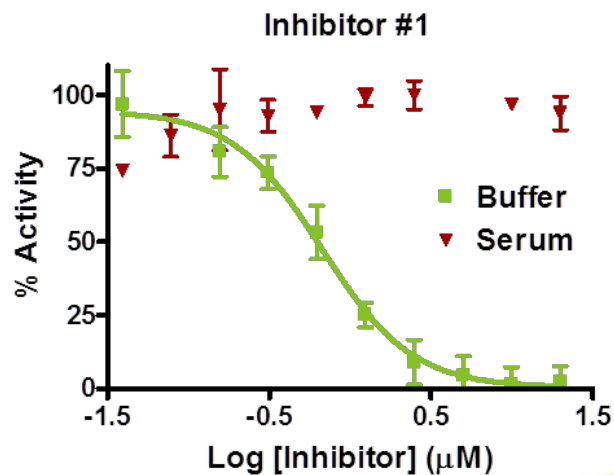
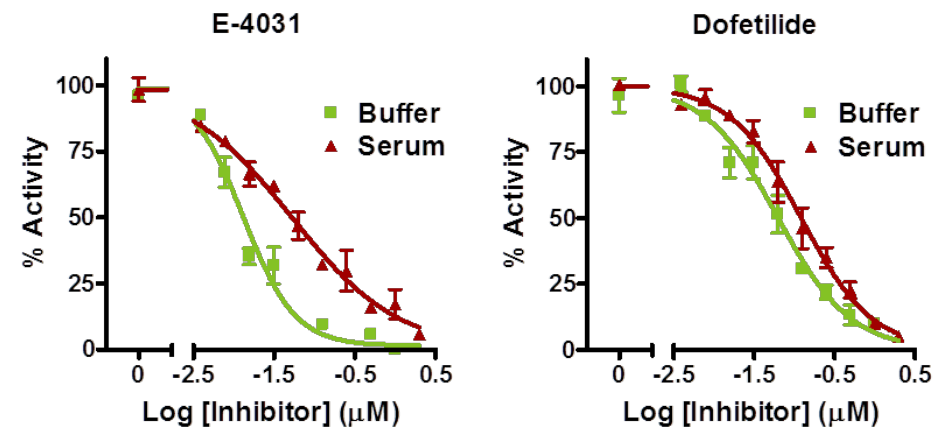
- Assay supports high DMSO
- XRpro<sup>®</sup> is only limited by cell tolerance

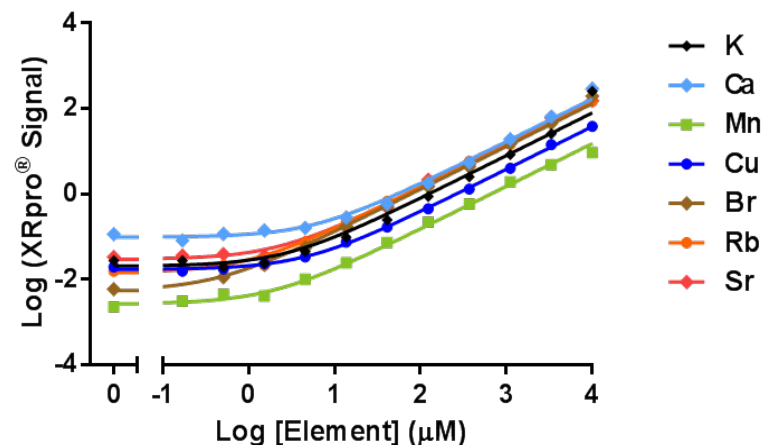
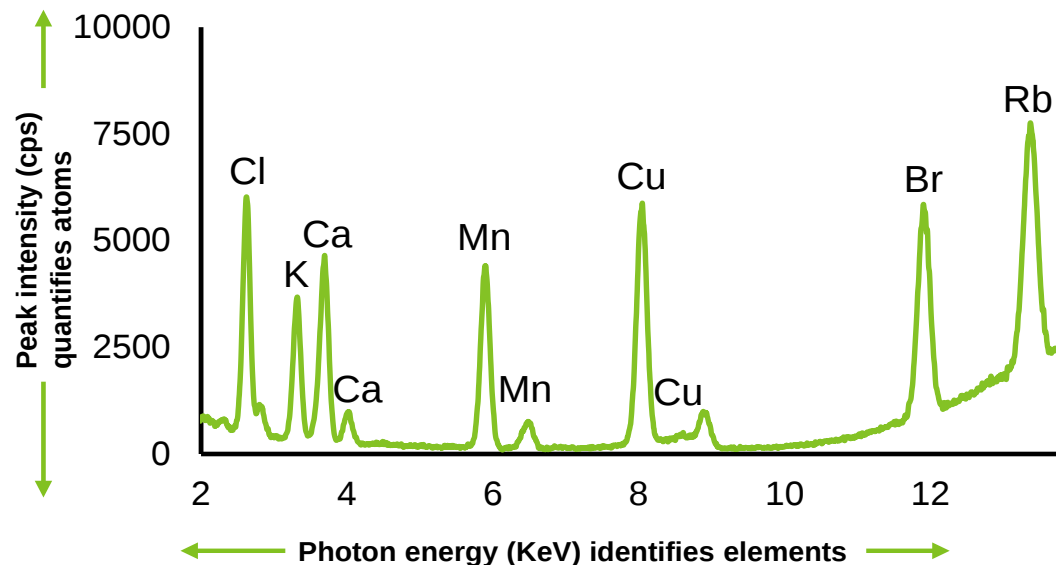
## hERG in 100% Serum

|            | Buffer                | 100% Serum            |
|------------|-----------------------|-----------------------|
|            | IC <sub>50</sub> (μM) | IC <sub>50</sub> (μM) |
| E-4031     | 0.014 ± 0.004         | 0.05 ± 0.01           |
| Dofetilide | 0.06 ± 0.01           | 0.12 ± 0.02           |

## K<sup>+</sup> Transporter in 100% Serum

|              | Buffer                | 100% Serum            |
|--------------|-----------------------|-----------------------|
|              | IC <sub>50</sub> (μM) | IC <sub>50</sub> (μM) |
| Inhibitor #1 | 0.5 ± 0.2             | '--                   |





## Method Detection Limits

| Element | $\mu\text{M}$  | pmol         |
|---------|----------------|--------------|
| K       | $1 \pm 0.4$    | $50 \pm 20$  |
| Ca      | $2 \pm 1$      | $100 \pm 40$ |
| Mn      | $0.7 \pm 0.2$  | $35 \pm 10$  |
| Cu      | $0.7 \pm 0.2$  | $30 \pm 10$  |
| Br      | $0.2 \pm 0.06$ | $9 \pm 3$    |
| Rb      | $0.4 \pm 0.1$  | $20 \pm 6$   |

## A Novel Technology Yielding New Insights

- HTS of ion channels and transporters
- Results match established values
- High Z' for HTS
- Fits current workflows
- Previously difficult or impossible measurements are now accessible, e.g.:
  - Assays of nonelectrogenic symporters
  - Quantify multiple ions simultaneously
  - In complex backgrounds: serum, buffers, DMSO, or opaque fluids

## In Your Lab or Ours...

- Fully compatible with lab automation
- Works with your cells, targets, and under your conditions

## XRpro<sup>®</sup> Science Staff

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## XRpro<sup>®</sup> Executive Team

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