Evaluation of a Novel Biogenic Amine Transporter Assay.

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A Novel Transporter Assay

- **Introduction**
  - Neurotransmitter transporters
  - Assays
- **Molecular Devices' Fluorescent Assay**
  - Overview
  - Validation
  - Summary

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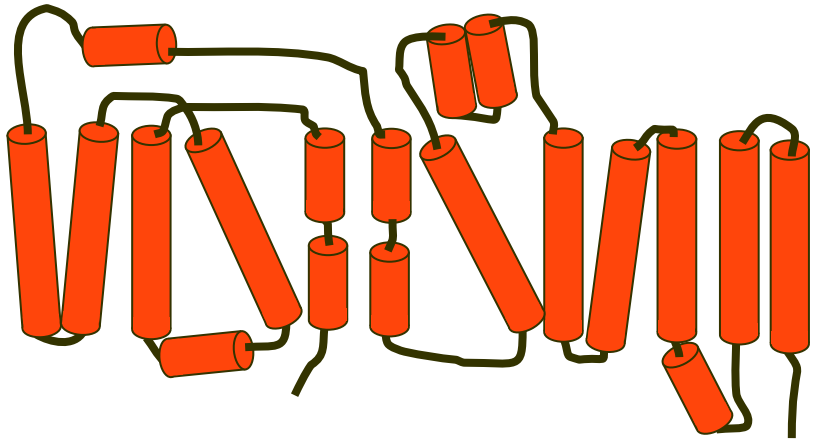
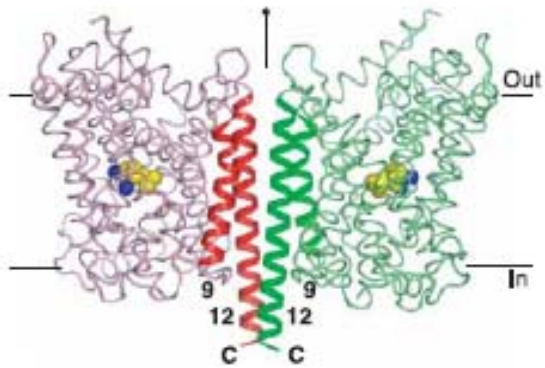
Neurotransmitter Transporters

- Transporters
  - Vesicular
  - Plasma Membrane
- SLC6 family
  - Na^+ Cl^- coupled transporters
  - Dopamine, 5HT, norepinephrine, glycine, GABA
  - Reduce synaptic concentrations of neurotransmitters
  - Terminate Signaling
  - Important targets for pharmaceuticals and drugs of abuse





Taken from Yamashita et al (2005) Nature 437 215-233

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Pharmacology of Biogenic Amine Transporters

Transporter	Endogenous Substrate(s)	Synthetic Substrates	Potent Inhibitors	Therapeutic Use/Potential
DAT	DA, NE,EPI	Amphetamines MPP+	Win35428, mazindol, nomifensine, cocaine	ADHD, Depression, PD, Tourettes, Schizophrenia
NET	DA, NE,EPI	Amphetamines MPP+	nisoxetine, nortripyline, desipramine, duloxetine	Depression, Pain
SERT	5HT	MDMA	sertraline, fluoxetine, duloxetine citalopram, imipramine,	Depression, Anxiety, OCD, Panic, Pain

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Assay Requirements

- High-throughput
- Sensitive
- Reproducible
- Homogeneous
- Non-radioactive
- Solvent compatible
- Compatible with multiple targets

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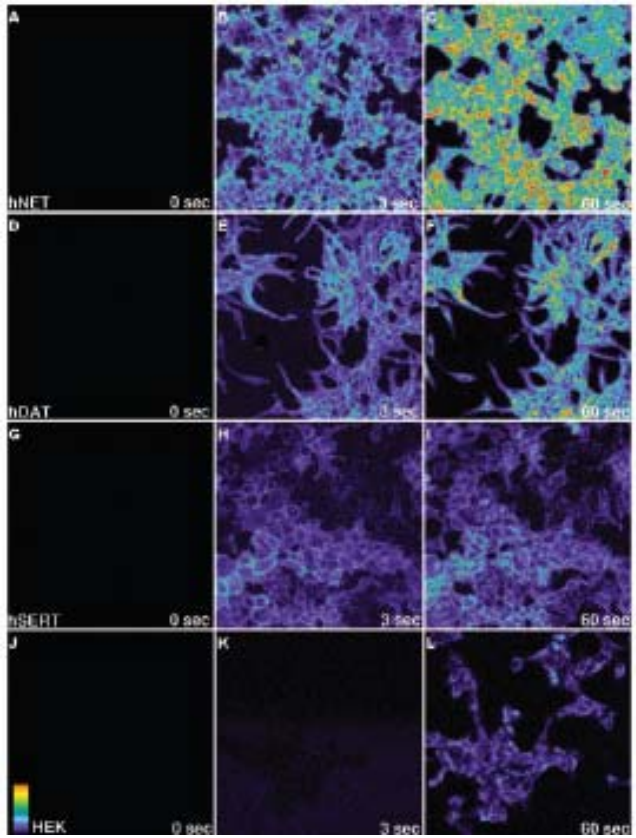
ASP+ is a Fluorescent substrate

- ASP+, fluorescent substrate for DAT and NET (1-3μM K_M values) Schwartz et al. 2003, Mason et al 2005
  - K_M for SERT 10-20μM Fowler et al. 2006
  - Trypan blue added to quench external fluorescence
- Valuable for transporters studies
  - Transfected cell lines
  - Synaptosomes
  - Primary cultures
- Compatible with different platforms
  - Fluorescent screening assays
  - High content assays
  - Confocal microscopy



Taken from Schwartz et al. 2003 JBC 278, 9768

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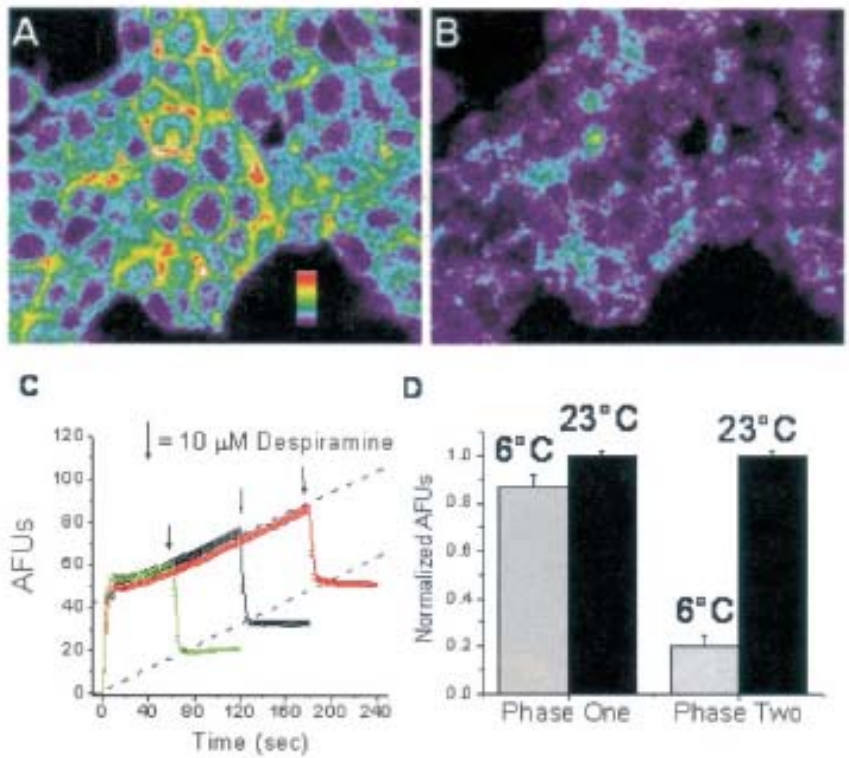
ASP+ Can Be Used For Mechanistic Studies

- ASP+ rapid on rate <1sec
  - Suitable for binding
- ASP+ Binding is Na^+ independent
  - Cell surface labeling
  - Temperature independent
  - Binding rate 100> transport rates



Taken from Schwartz et al. 2003 JBC 278, 9768

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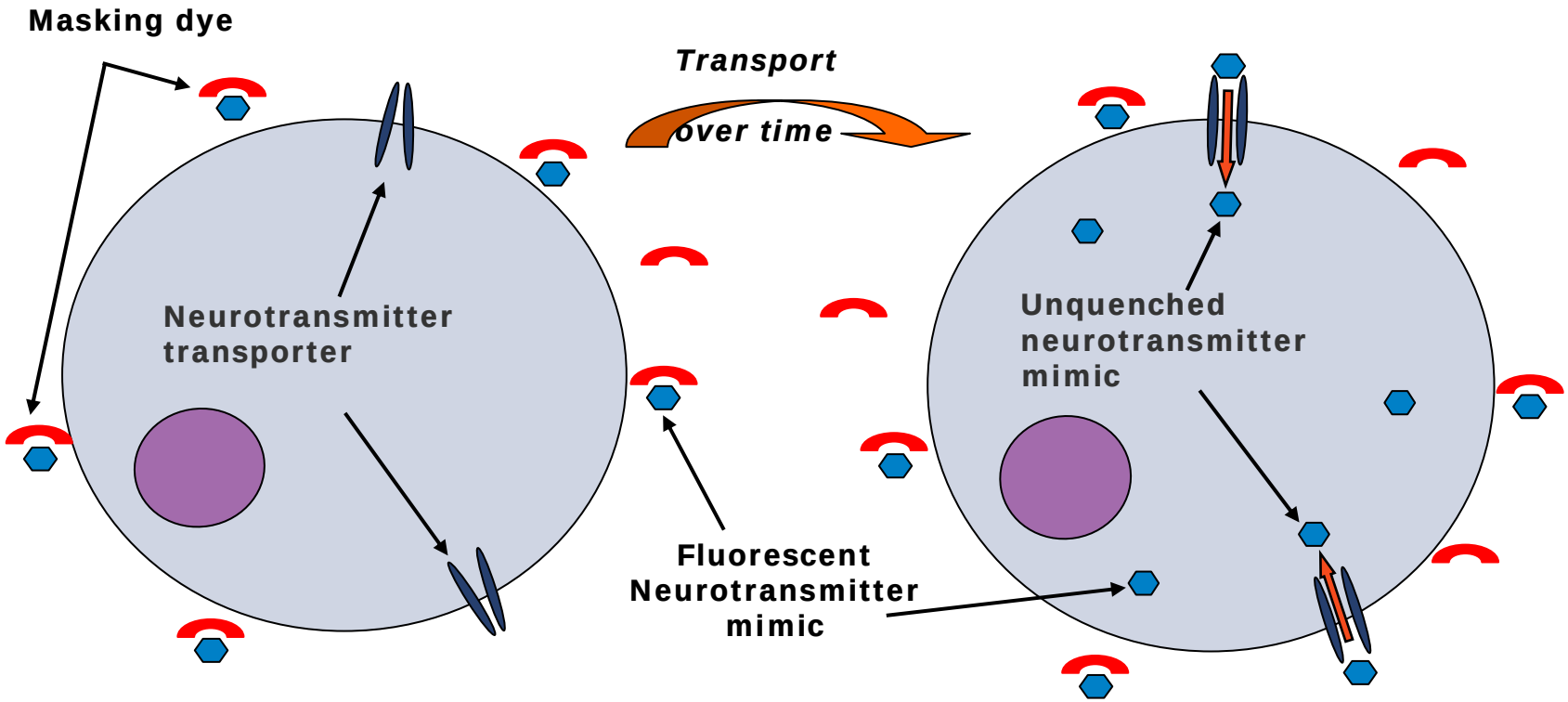
Neurotransmitter Transporter Uptake Assay Principle



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Fluorescent Assay is Simple

Plate cells 2 days before assay

↓ Replace growth media with assay buffer

+/- Test compound

↓ Pre-incubate

Add radioactive substrate

↓ Allow uptake reaction

↓ Quench w/inhibitor

Remove radiolabel & wash

↓ Dry plates

Add scintillation fluid

↓

Read

Plate cells 1day before assay

↓ Replace growth media with assay buffer

+/- Test compound

↓ Pre-incubate

+ Fluorescent substrate

↓

Read

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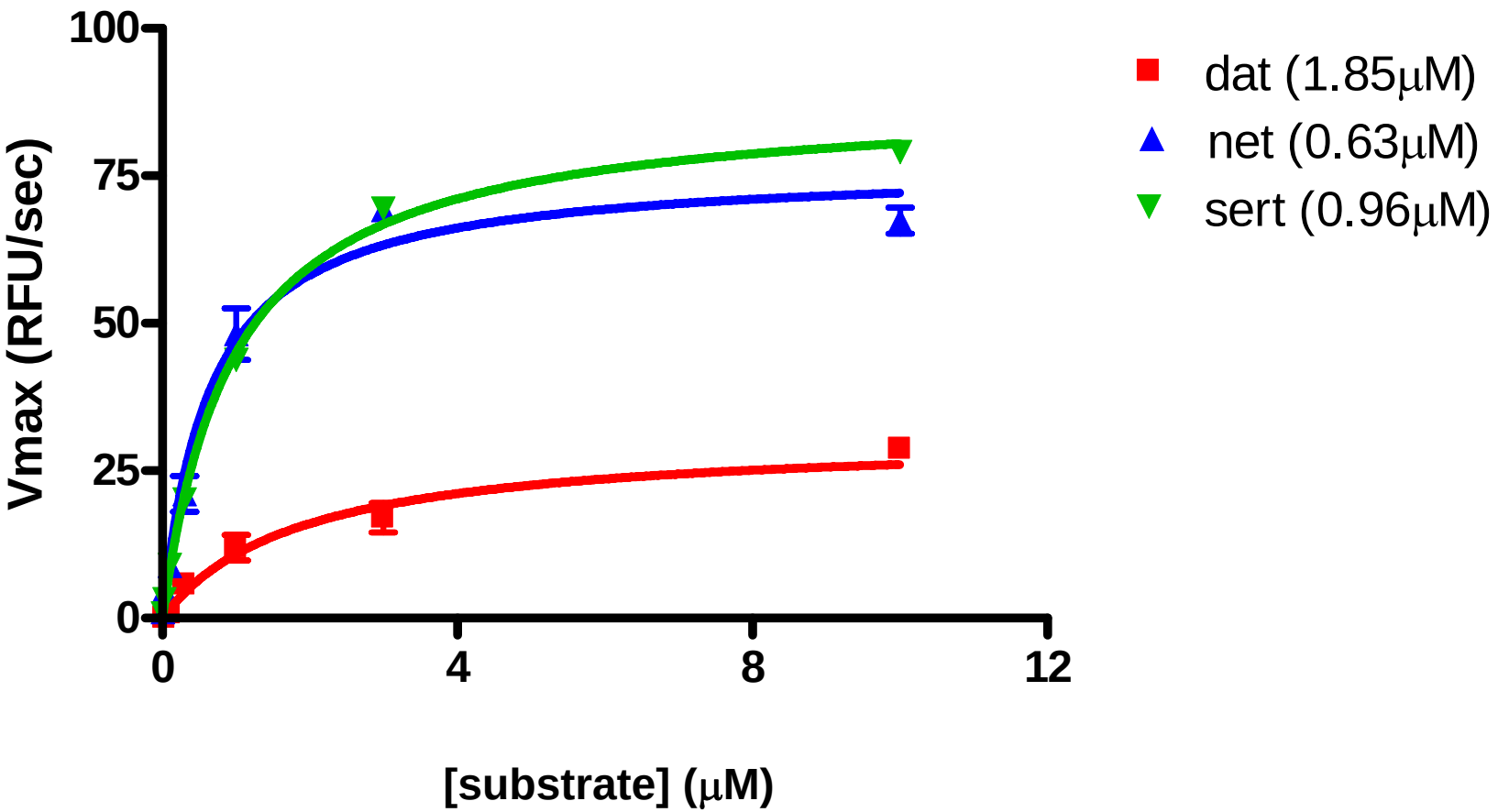
K_M is Similar for DAT, NET & SERT



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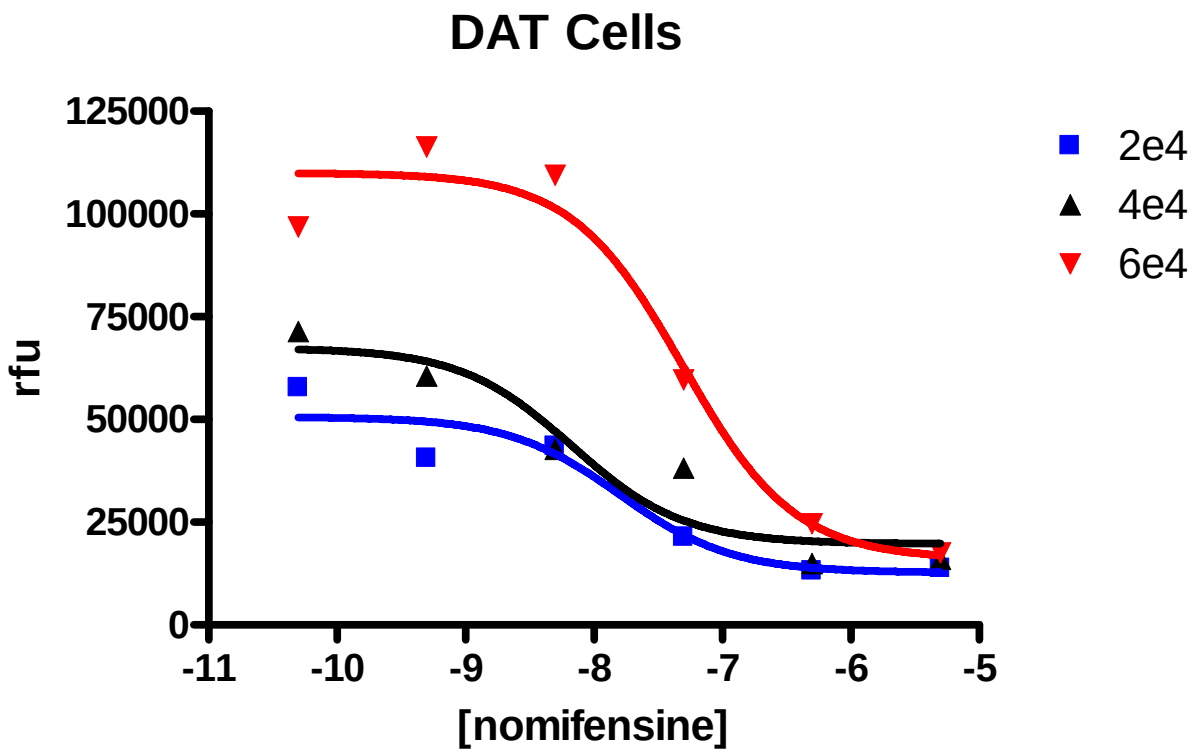
60,000 Cells/Well is Optimal (96 well)



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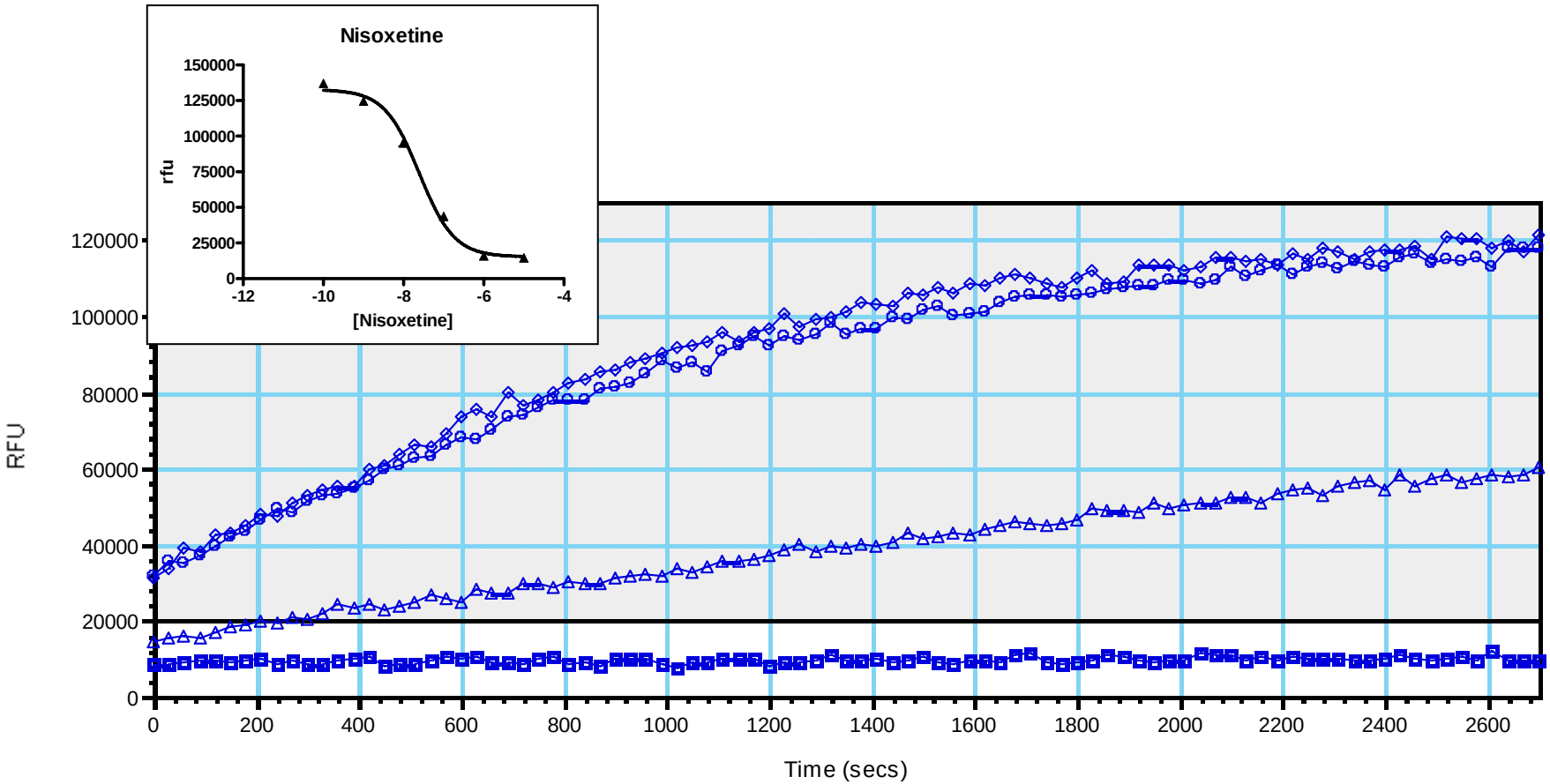
Representative Data



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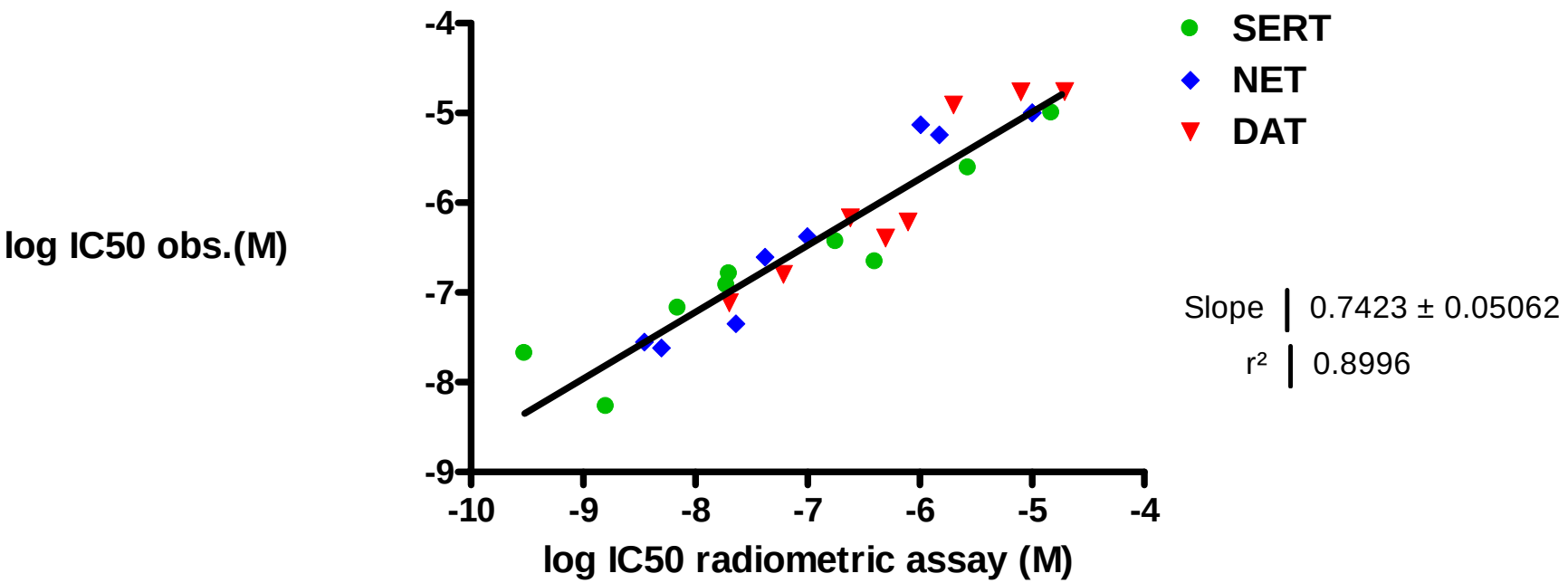
Assay Yields Expected IC50s



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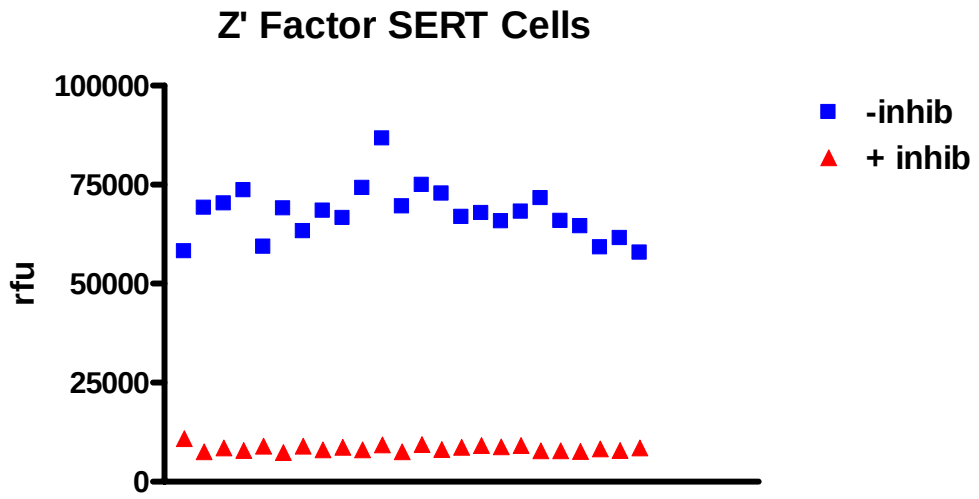
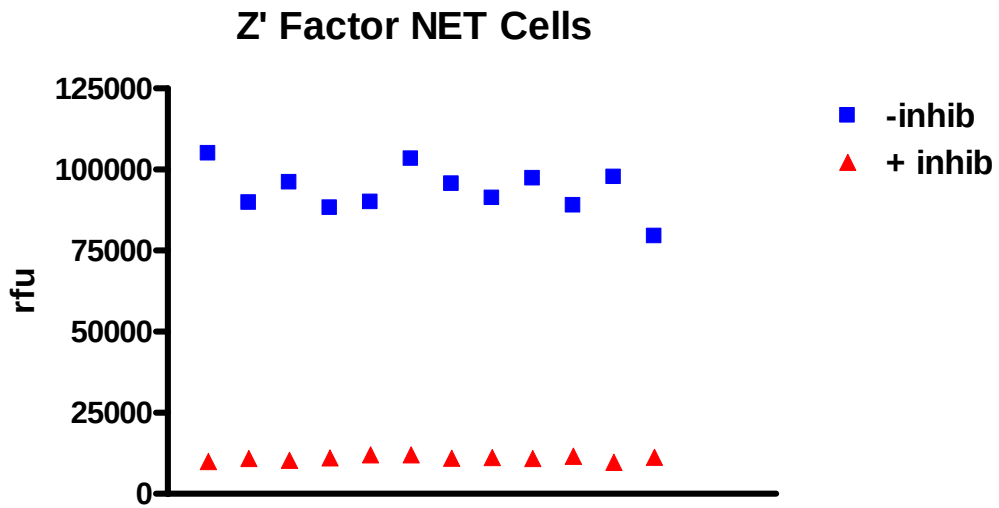
Assay is Reproducible

- **SERT**
  - Window 8.1 fold
  - Z’ factor = 0.64



- **NET**
  - Window 8.5 fold
  - Z’ factor = 0.72



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Comparison of Assays

Radioactive Assay		Fluorescent Assay
☑	Uses natural substrate	☐
☐	High-throughput	☑
☑	Sensitive	☑
☑	Reproducible	☑
☐	Homogeneous	☑
☐	Non-radioactive	☑
☑	Solvent compatible	☑
☐	Real-time analysis	☑
☐	Compatible w/ multiple targets	☑

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Summary

- Simple assay for all 3 transporters
- Homogeneous
- Sensitive (>8 fold window)
- Reproducible (Z' factors 0.64-0.72)
- IC_{50} values comparable to radioactive assay
- Amenable to high throughput
- Commercially Available!

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Acknowledgements

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