

# Data evaluation and interpretation – through case studies

**Krisztina Herédi-Szabó, PharmD, PhD**

Principal Scientist, ADME/Tox Services  
Study Manager

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# Good answers for good questions

- Assay type
- Assay setup
- Preliminary assays
- Special conditions
- **QUALITY DATA**



## Substrate testing

- Transporter uptake assays (for SLC and ABC transporters)
- Bidirectional permeability

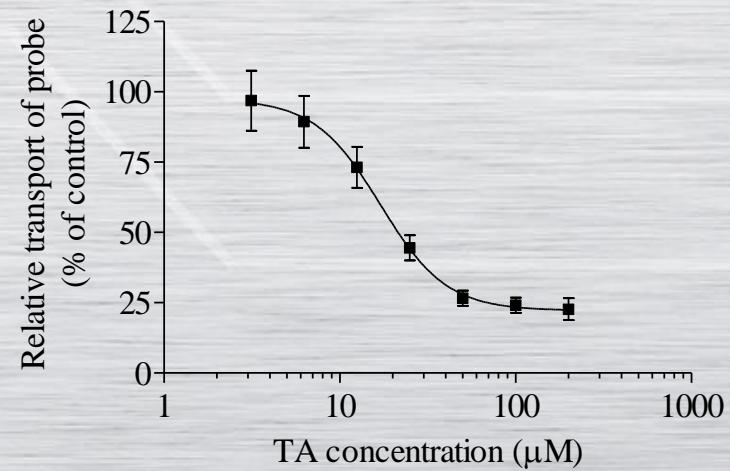
## Inhibition testing

- $IC_{50}$
- $K_i$

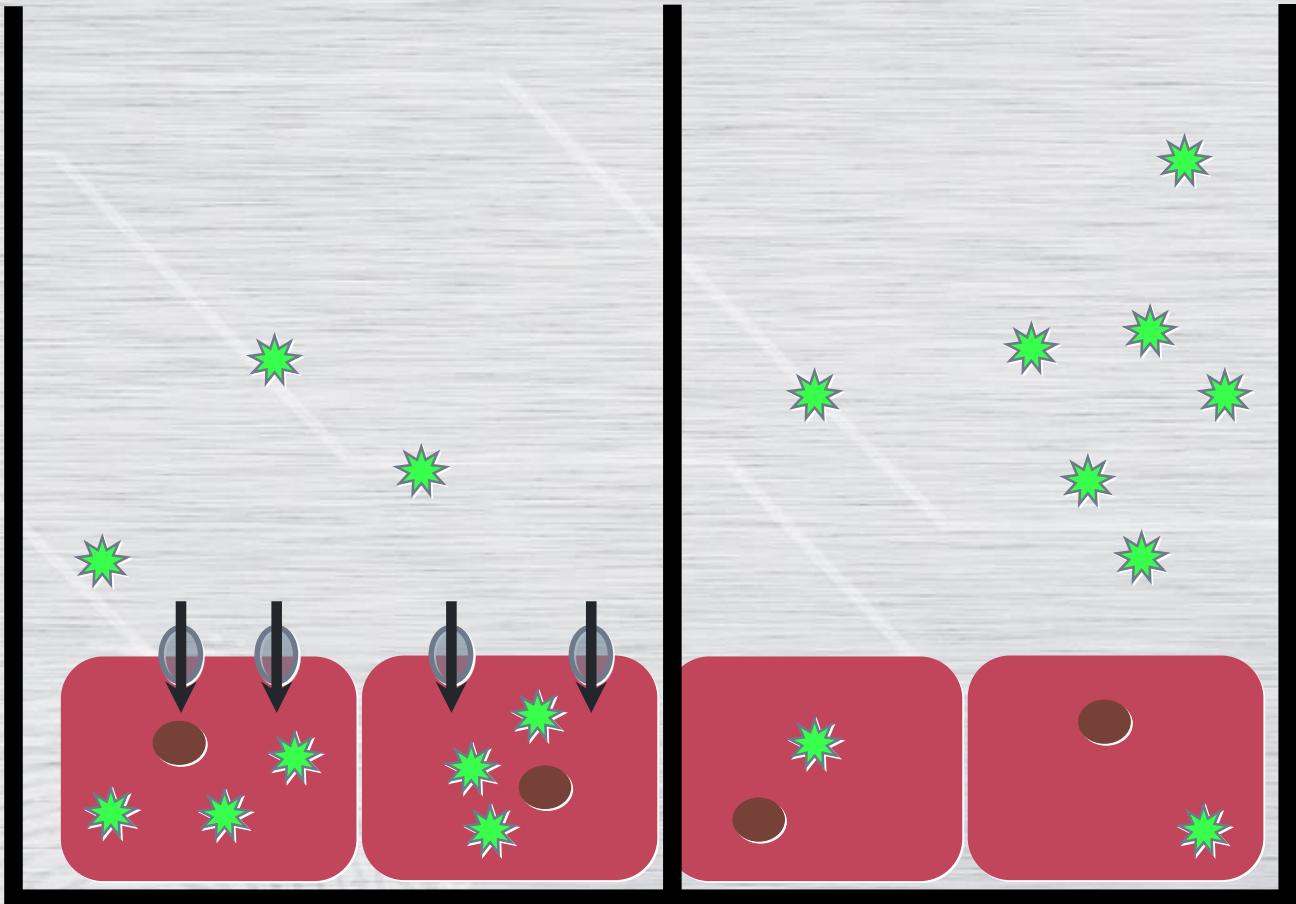
# Input of kinetic solubility

- Solubility assessment in assay buffers using microscopic evaluation
- Transporter assay performed in soluble concentration range w/ < 1% DMSO
- Precipitation influences obtained parameters

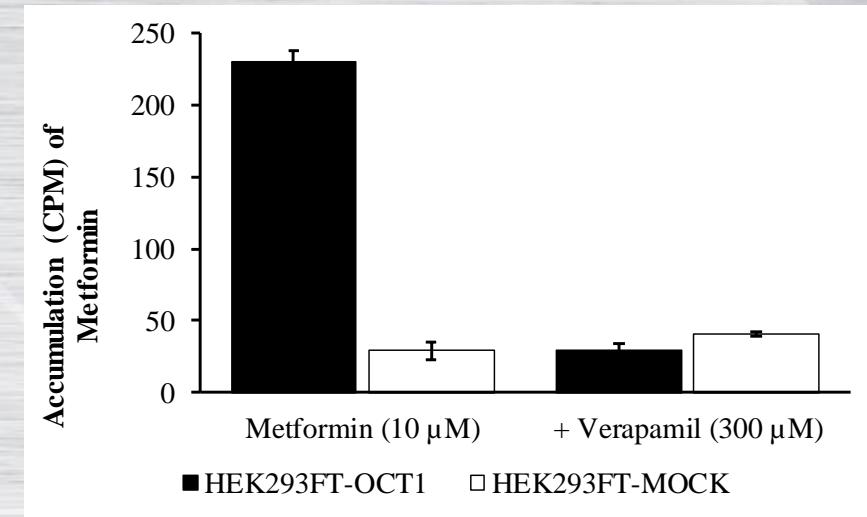
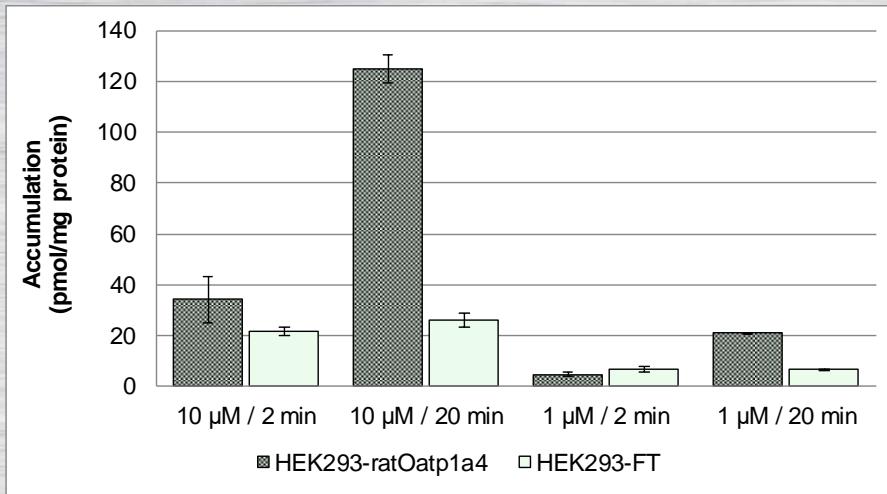
Concentration of TA ( $\mu$ M)	Accumulation (counts per minute; CPM)					
	with ATP			with AMP		
80.0	3019	3344	3003	1847	1315	1959
26.7	5049	5297	5344	1811	1819	1468
8.9	7154	6259	7595	1570	1569	1759
3.0	7876	8378	9911	1630	1616	1635
1.0	7904	7336	7455	1592	1685	1499
0.3	7707	7979	7702	1373	1468	1413
0.1	8887	9448	8854	1157	1106	1145
Vehicle <sup>a</sup>	8988	9783	9460	1318	1105	1004



# Uptake substrate assays

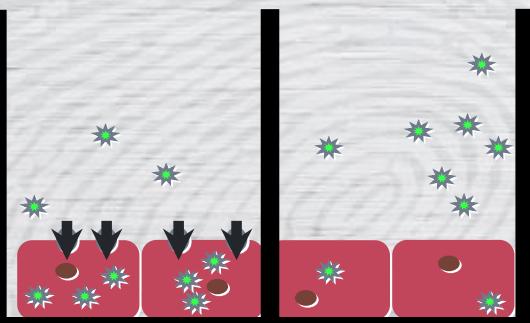


# Compound is a substrate

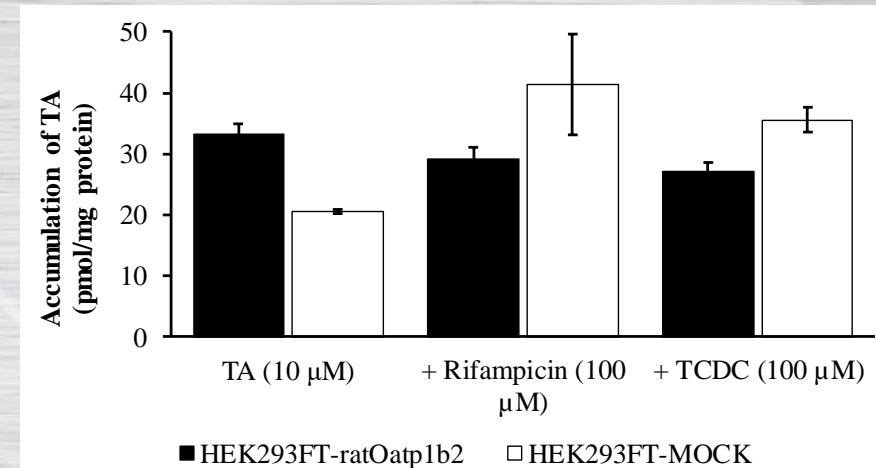
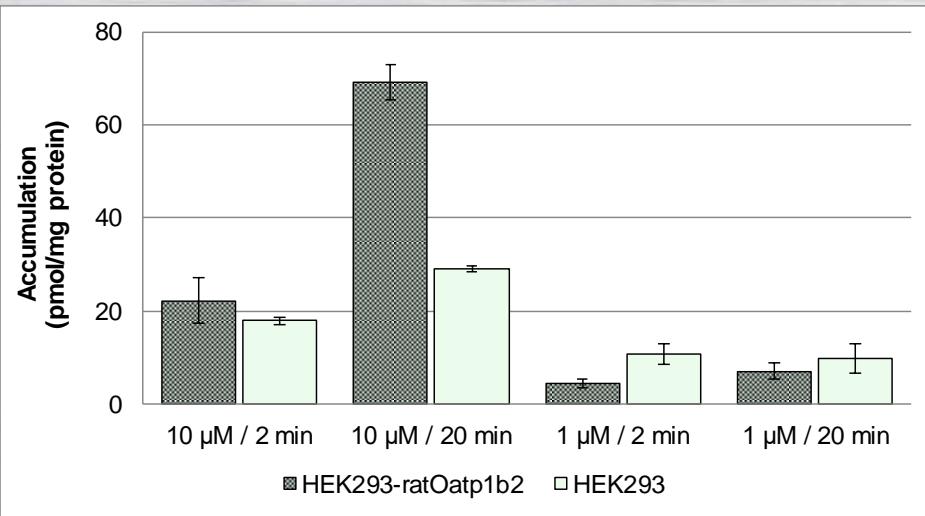


Transporter-specific accumulation > 2-fold  
(accumulation in tp cells / accumulation in ctrl cells)

Transport can be inhibited by ref inhibitor

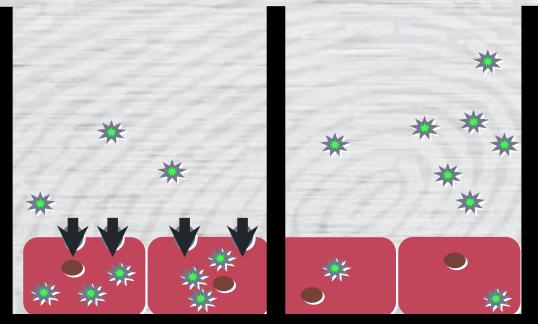


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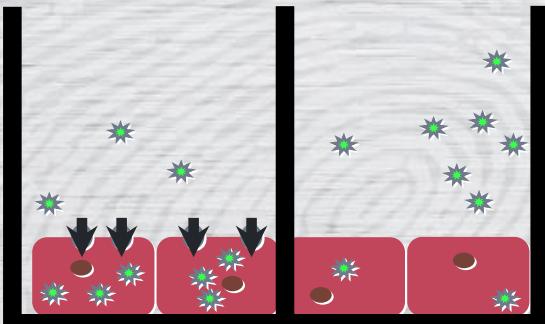
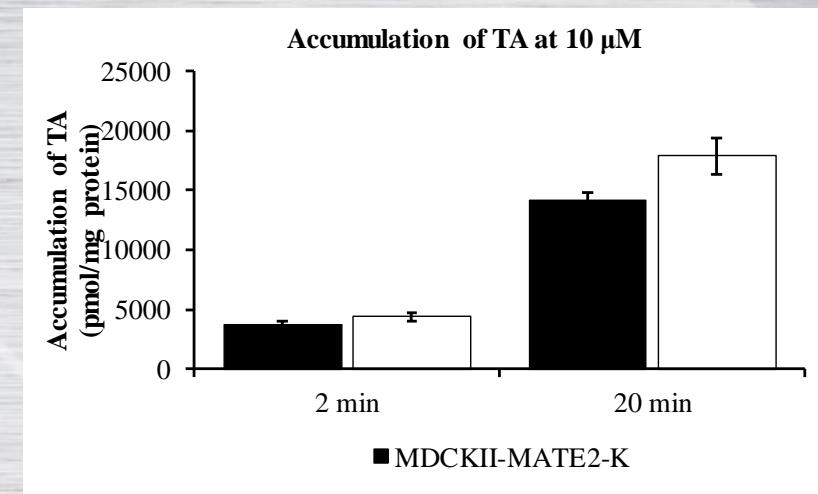
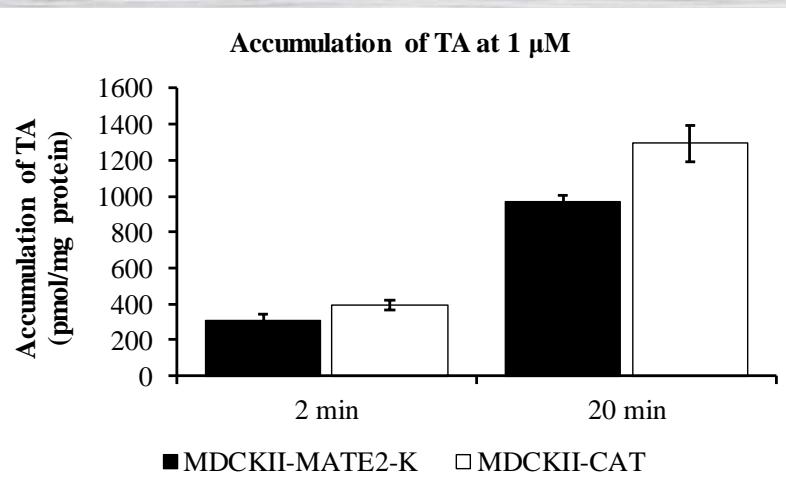


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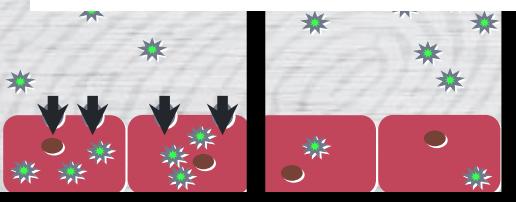
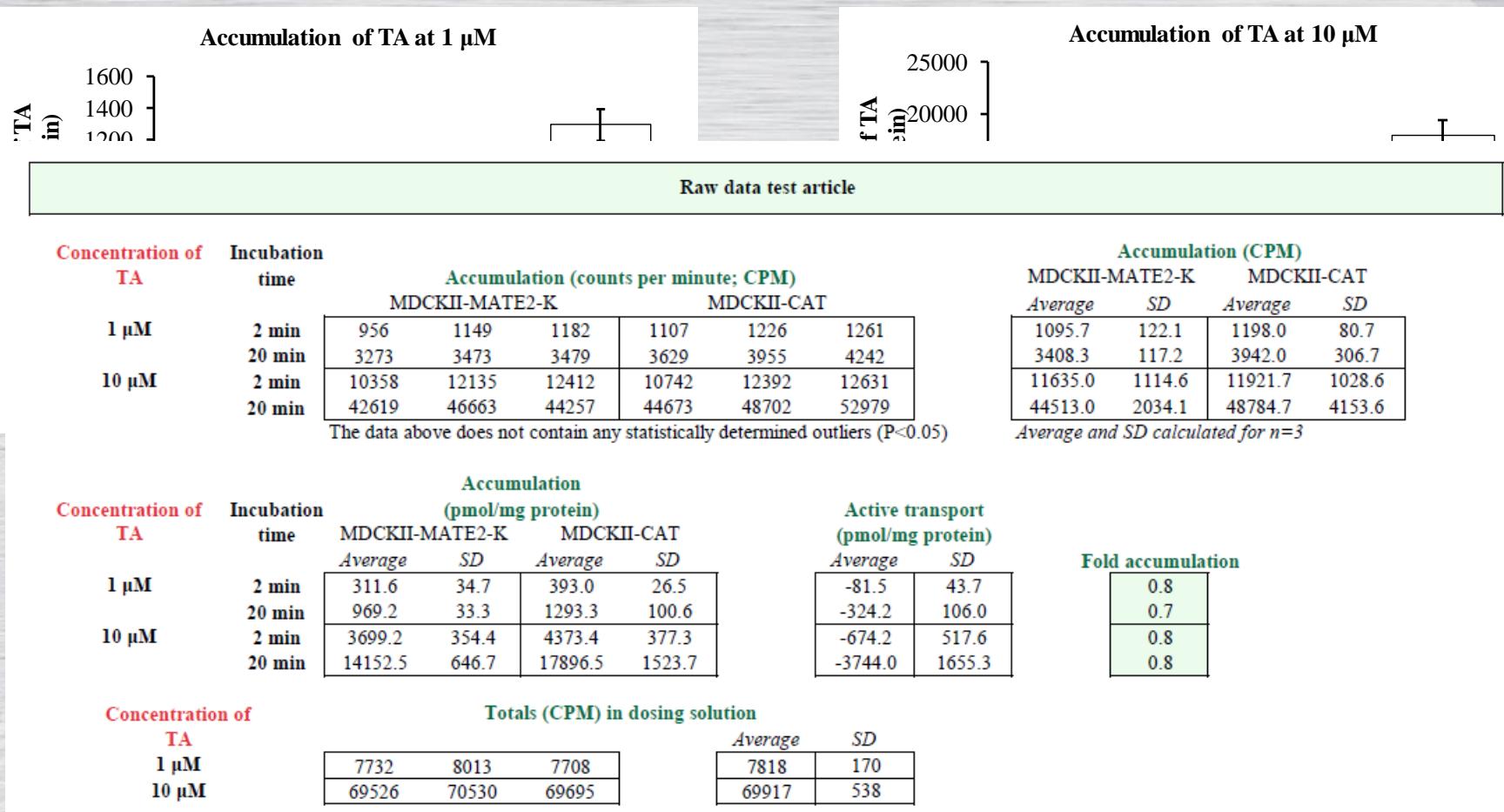
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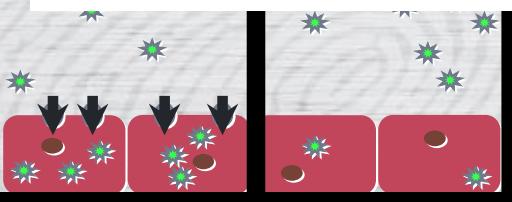
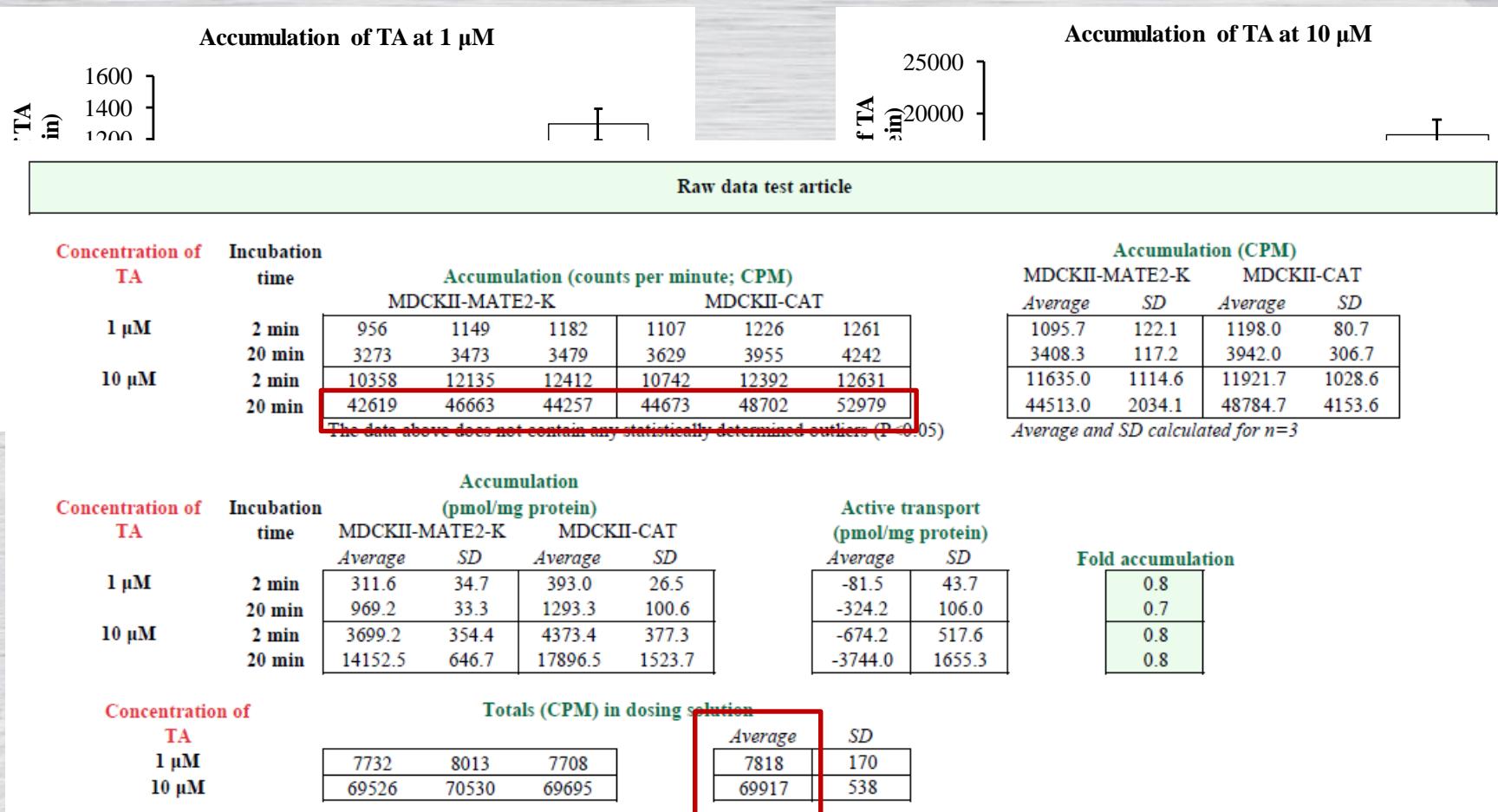
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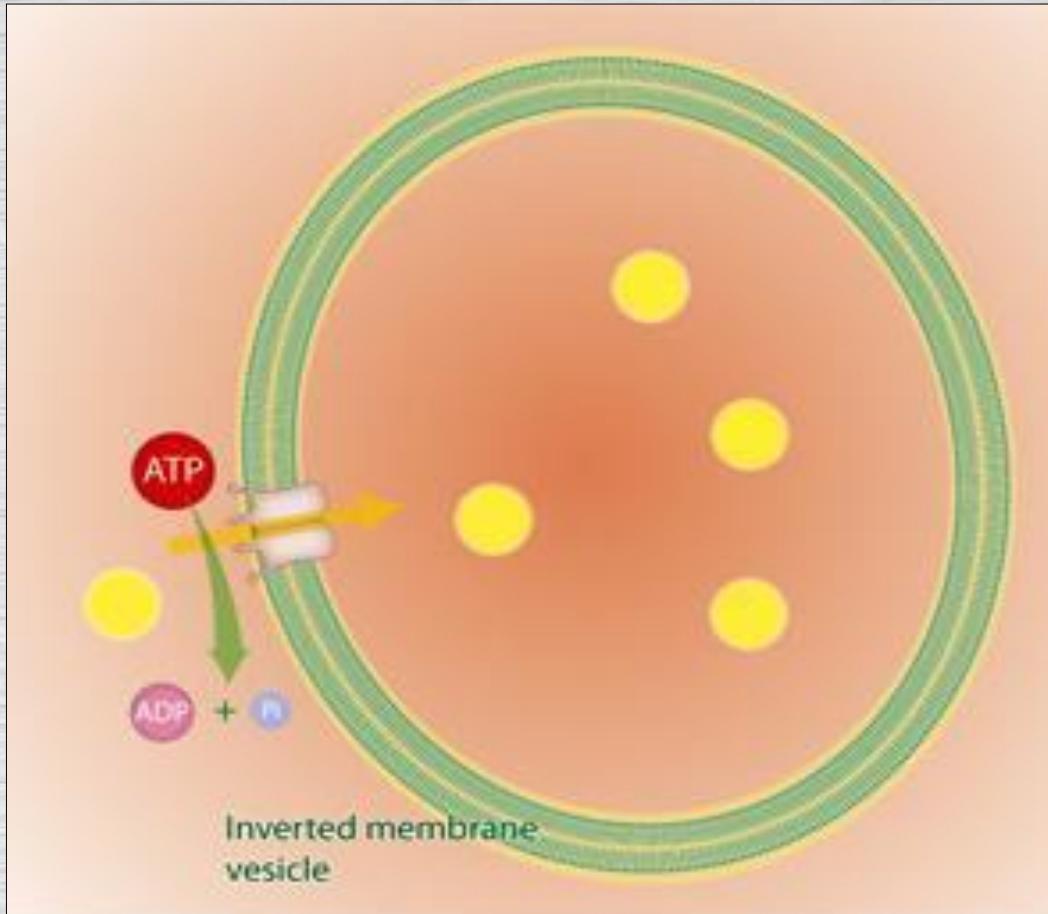
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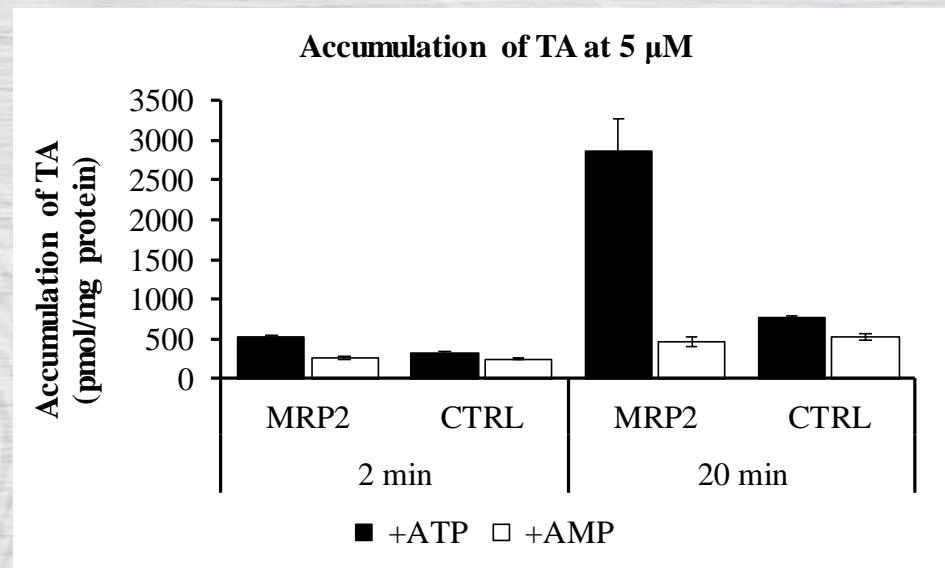
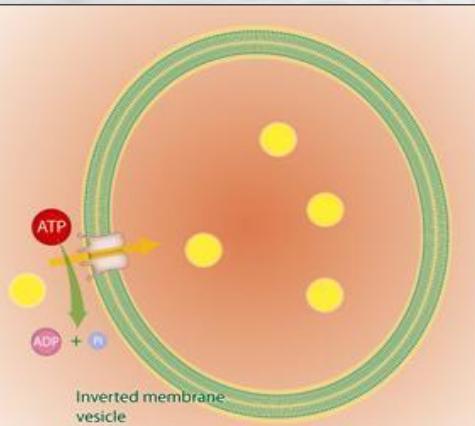
# Vesicular substrate assays



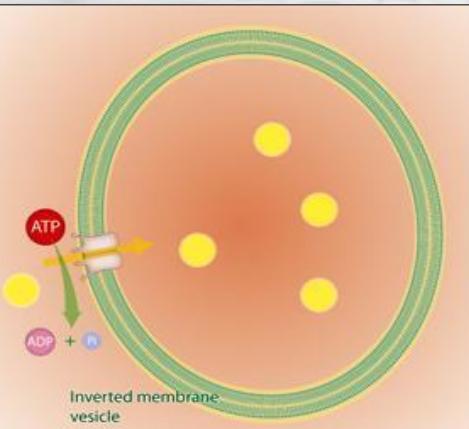
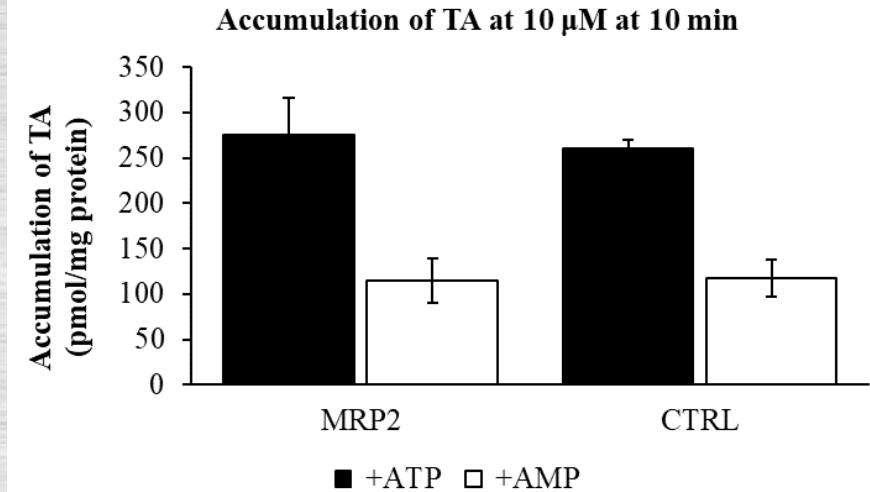
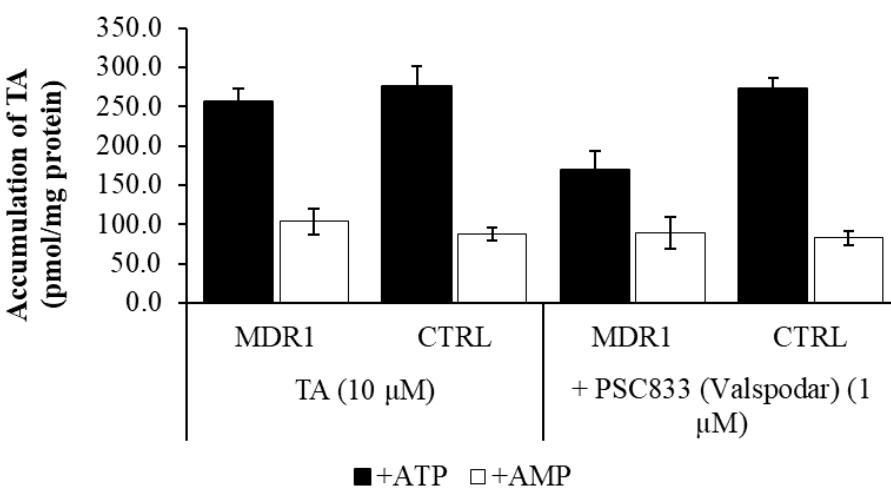
# Assay setup

**Table 1. Treatment groups for vesicular transport substrate assays**

Assay groups in the 96-well plate format	No. of samples
Part 1.1: TA at 2 concentrations with transporter expressing and control membrane vesicles, at 2 incubation times in triplicate + ATP or AMP, + Total count/C <sub>0</sub> in triplicate	2*2*2*3*2+6
Part 1.2 (optional): TA at 1 concentration, and 1 incubation time in triplicate, using transporter containing and control vesicles + ATP or AMP, +/- specific inhibitor, + Total count/C <sub>0</sub> in triplicate	1*1*3*2*2*2+3



# Compound is a substrate?



ATP-dependent accumulation > 2-fold (accumulation with ATP / accumulation with AMP)  
 Transporter-specific accumulation < 2-fold  
 (accumulation is comparable in transporter and CTRL vesicles)

Transport can be inhibited by ref inhibitor in both cases – endogenous transporter?

# Non-specific binding

- Estimates the loss of TA on plastic surfaces used
- Additional test included in 2017 FDA DDI guidance
  - Can be performed before (parameter optimization)
  - Or in parallel (assess actual concentrations) with the *in vitro* assay
  - Should be considered
    - If prior knowledge exists on the stickiness of the TA
    - If the physchem parameters suggest
  - Suspicious compounds:
    - High plasma protein binding
    - High cellular association
    - Hydrophobic compounds

# Sample protocol

Assay steps:

Stock solution in silanized glass inserts



Reaction tube ( $t_0$ )

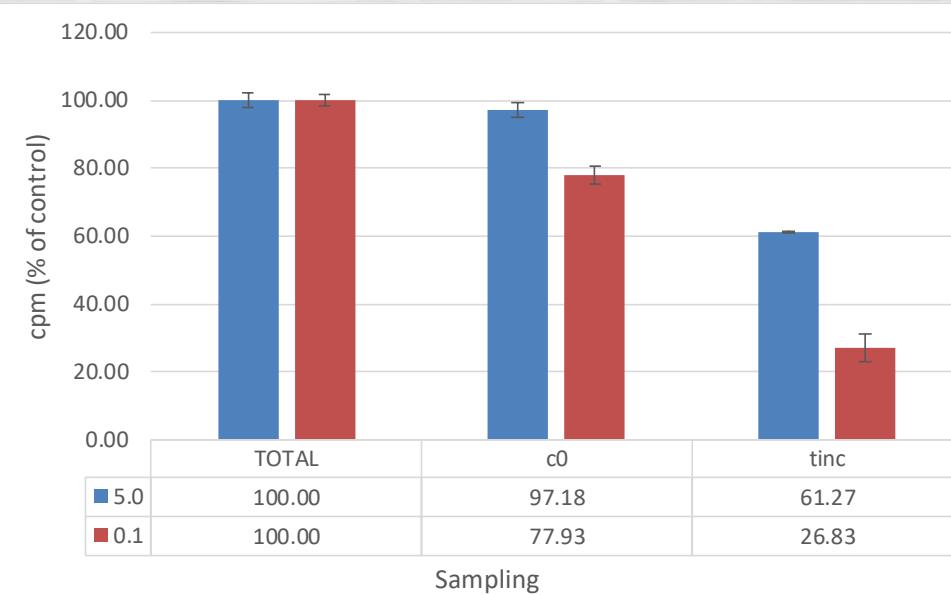
3.5  $\mu\text{L}$  aliquots added to 3500  $\mu\text{L}$  buffer, sampled after mixing ( $t_0$ )

Tissue culture plate ( $t_{\text{pre-inc}}$ )

300  $\mu\text{L}/\text{well}$  transferred after pre-incubation (10 min) and then sampled ( $t_{\text{pre-inc}}$ )

Tissue culture plate ( $t_{\text{inc}}$ )

300  $\mu\text{L}/\text{well}$  transferred after pre-incubation (10 min), further incubated for 20 min and then sampled ( $t_{\text{inc}}$ )

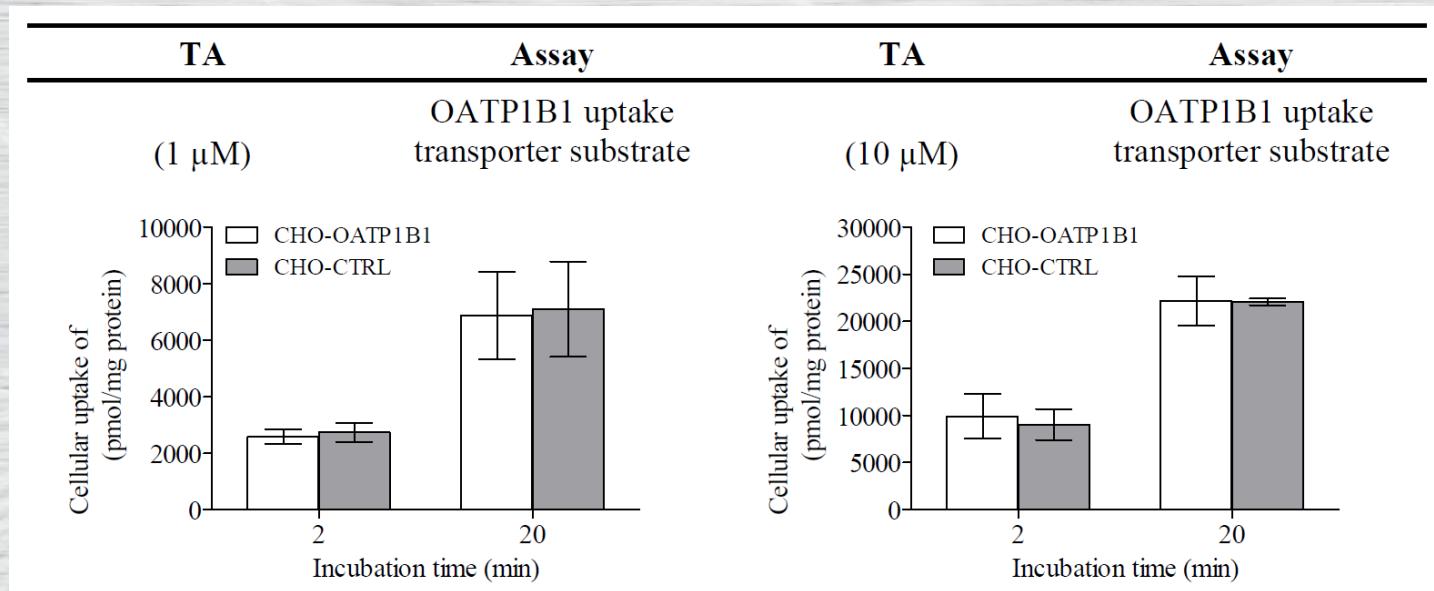


# How to reduce NSB?

- Optimize labware used
  - silanized glass tubes instead of PP tubes
  - skip plastic surfaces if possible (e.g. helper plate)
- Use BSA or serum in the buffer
  - Coating of NSB sites on plasticware
  - TA binds to protein rather than plastic
  - Only recommended for substrate assessment!

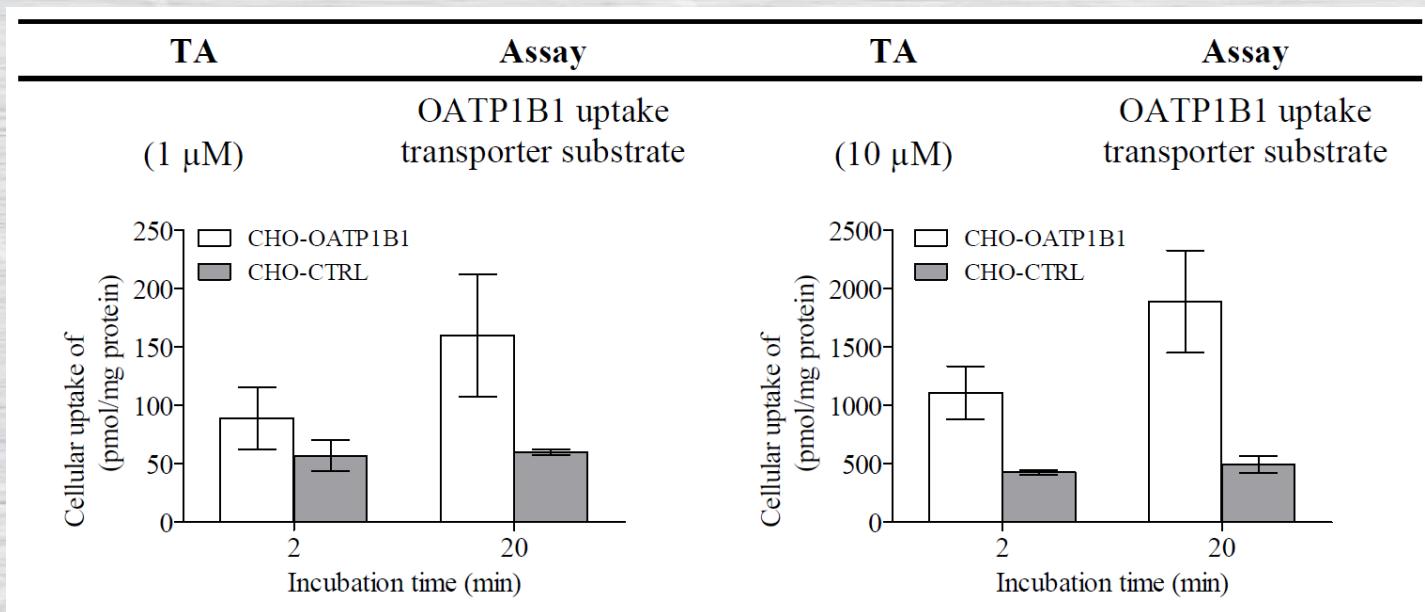
# NSB case study

- Cpd X
  - High solubility (>100 mg/ml), MW ~ 600 g/mol, high PPB (99.9%)



# NSB case study

- Assay was repeated with 10% serum

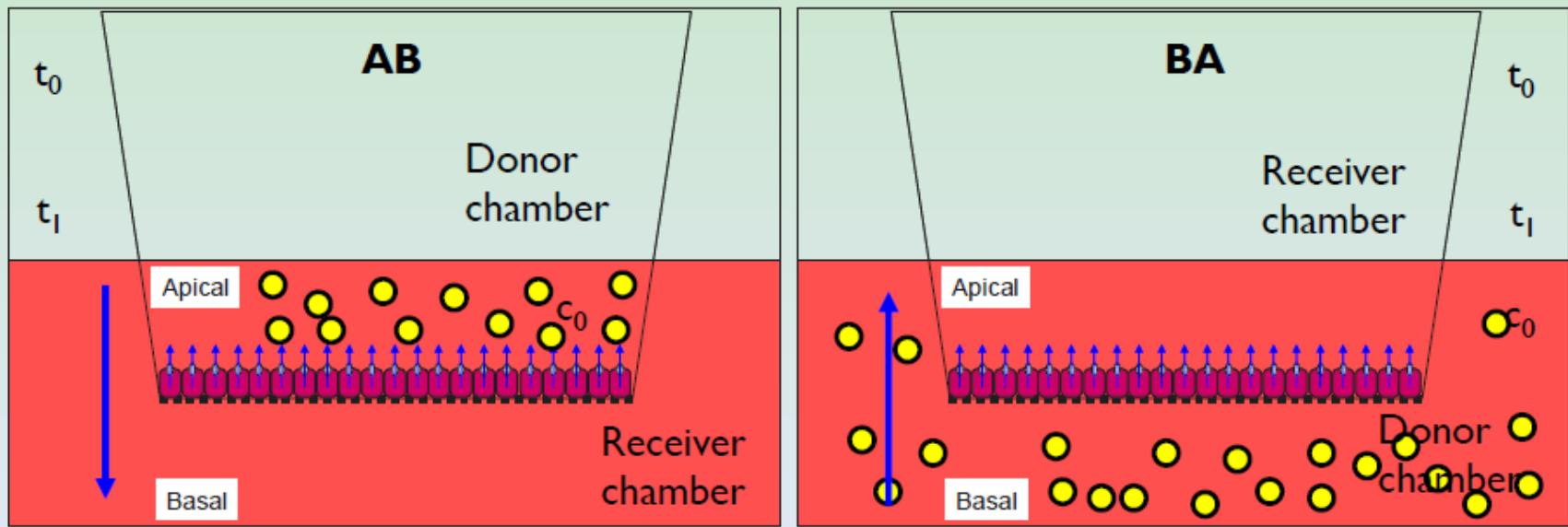


# NSB case study

- Assay was repeated with 10% serum

Test article	Assay	Fold accumulation	Conditions ( $\mu$ M / min)
OATP1B1 substrate		1.56	1 / 2
		2.67	1 / 20
		2.60	10 / 2
		3.84	10 / 20
+ cerivastatin	OATP1B1 substrate inhibition	2.41	1 / 20
		1.22	1 / 20
		1.30	1 / 20
E3S	OATP1B1 inhibition	3.47	0.1 / 10
E3S + cerivastatin	OATP1B1 inhibition	0.71	0.1 / 10

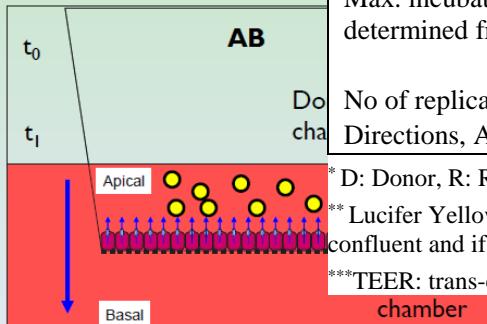
# Bidirectional permeability assay



# Bidirectional permeability assay – study design

**Table 1.** The assay conditions, controls and deliverables

Bidirectional permeability of TA in MDCKII and MDCKII-MDR1 cells		
Assay Conditions	Controls	Deliverables
<p>Three concentrations of TA (1, 10 and 100 µM);</p> <p>Sampling protocol (minutes):</p> <ul style="list-style-type: none"> <li>- <math>t_0</math>: D*</li> <li>- <math>t_{15}</math>: R</li> <li>- <math>t_{30}</math>: R</li> <li>- <math>t_{60}</math>: R</li> <li>- <math>t_{120}</math>: D and R</li> </ul> <p>No. of replicates: 3</p> <p>Directions, A-B and B-A‡</p>	<p>High permeability controls, A-B:</p> <ul style="list-style-type: none"> <li>- Antipyrine</li> </ul> <p>Low permeability control, A-B**:</p> <ul style="list-style-type: none"> <li>- Lucifer Yellow</li> <li>- Lucifer Yellow+ TA (100 µM)</li> </ul> <p>Functionality controls, A-B and B-A:</p> <ul style="list-style-type: none"> <li>- Digoxin</li> <li>- Digoxin + PSC833</li> </ul> <p>Monolayer confluence control:</p> <ul style="list-style-type: none"> <li>- TEER***</li> </ul>	<p>Recovery of the TA (%)</p> <p><math>P_{app(A-B)}</math>, (<math>10^{-6}</math> cm/sec)</p> <p><math>P_{app(B-A)}</math>, (<math>10^{-6}</math> cm/sec)</p> <p>TA ER, <math>\frac{P_{app(B-A)}}{P_{app(A-B)}}</math></p> <p>netER = <math>ER_T - ER_P</math></p>
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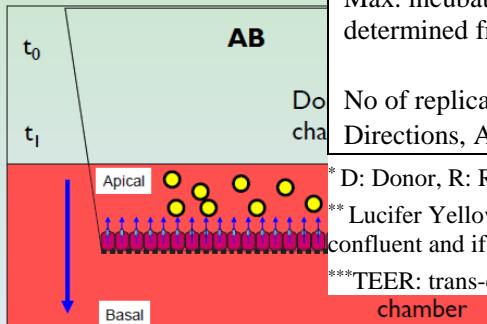
\*\* Lucifer Yellow (LY) control (A-B) will be incubated with and without the TA to determine whether the monolayer is confluent and if the TA can cause any disruption to the integrity of the monolayer at the highest concentration investigated.

\*\*\* TEER: trans-epithelial electrical resistance

# Bidirectional permeability assay – study design

**Table 1.** The assay conditions, controls and deliverables

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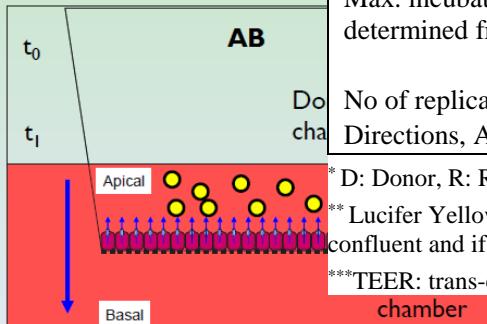
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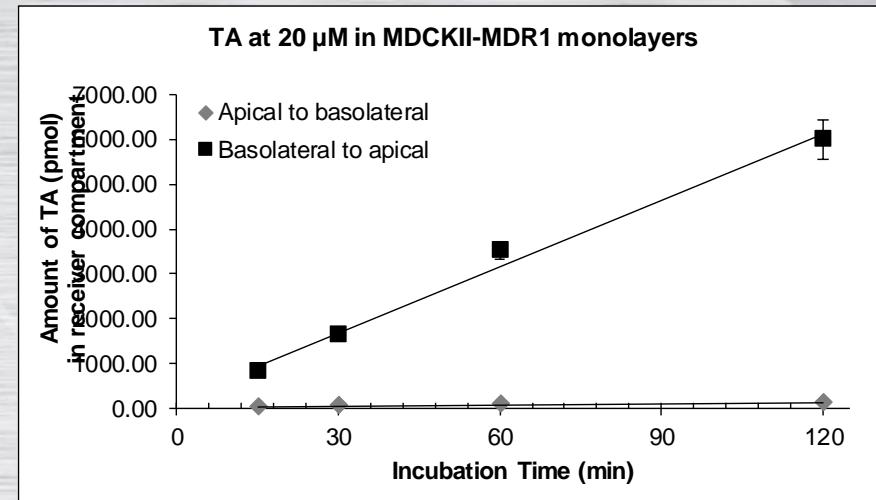
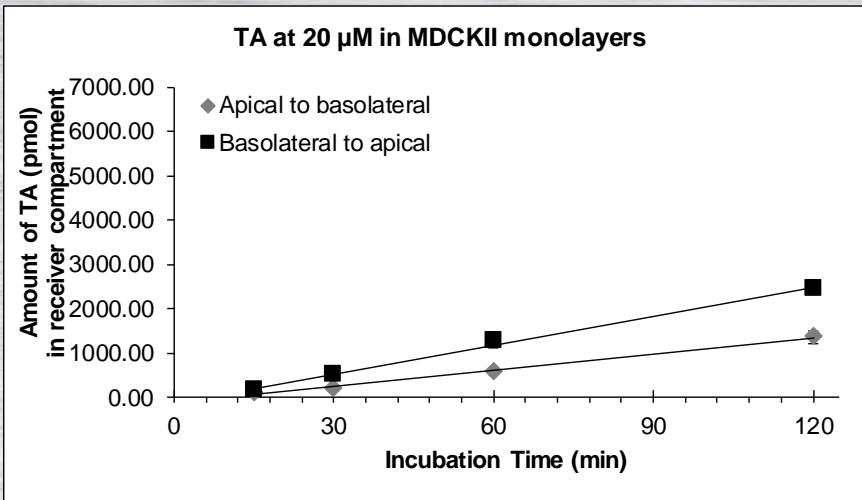


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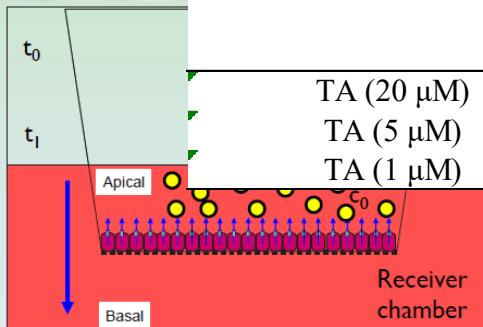
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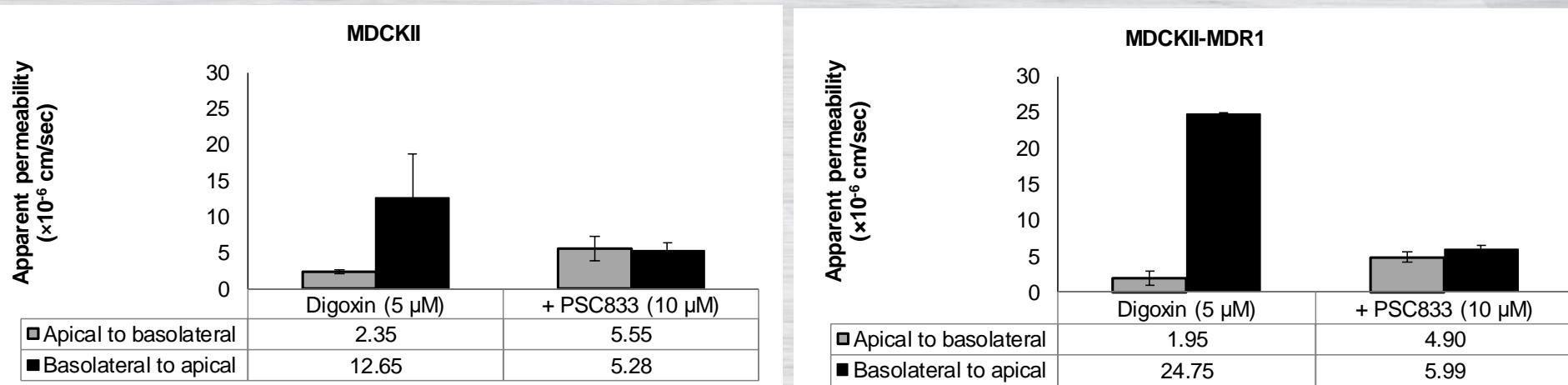


MDCKII			
	$P_{app\ A-B}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	$P_{app\ B-A}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	Efflux ratio <sup>b</sup> Average $\pm$ SD <sup>c</sup>
TA (20 $\mu$ M)	12.74 $\pm$ 0.82	24.05 $\pm$ 0.87	1.89 $\pm$ 0.14
TA (5 $\mu$ M)	7.66 $\pm$ 0.51	25.29 $\pm$ 0.60	3.30 $\pm$ 0.24
TA (1 $\mu$ M)	7.91 $\pm$ 1.23	35.72 $\pm$ 0.80	4.52 $\pm$ 0.71

	$P_{app\ A-B}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	$P_{app\ B-A}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	Efflux ratio <sup>b</sup> Average $\pm$ SD <sup>c</sup>	Net-efflux ratio <sup>d</sup> Average $\pm$ SD <sup>e</sup>
TA (20 $\mu$ M)	1.40 $\pm$ 0.11	61.70 $\pm$ 2.32	44.00 $\pm$ 3.82	42.11 $\pm$ 2.65
TA (5 $\mu$ M)	0.66 $\pm$ 0.04	55.39 $\pm$ 1.70	84.07 $\pm$ 6.09	80.77 $\pm$ 2.59
TA (1 $\mu$ M)	2.47 $\pm$ 0.29	56.69 $\pm$ 1.18	22.97 $\pm$ 2.77	18.46 $\pm$ 1.01

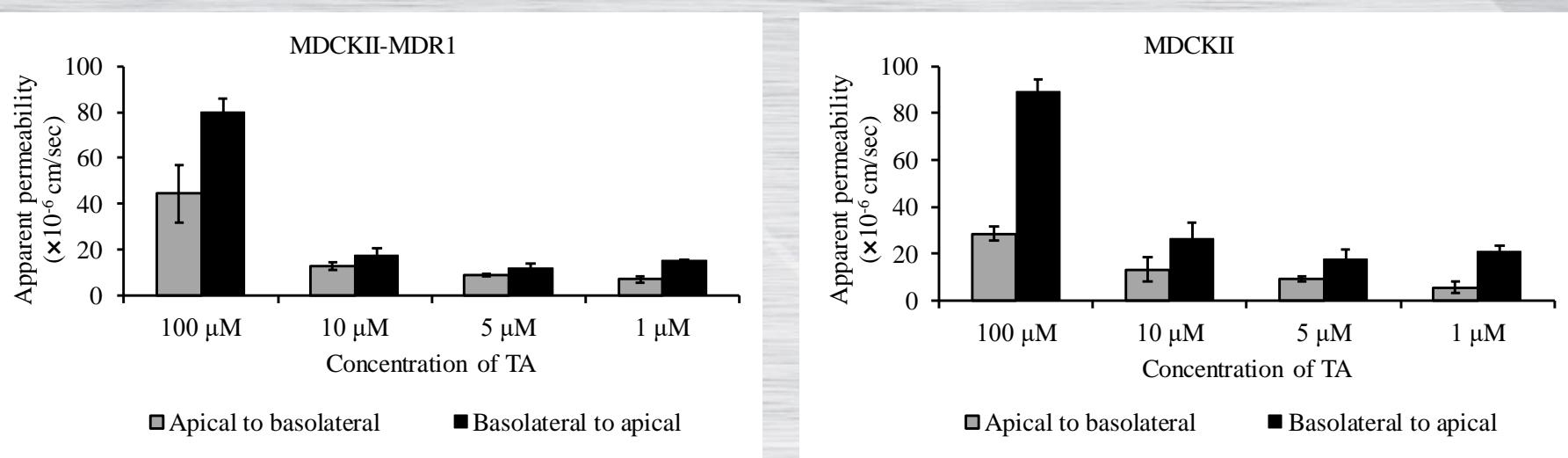


# Substrate follow up



MDCKII			
	$P_{app, A-B}^a (\times 10^{-6} \text{ cm/s})$ Average $\pm SD$	$P_{app, B-A}^a (\times 10^{-6} \text{ cm/s})$ Average $\pm SD$	Efflux ratio <sup>b</sup> Average $\pm SD^c$
Lucifer yellow (40 $\mu\text{g/mL}$ )	1.65 $\pm$ 0.26	NA	ND
Lucifer yellow + TA (20 $\mu\text{M}$ )	1.57 $\pm$ 0.03	NA	ND
Digoxin (5 $\mu\text{M}$ )	2.35 $\pm$ 0.22	12.65 $\pm$ 6.03	5.38 $\pm$ 2.61
Digoxin (5 $\mu\text{M}$ ) + PSC833 (10 $\mu\text{M}$ )	5.55 $\pm$ 1.69	5.28 $\pm$ 1.02	0.95 $\pm$ 0.34
MDCKII-MDR1			
	$P_{app, A-B}^a (\times 10^{-6} \text{ cm/s})$ Average $\pm SD$	$P_{app, B-A}^a (\times 10^{-6} \text{ cm/s})$ Average $\pm SD$	Efflux ratio <sup>b</sup> Average $\pm SD^c$
Lucifer yellow (40 $\mu\text{g/mL}$ )	1.64 $\pm$ 0.38	NA	ND
Lucifer yellow + TA (20 $\mu\text{M}$ )	1.62 $\pm$ 0.11	NA	ND
Digoxin (5 $\mu\text{M}$ )	1.95 $\pm$ 0.94	24.75 $\pm$ 0.17	12.67 $\pm$ 6.07
Digoxin (5 $\mu\text{M}$ ) + PSC833 (10 $\mu\text{M}$ )	4.90 $\pm$ 0.73	5.99 $\pm$ 0.51	0.27 $\pm$ 0.51

# Application of low permeability control



MDCKII-MDR1				
	$P_{app\ A-B}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	$P_{app\ B-A}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	Efflux ratio <sup>b</sup> Average $\pm$ SD <sup>c</sup>	Net-efflux ratio <sup>d</sup> Average $\pm$ SD <sup>e</sup>
TA (100 μM)	44.44 $\pm$ 12.50	79.98 $\pm$ 5.78	1.80 $\pm$ 0.52	-1.33 $\pm$ 0.18
TA (10 μM)	12.75 $\pm$ 1.66	17.64 $\pm$ 2.94	1.38 $\pm$ 0.29	-0.65 $\pm$ 0.36
TA (5 μM)	8.97 $\pm$ 0.70	11.87 $\pm$ 1.78	1.32 $\pm$ 0.22	-0.66 $\pm$ 0.20
TA (1 μM)	7.06 $\pm$ 1.28	15.09 $\pm$ 0.74	2.14 $\pm$ 0.40	-1.68 $\pm$ 0.28

MDCKII			
	$P_{app\ A-B}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	$P_{app\ B-A}^a$ ( $\times 10^{-6}$ cm/s) Average $\pm$ SD	Efflux ratio <sup>b</sup> Average $\pm$ SD <sup>c</sup>
TA (100 μM)	28.53 $\pm$ 3.14	89.24 $\pm$ 4.90	3.13 $\pm$ 0.39
TA (10 μM)	13.12 $\pm$ 5.29	26.65 $\pm$ 6.79	2.03 $\pm$ 0.97
TA (5 μM)	8.96 $\pm$ 1.10	17.80 $\pm$ 3.71	1.99 $\pm$ 0.48
TA (1 μM)	5.42 $\pm$ 2.44	20.70 $\pm$ 2.47	3.82 $\pm$ 1.78

# Application of low permeability control

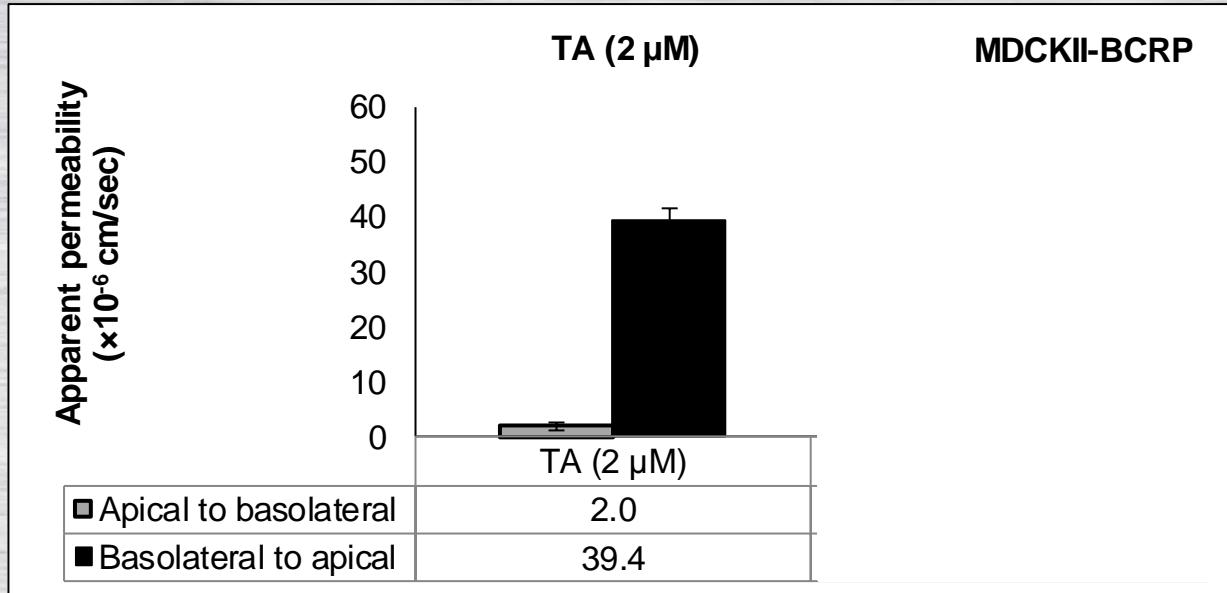
- Incubation of LY (A-B) with various TA concentrations
- Cutoff is  $2.0 \times 10^{-6}$  cm/sec
- Values above cutoff – indication of cell monolayer integrity problems

MDCKII-MDR1		
Compound	Concentrations	P <sub>app, A-B</sub> <sup>a</sup> ( $\times 10^{-6}$ cm/s) Average ± SD
Lucifer Yellow	40 µg/mL	0.9 ± 0.1
Lucifer Yellow + TA	40 µg/mL + 100 µM	25.0 ± 0.6
Lucifer Yellow + TA	40 µg/mL + 10 µM	1.0 ± 0.1
Lucifer Yellow + TA	40 µg/mL + 5 µM	0.9 ± 0.1
Lucifer Yellow + TA	40 µg/mL + 1 µM	1.1 ± 0.3

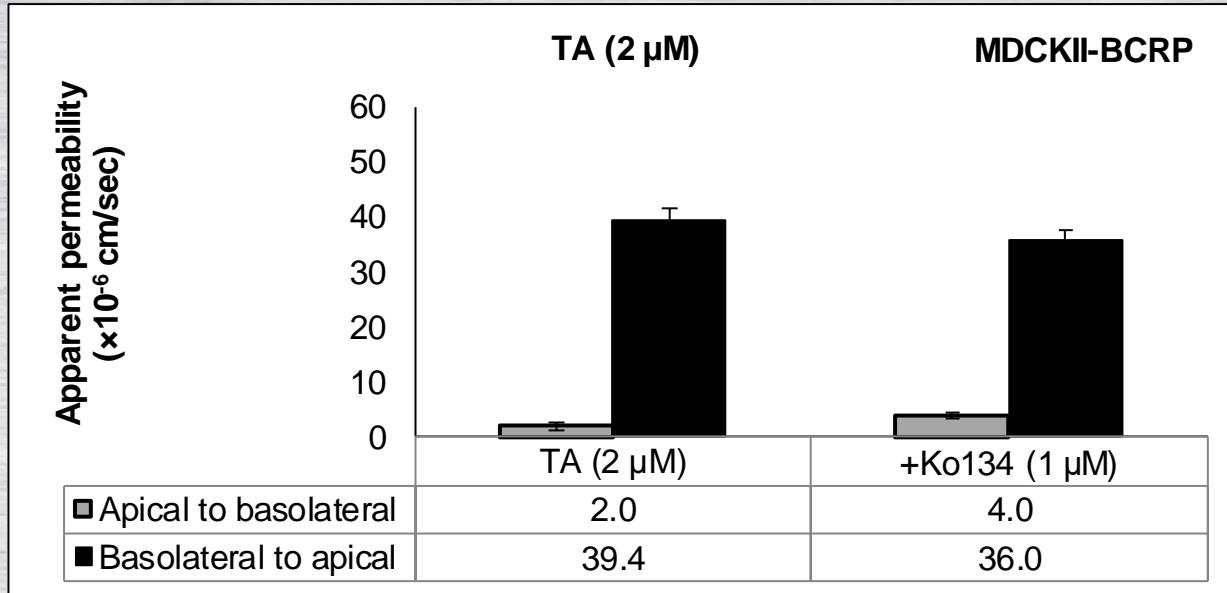
  

MDCKII		
Compound	Concentrations	P <sub>app, A-B</sub> <sup>a</sup> ( $\times 10^{-6}$ cm/s) Average ± SD
Lucifer Yellow	40 µg/mL	1.2 ± 0.3
Lucifer Yellow + TA	40 µg/mL + 100 µM	24.9 ± 0.6
Lucifer Yellow + TA	40 µg/mL + 10 µM	1.2 ± 0.7
Lucifer Yellow + TA	40 µg/mL + 5 µM	2.1 ± 1.2
Lucifer Yellow + TA	40 µg/mL + 1 µM	0.6 ± 0.1

# Case study on specificity

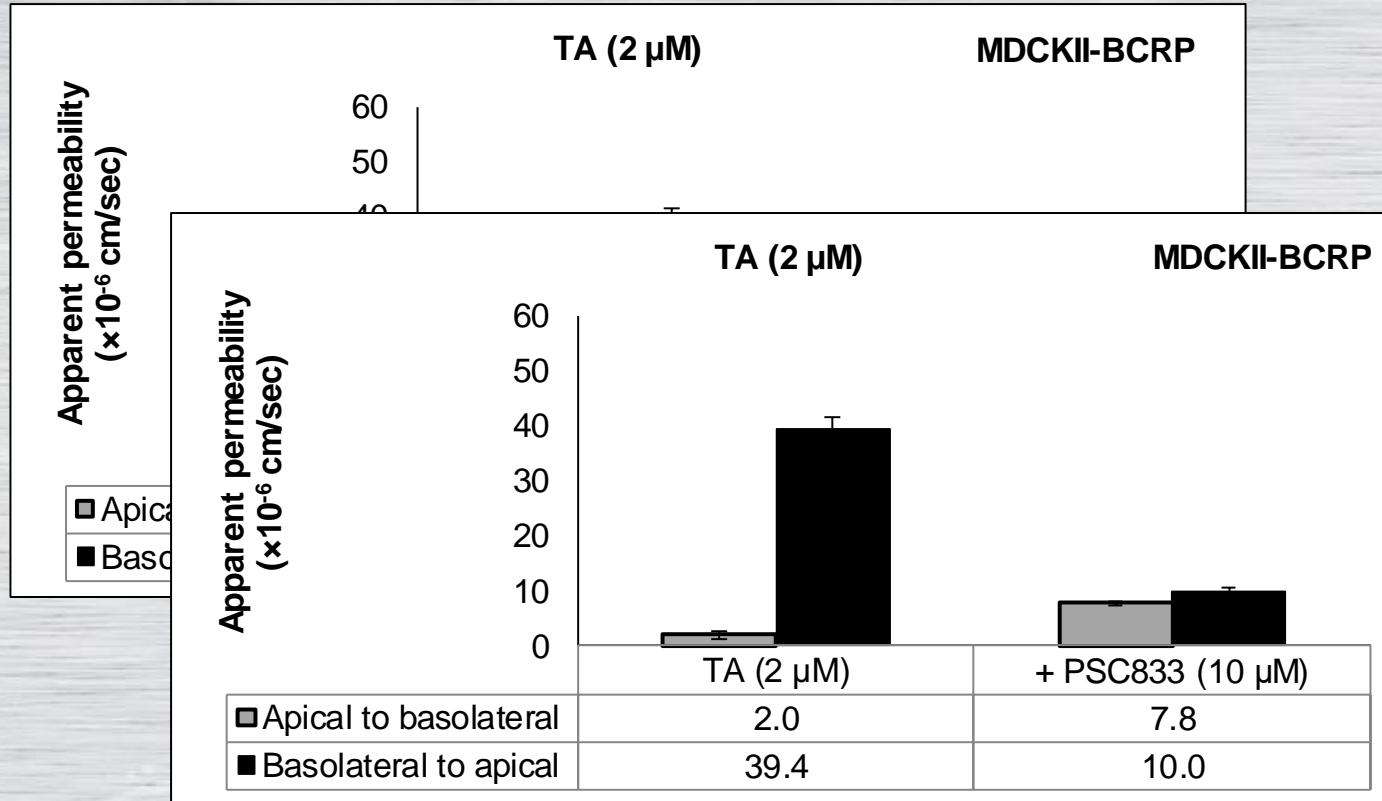


# Case study on specificity

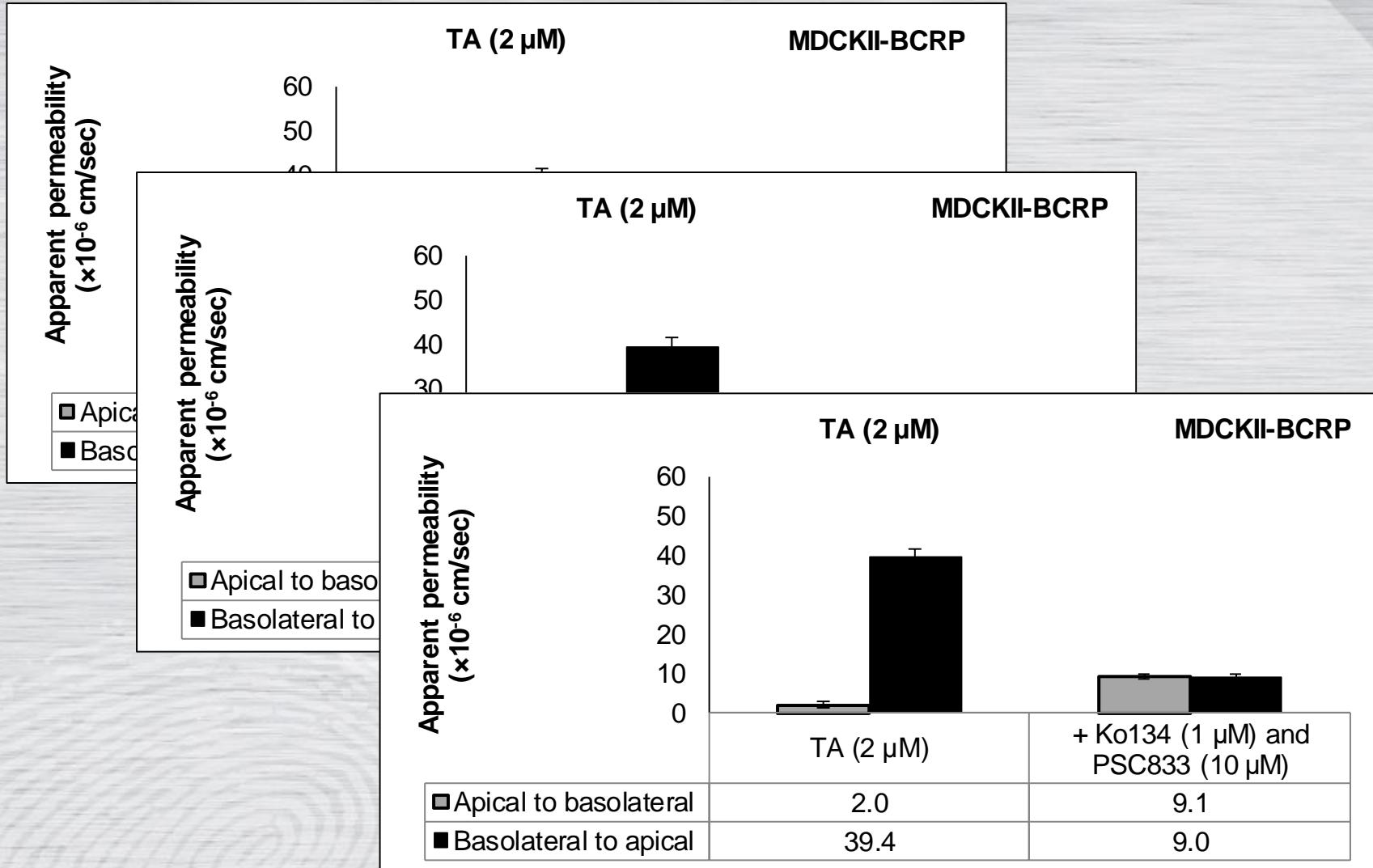


?

# Case study on specificity



# Case study on specificity



# Case study on specificity

- Conclusions
  - Compound is a known P-gp substrate
  - Also a substrate of canine P-gp
  - Seemingly a substrate of BCRP
  - Combination of inhibitors revealed that efflux is mediated by canine P-gp and not by BCRP

	MDCKII		
	$P_{app\ A-B}^a\ (\times 10^{-6}\ \text{cm/s})$	$P_{app\ B-A}^a\ (\times 10^{-6}\ \text{cm/s})$	Efflux ratio <sup>b</sup>
	Average $\pm SD$	Average $\pm SD$	Average $\pm SD^c$
TA (500 $\mu\text{M}$ )	9.7 $\pm$ 0.9	15.9 $\pm$ 0.2	1.6 $\pm$ 0.1
TA (2 $\mu\text{M}$ )	4.0 $\pm$ 0.4	67.6 $\pm$ 2.0	17.1 $\pm$ 1.7
TA (0.5 $\mu\text{M}$ )	4.6 $\pm$ 1.4	52.5 $\pm$ 1.0	11.5 $\pm$ 3.5

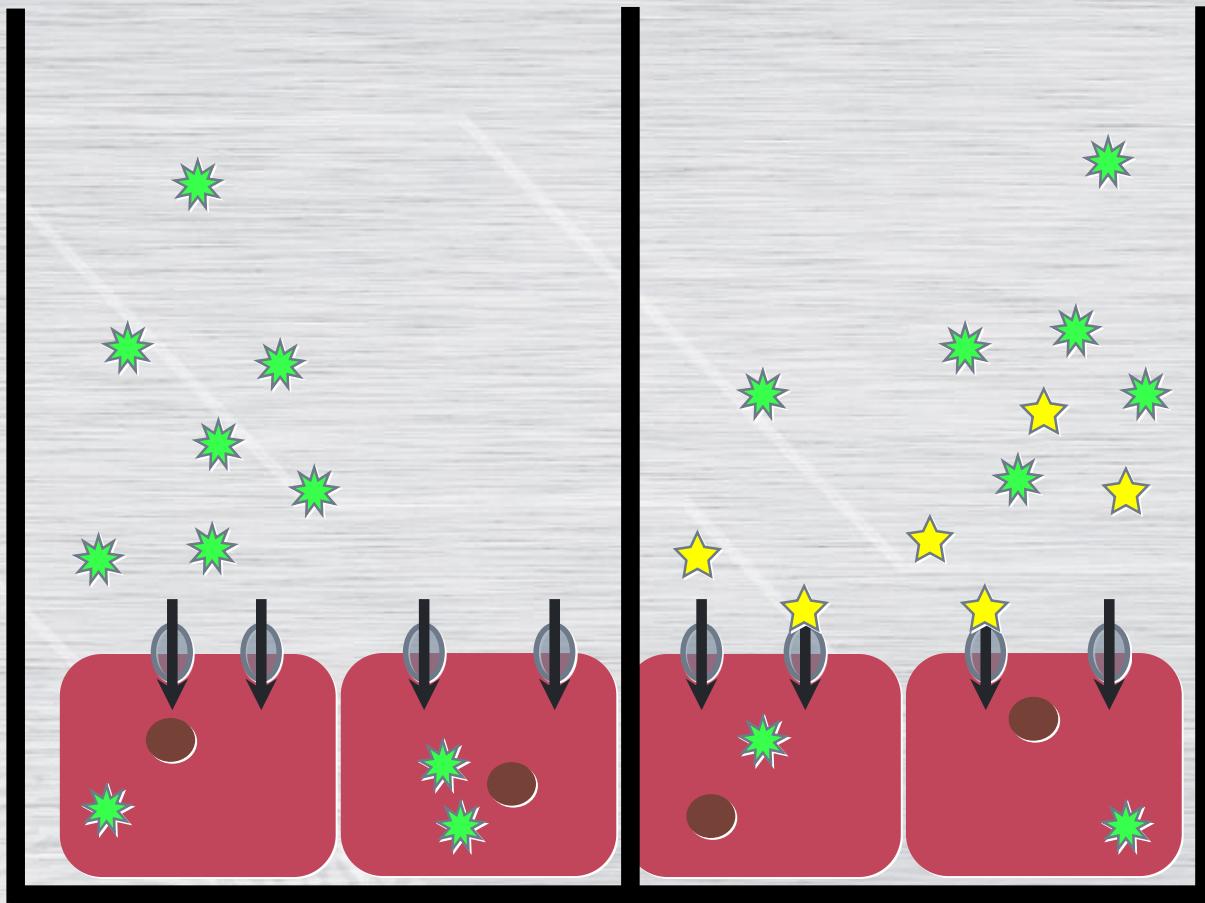
## Substrate testing

- Transporter uptake assays (for SLC and ABC transporters)
- Bidirectional permeability

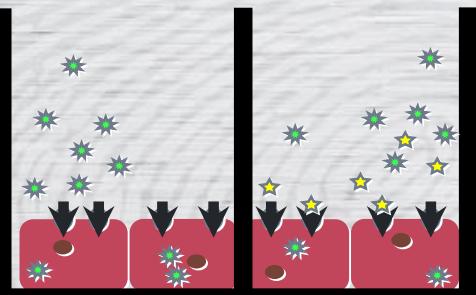
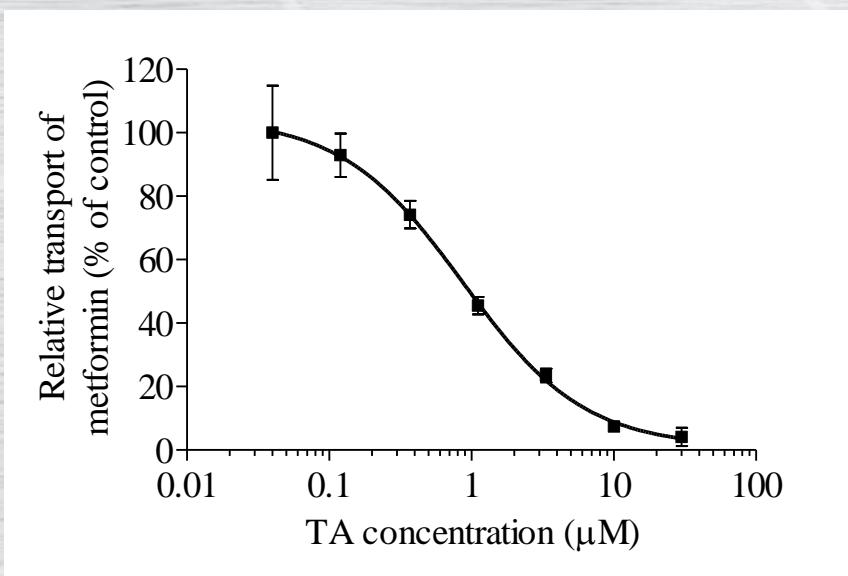
## Inhibition testing

- $IC_{50}$
- $K_i$

# Uptake inhibition assays



# Compound is an inhibitor



log(inhibitor) vs. response -- Variable slope

Best-fit values

0.8775

BOTTOM

104.6

TOP

-0.05977

LOGIC50

-1.018

HILLSLOPE

0.8714

IC50

103.8

Span

0.1767

Std. Error

2.122

BOTTOM

0.02803

TOP

0.07187

LOGIC50

3.354

HILLSLOPE

-4.746 to 6.501

IC50

97.88 to 111.4

Span

-0.1489 to 0.02942

95% Confidence Intervals

-1.247 to -0.7896

BOTTOM

0.7097 to 1.070

TOP

93.08 to 114.4

LOGIC50

Goodness of Fit

Degrees of Freedom

3

R2

0.9994

Absolute Sum of Squares

5.872

Sy.x

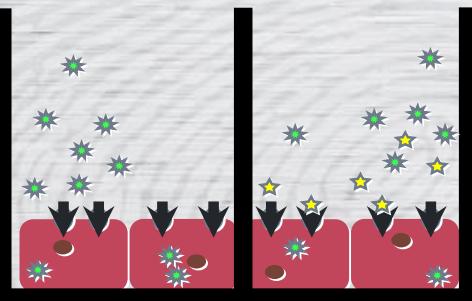
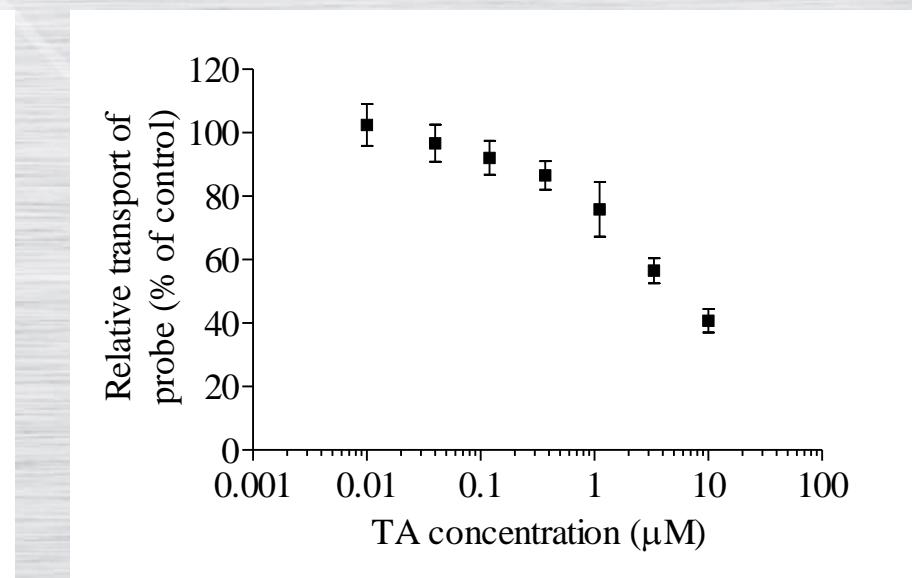
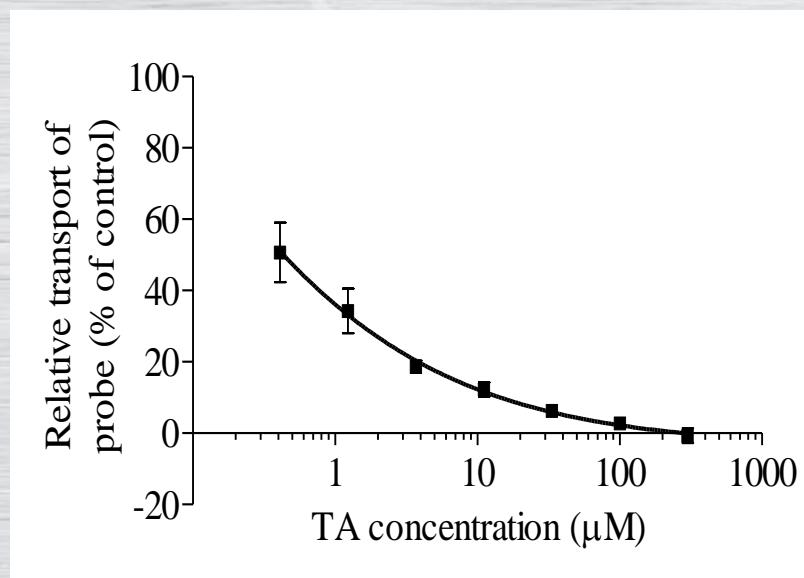
1.399

Number of points

7

Analyzed

# Partial curves



# Partial curves

log(inhibitor) vs. response -- Variable slope

Best-fit values

BOTTOM

-1.319

TOP

= 100.0

LOGIC50

-0.3554

HILLSLOPE

-0.6111

IC50

0.4411

Span

= 101.3

Std. Error

1.402

BOTTOM

0.03266

LOGIC50

0.04496

HILLSLOPE

0.04496

95% Confidence Intervals

-5.212 to 2.574

BOTTOM

-0.4461 to -0.2648

LOGIC50

-0.7359 to -0.4863

HILLSLOPE

0.3580 to 0.5435

IC50

0.3580 to 0.5435

Goodness of Fit

4

Degrees of Freedom

0.9966

R<sup>2</sup>

7.093

Absolute Sum of Squares

1.332

Sy.x

TOP

TOP = 100.0

7

Relative transport of

log(inhibitor) vs. response -- Variable slope

Best-fit values

BOTTOM

-12.13

TOP

103.6

LOGIC50

0.8551

HILLSLOPE

-0.5743

IC50

7.164

Span

115.7

Std. Error

51.92

BOTTOM

3.712

TOP

0.6488

LOGIC50

0.1869

HILLSLOPE

54.85

Span

-177.4 to 153.1

95% Confidence Intervals

91.77 to 115.4

BOTTOM

-1.209 to 2.919

TOP

-1.169 to 0.02048

LOGIC50

0.06177 to 830.7

HILLSLOPE

-58.82 to 290.2

IC50

0

Span

3

Goodness of Fit

0.9959

Degrees of Freedom

12.55

R<sup>2</sup>

2.046

Absolute Sum of Squares

7

Sy.x

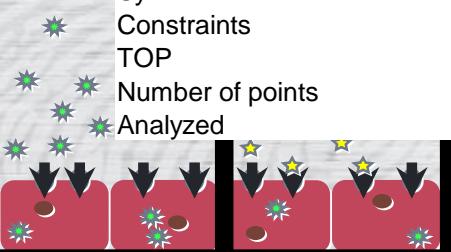
THE TRANSPORTER COMPANY

Number of points

ELVO®

Analyzed

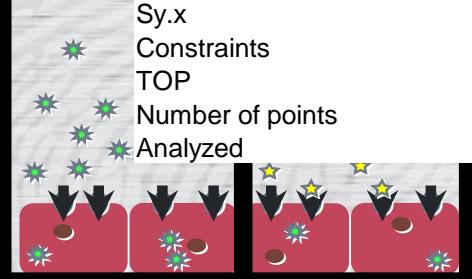
BIOTECHNOLOGY



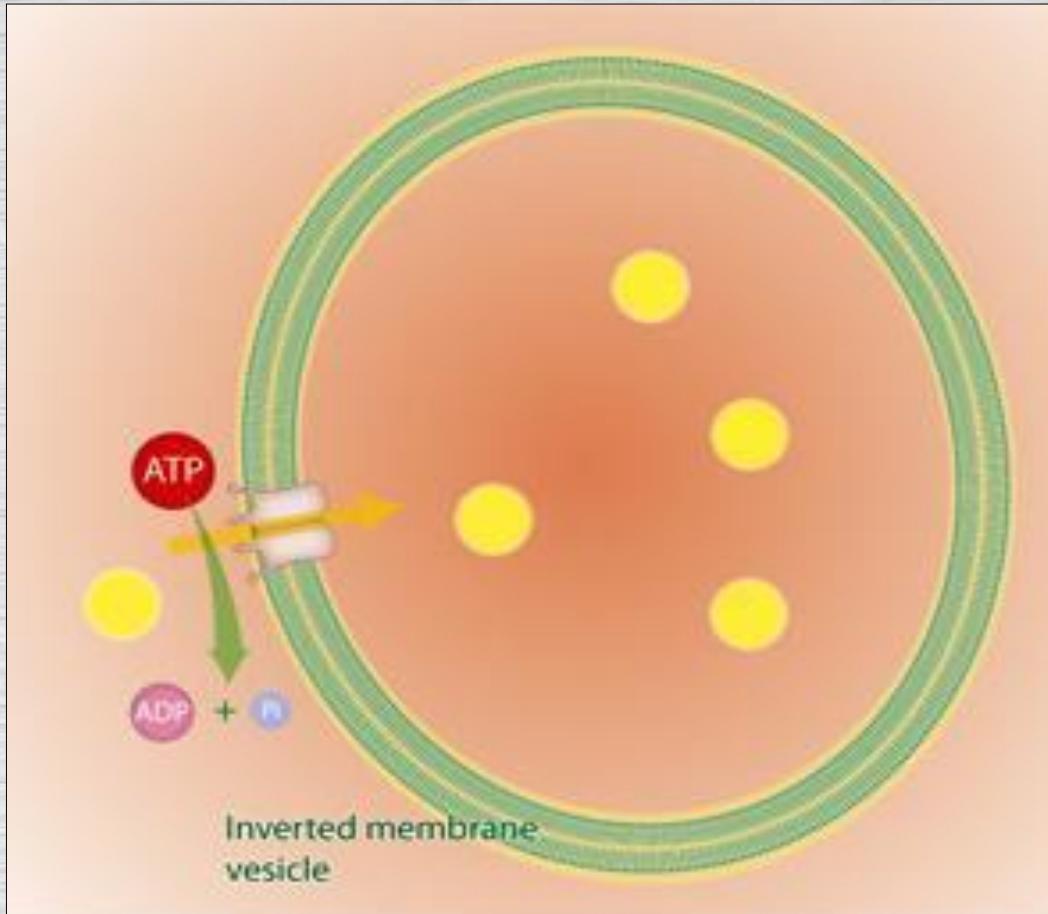
# Partial curves

log(inhibitor) vs. response -- Variable slope	
Best-fit values	
BOTTOM	-1.319
TOP	= 100.0
LOGIC50	-0.3554
HILLSLOPE	-0.6111
IC50	0.4411
Span	= 101.3
Std. Error	
BOTTOM	1.402
LOGIC50	0.03266
HILLSLOPE	0.04496
95% Confidence Intervals	
BOTTOM	-5.212 to 2.574
LOGIC50	-0.4461 to -0.2648
HILLSLOPE	-0.7359 to -0.4863
IC50	0.3580 to 0.5435
Goodness of Fit	
Degrees of Freedom	4
R <sup>2</sup>	0.9966
Absolute Sum of Squares	7.093
Sy.x	1.332
Constraints	
TOP	TOP = 100.0
Number of points	
Analyzed	7

Relative transport of	log(inhibitor) vs. response -- Variable slope
Best-fit values	
BOTTOM	= 0.0
TOP	102.8
LOGIC50	0.7000
HILLSLOPE	-0.6265
IC50	5.011
Span	= 102.8
Std. Error	
TOP	1.907
LOGIC50	0.04012
HILLSLOPE	0.04998
95% Confidence Intervals	
TOP	97.47 to 108.1
LOGIC50	0.5886 to 0.8113
HILLSLOPE	-0.7652 to -0.4877
IC50	3.878 to 6.476
Goodness of Fit	
Degrees of Freedom	4
R <sup>2</sup>	0.9958
Absolute Sum of Squares	12.85
Sy.x	1.793
Constraints	
BOTTOM	BOTTOM = 0.0
Number of points	
Analyzed	7

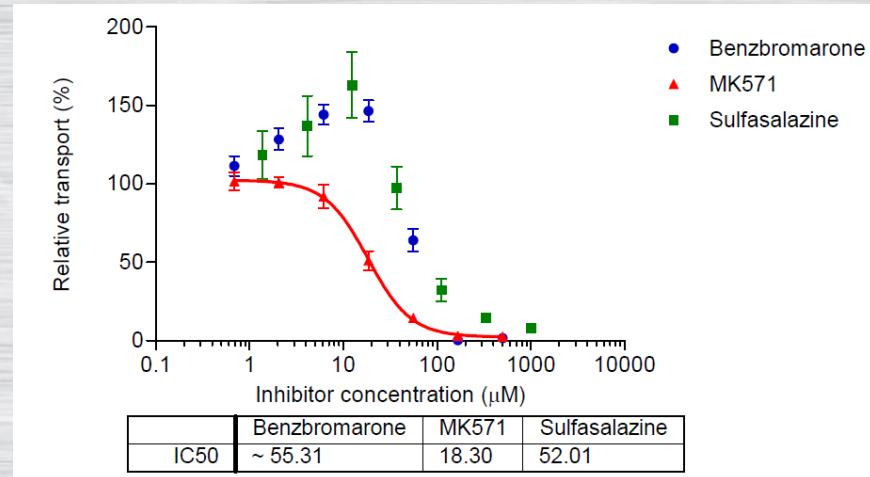


# Vesicular transport inhibition assays

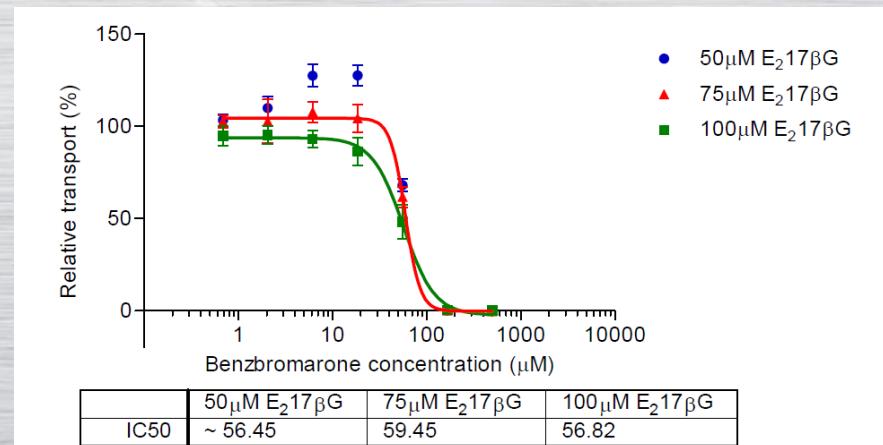


# MRP2 – is this an inhibitor?

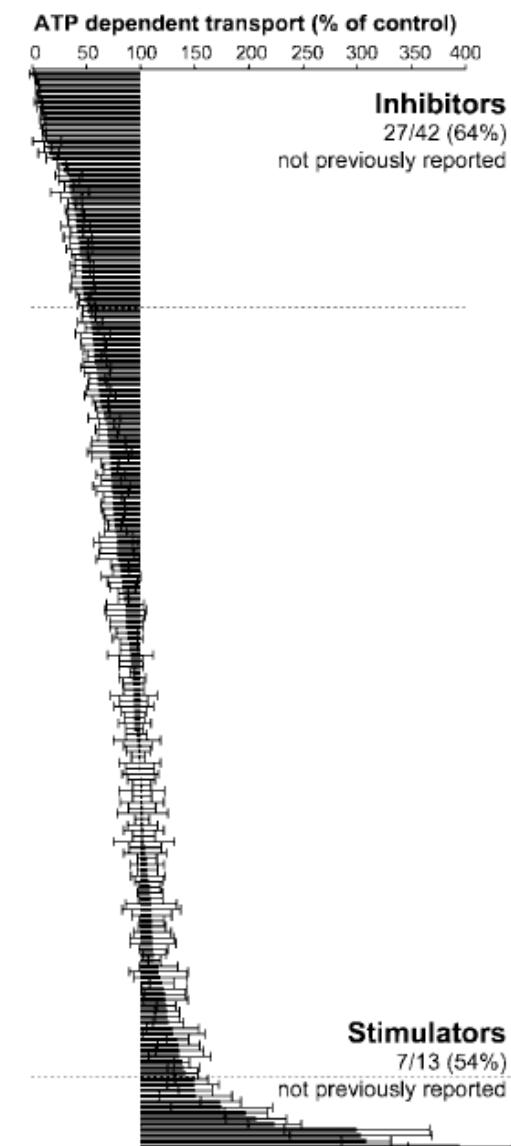
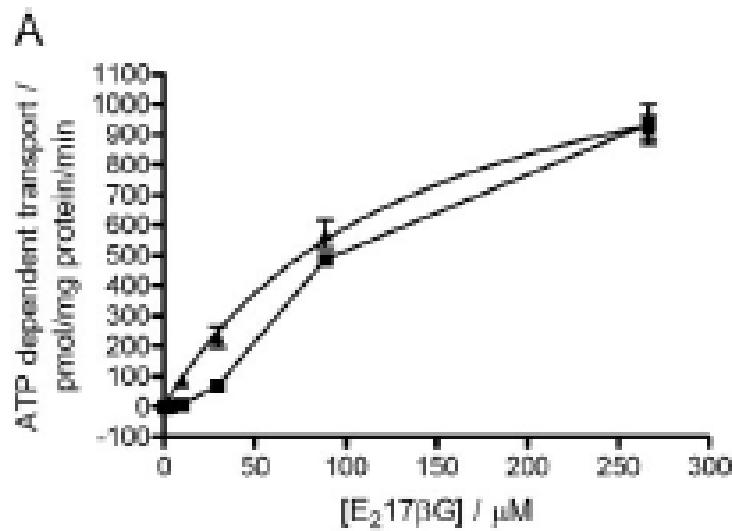
50  $\mu$ M  $E_2 17\beta G$



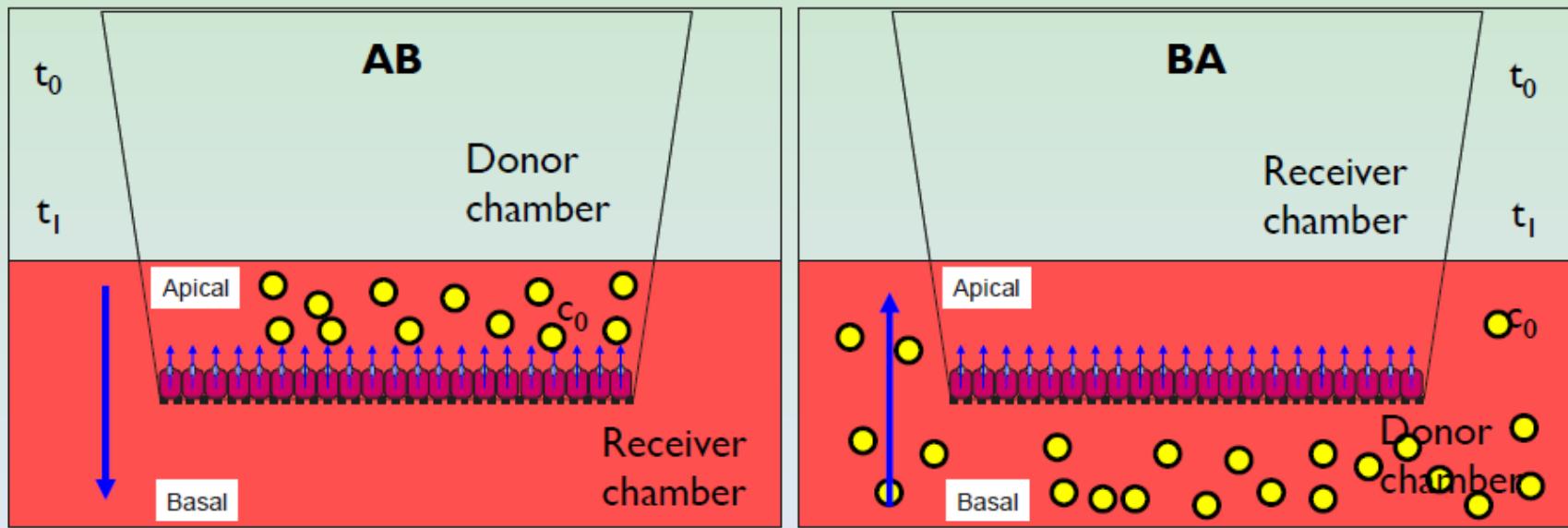
Multiple  $E_2 17\beta G$  cc



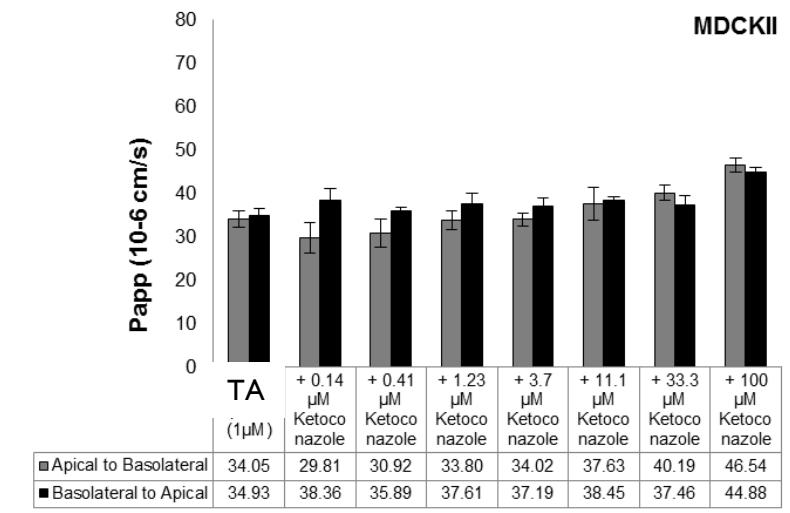
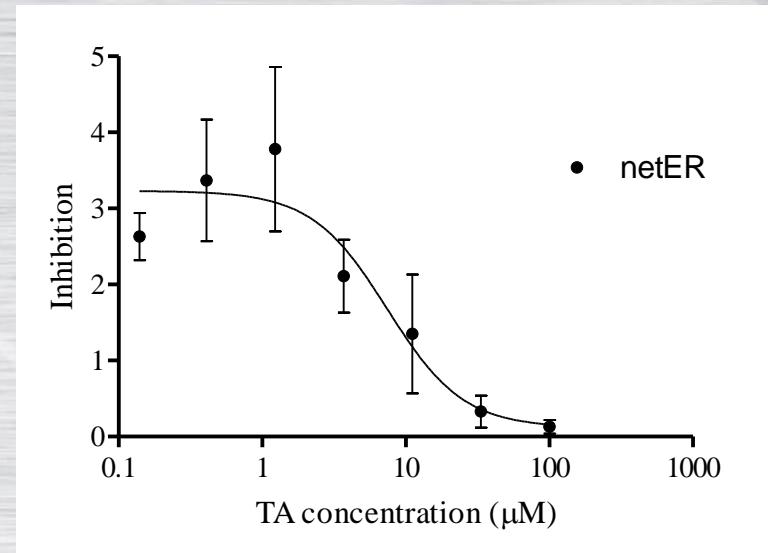
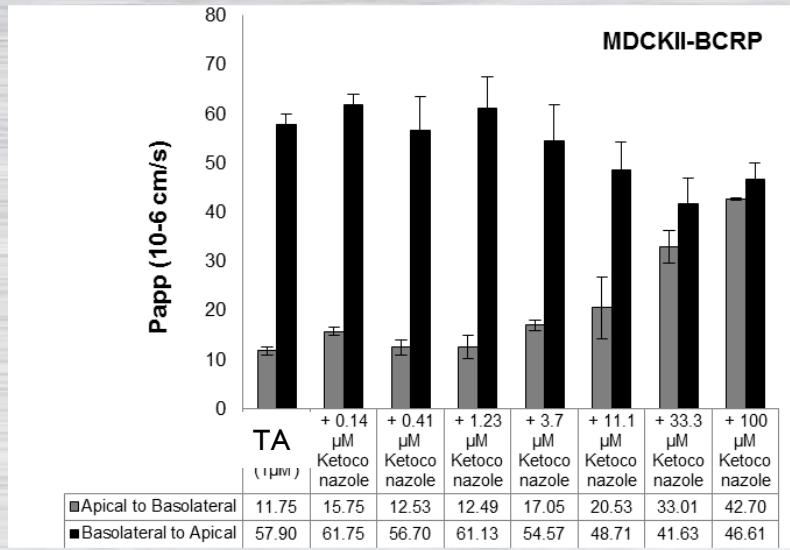
# MRP2 – is this an inhibitor?



# Bidirectional permeability assay

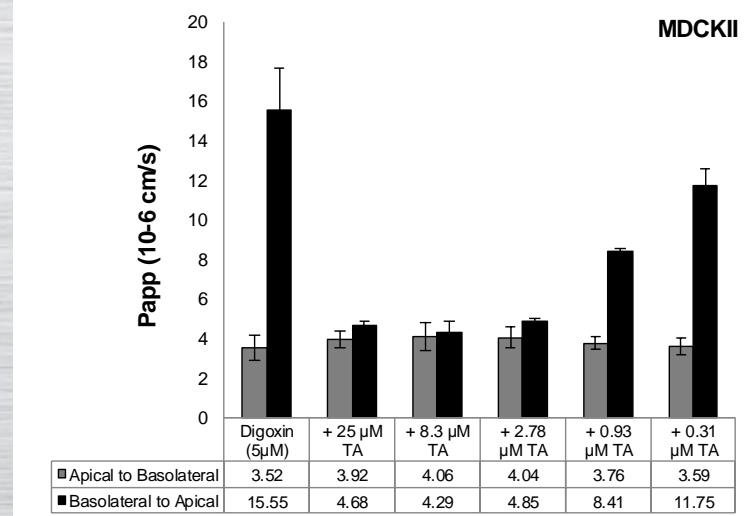
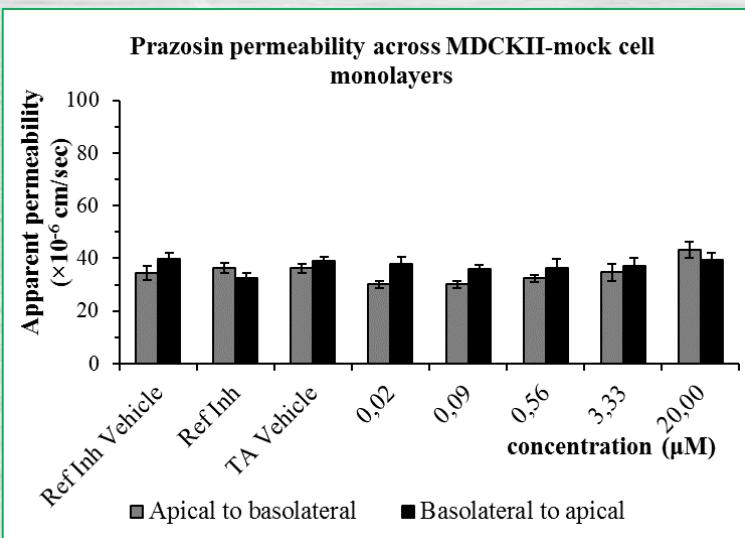
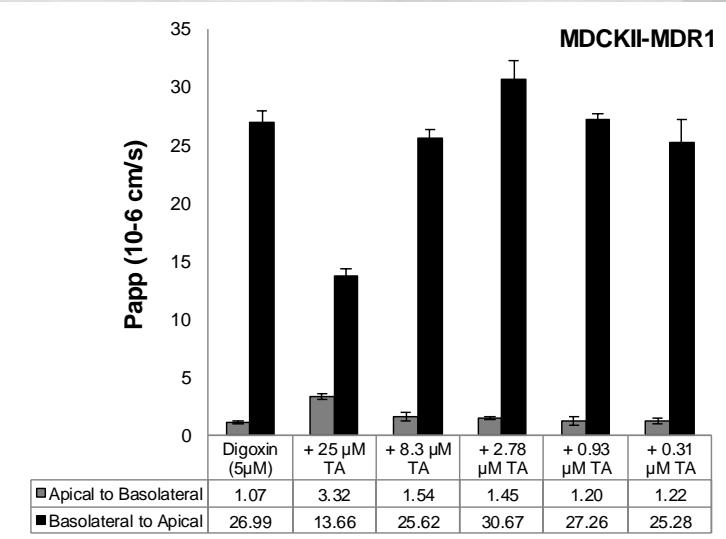
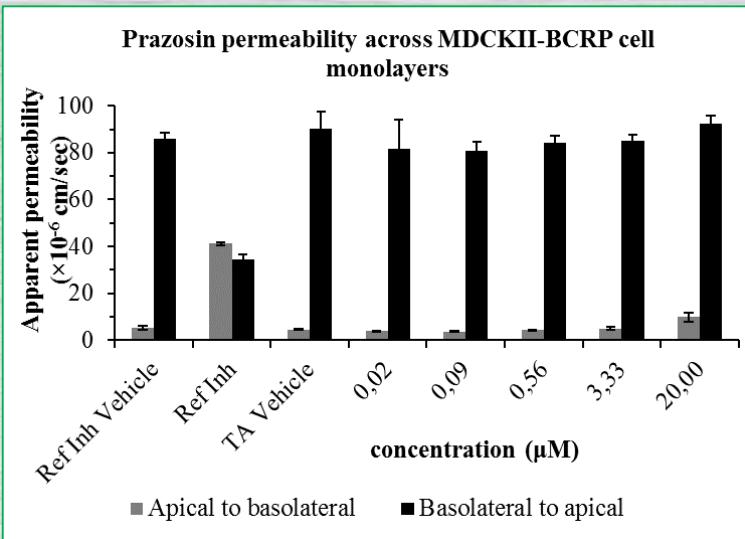


# Compound is an inhibitor

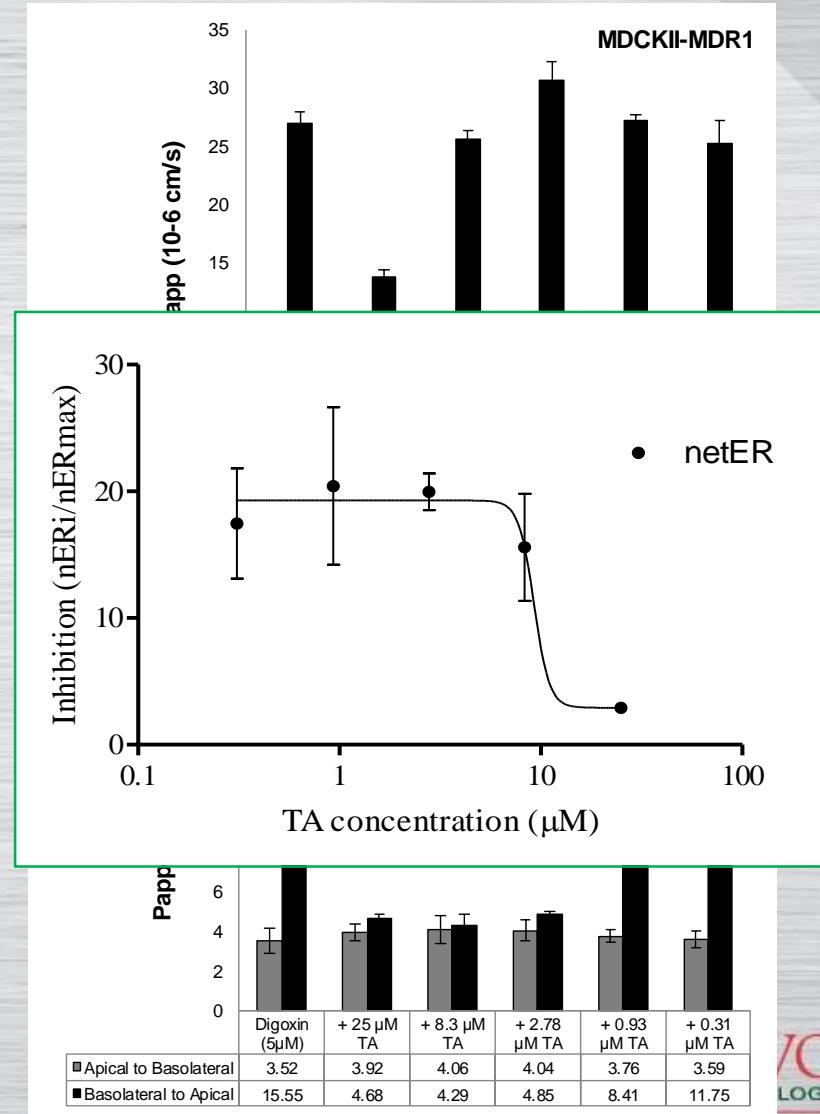
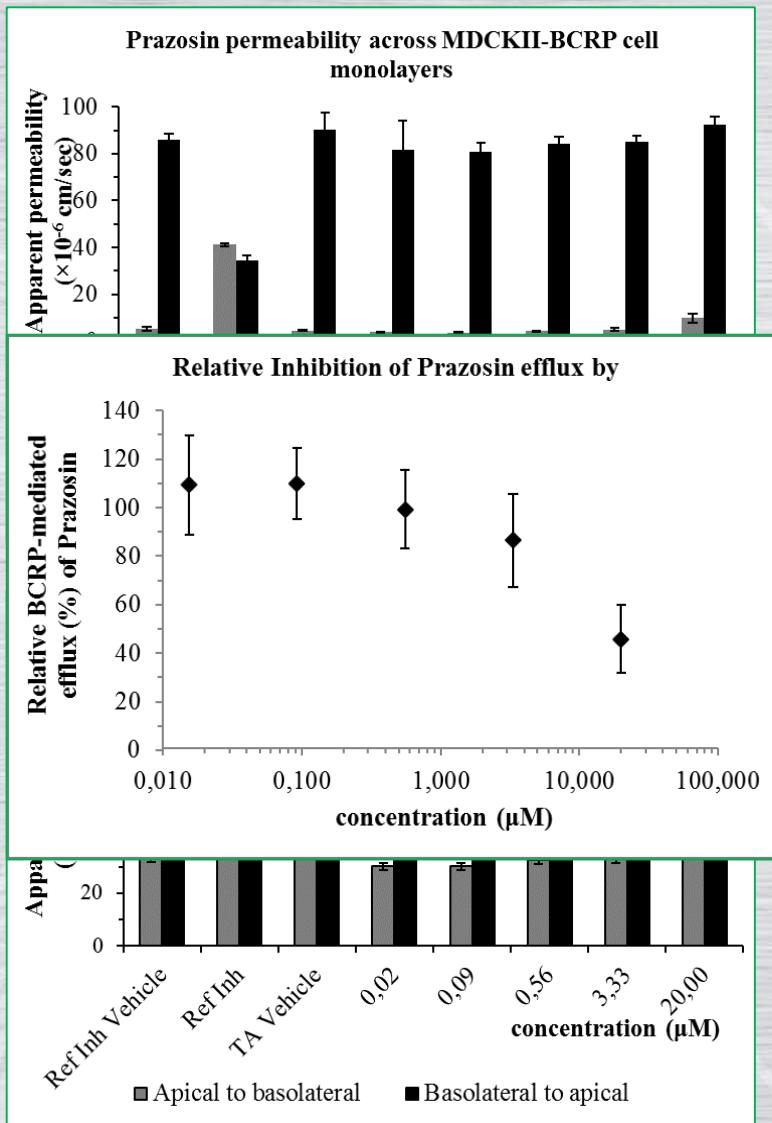


- Inhibition =  $nER_i / nER_{max}$

# Challenges



# Challenges



# Ways to calculate inhibition potential

TABLE 5  
Equations used to calculate remaining transport activity in presence of inhibitor in cell models

Eq. No.	Name	Equation <sup>b</sup>	Reference
A1 <sup>a</sup>	ER	$1 - [(BA_0 / AB_0) - (BA_i / AB_i)] / [(BA_0 / AB_0) - (BA_p / AB_p)]$ can be written as: $[(BA_i / AB_i) - (BA_p / AB_p)] / [(BA_0 / AB_0) - (BA_p / AB_p)]$	Balimane et al. (2008)
A2	ER (FDA)	$(BA_i / AB_i - 1) / (BA_0 / AB_0 - 1)$	US FDA/CDER (2006)
B1	Unidirectional flux (B>A)	$(BA_i - BA_p) / (BA_0 - BA_p)$	Tang et al. (2002)
B2	Unidirectional flux (A>B)	$(AB_i - AB_p) / (AB_0 - AB_p)$	Tang et al. (2002)
C1	KP (A>B)	$(AB_i / AB_0)[(AB_i - AB_p) / (AB_0 - AB_p)]$	Kalvass and Pollack (2007)
C2	KP (B>A)	$(BA_0 / BA_i)[(BA_i - BA_p) / (BA_0 - BA_p)]$	Adapted from Kalvass and Pollack (2007)
D	Net-secretory-flux	$(AB_i - BA_i) / (AB_0 - BA_0)$	Balimane et al. (2008)

ER, efflux ratio.

<sup>a</sup> Equation A2 is the FDA-recommended equation (US FDA/CDER, 2006). For this particular initiative, due to the limited number of inhibitor concentrations in the cell line experiments, the participants were asked to fit data to eq. A1 so that a no inhibitor control and positive control inhibitor could be included as data points (see Materials and Methods section).

<sup>b</sup> AB<sub>i</sub> Receiver A>B with inhibitor; BA<sub>i</sub> Receiver B>A with inhibitor; AB<sub>0</sub> Receiver A>B, without inhibitor; BA<sub>0</sub> Receiver B>A, without inhibitor; AB<sub>p</sub> Receiver A>B with positive control; BA<sub>p</sub> Receiver B>A with positive control.

# Determination of $K_i$

- EMA DDI Guideline suggest determination of  $K_i$  instead of  $IC_{50}$
- Advantages of  $K_i$ 
  - System-independent value
  - Nature of interaction can be learned (competitive, non-competitive or un-competitive)
- Experimental determination
  - Dixon plot (inhibitor concentration – response curves in the presence of various probe ccs)
  - $K_m$ - $V_{max}$  method (probe  $K_m$ ,  $V_{max}$  determined in the presence of various inhibitor ccs)
- Applied test systems
  - Uptake assay (SLC transporters)
  - Vesicular transport assay (ABC transporters)

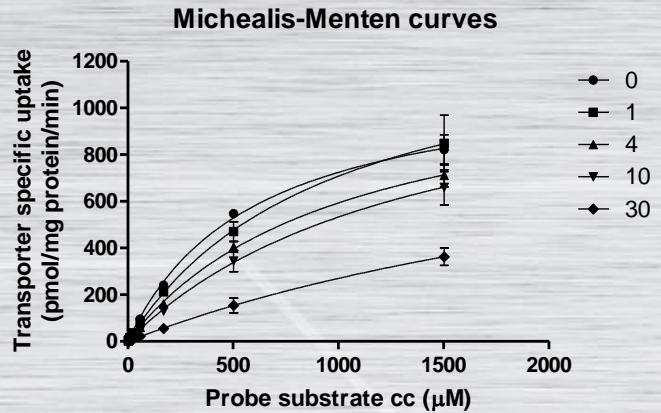
# ITC paper (Zamek-Gliszczynski et al, 2013)

- To allow unbiased parameter estimation regardless of the transporter inhibition mechanism (competitive or noncompetitive), this probe concentration needs to be significantly below its Km for the transporter of interest because under these conditions the IC<sub>50</sub> estimate would be equivalent to Ki for uptake transporters and for efflux transport experiments conducted with membrane vesicles.
- *Cheng-Prusoff equation*

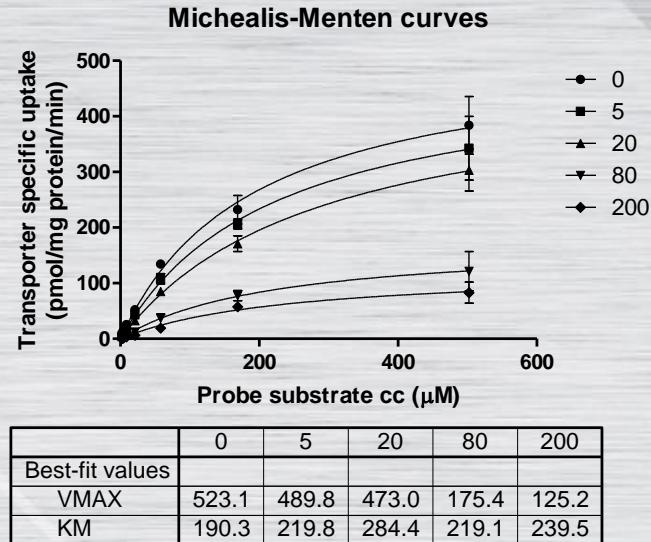
$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

- For efflux transporter inhibition assessment in polarized cell lines, there is not yet an agreed method for obtaining a Ki value, nor is there evidence that this extra effort is justified over simply determining an IC<sub>50</sub> value.
- For substrates such as digoxin that require two active processes, basolateral uptake and apical efflux, a change in digoxin B→A flux could come from inhibition of either apical efflux or basolateral uptake in which the measured IC<sub>50</sub> is a net value representing multiple transporters.

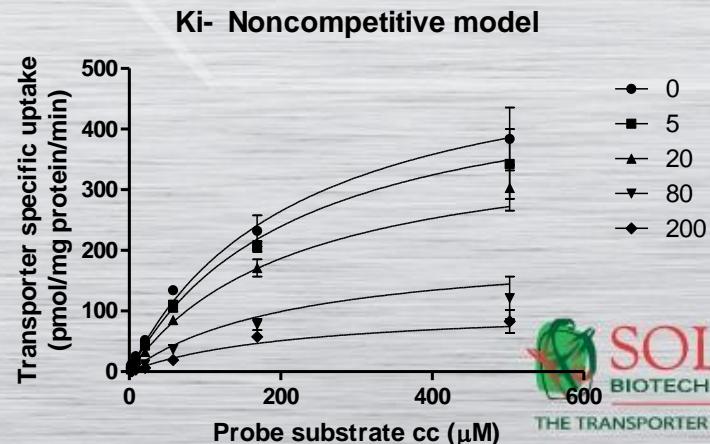
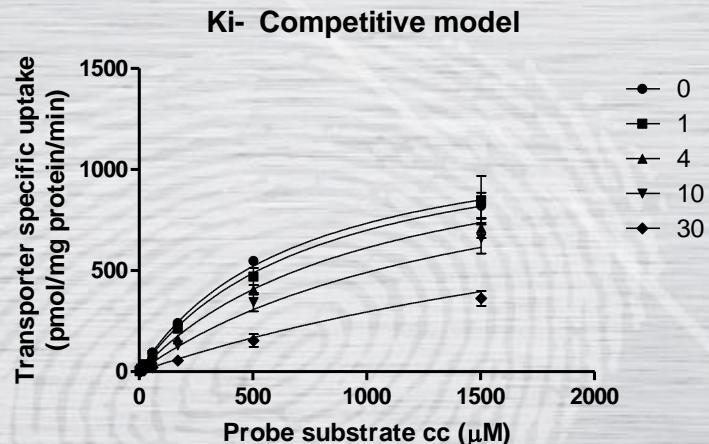
# Competitive vs non-competitive inhibition



$$K_i = 8.24 \mu\text{M}, IC_{50} = 4.92 \mu\text{M}$$

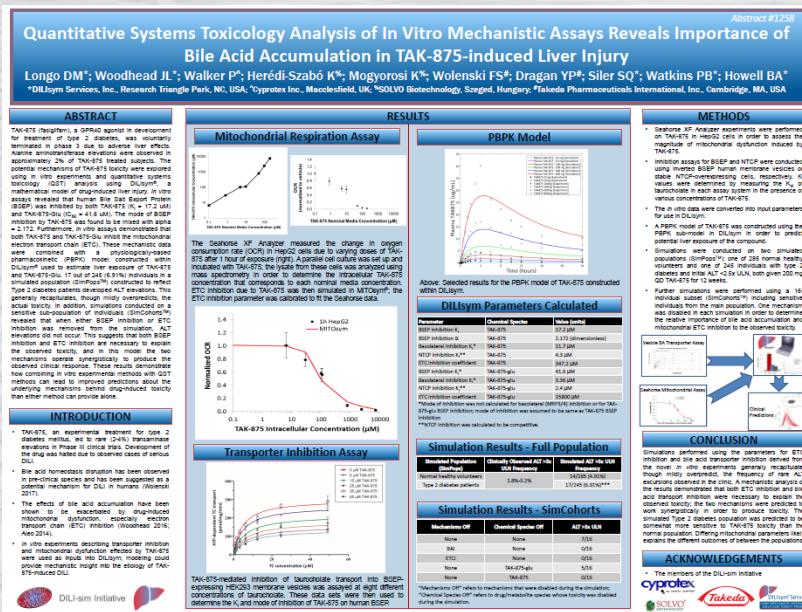


$$K_i = 47.9 \mu\text{M}, IC_{50} = 30.1 \mu\text{M}$$



# Why to determine a $K_i$ ?

- When probe substrate concentration is below  $K_m$ ,  $IC_{50}$  is a good estimate for  $K_i$
- Mode of inhibition
  - Alpha value determination
  - Mode of inhibition can be used for modeling (DILIsym)



- Possible alpha values:
- $\alpha \sim 1$  non-competitive inhibition (the inhibitor has an effect on transport capacity, not affinity)
- $\alpha > 1$  competitive inhibition (inhibitor decreases the affinity of substrate binding, capacity is not affected)
- $1 < \alpha \sim 10$  mixed-type inhibition (inhibitor decreases both the affinity and capacity of substrate transport)

# Conclusions

- 2017 FDA DDI guidance provide more detail on experimental design and practical considerations
- Physicochemical parameters of compound should be considered when choosing a test system
- A deeper understanding of the test system is needed for correct data interpretation (e.g. endogenous transporters)
- Appropriate controls should be applied to confirm the specificity of the interaction
- High quality *in vitro* data can be used as input for modeling

# Thank you for your attention!