Fluorosome-*trans*-pgp - Products and Services

December 12, 2013

Fluorosome®-*trans* are unilamellar lipid bilayer vesicles (diameter approximately 200 nm) with water-soluble, drug-binding fluorescent polymers encapsulated within their aqueous interiors. When drugs pass through their lipid bilayer membrane by passive diffusion, they bind (rapidly) to these polymers, causing a time-dependent change in the fluorescence signal. Fluorosomes®-*trans* were designed to measure passive permeability of drugs through lipid bilayer membranes "Determining P-glycoprotein-drug interactions: evaluation of reconstituted P-glycoprotein by a liposomal system and LLC-MDR1 polarized cell monolayers" by D.L. Melchior, F.J. Sharom, R. Evers, G.E. Wright, J.W.K. Chu, S.E. Wright, X. Chu and J. Yabut J. Pharm. Tox. Meth. 65: 64-74 (2012); doi: 10.1016/j.vascn.2012.02.002."

Fluorosome®-*trans*-pgp is a Fluorosome-*trans* variant containing reconstituted Pgp in the lipid bilayer membrane. They have been designed for P-glycoprotein (Pgp) inhibition assays - from single concentration inhibition to IC_{50} determinations. Fluorosome®-*trans*-pgp consists of Pgp-containing lipid vesicles with encapsulated drug-binding polymers, pre-equilibrated with the model Pgp substrate "S-RH". This compound is a substrate for both the H and R sites of Pgp (see Shapiro, A.B. and Ling, V. 1997 Eur. J. Biochem. 250, 130-137), and its inhibition captures all potential inhibitors of Pgp. (We also have available substrates that are selective for the H or the R site of Pgp.)

Plasma membrane preparations from CH^RB30 cells that overexpress Pgp (Doige, C.A. and Sharom, F.J. Protein Expr. Purif. 1991 2, 256-265) are used to purify Pgp to high (90-95%) purity, with very high ATPase activity (Liu, R., Siemiarczuk, A. and Sharom, F.J. 2000 Biochemistry 39, 14927-14938). After reconstitution into phospholipid proteoliposomes (Romsicki, Y. and Sharom, F.J. 1998 Eur. J. Biochem. 256, 170-178), this transport-competent Pgp (Lu, P, Liu, R and Sharom F.J., Eur. J. Biochem. 2001 268, 1687-1697) is incorporated into Fluorosomes by extrusion and gel filtration techniques.

Fluorosome®-*trans*-pgp has the following properties:

- Specificity - Fluorosome®-*trans*-pgp contains only the Pgp transporter.
- Low sample requirement – inhibition by a compound at 10 μM requires ~1 nanomole of compound.
- Speed – 30 seconds per inhibition assay, e.g. an 8 point IC_{50} + 2 references takes only 5 minutes to run.
- Convenience - real time measurement and full analysis in a fluorescence plate reader.
- Popular format - 96 well half-well or 384 well microplates - one well per inhibition assay.
- Full coverage - inhibition at both the H and R sites of Pgp is measured.
- Wide dynamic range - limited only by the solubility of the test compound.
- Simple assay procedure (see below).

Fluorosome®-*trans*-pgp: typical assay procedure

1. Aliquot 20 - 50 μL of Fluorosome®-*trans*-pgp suspension (pre-equilibrated with the substrate S-RH) into wells of a microtiter plate.
2. Add the desired volume of test compound as DMSO stock solution to appropriate wells; up to 5% DMSO is tolerated.
3. After 5 minutes at ambient temperature, run the plate in an injecting fluorescence plate reader. For each well, the reader is programmed to:

   a. read the sample for 5 seconds to establish a baseline (no transporter activity).
   b. inject ATP from stock solution into the sample (initiate transport of S-RH by Pgp).
   c. acquire fluorescence for approximately 20 seconds (measure Pgp transport activity).
4. Following run completion (approximately 30 seconds per well), instrument software immediately determines the slope of the fluorescence change for that concentration of test compound, which indicates the relative level of Pgp transport activity. Percent inhibition is the ratio of the slope obtained for a well containing a certain concentration of test compound, relative to that for control wells containing only DMSO.
5. An inhibition plot is generated by measuring relative Pgp transport activity at (typically) 8 different concentrations of the test compound, and fitting the data to obtain an IC_{50} value for that compound.

The IC_{50} values for representative drugs, tested for inhibition at 8 concentrations under the above assay conditions, are summarized in Table 1. These values compare favorably with those obtained using traditional, cell-based methods, including those utilizing Pgp-expressing cells.

Table 1. Data for correlation of IC_{50} values for Fluorosome-*trans*-pgp and Pgp drug binding affinity

Drug	Fl-*trans*-pgp IC_{50} (μM)	Pgp binding K_d (μM)	Drug	Fl-*trans*-pgp IC_{50} (μM)	Pgp binding K_d (μM)
Telmisartan	0.10		Troglitazone	5.2	
Elacridar	0.12	0.059[3]	Nitrendipine	5.8	7.1[3]
Ritonavir	0.25	0.78[3]	Isradipine	6.6	13[3]
Valspodar	0.25	0.08[3]	Mibefradil	7.1	
Reserpine	0.31	0.73[2]	Verapamil	9.1	2.4[2]
Zosiquidar	0.59	0.055[3]	Vinblastine	9.52	0.77[2]
Nelfinavir	0.59	0.98[3]	Felodipine	16.5	4.3[3]
Cyclosporin A	0.87	0.30[1]	Diltiazem	26.1	
Nicardipine	0.9	6.5[3]	Sertraline	29	18[3]
Indinavir	0.93	0.94[3]	Ranolazine	64.1	
~~Atorvastatin~~	~~1.1~~	~~55~~[5]	Digoxin	80	53[3]
Paclitaxel	1.2	0.038[4]	ALLN	142	138[4]
Quinidine	1.9	7.8[1]	Naloxone	206	54[3]
Ketoconazole	1.96	1.5[3]	Colchicine	218	158[2]
Nifedipine	2.55	48[3]	Captopril	429	
Amiodarone	2.6	2.0[3]	Digoxigenin	633	263[3]
Carvedilol	3.8				

[1] Liu, R., Siemiarczuk, A., and Sharom, F.J. 2000 Biochemistry 39, 14927-14938
[2] Sharom, F.J. (unpublished data)
[3] Liu, R., and Sharom, F.J. 1996 Biochemistry 35, 11865-11873
[4] Sharom, F.J., Liu, R., Romsicki, Y., and Lu, P. 1999 Biochim. Biophys. Acta 1461, 327-345
[5] Goard, C.A., Mather, R.G., Vinepal, B., Clendening, J.W., Martirosyan, A., Boutros, P.C., Sharom, F.J. and Penn, L.Z. 2010 Int. J. Cancer Mar 3. (e-pub ahead of print)

Validation of Fluorosome-*trans*-pgp inhibition data

The IC_{50} values for a panel of important drugs were compared with the binding affinities (K_d) determined for some of them by fluorescence spectroscopic techniques using Pgp in detergent solution (see Table 1). The plot of IC_{50} vs. K_d shown in Figure 1 illustrates the strong correlation between these two parameters over almost 4 orders of magnitude (IC_{50} values ranging from 0.1-633 μM), confirming the validity of the Fluorosome-*trans*-pgp assay.

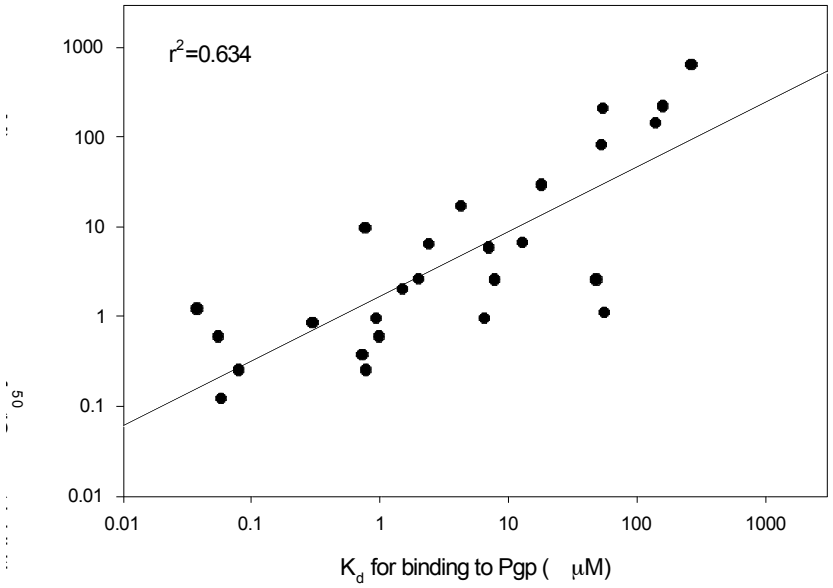


Figure 1. Comparison of drug IC_{50} values for transport inhibition in Fluorosome-*trans*-pgp and their K_d values for binding to Pgp determined by fluorescence spectroscopy

Correlation *(in vitro-in vivo)* of Fluorosome-*trans*-pgp inhibition data

Further validation of the results of Fluorosome-*trans*-pgp inhibition assays was carried out using *in vivo* data for the effects of various co-administered second drugs ("inhibitors") on oral absorption (AUC) and plasma levels ($C_{max,ss}$) of digoxin in human patients. Relevant data for digoxin, AUC and AUC_I and $C_{max,ss}$ and $C_{max,ss,I}$, in the absence and presence of inhibitor I, respectively, were taken from Fenner et al. (Fenner, K.S., Troutman, M.D., Kempshall, S., Cook, J.A., Ware, J.A., Smith, D.A., and Lee, C.A. 2009 Clin. Pharmacol. Ther 85**,** 173-181). [I] is the reported plasma concentration of drug (inhibitor) I, and $[I_2]$ is the estimated intestinal concentration of drug (inhibitor) I (compiled in Fenner et al., 2009). The ratios AUC_I/AUC and $C_{max,ss,I}$ /$C_{max,ss}$ represent the effect on absorption and plasma levels, respectively, of digoxin by drug (inhibitor) I. $IC_{50,Fl}$ values are half maximal-Pgp inhibitory concentrations of drugs from Fluorosome-*trans*-pgp, each obtained in approximately 10 minutes by the standard 96-well plate assay. Table 2 summarizes the data used in the correlations.

Table 2. Data for correlations of Fluorosome-*trans*-pgp IC_{50}s with effects on digoxin disposition							
	Digoxin *in vivo*		**"inhibitor" *in vivo***		**Fl-*t*-pgp**		
	AUC_I/AUC	**$C_{max,ss,I}/C_{max,ss}$**	**[I]***	**$[I_2]$†**	**$IC_{50,Fl}$‡**	**$[I]/IC_{50,Fl}$**	**$[I_2]/IC_{50,Fl}$**
Diltiazem	1.44	1.38	0.7	532	26.1	0.0268	20.383
Quinidine	1.76	1.75	3.54	3397	1.3	2.723	2613
Telmisartan	1.22	1.58	1.11	933	0.1	11.1	9330
Troglitazone	1.04	10.5	3.62	3264	5.2	0.696	627.7
Verapamil	1.51	1.44	1.2	652	11.9	0.101	54.79
Carvedilol	1.56	1.38	0.13	61.5	3.8	0.0342	65.3
Felodipine	1.18	1.34	0.03	104	16.5	0.00182	6.303
Mibefradil	1.31	1.41	2.42	1271	7.1	0.341	179.0
Nicardipine	1.06	1.06	0.18	248	0.9	0.2	275.6
Ranolazine	1.6	1.46	8.4	9356	64.1	0.131	145.96
Amiodarone	1.68	1.84	2.2	4693	2.6	0.846	180.5
Isradipine	1.11	1.26	0.02	161.5	6.6	0.003	24.47
Sertraline	1.1	1.05	0.39	2612	29	0.013	90.07
Captopril	1.39	1.59	0.23	230	*ca.* 425	0.00054	0.54
Nifedipine	1.23	1.06	0.23	115.5	2.55	0.09	45.29
Nitrendipine	1.15	1.57	0.03	222.0	5.8	0.0052	38.28

* μM plasma concentration of drug ("inhibitor") at steady state.
† estimated μM intestinal concentration of drug ("inhibitor") after oral dose.
‡ μM concentration for half-maximal inhibition of drug ("inhibitor") transport by Fluorosome-*trans*-pgp.

Plots of AUC_I/AUC and $C_{max,ss,I}/C_{max,ss}$ vs. I/IC_{50} and I_2/IC_{50}, where IC_{50} values are measured with Fluorosome-*trans*-pgp, demonstrate the correlations (see next page). It is assumed that a change in digoxin absorption or plasma concentration of >25%, i.e. AUC_I/AUC and $C_{max,ss,I}/C_{max,ss}$ >1.25, would represent a potentially toxic effect caused by the second drug (inhibitor). It is also assumed that values of $[I]/IC_{50} < 0.1$ and $[I_2]/IC_{50} > 10$ are cutoffs for significant changes in digoxin disposition (The International Transporter Consortium. 2010 Nature Rev. Drug Disc. **9**, 215-236). In all cases, compounds in the upper left quadrant are "false negatives" and those in the lower right quadrant are "false positives".

Using IC_{50} values measured with Fluorosome-*trans*-pgp for 16 of the 19 drugs reported by Fenner et al. (2009), the resulting plots are equivalent or superior to those based on net secretory flux results in Caco-2 cell monolayers reported in that paper. In particular, the plot of AUC_I/AUC vs. $[I_2]/IC_{50}$ (Figure 3) leaves a single false negative (captopril), and the plot of $C_{max,ss,I}/C_{max,ss}$ vs. $[I_2]/IC_{50}$ (Figure 5) leaves two false negatives (captopril and felodipine). Fewer false positives are seen in both correlations with $[I]/IC_{50}$ (Figures 2 and 4) compared with those of Fenner et al.

Figure 2

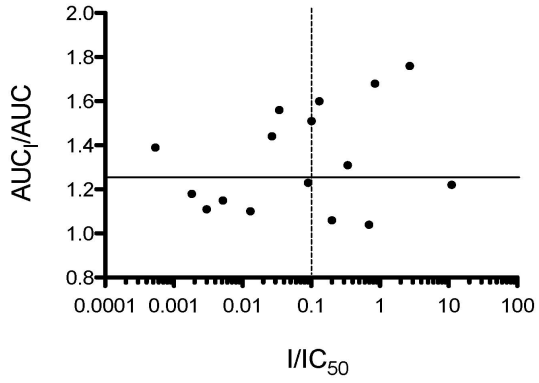


Figure 3

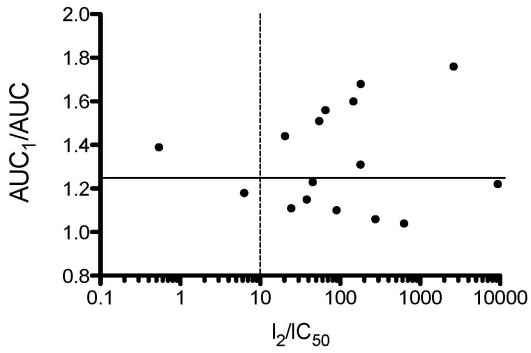


Figure 4

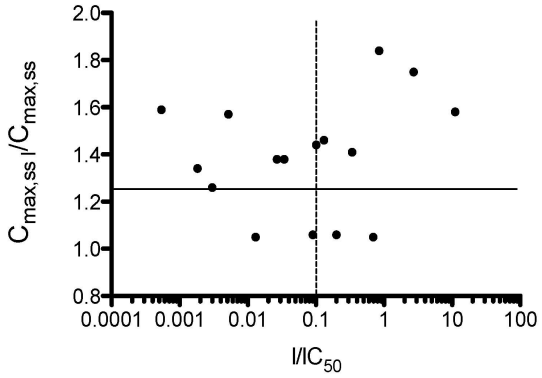
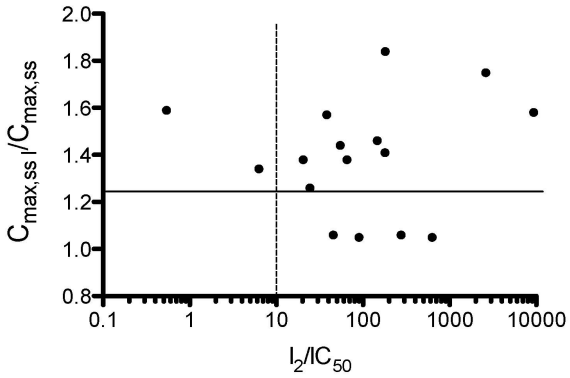


Figure 5



For further information see additional data on the website www.fluorosome.com or contact:

Praveen Bansal
Phone: 617-842-6663
Email: praveen.bansal@fluorosome.com

George E. Wright
Phone: 508 7546700, ext. 102
Email: george.wright@fluorosome.com

Donald L. Melchior
Phone 508 7546700, ext 105
Email: donald.melchior@fluorosome.com

TFC Biosciences
One Innovation Drive
Worcester, MA 01605