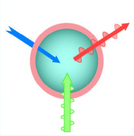

Reconstituted P-glycoprotein in Fluorosome® lipid bilayer vesicles - basis for an *in vitro* P-glycoprotein assay.

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1. Introduction

The need for a simple, direct method to characterize P-glycoprotein (Pgp, MDR1) substrates and inhibitors led us to combine two methodologies:

Fluorosome®-trans;

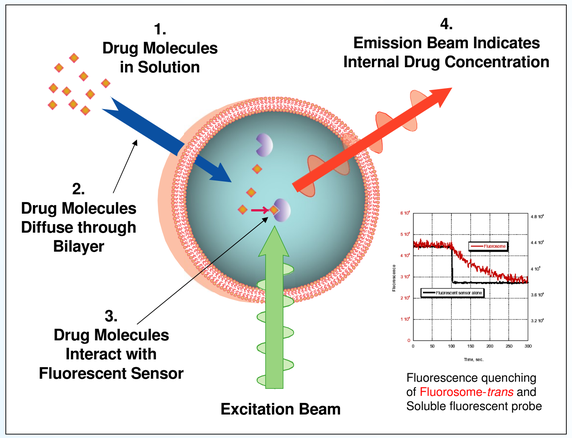
Pgp isolation and membrane reconstitution.

to produce a prototype *in vitro* Pgp assay -

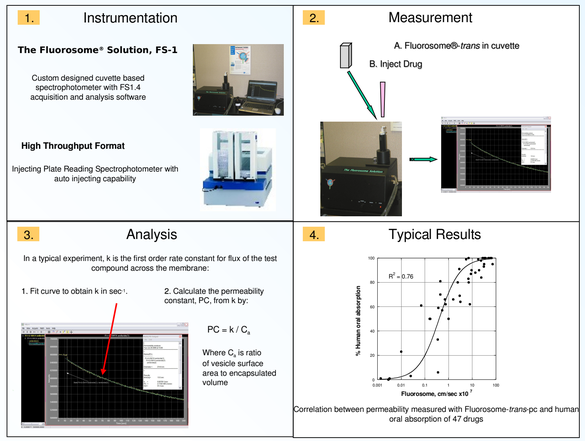
"Fluorosome-*trans*-pgp".

Fluorosome®-*trans* is a fluorophore-containing liposome developed to measure the passive diffusion (permeability) of drugs across membrane bilayers. The incorporation of reconstituted Pgp into the bilayers of Fluorosomes results in a system capable of measuring active, Pgp mediated, ATP-dependent transport of small molecules and to characterize inhibitors of the Pgp.

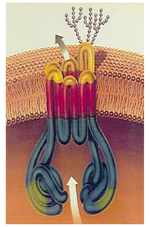
2. The Fluorosome-*trans*-vesicle



3. The Fluorosome-*trans* Assay
Trans-membrane Passive Drug Diffusion



4. The Pgp Molecule



P-glycoprotein (Pgp, MDR1, ABCB1) is a member of the ATP-binding casette (ABC) superfamily. It is a 170 kDa intrinsic membrane protein. Structural models suggest that 2 nucelotide-binding domains are closely associated to form a nucleotide sandwich dimer. Pgp is an outwardly directed flippase for fluorescent phospholipid and glycosphingolipid derivatives, which suggests that it may also translocate drug molecules from the inner to the outer membrane leaflet. The ATPase catalytic cycle of the protein is thought to proceed via an alternating site mechanism, although the details are not clear. The membrane lipid bilayer plays an important role in Pgp function and may regulate both the binding and transport of drugs[1].

[1]Frances J. Sharom in "Drug Transporters: Molecular Characterization And Role in Drug Disposition" eds. G. You, M.E. Morris, Chapter 10, pgs. 223-262, Wiley Interscience, 2007.

5. Fluorosome-*trans*-pgp manufacture

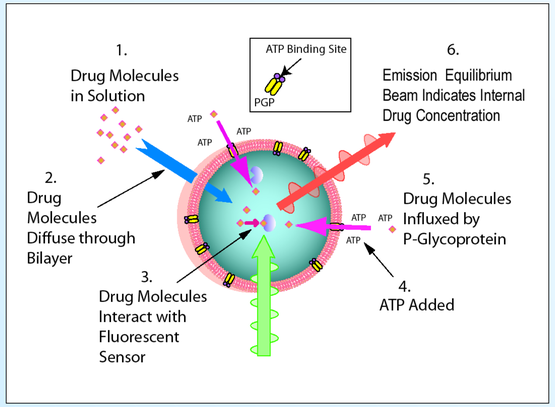
Isolation and Purification of Pgp

Pgp is isolated from Pgp-overexpressing CHRB30 cells by detergent (CHAPS) extraction followed by affinity chromatography on Con A-Sepharose. This micellar form of Pgp is reconstituted into lipid bilayers by being mixed in solution with CHAPS solubilized micellar phospholipid and the detergent removed by gel filtration. The resulting reconstituted pgp is aliquoted and stored frozen at -70°C.

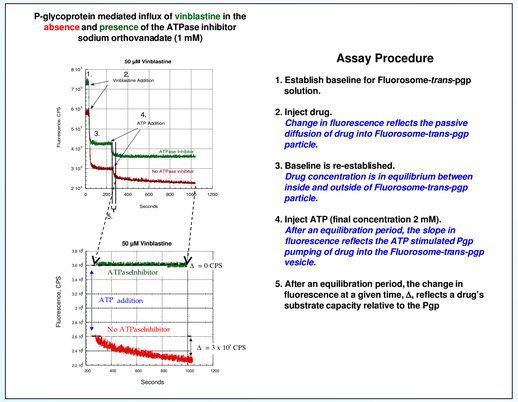
Manufacture of Fluorosome-*trans*-pgp

Frozen reconstituted Pgp is thawed and mixed in buffer with fluorophore (BSA-fluorescein) then converted into Fluorosome-*trans*-pgp by extrusion. The resulting unilamellar vesicles are separated from unencapsulated fluorophore by gel exclusion chromatography. The Fluorosome-*trans*-pgp is then subjected to Quality Control Criteria; size,fluorescence, passive permeability, ATPstimulated control substrate transport.

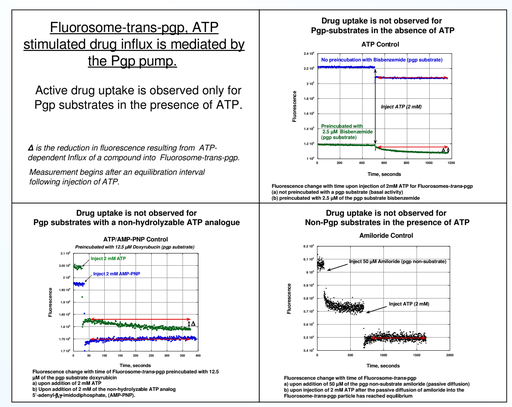
6. The Fluorosome-*trans*-pgp Vesicle



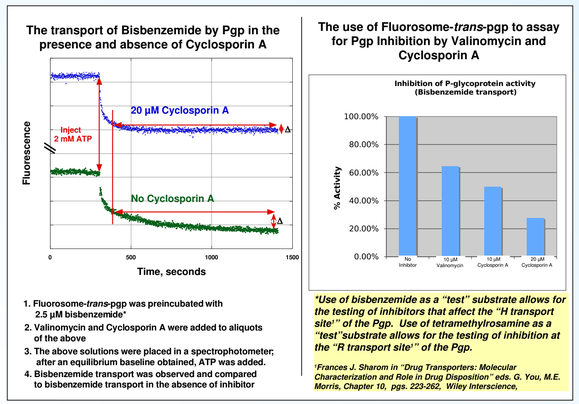
7. The Fluorosome-*trans*-pgp Assay
Pgp-mediated active Drug Transport



8. Fluorosome-*trans*-pgp
Assay Controls



9. Fluorosome-*trans*-pgp as a Pgp inhibitor assay



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