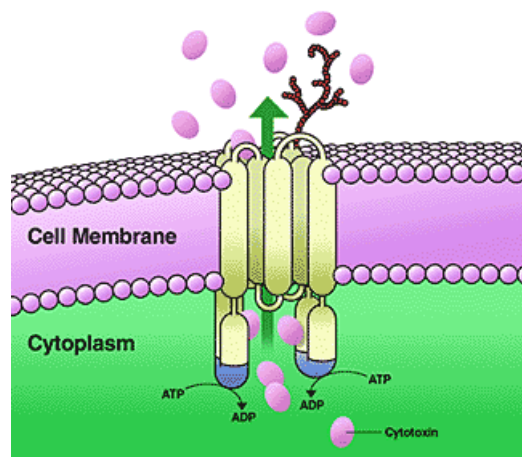
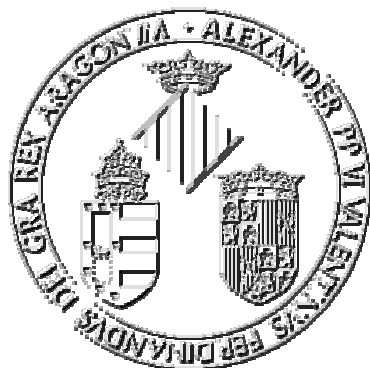




# memtrans



**Membrane transporters:  
In vitro models for the study of  
their role in drug fate  
(FP6 LSHB-CT-2006- 518246).**



*Assoc Prof. Marival Bermejo  
Depto. Farmacia y Tecnología Farmacéutica  
Facultad de Farmacia.  
Universidad de Valencia  
España*

*Image from: <http://www.glycoscience.com/glycoscience/images/ImgD004-A.gif>*

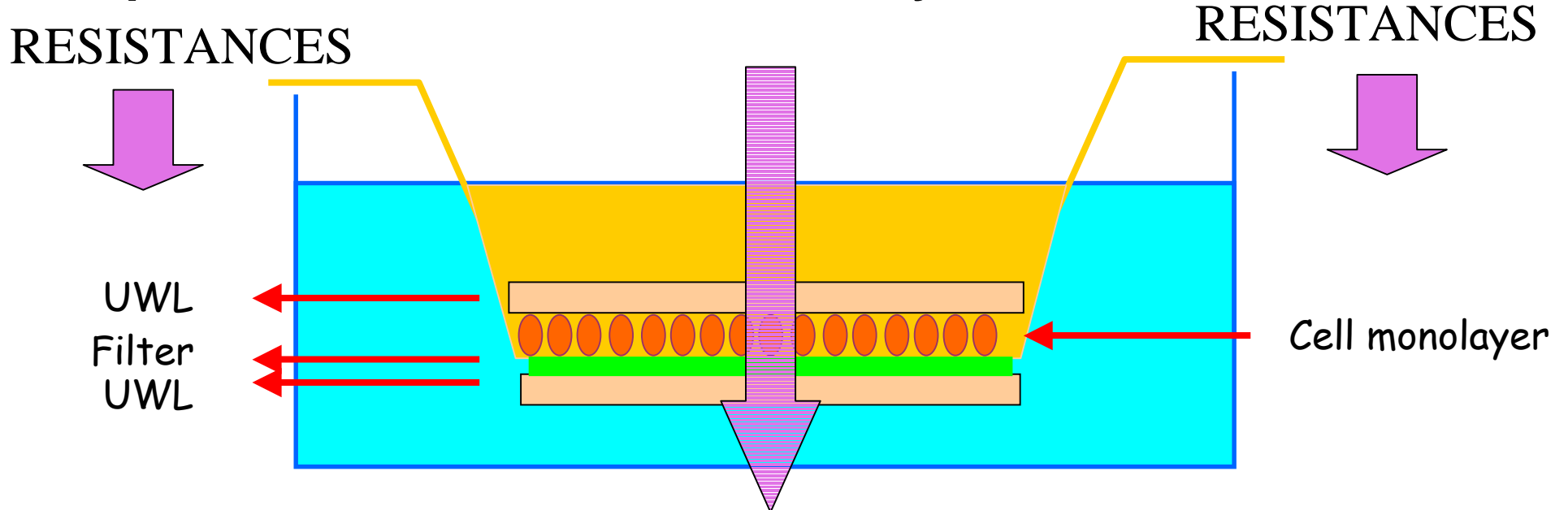


**Dept. of Pharmacy and Pharm. Tech.**

## GOAL

- To optimize and pre-validate *in vitro* cultured cell models to predict oral absorption and pharmacokinetics of efflux systems substrates (P-gp),

# Components of effective Permeability



Permeation Routes

Transcellular

Paracellular

Factors

- Membrane
- Transporter levels
- UWL
- Media comp

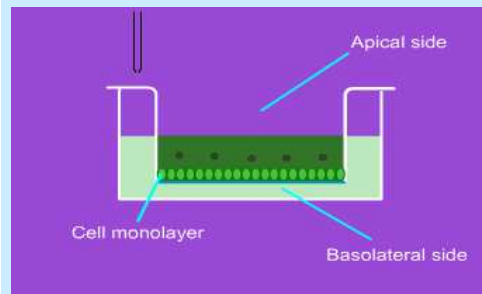
- Tight junctions
- Pore size, density
- UWL
- Media comp.

## Experimental Conditions affecting Permeability

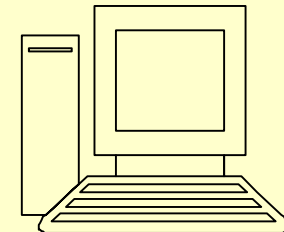
- Pre - experimental factors



- Experimental conditions



- Post-experimental factors: Calculations



$$\frac{d[ATP]}{dt} = k_{in} - k_{out} \cdot [ATP] = 0$$

$$\frac{d[ATP]}{dt} = k_{in} - k_{out} \cdot [ATP]$$

Total Effective Permeability  $P_{effT}$

Paracellular Permeability  $P_{para}$

Transcellular Permeability  $P_{trans}$  → Passive diffusion  $P_{diff}$  Carrier Mediated  $P_c$

Total Monolayer Permeability  $P_{cells}$

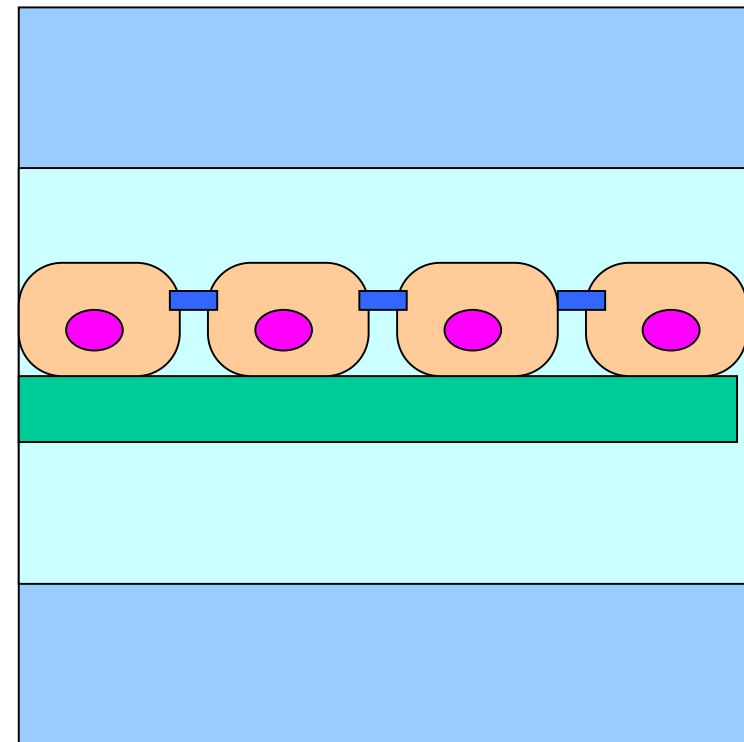
Unstirred Water Layer Permeability  $P_{UWL}$

Filter Permeability  $P_{filter}$

$$\frac{1}{P_{effT}} = \frac{1}{P_{UWL}} + \frac{1}{P_{cell}} + \frac{1}{P_{filter}}$$

$$P_{cell} = P_{trans} + P_{para}$$

$$P_{trans} = P_{diff} (\pm) \frac{V_{max}}{K_m + C_i?}$$



## Standardization of results

- Option A: to standardize of all the experimental factors affecting the individual resistances in total permeability.
- Option B: To use markers of individual resistances and to “weight” or normalize values to combine data coming from different experimental set ups.

## Technological objectives: Expected achievements.

- Prevalidation of different methods to predict gastrointestinal absorption of secretion transporters substrates.
- Standard operation procedures for maintaining and working with the developed models in order to reduce inter-laboratory variability
- Mathematical models to predict from *in vitro* data the *in vivo* intestinal efflux characteristics and its impact in drug ADME.

- WP1. Selection of model compounds and cell lines
- WP2 validation of analytical methods. Design of SOP for cell cultures and transport experiments
- WP3 Development of permeability experiments-analysis of samples
- WP4 modelling of transport for individual compounds, in each cell line
- WP5 development of prediction models.

# WP1

- Cell lines:
  - Caco-2,
  - MDCKII-WT and
  - MDCKII-MDR.
  
- Compounds:
  - Celiprolol,
  - Fexofenadine,
  - Quinidine,
  - Loperamide,
  - Talinolol,
  - Saquinavir,
  - Paclitaxel

## WP2: Prevalidation of cell lines and SOP's

- Demonstration of low paracellular permeability with either  $^{14}\text{C}$ -Mannitol or Lucifer Yellow
- Demonstration of high transcellular permeability with Metoprolol or  $^3\text{H}$ -Metoprolol.
- Demonstration of Pgp efflux using Rhodamine 123

## Part 1 conclusions

- Variability within and between laboratories is very high (60 to 30%) even with standardized protocols.
- Normalization of drug  $P_{eff}$  values with the  $P_{eff}$  of markers would reduce this variability.
- Standardization of calculation methods is essential: More than 50% of differences in  $P_{eff}$  estimations using SAME data.