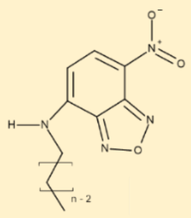
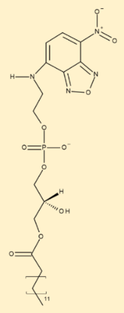
Interaction of a homologous series of amphiphiles with P-glycoprotein in a membrane environment: Contributions of polar and non-polar interactions

Maria João Moreno,[1,2] Hugo AL Filipe,[1,2] Susana VP Cunha,[1] Cristiana Ramos,[1] Patrícia AT Martins,[1] Biebele Abel,[3] Luís MS Loura,[1,2,4], Suresh V Ambudkar,[3]

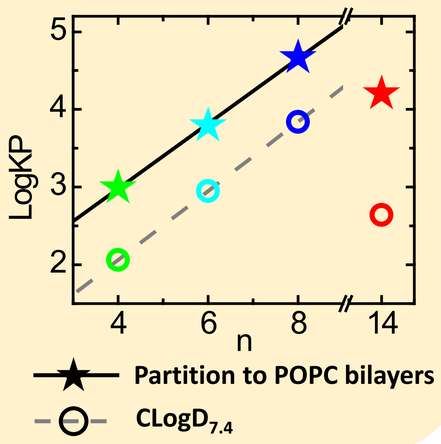
[1] Coimbra Chemistry Center, Chemistry Department, Faculty of Sciences and Technology, University of Coimbra, 3004-535 Coimbra, Portugal;
[2] CNC - Center for Neuroscience and Cell Biology, University of Coimbra, 3004-535 Coimbra, Portugal
[3] Laboratory of Cell Biology, CCR, National Cancer Institute, NIH, Bethesda, MD 20892, USA;
[4] Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal

The ligands studied have different lengths of alkyl chains and different lipophilicity



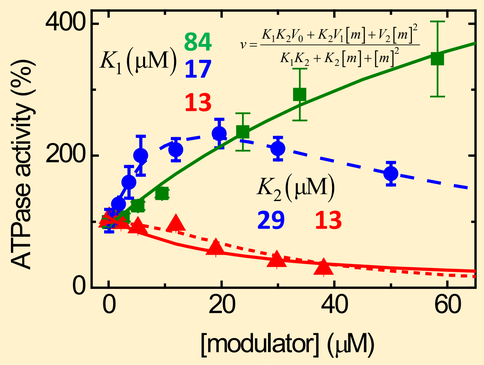


NBD-C4 (n=4)
NBD-C6 (n=6)
NBD-C8 (n=8)
NBD-LysoMPE



The usual assay and analysis leads to the expected results

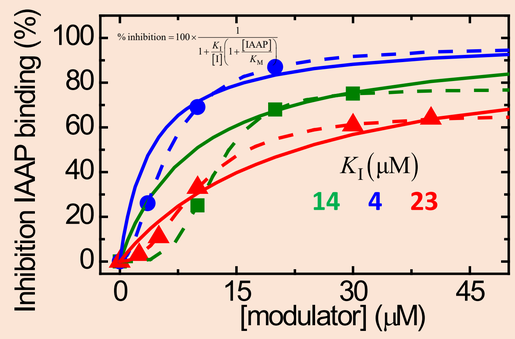
P-gp ATPase activity (0.1gP/L)



↑ lipophilicity ⇒ ↑ **Apparent** affinity

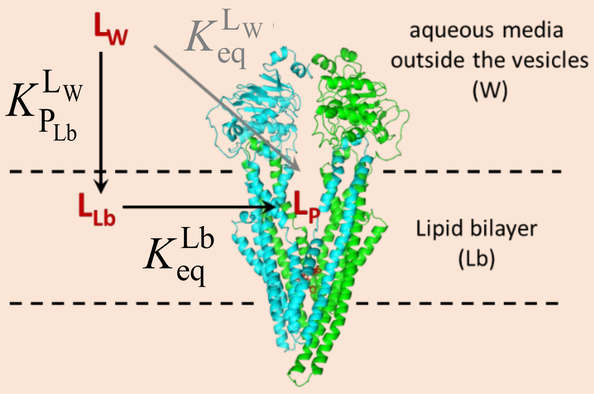
Different assays give different results…

inhibition of [^{125}I] IAAP binding (1gP/L)



Similar **Apparent** affinity for all

The distribution of the ligand in the distinct media MUST be included in the analysis



when assuming…

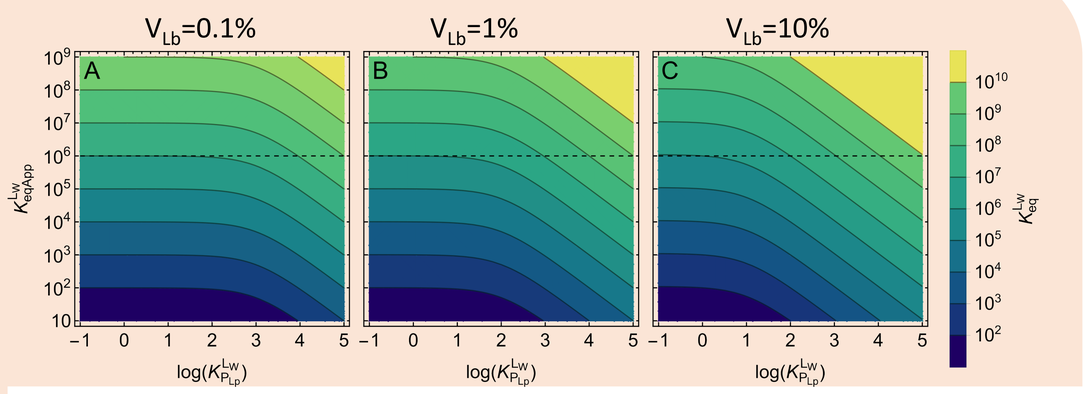
$[L_W] \cong [L_T] \Rightarrow K^{L_T}_{eqApp}$

$[L_W] \cong [L_T] - [L_P] \Rightarrow K^{L_W}_{eqApp}$

$[L_W] = [L_T] - ([L_P] + [L_{Lb}])$

$K^{L_W}_{eq}$

Intrinsic Binding Affinity

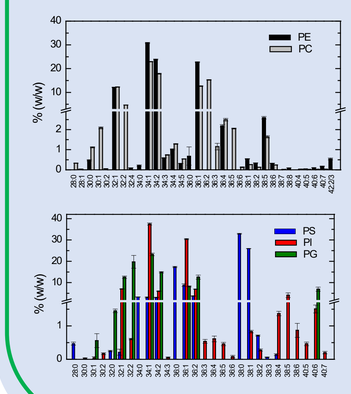


$K^{L_W}_{eqApp}$ = 10^6 M^{-1} may correspond to $K^{L_W}_{eq}$ from 10^6 to 10^{10} M^{-1}

Partition coefficient of the ligands to the lipid bilayer and to the whole membrane

Lipidome

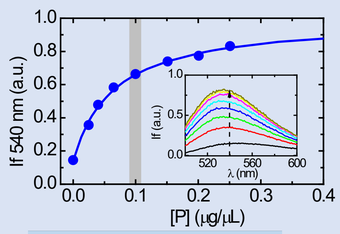
membranes from High Five insect cells overexpressing P-gp

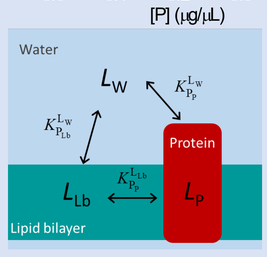


0.6 ± 0.1 gLipid/gProtein

POPE:POPC:POPS 45:35:20

I_F from NBD-group increases when in the membrane





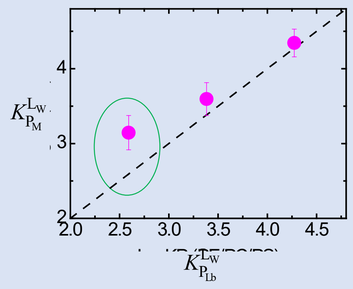
$K^{L_W}_{P_M} = K^{L_W}_{P_{Lb}} \frac{V_{Lb}}{V_M} + K^{L_W}_{P_P} \frac{V_P}{V_M}$

$K^{L_W}_{P_P} = K^{L_W}_{P_{Lb}} K^{L_{Lb}}_{P_P}$

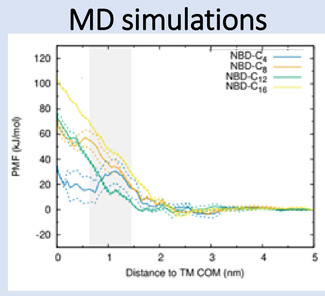
↑ Lipophilicity ⇒ ↓ relative affinity for P-gp $K^{L_{Lb}}_{P_P}$

Log	$K^{L_W}_{P_M}$	$K^{L_W}_{P_{Lb}}$	$K^{L_W}_{P_P}$	$K^{L_{Lb}}_{P_P}$
NBD-C4	3.1	2.6	4.8	2.2
NBD-C6	3.6	3.4	5.0	1.6
NBD-C8	4.3	4.3	5.4	1.1

NBD-C4 has the highest relative affinity for P-gp



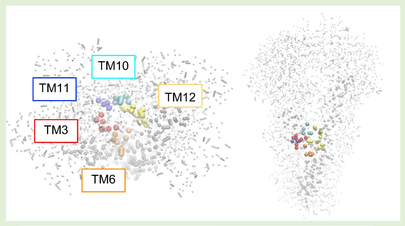
Same conclusion from MD simulations

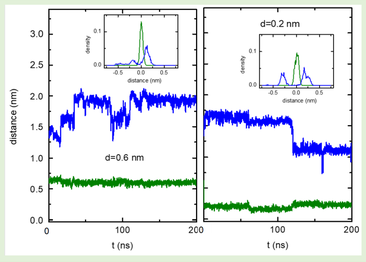


Re-analysis of P-gp's ATPase activity and IAAP displacement assays

many binding sites in P-gp's binding pocket

MD simulations





$L_W \xleftrightarrow{K_P^{W \to Lb}} L_{Lb} \quad [L_{Lb}] = K^{L_W}_{P_{Lb}} V_{Lb}/V_W [L_W]$

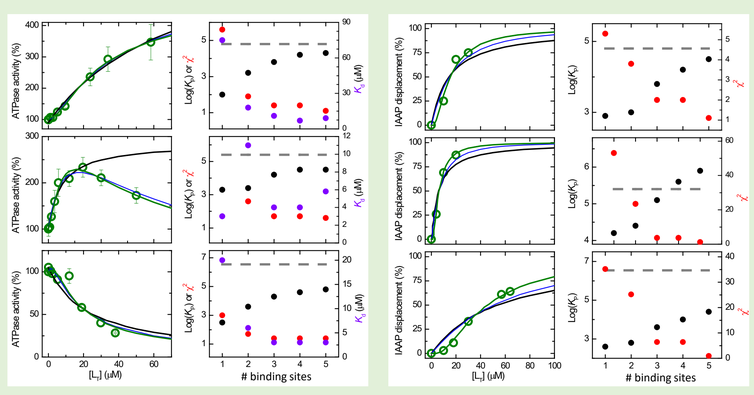
$L_W + P \xleftrightarrow{\beta_1} PL_1 \quad [PL_1] = \beta_1 [P][L_W]$

$i\,L_W + P \xleftrightarrow{\beta_i} PL_i \quad [PL_i] = \beta_i [P][L_W]^i$

$n\,L_W + P \xleftrightarrow{\beta_n} PL_n \quad [PL_n] = \beta_n [P][L_W]^n$

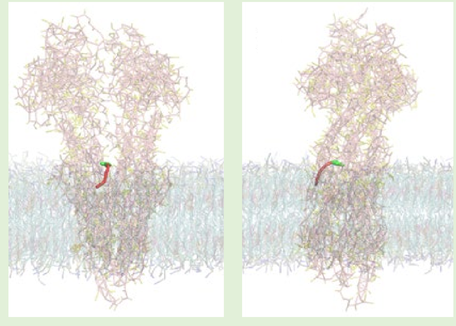
ATPase activity **IAAP displacement**

NBD-C4
NBD-C8
NBD-LysoMPE



Similar binding affinity for all ligands
Several ligands required for IAAP displacement

Inhibition of ATPase activity by long chain amphiphiles



The alkyl chain remains in the lipid bilayer

Conclusions

Increasing ligand lipophilicity increases its association with the lipid bilayer <u>but not with P-gp</u>

P-gp's binding pocket can simultaneously bind several ligand molecules

Very lipophilic ligands inhibit P-gp's ATPase activity due their anchoring in the lipid bilayer

Acknowledgements

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Marvin Sketch was used to calculate ligand's molecular descriptors. http://www.chemaxon.com