

*Supplementary Information for*

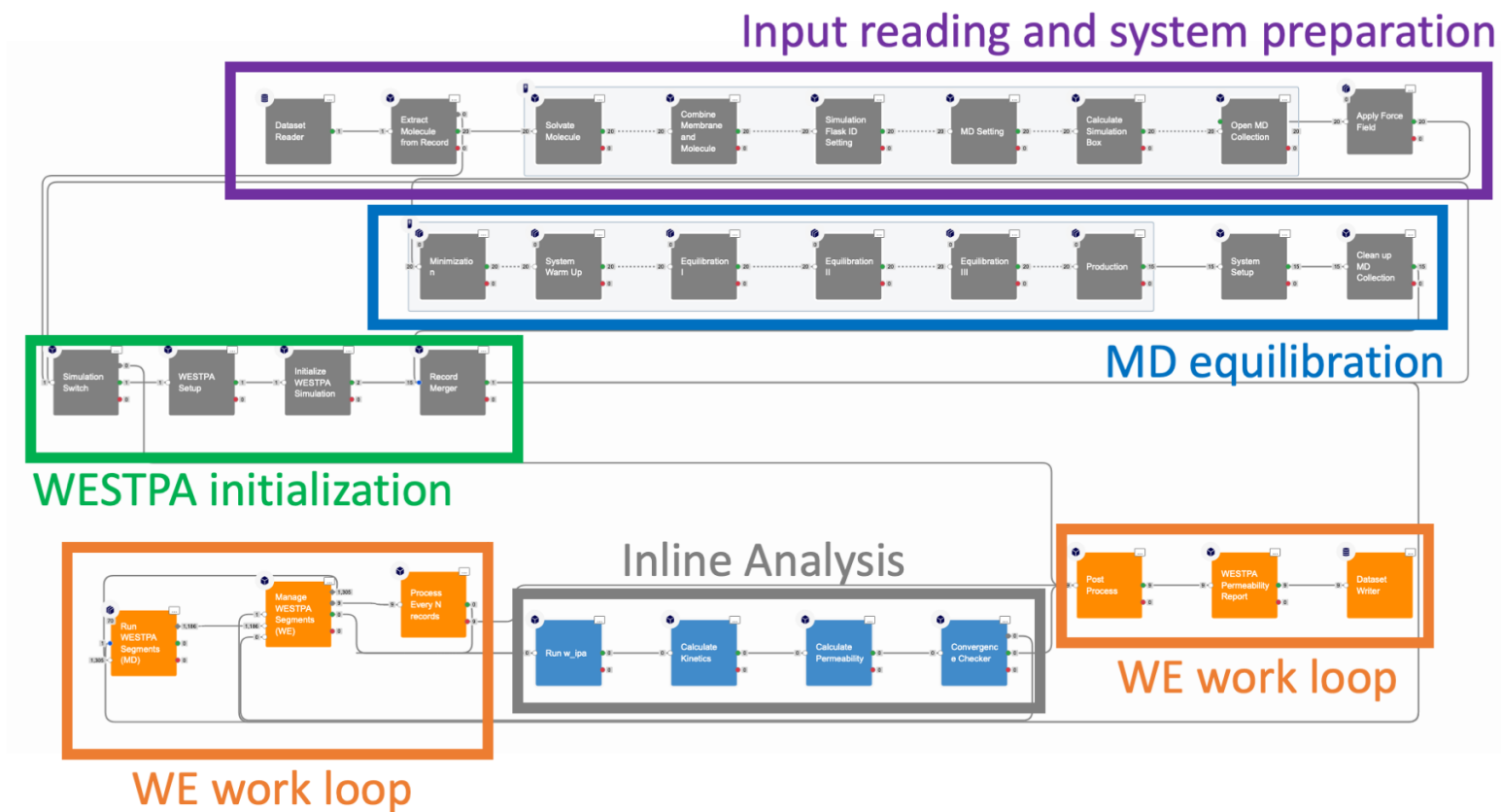
**Mechanistic Insights into Passive Membrane Permeability of Drug-Like Molecules from a Weighted Ensemble of Trajectories**

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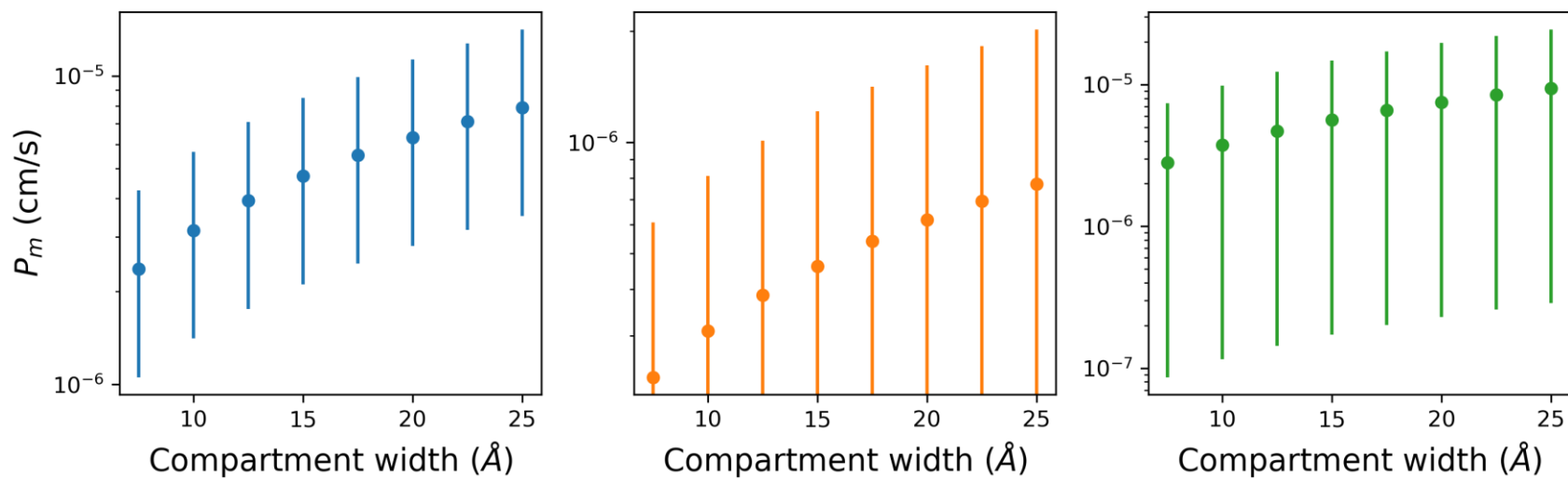
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**Figure S1. Structural layout of the OpenEye Permeability Floe**, which can be broken into 5 logical phases: (1) Preparation of input the permeate system (purple); (2) Equilibration using a standard MD protocol of the aqueous ligand/membrane system (blue); (3) Initialization of the WESTPA toolkit (green); (4) Loop of segment trajectory splitting/recycling events using the WE algorithm, and output trajectory storage (orange); (5) Inline analysis of the permeability simulation for kinetics and convergence criteria (gray).



**Figure S2. The estimated permeability as a function of the compartment width.** The upper limit, 25 Å, is the full length of the water compartment in our simulation ( $(L_z - 40\text{Å})/2$ ). The lower limit, 7.5 Å, roughly corresponds to the thickness of the water layer near the membrane surface.

### Permeability - Run Permeability Simulation (GPU) (Permeability)

A floe to calculate the passive membrane permeability of a drug-like molecule on GPUs

**Job Properties**

Name:

Email me when this job completes:  Yes

Output path: *The folder where this job's output will be saved*

**Job Cost Limits** Email: not set Terminate: not set

**Promoted Parameters**

**Inputs**

Required input parameters

Input Dataset: *The dataset(s) to read records from*  \* Value is required

**Outputs**

Required output parameters

Output Dataset:  \* Value is required

**System Preparation Parameters**

**Weighted Ensemble Parameters**

Number Of Basis States:  *A set of Omega conformers used as input to permeability*

MAB:  On *Whether enable the Minimal, Adaptive Binning (MAB) scheme*

Iteration Interval (Tau):  *Length of each WESTPA iteration in picoseconds*

Iterations:  *Number of iterations for the WESTPA simulation*

Restart Simulation:  Off *Restart (instead of continue) the simulation if it is unfinished*

Reweighting:  On *Whether or not reweighting of the walkers should occur to reduce the relaxation time in steady-state simulations.*

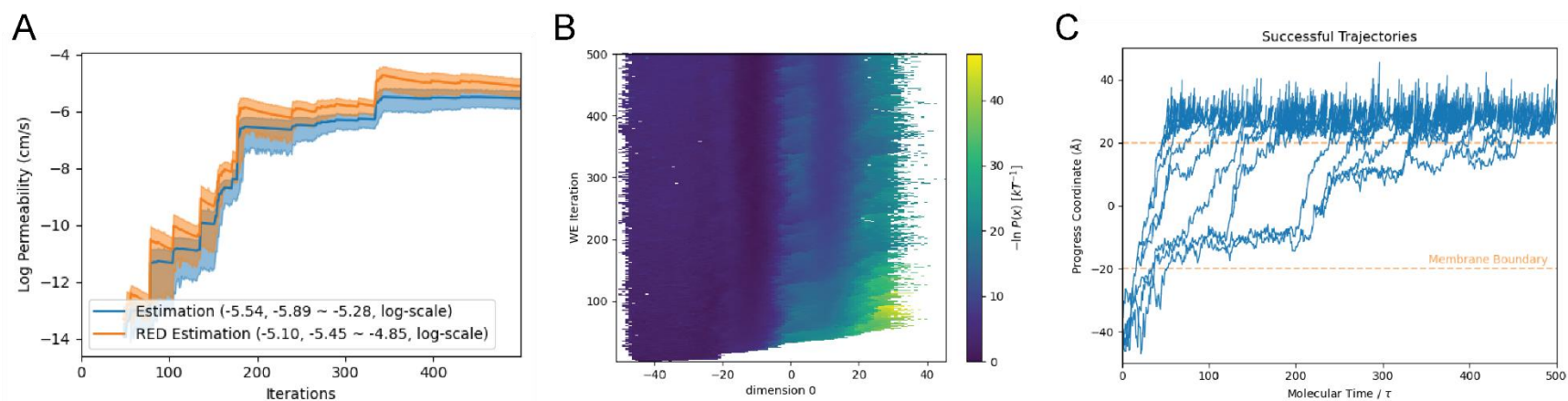
Convergence Detection:  Off *Whether or not automatically detect convergence of the simulation.*

**Advanced Weighted Ensemble Parameters**

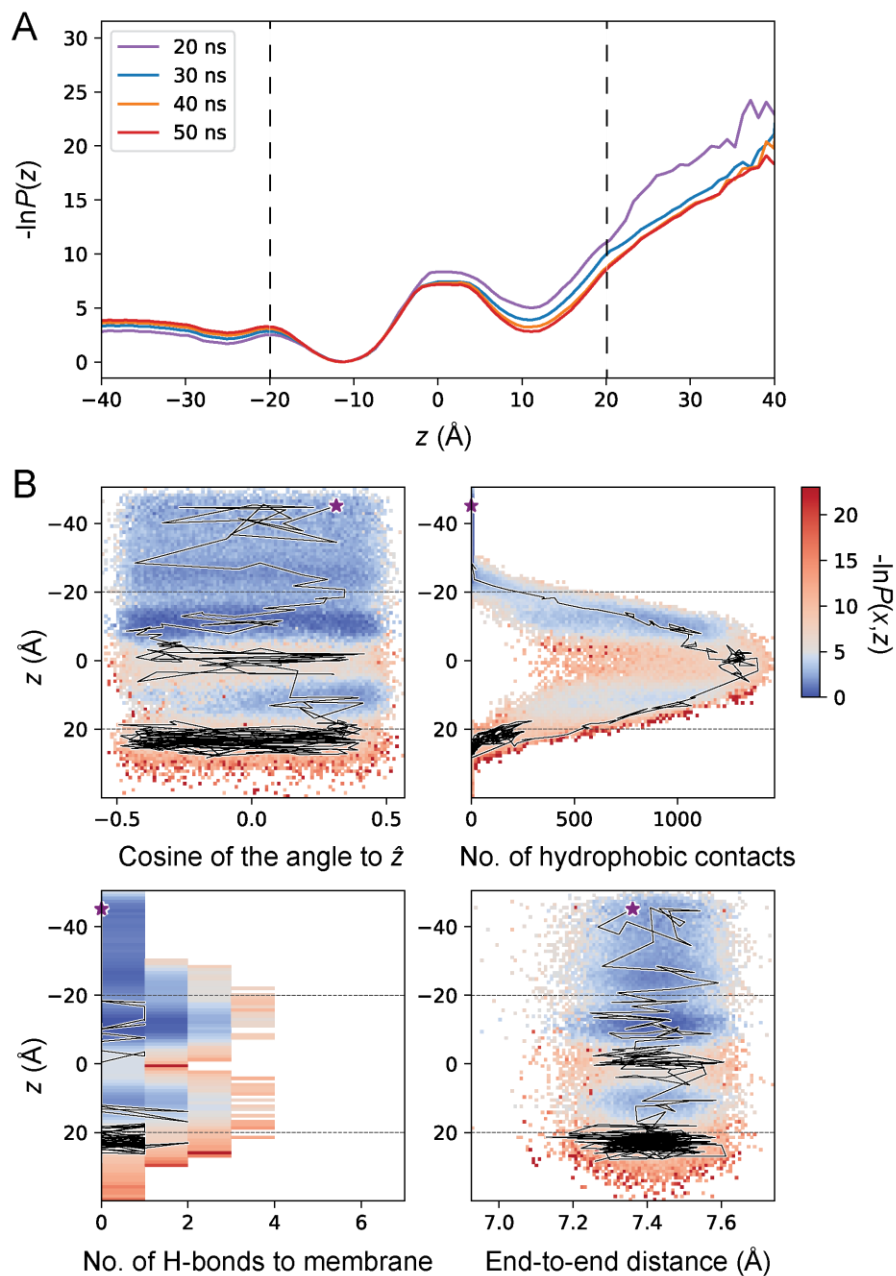
**Miscellaneous Parameters**

Show cube parameters:  No

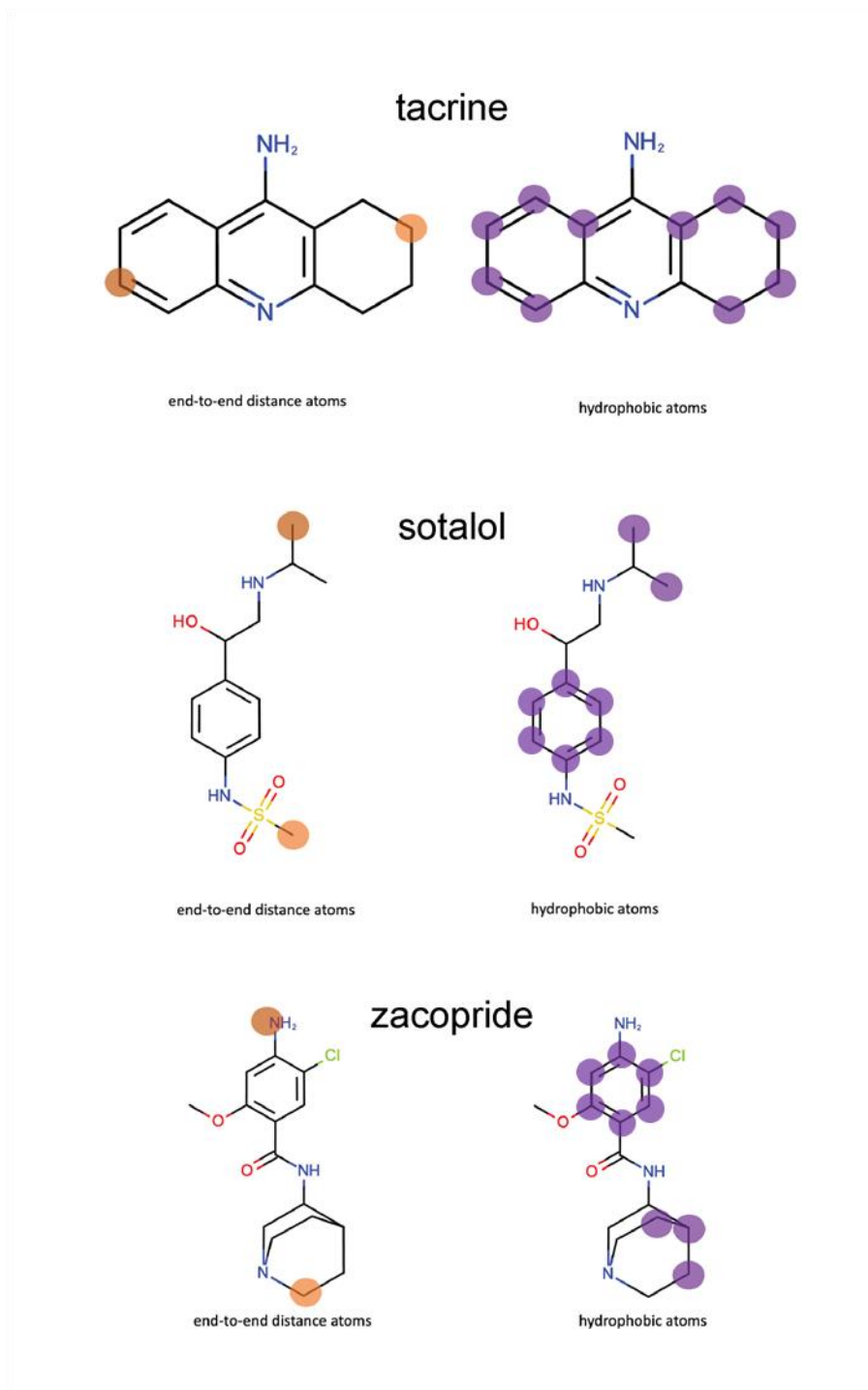
**Figure S3. The Floe setup GUI of the OpenEye Permeability Floe.** All the parameters have a brief description of their function and are grouped with other parameters of the same component (e.g., system preparation, WE simulation, etc.) for easier navigation on the user's end.



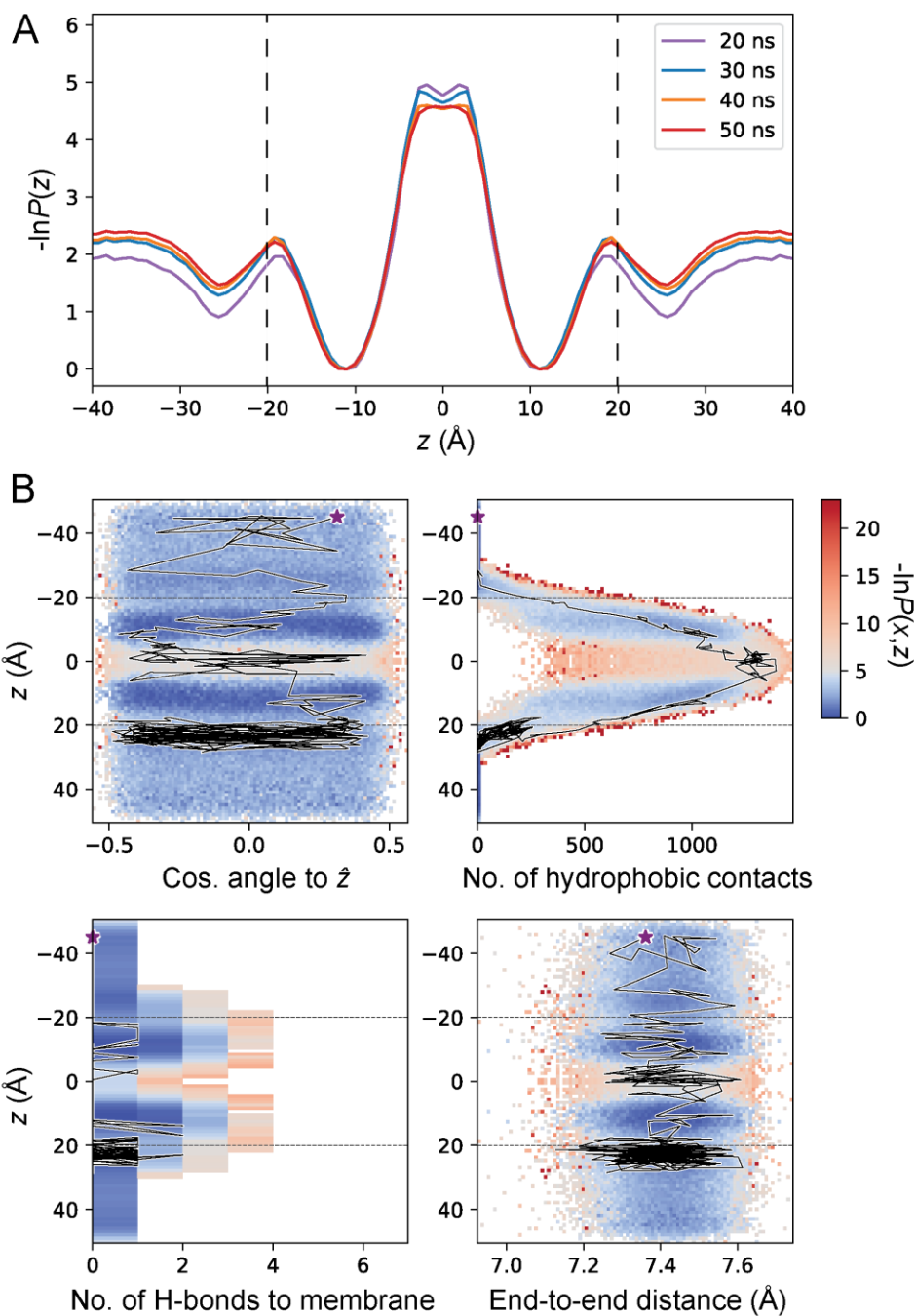
**Figure S4. Simulation report figures generated by OpenEye Permeability Floe.** **A)** The time (iteration=molecular time/ $\tau$ ) evolution of the permeability estimate (*blue*) and that estimated using RED (*orange*). The curves represent the mean estimates, and the shaded areas represent 95% CI. **B)** The time evolution of the probability distribution (in units of  $k_B T$ ). **C)** All the recycled trajectories in the regular WE with fixed binning scheme run represented by the progress coordinate,  $z$  (y-axis), versus the molecular time in terms of the number of iterations (x-axis).



**Figure S5. The original, unsymmetrized (inverted) probability distribution along the lipid normal ( $\hat{z}$ ) for tacrine using the reweighting WE protocol (WESS). Note that, due to the recycling condition imposed by the WE steady-state protocol, this is not a free energy profile. See [Figure 3](#) legend for details.**



**Figure S6. Chemical structures of tacrine, sotalol, and zacopride.** Atoms that were chosen to calculate the end-to-end distances were marked by orange circles, and the hydrophobic carbons were marked by purple circles.



**Figure S7.** Free energy profiles along the lipid normal ( $\hat{z}$ ) and auxiliary coordinates for tacrine using the MAB scheme and reweighting WE protocol (MAB + WESS). See [Figure 3](#) legend for details.



**Table S1. Predicted and experimentally determined permeabilities.**

<b>Compound</b>	<b>MAB Adaptive binning</b>	<b>WESS Reweighting</b>	<b>Platform</b>	<b>Predicted Log <math>P_m</math> (cm/s)</b>	<b>Expt. Log <math>P_m</math> (cm/s)</b>
Tacrine			GPU	$-5.54 \pm 0.13$	-4.64, -5.03 $\pm$ 0.2,
		×	GPU	$-3.23 \pm 0.09$	
	×		GPU	$-6.96 \pm 0.16$	
	×	×	GPU	$-4.27 \pm 0.24$	
	×	×	CPU	$-5.20 \pm 0.28$	
Sotalol	×	×	GPU	$-5.32 \pm 0.22$	-6.02, -5.58 (TtoB), -6.74 (BtoT)
Zacopride	×	×	GPU	$-6.35 \pm 0.22$	-5.23