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Drug (Re)Design guided by biophysical characterization of interactions with biomimetic membranes

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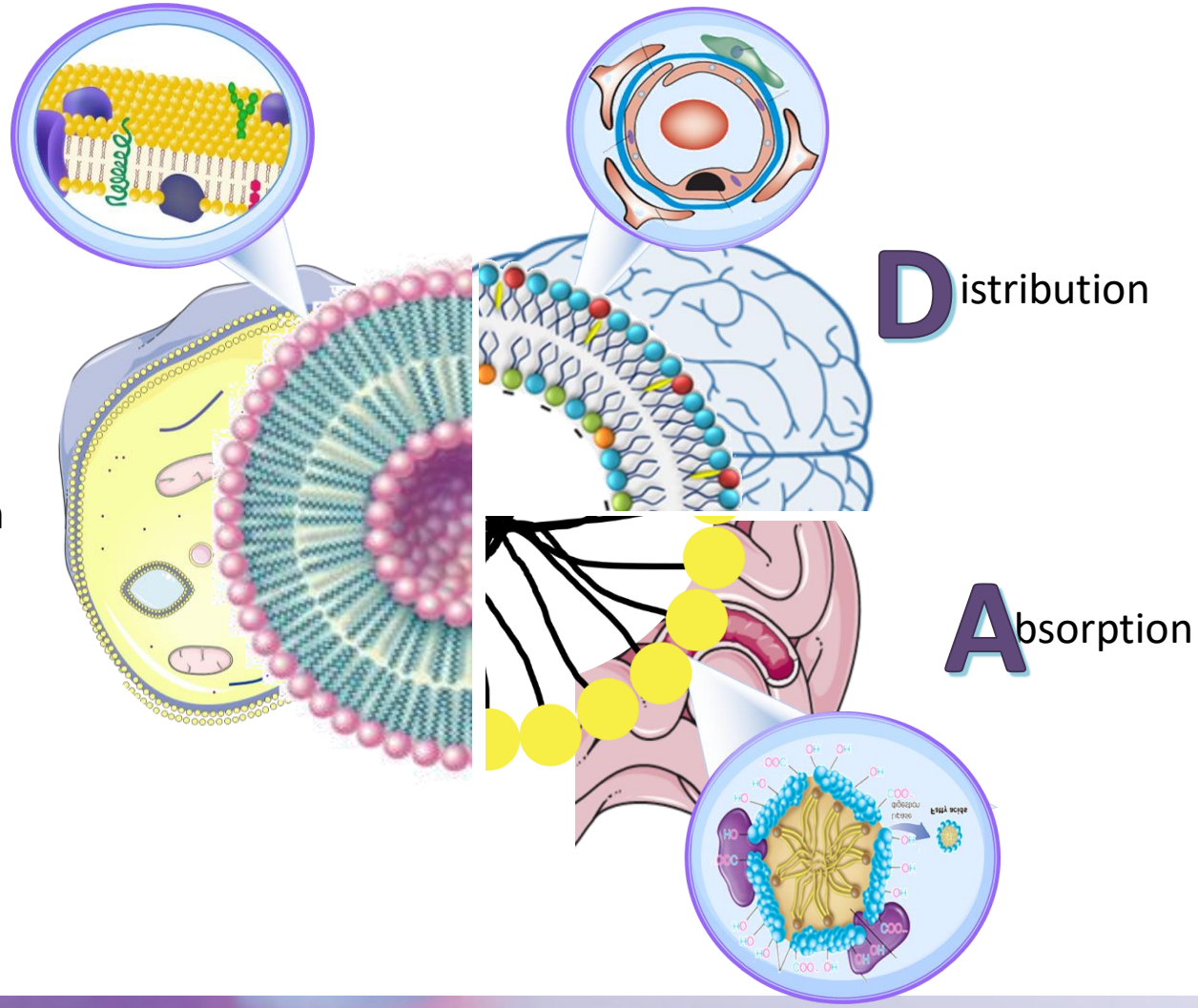
Drug (Re)Design guided by biophysical characterization of interactions with biomimetic membranes

Absorption

Distribution

Toxicity



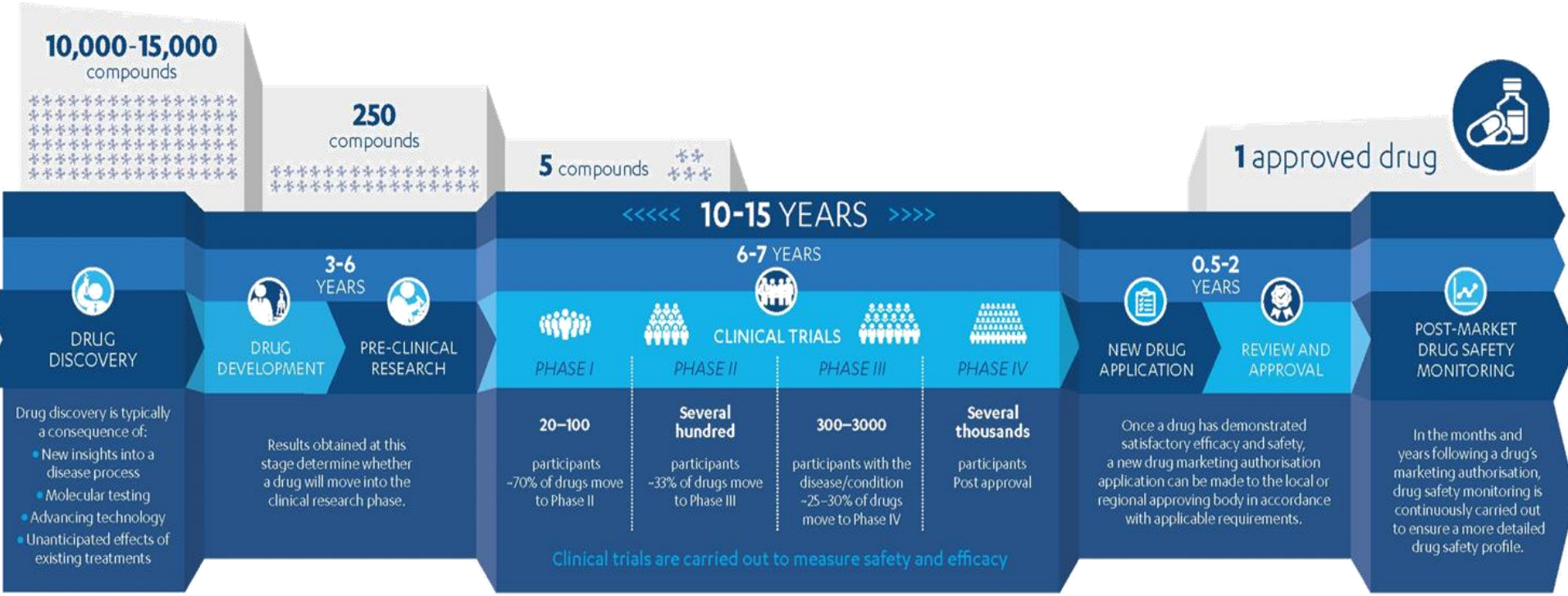
Distribution

Absorption

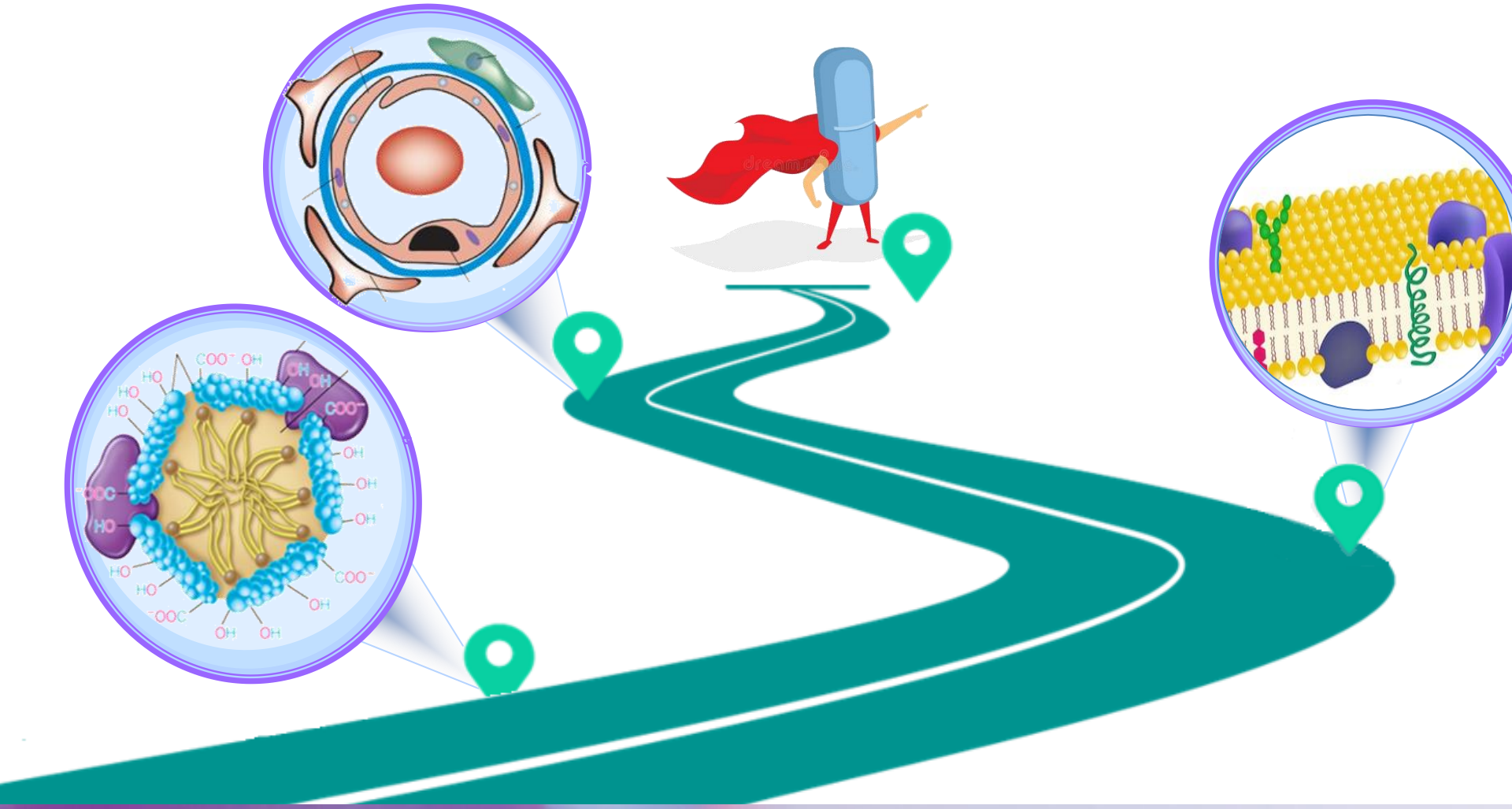
Abstract:
Successful drug development requires not only the optimization for specific and potent recognition by its pharmacodynamical targets, but also efficient delivery to these target sites. Drug-biomembrane reciprocal interactions are a key determinant to understand how a compound performs at a barrier with relevant implications in its pharmacokinetic behaviour especially in Absorption, Distribution, Metabolism and Excretion (ADME). Concerning this, a rational drug design, where medicinal chemists can envision how a structure can be optimized aiming an improved pharmaceutical profile, can be the solution to avoid bigger investments in drugs that might not be effective. Lipid biomimetic membrane models with different lipid constitution are increasingly employed as alternative platforms with very well defined and controlled conditions to predict structural, biophysical and chemical aspects involved in the compounds' penetration and/or interaction with biomembranes. As a proof-of-concept, in this study several biomimetic membrane models (cell membrane and epithelial membrane of blood-brain barrier) were used and different biophysical techniques (derivative spectroscopy; quenching of steady-state and time-resolved fluorescence; dynamic light scattering; differential scanning calorimetry and small and wide angle x-ray diffraction) were applied to characterize the pharmacokinetic profile of a newly synthesized drug in order to support drug screening process decisions.

Keywords: pharmacokinetics; ADME; biophysics; biomimetic membrane models; drug design; newly-synthesized drug

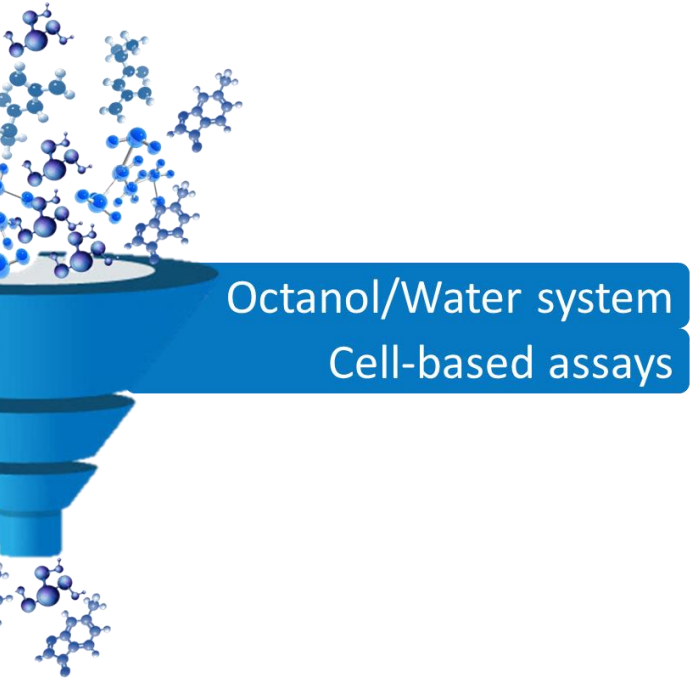
DRUG DEVELOPMENT



DRUG DEVELOPMENT



DRUG SCREENING: PHARMACOKINETICS



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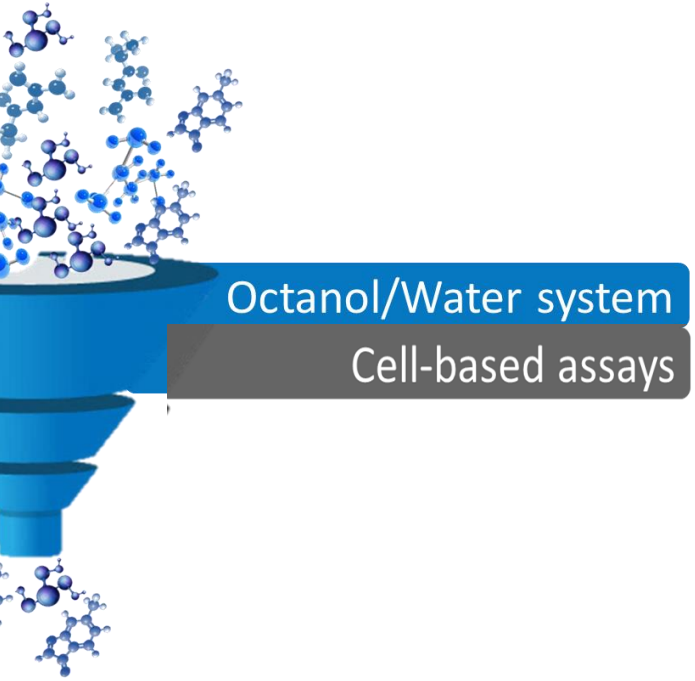

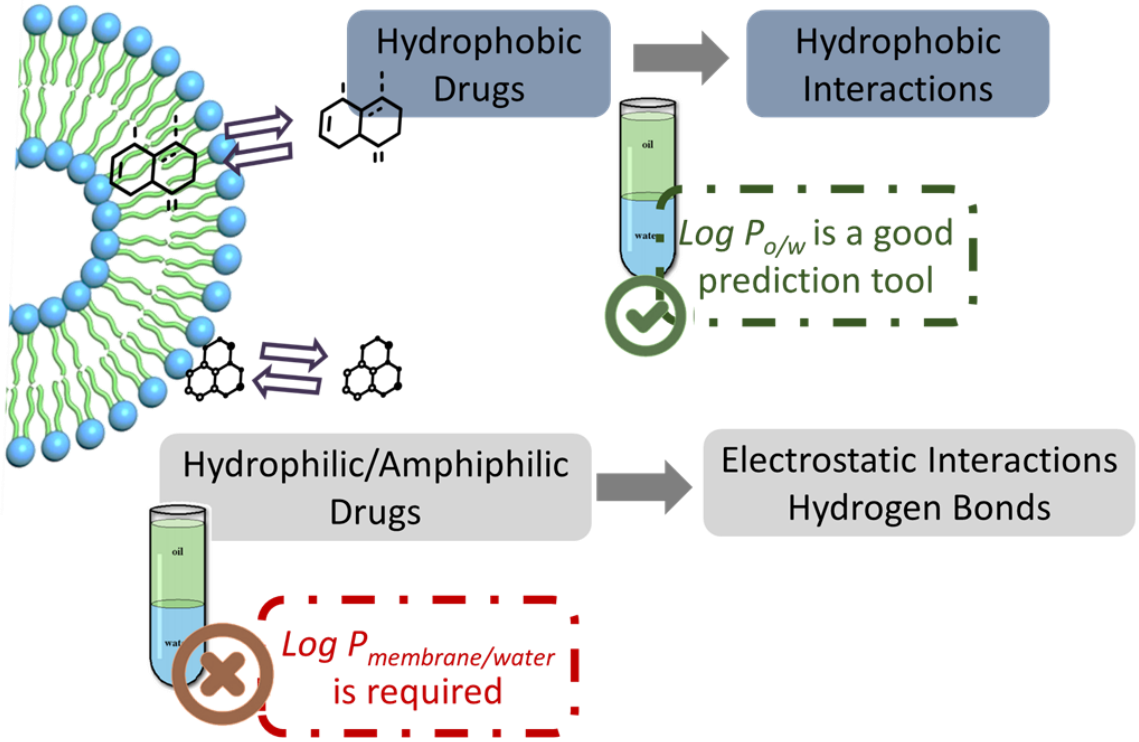

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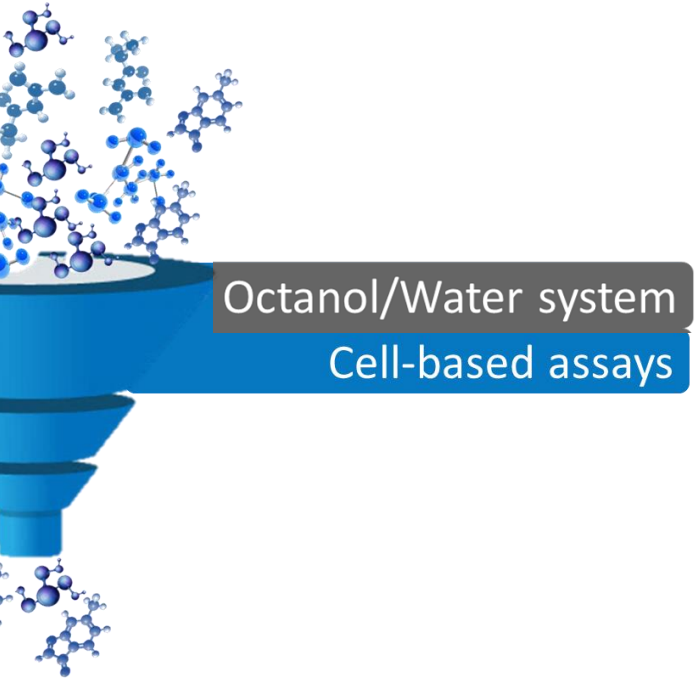
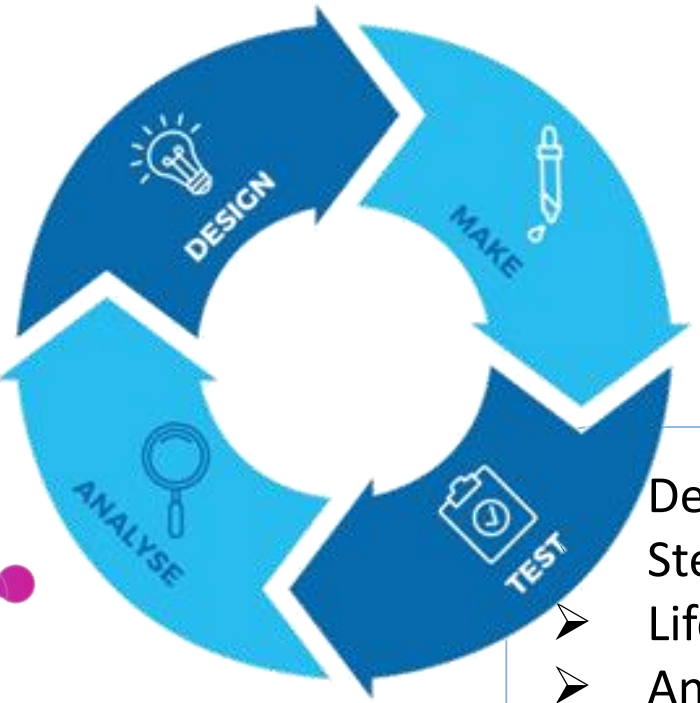
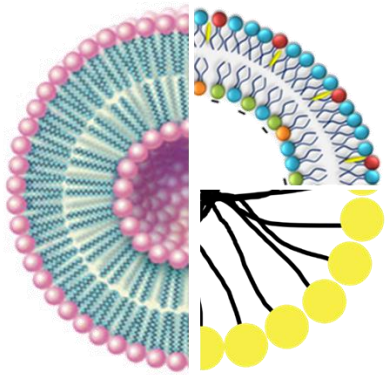


Relevant Information
Better Prediction

Expensive as drug screening process

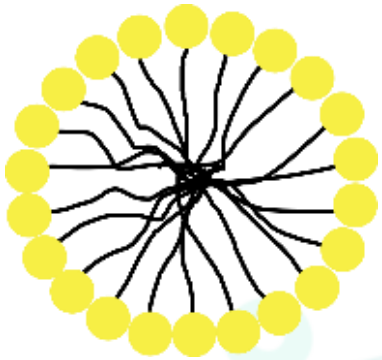
THE DRUG-BIOMEMBRANE APPROACH





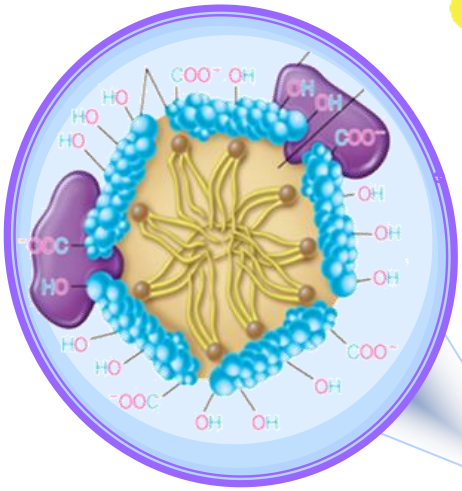
- Derivative Spectrophotometry
- Steady-State Fluorescence
- Lifetime Fluorescence
- Anisotropy
- Dynamic Light Scattering
- Small- and wide- angle X-ray Scattering

INTESTINAL ABSORPTION

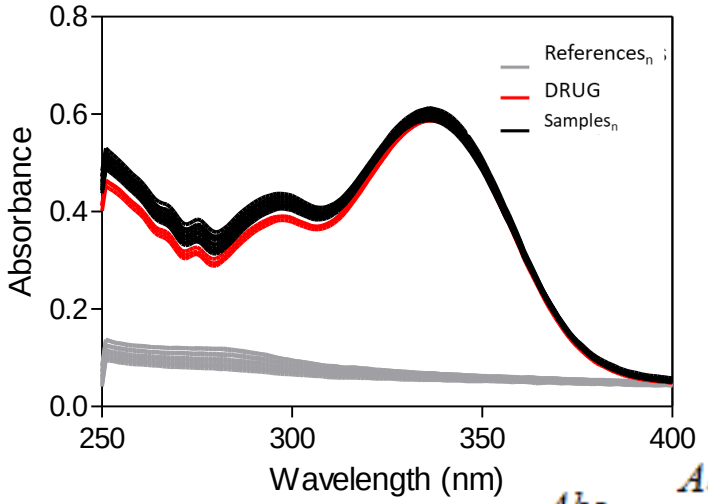


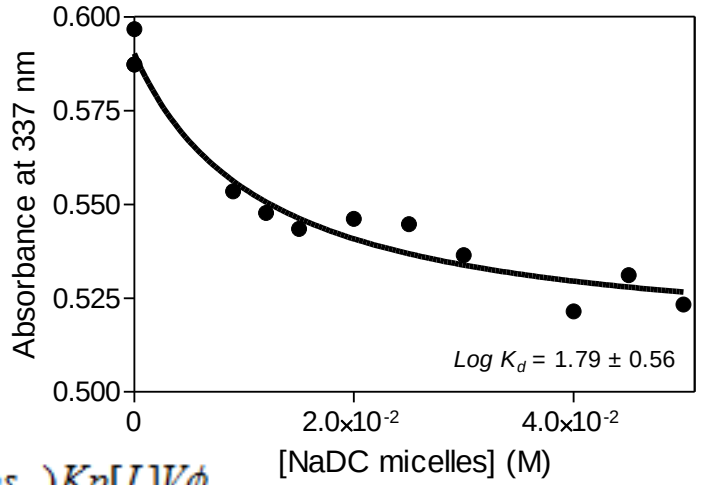
BIOMIMETIC MODEL Sodium Deoxycholate

BIOPHYSICAL TECHNIQUE Partition Coefficient by Derivative Spectrophotometry



INTESTINAL ABSORPTION

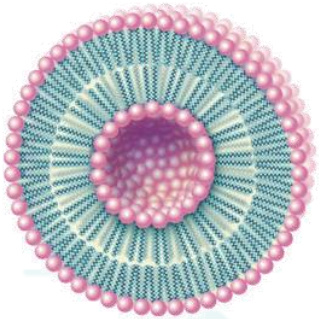


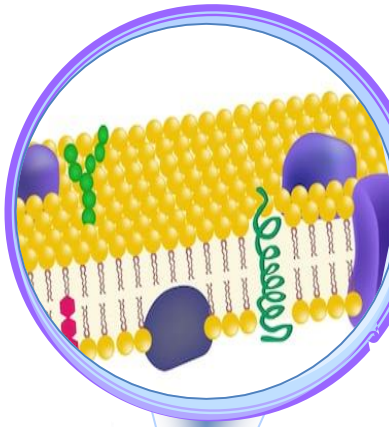


$$Abs_T = \frac{Abs_w + (Abs_m - Abs_w)Kp[L]V\phi}{1 + Kp[L]V\phi}$$

- ✓ Good solubilization of the drug at small intestine level by mixed micelles of intestinal surfactants
- ✓ Transport route to systemic circulation is predicted to occur by transcellular pathway

DRUG DISTRIBUTION

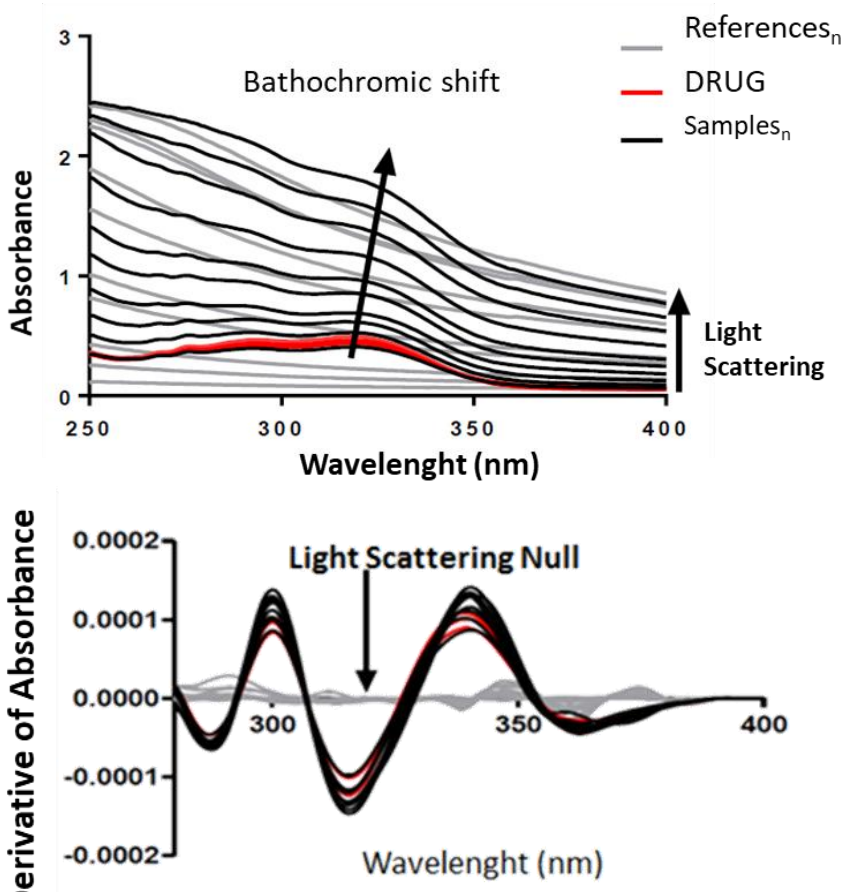




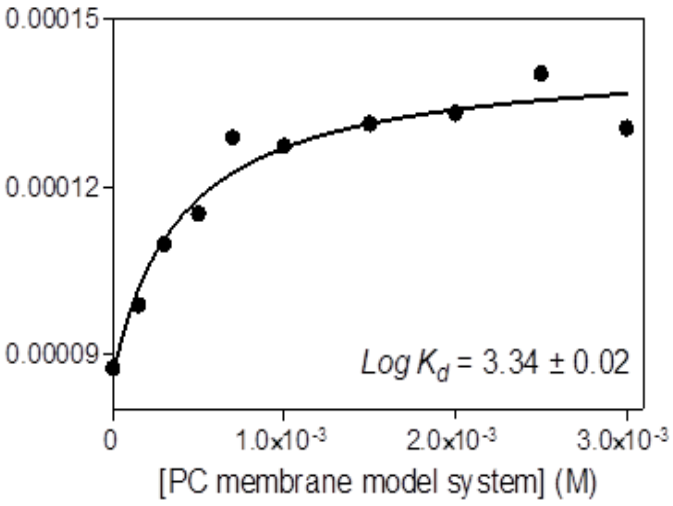
BIOMIMETIC MODEL Dimyristoylphosphatidylcholine (DMPC)

BIOPHYSICAL TECHNIQUE Partition Coefficient by Derivative Spectrophotometry

DRUG DISTRIBUTION



$$D = D_w + \frac{(D_m - D_w)Kp[L]V\phi}{1 + Kp[L]V\phi}$$



DRUG DISTRIBUTION

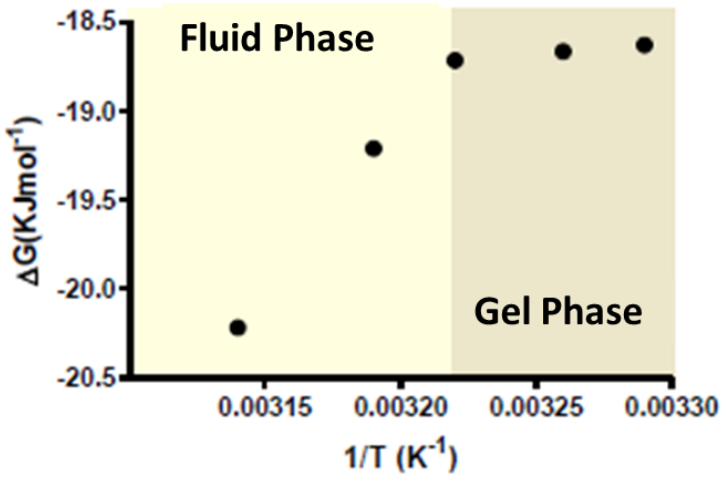
$$K_{bioaccumulation} = \frac{Q}{VK_d}$$

- Adrenal Glandes
- Tyroid
- Kidneys

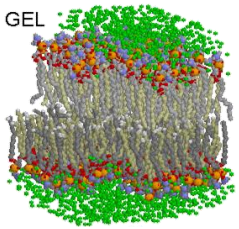
✓ Moderate to high lipophilicity – good balance of solubility and permeability

✓ Tendency for bioaccumulation in peripheral tissues

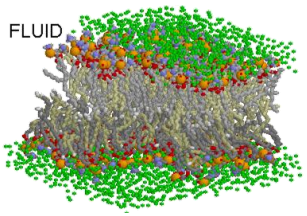
DRUG DISTRIBUTION



pH	*Log* K_d
2.00	3.98 ± 0.22
3.00	3.93 ± 0.43
4.00	3.98 ± 0.63
5.00	3.02 ± 0.14
6.00	3.46 ± 0.25



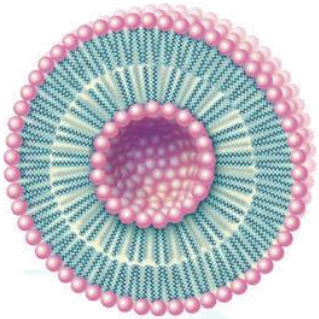
$\Delta S > 0$
$\Delta H < 0$
$\Delta G < 0$

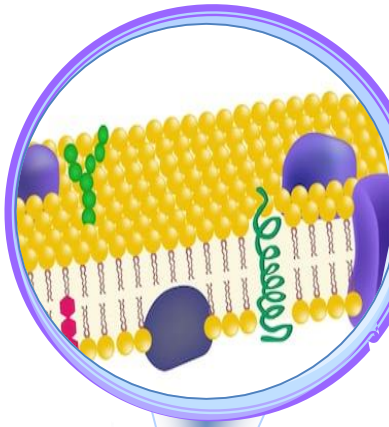


ΔS
ΔH > 0
$\Delta G < 0$

- ✓ Non-ionized drug
- ✓ Drug partition is a spontaneous process and van der Walls interactions are stablished

DRUG DISTRIBUTION

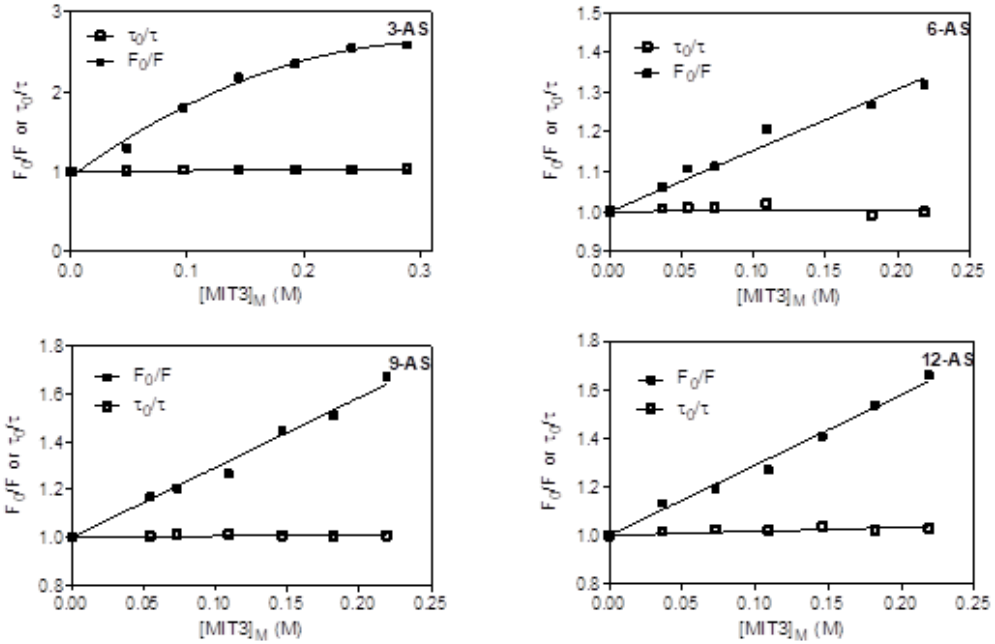




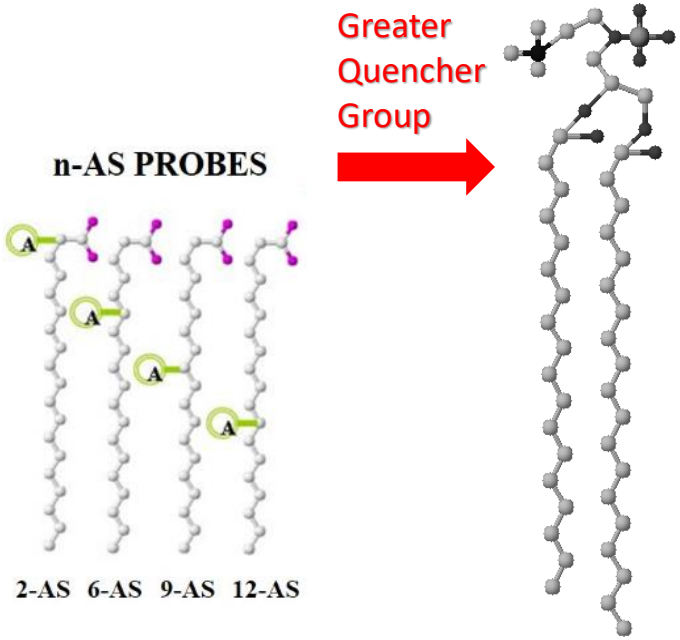
BIOMIMETIC MODEL Dimyristoylphosphatidylcholine (DMPC)

BIOPHYSICAL TECHNIQUE Membrane Location by Steady-State and Lifetime Fluorescence

DRUG DISTRIBUTION

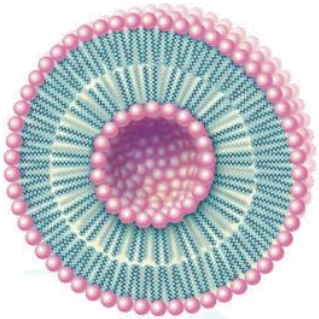


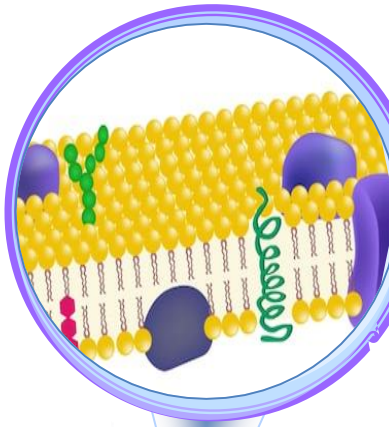
Probe	3-AS	6-AS	9-AS	12-AS
K_{SV} $(M^{-1})^{(a)}$	11.25	1.54	2.99	2.90
K_q $(M^{-1} \cdot s^{-1})$	1.36 x 10^6	1.73 x 10^5	2.71 x 10^5	2.52 x 10^5



✓ Extended molecular orientation of MIT3 parallel to the membrane phospholipids

MEMBRANE TOXICITY

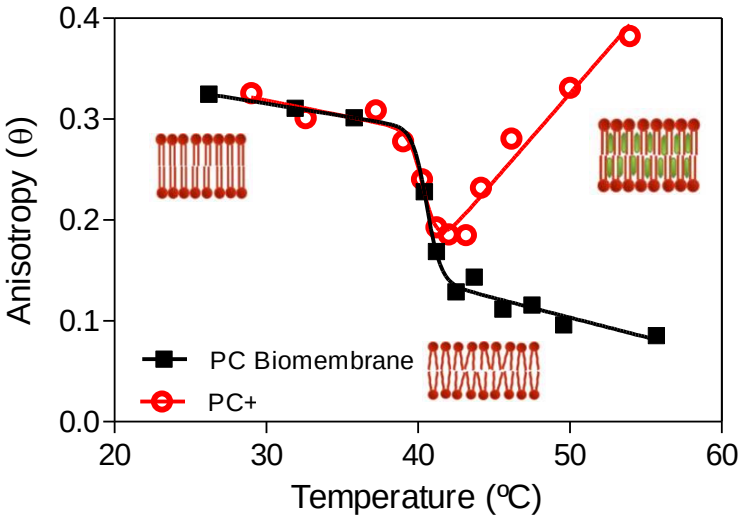




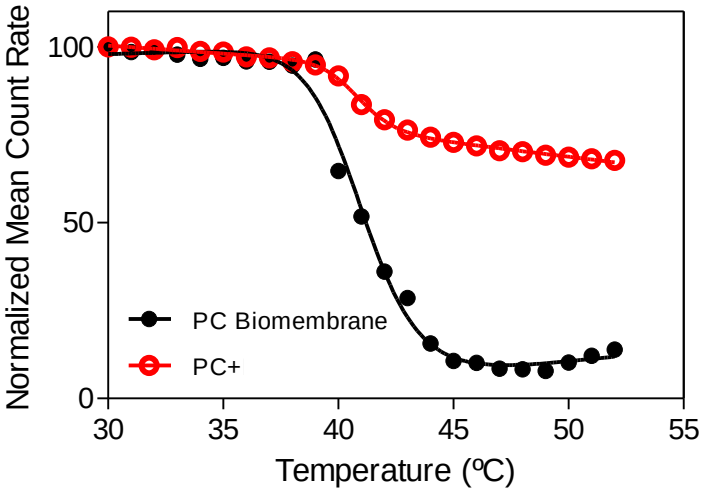
BIOMIMETIC MODEL Dipalmitoylphosphatidylcholine (DPPC)

BIOPHYSICAL TECHNIQUE Membrane Microviscosity by Anisotropy Fluorescence and Dynamic Light Scattering (DLS)

MEMBRANE TOXICITY



$$TCM = r_{s1} + p_1 T + \frac{r_{s2} - r_{s1} + p_2 T - p_1 T}{1 + 10^{B\left(\frac{1}{T} - \frac{1}{T_m}\right)}}$$

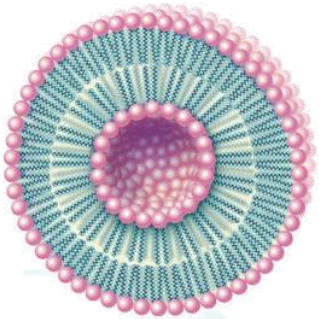


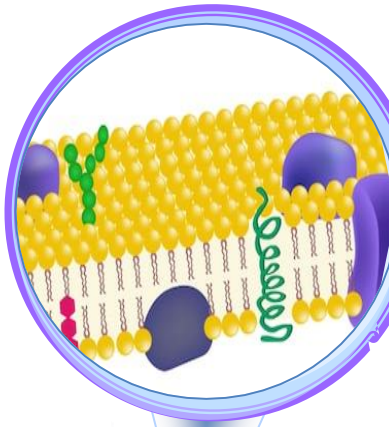
- DPH PROBE
- Tm ≈ K

- Tm ≈ K
- Cooperativity (B) ↑

- ✓ The drug location/orientation parallel to the acyl chains of the hydrophobic core of phospholipids promotes the membrane stiffness;
- ✓ No signs of toxicity are identified.

MEMBRANE TOXICITY

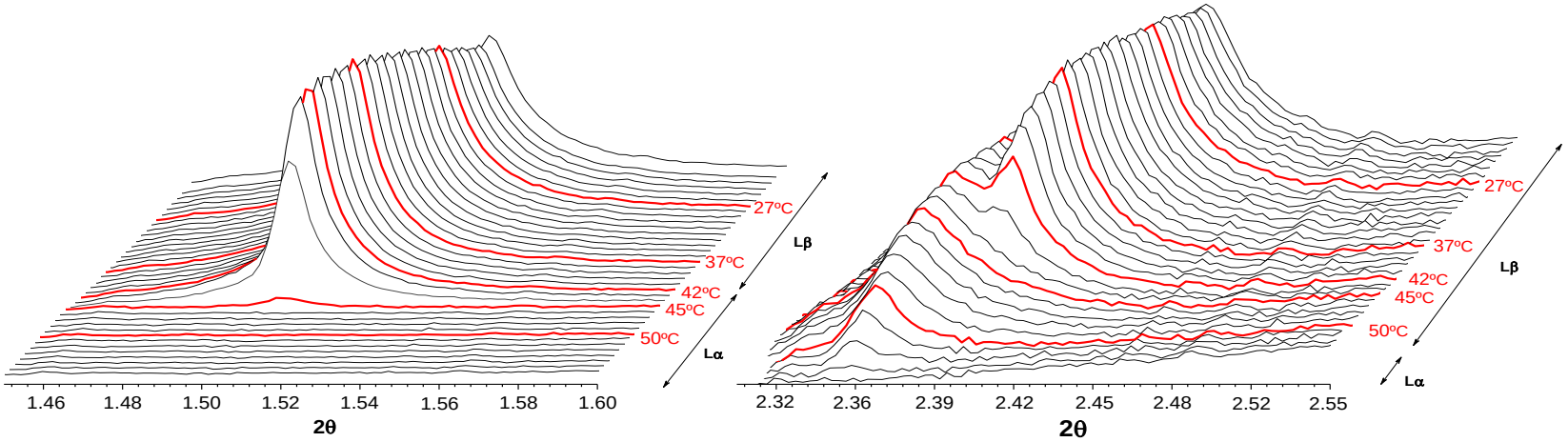


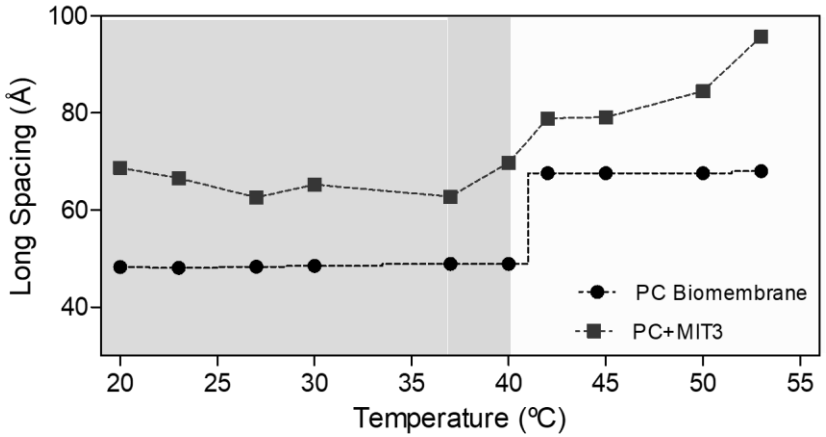


BIOMIMETIC MODEL Dipalmitoylphosphatidylcholine (DPPC)

BIOPHYSICAL TECHNIQUE Membrane order/packing changes by Small- and Wide-angle X-ray Scattering (SAXS and WAXS)

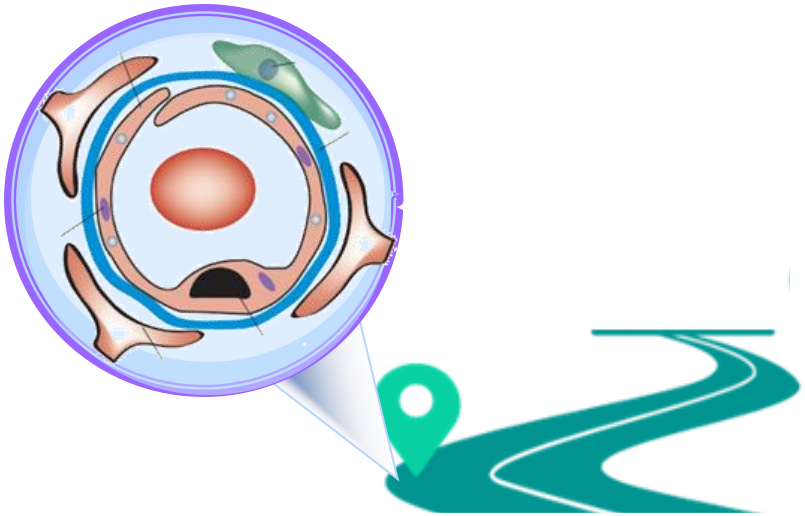
MEMBRANE TOXICITY

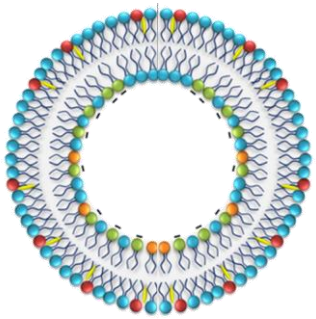




- ✓ Membrane stiffness due to its intercalation in the hydrophobic region of the bilayer were corroborated by the signal in WAXS for T > 45˚C
- ✓ No membrane toxicity signs

TARGET DISTRIBUTION

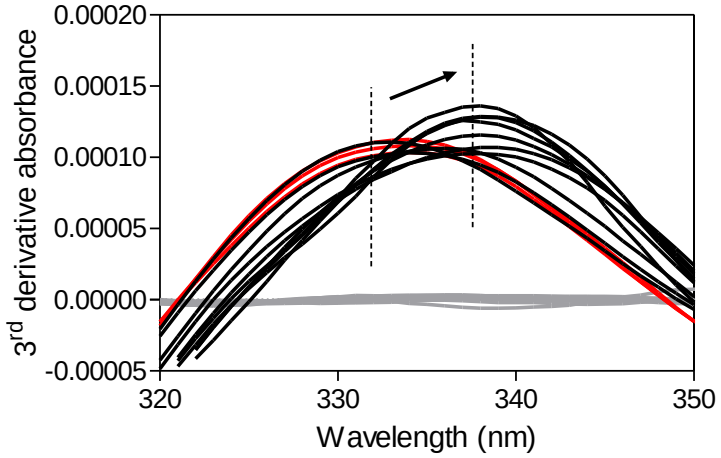


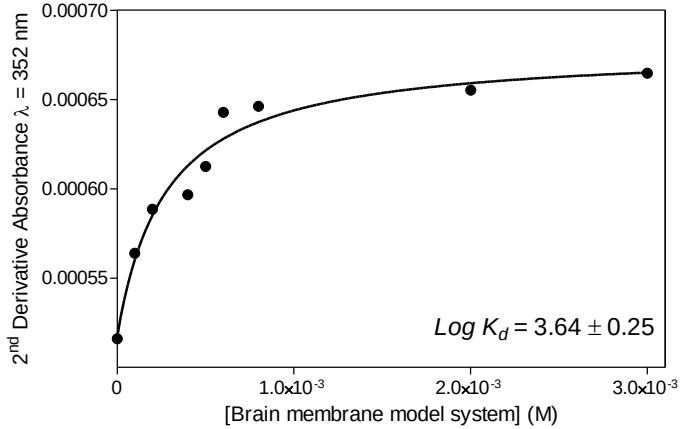


BIOMIMETIC MODEL Brain Polar Lipids Extract

BIOPHYSICAL TECHNIQUE Partition Coefficient by Derivative Spectrophotometry

TARGET DISTRIBUTION





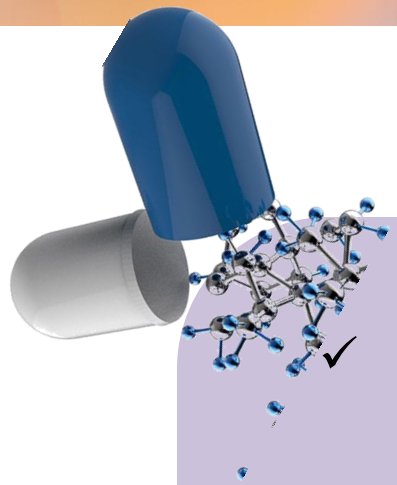
$$LogBB = 0.388Log\ K_{d\ (BBB)} - 0.00618V_M + 1.359$$

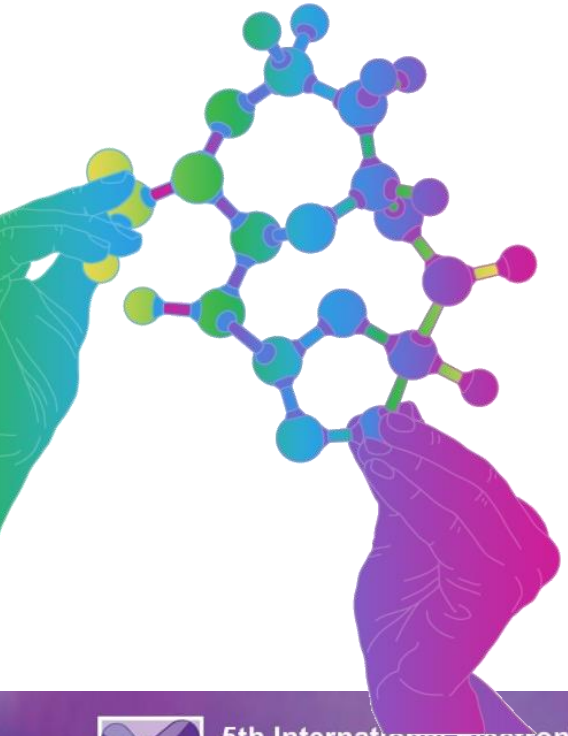
$$Log\ PS = -2.19 + 0.262Log\ K_{d\ (BBB)} + 0.0583VWSA\_B - 0.00897PSA$$

LogBB = 2.77 ± 0.10

Log PS = -1.88

- ✓ The drug is classified as BBB+
- ✓ The drug is able to pass through BBB





- ✓ Good intestinal absorption by transcellular route predicted
- ✓ Ability to reach the therapeutic target
- ✓ Bioaccumulation in off-target tissues is expected
- ✓ Membrane location parallel to phospholipids
- ✓ No signs of membrane toxicity
- ✓ MIT3 promotes the membrane stiffness.

Acknowledgments

CF-UM-UP

Universidade do Minho

U.PORTO

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D2CIQUP – University of Porto

COMPETE 2020

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