Impact of cyclization and methylation on peptide penetration through droplet interface bilayers

Vincent Faugeras[1,3], Olivier Duclos[2], Didier Bazile[3], Abdou Rachid Thiam[1]

[1]Laboratoire de Physique de l'École Normale Supérieure, ENS, Université PSL, CNRS, Sorbonne Université, Université de Paris, F-75005 Paris, France

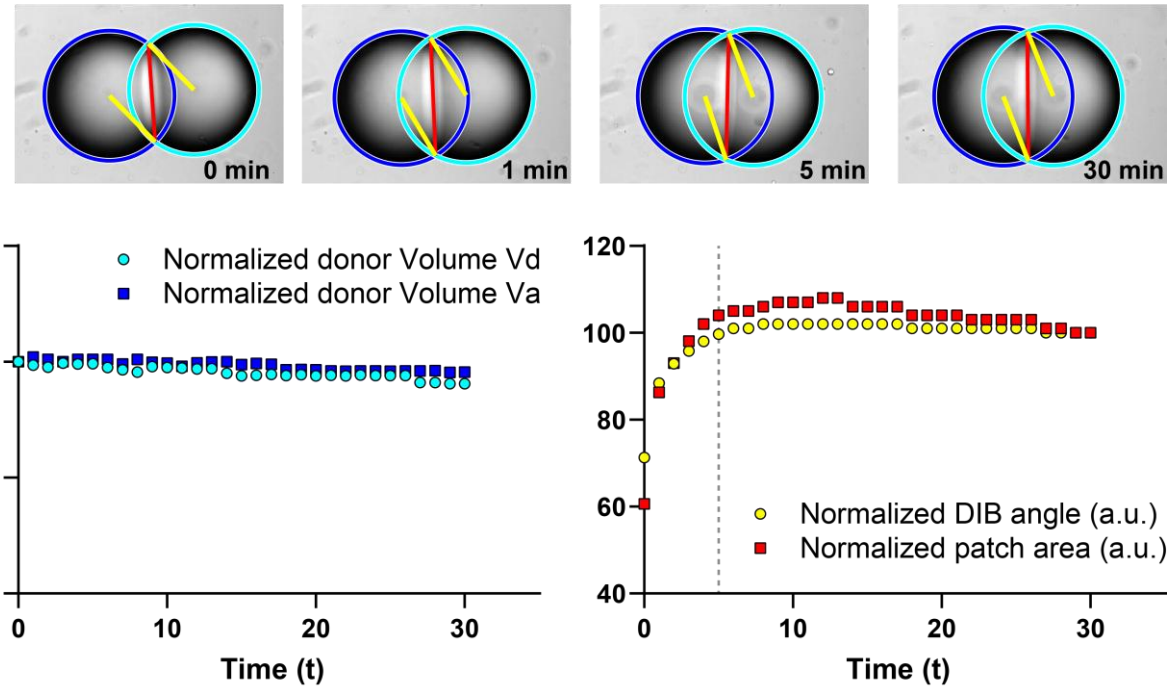
[2]Integrated Drug Discovery Platform. Sanofi R&D, Chilly-Mazarin, France.

[3]Pharmaceutics Development Platform. Sanofi R&D, Gentilly, France,

(correspondence to thiam@ens.fr)

Supplementary figures

a



b **Unstirred Water Layer (UWL) permeability assessment**

$$p_{USL} = \frac{1}{2}\frac{D_{sol}}{\delta_{USL}}$$

$D_{sol} \approx 10^{-5}\ cm^2.s^{-1}$

$\delta_{USL} < 10^{-2}\ cm$

$p_{USL} \approx 10^{-3}\ cm.s^{-1} >> p_{bilayer}$

Figure S1: a) Top: Brightfield microscopy pictures of DIBs over time. Bottom left: Normalized donor and acceptor volume as a function of time, the normalization is relative to value at t_0. Bottom right: Normalized contact angle and patch area as a function of time, the normalization is relative to final value at 30 minutes. Note that evaporation is almost negligible with more than 95% of initial volume after 30 minutes assay. After about 2 minutes, contact angle and patch area values represent about 90% of final values; after 5 minutes, morphological parameters are identical to final values. b) Evaluation of unstirred water layer permeability which is negligible compared to bilayer permeability.

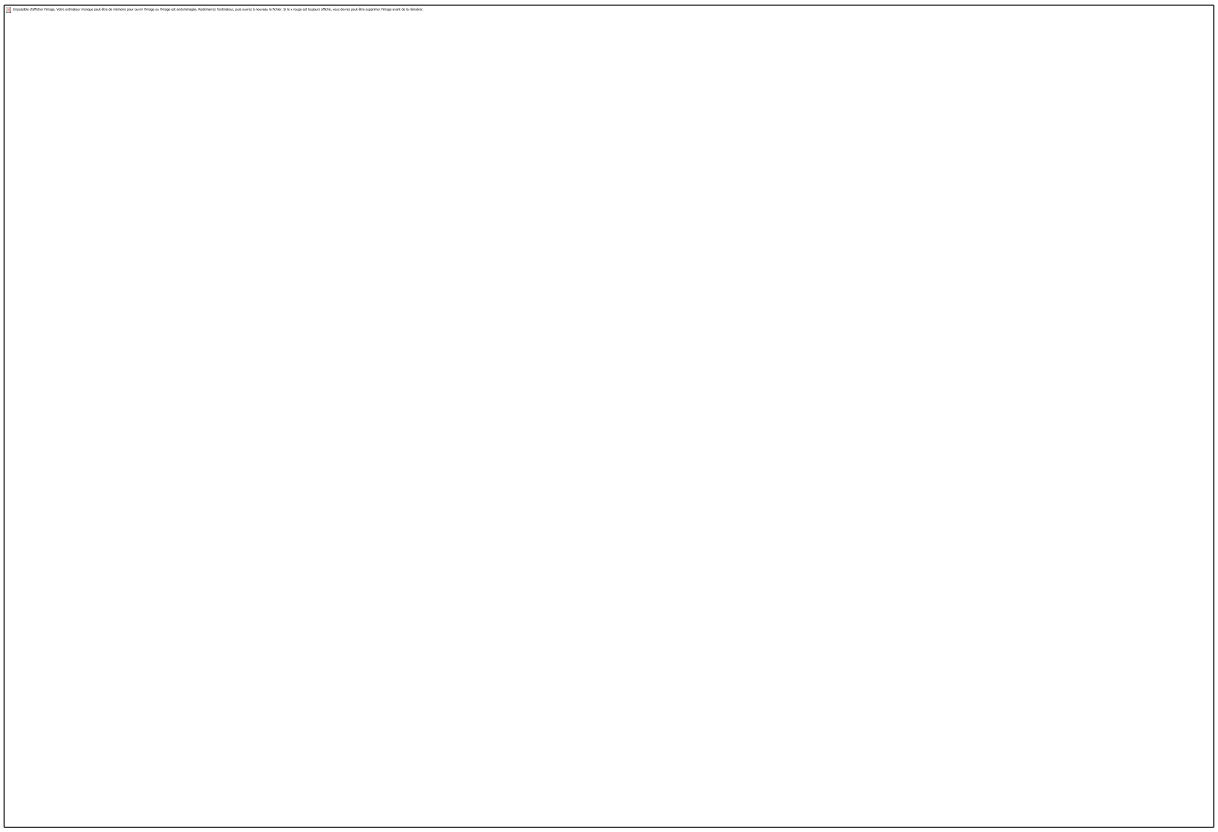


Figure S2: a) Chemical representations of peptide L1, C1 and C1m. L1 features protected C- and N-terminus to feature the same net charge as cyclic peptides at physiological pH b) Peptide details are provided. Peptide sequences are given with the one-letter code, D- amino acids are written in red; MW is calculated from the peptide sequence, without considering potential counter ions; Net charge value is calculated at physiological pH 7.4, the first value accounts for the peptide sequence without the fluorescent probe attached, the second value accounts for the complete molecular assembly, A hydrophilicity (average hydrophilicity) is obtained from BACHEM peptide calculator (https://www.bachem.com/knowledge-center/peptide-calculator/), this parameter estimates the hydrophilicity of every peptides from the relative hydrophilicity of each amino acids.

a

Peptides id	Type	Sequence	MW	Net Charge	Labeling	Av hydrophilicity	# of methylation
TAT	Polycationic	YGRKKRRQRRR	1918	8 / 6	FAM (f)	2.0	NA
Pen	Amphipathic	RQIKIWFQNRRMKWKK	2604	7 / 5	FAM (f)	0.5	NA
C1(f)	Hydrophobic cycle	L D-L D-L D-K P Y	1118	0 / -2	FITC (f)	-0.8	0
C1m(f)	Hydrophobic methylated cycle	Met-L Met-D-L D-L D-K P Y	1148	0 / -2	FITC (f)	-0.8	2

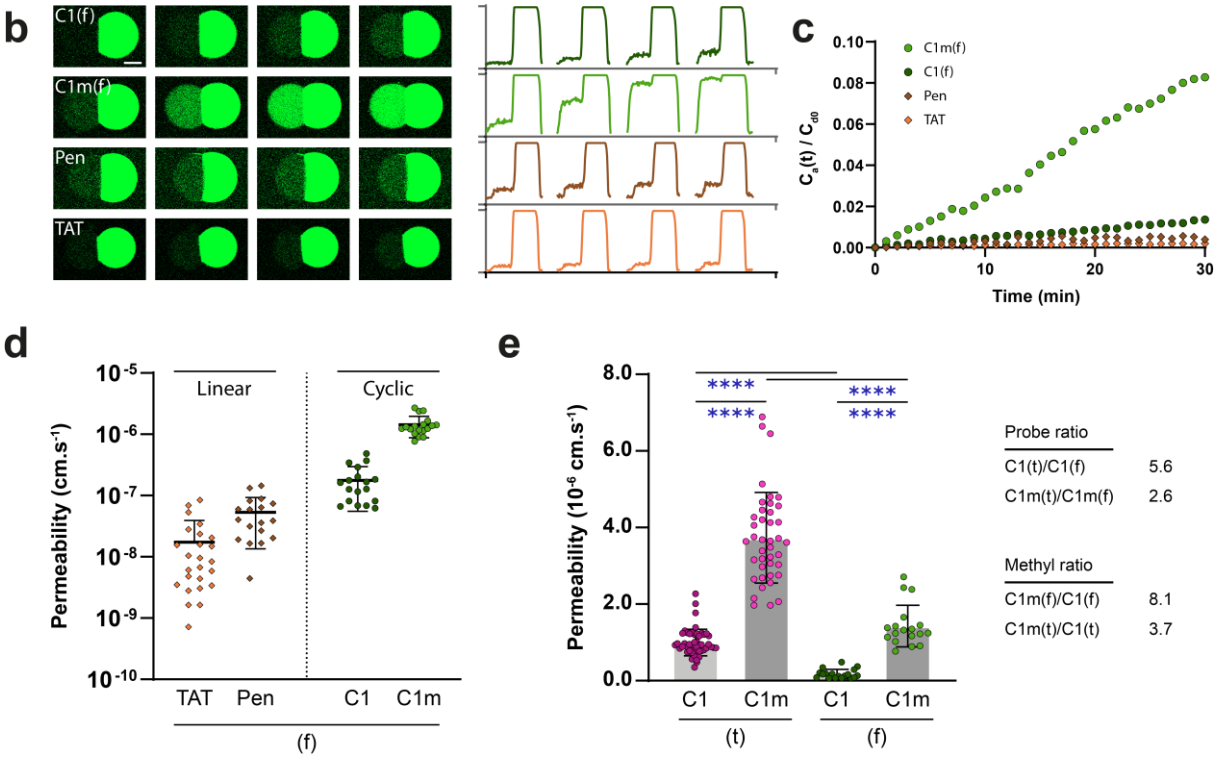


Figure S3: Permeability studies of FAM and FITC-labelled Penetratin, TAT and cyclic peptides analogues C1(f) and C1m(f). a) Peptide details for C1(f), C1m(f), Pen and TAT peptides. Peptide sequences are given with the one-letter code, D- amino acids are written in red; MW is calculated from the peptide sequence, without considering potential counter ions; Net charge value is calculated at physiological pH 7.4, the first value accounts for the peptide sequence without the fluorescent probe attached, the second value accounts for the complete molecular assembly, A hydrophilicity (average hydrophilicity) is obtained from BACHEM peptide calculator (https://www.bachem.com/knowledge-center/peptide-calculator/), this parameter estimates the hydrophilicity of every peptides from the relative hydrophilicity of each amino acids. b) Confocal microscopy pictures of DIB permeation assay for C1(f), C1m(f), Pen and TAT peptides over time. The same signal enhancement correction was applied to every single image to be able to detect significative signal of translocation for peptides. Corresponding fluorescence profiles are provided. Scale bars, 100 µm. c) Permeation kinetics for C1(f), C1m(f), Pen and TAT peptides. Ca(t)/Cd0 data are shifted to 0 at t0 to appreciate differences in permeation between these four peptides d) Calculated permeability for C1(f), C1m(f), Pen and TAT peptides (defined as permeation parameter p_{30}) e) Comparison of permeability for TAMRA- and FITC-labelled C1 and C1m peptides, corresponding ratio are provided. (mean value, error bars represent the SD; **** indicates $p < 0.0001$).

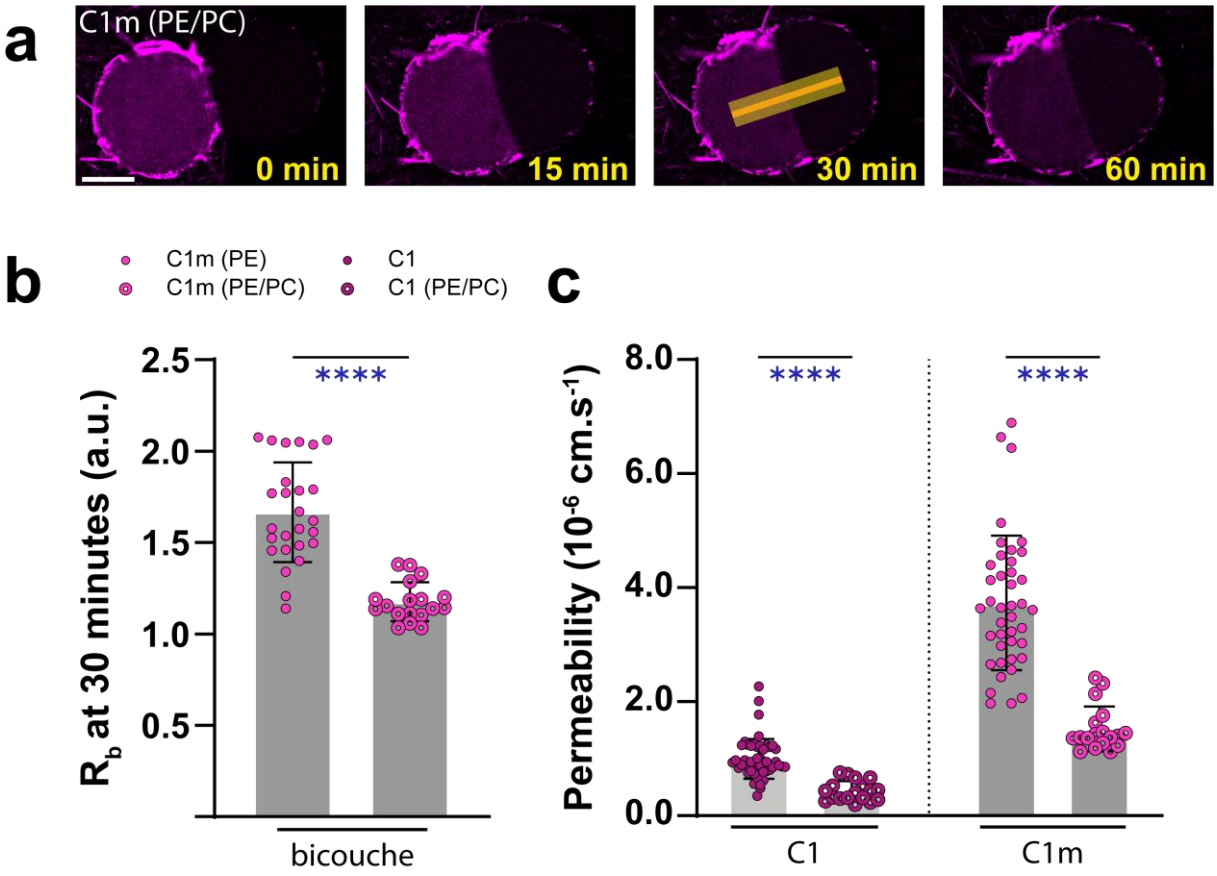


Figure S4: Impact of bilayer composition (addition of PC phospholipids) on peptide recruitment on interfaces and translocation rate. a) Confocal microscopy pictures of DIB permeation assay for C1m peptide with DOPE:DOPC 1:1 DIB phospholipid composition. b) R_b at 30 minutes for C1m as a function of bilayer composition. c) Calculated permeability for C1 and C1m as a function of bilayer composition.

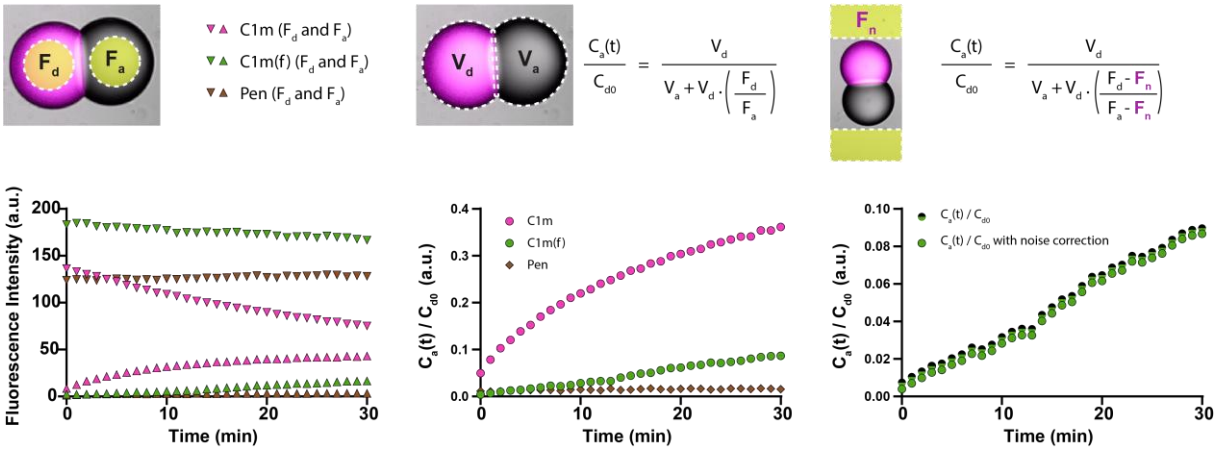


Figure S5: Left: fluorescence raw data of acceptor and donor droplets for three peptide C1m, C1m(f) and Pen showing various permeation kinetics. Middle: corresponding Ca(t)/Cd0 evolution over time. Right: modulation of Ca(t)/Cd0 when considering noise. Noise subtraction has a significant impact on permeation values when the translocation rates of peptides are really low (for example for L1, L1m or Pen and TAT peptides) and when noise is shifted over time.