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Negatively charged lipid membranes enhance adsorption of TAT-decorated ELPs micelles

Vivien Walter^{1,2}, Tatiana Schmatko^{1,*}, Pierre Muller¹, André P. Schroder^{1,3}, Sarah R. MacEwan^{4,5}, Ashutosh Chilkoti⁴, and Carlos M. Marques^{1,6,*}

¹Institut Charles Sadron CNRS (UPR22) & Université de Strasbourg, France

²Current address: Department of Engineering, King's College London, UK

³Current address: INSA Lyon, Villeurbanne, France

⁴Department of Biomedical Engineering, Duke University, Durham, North Carolina, USA

⁵Current address: Division of General Internal Medicine, College of Medicine, Ohio State University, Ohio, USA

⁶Current address: University of Lyon, ENS-Lyon, CNRS UMR 5182, Chem. Lab., 69342 Lyon, France

*Correspondence: tatiana.schmatko@ics-cnrs.unistra.fr

ABSTRACT

A cell penetrating peptide (CPP) is a short amino-acid sequence capable of efficiently translocating across the cellular membrane of mammalian cells. However, the potential of CPPs as a delivery vector is hampered by the strong reduction of its translocation efficiency when it bears an attached molecular cargo. To overcome this problem, we used previously developed deblock copolymer elastin-like polypeptides (ELPBC) end-functionalized with TAT, an archetypal CPP built from a positively charged amino acid sequence of the HIV-1 virus, that assemble into micelles at a specific temperature and present the TAT peptide on their corona. These micelles can recover the lost membrane affinity of TAT and can trigger interactions with the membrane despite the presence of a molecular cargo. Herein, we study the influence of membrane surface charge on the adsorption of TAT-functionalized ELP micelles onto Giant Unilamellar Vesicles (GUVs). We show that the TAT-ELPBC micelles show an increased binding constant towards negatively charged membranes compared to TAT without cargos. The affinity of the TAT-ELP_{BC} micelles for the GUV displays a step-wise dependence on the lipid charge of the GUV, which, to our knowledge, has not been reported previously for peptide-lipid membrane interactions. By unveiling the mechanisms of control over the interaction of an archetypal CPP with lipid membranes, through regulation of the charge of the lipid bilayer, our results pave the way for a better design of delivery vectors based on CPPs.

INTRODUCTION

Cell penetrating peptides were discovered in the early eighties, along with the Trans Activator of Transcription domain (TAT) of the HIV-1 virus (1, 2). Other CPPs were discovered later, such as penetratin from the *Drosophila antennapedia* HOX gene (3) or transportan (4). More specifically for TAT, the purely cationic sequence, from amino acid 47 to 57, was found to be responsible of the translocation of the viral host in the infected cell.

Currently, TAT and other CPPs sequences are mainly used as drug delivery vectors (5). Several translocation mechanisms have been proposed (6, 7), including active and non-active pathways (8–10). To better assess the translocation efficiency of TAT in the absence of active, energy-driven processes, studies have been performed in lipid bilayer models (11–13).

On giant unilamellar vesicles (GUVs) made of ternary mixtures of 1,2 dioleoyl, sn-glycero phosphocholine (DOPC), 1,2 dipalmitoyl sn-glycero phosphocholine (DPPC) and cholesterol, Ciobanasi *et al.* showed that TAT binding to the surface of GUVs induces the leakage of small dyes, but without CPP translocation (14). The inclusion of a small proportion of negatively charged lipids in the membrane composition increased the affinity of TAT for the membrane but did not promote translocation. They concluded from these experiments that pores of nanometric size are formed. The same authors also found that TAT has a higher mobility than the membrane lipids with which they interact (15). They propose that CPPs float above the lipid membrane, although this fast diffusion coefficient might be the signature of a more fluid membrane, although a fast diffusion coefficient suggests that CPPs may increase the fluidity of membranes they interact with. Indeed, Akabori *et al.* (16) have since found by SAXS experiments on supported lipid bilayers that once TAT is bound to the membrane, the average area per lipid head is increased along with a decrease of the membrane bending modulus. On neutral membranes, SAXS electron density profiles show that TAT peptides, despite their cationic nature, are inserted close to the aliphatic chains such that they bind to and drag along the lipid phosphate groups. The incorporation of anionic lipids in the supported bilayer keeps TAT peptides further away from the aliphatic chains, likely due to the stronger affinity for the charged head groups. Wong and coworkers have shown that TAT penetrates GUVs only when the lipid composition contains more than 20% 1,2 dioleoyl sn-glycero phosphoethanolamine (DOPE), a lipid with an inverted conical shape that induces a negative spontaneous curvature of the lipid leaflets. They also performed SAXS experiments on liposomes exposed to TAT and show that upon exposure to TAT, the lipid undergoes a drastic structural change from a lamellar phase to a cubic double diamond phase (17, 18). More recently, Allolio and coworkers have shown that GUVs self-assembled from a ternary mixture of DOPC/DOPG/DOPE leak small dyes and fuse when exposed to TAT. TEM experiments performed on liposomes by the same authors have shown that the fusion is

associated with the multilamellar structure of the liposomes. With the help of molecular dynamic simulations, the authors

proposed a membrane fusion model based on pore formation (19). Along the same line, Sakamoto *et al.* have shown that osmotic pressure shocks or chaotropic solutes could also induce curvature changes on a egg-PC membrane and promote CPP entry (20).

We have recently designed a family of temperature responsive diblock copolymer elastin-like polypeptides (ELP_{BC}). Above a critical micellar temperature (CMT), ELP_{BC} self assemble into spherical micelles. ELP_{BC} have been end-functionalized with a TAT sequence on one end and tagged with a fluorophore on the other end. Above the CMT, the fluorescent dye is located inside the hydrophobic core of the micelle, while the TAT moieties are presented on the corona of the micelle. The TAT-ELP_{BC} can be considered a model of a cargo—the ELP_{BC}— that is carried into the cell by TAT that is useful to understand the basis of the interactions between this class of molecules and model membranes. Although the presence of cargo usually reduces the translocation efficiency of TAT (21, 22), we have recently reported that the self-assembly of ELP_{BC} into micelles recovers affinity of the TAT peptides for lipid membranes (23). Indeed, the unimeric forms of TAT-ELP_{BC} do not display any adsorption on neutral membranes, whereas TAT-ELP_{BC} micelles show significant adsorption on the lipid membrane.

In this paper, we study the effect of the charge of the lipid membrane on the adsorption behavior of TAT-functionalized ELP unimers and micelles. By measuring adsorption amounts for a range of TAT-ELP_{BC} bulk concentrations and for surface molar charges between 0 and 20%, we extract affinity constants and compare our results with those obtained for cargo-less TAT sequences. We also study the effect of temperature on the adsorption amounts and witness a drastic increase of TAT-ELP_{BC} binding as the temperature rises.

MATERIALS AND METHODS

Materials

The Phospholipids used in this work, namely DOPC and 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG) were purchased from Avanti Polar Lipids (Alabaster, AL) already dissolved in chloroform and stored at -18 °C. Chloroform, acetone and ethanol for cleaning purposes were purchased from Carlo Erba Reagents. Other reagents, such as glucose, sucrose and PBS tablets were obtained from Sigma Aldrich and used without further purification unless specified in the text. The CPPs used in this work are TAT₄₇₋₅₇ terminated elastin-like block copolymer polypeptides (TAT-ELP_{BC} and TAT-ELP_{BC}-AlexaFluor488). Their synthesis has been reported in ref (24). The ELP_{BC} consists of two different blocks of 60 pentapeptides of Val-Pro-Gly-Xaa-Gly (VPGXG) derived from native tropoelastin where X is a guest residue, being any amino acid except proline. ELPs exhibit lower

critical solution temperature (LCST) phase separation behavior in aqueous solvents, wherein a solution of an ELP will phase separate above a critical cloud point temperature into an ELP-rich dense phase and an ELP-depleted dilute phase. The phase diagram of an ELP can be tuned by its composition (X residues in the VPGXG repeat) and its chain length (25) (26). In the hydrophobic block of the ELP_{BC} used herein, Xaa is Val. In the hydrophilic block, Xaa is Gly and Ala with a 1:1 ratio. For some diblocks a maleimide derivative of the Alexa488 fluorescent dye (Molecular Probes) was covalently conjugated to the end of the hydrophobic block at a terminal cysteine residue, see Fig. 1 for a schematic depiction of the ELP copolymers. The ELP_{BC} were dissolved in PBS (ready to use tablets from Sigma Aldrich) at the desired concentrations. To prepare fluorescently labeled ELP_{BC}, unlabeled ELP_{BC} were mixed with Alexa488-labeled ELP_{BC} at a 100:1 ratio. At their CMT (34 °C), these polypeptides self-assemble into micelles with the TAT sequences exposed at the corona of the micelle, and the fluorescent marker trapped inside the core. The ELP_{BC} and their fluorescently tagged versions were aliquoted and stored at -18 °C. Before each experiment, one aliquot (500 μ L, 25 μ M) was thawed overnight at 4 °C under constant agitation, then centrifuged for 1 minute at 22,500 g at 4 °C.

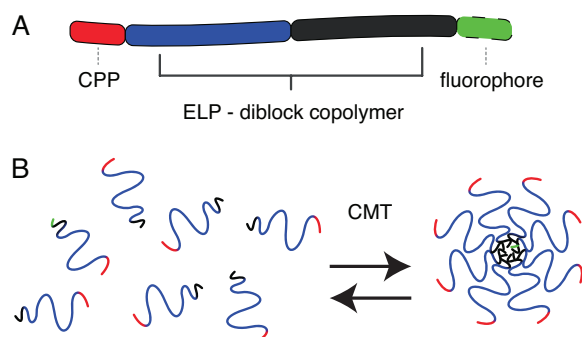


Figure 1: (A) Schematic representation of the diblock polypeptide ELP_{BC}. The hydrophobic block (in black) is composed of 60 repeats of the VPGVG. A small fraction is functionalized with an Alexa 488 fluorescent dye (in green). The hydrophilic block (in blue) is composed of 60 repeats of the VPGXG where X is G or A in a 1-1 ratio. This block is fused to the CPP sequence TAT₄₇₋₅₇ (in red) as its C-terminus. (B) Sketch of the self-assembly of ELP_{BC} into micelles above its CMT

Preparation of GUVs

Giant vesicles were obtained by the electroformation technique (27). Sucrose and glucose were dissolved in milliQ water (Millipore), the solution osmolarity was measured with an osmometer (Osmomat 030, Gonotec, Berlin, Germany) and adjusted to 280 mOsm.L⁻¹, then filtered with a syringe with a 0.2 μ m mesh size filter. Syringes were mounted with a new sterile filter unit to be stored at 4 °C. Once prepared, sugars

solutions were used within a week. 5 μ L of a lipid solution at 1 mg.mL⁻¹ in chloroform (HPLC grade) was deposited on each electrode of a homemade electroformation chamber. A moderate vacuum was applied to evaporate chloroform in order to obtain lipid stacks. The electro-formation chamber was then filled with 1 mL of sucrose and closed. An oscillatory electric field was applied across the chamber for 4-15 hours at room temperature. Its amplitude and frequency were set respectively to 1 V peak to peak and 10 Hz. The obtained GUVs were diluted with 1 mL of iso-osmotic glucose and left to settle down for 15 to 30 minutes before use.

Sample preparation

To avoid GUV adhesion on glass, microscope slides were functionalized with polyethylene glycol (PEG) with a molecular weight of 5000 Da using a variation of the protocol described by Perret *et al.* (28). Glass slides were washed with chloroform, acetone and ethanol successively, and dried under a nitrogen flow. The silanol groups at the glass surface were then activated by UV-ozone cleaning (Novascan Technologies, USA) for approximately 1 hour in total for both sides. Silanization was performed at room temperature in a mixture of 99.7 % HPLC grade ethanol, 0.2 % ultra pure water (Milli-Q systems by Millipore) and 0.1 % Mercapto Propyl Triethoxy Silane (MPTS). To control the water concentration necessary for gentle silane hydrolysis at the surface, special precautions were taken: before each experiment, MPTS was freshly distilled under vacuum and a new sealed bottle of ethanol (HPLC grade) was used as high water amounts would give rise to 3D bulk polymerization of the silane. Clean slides were immersed in the silanization solution for 1 hour under argon atmosphere and then rinsed twice in fresh ethanol. After drying under nitrogen, slides were cured in an oven at 110 °C for 10 minutes to enhance covalent bonding. They were sonicated for 30 s again in ethanol to remove physisorbed MPTS. Then, they were dried under flowing nitrogen and placed in a sandwich like configuration in contact of 25 μ L of a Mal-PEG₅₀₀₀-methoxy in 140 μ M of PBS, pH 6.5), for at least 24h. The above environment was kept saturated with water to prevent evaporation. Sandwiches were opened just before use; slides were rinsed extensively with milliQ water, sonicated for 30 s and dried under flowing nitrogen.

The microscopy observation cell, was made of two circular pegylated slides (as described above) separated by a ring-like inox spacer. A thin layer of grease (Apiezon) was used to seal the bottom slide to avoid leakage. 80 μ L of the GUVs in 1:1 glucose/sucrose solution was pipetted and put in the observation chamber taht contained 320 μ L of a 25 μ M solution ofth eELP_{BC} in PBS buffer at 280 mOsm.L⁻¹, leading to a final peptide concentration of 20 μ M in PBS/glucose/sucrose. The observation chamber was closed and heated up at the desired temperature for 90 min before starting the microscopy experiments. We used a home made heating plate and a heating objective mantel (ALA Scientific) to precisely control the

temperature.

Confocal microscopy and image processing

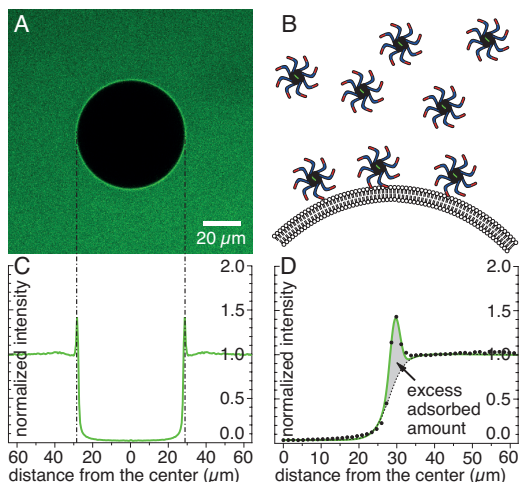


Figure 2: (A) Typical confocal laser scanning microscopy image of a GUV immersed in a solution of TAT-ELP_{BC} interacting with the membrane. (B) TAT-ELP_{BC} adsorb on the membrane as micelles as depicted on the sketch. (C) The radial intensity profile can be fitted by a Gaussian function added to a sigmoid. (D) The grey area corresponds to $rdr(I_r(r) - I_s(r))$ *i.e.* the number of CPP-ELP_{BC} adsorbed per unit of membrane surface

Fluorescence imaging was performed using confocal laser scanning microscopy (CLSM). An inverted TE-2000 microscope (Nikon, Japan) equipped with a 60X WI/1.2NA Plan Apo DIC objective and a Nikon C1 scan head was used in our experiments. Images were captured using EZ-C1 software (Nikon, version 3.50). AlexaFluor488 labelled TAT-ELP_{BC} were excited using an argon-ion laser (Melles-Griot) at 488 nm. Quantitative fluorescence intensity analysis was performed on the confocal images using the "radial profile extended" plug-in from ImageJ. Briefly, for each GUV, we plotted its averaged radial intensity profile from which we extracted the excess of fluorescence on the membrane due to peptide adsorption, as describe before (23).

In the range of concentrations for which intensities are proportional to the number of fluorescent species per unit volume, we calculate absolute local concentrations using the bulk peptide concentration C_b . Hence, as the left top panel of Fig. 2 shows, an empty GUV immersed in an solution of fluorescent peptides which adsorb on the membrane appears as a black disk with a bright corona, surrounded by a homogeneous bulk fluorescent background. The radial profile can be fitted by a Gaussian curve added to a sigmoid. For each radial coordinate r , we subtracted the sigmoid intensity I_s from the total radial intensity I_r and integrate it over r . The

obtained value is normalized with the bulk intensity $I_{b,\infty}$ and the mean radius R of the vesicle considered. The result of this computation is a number of peptides adsorbed per unit of membrane surface which, assuming a lipid surface in a bilayer $A_H = 0.65 \text{ nm}^2$ (29), can be converted in a number of peptides adsorbed per thousand lipids (N_{PTL}) as follows:

$$N_{PTL} = 1000A_H \frac{C_b}{R \cdot I_{b,\infty}} \int_0^{2\pi} d\theta \int_0^\infty r dr (I_r(r) - I_s(r)) \quad (1)$$

RESULTS

Adsorption as a function of bulk concentration and surface charge

Adsorption of TAT-ELP_{BC} on GUVs with various charge fractions X_q of DOPG in DOPC has been investigated as a function of the TAT-ELP_{BC} concentration C_b . Experiments were performed at 35° C, a temperature at which TAT-ELP_{BC} not only form micelles in bulk, but also keep their micellar structure upon adsorption on the membrane (23). Adsorption of TAT-ELP_{BC} on GUVs is presented as a surface plot on Fig. 3A, where N_m , the number of adsorbed micelles per thousand lipids is given as a function of C_m , the micellar concentration and X_q the fraction of charged lipids in the membrane. Each value of N_m is an average over at least 30 GUVs. Five membrane compositions $X_q = 0, 5, 10, 15$ and 20 %, and several bulk concentrations C_b ranging from 7.5 to 60 μM were explored, corresponding to a micelle concentration range $C_m \approx 100$ to 800 nM. C_m is calculated as $C_m = C_b/p$, with $p = 73$ the micelle aggregation number at 35° (30). For each prescribed charge fraction X_q , the adsorbed amount of micelles N_m as a function of micelle bulk concentration C_m is well described by the Langmuir isotherm (31):

$$N_m = N_m^{max} \frac{K_m C_m}{1 + K_m C_m} \quad (2)$$

where N_m^{max} expresses the maximum value of adsorbed micelles per thousand lipids, and K_m is the adsorption constant of TAT-ELP_{BC} micelles, which depends on X_q .

Dependence of N_m on membrane charge X_q is shown on a 2D plot on Fig. 3B for the three micelle bulk concentrations $C_m = 0.14, 0.27$ and $0.62 \mu\text{M}$. They all show a similar tendency, *i.e.* a sigmoidal increase of N_m with X_q around some characteristic charge fraction X_q^0 . Hence, the whole set of data has been fitted using the Langmuir Eq. (3) with a sigmoidal empirical form for the X_q dependence of K_m

$$K_m(X_q) = K_m^0 \left(1 + \frac{J - 1}{1 + \exp\left(-\frac{X_q - X_q^0}{\Delta X_q}\right)} \right), \quad (3)$$

where K_m^0 is the adsorption constant of TAT-ELP_{BC} micelles on a neutral membrane, X_q^0 the charge fraction threshold. J

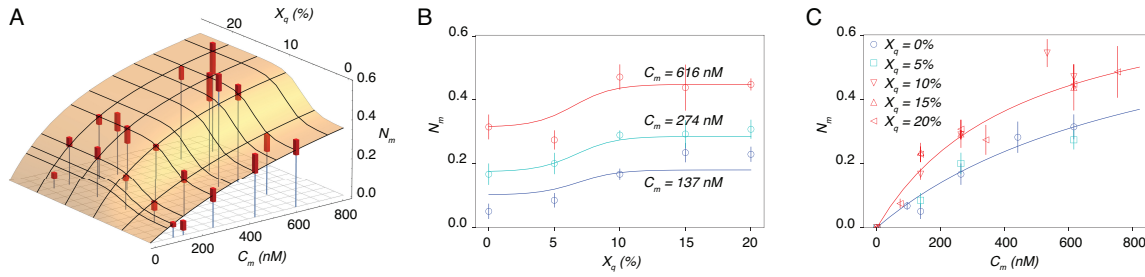


Figure 3: TAT-ELP_{BC} adsorption on GUVs made of various DOPG/DOPC compositions. $C_m = C_b/p$ is for bulk molar concentrations of TAT-ELP_{BC} micelles with aggregation number p , X_q is for DOPG molar fraction. A) 3D representation of the data, with a surface plot corresponding to the best fit, obtained as explained in the text. B) 2D representation of the data as a function of X_q only, for three different values of the micelle concentration C_m . Same best fit as in A), represented by full lines. C) 2D representation of the data as a function of C_m only, for five different values of the charge fraction X_q . Same best fit as in A), represented by full lines for the low (in blue) and high (in red) charge fractions ($X_q \leq 5\%$ and $X_q \geq 10\%$).

and ΔX_q are respectively the numerical factors describing the amplitude and the width of the empirical function.

The surface plot in Fig. 3A, which represents the best fit to the data using eqs. (2) and (3), is obtained for the set of values $N_m^{max} = 0.78$, $K_m^0 = 1.1 \times 10^6 \text{ M}^{-1}$, $X_q^0 = 7\%$, $J = 2.2$ and $\Delta X_q = 1.5\%$. Continuous lines in figs. 3A, 3B and 3C correspond to this best fit.

The existence of the two distinct charge regimes, implicitly assumed by the choice of the sigmoidal Eq. (3), is better shown in Fig. 3C by the Langmuir isotherms that have been separated into a *low charge* group ($X_q \leq X_q^0 = 7\%$) (blue symbols), and a *high charge* group ($X_q \geq X_q^0$) (red symbols). The charge on the membrane (above X_q^0) roughly doubles the value of the binding constant K_m , which varies from $K_m^0 = 1.1 \times 10^6 \text{ M}^{-1}$, for $X_q \leq X_q^0$, to $K_m^0 = 2.4 \times 10^6 \text{ M}^{-1}$ for $X_q \geq X_q^0$. Affinity constants of short TAT moieties (referred hereafter as K) interacting with the surfaces of liposomes have been measured in the literature by several groups on bilayers assembled from lipids with similar headgroups, phosphatidylcholine (PC) and phosphatidylglycerol (PG), albeit with different lipid tails. For neutral membranes Rao et al (13) obtain a value of $K = 3.1 \times 10^3 \text{ M}^{-1}$. For charged membranes, Ziegler and coworkers (32) get $K = 6 \times 10^4 \text{ M}^{-1}$ on 20% charged lipids, and Rao et al report $K = 1.3 \times 10^5 \text{ M}^{-1}$ with 100% charged lipids. Hence K_m remains higher than K especially on neutral membranes, reflecting a higher adsorption affinity of TAT-ELP_{BC} micelles to membranes of any charge density compared to TAT without cargos. The K_m of micelles ranges from 10 to 1000 fold that of short chains, for charged and neutral membranes respectively. Note however that bulk molar concentrations (C_b) of TAT moieties in micellar solutions are ~ 70 fold greater than those of micelles. Thus, half adsorption isotherms for TAT-ELP_{BC} are reached for C_b values ranging between 7 to 0.07 fold of those corresponding to short chain adsorption.

Considering a molar mass $M_w = 49.6 \text{ kDa}$ for TAT-ELP_{BC}, and an average area per lipid $A_H = 0.65 \text{ nm}^2$, $N_m^{max} = 0.78$ corresponds to a mass coverage of 7.2 mg.m^{-2} , larger than

typical values of adsorbed polymer monolayers ($\approx 1 \text{ mg.m}^{-2}$), but consistent with the adsorption of copolymer micellar assemblies. A model of hard spheres packing in an hexagonal fashion would lead to a mean distance between micelles in close contact of $d_{mic} \approx 32 \text{ nm}$, which needs to be compared to their hydrodynamic radius $R_h = 25 \text{ nm}$ at 35° (30). Hence, the measured N_m^{max} value corresponds to a density of TAT-ELP_{BC} on the membrane 50% larger than the one from the compact packing of hard spheres. This suggests that at high C_b , TAT-ELP_{BC} micelles adsorb on the membrane with a certain degree of inter-penetration, as sketched in Fig. 4.

Adsorption as a function of temperature

Adsorption on neutral and on 20% charged membranes has also been studied as a function of the temperature, from 25 to 42 °C, for a fixed bulk peptide concentration $C_b = 20 \mu\text{M}$. The results are displayed on Fig. 5. Adsorption of TAT-ELP_{BC} is triggered by self assembly at a temperature above the CMT and is significantly increased by raising the temperature above the CMT.

The formation of ELP_{BC} micelles is a complex phenomenon that has been studied in detail by Garanger *et al.* (30). Their experiments show that the micelle aggregation number p increases gradually from 73 at 35 °C to 127 at 45 °C. Micellization of TAT-ELP_{BC} is well described by a model developed by Hassounh *et al.* (33). The model refers to micellization in these systems as weak micellization, due to the low interfacial tension at the surface of a hydrophobic core that retains a significant amount of solvent. As the temperature increases, the core further collapses expelling solvent, thus increasing the interfacial tension between the core and corona blocks. This results in increasing aggregation number as the temperature increases above the CMT and changes the micelle core and corona sizes. From the point of view of adsorption of the micelles on lipid membranes in this paper, this implies that a direct comparison of adsorption isotherms at different temperatures is a complex task, as the adsorbing units—the

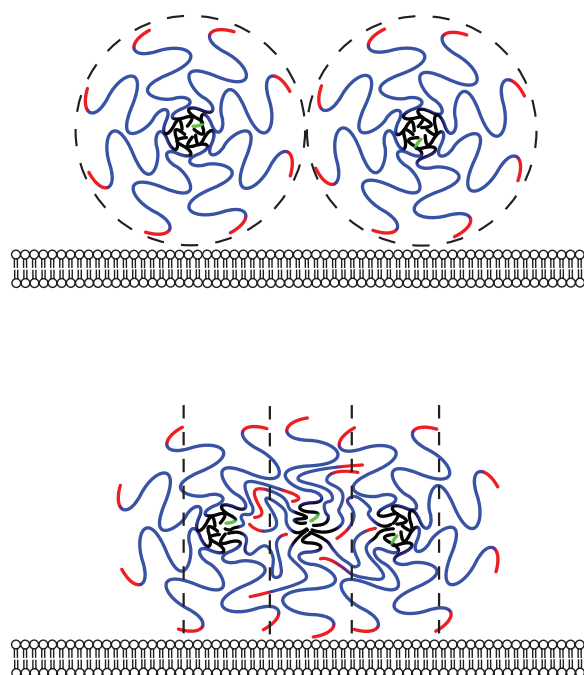


Figure 4: Sketch of TAT-ELP_{BC} micelles inter-penetration upon adsorption. If micelles do not inter-penetrate (top) N_m^{max} is smaller than the measured value. The latter is reached for a micelle-micelle distance $d = 32$ nm, corresponding to an increment of the projected density of TAT-ELP_{BC} of $\sim 50\%$.

micelles—are different objects at different temperatures; they have different aggregation number, sizes, and presumably also different affinities to the membrane surface.

Despite the underlying variation of the structure of the ELP_{BC} micelles with temperature, several important trends can be observed in Fig. 5. As the figure clearly shows, there is no adsorption below 31 °C. For neutral membranes there is no adsorption up to 33 °C, at higher temperature the N_{PTL} value increases almost linearly with T . For the 20 % charged membranes the onset of adsorption is at 31 °C, followed by an increase with temperature similar to, but generally above, the variation of adsorption on neutral membranes. Micelle adsorption affinities to phospholipid membranes appear therefore to be in general enhanced by the presence of charges on the membranes. A precise measurement of such affinities would require the determination of the adsorption isotherms for each of the studied temperatures, a significant undertaking well beyond the scope of this work.

DISCUSSION AND CONCLUSION

We have measured the affinity of TAT-ELP_{BC} acting as TAT-coupled cargo molecules of a diblock polypeptide,—ELP_{BC}—on model membranes at physiological charge densities. Below the CMT of the ELP_{BC}, the affinity of the ELP_{BC} unimers for the membrane is suppressed by the presence of the attached cargo, but above the CMT, the micelle structure provides a

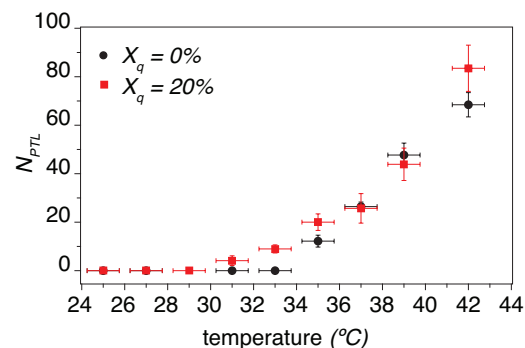


Figure 5: N_{PTL} as a function of the temperature for a neutral membrane ($X_q = 0\%$) and a charged membrane $X_q = 20\%$ at bulk concentration $C_b = 20$ μ M. The TAT-ELP_{BC} critical micellar temperature (CMT) is at 32 ± 1 °C as measured by optical density.

greater collective—multivalent—affinity for the lipid bilayer, restoring the ability of the peptide to adsorb on the lipid bilayer. Increasing the membrane charge density by modulating the composition of charged lipids leads, as expected, to an increased affinity for the ELP_{BC} micelles. Interestingly, the functional dependence of the affinity with lipid charge displays a step-shape which, to our knowledge, has not been reported before for the interaction between polymer and lipid membranes.

Such shape, that corresponds to the the existence of two adsorption regimes separated by a crossover membrane charge fraction $X_q^0 = 7\%$, independent of micelle concentration, is indicative of a Bragg-Williams adsorption process of the charged lipids on micelle surface exposed to contact with the membrane (31). Complementary to the simple Langmuir isotherms describing the adsorption of ideal particles from the bulk to the surface due to particle-surface affinity, Bragg-Williams adsorption accounts also for repulsive interactions between the adsorbed particles. For large enough repulsions, there are two adsorption states. The first occurs at low bulk concentrations, when the amount of particles on the surface is vanishingly small. The second state, above a crossover concentration that depends on the microscopic description of the repulsions, corresponds to a surface saturated by adsorbed particles. The transition between these two states is first-order for large enough repulsions. In our case, if we identify the membrane as a 2D reservoir of charged lipids, with attractive interactions to the oppositely charged micelles sitting on the membrane and repulsive interactions between charges, then the *lower charge* regime would correspond to micelles with no adsorbed charged lipids, and the *high charge* regime to micelles that have collected (adsorbed) as much charged lipids as they can (saturation).

The plausibility that such a mechanism controls adsorption of TAT-ELP_{BC} micelles on charged membranes surfaces is reinforced by the measured X_q^0 value. Indeed, from a micelle

gyration radius $R_g = 20$ nm, from an aggregation number of 73 at 35 °C (30), and considering 8 charges per peptide (6 arginines and 2 lysines), one finds that each micelle of TAT-ELP_{BC} has a positive surface charge density of the order of 0.1 elementary charges (e) per nm². Perhaps unsurprisingly, one finds that a negative charge density matching these conditions is reached when the membranes contain a charged lipid fraction $X_q^0 = 7\%$. Refinements of this picture, as for instance considering other values of the micelle radius, different partial ionization degrees for the charged micelles and membrane, charge screening or membrane wrapping, can change the precise values without compromising the description.

Our measurements of adsorbed amounts as a function of temperature bring new information to a field where the adsorption from micellar solutions of non-ionic and ionic surfactants to the air-water and solid-water interfaces have been extensively studied. At the air-water interface, standard adsorption from non-ionic surfactant solutions occurs by monomer adsorption, even above the CMC, and displays a larger affinity and surface coverage as the temperature is increased (34). Also, at the air-water interface, monomeric adsorption from ionic-surfactant solutions displays increased affinities with temperature but decreasing surfactant densities (35). Our data, which cuts through the general Langmuir adsorption diagram (N_m, C_m, T) at constant monomer concentration – and potentially at non constant micellar concentration C_m – displays **thus???** features compatible with increased adsorption affinities at the air-water interface. This is in contrast with the behavior of **what** at the solid-liquid interface where the loss of affinity of **what** with temperature is reported (36), and where adsorption from micellar solutions implies often a strong re-organization of the structure of the aggregates, even for polymeric surfactants (37). The case of the lipid bilayer interface appears thus as an interesting situation intermediate between the air-water and solid-water interfaces, where affinity can be gained by raising the temperature – related in all cases to lower critical micellar concentrations – but where micellar integrity can be preserved.

AUTHOR CONTRIBUTIONS

Experiments were designed by CM, VW and TS. The experiments shown in this work were performed by VW, analyzed and interpreted by VW, TS, AS, PM and CM. The ELP_{BC} used in this work were designed and synthesized by AC and SRM. CM, VW and TS wrote the manuscript. All authors reviewed and corrected the manuscript.

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LIST OF FIGURES

- 1 (A) Schematic representation of the diblock polypeptide ELP_{BC}. The hydrophobic block (in black) is composed of 60 repeats of the VPGVG. A small fraction is functionalized with an Alexa 488 fluorescent dye (in green). The hydrophilic block (in blue) is composed of 60 repeats of the VPGXG where X is G or A in a 1-1 ratio. This block is fused to the CPP sequence TAT₄₇₋₅₇ (in red) as its C-terminus. (B) Sketch of the self-assembly of ELP_{BC} into micelles above its CMT 3
- 2 (A) Typical confocal laser scanning microscopy image of a GUV immersed in a solution of TAT-ELP_{sBC} interacting with the membrane. (B) TAT-ELP_{sBC} adsorb on the membrane as micelles as depicted on the sketch. (C) The radial intensity profile can be fitted by a Gaussian function added to a sigmoid. (D) The grey area corresponds to $rdr(I_r(r) - I_s(r))$ i.e the number of CPP-ELP_{BC} adsorbed per unit of membrane surface . . . 4
- 3 TAT-ELP_{BC} adsorption on GUVs made of various DOPG/DOPC compositions. $C_m = C_b/p$ is for bulk molar concentrations of TAT-ELP_{BC} micelles with aggregation number p , X_q is for DOPG molar fraction. A) 3D representation of the data, with a surface plot corresponding to the best fit, obtained as explained in the text. B) 2D representation of the data as a function of X_q only, for three different values of the micelle concentration C_m . Same best fit as in A), represented by full lines. C) 2D representation of the data as a function of C_m only, for five different values of the charge fraction X_q . Same best fit as in A), represented by full lines for the low (in blue) and high (in red) charge fractions ($X_q \leq 5\%$ and $X_q \geq 10\%$). 5
- 4 Sketch of TAT-ELP_{BC} micelles inter-penetration upon adsorption. If micelles do not inter-penetrate (top) N_m^{max} is smaller than the measured value. The latter is reached for a micelle-micelle distance $d = 32$ nm, corresponding to an increment of the projected density of TAT-ELP_{BC} of $\sim 50\%$ 6
- 5 N_{PTL} as a function of the temperature for a neutral membrane ($X_q = 0\%$) and a charged membrane $X_q = 20\%$ at bulk concentration $C_b = 20 \mu\text{M}$. The TAT-ELP_{BC} critical micellar temperature (CMT) is at $32 \pm 1^\circ\text{C}$ as measured by optical density. 6