Supporting Information

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Abstract: An *ad hoc* electrostatic self-assembly method has been developed to obtain nanostructures containing an aqueous lumen and an addressable, cross-linked nanomembrane from non-assembling polymers. The essence of this method involves the use of divalent counterions to temporarily perturb the packing features of ionic groups in a hompolymer, which results in a vesicle-like structure that is captured *in situ* through a simple crosslinking reaction. The fidelity of the assembly has been tested for molecular transport across the nanomembrane, both for the molecules encapsulated in the lumen and for those trapped in the membrane itself. The membranes are addressable for robust multifunctionalization of their surfaces and for tunable transmembrane molecular transport.

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Experiment procedures
Result and discussion
Figure S1-S13
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Experimental Procedures

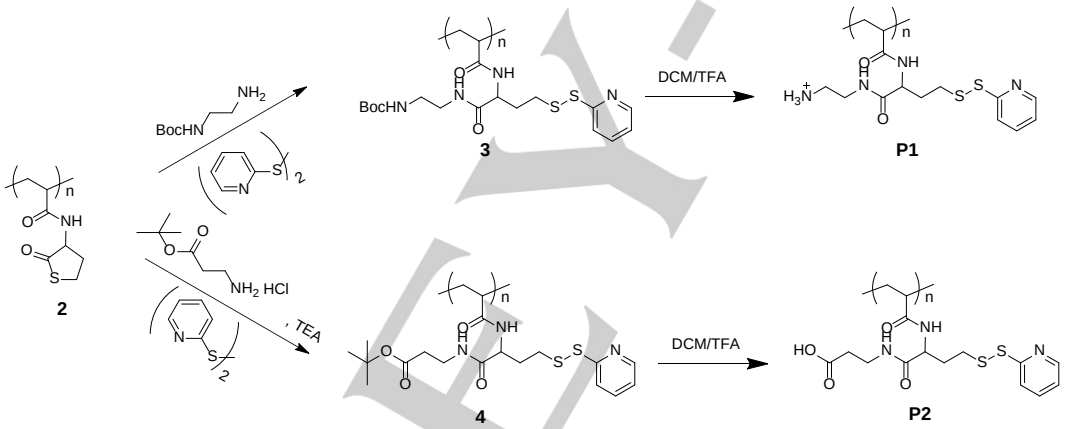
Instrumentation

^{1}H-NMR and ^{13}C-NMR spectra were recorded on an Avance 400 NMR spectrometer. Molecular weight of the polymers was measured by gel permeation chromatography (GPC, Waters) using a PMMA standard with a refractive index detector. DMF was used as eluent with a flow rate of 1 mL/min. Dynamic light scattering (DLS) measurements were performed using a Malvern Nanozetasizer. SLS measurements were performed on an ALV/SP-125 goniometer instrument. TEM images were recorded in JEOL 2000FX transmission electron microscope.

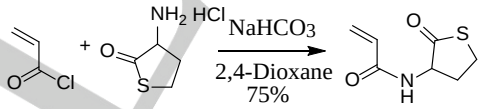
Chemicals

All reagents and solvents are purchased from commercial sources and used as received unless otherwise mentioned.

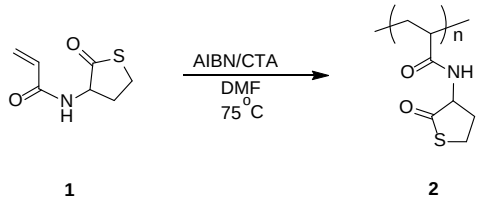
Material synthesis



Scheme 1. Synthetic strategy to prepare targeted amphiphilic homopolymers

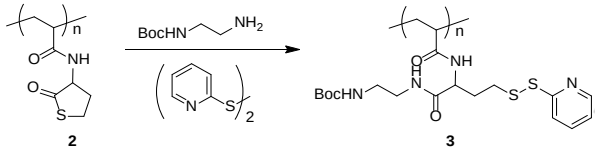


*Synthesis of Thiolatoneacrylamide, **1**.* Thiolatone acrylamide monomer was synthesized following the reported procedure.[1] Homocysteine thiolactone hydrochloric acid (45.6 mmol, 7.0 g) was dissolved in 100 mL of 1,4-dioxane and H_2O (1:1) mixture. Then 10 eq. of $NaHCO_3$ was weighed to the mixture followed by the slow addition of acryloyl chloride under ice bath. The reaction mixture was concentrated and extracted with ethyl acetate and H_2O after 12 hours. The crude product was obtained after drying the organic layer in anhydrous Na_2SO_4 followed by rotovapping. The crude product was further purified by recrystallization in DCM. Yield: 6.4 g, 82%. ^{1}H NMR (400 MHz, DMSO) δ (ppm): 8.43-8.49 (NH), 6.05-6.30 (2H), 5.60-5.72 (1H), 4.63-4.76 (1H), 3.26-3.50 (2H), 2.40-2.50 (1H), 2.0-2.17(1H).

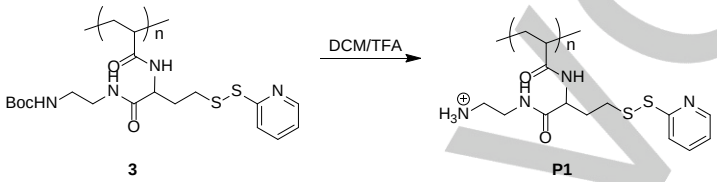


*Polymerization of Thiolactone acrylamide, **2**.* Thiolatoneacrylamide monomer, **1** (2.91 mmol, 500 mg), AIBN (0.0058 mmol, 0.954 mg) and 2-(Dodecylthiocargonothioylthio)-2-methylpropionic acid (0.0388 mmol, 14.1 mg) were weighed into a glass vial and dissolved in

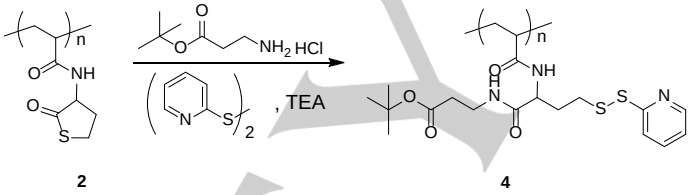
1 mL of DMF. The solution was freeze-pump-thaw for 3 times. The polymerization was carried out at 75 degree by immersing the glass vial in a preheated oil bath. The polymerization was then quenched by liquid nitrogen cooling after 4 hrs. The polymerization mixture was concentrated to remove majority of DMF. Then polymer was purified by precipitation in MeOH. The precipitate was redissolved in DCM and precipitated in MeOH for two more times. 450 mg of pure polythiolactoneacrylamide, **2** was obtained after drying. Yield: 450 mg, 88%. GPC (DMF): 14.0 kDa, PDI: 1.26. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 6.98-7.70, 4.30-5.11, 3.11-3.52, 2.50-2.76, 2.0-2.50, 1.31-2.0, 1.0-1.29.



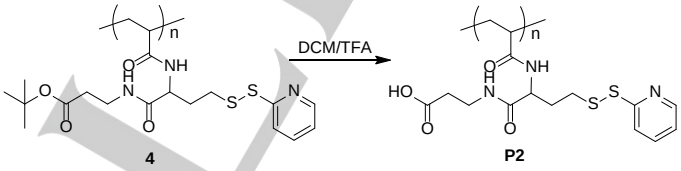
*Synthesis of **3**.* 100 mg of **2** (0.582 mmol repeat unit) with 10 eq. of aldithiol (1282.2 mg, 5.82 mmol) were dissolved in 1 mL of DMF. To the mixture 5 eq. of N-Boc ethylenediamine (466.2 mg, 2.91 mmol) was added dropwise after being purged with argon for 20 minutes. The reaction stands overnight. The reaction was repeated to accomplish the full conversion if needed. The product, **3** was purified by dialysis against DCM/MeOH and concentrated. Yield: 198 mg, 72%. GPC (DMF): 29.0 kDa, PDI: 1.25. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.26-8.51, 7.52-7.80, 6.96-7.24, 4.02-4.63, 2.50-3.74, 1.75-2.46, 1.0-1.68.



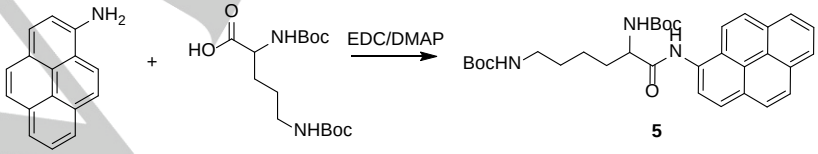
*Synthesis of **P1**.* 170 mg of **3** was dissolved in 1 mL of DCM followed by the addition of 1 mL of TFA. DCM and TFA were removed after reaction for overnight. The concentrate was precipitated in cold diethylether. Then precipitate was rinse with diethylether for 3 more times to afford **P1**. Yield: 150 mg. 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.29-8.48, 7.78-7.96, 7.08-7.29, 4.11-4.69, 2.50-3.25, 1.75-2.48.



*Synthesis of **4**.* 100 mg of **2** (0.582 mmol repeat unit) with 10 eq. of aldithiol (1282.2 mg, 5.82 mmol)were dissolved in 1 mL of DMF. 5 eq. of B-Alanine t-butyl ester hydrochloric acid (528.6 mg, 2.91 mmol) with TEA (520 µL, 2.91 mmol) in 1 mL in MeOH was added after being purged with argon for 20 minutes. The reaction stands overnight. The reaction was repeated to accomplish the full conversion if needed. The reaction mixture was purified by dialysis against DCM/MeOH and concentrated to afford **4**. Yield: 140 mg, 54%. GPC (DMF): 27.0 kDa, PDI: 1.33. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.34-8.51, 7.59-7.87, 6.96-7.24, 4.19-4.78, 3.12-3.89, 2.68-3.06, 2.25-2.68, 1.25-1.58.



*Synthesis of **P2**.* 140 mg of **4** was dissolved in 1 mL of DCM followed by the addition of 1 mL of TFA. DCM and TFA were removed after reaction for overnight. The concentrate was precipitated in cold diethylether. Then precipitate was rinse with diethylether for 3 more times to afford **P2**.Yield: 122 mg, 99%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 8.34-8.51, 7.59-7.87, 6.96-7.24, 4.19-4.78, 3.12-3.89, 2.68-3.06, 2.25-2.68, 1.25-1.58.



*Synthesis of Boc protected lysinyl pyrene, **5**.* Boc protected lysine (582.9 mg, 1.106 mmol), EDC (212 mg, 1.106 mmol) and DMAP (22.5 mg, 0.184 mmol) were dissolved in dry 50 mL of DCM. After 20 minutes, amino pyrene (200 mg, 0.922 mmol) in 5 mL DCM was added to the mixture. The reaction was kept for overnight. The reaction mixture was extracted using DCM and H_2O. The organic layer was collected and concentrated to obtain the crude product. The pure product, **5** was obtained after purification by flash chromatography. Yield: 180mg, 36%, ^{1}H NMR (400MHz, DMSO-d6): 10.31 (NH), 8.06-8.42 (9H), 7.15 (NH), 6.83 (NH), 4.29 (1H), 2.96 (2H), 1.65-1.90 (2H), 1.45 (13H), 1.38 (9H). ^{13}C NMR (100MHz, DMSO-d6): 172.83, 156.21, 156.09, 132.11, 131.98, 131.27, 130.94, 128.83, 127.71, 127,48, 127.11, 126.88, 125.71, 125.42, 124.81, 124.31, 124.03, 122.93, 112.49, 79.88, 79.64, 79.44, 78.62, 77.82, 55.38, 31.83, 29.82, 28.75, 23.58 FAB/MS: m/z 546.29$[M+H]^+$ (expected m/z 545.30)

5 **6**

*Synthesis of lysinyl pyrene, **6**.* To 75 mg (0.138 mmol) of **5** in 1 mL of DCM solution, 1 mL of TFA was added. The reaction mixture was concentrated after 1 hour. The concentrated mixture was precipitated in cold diethylether for 3 times to afford the product. Yield: 72 mg, 82%. ^{1}HNMR (400MHz, DMSO-6d): 8.05-8.45 (9H), 4.26 (1H), 2.84 (2H), 2.01 (2H), 1.49-1.70 (4H). ^{13}C NMR (400MHz, DMSO-6d): δ(ppm) 168.55, 158.33, 157.88, 130.80, 130.40, 130.31, 128.87, 127.51, 127.22, 127.09, 126.63, 125.60, 125.24, 125.04, 124,24, 123.76, 123.52, 122.06, 112.03, 79.43, 78.99, 78.55, 52.70, 30.80, 26.75, 21.50. ESI/MS: m/z 346.18 $[M+H]^+$ (expected m/z 346.19)

Synthesis of thiol modified FITC. The thio modified FITC was synthesized following the report procedure. [2]

Polymer stock solution preparation. 15mg of P1 or P2 was directly dissolved in 2 mL of milliQ water. To make P2 dissolved, 1.5 eq. of NaOH was added to the solution. The obtained solution was then dialyzed against DI water using membrane with a MWCO of 3500 Da. The final concentration of solution was fixed to 5mg/mL by adding milli Q water.

Polymersome and crosslinked vesicle formation. Calculated amount of salt stock solution (100 mM) was added to 300 µL of milliQ water. To the above slat solution, 200 µL of P1 stock solution was added to make final polymer solution with a concentration of 2 mg/mL. For P2, 400 µL of salt solution was prepared and added with 100 µL of P2 stock solution giving the polymer solution with a concentration of 1 mg/mL. The final polymer solutions were left for 3 hours to form polymersomes. The crosslinked vesicles were obtained by cross-linking the polymersome solution through the addition of calculated amount of DTT. The cross-linking reaction was allowed to undergo for 4 hours. The crosslinked vesicle was then purified by dialysis against water.

Hydrophobic and hydrophilic guest encapsulation. For hydrophobic guest encapsulation, 1wt% of DiI solution (1mg/mL) in acetone was added to the polymersome solutions followed by the addition of DTT. The unloaded guest molecule was removed by filtration using syringe filter with a pore size of 0.4 um. The hydrophilic guests were dissolved in the salt solution with a desired salt and guest concentration. To the hydrophilic guest molecule solution, polymer stock solutions were added to form polymersomes which encapsulate the hydrophilic guest *in situ*. The free guest was removed by extensive dialysis against water after DTT crosslinking.

Myoglobin encapsulation. 800 µL of myoglobin stock solution in milliQ water (2mg/mL) was added with 42 µL of 100 mM $MgCl_2$ solution. 200 µL of P2 solution (5mg/mL) was added to myoglobin- $MgCl_2$ solution. 0.1 equivalent of DTT was added to the mixture after 2 hrs. The free myoblobin was removed 4 hours later after the addition of DTT by dialysis against water using membrane with MWCO of 100 kDa.

Lysozyme encapsulation. To 600 µL of lysozyme stock solution (5mg/mL), 20 µL of 100 mM Na_2HPO_4 solution was added. Then, 400 µL of P1 stock solution (5mg/mL) was added into lysozyme solution followed by the addition of 0.1 eq of DTT after 2 hours. The cross-linking reaction was allowed to undergo for 4 hrs. The unloaded lysozyme was removed by extensively dialysis against water using membrane with MWCO of 100 kDa.

Trypsin digestion

Free lysozyme: 50 µL of 100 µM lysozyme solution was added to 50 µL of 50 mM Tris buffer (pH 8.0) with 1 mM of $CaCl_2$. Then 1 µL of 1 M DTT was added to lysozyme solution. The mixture was incubated for 2 hours at 37 ^{o}C. 10 µL of ACN was added to the mixture followed by heating at 55 ^{o}C for 15 min. After cooling to room temperature, to above solution 50 µL of 0.1 µg/µL trypsin stock solution was added. The mixture was incubated at at 37 ^{o}C for 18 hours before being submitted to MALDI.

Trypsin digestion of released lysozyme from crosslinked vesicle

50 µL of lysozyme loaded crosslinked vesicle solution was added to 50 µL of 50 mM Tris buffer (pH 8.0) with 1 mM of $CaCl_2$. Then 1 µL of 1 M DTT was added to lysozyme solution. The mixture was incubated for 2 hours at 37 ^{o}C. 10 µL of ACN was added to the mixture followed by heating at 55 ^{o}C for 15 min. After cooling to room temperature, to above solution 50 µL of 0.1 µg/µL trypsin stock solution was added. The mixture was incubated at at 37 ^{o}C for 18 hours before being submitted to MALDI.

Trypsin digestion of lysozyme inside crosslinked vesicle

50 µL of lysozyme loaded crosslinked solution was added to 50 µL of 50 mM Tris buffer (pH 8.0) with 1 mM of $CaCl_2$. Without the addition of DDT solution, the mixture was directly incubated for 2 hours at 37 ^{o}C. 10 µL of ACN was added to the mixture followed by heating at 55 ^{o}C for 15 min. After cooling to room temperature, to above solution 50 µL of 0.1 µg/µL trypsin stock solution was added. The mixture was incubated at at 37 ^{o}C for 18 hours before being submitted to MALDI.

Crosslinked vesicle functionalization. 1 mL of 2 mg/mL of cationic crosslinked vesicle solution was added to 1 mL of DMSO. Then, the solution was adjusted to pH 9 by the addition of $NaHCO_3$ solution. 0.2 equivalent of TRITC (to the amine) in DMSO was added to the nanoparticle solution. After 24 hours, 0.2 equivalent thiol functionalized fluorescein solution in DMSO was also added to the reaction mixture. The reaction mixture was dialysis against MeOH using membrane with MWCO of 11000 Da after 24 hours.

PEGylation of cationic Crosslinked vesicle. To 500 µL of 2 mg/mL cationic crosslinked vesicle solution (0.0022 mmol repeat unit), 500 µL of DMSO was added with 0.95 µL of triethylamine (0.0066 mmol, 3 equivalents). 8.8 mg (0.0044 mmol, 2 equivalents) of PEG-NHS ester (Mn: 2000 Da) was dissolved in 500 µL of DMSO and then added to the crosslinked vesicle solution. The reaction was allowed to go overnight. The free PEG was removed by dialysis against water.

PEGylation of anionic Crosslinked vesicle. To 2 mL of 1 mg/mL of anionic crosslinked vesicle solution (0.0054 mmol repeat unit), 2.07 mg EDC (0.0108 mmol, 2 equivalents) was added. Then, 6 mg (0.0108 mmol, 2 equivalents) of PEG-NH_2 (Mn: 550 Da) in 500 µL of water solution was added to crosslinked vesicle solution. The reaction was kept for 24 hrs. The free PEG was removed by dialysis against water.

Quantification of the amount of amine on P1 and cationic crosslinked vesicle before and after PEGylation. 200 µL of P1 solution (5 mg/mL in water) was mixed with 300 µL of H_2O and 1 mL of DMSO to make P1 stock solution with a concentration of 0.333 mg/mL in DMSO/water mixture(2:1). To a 96 well plate, different volume of P1 stock solution was added to the well with 50 µL of fluorescamine (2.5 mg/mL in DMSO). The final volume of solution in each well was adjusted to 200 µL by the addition of corresponding amount of DMSO. Each sample was repeated in triplicates. The mixture was allowed to stand for 2 hours at room temperature. The fluorescence was record in SpectraMax M5 plate reader with 400 nm excitation filter and a 460 nm emission filter. The calibration curve can be achieved by ploting fluorescence intensity against number of moles of amine in P1. The total amount of amine possessed by crosslinked vesicle is presumable to be the same as equal amount of P1. Similarly, 50 µL of VesGel solutions before and after PEGylation (0.333mg/mL in 2:1 DMSO/water mixture) was added with with 50 µL of fluorescamine (2.5 mg/mL in DMSO) and 100 µL of DMSO. The mixture was submitted to fluorescence measurement after 2 hours. The amount of amine accessible for fluorescamine on crosslinked vesicle before and after PEGylation can be obtained by fitting the fluorescence intensity in calibration curve.

Rg measurement by Static light scattering (SLS). The same solution, prepared for DLS was also used for SLS study. Here, data was collected for different angles of incident light source, keeping the concentration of the solution same in all the measurements. The radius of gyration (R_g) was estimated from the slop of Guinier plot using the equation shown below.

$$\ln\left(\frac{I_{(q)}}{I_{(0)}}\right) = -\frac{R_g^2}{3} * q^2$$

Results and Discussion

The entire simulation work was conducted using the AMBER 12 software.[3] All models for the **P2** polymers simulated in this study were built and parametrized according to a validated procedure adopted in precedence for similar studies.[4-8] In particular, the **P2** monomer was parameterized with the "general AMBER force field (GAFF)" (gaff.dat).[9] Initially, the **P2** 10-mer models were built in two different ways – *i.e.*, with monomers mounted in a facial fashion, or alternated on the polymer backbone. However, as already seen in the case of amphiphilic dendrons,[4,5] also in this case preliminary MD simulation (200 ns) of the two different starting configurations in presence of explicit TP3P[10] water molecules and Na^+ neutralizing ions did not result in any appreciable difference, demonstrating that the behavior of the **P2** model in solution is substantially invariant on how the latter is built (Figure S11 a-f).

A molecular mode for a 70-mer **P2** chain, consistent with the average experimental length, was also built and equilibrated through a 1 µs of MD simulation in explicit solvent molecules (Figure S11 g-j). The per-monomer enthalpy *H* of the **P2** 70-mer system extracted from the MD and calculated as the sum of solute-solute and solute-solvent interactions ($H = E_{gas} + E_{solv}$, see below) is a good indicator of the solubility of the polymer in water.[7] The latter was found equal in the 70-mer and in the 10-mer, demonstrating that the reduced **P2** 10-mer system constitutes a reliable model to study polymer self-assembly phenomena in water solution.

After initial minimization, all system were preliminary simulated for 50 ps of NVT (constant N: number of atoms, V: volume and T: temperature) to reach the experimental temperature of 25 °C (298 K). During this step, the polymer chains were maintained as fixed. Then, all restraints were removed, and all systems were equilibrated for 200 ns of MD simulations (except for the **P2** 70-mer, for which longer simulation time, 1µs, was necessary for the equilibration) conducted in NPT (constant N: number of atoms, P: pressure and T: temperature) periodic boundary conditions at the temperature of 25 °C and pressure of 1 atm. All MD simulations used a time step of 2 fs, the Langevin thermostat and a 10 Å cutoff. Long-range electrostatic effects were according to the particle mesh Ewald[11] approach, while the SHAKE algorithm was used on all bonds involving Hydrogen atoms.[12] Several parameters including systems energy (*H* or ΔE_{ass}, see further), the root mean square displacement (RMSD) and the systems radius of gyration (R_g) were used to assess the equilibration of the simulated systems in the MD regime. All structural analyses were conducted with the *ptraj* module of AMBER 12.

Consistent to our previous studies of similar systems, energy analysis was performed according to the MM-PBSA approach.[4,5,13,14] In particular, the average molecule self-assembly energies ΔE_{ass} were calculated from the MD simulations as:

$$\Delta E_{ass} = E_{assembled} - E_{disassembled} \quad (1)$$

$$\Delta E_{ass} = \Delta E_{gas} + \Delta E_{solv} \quad (2)$$

Where $E_{assembled}$ is the energy of the assembled system divided for the number of **P2** 10-mer chains in the system, and $E_{disassembled}$ is the energy of one **P2** 10-mer chain in solution. ΔE_{ass} is composed of the total gas-phase *in vacuo* non-bond energy (ΔE_{gas}), and of a solvation term ($\Delta E_{solv} = \Delta E_{PB} + \Delta E_{NP}$)[15] as described in Eq. (2). The polar component of ΔG_{PB} was evaluated using the Poisson-Boltzmann[16] (PB) approach with a numerical solver implemented in the *pbsa* program of AMBER 12.[17] The non-polar contribution to the solvation energy was calculated as $\Delta E_{NP} = \square$ (SASA) + $\square$, in which $\square$ = 0.00542 kcal/Å^2, $\square$ = 0.92 kcal/mol, and SASA is the solvent-accessible surface estimated with the MSMS program.[18] For prompt comparison of the stabilization induced by Mg^{2+} or inefficiency of Na^+, ΔE_{ass} was set to 0 at the beginning of each MD simulations in the plots reported in the main paper, while the evolution of the ΔE_{ass} values was monitored as a function of the simulation time. Notably, ΔE_{ass} drifting to <0 values during the MD run indicate that the ions are capable of successfully stabilizing the assembly, while evolving to ΔE_{ass}>0 values demonstrate that the ions are inefficient and that the initial configuration for the assemblies is destabilized during the MD (see Figure 2 and Figure S10).

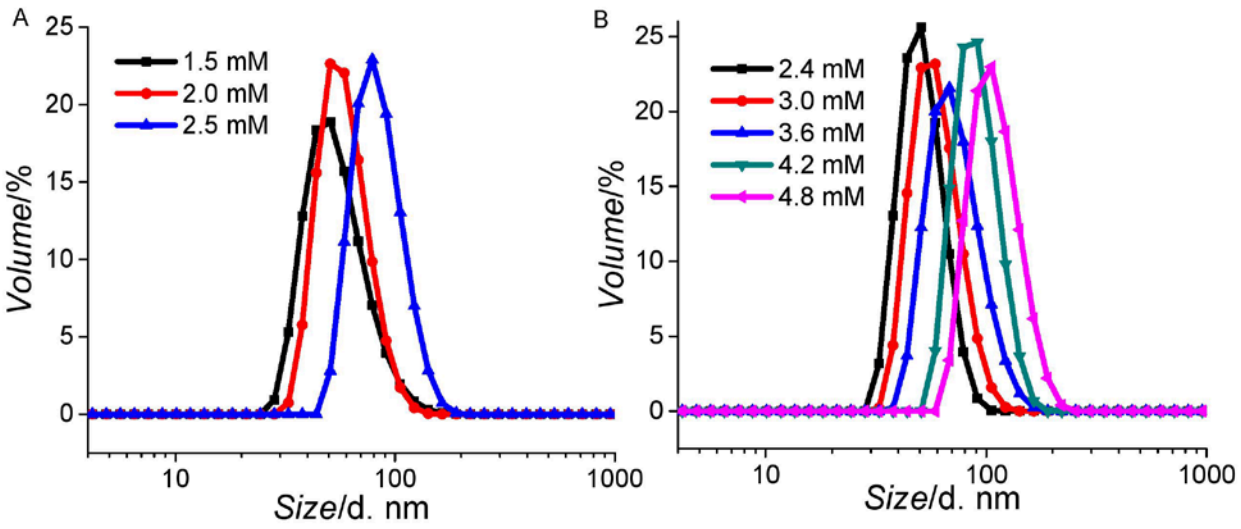


Figure S1. Salt concentration dependent assembly of homopolymers before crosslinking. A, P1 assembly in Na_2HPO_4 solution. B, P2 assembly in $MgCl_2$ solution on.

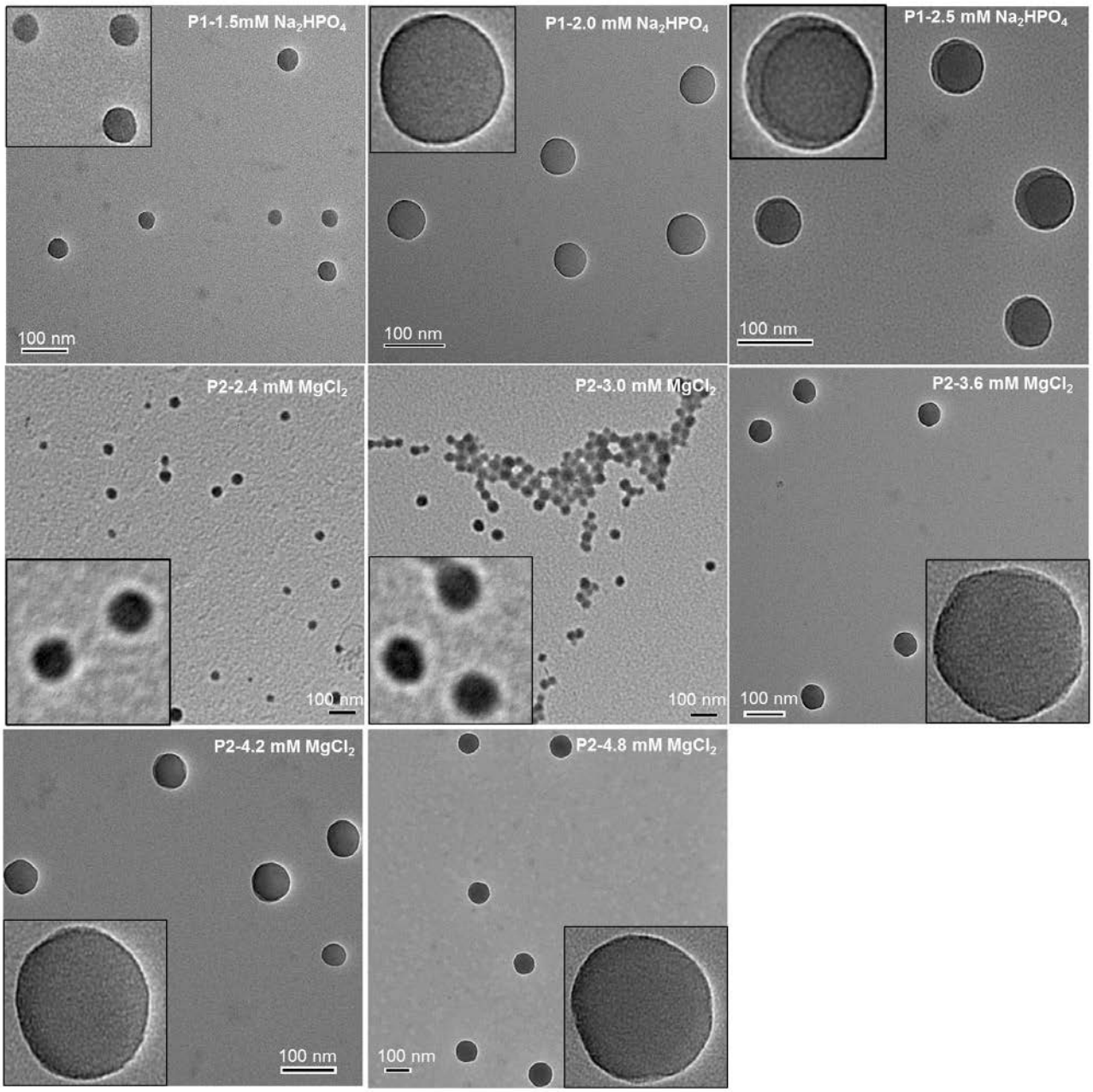
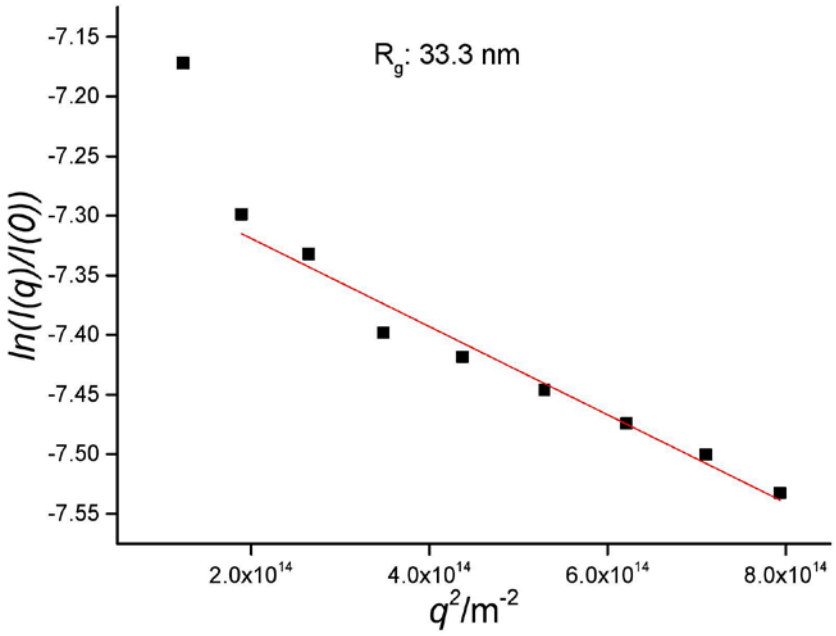


Figure S2. Salt concentration dependent morphological evolution followed by TEM. Top: P1 assembly in Na_2HPO_4 solution. Middle & bottom: P2 assembly in $MgCl_2$ solution (scale bar: 100 nm



FigureS3. Measurement of R_g of P2 assembly by static light scattering. (Crosslinked assembly forms at 2.4 mM $MgCl_2$).

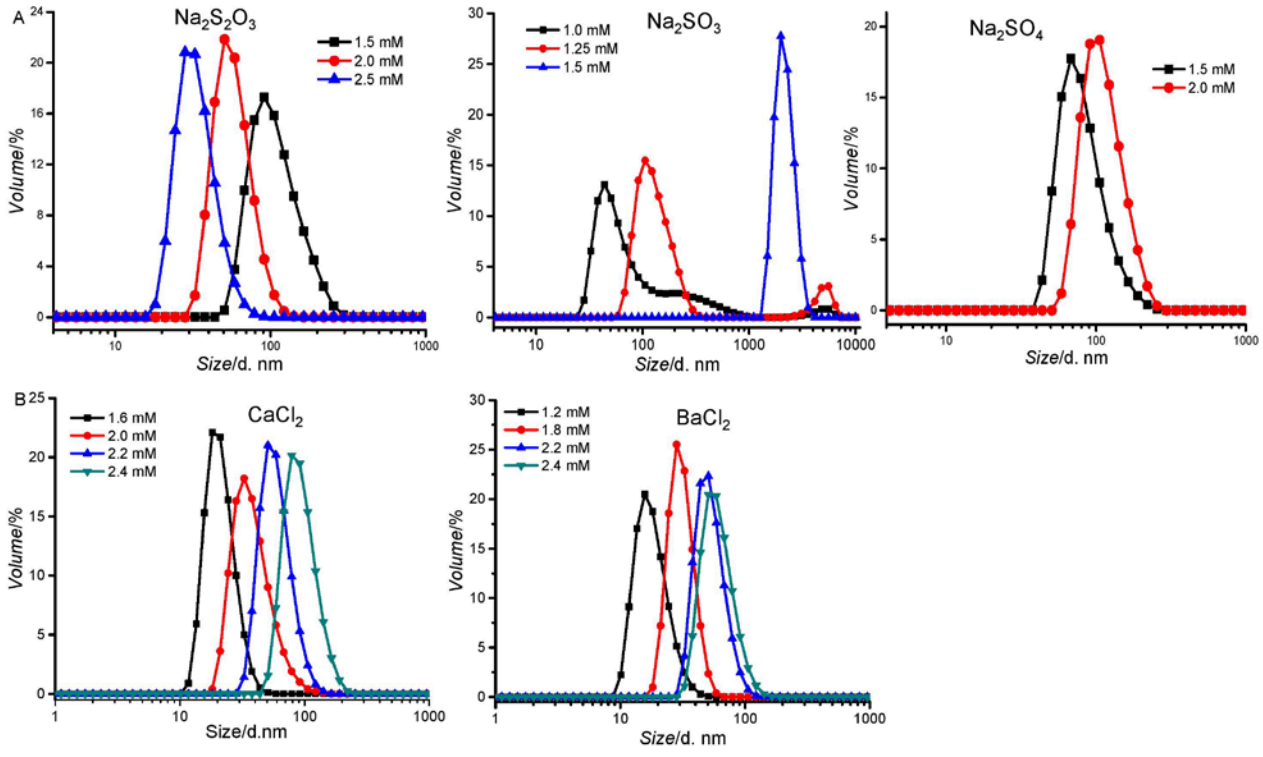


Figure S4. Salt concentration dependent self-assembly of P1 and P2 in a variety of divalent salts. A, Size of P1 assemblies in a variety of concentrations of $Na_2S_2O_3$, Na_2SO_3, and Na_2SO_4 after crosslinking. B, Size of P2 assemblies in a variety of concentrations of $CaCl_2$ and $BaCl_2$ after crosslinking

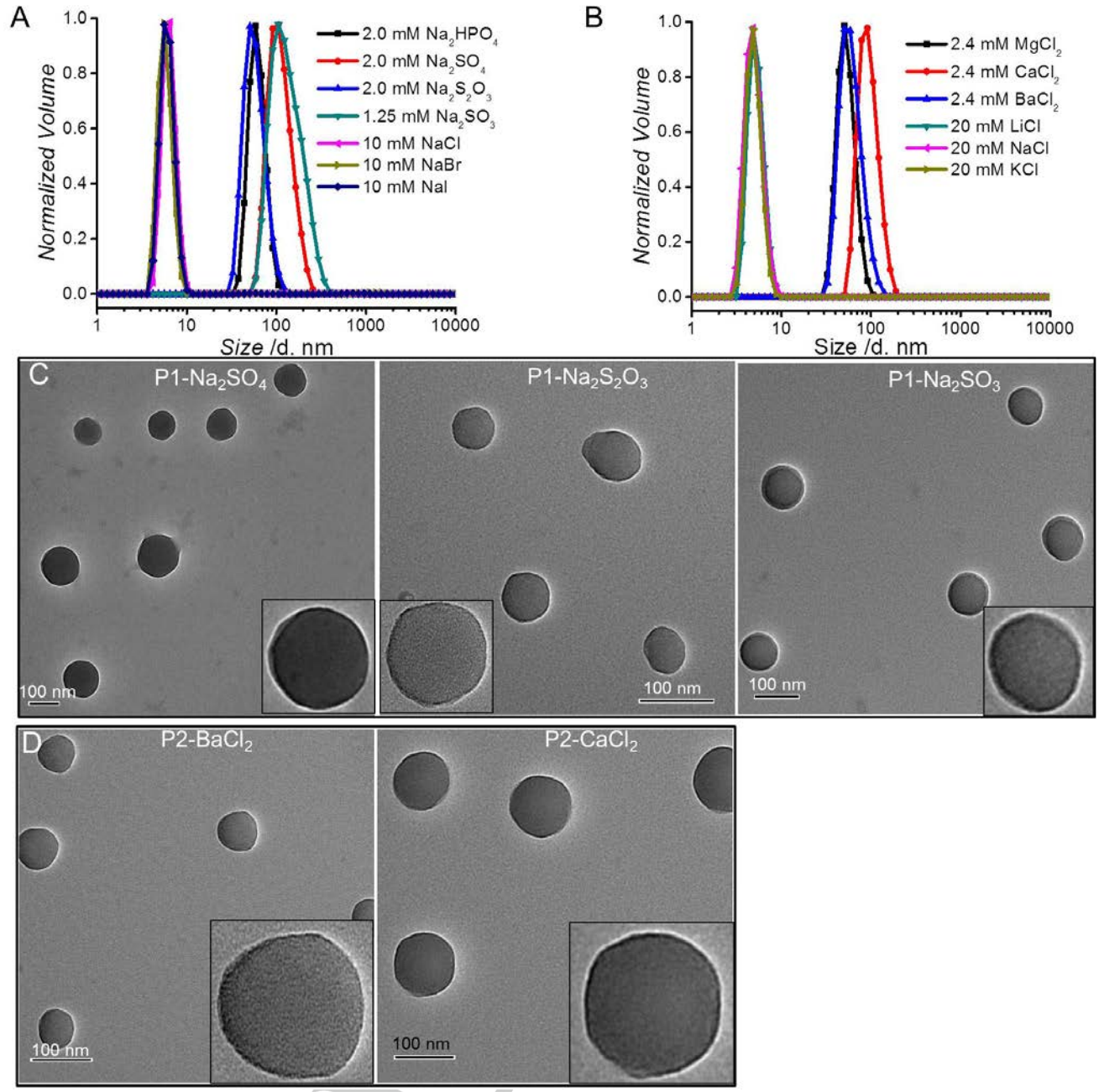


Figure S5 Illustration of the need for multivalent counterions for vesicular assembly formation from P1 and P2 A, The effect of counterion valence on the size of assemblies from P1; B, The effect of counterion valence on the size of assemblies from P2; C. TEM images of crosslinked vesicles prepared from P1 in the presence of corresponding divalent anions (scale bar: 100 nm); D, TEM images of crosslinked vesicles prepared from P2 in the presence of corresponding divalent cations

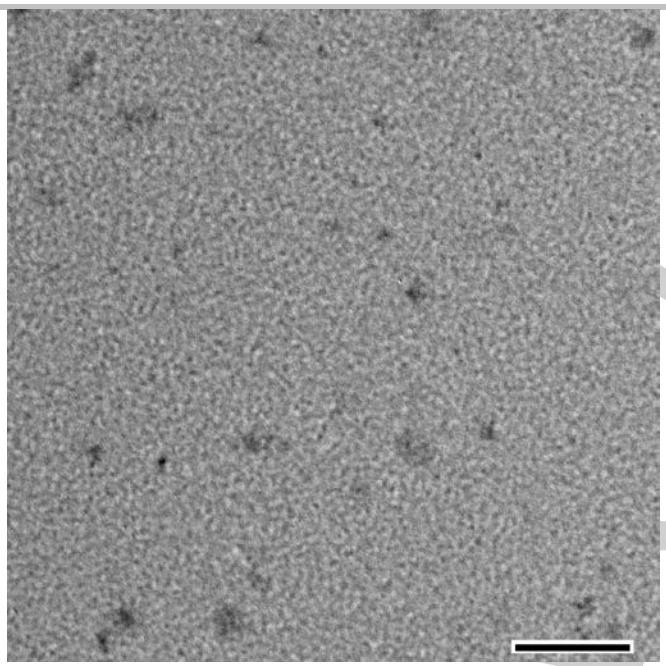


Figure S6. TEM image of P1 without divalent salt addition. Scale bar: 100 nm

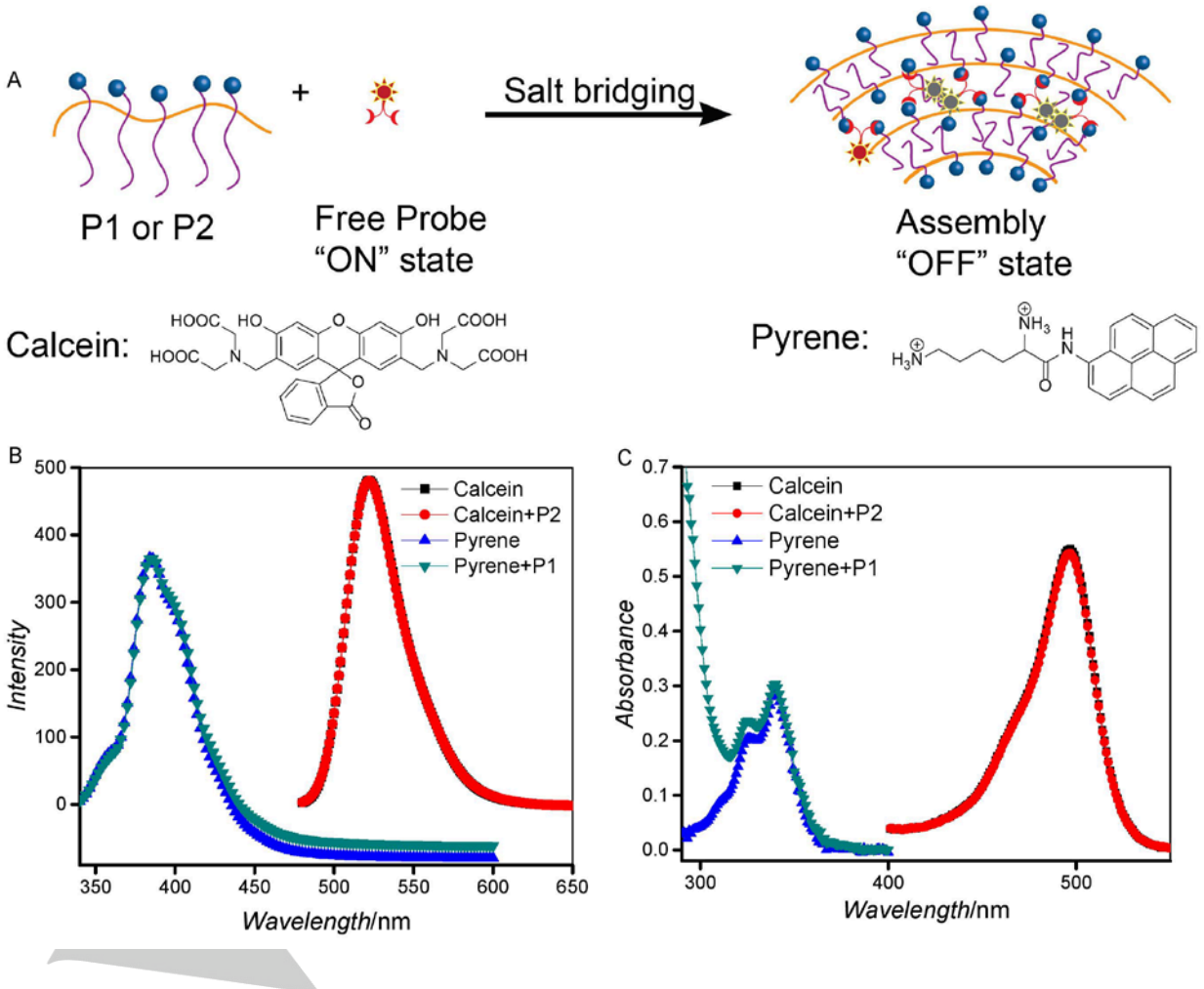


Figure S7. Salt bridging mechanism of self-assembly. A, Schematic illustration of salt-bridging mechanism probed by fluorescent dyes; B, effect of mixing fluorescent probes with like-charged polymers on fluorescence; C, absorbance-matching of the fluorescent probes in the presence and absence of the like-charged polymers shown in (b).

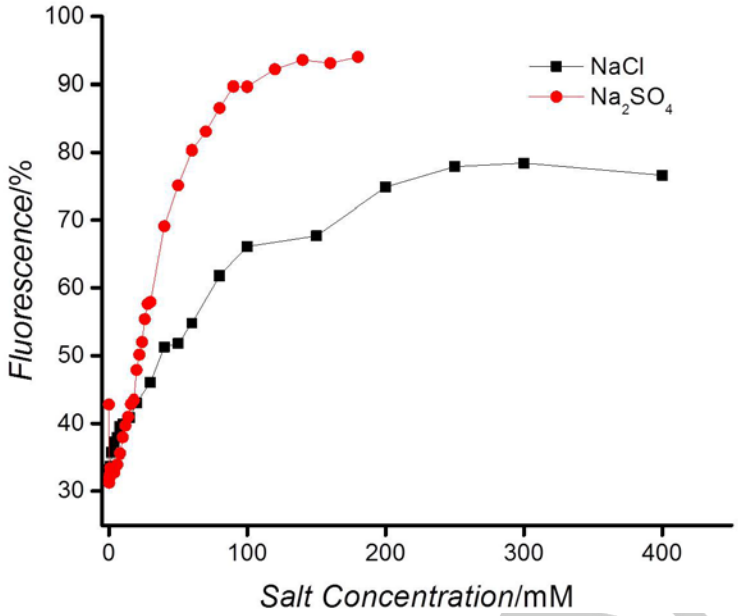


Figure S8. Fluorescence recovery from calcein-P1 complex emission titrated by Na_2SO_4 and NaCl

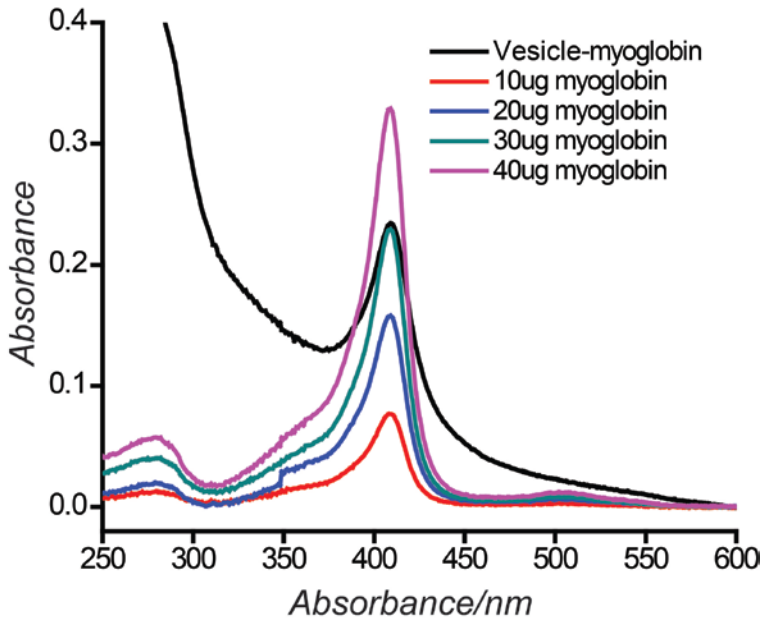


Figure S9. Absorbance spectra of myoglobin for calibration and myoglobin loaded in anionic vesicle.

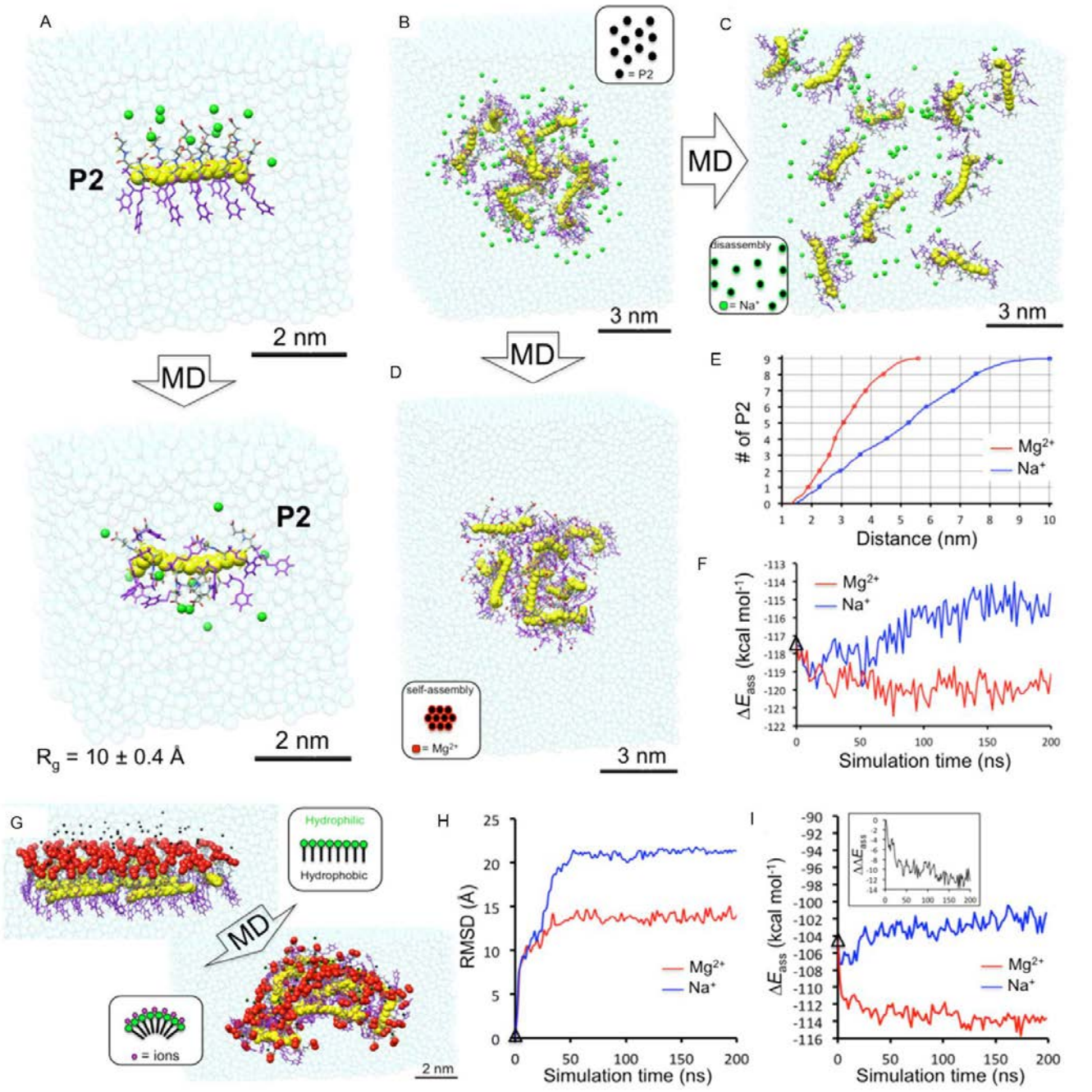


Figure S10 MD simulations of polymers self-assembly and the effect of divalent ions. A, Starting and equilibrated snapshots taken from the MD simulation of a single P2 10-mer chain in solution. B, Starting configuration of the complex system containing ten P2 chains in solution. (C,D) Final configurations (200 ns) of the same complex system in the presence of Na^+ (C) or Mg^{2+} cations (D) in solution. The hydrophobic moieties of P2 are colored in purple, the hydrophilic groups are colored per-atom (C: grey, O: red, N: blue and H: white), Na^+ ions in green and Mg^{2+} in red. Water molecules (O atoms) are colored in transparent cyan and the backbones of P2 polymers are represented as yellow spheres. E, Number of P2 neighbors in space as a function of the distance (each P2 chain has a maximum of nine neighbors). F, Self-assembly energy (ΔE_{ass})), set to 0 kcal mol^{-1} at the start of the simulation and monitored as a function of simulation time (values per-monomer) during the MD runs. ΔE_{ass} drifting to <0 or >0 values mean that the ions respectively can or cannot successfully stabilize the initial configuration for the assembly during the MD. G, Starting and equilibrated snapshots of the P2 monolayer. H, Distortion (root mean square deviation data, RMSD) of the system from the initial monolayer configuration. I, Self-assembly energy (ΔE_{ass}) and self-assembly energy differences ($\Delta\Delta E_{ass}$ = $\Delta E_{ass}(Mg^{2+}) - \Delta E_{ass}(Na^+)$) in kcal mol^{-1} as a function of simulation time (values per-monomer).

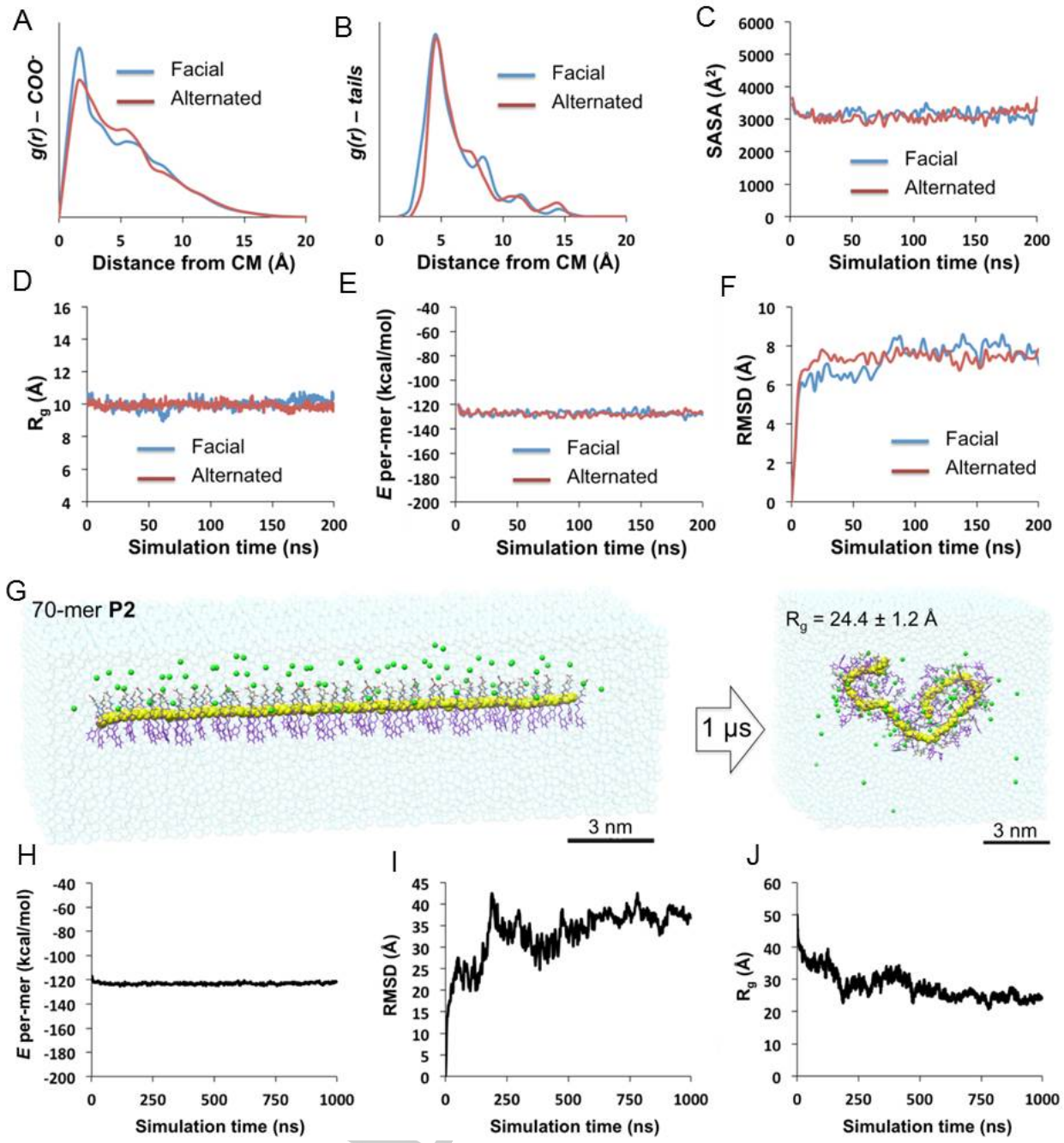


Figure S11. Comparison between facial and alternated P2 10-mers (A-F) and MD simulation of the P2 70-mer(g-j). radial distribution functions *g(r)* of the COO^- groups (A) and hydrophobic tails (B) calculated respect to the P2 10-mers centers of mass (CM). C, Solvent accessible surface areas (SASA) as a function of simulation time. D, radii of gyration, R_g. E, Total energy of the 10-mers in solution (sum of solute-solute and solute-solvent interactions) per-monomer. F, Root mean square displacement data (RMSD) as afunction of simulation time. G, Starting and equilibrated (1 µs) configuration from the MD for 70-mer. H, Enthalpy *H* per-monomer. I, Root mean square deviation, RMSD, data. J, Radius of gyration, R_g.ption.

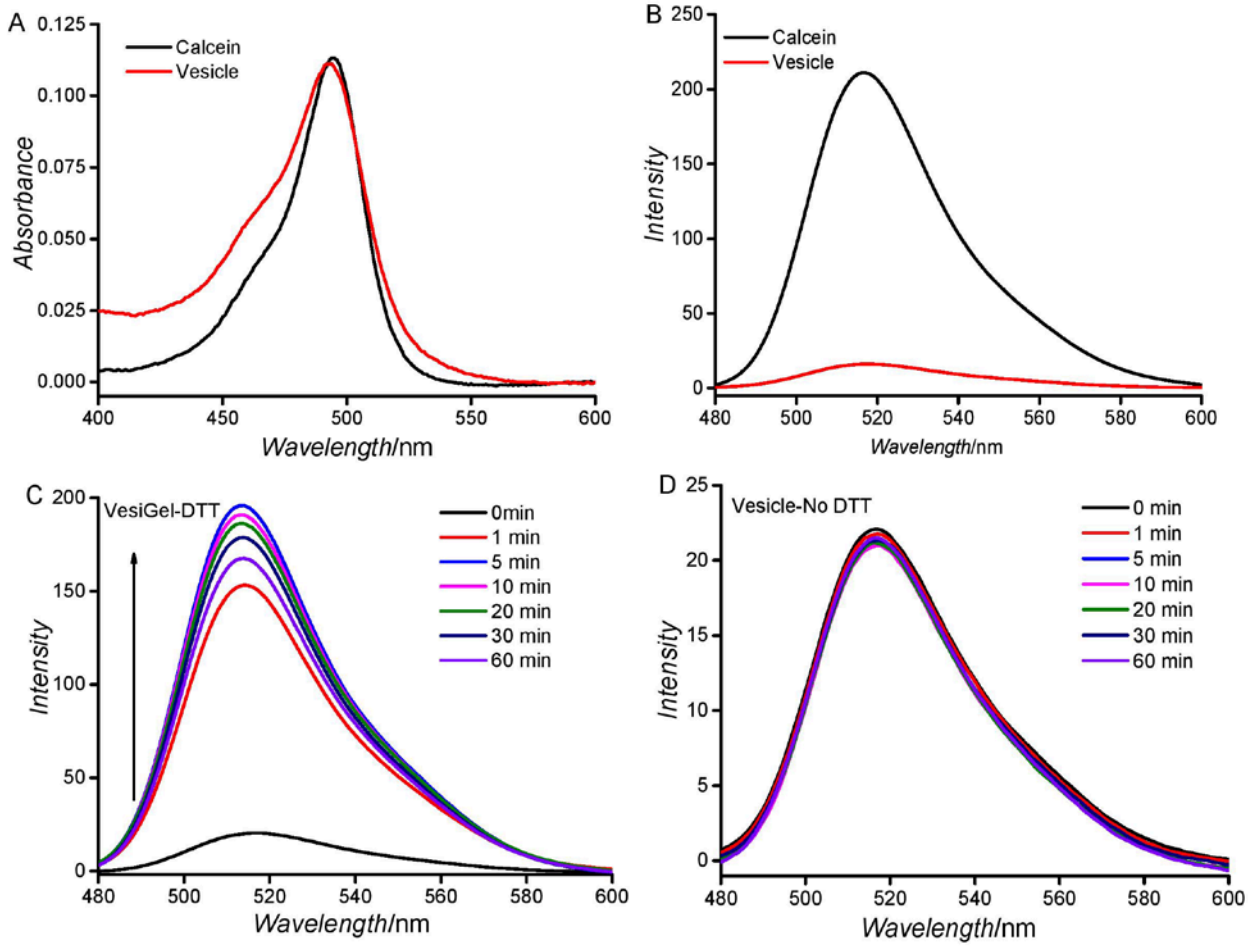


Figure S12. Calcein encapsulation in anionic crosslinked vesicle and redox-triggered release from Crosslinked vesicle. A, Absorbance matching of free calcein and calcein loaded crosslinked vesicle. B, Corresponding fluorescence of free calcein and encapsulated calcein. C, Calcein release from disrupted anionic crosslinked vesicle (0.05 mg/mL) in the presence of 5 mM DTT. D, crosslinked vesicle (0.05 mg/mL) without DTT addition.me Caption.

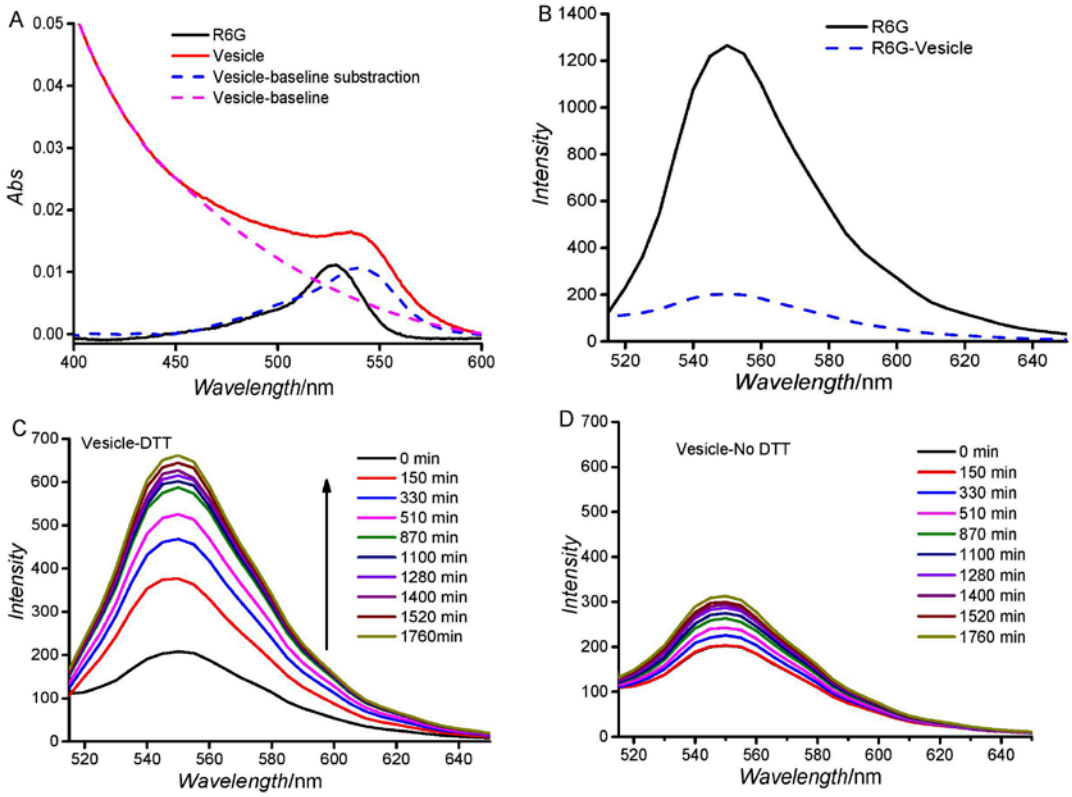


Figure S13. R6G encapsulation in cationic crosslinked vesicle and redox-triggered release from Crosslinked vesicle. A, Absorbance matching of free R6G and R6G loaded Crosslinked vesicle. B, Corresponding fluorescence of free calcein and encapsulated R6G. C, R6G release from disrupted anionic crosslinked vesicle (0.1 mg/mL) in the presence of 5 mM DTT. D, crosslinked vesicle (0.1 mg/mL) without DTT addition.

References

[1] S. Reinicke, P. Espeel, M. M. Stamenovic, F. E. Du Prez, *ACS Macro Lett.* **2013**, *2*, 539-543

[2] J.-H. Ryu, S. Jiwpanich,R. Chacko, S. Bickerton, S.Thayumanavan, *J. Am. Chem. Soc.* **2010**, *132*, 8246-8247

[3] D. A. Case, et al. AMBER 12 University of California (2012).

[4] D. AmadoTorres, M. Garzoni, A. V. Subrahmanyam, G. M. Pavan, S.Thayumanavan, *J. Am. Chem. Soc.* **2014**, *136*, 5385-5399

[5] O. Munkhbat, M. Garzoni, K. R. Raghupathi, G. M. Pavan, S. Thayumanavan, *Langmuir* **2016**, *32*, 2874-2881.

[6] M. B. Baker, L. Albertazzi, I. K. Voets, C.M.A. Leenders, A.R.A. Palmans, G. M. Pavan, E. W. Meijer, *Nat. Commun.* **2015**, *6*, 6234.

[7] A. E. Enciso, M. Garzoni, G. M. Pavan, E. E. Simanek, *New J. Chem.*, **2015**, *39*, 1247-1254.

[8] E. Beltran, M. Garzoni, B. Feringan, A. Vancheri, J. Barbera, J. L. Serrano, G. M. Pavan, R. Gimenez, T. Sierra, *Chem. Commun.* **2015**, *51*, 1811-1814..

[9] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, *J. Comput. Chem.* **2004**, *25*, 1157-1174.

[10] W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, M. L. Klein, *J. Chem. Phys.* **1983**, *79*, 926-935 .

[11] T. Darden, D. York, L. Pedersen, *J. Chem. Phys.* **1993**, *98*, 10089-10092.

[12] V. Kräutler, W. F. van Gunsteren, P. H. Hunenberger, *J. Comput. Chem.* **2001**, *22*, 5385-5399.

[13] P. A. Kollman, I. Massova, C. Reyes, B. Kuhn, S. H. Huo, L. Chong, M. Lee, T. Lee, Y. Duan, W. Wang, O. Donini, P. Cieplak, J. Srinivasan, D. A. Case, T. E. Cheatham, *Acc. Chem. Res.* **2000**, *33*, 889-897.

[14] J. Srinivasan, T. E. Cheatham, P. Cieplak, P. A. Kollman, D. A. Case, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 9401-9409.

[15] B. Jayaram, D. Sprous, D. L. Beveridge, D. L. *J. Phys. Chem.* **1998**, *102*, 9571-9576

[16] D. Sitkoff, K. A. Sharp, B. Honig, *J. Phys. Chem.* **1994**, *98*, 1978-1988.

[17] R. Luo, L. David, M. K. Gilson. M. K. *J. Comput. Chem.* **2002**, *23*, 1244-1253.

[18] M. F. Sanner, A. J. Olson, J. C. Spehner, J. C. *Biopolymers* **1996**, *38*, 305-320.

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Author Contributions

S.T. and J. Z. designed the experiments. J. Z., D. A.-T. and A. P. performed the experiments. G. M. P. designed the computational work. G. M. G. and M. G. performed the simulation. S.T., J. Z., G. P., M.G. analyzed the data. J.Z. and S. T. wrote the paper and all the authors read and approved the manuscript prior to submission.