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SUPPORTING INFORMATION

Elucidating the stability of bolaamphiphilic polypeptide nanosheets using atomistic molecular dynamics

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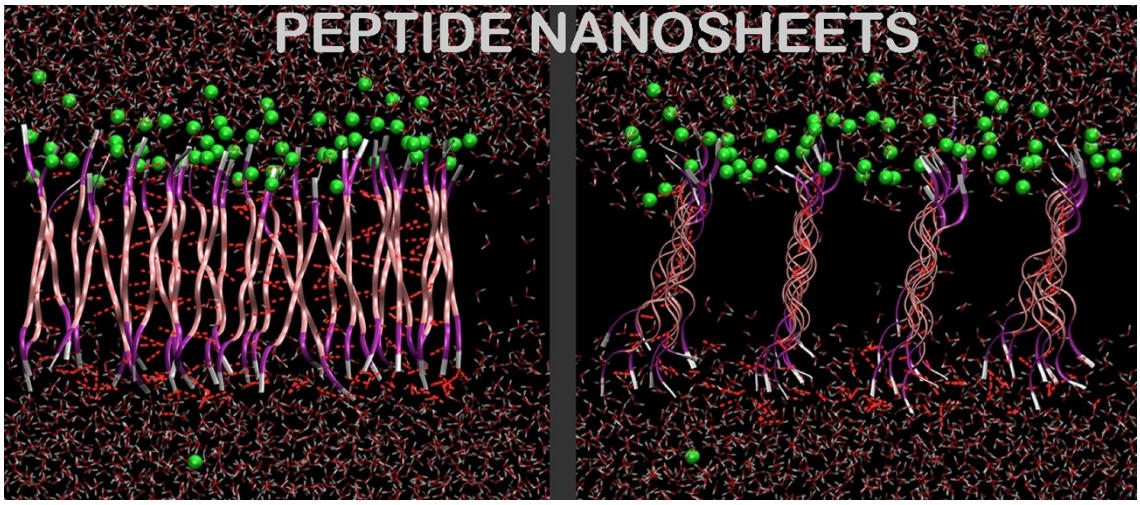

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Generation of the pre-assembled RFL_4FR and EFL_4FE nanosheets

The two nanosheets were pre-assembled by adjusting the RFL_4FR or EFL_4FE molecules in a matched dimer (Figure S1).

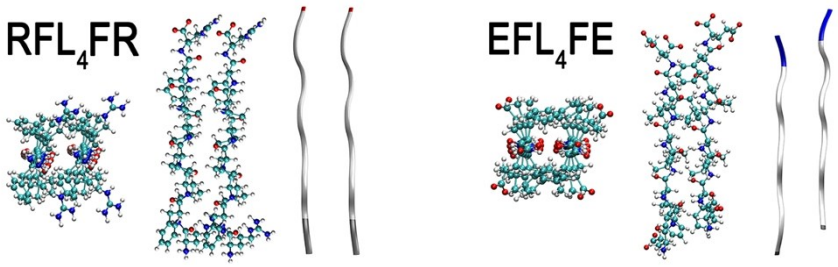

Figure S1: RFL_4FR and EFL_4FE starting dimer used for the nanosheets generation. The initial dimer was replicated in x and y directions. Atom representation: Blue = Nitrogen; Green = Carbon; White = Hydrogen; Red = Oxygen. Ribbon representation: Blue and Red = E and R charged residue; Grey = uncharged residue; White = FL_4F residues. E = Glutamic acid (GLU); F = Phenylalanine (PHE), L = Leucine (LEU), R = Arginine (ARG).

After match, the dimer was replicated in the x and y directions, resulting in a homogeneous and compact structure with 32 polypeptides. After this process, the simulation box was extended on its z-axis (normal to the nanosheet) and filled with water (using the *gmx solvate* tool). The ions that neutralize the total charge of the system were included along with the polypeptides, placed at a distance of about 0.2-0.4 nm from the charged end of the polypeptide. The initial configurations generated by this procedure are shown in Figure S2.

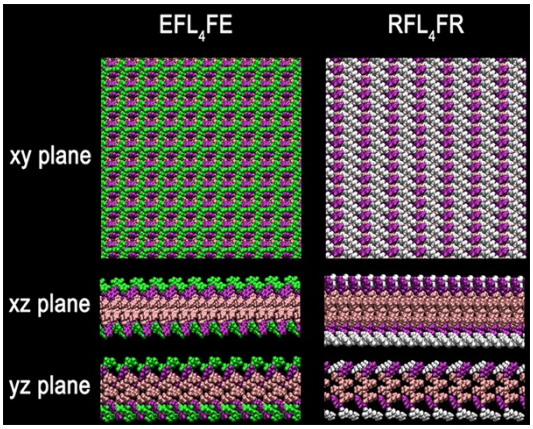

Figure S2: Initial configurations for EFL_4FE and RFL_4FR nanosheets (water and ions were omitted). E is Glutamic acide (GLU in Green); F is Phenylalanine (PHE in Purple); L is Leucine (LEU in Brown); R is Arginine (ARG in White).

The stability of the nanosheets, in special EFL_4FE, was very sensitive to the initial conditions, which required a refined minimization and thermalization procedure composed of a series of steps, summarized in Table S1.

Run	Restraints	Integrator	# of steps	Time (ns)	Thermostat	Barostat
1	Yes	steep	10k	0.02	v-rescale	no
2	No	steep	10k	0.02	v-rescale	no
3	No	md	20k	0.04	v-rescale	no
4	No	md	20k	0.04	v-rescale	Par-Rah
5	No	md	2500k	5.00	v-rescale	no
6	No	md	2500k	5.00	v-rescale	Par-Rah
7	No	md	25000k	50.00	v-rescale	no
8	No	md	50000k	100.00	v-rescale	Par-Rah

Table S1: Main characteristics of the thermalization steps used to equilibrate the nanosheets and to obtain the configurations for the production stage.

Firstly, for removal of bad contacts and minimization the structures were submitted to steps 1 and 2. This process prepared the initial configurations for steps 3, where the simulation box is subjected to a dynamic at constant volume. Next, a sequential equilibration alternating the NVT and NPT runs was employed (steps 4-8). For some attempts the EFL_4FE nanosheet showed to be unstable in one of steps 4-8, being dismantled by interaction with the water molecules. A gap in the EFL_4FE nanosheet structure, characterized by the breakdown of the van der Waals interactions as well as the hydrogen bonding network, leading to a high concentration of water in the interior of nanostructure. Figure S3 shows one configuration that were not successful during the simulation process due to failures in the interaction between the peptides favoring the excessive infiltration of water inside the nanostructure. In these cases, the configuration was discarded and the whole equilibration process was restarted from a new initial structure, slightly modified. This modification was made relative to the relative positions of the peptides in the dimer that gives rise to the nanosheet.

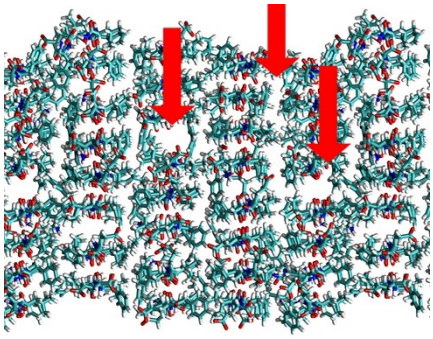

Figure S3: Failed initial configuration (water and ions were removed). We highlight the spaces with flaws in the interactions between polypeptides that lead to the disorganization of the nanosheet. The empty spaces favor the excessive infiltration of water inside the nanosheet.

A relatively extensive NVT run (step 7) was necessary to ensure the stability of the EFL_4FE structure since in many attempts it was destabilized only after several nanoseconds of the NPT simulation (step 8), which was the last step of the equilibration process. Once the proper stability of the nanosheet was reached at

constant volume, the last step of the process assured us the stabilized final configuration by a NPT run of 100 ns.

Pairwise energy analyses

Tables S2 and S3 present the pairwise energies between all the peptide residues and also between the peptides and water/ions. In order to have a reference on the energy per peptide, we also present the energy analysis obtained for a single hydrated monomer.

	Coulomb			Lennard-Jones		
RFL_4FR - Monomer	Average	Err. Est.	RMSD	Average	Err. Est.	RMSD
ARGC-ARGC	481.03	0.34	4.61	-16.23	0.16	0.92
ARGN-ARGC	0.00	0.00	0.00	0.00	0.00	0.00
ARGN-ARGN	194.96	3.44	10.11	-6.85	0.27	1.80
ARGC-PHE	-158.65	0.66	1.98	-16.28	0.28	0.99
ARGC-LEU	0.88	0.10	0.39	-9.83	0.19	0.70
ARGC-Ion	-399.64	3.44	17.07	25.61	0.34	2.83
ARGC-SOL	-203.65	2.56	14.76	-1.95	0.38	2.90
ARGN-PHE	-169.96	0.41	1.94	-20.48	0.17	0.87
ARGN-LEU	-0.32	0.10	0.47	-9.69	0.11	0.59
ARGN-Ion	-0.68	0.19	1.64	0.03	0.01	0.15
ARGN-SOL	-345.41	5.94	16.81	2.29	0.34	2.97
PHE-PHE	-166.58	0.38	2.27	-31.90	0.10	0.78
PHE-LEU	-391.72	0.53	2.05	-79.03	0.56	1.83
PHE-Ion	-8.33	0.59	2.22	-2.57	0.07	0.49
PHE-SOL	-42.73	0.56	3.41	-19.76	0.50	1.74
LEU-LEU	-641.06	0.63	3.50	-100.17	0.08	1.72
LEU-Ion	-2.03	0.12	0.43	-1.24	0.04	0.17
LEU-SOL	-44.19	0.72	2.87	-12.65	0.38	1.62

Table S2: Energy analyses for $\underline{RFL_4FR}$ nanosheet in water [kJ mol^{-1}]. Energies were normalized by number of peptides. ARGC and ARGN stand for the arginine residue with charged and neutral termini, respectively.

	Coulomb			Lennard-Jones		
RFL_4FR - Monomer	Average	Err. Est.	RMSD	Average	Err. Est.	RMSD
GLUC-GLUC	-738.69	1.56	6.35	-7.92	0.02	0.54
GLUC-GLUN	0.00	0.00	0.00	0.00	0.00	0.00
GLUN-GLUN	-830.26	0.81	8.18	7.05	0.16	1.83
GLUC-PHE	-165.16	0.21	1.73	-16.32	0.10	0.54
GLUC-LEU	-3.64	0.02	0.58	-6.35	0.05	0.40
GLUC-Ion	-364.94	5.94	28.64	16.51	0.41	2.60
GLUC-SOL	-583.67	2.91	22.77	28.00	0.19	3.49
GLUN-PHE	-171.46	0.56	2.71	-14.78	0.13	0.87
GLUN-LEU	-7.32	0.05	0.66	-7.60	0.11	0.56
GLUN-Ion	-0.61	0.10	1.60	0.03	0.01	0.12
GLUN-SOL	-362.98	1.56	15.36	14.84	0.22	2.99
PHE-PHE	-161.98	0.81	2.91	-24.28	0.03	0.81
PHE-LEU	-397.40	0.41	2.06	-83.69	0.25	1.47

PHE-Ion	-26.33	0.30	4.57	2.11	0.05	0.75
PHE-SOL	-35.27	0.81	4.94	-30.54	0.18	1.60
LEU-LEU	-631.25	0.47	3.31	-97.80	0.11	1.67
LEU-Ion	-0.24	0.16	1.35	-0.01	0.01	0.19
LEU-SOL	-57.96	0.25	2.81	-12.97	0.23	1.58

Table S3: Energy analyses for EFL$_4$FE nanosheet in water [kJ mol^{-1}]. Energies were normalized by number of peptides. GLUC and GLUN stand for the glutamic acid residue with charged and neutral termini, respectively.

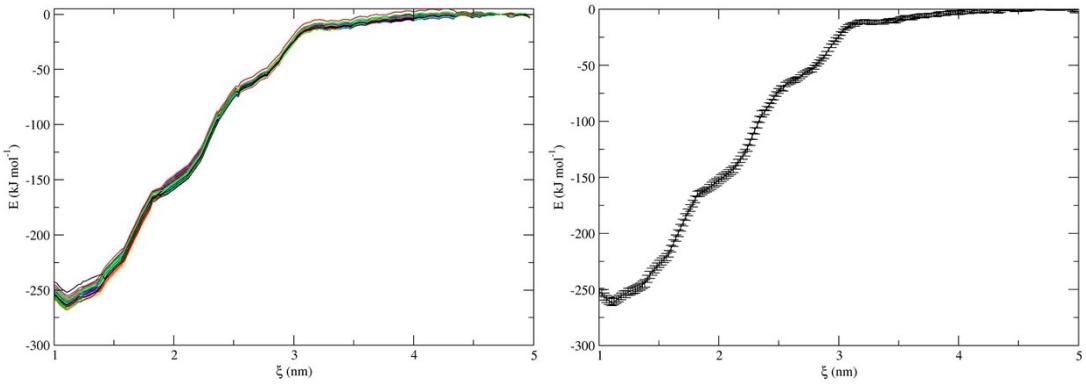

Figure S4: Bootstraps analysis and average profile (and standard deviation), obtained for the RFL$_4$FR peptide extraction from the charged surface. For the other three PMF we obtained similar results.