Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Two Dimensional Materials are Non-nanotoxic and Biocompatible Towards Cyclotides: Evidence from Classical Molecular Dynamics Simulations

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Table of contents:

1. Minimization-equilibration protocol for the adsorption simulations:

First, each of the systems was minimized for 20000 steps while keeping the peptide atoms constrained by a harmonic force constant of 100 kcal/mol/Å^2 and another minimization was performed for 20000 steps without any constraint. Then, each of the systems were equilibrated for 50 ns at 100 K in canonical (NVT) ensemble, again constraining the peptide atoms by a force constant of 100 kcal/mol/Å^2. Further, the force constant was reduced to 10 kcal/mol/Å^2 and heating was performed to 300 K over the period of 50 ns. Finally, constraining forces over protein atoms were removed and the systems were equilibrated for another 20 ns in isothermal – isobaric (NPT) ensemble at 300 K and 1 atmospheric pressure. In all the above simulations, each atom of the 2D materials was harmonically constrained by a force constant of 10 kcal/mol/Å^2. Finally, production simulations were carried out with structures obtained at the end of the equilibration simulations using at least two different initial configurations for each system, at 300 K in NVT ensemble. Also, for the simulations of peptides in the absence of any of the 2D materials (blank simulations), the above-mentioned protocol was followed for minimization, equilibration and production simulations.

2. Details of PMF calculations:

To calculate the PMF's for the single cyclotide molecules, center of mass (COM) distances (d) between the cyclotides and the 2D materials were chosen as the reaction coordinates which were divided into several overlapping windows of 0.1 nm widths. Each of these windows was further divided into small bins of 0.02 nm widths. A harmonic force of 100 kcal/mol/Å was applied to the upper and lower boundaries of the reaction coordinates and 20 ns production ABF simulations were performed for each window at 300 K. To reduce sampling issues and avoid memory effects, we performed each PMF calculations at least two times and the averages are reported. Since the peptides are adsorbed at different COM distances on the 2D materials, therefore, for a direct comparison between the PMF curves, we substituted the reaction coordinate d with δ, where δ is defined as $(d - d_0)$, d_0 and d being the COM distances initially and at any instant during PMF calculations respectively. Free energy of the completely desorbed structure is set to zero, where the peptides are non-interacting with the surfaces. For the cyclotide aggregates, the adsorbed structures at the end of 500 ns adsorption simulations were taken as the initial structures and the same protocol was adopted for the calculations of the adsorption free energies.

3. Deviation of the cyclotide structures during equilibration:

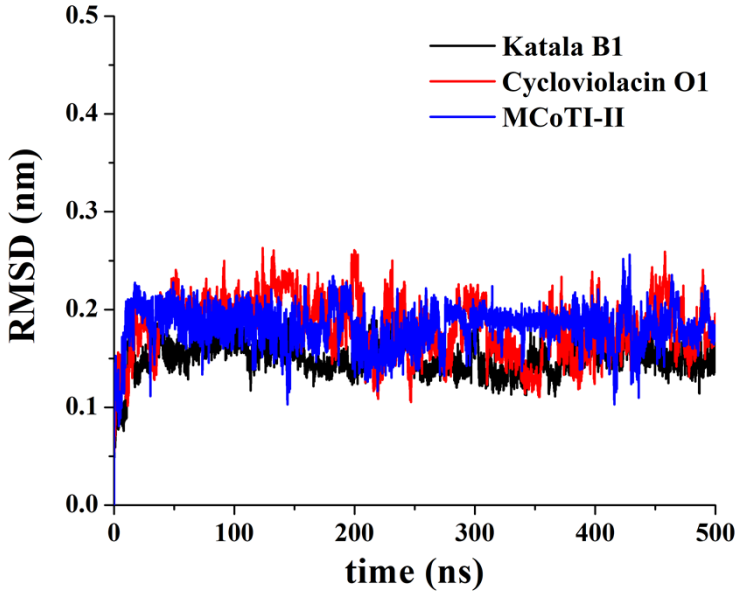

Figure S1: *Deviation of the cyclotide structures in terms of the root mean squared displacement (RMSD) plots of the cyclotides in water for the entire duration of the equilibration simulations, taking the crystal structure of the peptides as the reference.*

4. Decomposition of the interaction energy between *h*-BN and cyclotides into electrostatic and van der Waals components.

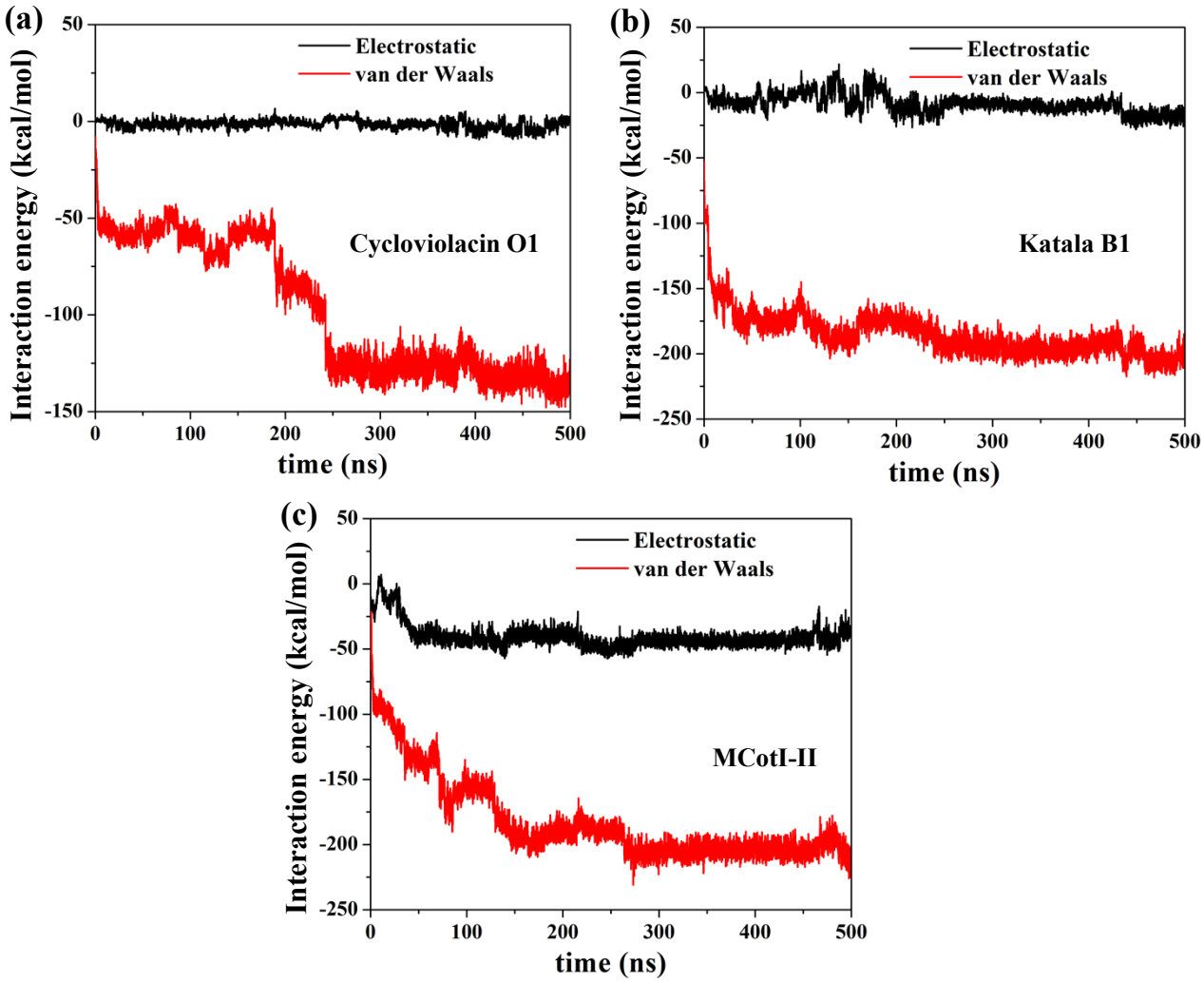

Figure S2: *Decomposition of the interaction energy into electrostatic and van der Waals components between single cyclotide molecules and 2D materials for (a) Cycloviolacin O1, (b) Katala B1, and (c) MCotI-II, during the entire course of the adsorption simulations.*

5. Interactions between peptides within the aggregates:

Peptides are aggregated via various non-covalent interactions, such as H-bonding, vdW, and electrostatic interactions. We have now plotted the inter-peptide hydrogen bonds (H-bonds) for all three peptides during the last 400 ns of the 500 ns long aggregation simulations. The first 100 ns are not provided because during that duration, the peptides randomly move in water and then come close to each other, initiating the process of aggregation. Figure S3(a) shows that there are more than 30 H-bonds between the any of the peptide aggregates, and the number of H-bonds do not decrease during the rest of the simulations, rather H-bonds tend to increase and reach a nearly asymptotic value around 250 ns. Therefore, clearly hydrogen bonds are one of the important modes of aggregation. In addition, we plotted the electrostatic and van der Waals interaction energies (Figure S3(b-d)) between the peptides, which clearly show that although there are small fluctuations in the interaction energies, however, both of the interaction energies are stabilizing during the entire duration of the aggregation simulations. Thus, electrostatic attraction between the charged groups of the different peptides actively take part in the process of aggregation, while the contribution from the van der Waals interactions between the amino acid residues is nearly equal. It is worthwhile to mention that the fluctuation in the interaction energies can be attributed to the flexibility of the individual peptide molecules, which in turn, momentarily changes the relative spatial orientation of the amino acid residues even in the aggregated form, thereby changing the interaction energy. However, this does not hamper the overall stability of the aggregates and the interaction energy gets restored over the time.

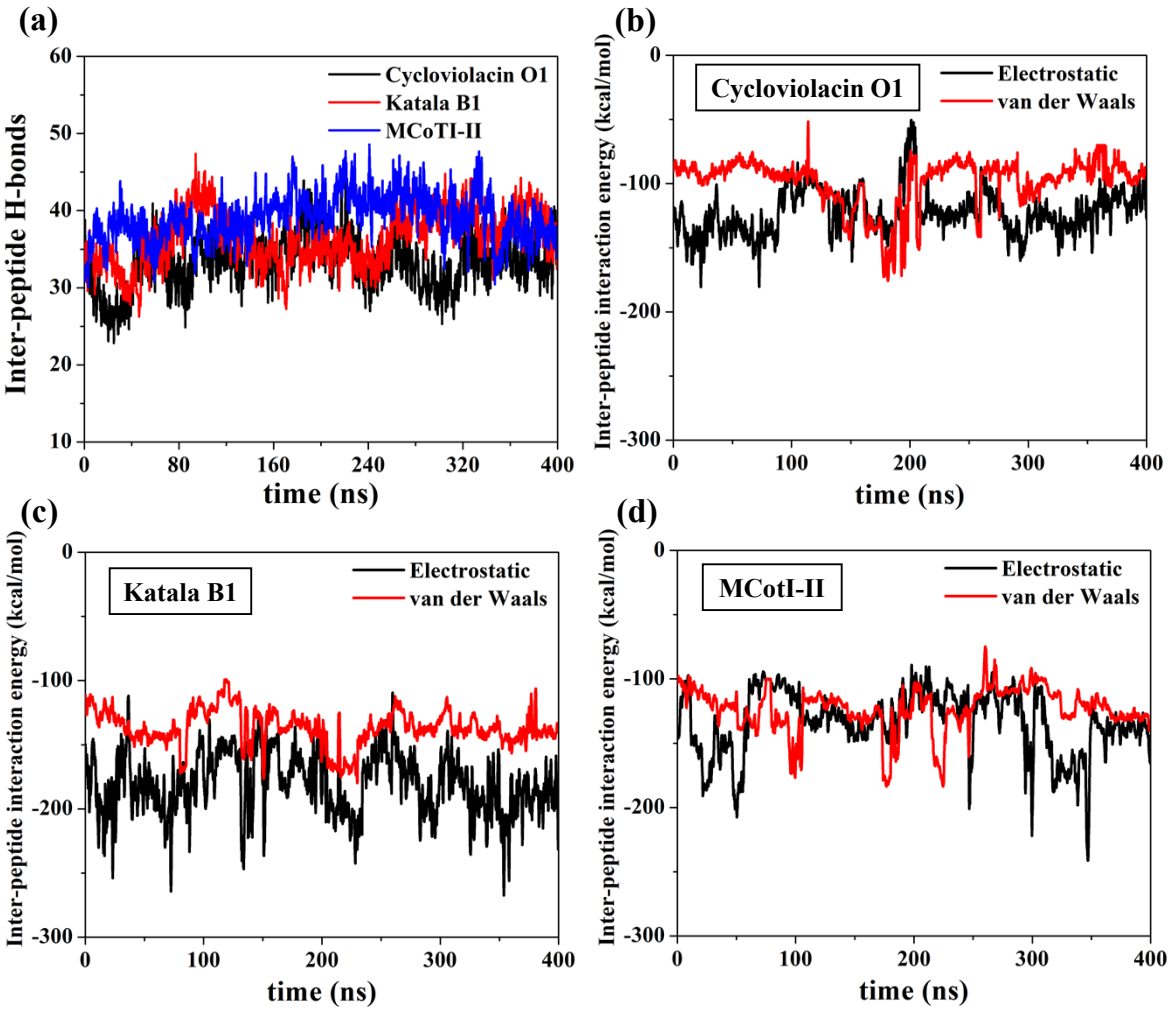

Figure S3: *(a) Inter peptide H-bonds, and inter-peptide interaction energies for (b) Cycloviolacin O1, (c) Katala B1, and (d) MCoTI-II for the last 400 ns of the 500 ns aggregation simulations. The interaction energies are divided into electrostatic and van der Waal components.*

6. PMF profiles representing the Gibbs free energy of adsorption of the cyclotide aggregates on graphene and *h*-BN:

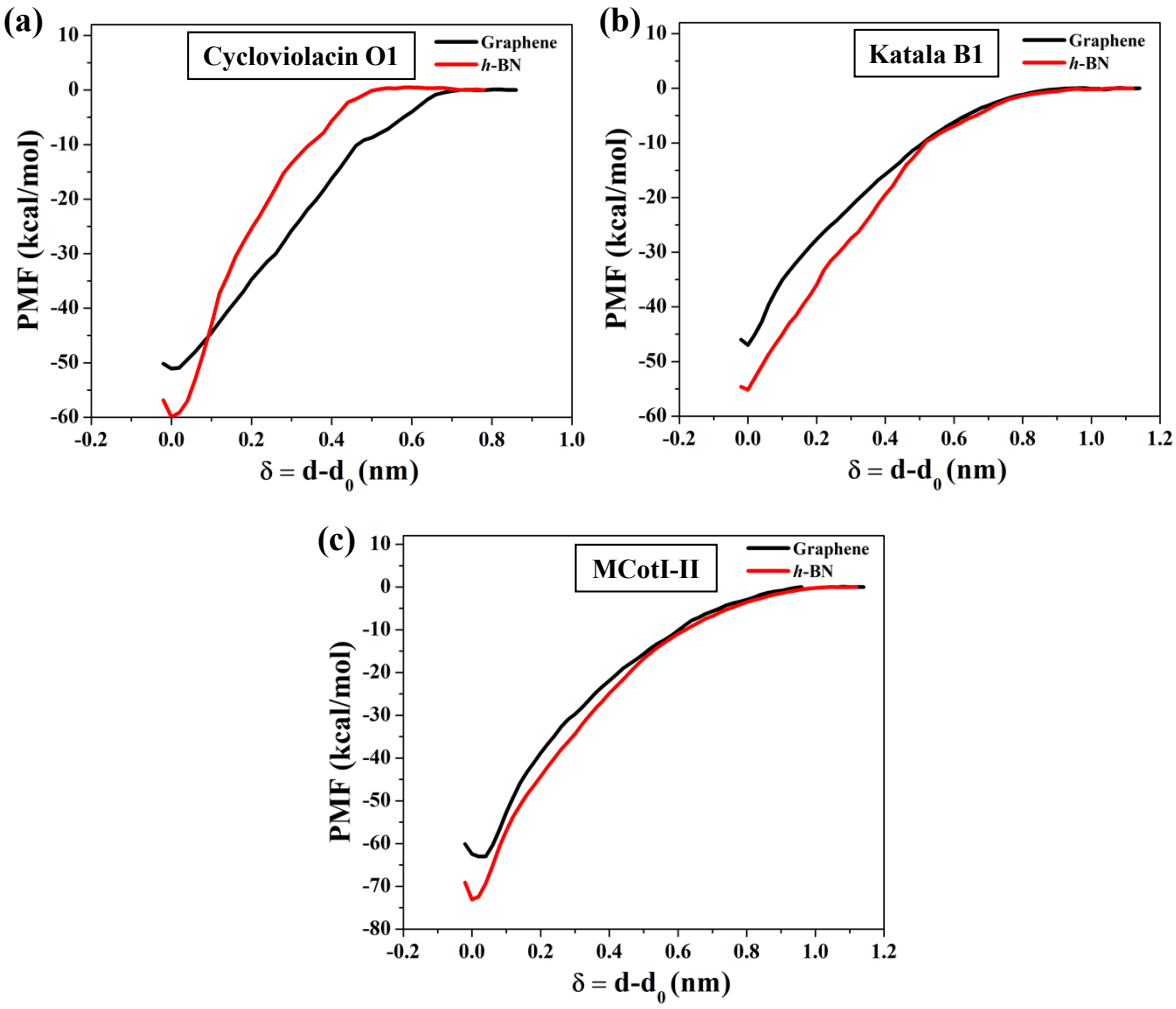

Figure S4: *Free energy of adsorption (in kcal/mol) of the three cyclotide aggregates (a) Cycloviolacin O1, (b) Katala B1, and (c) MCotI-II on graphene, and h-BN in terms of potential of mean forces (PMF's). The PMF profiles are obtained at 300 K and at least two independent PMF simulations are carried out to check the reproducibility and produce an average profile. Since cyclotide aggregates are adsorbed at different COM distances with respect to the 2D materials, we substituted the reaction coordinate with* $\delta = (d - d_0)$, d_0 *and d being the COM distances initially and at any instant during PMF calculations respectively. Free energy of the*

completely desorbed structure is set to zero, where the cyclotide aggregate is non-interacting with the surfaces.