Supporting Information

Investigating the Complexation Propensity of Self-Assembling Dipeptides with the Anticancer Peptide-Drug Bortezomib: A Computational Study

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Experimental materials and methods

The Bortezomib peptide was purchased from Merck in form of lyophilized powder with purity greater than 95 %. The water solvent used was nanopure purified, filtered and sterilized. Sample solutions were prepared by dissolving the Bortezomib powder into water at room temperature following sonication at c=4 mg/ml. Different concentrations were tested as well as briefly heating Bortezomib up to 55 °C, for a couple of seconds, to facilitate dissolution.

For FESEM observation, a sample of 10 μL placed in a slide to be dried. After the samples were dried, sputtering was carried out as follows: The samples were placed on a slide with the help of conductive carbon adhesive tape. The slide was subsequently placed on a gold sputtering machine and a 15 nm thick gold (Au) layer was deposited for 64 sec at 40 mA. For the observation of the samples FESEM JEOL 7000 field emission scanning electron microscope was used, operating at 15 kV.

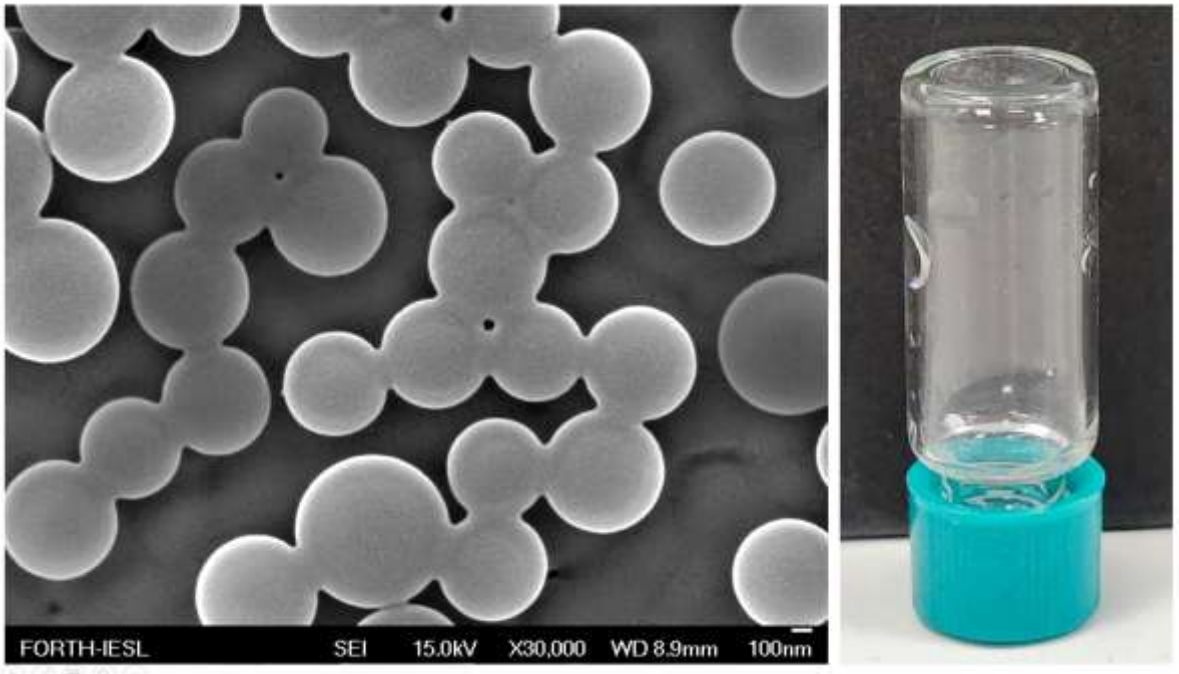

Figure SI-1: FESEM pictures of bortezomib spheres, following H_2O evaporation after 24h incubation at room temperature, at a concentration of 4 mg/ml, along with an inverted vial showing that the solution flows to the bottom and does not form a self-supporting gel.

In the case of Cyclo-LF, Z-FF and Fmoc-FF with and without Bortezomib the two-solvent ethanol/water (3:7) approach was used. Following ethanol addition, the mixture was vortexed and placed in a water bath thermostat with a temperature of T=50 °C. Sonication was performed for 4 seconds with 20 seconds intervals for 15 minutes until complete dissolution was achieved, after which water addition took place to induce self-assembly. When mixed with Bortezomib, it was done in a 1:1 peptide/BTZ ratio. The final concentration in all cases was c=4 mg/ml, although lower concentrations were also tested. The above was also tested with Bortezomib's addition without heating, without observing discernible alterations in the resultant self-assembled structures. Bortezomib on its own was also examined in the EtOH/H_2O 3:7 system, at c=4mg/ml, without any apparent deviation in the spherical conformations it adopted.

For field emission scanning electron microscopy (FESEM) observations, once more, 10 μL of the sample was placed on a glass slide and allowed to dry. Subsequently, sputtering was conducted as follows: The samples were affixed to the slide using conductive carbon adhesive tape and subjected to a gold sputtering procedure, depositing a 15 nm thick gold (Au) layer for 64 seconds at 40 mA. FESEM analysis was performed using a JEOL 7000 field emission scanning electron microscope, operating at 15 kV, to examine the structural characteristics of the samples.

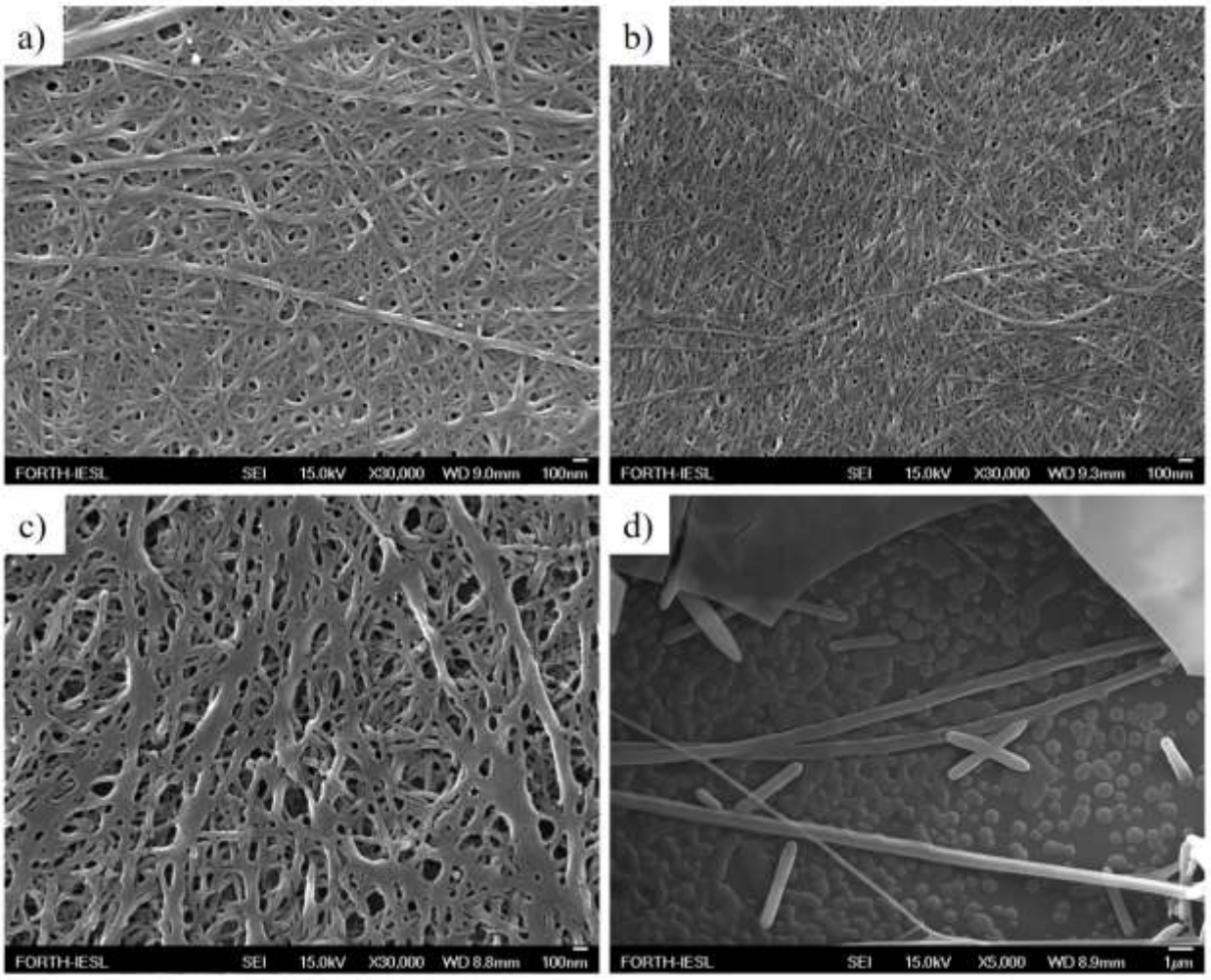

Figure SI-2: FESEM images peptides in (EtOH/H_2O 3:7) with BTZ at a 1:1 ratio at a concentration of c= 4 mg/ml after 24h at room temperature. Scale bars are 1 μm. a) Z-FF with BTZ b) Fmoc-FF with BTZ c) Cyclo-LF with BTZ and d) LF with BTZ.

As depicted in the Field Emission Scanning Electron Microscopy (FESEM) images, it is evident that all examined peptides, with the exception of LF (Figure SI-2d), exhibit the capacity to yield hydrogels, even upon the introduction of BTZ (Bortezomib). Among these, Fmoc-FF demonstrates the highest degree of hydrogel stability in the presence of BTZ (Figure SI-2b), forming intricate and densely interwoven fibril networks. Z-FF, in conjunction with BTZ (Figure SI-2a), constitutes the second most robust hydrogel, characterized by substantial fibril networks. In contrast, CycloLF exhibits the lowest hydrogel stability in the presence of BTZ (Figure SI-2c) among the three peptides, and a notably slower rate of hydrogel formation.

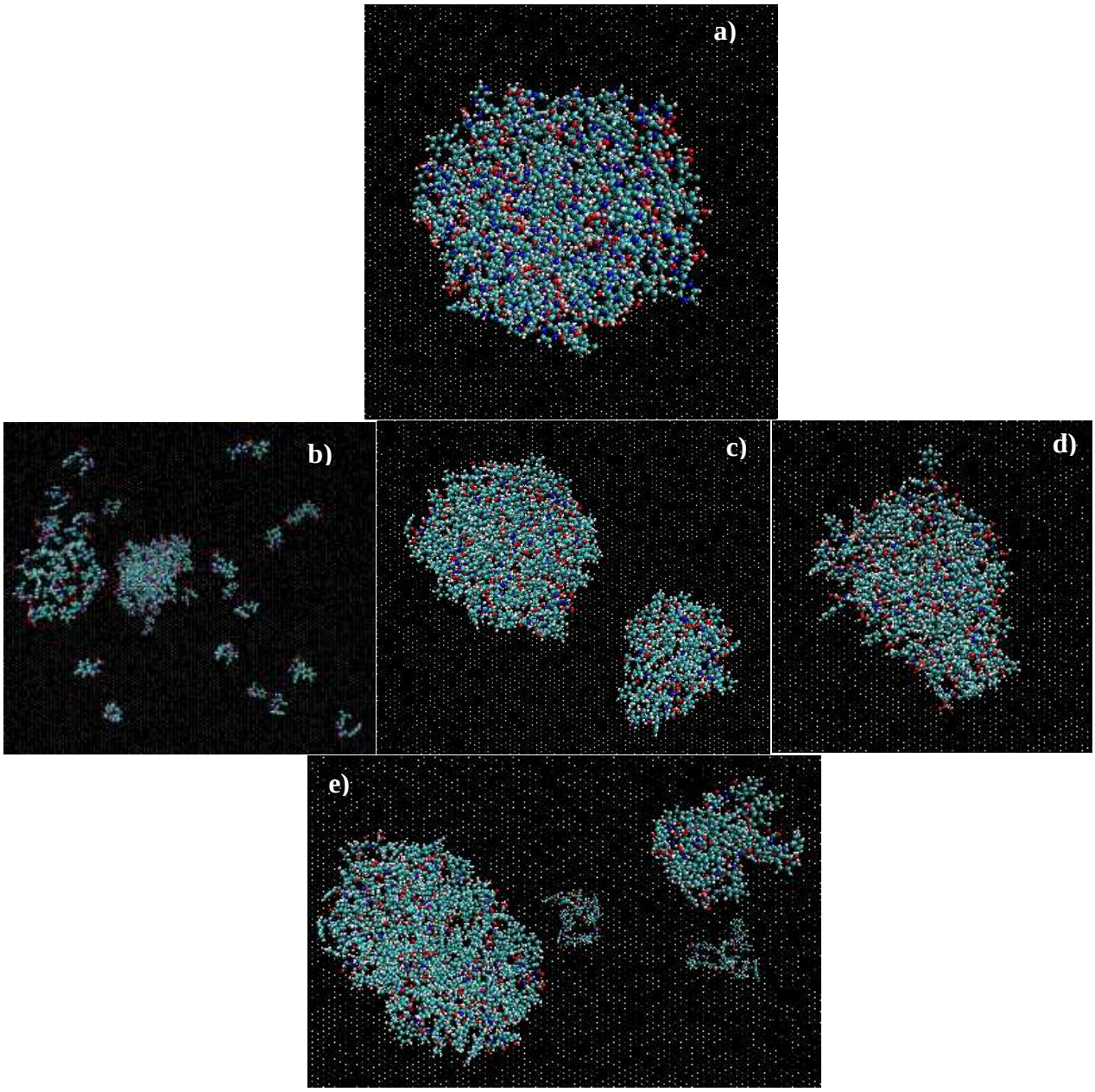

Figure SI-3: Characteristic snapshots of clusters (a) BTZ; (b) LF; (c) z-FF; (d) Cyclo-FF; (e) Fmoc-FF. Water molecules are presented as ghost for clarity.

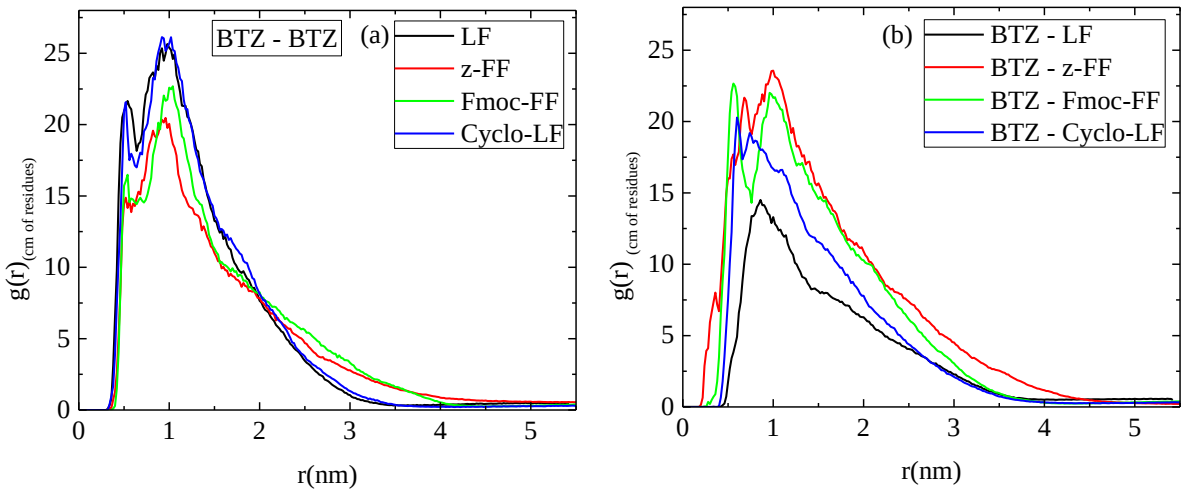

Figure SI-4: Pair radial distribution functions between (a) Bortezomib molecules and (b) Bortezomib-dipeptide calculated for the cm of molecules in the aqueous solutions at T=300K.

Table SI-1: Average number of hydrogen bonds between the three groups of hydrogen bonding active sites of BTZ and dipeptide molecules.

Systems	(OH-DIPEP.)/BTZ	(NH-DIPEP.)/BTZ	(CO-DIPEP.)/BTZ
LF	0.08±0.05	0.02±0.01	0.06±0.03
Cyclo-LF	0.12±0.01	0.29±0.01	0.15±0.01
Fmoc-FF	0.17±0.06	0.35±0.06	0.14±0.05
Z-FF	0.16±0.05	0.33±0.06	0.27±0.05

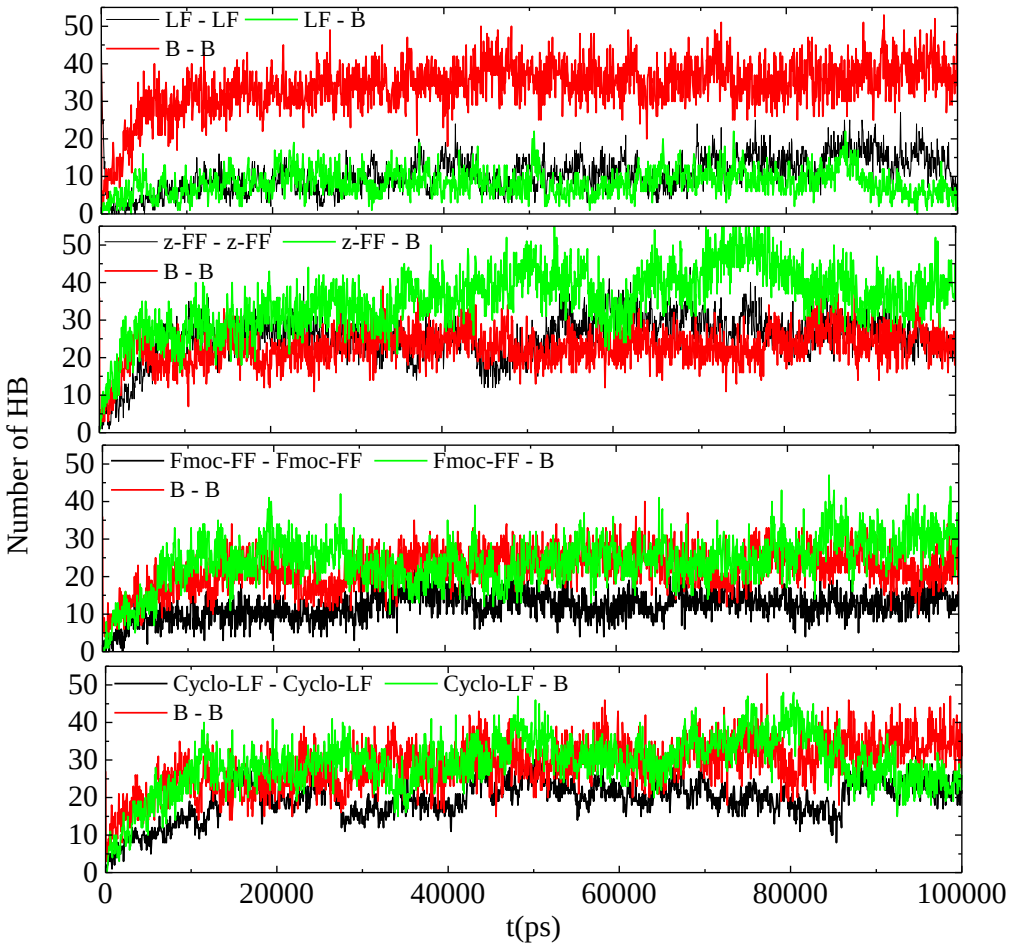

Figure SI-5: Hydrogen bonds, between the BTZ and dipeptide molecules, as a function of time.

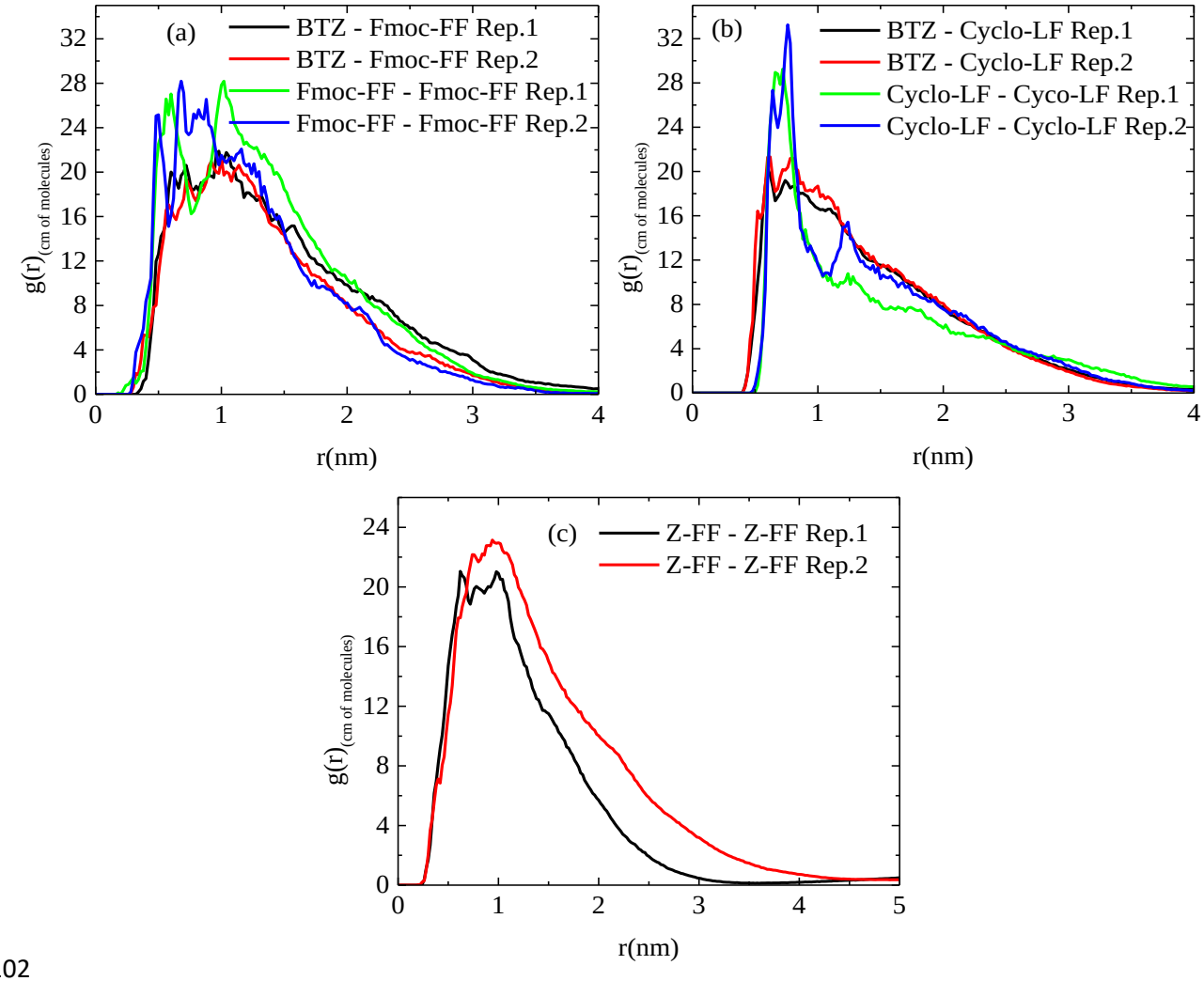

Figure SI-6: Pair radial distribution functions between dipeptide molecules and Bortezomib-dipeptide molecules calculated for the cm of molecules in the aqueous solutions (a) Fmoc-FF with BTZ; (b) Cyclo-LF with BTZ; (c) Z-FF bulk system.