Self-assembling dipeptides as vehicles for the delivery of peptide therapeutics

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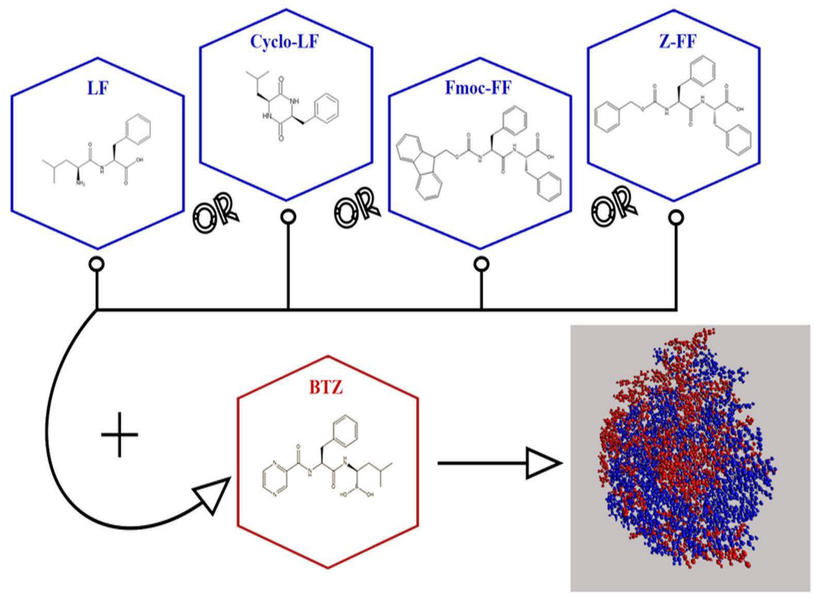
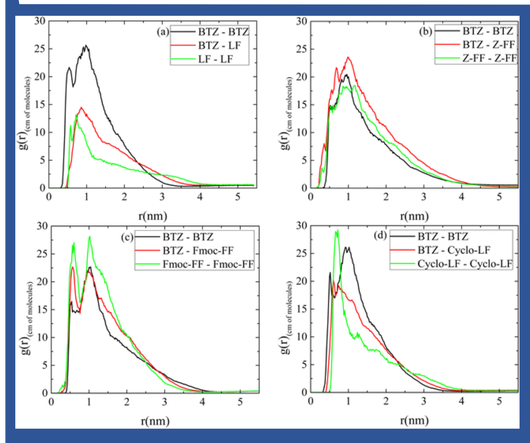
ABSTRACT

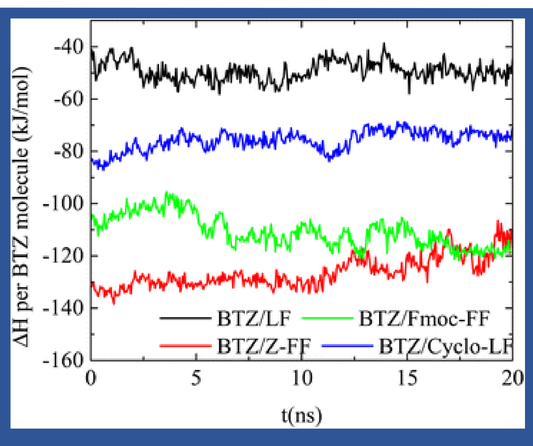
Self-assembling peptides consist of short chains of amino acids capable of spontaneously forming higher-order structures such as fibers, tubes, and hydrogels. Combination of computational and experimental approaches allow rational design of peptide materials with increasing complexity. While peptides ranging from 6-12 amino acids allow the insertion of targeting functionalities, their length results in increased cost and limits wider applicability. On the other hand, ultrashort peptides ranging from 2-6 amino acids, are more tractable and still amenable to interesting applications. One such application is the controlled delivery of peptide therapeutics from structurally similar hydrogelating peptides. **In recent studies we combined both theoretical and experimental approaches for the thorough investigation of the structural and conformational properties of biocompatible aliphatic-aromatic and aromatic dipeptide hydrogels for the controlled release of the peptide drug Bortezomib**. Bortezomib is a modified, Phenylalanine-BoroLeucine dipeptide (N-pyrazino-Phe-BoroLeu) that acts as proteasome inhibitor for the treatment of multiple myeloma, approved by the U.S. Food and Drug Administration (FDA) in 2003. It has also shown potential in the treatment of other malignancies. The self-assembly of Bortezomib in water was examined computationally for the first time, as well as its tendency to bind to similar dipeptides with hydro-gelating capacities, using a series of all-atom molecular dynamics simulations [1]. Our aim was to obtain a quantitative prediction of the Bortezomib-Peptides conformational, dynamical and structural properties at the molecular level, while also performing a comparison between dipeptides in order to find the most suitable candidates for Bortezomib controlled release. **Our results suggested that the protected Phe-Phe category is qualified and between its two members, Fmoc-Phe-Phe looks more promising. This study paves the way towards optimal peptide carrier selection for the encapsulation and controlled delivery of Bortezomib in future experimental studies.**



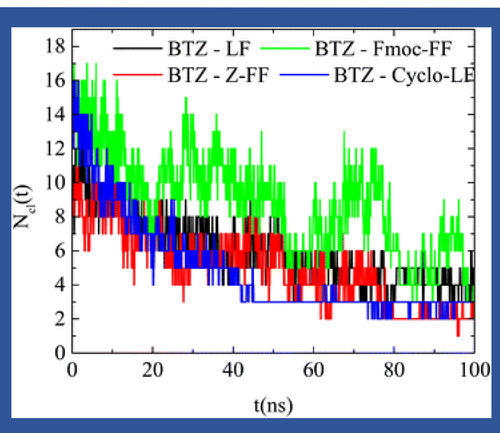
SIMULATION DETAILS



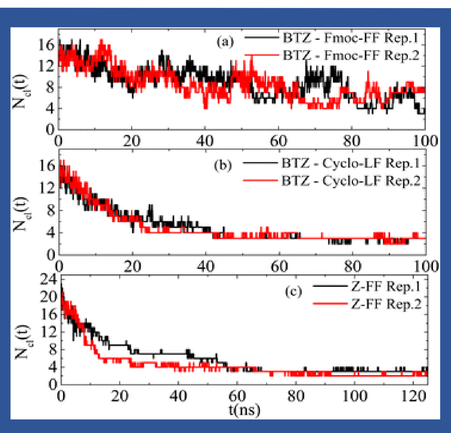
Pair radial distribution functions between the centers of mass of molecules for the pairs: Bortezomib–Bortezomib; dipeptide–dipeptide and Bortezomib–dipeptide in the aqueous solutions at T = 300 K in all systems: (a) LF system; (b) Z-FF system; (c) Fmoc-FF system; and (d) Cyclo-LF system.→ No big differences observed amongst the 3 curves for the systems except LF.



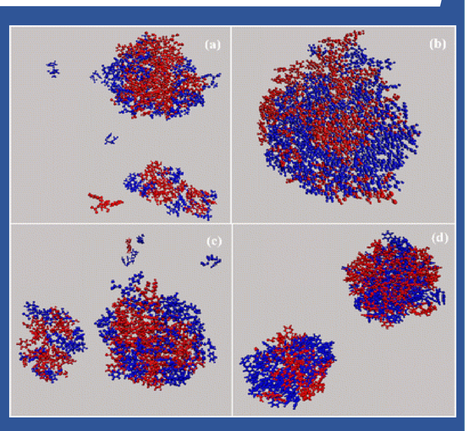
Binding enthalpy per BTZ molecule as a function of time between the dipeptides and BTZ.→ lowest energy indicates the energetically most stable association to BTZ which is Z-FF, followed closely by Fmoc-FF.



Number of clusters formed by BTZ and dipeptides as a function of time in the examined systems.→ Large fluctuations imply a dynamic equilibrium between the association & dissociation of molecules in the formed clusters. Fluctuations are most pronounced for the Fmoc-FF/BTZ system, indicating a continuous restructuring of the clusters.

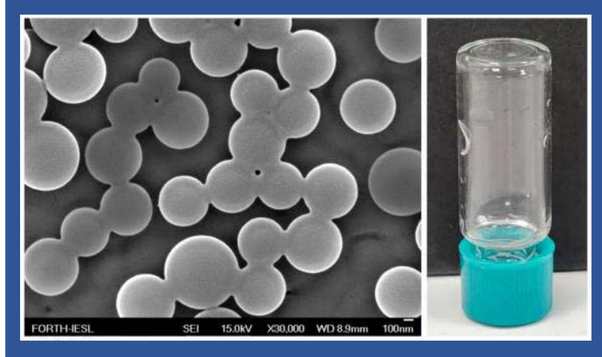


Number of clusters formed by molecules as a function of time in the (a) Fmoc-FF with BTZ; (b) Cyclo-LF with BTZ; and (c) Z-FF bulk systems.→ Accounting for the trapping in metastable states, the number of clusters formed by BTZ / dipeptides as a function of time has been recalculated with new replicas, showing similar pathway towards the “equilibrated state”. Hence physicochemical association characteristics are captured whether or not the system reaches a local (metastable state) or the global (final state) energetic minimum.

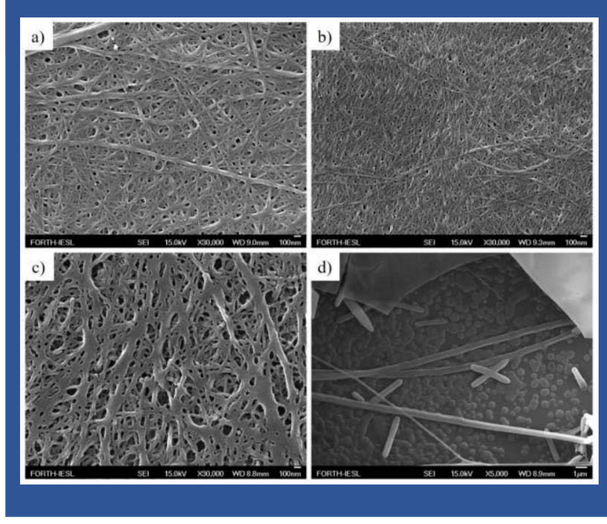


Characteristic snapshots of dipeptide–BTZ complexes (a) LF; (b) Z-FF; (c) Cyclo-LF; and (d) Fmoc-FF. Bortezomib molecules are presented in red and dipeptides in blue color. Water molecules are omitted for clarity.→ Clusters of various sizes are observed, which are classified in one or two clusters case with few individual molecules dispersed in the solution.

EXPERIMENTAL STUDIES



FESEM pictures of bortezomib spheres, following H2O evaporation after 24h incubation at room temperature, at a concentration of c=4 mg/ml, along with an inverted vial showing that the solution flows to the bottom and does not form a self-supporting gel.



FESEM images peptides in (EtOH/H2O 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.

CONCLUSIONS

Evaluating all the measures, we can claim that the protected FF category is qualified and between its two members, Fmoc-FF looks more promising. However, Z-FF also has various features that could make it a potential candidate carrier for Bortezomib and a promising candidate for future studies.

REFERENCES

[1] P. Divanach, E. Fanouraki, A. Mitraki, V. Harmandaris, and A. N. Rissanou, Soft Matter, 2023, 19, 8684-8697

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