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# Insights into the Self-Assembly of Phenylalanine Oligopeptides by Replica Exchange MD Simulations with the GBSW Implicit-Solvent Model

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The diphenylalanine peptide (FF), the core recognition motif of the Alzheimer's $\beta$-amyloid peptide, self-assembles into tubular structures of high stability. We have studied the aggregation properties of FF and the related triphenylalanine peptide (FFF) by 0.4-$\mu$s implicit- solvent Replica Exchange MD simulations of aqueous FF and FFF solutions. The FF and FFF peptides form ellipsoidal aggregates with a similar density and shape in the simulations. Within each aggregate, we observe structural features, which are consistent with the properties of L-Phe-L-Phe crystals. In particular, the aromatic planes of interacting sidechains are mainly oriented perpendicular to each other and the backbone moieties of several (2-6) adjacent peptides interact frequently by head ($NH_3^+$) -to-tail ($^-OOC$) hydrogen bonds, forming open or closed (ring-like) linear networks. The ring networks of six peptides observed in the FF simulations are reminiscent of the hexagonal FF rings in the L-Phe-L-Phe crystals. The rings are energetically more stable than the open networks, due to both non-polar and polar interactions. The network propensity is higher in the FFF solution, mainly due to stronger non-polar and to a smaller extent due to stronger polar interactions in the networks of the FFF aggregate; in line with this observation is the somewhat higher stability of the FFF aggregate, observed in the simulations.

## 1 Introduction

The ability of short peptide fragments to self-assemble into amyloids[1], nanotubes[2] and systems responsive to external stimuli (pH, temperature, concentration of specific solutes)[3] is the focus of intense experimental and computational studies in recent years, as it can provide insights on the formation of amyloid fibers and has potential applications in biomaterial synthesis, nanodevice fabrication and tissue engineering.

The diphenylalanine peptide ($NH_2$-L-Phe-L-Phe-COOH, FF), the core recognition motif of the Alzheimer's $\beta$-amyloid peptide was already been crystallized[4]. Under certain conditions the diphenylalanine peptide self assembles into nanotubes of remarkable stiffness[5], which can serve as casts for the fabrication of silver nanowires[6]. In the crystals, the FF peptides are hydrogen-bonded head-to-tail, forming helical chains with six peptides per helical turn and a 10-Å van der Waals diameter[4]. Adjacent helices are oriented parallel to each other and interact extensively via an intricate three-dimensional stacking arrangement of the aromatic side chains. This structural information provides some hints for the molecular organization of the peptide nanotubes[7]. Nevertheless, the understanding of the key factors responsible for the nanotube stabilization is still not complete. For example, the peptide Ac-Phe-Phe-$NH_2$ forms highly-ordered tubular structures despite the

lack of charge in its terminal ends, suggesting that the interactions between the aromatic sidechains rather than the electrostatic backbone interactions play the key role in the self-assembly process. At the same time, chemical modifications of the FF terminal ends cause the formation of macroscopic hydrogels[8] or amyloid-like fibers[9], suggesting that the nanostructures formed by FF depend also on the chemical nature and interactions of its terminal ends.

To obtain further insights on the aggregation properties of these systems, in the present study we investigate by MD simulations the properties of aqueous solutions formed by FF and the related system, triphenylalanine peptide ($NH_2$-FFF-COOH).

## 2   Systems and Methods

All simulations were performed with the CHARMM program, version c35a1[10]. We simulated two aqueous solutions, consisting of 12 FF dipeptides and 8 FFF tripeptides, respectively. The peptides were placed in a 57-Å cubic box, modeling 34 mgr/ml (FF) and 33 mgr/ml (FFF) solutions. The box was replicated by periodic boundary conditions. The peptide atomic charges, van der Waals and stereochemical parameters were taken from the CHARMM27 all-atom force field[11, 12]. The aqueous solvent effects were modeled implicitly by the Generalized Born approximation GBSW[13, 14]. To improve the conformational sampling, each solution was simulated by the replica-exchange scheme, with 10 replicas spanning the temperature ranges 289-405 K (FF) and 288-416 K (FFF). The replica temperatures were optimized iteratively in the beginning of the simulations as in ref. 15, 16, targeting a uniform exchange probability of 18-20% among adjacent replicas. The simulation length for each temperature was 40 ns, yielding a total simulation time of 0.4 $\mu$s for the 10 replicas. Replica exchanges were attempted at 10-ps intervals. The analysis was done with the CHARMM modules and in-house FORTRAN programs.

## 3   Results and Discussion

*Geometrical Analysis*: In both solutions the peptides form approximately ellipsoidal aggregates, which are stable at 300 K. The geometrical properties of the aggregates in the 300-K simulations are summarized in table 1.

| Aggregate Properties | FF | FFF |
|---|---|---|
| Radius of gyration (Å) | 9.48±0.41 | 9.25±0.30 |
| Volume (Å$^3$) | 3495.77±14.53 | 3422.04±13.28 |
| Density (gr/ml) | 1.779±0.007 | 1.782±0.006 |
| I1/I2, I1/I3, I2/I3 | 0.76±0.12, 0.66±0.10, 0.88±0.06 | 0.76±0.11, 0.66±0.09, 0.86±0.06 |
| PSA (%) | 29.00±6.04 (42.18±1.44) | 24.40±4.48 (34.64±3.41) |

I1, I2 and I3 are the principal moments of inertia of the aggregate. PSA is the polar accessible surface area. The numbers in parentheses correspond to the average PSA of FF and FFF monomers, computed by independent monomer simulations[17].

Table 1. Geometrical characteristics of FF and FFF aggregates at 300 K.

The FF and FFF aggregates have similar shapes (indicated by the comparable moment-of-inertia ratios) and densities. The radius of gyration of the FFF aggregate has a somewhat smaller mean and standard deviation (sd), reflecting the fact that the FFF aggregate is somewhat more stable. In line with this observation, the radius of gyration of the FF aggregate increases faster with temperature[17]. The solvent-accessible surface area of the FF and FFF peptides has a mixed character, due to the non-polar sidechains and the polar (charged) terminal ends and interior peptide bonds. The average fraction of the FF and FFF peptide polar solvent accessible surface area (PSA) is 42.2% and 34.6%, respectively, at 300 K, as computed by simulations of the FF and FFF monomers (with the same implicit model GBSW[13,14]). The PSA ratio is reduced in the FF and FFF aggregates, reflecting the fact that the polar groups participate in several interactions. Indeed, the peptides form network structures in the aggregates, as we analyze below.

*Network Structures*: The backbone moieties of several (2-6) adjacent peptides are frequently arranged into open or closed (ring-like) linear networks, in which adjacent peptides interact by head ($NH_3^+$)-to-tail ($^-OOC$) hydrogen bonds and the aromatic planes of interacting sidechains are mainly oriented perpendicular to each other. A typical, six-peptides ring of the FF simulations is shown in fig. 1, along with the hexagonal ring pattern observed in the L-Phe-L-Phe crystals[4]. The open networks are more frequent due to entropic reasons; nevertheless, the closed networks are more stable energetically, due to both non-polar and polar interactions[17]. The network propensity is higher in the FFF solution. Energetic analysis shows that the non-polar and electrostatic interactions are stronger in the networks of the FFF aggregate[17]; this is in accord with the higher network propensity and the higher stability of the FFF aggregate. The frequency of the peptide networks decreases with temperature, in agreement with the increase in the radius of gyration and the loss of stability[17].
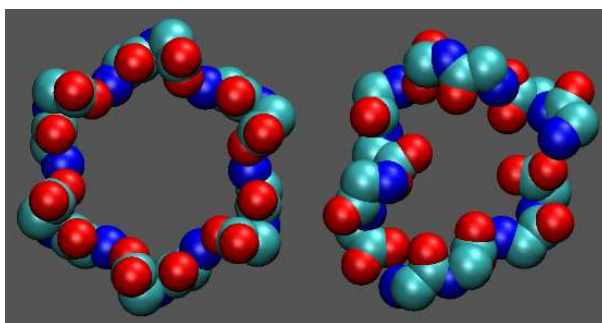


Figure 1. Left: The hexagonal ring network observed in FF crystals[4]. Right: A typical, six-peptides ring network, observed in the FF simulations.

## 4  Concluding Remarks

We have investigated the conformational properties of aqueous FF and FFF solutions by implicit-solvent Replica Exchange MD simulations. In both solutions, the peptides form stable ellipsoidal aggregates. Within the aggregates the peptides are arranged into open and closed linear networks, which are stabilized by head-to-tail and sidechain interactions and have some of the structural features observed in the FF crystals[4]. The intermolecular interactions are stronger in the FFF system, in line with the higher frequency of inter-peptide networks and the increased stability of the FFF aggregate in the temperature range of the simulations. An energetic analysis shows that the side chains contribute to the aggregate stability by forming direct interactions and by modulating the screening of the termini electrostatic interactions by solvent.

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