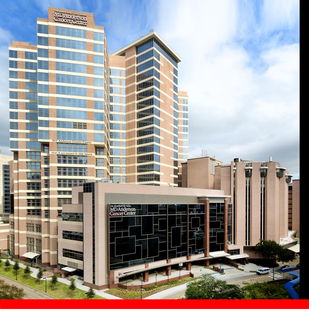

Molecular Dynamics Simulations Testing Suitability of EYKY for Diagnostic Imaging

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Introduction

In diagnostic imaging, peptide-functionalized imaging agents are instrumental to the implementation of sensitive and selective imaging procedures[1]. Peptides are frequently used as targeting moieties in the design of imaging probes[2], owing to a host of their biologically favorable properties,[1] as well as their structural simplicity[3]. Key among these properties are peptides' high binding affinity and specificity and their quick clearance from non-target tissues[1].

Many biomedical applications requiring peptides exploit peptides' ability to self-assemble into a diverse range of useful nanostructures[3]. In order to choose a peptide for use in imaging applications, it is helpful to have a correspondence between the properties of self-assembled peptide structures and the properties of their constituent peptides. However, the vast number of possible peptides presents difficulty in experimentally investigating the properties of self-assembled peptide structures[3]. In practice, molecular dynamics (MD) simulation has arisen as a flexible and inexpensive means of determining the assembly mechanism and structural features of peptides.

Recently, some MD simulation has been performed to investigate the viability of peptides for use in gold nanoparticle (GNP) formulations, which have shown promise in diverse applications to biotechnology[4]. Peptides have previously been shown to aid both the passivation and functionalization of GNPs[4]. In this work, we investigate the potential of peptides to enhance the *in-vivo* stability and biocompatibility of GNPs through self-assembled peptide monolayers formed around the surface of GNPs (**Figure 1**). As a starting point, one peptide that might be of interest for this purpose is EYKY (Glutamic acid-Tyrosine-Lysine-Tyrosine). Experimental work with similar peptides suggests that the glutamic acid residue might show responsiveness to the polarizable surface of the GNPs[5,6]. The purpose of this study, therefore, is to use MD simulation to explore the self-assembly behavior of EYKY. If EYKY can be shown to self-assemble into conformations with suitable chemical properties – for example, beta-sheet formation and inter-peptide bonding – then it might merit further investigation for use in coating GNPs for diagnostic imaging and drug delivery applications.

Theory and Methods

To model interatomic interactions in EYKY, a force field is employed in the MD software. The force field models several interatomic forces, including electrostatic forces, van der Waals forces, and forces that stabilize bond lengths and angles[7]. Additional terms can be added to the force field to model temperature and pressure constraints[8]. Using Newtonian physics, the force field can generate an equation of motion for every point particle in the system. MD simulation consists in numerical integration of these equations, which yields the final structure of the system.

In this work, MD simulation is performed using GROMACS software[9]. To simulate, 100 EYKY peptides (8,200 atoms) are put into an aqueous solution of approximately 39,000 water molecules. The simulation uses a coarse-grained (CG) force field[10-12], which represents atoms and molecules by a smaller number of point particles, thus reducing the computational cost of the simulation. An example of coarse-graining is shown in **Figure 2**, which displays the EYKY molecule. The CG force field maps EYKY's 82 atoms into a simpler representation containing 13 beads. The EYKY-water system is simulated at a temperature of *303 K* and a pressure of *1 bar.* The simulation uses a timestep of *25 femtoseconds* and lasts for *5 μs* in total.

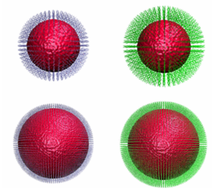

Figure 1 shows examples of peptide monolayers formed around GNP surfaces. As shown in these examples, it is desirable that the peptide coats the GNP surface fully. Adapted from Colangelo E, Chen Q, Davidson A, et al.

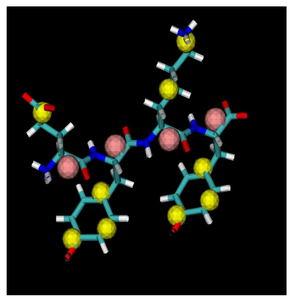

Figure 2 shows an EYKY molecule with all of its atoms (the blue, red, cyan, and white lines) and its simplified, CG representation (the pink and yellow spheres). The CG representation allows for reduced computational burden during simulation.

Figure 3 shows the EYKY molecules after *500 ns* of simulation. The peptides have begun to aggregate but have not yet assembled into a well-defined shape.

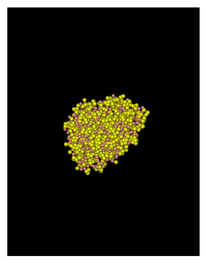

Figure 4 shows the EYKY molecules after *5 μs* of simulation. The system is only slightly more aggregated here than after *500 ns*, and the peptides also do not possess any obvious pattern of orientation.

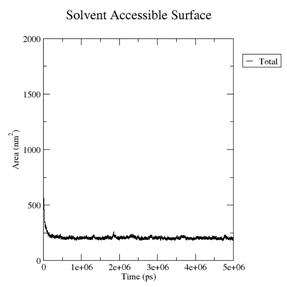

Figure 5 shows the *SASA* of the peptides as a function of time. The graph shows that the *SASA* begins relatively large but quickly falls off to around *200 nm²*, at which it stays roughly constant for the remainder of the simulation.

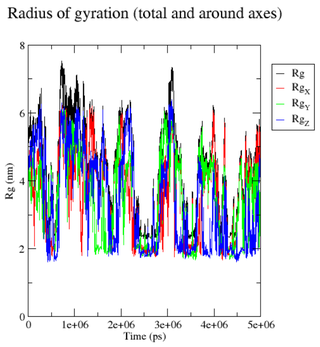

Figure 6 shows the R_g of the peptides as a function of time. Unlike the *SASA* value, the R_g value does not converge during the simulation; rather, R_g oscillates with no obvious pattern.

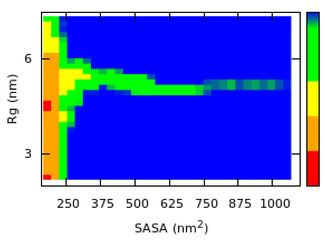

Figure 7 shows the system's free energy landscape as a function of *SASA* and R_g. Here, red colors represent low free energy values, and blue colors represent high free energy values. The system's free energy is lowest when its *SASA* value is lowest, essentially independent of R_g.

Analysis

To quantify the extent of peptide aggregation, the ratio of the peptides' solvent-accessible surface area (*SASA*) is computed. After undergoing energy minimization, the system's *SASA* value is S_1 = *1084.41 nm²*. After *500 ns* of simulation, the *SASA* value S_2 = *249.95 nm²*, and after *5 μs* of simulation, the *SASA* value S_3 = *187.592 nm²*. The structures possessing S_2 and S_3 are shown in **Figure 3** and **Figure 4**, respectively. These results show that the peptides aggregate substantially. Overall, the system's *SASA* value decays quickly over time and stabilizes around *190 nm²*, suggesting that the peptides quickly form a well-aggregated, stable structure.

The peptides' radius of gyration R_g is also used to quantify peptide aggregation. Like *SASA*, R_g is expected to decay and then stabilize as the peptides form a stable aggregate. However, as shown in **Figure 6**, the peptides' R_g value oscillates throughout the entire simulation and shows no obvious decay. These results, contrary to the *SASA* measurements, suggest that the final structure is unstable.

Figure 7 shows the system's free energy landscape as a function of R_g and *SASA*. The data show that the system has the lowest free energy when its *SASA* value is lowest, essentially independent of R_g. Since the low *SASA* values correspond to well-aggregated peptide structures, like those in **Figure 3** and **Figure 4**, the free energy landscape suggests that the aggregated structures possess a smaller free energy than the disaggregated structures.

Conclusions and Future Work

In conclusion, more work is needed to determine the suitability of EYKY as a monolayer coating for GNPs. In this work, the EYKY peptides do not assemble into a nanofiber configuration and thus don't show signs of possessing chemical properties desirable for use with GNPs. In the future, this work could be enhanced by simulating different peptides – for example, by replacing the 'E' and 'K' amino acids of EYKY. Simulations with different peptides may yield insights concerning the structural characteristics required of peptides to form monolayers on GNPs with desirable properties.

Acknowledgement

JM was funded by the AAPM Education and Research Fund.

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