Tuning the Inter-nanofibril Interaction To Regulate the Morphology and Function of Peptide/DNA Co-assembled Viral Mimics

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Abstract: The ability to tune the inter-subunit interaction within the virus capsid may be critical to assembly and biological function. This process was extended here with peptide/DNA co-assembled viral mimics. The resulting co-assemblies, formed and stabilized by both peptide nanofibril–DNA and peptide nanofibril–nanofibril interactions, were tuned through hydrophobic packing interactions of the peptide sequences. By strengthening peptide side-chain complementarity and/or elongating the peptide chain (from 4 to 8 residues), we report strengthening the inter-nanofibril interaction to create stable nanococoons that give high gene-transfection efficacy.

In naturally occurring protein-based hierarchic assemblies, highly ordered structures underpin their unique functional capability. For example, viruses, the most efficient gene-transfection agents in nature, have the viral genome wrapped in an orderly packed protein capsid.[1] The virus capsid is composed of multiple copies of the capsid proteins that assemble through non-covalent interactions on two levels: 1) intra-subunit interactions that assemble several protein monomers into a subunit, and 2) inter-subunit interactions that tie the subunits together to form the capsid.[2] During viral infection, the capsid disassembles through inter-subunit dissociation (instead of protein monomer dissociation) to achieve efficient genome release and transfection.[3] Learning these rules for hierarchical assembly and subunit interactions could be critical for the development of novel biomimetics with tuneable stability and function.[4]

Recently, peptide- and small-protein-based structural virus mimics have been extensively developed through de novo synthetic approaches, including the filamentous tobacco mosaic virus (TMV) mimics.[5] These constructs were formed either through the co-assembly of peptide/protein with plasmid DNA, or through the absorption of siRNA onto the surface of preformed short filamentous peptide nanoribbons.[5] In these viral mimics, the formed peptide β-sheets or α-helical coiled coils provided the core of the “capsid”, contributing to both the intra- and inter-subunit interactions and stabilizing the whole capsid. In addition to filamentous viral mimics, spherical synthetic virions have also been constructed.[6] One example is the small spherical viral mimics formed through the co-assembly of α-helical peptides and RNA. Here, the trifurcated coiled coils are involved in the inter-subunit interaction in the peptide shell.[6] However, the length of the peptide/protein sequence (30–531 amino acids) involved in these viral mimics has limited studies on the stability and function of the inter-subunit interactions.

Recently, our laboratory developed cocoon-like viral mimics called nanococoons through the co-assembly of a short designed peptide K3C6SPD (KKKC<sub>6</sub>-WLFFAQ-GSPD) with plasmid DNA (Figure 1).[7] The short peptide strand contains three main segments: 1) an N-terminal...
cationic segment for DNA binding; 2) a central β-sheet-forming segment for peptide assembly and stability of the nanococoons; and 3) a C-terminal hydrophilic segment for dispersion of the nanococoons in aqueous media. The structural characterization of nanococoons has led to a model for co-assembly that involves: 1) the peptide strands pre-organize along the DNA backbone through electrostatic interactions; 2) the peptides self-assemble into nanofibrils; and 3) inter-nanofibril association places the DNA inside the nanococon (Figure 1A). The intra- and inter-nanofibril interactions involved in nanococon formation imitate the intra- and inter-subunit interactions within virus capsids. Therefore, nanococoons provide a simple model system to investigate inter-subunit interactions, and to study the impact of these interactions on the hierarchic architecture and biological function of peptide/protein assemblies.

According to the structural model of the β-sheet nanofibrils (Figure 1A), the central segment contributes to β-sheet formation. The surface-exposed residues on the β-sheet region are also involved in inter-nanofibril associations. Therefore, the properties of these central residues appear to determine the driving force for inter-nanofibril association through hydrophobic interactions between the subunits. Therefore, this driving force was selected to tune the inter-nanofibril interaction in nanococoons.

To extend the hydrophobic interaction between nanofibrils, the eight residues within the β-sheet segment of K3C6SPD were substituted with peptide segments composed of leucine (L) and/or alanine (A). The reasons for this choice include: 1) both L and A have hydrophobic aliphatic side chains, which display dramatic differences in volume (92 vs. 168 Å³), accessible surface area (67 vs. 137 Å²), and normalized hydrophobic index (97 vs. 41); and 2) both residues show a propensity for β-sheet formation in peptide self-assembly. Accordingly, the range of L and/or A substitutions with lengths ranging from 4 to 8 were designed from the original central β-sheet segment of K3C6SPD (Figure 1B). These new peptides were synthesized on a solid-phase peptide synthesizer, purified on HPLC, and confirmed by MALDI-MS (see supporting information).

The sequence variation generally did not change the assembled morphology or secondary structure (Figure 2 and Figure S1, S2). At neutral pH in aqueous solution, all of the peptides assembled into filamentous nanofibrils (Figure 2 and Figure S1). As shown in the Fourier transform infrared (FTIR) spectra in Figure S2, all of the nanofibrils display the characteristic amide I band around 1628 cm⁻¹, which is consistent with the presence of antiparallel β-sheets within these nanofibrils.

These β-sheet nanofibrils predict the existence of distinct surface-exposed residues suitable for inter-nanofibril interactions in peptide/DNA co-assemblies. At a peptide-to-DNA charge ratio (N/P ratio; defined as the ratio of the number of positive charges from the amines of the peptide over the number of negative charges from the phosphates of the DNA) of 20, the peptides (0.2 mM) co-assemble with DNA (10 μg/mL) into distinct morphologies (Figure 3 and Figure S3). L8/DNA, L6/DNA, L4/DNA, and (L2A2)2/DNA form nanococoons with striped surfaces. Specifically, L8/DNA nanococoons coexist with dense short nanofibrils, thus suggesting that L8 maintains the ability to self-assemble as well as co-assembly with DNA. L6/DNA, L4/DNA, and (L2A2)2/DNA co-assemblies gave nanococoons without nanofibrils, thus indicating that the DNA templates overwhelmed peptide self-assembly. A8/DNA contained a dense nanofibril/DNA network, thus implying that the interaction between small alanine residues is too weak to drive nanofibril lateral association and the non-specific charge interaction between nanofibrils and DNA led to the formation of the disordered nanofibril/DNA network. The shorter A6 failed to complex DNA and only loosely tangled DNA was found under TEM. The peptide strands within these co-assembled nanostructures mainly adopted a β-sheet secondary structure, similar to that found in nanofibrils. As shown in the FTIR spectra in Figure 4A, the typical amide I stretching mode of antiparallel β-sheets around 1626–1630 cm⁻¹ and the transition at 1690 cm⁻¹ were found in the L8/DNA, L6/DNA, A8/DNA, A6/DNA, and (L2A2)2/DNA nanostructures. The L4/DNA assemblies contain two amide I transitions at 1643 cm⁻¹ and 1680 cm⁻¹, respectively, which are different from those in L4 fibrils, thus suggesting that DNA templates affected L4 strand arrangement in L4/DNA complex. Taken together, the side-chain hydrophobic interactions between nanofibrils significantly impact on the nanofibril lateral association and peptide/DNA co-assembled morphology.

To further understand the impact of the inter-nanofibril interactions on the co-assembled morphology, three peptides (L8, (L2A2)2 and A8) of the same length were studied at N/P ratios of 5, 10, with the same DNA concentration of 10 μg/mL but with a lower peptide concentration. As shown in Figure 3 and Figure S3, at both the lower N/P ratios (5 and 10), L8/DNA gave nanococones and short nanofibrils, thus confirming the strong assembly propensity of L8. (L2A2)2/DNA formed exclusively well-dispersed nanococones, and for A8/DNA, no co-assembled nanostructure are observed. This co-existence of nanofibrils and DNA suggests that the non-specific interaction between A8 nanofibrils and DNA is weak at both N/P ratios. The significant
morbidity difference between various peptide/DNA co-assemblies, such as the ones at an N/P ratio of 5, further confirms the substantial effect of peptide side-chain hydrophobicity on inter-nanofibril interaction and the final peptide/DNA co-assembled morphology.

The nucleic acid condensation and protection capability of these co-assembled nanococoons at an N/P ratio of 20 were assessed through gel migration and DNase I digestion assays (Figure 4B). In the absence of DNase I, L8/DNA and L6/DNA nanococoons, and A8/DNA nanofibril networks, completely retarded DNA gel migration, thus supporting formation of a stable complex. (L2A2)2/DNA nanococoons only partially retarded DNA migration, thus suggesting a weaker interaction between (L2A2)2 nanofibrils and DNA. And finally, the DNA in L4/DNA and A6/DNA completely migrated forward as the free DNA did, thus indicating the weakest complex. Upon DNase I treatment, the L8/DNA and L6/DNA nanococoons protected against DNA digestion, while the DNA in A8/DNA, (L2A2)2/DNA, L4/DNA, and A6/DNA is completely digested. The degree of DNA protection by these peptide/DNA nanostructures suggests that nucleic acid encapsulation/protection requires tight binding, not only between DNA and peptide, but also between peptide nanofibrils. The hydrophobicity of the central peptide β-sheets drives the formation of the nanococoon shell, which protects the DNA cargo from enzymatic degradation.
These co-assembled nanostructures with peptide concentration of 10–40 μM, the final concentration in the cell culture medium, are safe to cells (Figure S4), thus allowing the biological function of these nanostructures (10 μM) to be evaluated through the delivery of GFP-encoded plasmids into HEK 293 cells for GFP expression. As shown in Figure 5 A, at an N/P ratio of 20, L8/DNA and L6/DNA nanococoons gave fluorescent cells, thus confirming delivery of the plasmid into the cell for expression. L4/DNA, A8/DNA, A6/DNA, (L2A2)2/DNA nanostructures and the control naked DNA did not induce GFP expression (Figure S5). Therefore stable co-assembly is required for cellular entry, but it should not be so stable as to prevent access and expression of the encoded information.

To understand the different gene-transfection efficacy of these nanostructure, their cellular uptake was assessed with Rhodamine (Rh)-containing samples. When Rhodamine is conjugated at the N-terminus of the peptide through solid-phase peptide synthesis (Figure 5), 1% added Rh-peptide did not affect either the co-assembled morphology (data not shown) or cellular uptake (Figure S6). When incubated with HEK 293 cells for 4 h, the degree of cellular internalization was evaluated by using confocal microscopy imaging. As shown in Figure 5 B, cells treated with L8/DNA and L6/DNA are homogeneously fluorescent, thus suggesting that L8/DNA and L6/DNA nanococoons are internalized. In contrast, L4/DNA, A8/DNA, A6/DNA, and (L2A2)2/DNA-treated cells display much weaker fluorescence intensity (Figure S6), which might be attributed to either low peptide/DNA co-assembly formation in the solution or limited uptake of the peptide/DNA co-assemblies into cells. Again, the DNA encapsulation/protection and cellular uptake, which are regulated by peptide side-chain interaction, are prerequisites for the overall biological function of these co-assembled nanostructures.

In conclusion, we were able to tune the morphology, stability, and function of peptide/DNA co-assembled viral mimics by regulating the inter-nanofibril hydrophobic interactions. We found that increasing the peptide side-chain hydrophobicity (from A to L) or the peptide length (from 4 to 8) within the β-sheet segment gradually enhances inter-nanofibril association and controls the final co-assembled morphology. At the same N/P ratio of 20, distinct morphology differences can be observed with different peptides. These differences range from DNA/peptide tangles (A6/DNA) and non-specific electrostatic interaction-driven nanofibril/DNA network (A8/DNA) to striped nanococoons (L4/DNA, (L2A2)2/DNA and L6/DNA) and the co-existence of nanofibrils and nanococoons (L8/DNA). The strength of the inter-nanofibril interactions also regulate the stability, nucleic acid protection, and cellular internalization of the formed nanostructures. Stronger inter-nanofibril interactions gave more stable nanococoons for higher cellular uptake, but this stability did not appear to negatively impact gene transfection efficiency. Overall, these results establish that DNA/peptide co-assembly can be optimized through simple hydrophobic packing of peptide side chains, and the resulting stability can significantly influence the function of these co-assemblies in DNA cellular delivery.

Acknowledgements

The authors thank the Hong Kong Research Grant Council (GRF 16305815) and Hong Kong University of Science and Technology (initiation grant N1574) for their financial support.

Conflict of interest

The authors declare no conflict of interest.

Keywords: hydrophobic interactions · nanofibrils · peptide/DNA assemblies · virus mimics · β-sheets

How to cite: Angew. Chem. Int. Ed. 2017, 56, 9356–9360
              Angew. Chem. 2017, 129, 9484–9488


