Drug Delivery

DOI: 10.1002/anie.201005548

A Hierarchical Assembly Process to Engineer a Hydrophobic Core for Virus-like Particles**

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DNA amphiphiles · drug delivery · self-assembly · viruses · virus-like particles

Modern gene- and drug-delivery vehicles are evolving into elaborate machines with multiple targeting moieties and efficient cargo-loading capacities. These functionalities need to be localized such that the targeting motifs are on the exterior with high surface accessibility whereas the cargo is protected in the interior. To address this need for incorporating a variety of functionalities with spatial selectivity, multivalent protein assemblies, viruses, and virus-like particles (VLPs) have recently been considered as ideal scaffolds.[1,2] The advantages of viral systems include simple production, uniform size and structure, and a surface, which molecular cloning and protein-conjugation strategies, can display a variety of functional groups with molecular precision. In the past decade, myriad viruses and VLPs have been genetically and chemically reprogrammed to function as drug/gene-delivery vehicles,[2,3] vaccine carriers,[4] imaging probes,[5] and composite materials.[6]

The clustering of targeting units on the surface of viruses with control over the spacing and orientation of those groups allows for multivalent ligand–receptor interactions, a principle of multivalency, which can improve binding significantly.[7] For example, the multivalent display of tumor-targeting molecules, such as folic acid, on the virus surface has been shown to enhance the uptake of the viruses by cancer cells.[8] However, it is a challenge to load small-molecule drugs, which are often hydrophobic, within viral particles for delivery purposes on account of the hydrophilic environment inside the particle. An even greater challenge is the retention of drugs because it is difficult to control the porosity of the protein shell (capsid) of the viral particle. To address these issues, a wide range of methods from conventional protein-modification reactions to nonconventional bioconjugation strategies have been developed to covalently attach drugs to viral capsids.[8–10] In this case, a carefully designed linker is necessary to achieve controlled drug release, and orthogonal bioconjugation reactions should be employed to decorate the exterior surface with targeting motifs as well as to load the drugs inside the viral capsid (Figure 1 A).[10] However, the loading capacity of viruses is still very low compared to that of other nanoscale delivery vectors. To improve drug loading, intact viral particles have been co-assembled with polymers or liposomes to generate a composite core–shell nanoassembly,[11] which can entrap hydrophobic drugs (Figure 1 B, C). Thus the immobilized viral particles can serve as scaffolds displaying targeting motifs in the same way as free viral particles.

Recently, Kwak et al. described the use of DNA amphiphiles as templates to modulate the self-assembly of coat proteins (CPs) of a plant virus, cowpea chlorotic mottle virus (CCMV), resulting in a nanocarrier for both hydrophobic and hydrophilic drugs (Figure 1 D).[12] The idea is based on derivatizing small oligodeoxynucleotides (ODNs) with hydrophobic units to induce their self-assembly into micellar structures. These can serve as templates for further hierarchical assembly of viral CPs to form VLPs. This general and facile strategy can be used to adapt virus-based vehicles for drug-delivery applications.

Two classes of DNA amphiphiles known to self-assemble into larger aggregates were used in this study. The first class consists of ODNs with attached low-molecular-weight hydrophobic chains (i.e. dodecynyl), and the other class of DNA amphiphiles is diblock copolymers of ODNs and hydrophobic polymers (polypropylene oxide in this case; Scheme 1). Kwak and co-workers found that the amphiphiles can readily form micelles at room temperature with sizes in the range of 7–11 nm. Owing to the hydrophobic nature of the cores of the micelles, hydrophobic compounds accumulate within them. Alternatively, a hydrophilic compound can be first conjugated to a single-strand DNA complementary to the ODN of the amphiphile, and then loaded to the micelles upon DNA hybridization. Further incubation of the micelles, loaded with either a hydrophilic or a hydrophobic compound, with CCMV CPs at pH 7.5 and 4 °C for 30 min led to the formation of spherical VLPs with a size range of (19.9 ± 3.1) nm based on

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[**] Z.S. thanks the National Natural Science Foundation of China (50921062) for support. Q.W. acknowledges the NSF-CAREER program, DoD-W911NF-09-1-236, DoE-BES, the Alfred P. Sloan Foundation, and the Camille Dreyfus Teacher Scholar Awards Program for financial support. Q.W. is also grateful to CIAC for a guest professorship.

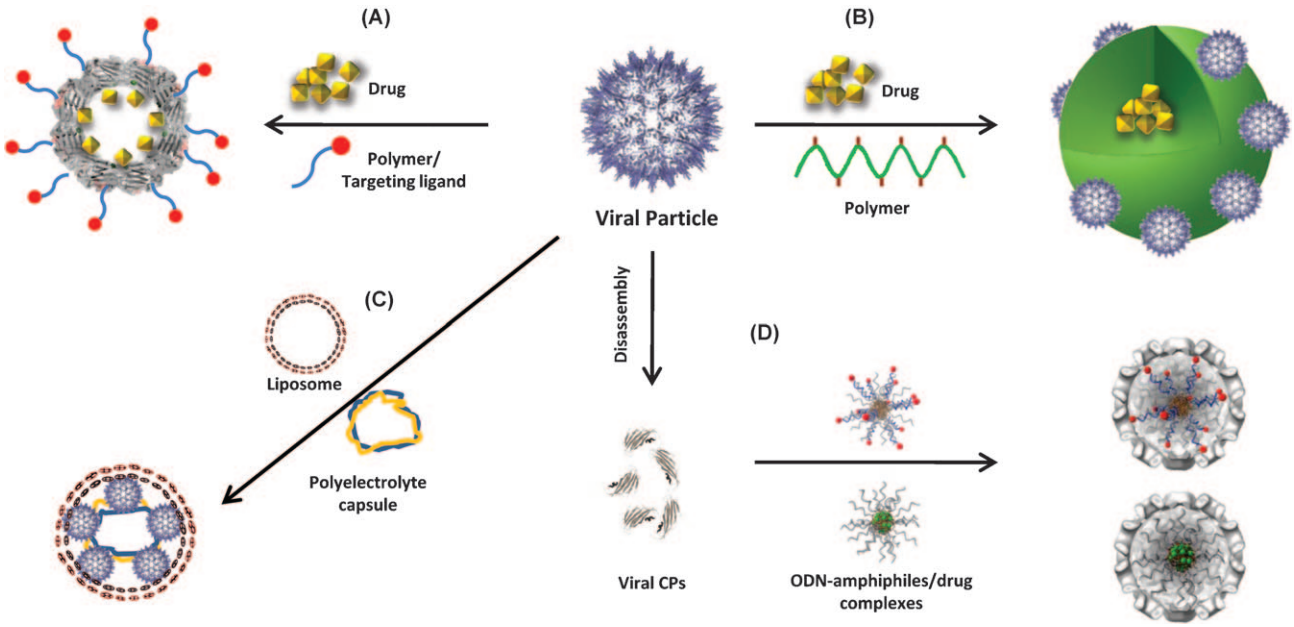
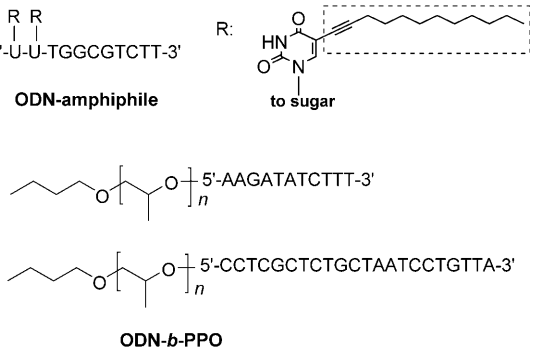


Figure 1. A) Loading a viral particle with drugs and targeting motifs through bioconjugation reactions. B) Loading hydrophobic drugs within a polymer–virus core–shell assembly. C) Constructing a virus–polyelectrolyte–liposome complex. D) Using DNA amphiphiles to induce the assembly of viral coat proteins to encapsulate drugs.



Scheme 1. Structures of ODN amphiphiles used in producing VLPs.

transmission electron microscopy (TEM) analysis. In some situations, smaller particles could also be detected.

Although it is well known that ODNs and other polyelectrolytes can be employed to modulate the assembly of virus CPs to form VLPs,[13] the work by Kwak et al. has given a new flavor to this existing technique. We can envision this strategy for encapsulating a variety of drugs or other small molecules. Moreover, since the morphology of the DNA–amphiphile assembly can be varied by more sophisticated supramolecular design, a broad array of nanoarchitectures can be constructed using virus CPs mediated by the DNA–amphiphile assemblies. For example, the same group has reported that nanotubes can be produced by self-assembly of CPs of CCMV with one-dimensional DNA–hybrid complexes.[14]

The work by Kwak et al. offers an exciting new way to construct virus-based targeted therapeutics, yet some fundamental questions still remain about the method. For instance, the potential loading capacity for hydrophobic drugs is not clear. The DNA–amphiphile structure may need to be further optimized in order to improve the encapsulation capacity of hydrophobic drugs. Similarly, additional designs are necessary for increasing the incorporation efficiency of hydrophilic drugs. In addition, since not many viruses have the same in vitro assembly potential as CCMV, it would be a challenge to expand this approach to other viral systems as well as to chemically tailored viral CPs. Nevertheless, even though this work focused on using VLPs as delivery vehicles, it still represents a small step toward largely uncharted territory: can we decode protein assembly and create a de novo designed template to control it? The combination of supramolecular chemistry along with an in-depth understanding of structural biology and the principles of self-assembly will certainly point us in the right direction.

Received: September 4, 2010
Published online: November 25, 2010

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