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A simple RNA-DNA scaffold templates the assembly of functional virus-like particles

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Abstract

Using the components of a particularly well-studied plant virus, cowpea chlorotic mottle virus (CCMV), we demonstrate the synthesis of virus-like particles (VLPs) with one end of the packaged RNA extending out of the capsid and into the surrounding solution. This construct breaks the otherwise perfect symmetry of the capsid and provides a straightforward route for monofunctionalizing VLPs using the principles of DNA nanotechnology. It also allows physical manipulation of the packaged RNA, a previously inaccessible part of the viral architecture. Our synthesis does not involve covalent chemistry of any kind; rather, we trigger capsid assembly on a scaffold of viral RNA that is hybridized at one end to a complementary DNA strand. Interaction of CCMV capsid protein with this RNA-DNA template leads to selective packaging of the RNA portion into a well-formed capsid, but leaves the hybridized portion poking out of the capsid through a small hole. We show that the RNA-DNA protruding from the capsid is capable of binding DNA and DNA-functionalized colloidal particles. Additionally, we show that the RNA-DNA scaffold can be used to nucleate virus formation on a DNA-functionalized surface. We believe this self-assembly strategy can be adapted to viruses other than CCMV.

Graphical abstract

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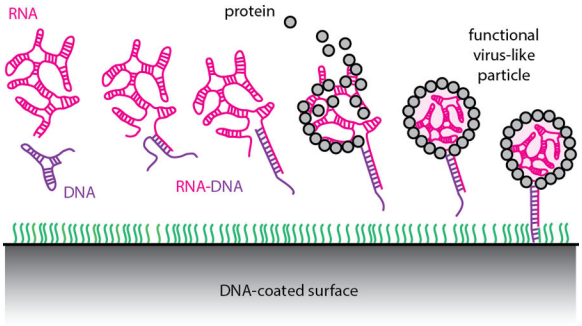
ASSOCIATED CONTENT

Supporting Information. Procedures, data from control experiments, and examples of CP-AuNP aggregation are available free of charge via the Internet at http://pubs.acs.org.



Small RNA viruses consist entirely of genomic RNA packaged inside a one-molecule-thick protective protein capsid (Fig.1). In addition to making up a large fraction of the world's viral pathogens, small RNA viruses are helping to define new fields of applied science through their use as functional nanoparticles[1]. For example, they have been exploited as contrast agents for biomedical imaging[1–1], as vectors for the delivery of small molecules and genes to cells[6–11], and as nanoscale building blocks for the formation of superstructures with unique material, optical and dynamic properties[12–17].

Much of the utility of small RNA viruses derives from their symmetric capsids[18] which can be engineered to display a high density of functional moieties. Indeed, an arsenal of functionalization strategies[20–31] have been developed that combine molecular biology (cloning) and/or selective covalent chemistries to isotropically label the various polyvalent surfaces (exterior, interior, and interfacial[1]) of the capsid. However, in situations where a high degree of labeling is not desired, monofunctional particles that display only a single copy (or a specific limited arrangement) of a particular functional group are needed. Monofunctional virus particles are difficult to produce[32–34] in a controlled way due to the inherent symmetry of the capsid and its abundance of equivalent binding sites.

Lying just beneath the capsid, the viral RNA contains a wealth of inequivalent binding sites that could in principle be selectively targeted using the methods of DNA nanotechnology[35–40]. Unfortunately, the capsid is impermeable these techniques – the main evolutionary purpose of the capsid is to protect the RNA from unfavorable interactions with macromolecules from the outside world. Our work bypasses this inaccessibility through the synthesis of virus-like particles (VLPs) with a portion of one end of the RNA extending outside of the capsid (Fig. 2). With the symmetry of the particle broken by the exposed RNA, we generate robust monofunctionalization through the conjugation of desired moieties using only Watson-Crick basepairing.

Our synthesis (Fig. 2A) does not require genetic modification nor covalent chemistry, but instead relies on the ability of a particularly well-studied small RNA virus, CCMV, to be disassembled and reconstituted by self-assembly *in vitro*[41]. Additionally, we exploit the qualitative structural differences between single-stranded (ss) and double-stranded (ds) nucleic acid to reshape the viral RNA that templates the assembly. Due to extensive intramolecular base pairing, ss-RNA of the length naturally packaged by CCMV (about 3 kb) is a highly branched, flexible, compact object that has physical dimensions comparable to the capsid interior[42] (22-nm-internal diameter). In contrast, the same length (3 kbp) of ds-

DNA occupies a much larger volume due to its increased stiffness (~50 nm persistence length) and lack of branching. As a result, ds-DNA longer than about 75 bp cannot be accommodated within the interior volume of the CCMV capsid and does not function as a template for normal capsid assembly[43,44].

By hybridizing the first 185 bases at the 5′-end of a 3.2 kb ss-RNA with a complementary ss-DNA strand, we dramatically stiffen the 5′-end of the RNA. [We use the 5′-end of the 3234-base RNA molecule ("B1") of the tripartite genome of brome mosaic virus (BMV), although either end of any similar-length sequence will likely do.] Here, the DNA strand (see SI) acts as a molecular splint. The resulting RNA-DNA hybrid can be expected to behave as a compact, flexible, branched 3-kb ss-RNA connected to a rigid, linear, 185-base ds-RNA-DNA appendage (see second-from-left cartoon in Figure 2A). The physical length of the ds portion is about 50 nm. It is very stable (85 °C melting temperature) owing to its perfect sequence complementarity. The *in vitro* packaging of this RNA-DNA hybrid by CCMV capsid protein (CP), using the same protocol developed earlier for pure RNA,[45–47] results in the selective encapsidation of the ss-RNA portion and leaves the ds-RNA-DNA appendage poking out of the capsid and into solution. We refer to this final construct as a "cherry bomb" because of its structural resemblance (Figure 2B, additional images shown in SI, Figure S1) to the well-known explosive firework.[48]

While the structure of the hole that passes the ds-RNA-DNA through the capsid is not known, previous *in vitro* packaging studies have shown ss and ds nucleic acid traversing the capsids of CCMV and the closely related BMV. We previously observed[49] that ss-RNA molecules significantly longer than wild-type are packaged by multiple CCMV capsids ("multiplets") that each share a portion of the overlong RNA (Figure 3). And a separate study[44] found that long ds-DNA is packaged by a contiguous string of many BMV capsids if ss-RNA fragments are also present. In both cases, nucleic acid is shared by connected capsids, passing through one or more holes in each capsid that are too small to be seen by negative-stain transmission electron micrograph (TEM). It is likely that the ds-RNA-DNA appendage of the cherry bomb exits the capsid through a similar hole.

To test whether the exposed RNA-DNA appendage can be used to bind nucleic acids in solution, we designed the DNA splint with a 3' poly-A_{15} overhang in addition to the 185 bases that complement the 5'-end of the RNA (see right-most cartoon in Fig. 2A). Once formed, cherry bombs were combined with fluorescent (green) poly-T_{15} DNA strands and analyzed by native agarose gel electrophoresis (Fig. 4). The capsids co-migrated with the fluorescent poly-T_{15} (Fig. 4, lane 3), confirming that the poly-A_{15} sticky end of the RNA-DNA appendage binds its complementary strand in solution. Control experiments in which *already*-assembled VLPs containing only 3.2 kb RNA (B1) were added to the splint DNA and fluorescent poly-T_{15} showed no nonspecific binding (Fig. 4, lane 4).

The ability of the cherry bomb to bind a functionalized surface was demonstrated by direct imaging of a mixture of these capsids with 30-nm gold nanoparticles (AuNPs) that had been previously decorated with a high density of poly-T_{25} DNA strands[50] (Fig. S2). Negative-stain TEM (Fig. 5) shows a high concentration of capsids at the AuNP surface, and an exceptionally well-stained image reveals the RNA-DNA appendage linking the capsids to

the gold surface (Fig. 5, arrows). Control experiments between B1 VLPs and functionalized AuNPs showed no non-specific binding (Fig. S3).

Separately, we tested whether capsids could be assembled around RNAs that were already tethered to a functionalized surface (Fig. 6A). Here, the hybrid RNA-DNA scaffold was prepared and equilibrated with 30-nm poly-T_{25}-coated AuNPs at a molar ratio of 10:1 (RNA:AuNP). After hybridization of the RNA to the AuNPs, CP was added at a mass ratio of 10:1 (CP:RNA), equilibrated for 5 min on ice, and imaged by negative-stain TEM (Fig. 6B). The presence of well-defined capsids at the Au particle surface demonstrated assembly of cherry bomb capsids around the immobilized RNA-DNA. Some aggregation of CP in the presence of AuNPs was also observed (Fig. S4).

While several elegant methods have recently been described for the monofunctionalization of tobacco mosaic virus particles,[51–53] they most likely cannot be applied to other viruses; they require either controlled disassembly of one end of the rod-like capsid or self-assembly of capsids on a substrate bound RNA that contains a specific packaging sequence. The homogeneous assembly pathway described here provides a general strategy for monofunctionalizing icosahedral particles. Here we note that the ability to form cherry bomb structures is probably not limited to the plant virus CCMV; multiplet capsids have been observed in the packaging of overlong RNAs by the CP of the bacterial virus fr[54] and of the mammalian virus SV40,[55] indicating that they too have the potential to form cherry bombs.

In addition to offering a single, highly specific binding modality for building functional viral-based materials, our method for (i) physically binding and manipulating one end of the packaged genome and (ii) nucleating capsid assembly at a surface will enable new single-particle measurements that might reveal how RNA gets into and out of viral capsids during infection. Examples of such measurements include time-resolved studies of capsid assembly and force-pulling experiments[56,57] that measure the work required to pull viral RNA out of its capsid.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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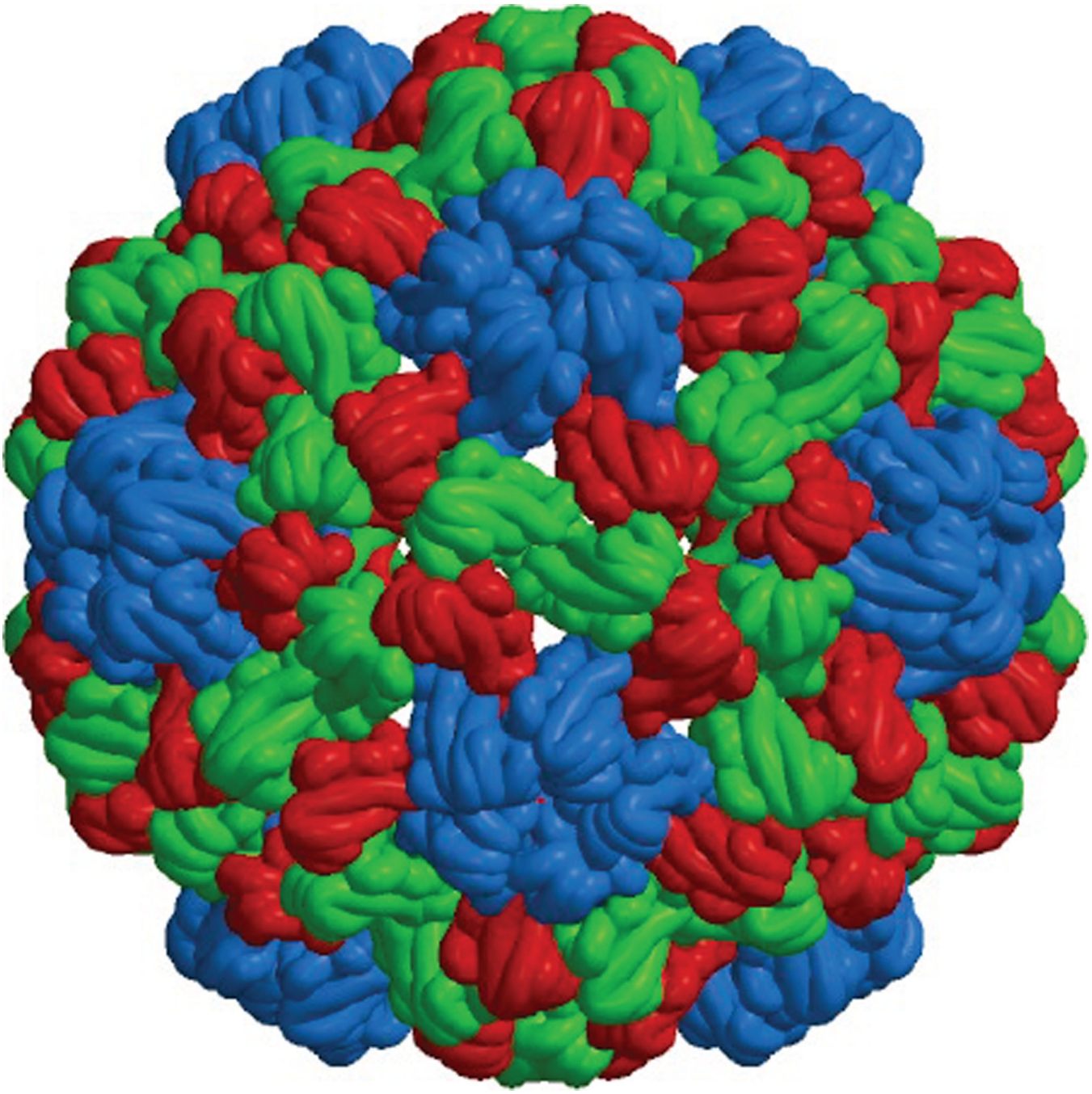

Figure 1.
The capsid of cowpea chlorotic mottle virus (CCMV), like many small RNA viruses, has icosahedral symmetry and consists of 180 copies of its capsid protein. Diameter is 28 nm. Taken from VIPERdb[18].

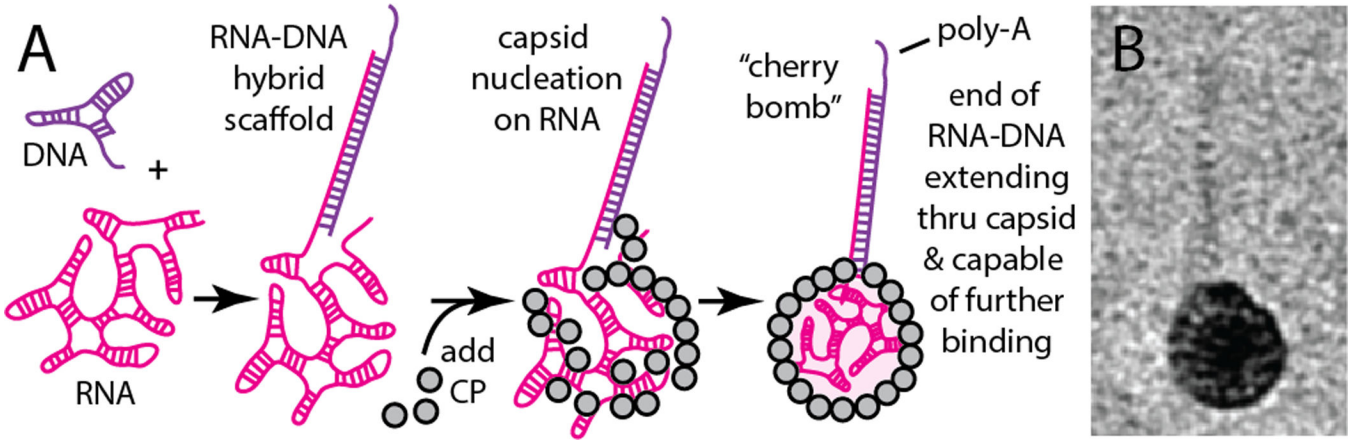

Figure 2.

(A) Schematic illustration of assembly of the "cherry bomb". (B) Positive-stain transmission electron micrograph (TEM) of a cherry bomb capsid (dark sphere measuring 26 nm) and its RNA-DNA appendage (lighter strand extending upward).

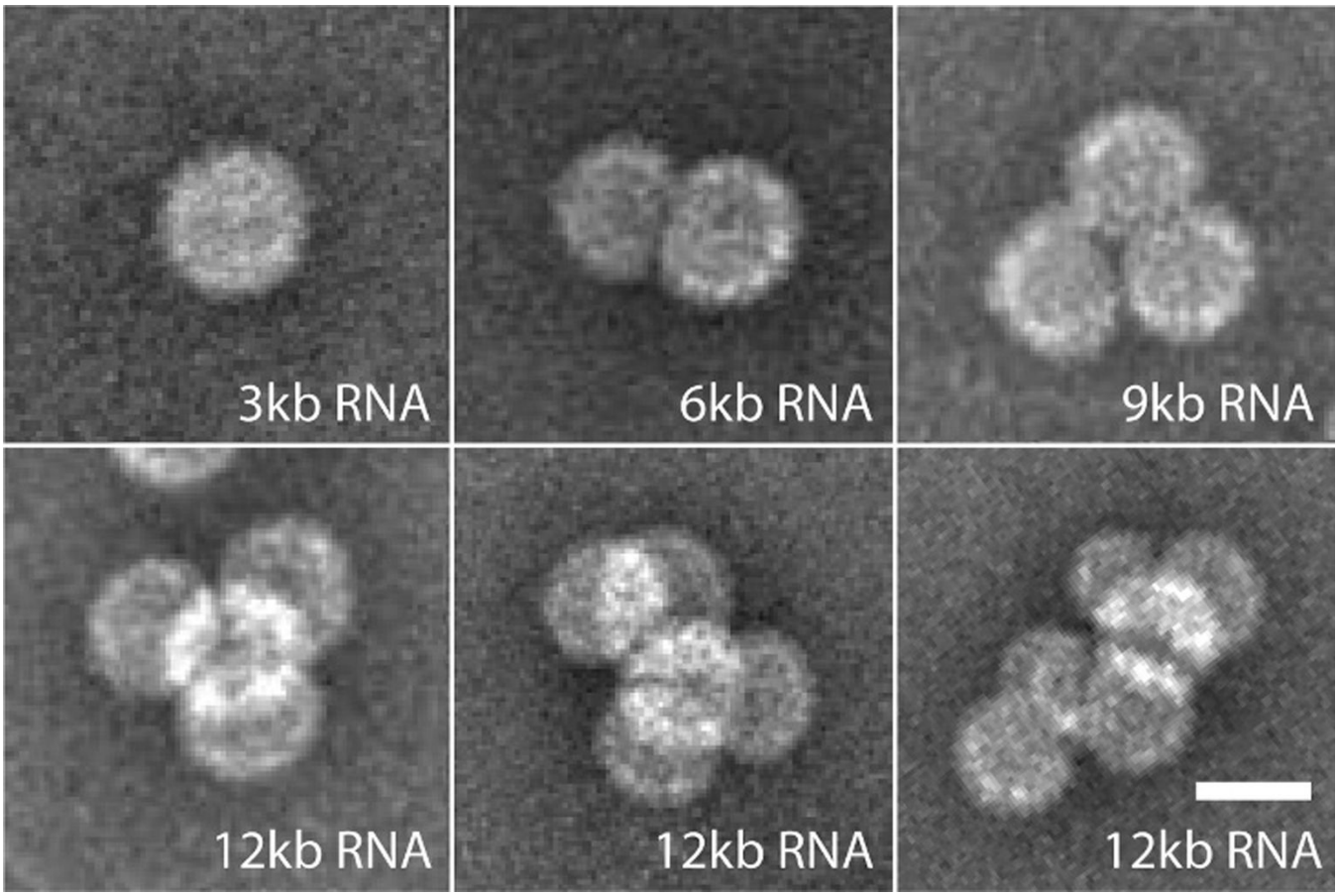

Figure 3.

Single ss-RNA molecules progressively longer than wild-type (3 kb) are shared by two or more CCMV capsids; multiplets. Scale bar shows 25 nm. Adapted from J. Virol. 2012, 86, 3322, doi: 10.1128/ JVI.06566-11. Copyright American Society for Microbiology.

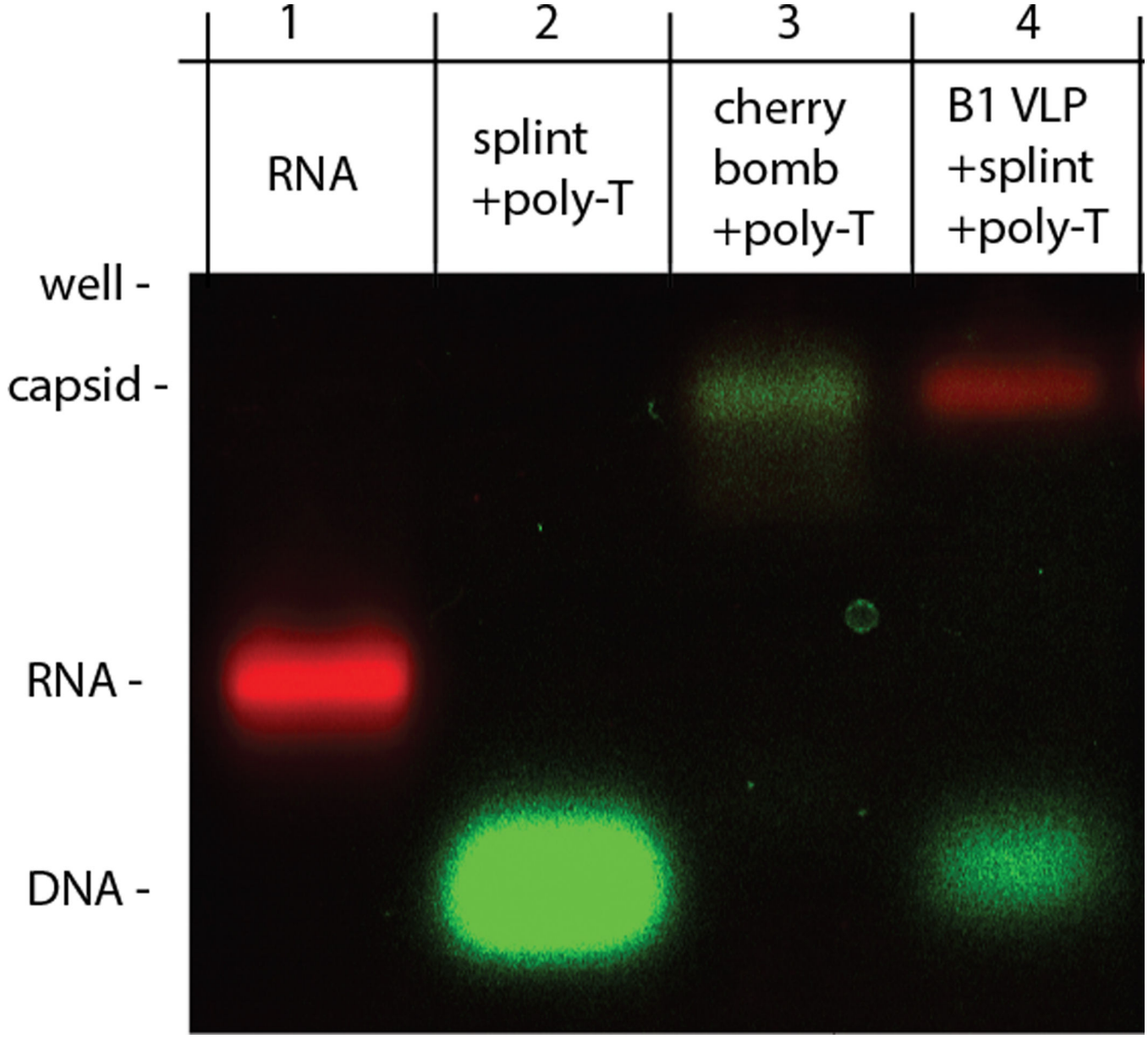

Figure 4.
Native agarose gel electrophoresis shows cherry bombs selectively bind fluorescently labeled poly-T_{15} DNA strands (green). Fluorescently labeled RNA shown in red.

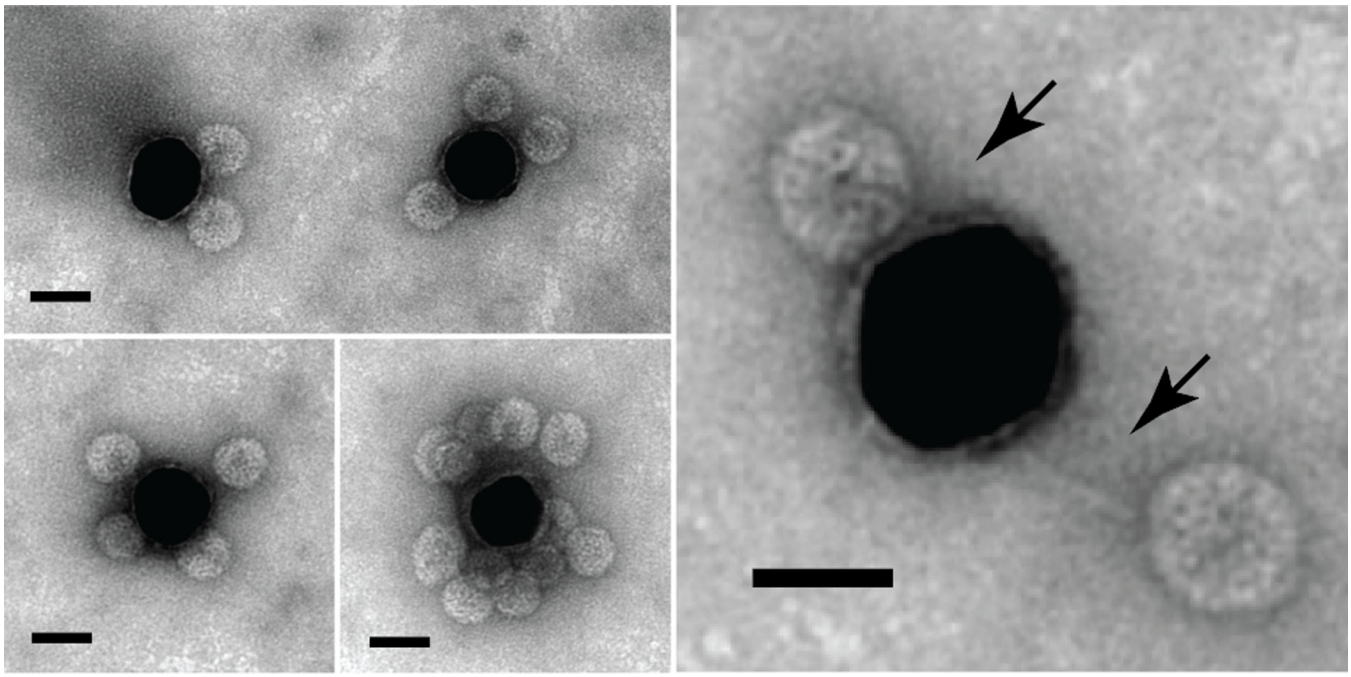

Figure 5.

Cherry bomb capsids bind the surface of DNA-functionalized 30-nm AuNPs. A zoomed-in image resolves the RNA-DNA duplex (arrows) linking the capsids (light spheres) and the AuNPs (dark sphere). Scale bars show 25 nm.

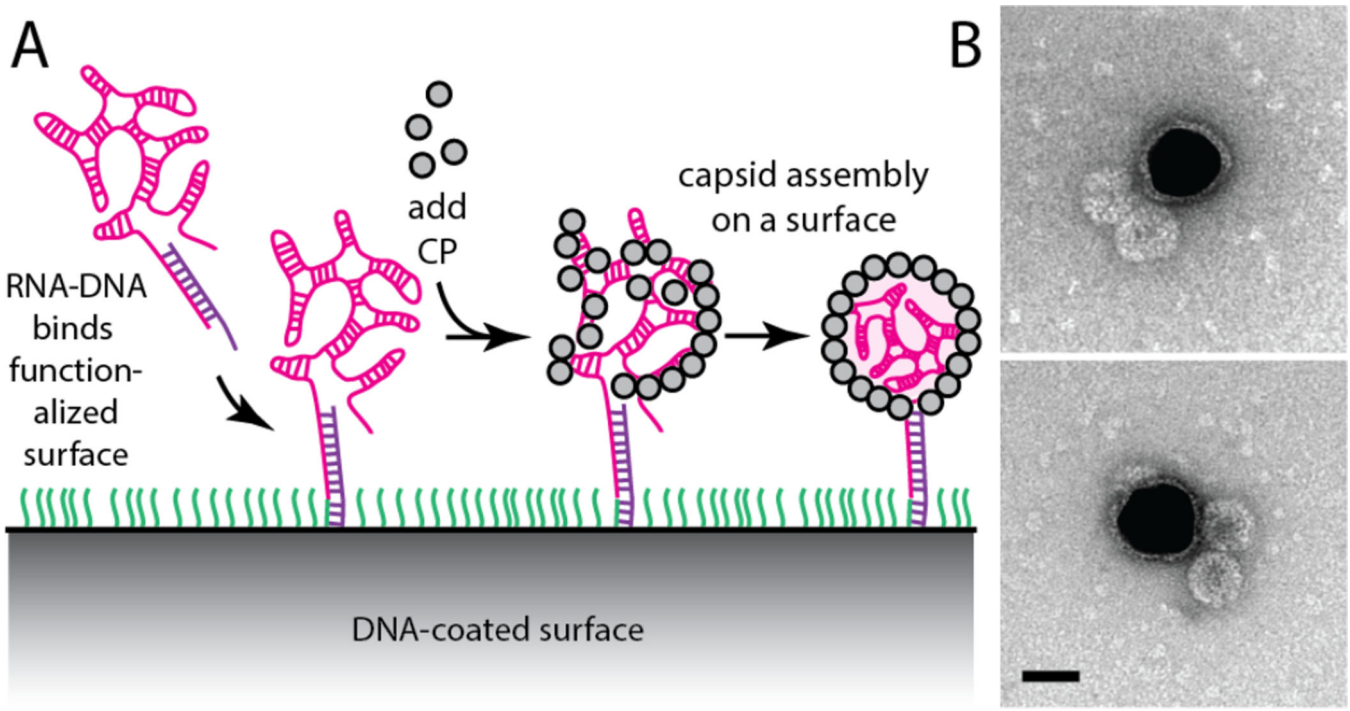

Figure 6.

(A) Schematic showing the assembly of VLPs on a DNA-functionalized (green strands) surface. (B) Cherry bomb capsids (light spheres) were grown on 30-nm AuNPs (dark spheres) as shown in (A). Scale bar shows 25 nm.