Engineering of bacterial virus phi29 motor pRNA as self-assembling nanomolecular building blocks to form conjugates, supramolecular structures and three dimensional arrays

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DNA packaging in bacterial virus phi29 is accomplished by an ATP-driven motor complex with an RNA molecule (pRNA) that plays an essential role in the packaging process and exhibits unique structural features. Biological macromolecules including protein and DNA with their intrinsic nanoscale properties were reported as powerful building blocks for nanotechnology. However, RNA, in spite of the attractive features of this class of biomolecules, has remained poorely explored by nanoscientists. The objective of this work is to design engineered RNA molecules and investigate their potential for applications in nanobiotechnology.

pRNA of bacteriophage phi29 functions by forming a hexameric ring as part of the DNA packaging motor. Ring formation involves hand-in-hand interaction by Watson-Crick base pairing of four nucleotides from left and right RNA loops, and the assembly pathway is from dimer to hexamer. By making complementary mutations in the interacting loops, we engineered pRNA molecules that can form stable dimers and trimers in a protein-free environment with nearly 100% efficiency. The dimers and trimers could be isolated by ultracentrifugation or by purification from native polyacrylamide gels. The 5'/3' paired ends of pRNA form a double helix which is essential for phi29 DNA packaging, but not involved in the assembly of dimers, trimers and hexamers. Therefore, modifications including extensions, deletions and circular permutations of this domain gave rise to defined self-assembled nanostructures of various sizes and shapes. Furthermore, pRNA twins were formed by connecting the 5'/3' paired regions end to end. The Mg^{2+} dependent loop-loop interactions allowed self-assembly of these twins into micrometer-size, three dimensional arrays. Dimers, trimers and arrays were found to be stable between pH 4 and 11, -70°C and 80°C, and at high salt concentration including 2M NaCl and 2M $MgCl_2$.

Our results with bacteriophage phi29 pRNA demonstrate that RNA molecules have the potential to serve as versatile building blocks in nanobiotechnology. The attractive features of RNA for designing biomolecular nanostructures include its complex, well-defined yet versatile structure, the relative ease with which it can be produced, engineered and manipulated, and its self-assembly properties which can be be programmed by designing the base-pairing interactions of complementary molecules.

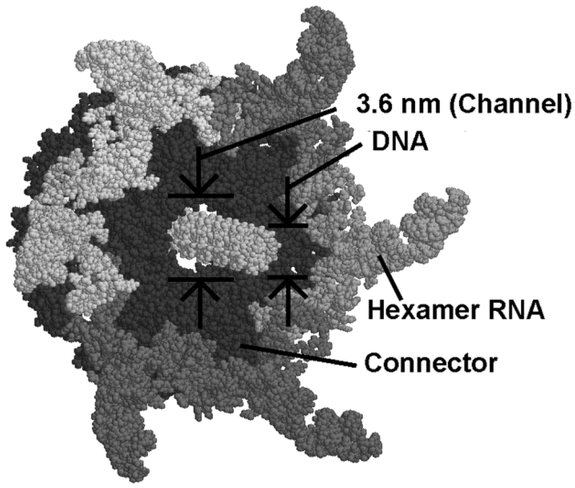

Fig. 1: Model of the DNA packaging motor of bacterial virus phi29.

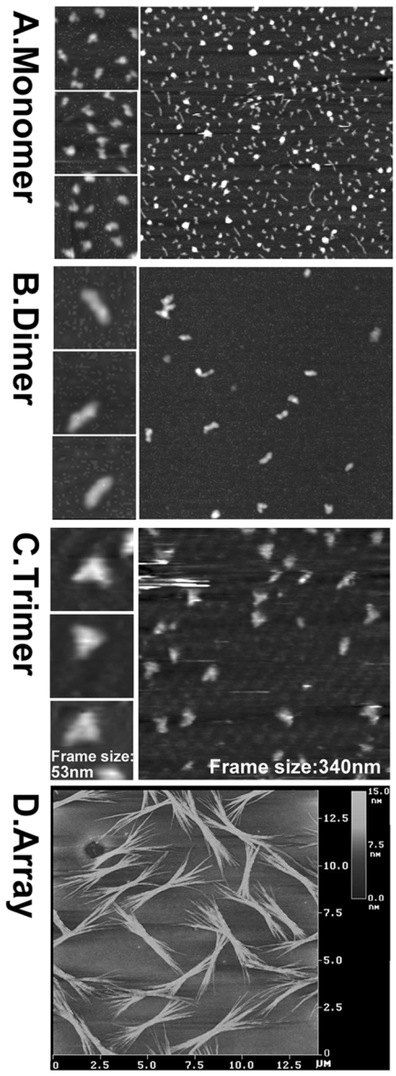

Fig. 2: AFM images of pRNA structures and array.