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Construction of a switchable 30-nm phi29 DNA packaging nanomotor driven by six ATP-binding RNAs

Dan Shu, Wulf-Dieter Moll and Peixuan Guo

Department of Pathobiology and Purdue Cancer Center; Purdue University, West Lafayette, IN 47907, USA.
http://www.vet.purdue.edu/PeixuanGuo/

The construction of nano-scale artificial motors by chemical synthesis is an intriguing endeavor in contemporary technology. We show here that a 30-nanometer motor can be assembled *in vitro* with purified recombinant proteins and artificially designed synthetic RNAs. The motor mimics the DNA-packaging motor of bacterial virus phi29, which features the strongest biomolecular nanomotor characterized to date. It uses ATP as fuel to interconvert motor motion to DNA translocation with up to 57 pico-Newton of force. A central component of the motor is a 120 nucleotide RNA molecule termed pRNA. pRNA forms a hexameric ring by hand-in-hand interaction of pRNA loops. We have engineered pRNAs with complementary mutations in the loop sequences, allowing us to control hexamer assembly in a precisely defined way. pRNA can also be modified to carry additional biomolecules or inorganic nanoparticles. A key feature of pRNA is ATP binding, and the wild-type ATP binding region can be replaced with a synthetic ATP binding aptamer. The motor's symmetry mismatch between hexameric pRNA and the pentameric protein scaffold which forms the motor bearing suggests 12° increments of rotation. DNA translocation occurs via a mechanism similar to a hex nut driving a bolt. The DNA can also be modified to carry cargo. The motor can be turned off by γ-S-ATP or EDTA, and turned on again with ATP or magnesium. The ease of RNA manipulation and engineering coupled with pRNA's programmable self-assembly properties make this motor a viable option to perform mechanical work in nanotechnology.

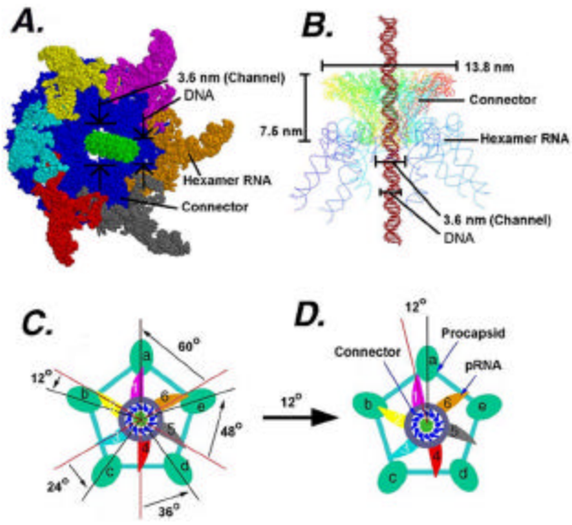

Fig. 1: Construction of a controllable 30-nm DNA-packaging motor of bacterial virus phi29. The motor is driven by an ATP-binding RNA (pRNA) hexamer, similar to the driving of a bolt with a hex nut. Conformation change and sequential action of the RNA with five-fold (viral capsid)/six-fold (pRNA hexamer) mismatch could ensure continuous rotation of the motor with ATP as energy. The figure shows the tertiary bottom view (A) and side view (B) of phi29 DNA packaging motor, which is embedded in a pentagonal capsid to enable the sequential action of pRNA, in a manner similar to the sequential action of six cylinders of a car engine (Guo, P. (2002): Structure and Function of phi29 Hexameric RNA that Drives the Viral DNA Packaging Motor: Review. Prog. Nucleic Acid Res. Mol. Biol. 72: 415-472; Chen, C. and Guo, P. (1997): Sequential Action of Six Virus-Encoded DNA-Packaging RNAs during Phage phi29 Genomic DNA Translocation. J. Virol. 71: 3864-3871).