# Explaining the size-dependent kinetics of Phi29 polymerase rolling circle amplification


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Poster Abstract


Rolling circle amplification (RCA) is an important method of efficiently synthesising many ssDNA strands with the same sequence, each complementary to a circular template. It is used both in diagnostic essays as well as for the synthesis of strands for DNA nanostructures [1].

Experimental work presented here has shown a clear size-dependence for the efficiency of bacteriophage Phi29 DNA polymerase RCA for a range of circular template sizes. A periodic pattern of amplification efficiency is observed: circular templates of half-integer pitch lengths are copied roughly three times more quickly as those of integer pitch lengths. Pronounced variation in the rate of transcription with the size of the circular template is a potentially important factor in the above-mentioned applications.

Simulations with a coarse-grained model of DNA, oxDNA [2], were performed to determine the fraying propensity of terminal base pairs in the direction of precession of the enzyme. The same periodic pattern was found for the fraying propensity as was experimentally determined for the efficiency of amplification. A further amplification of the signal was achieved by constraining the simulated DNA to adopt a similar configuration to that in the enzyme, thus increasing the stress on the base pairs downstream of the enzyme. A particularly marked variation in the propensity to fray the two terminal base pairs was identified, with the probability of fraying increasing by up to a factor of six for circular templates differing in circumference by merely five nucleotides.


***References***


1. M. M. Ali et al., *Chem. Soc. Rev.* 43, 3324, **2014**
2. B. E. K. Snodin et al., *J. Chem. Phys.* 142, 234901 **2015**