

# Investigating the affinity of molecular interactions of the $\phi$ 29 packaging motor

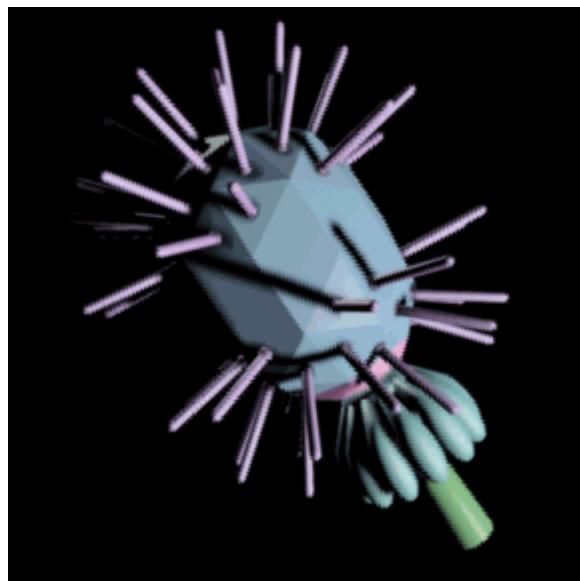
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## Introduction

$\phi$ 29 is a bacteriophage responsible for the infection of *Bacillus* species and its proposed mode of action of DNA packaging has stimulated much recent interest. Commonly, biologically endogenous molecular motors display either a rotary motion e.g. bacterial flagella, or a linear movement e.g. muscle contraction. The  $\phi$ 29 molecular motor consists of a protein/RNA complex and the action of this motor results in packaging of the double-stranded genomic DNA into preformed procapsids. A novel RNA-RNA multimerisation event is thought to be involved, resulting, in combination with ATPase activity, in possible rotation of the molecular motor and concomitant translation of genomic DNA through the connector complex.

## Results

The rules governing this protein/RNA interaction, for example the possible existence of sequence specificity, are not known and the aim of this current work is directed towards developing an understanding of this system. Both RNA (pRNA) and connector protein components form multimeric complexes and the multimerisation of pRNA has been previously investigated in this laboratory by analytical ultracentrifugation (AUC) and light scanning experiments. These experiments indicated that pRNA undergoes  $Mg^{2+}$ -dependent formation of monomeric, dimeric and trimeric species, which may act as components of the ultimate higher order species.



**Fig. 1:** Cartoon representation of  $\phi$ 29 produced by Katie Radcliffe  
The phage procapsid is shown in blue, spikes in purple and the head-tail connector in pink.  
pRNA is not shown as it is not present in an assembled phage particle.

Current work has involved the chemical and enzymatic synthesis of novel pRNA species – hairpin loop domains and full length molecules, both wild-type and mutant. We have shown that the affinity of the pRNA-pRNA interaction is  $\mu M$ , whereas the  $K_D$  of pRNA-connector binding is nM by surface plasmon resonance studies. Connector binding affinity is increased in the presence of magnesium ions, however, this affinity does not seem to be dependent on pRNA multimerisation (although pRNA multimerization is essential for DNA packaging). Differences in the magnitude of affinity between components of the motor lead us to deduce that it is unlikely that there is a rotation between the connector and pRNA during DNA

packaging, and we propose that magnesium-induced conformational changes could drive the packaging event.

### **Collaborators**

Neil Thomson

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### **Publications**

Wood J.P.A, Capaldi, S.A, Robinson, M.A., Baron, A.J. and Stonehouse, N.J. (2005) RNA multimerisation in the DNA packaging motor of bacteriophage  $\phi$ 29. *Journal of Theoretical Medicine*. In press.

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