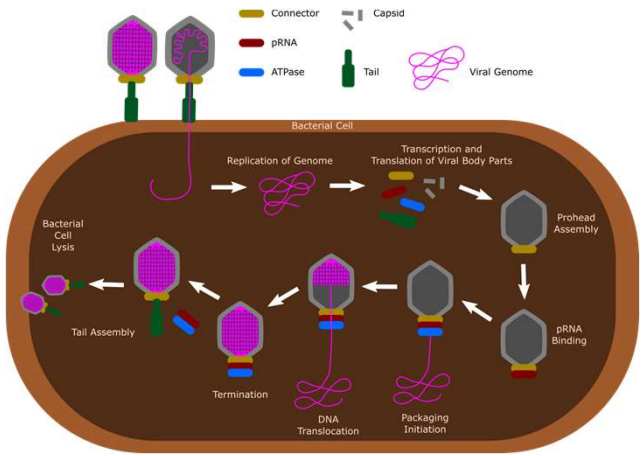
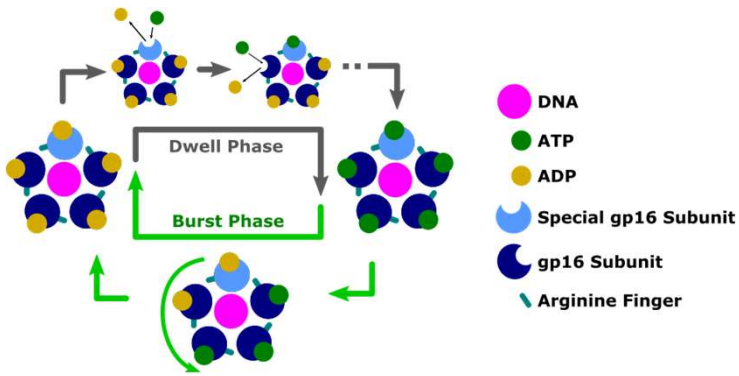
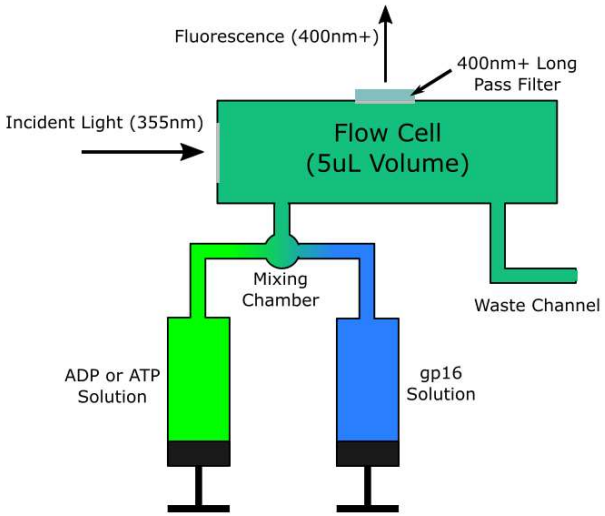
Kinetics of Nucleotide Binding to the gp16 ATPase

Lab symbolics or author photo

Aaron Morgan[1] , Allen Eastlund[2] , Christopher Fischer[1], Paul Jardine[2]
[1]Department of Physics and Astronomy, University of Kansas
[2]Department of Diagnostic and Biological Sciences, University of Minnesota







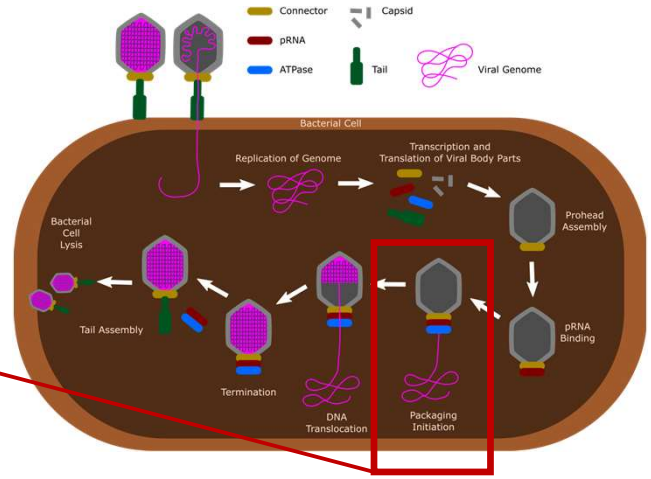
Mant-ADP Binding

k_{on}	$(2.30 \pm 0.07) \times 10^4\ M^{-1}s^{-1}$ ***Upper Limit***
K_d	**30 μM *Constrained Lower Limit***
c_{PA}	$(1.25 \pm 0.02) \times 10^8\ VM^{-1}$ ***Lower Limit***

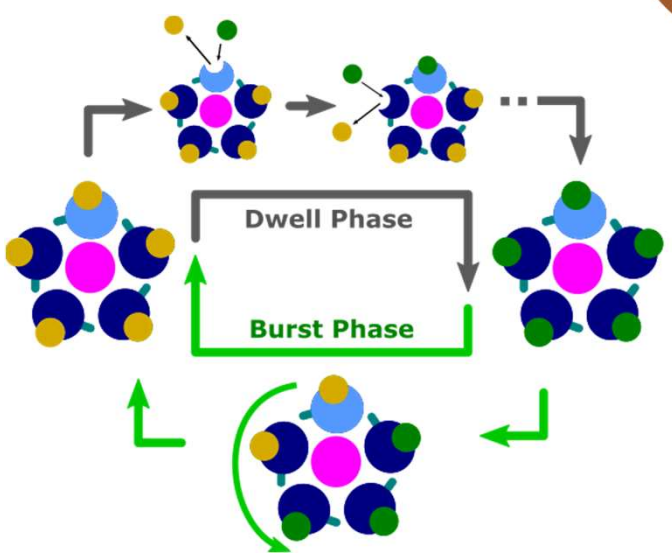
Mant-ATP Binding

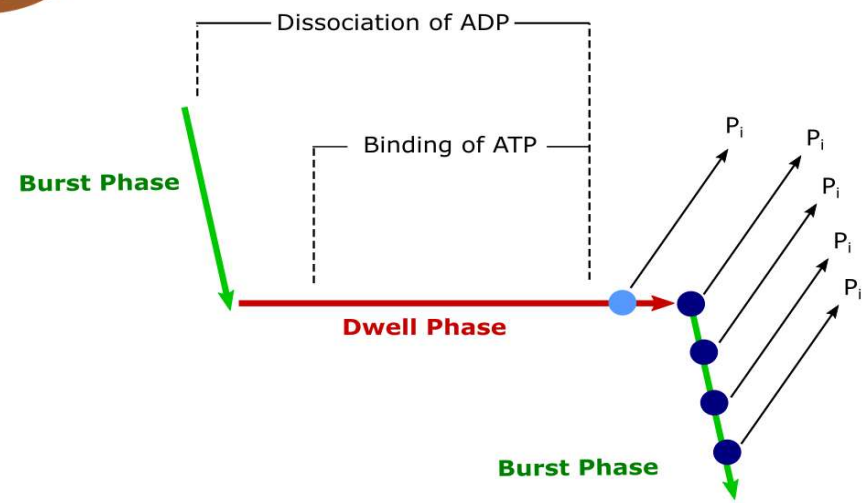
k_{on}	$(2.5 \pm 0.8) \times 10^6\ M^{-1}s^{-1}$
K_d	$523.8 \pm 247.3\ nM$
c_{PA}	$(1.7 \pm 0.5) \times 10^6\ VM^{-1}$

Dwell-Burst cycle of Φ29 Motor

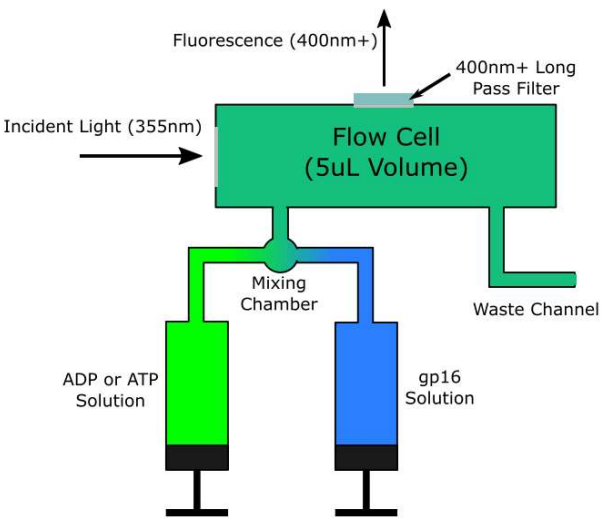


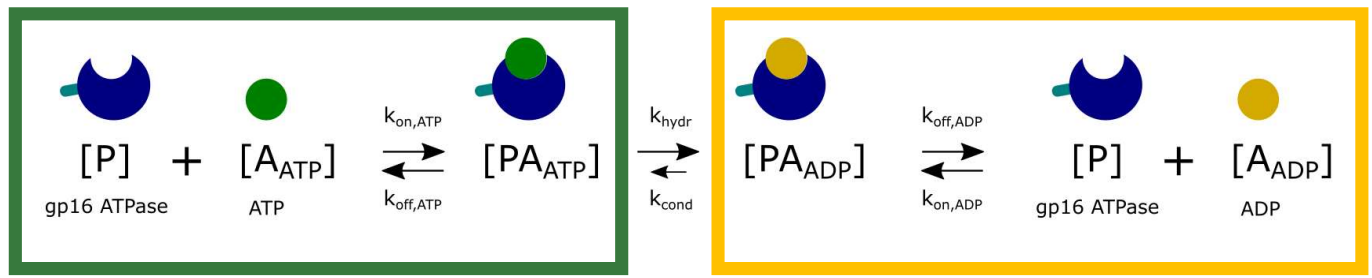
We are here





Binding Fluorescence Assay and Modelling





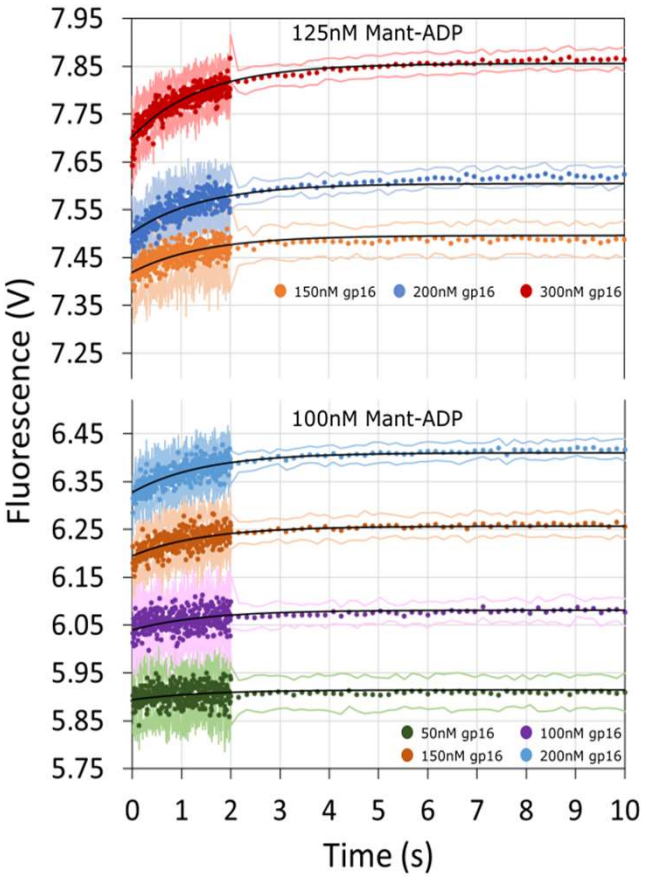
$$Fluorescence = c_i + c_{PA}[PA]$$

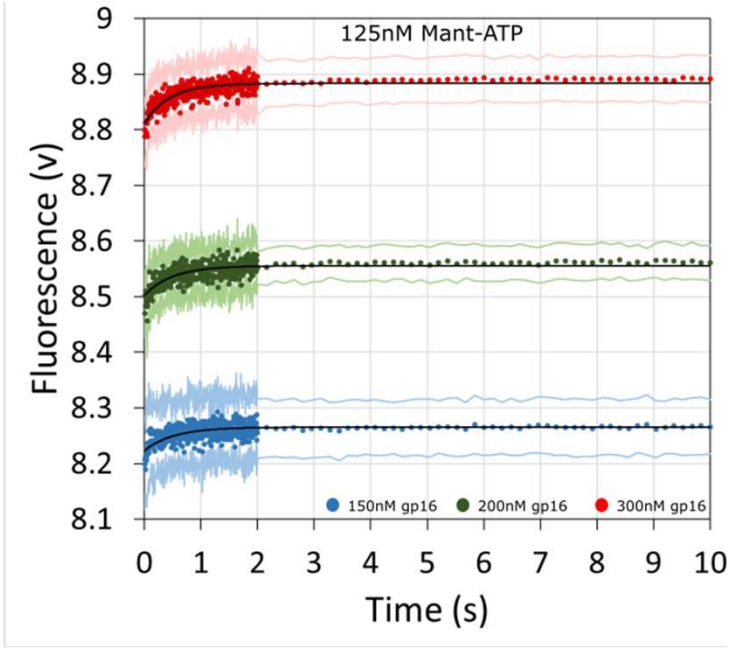
k_{on}	Association Rate Constant
K_d	$\frac{Dissociation\ Rate\ Constant}{Association\ Rate\ Constant}$
c_i	Initial Fluorescence of *i*th trace
c_{PA}	Fluor. Enhancement of Mant-ADP/ATP bound gp16

$$[PA] = \frac{2[A]_0[P]_0}{[A]_0+[P]_0+K_d + z_{PA}\coth(\frac{1}{2}z_{PA}k_{on}t)}$$

$$z_{PA} = \sqrt{([A]_0 - [P]_0)^2+K_d^2 + 2K_d([A]_0 + [P]_0)}$$

Binding Fluorescence Assay Results





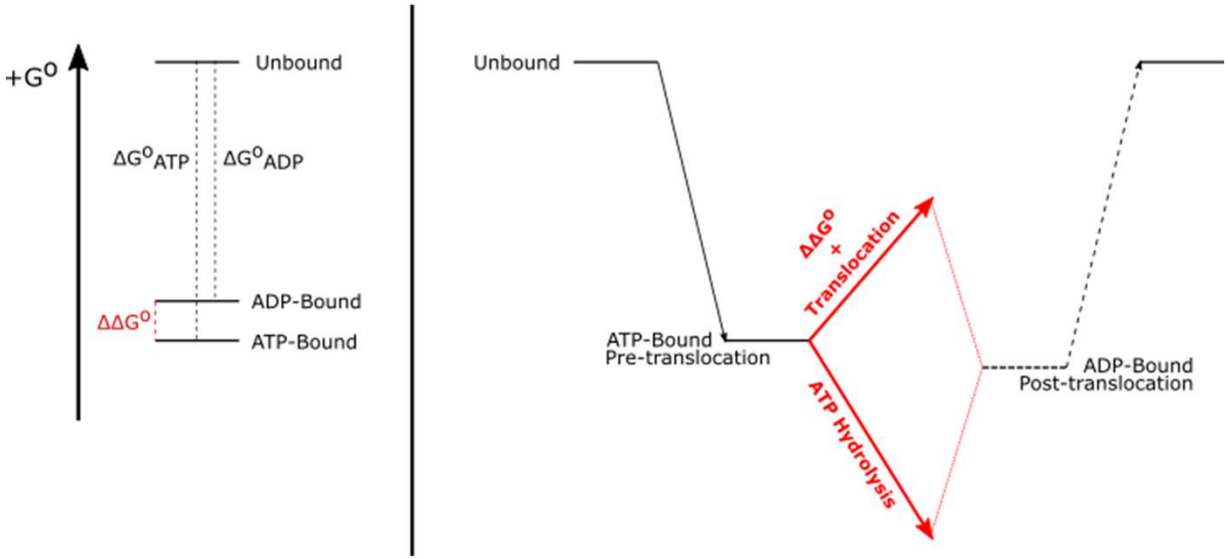
Mant-ADP Binding

k_{on}	$(2.30 \pm 0.07) \times 10^4\ M^{-1}s^{-1}$ ***Upper Limit***
K_d	**30 *μM Constrained Lower Limit***
c_{PA}	$(1.25 \pm 0.02) \times 10^8\ VM^{-1}$ ***Lower Limit***

Mant-ATP Binding

k_{on}	$(2.5 \pm 0.8) \times 10^6\ M^{-1}s^{-1}$
K_d	$523.8 \pm 247.3\ nM$
c_{PA}	$(1.7 \pm 0.5) \times 10^6\ VM^{-1}$

Gibbs Free Energy Perspective



- This motor can exert forces beyond 60pN (Smith et al.,2001). To translocate ~10bp of B-DNA against this force, with a length of ~3,300pm, approximately $20 \times 10^{-20} J$ of work must be done.

- Let's use the approximation that hydrolysis of ATP stands to deliver 50-70 kJ/mol of free energy, as this is the range of energy delivered by ATP hydrolysis in mammalian liver, brain, and muscle cells (Bergman *et al*., 2010). For five hydrolysis events, this is $\sim 40 - 60 \times 10^{-20}\ J$ of energy.

- Going from five gp16 molecules bound with ATP to five gp16 bound with ADP "costs"

$$\Delta\Delta G^0 = -5k_B(295K)ln\left(\frac{K_{d,ADP}}{K_{d,ATP}}\right) = 8.24 \pm 0.96 \times 10^{-20} J.$$

Timescales

- In yet unpublished ATPase assays performed by our collaborators at the University of Minnesota (Eastlund, A.; Jardine, P., 2019), using fully functional Φ29 Bacteriophages in the presence of DNA, an ATPase rate of 15-20 ATP/gp16/s is observed.
- This corresponds to a maximum average time to hydrolyze of ~0.05s. The average time for a **single** Mant-ATP to dissociate from a gp16 monomer is

$$t_{dis,ATP} = \frac{1}{k_{off,ATP}} = \frac{1}{(K_{d,ATP})(k_{on,ATP})} = 0.76 \pm 0.44\ s.$$

- Single molecule experiments observe a dwell time for the motor at low capsid filling of less than 0.15s.
- Along with this data, the model proposed for the cyclical operation of the motor states that the dissociation of ADP molecules from the motor happens during the dwell phase (Bustamante et al., 2014).
- Assuming the **most efficient** case, where all five ADP molecules are allowed to dissociate at once, the minimum average time for all **five** Mant-ADP to dissociate is

$$t_{dis,ADP} = \frac{5}{k_{off,ADP}} = \frac{5}{(K_{d,ADP})(k_{on,ADP})} = 1.45 \pm 0.04\ s.$$

- This far exceeds the shortest dwell times observed in single molecule translocation experiments. One possible explanation for this discrepancy is that there is a certain amount of cooperativity in the system when the motor is intact.

Acknowledgements

Smith, D. E. *et al.* (2001) ‘The bacteriophage straight phi29 portal motor can package DNA against a large internal force.’, *Nature*. doi: 10.1038/35099581.

Morais, M. C. *et al.* (2001) ‘Cryoelectron-microscopy image reconstruction of symmetry mismatches in bacteriophage phi29.’, *Journal of structural biology*. doi: 10.1006/jsbi.2001.4379.

Bustamante, C. *et al.* (2014) ‘A Viral Packaging Motor Varies Its DNA Rotation and Step Size to Preserve Subunit Coordination as the Capsid Fills.’, *Cell*. doi:10.1016/j.cell.2014.02.034.

Mao, H. *et al*. (2016) ‘Structural and Molecular Basis for Coordination in a Viral DNA Packaging Motor.’, *Cell Rep*. doi: 10.1016/j.celrep.2016.01.058.

Morais, M. C. *et al.*(2012) ‘The dsDNA packaging motor in bacteriophage ø29.’ *Adv Exp Med Biol*. doi: 10.1007/978-1-4614-0980-9_23.

Bergman, Christian, Yoshihiro Kashiwaya, and Richard L. Veech. "The Effect of PH and Free Mg2 on ATP Linked Enzymes and the Calculation of Gibbs Free Energy of ATP Hydrolysis." *Journal*