Supplemental Data

Single stranded DNA translocation of E. coli UvrD monomer is tightly coupled to ATP hydrolysis

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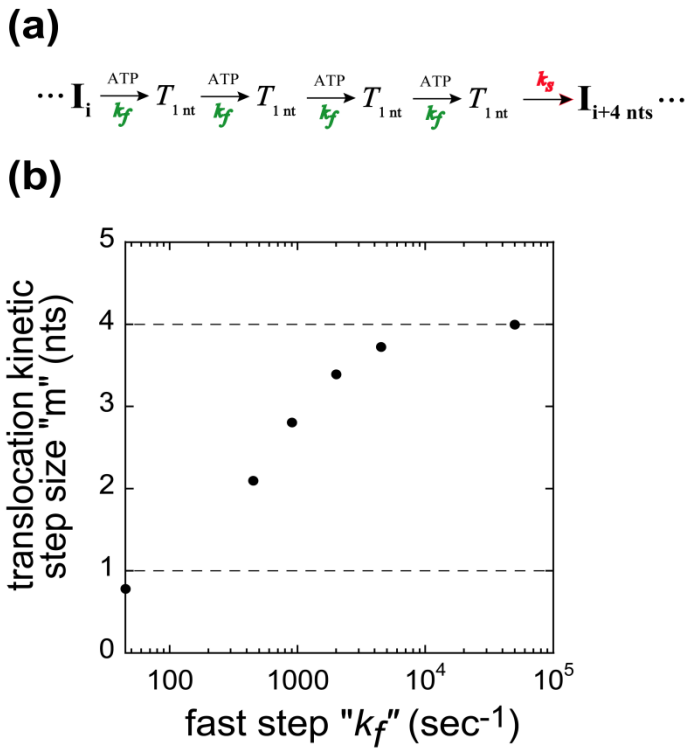


Figure S1. Discontinuous stepping model for UvrD monomer ssDNA translocation. (**A**)- UvrD monomer rapidly translocates (k_f) in ~4, 1nt steps each resulting in the hydrolysis of one ATP followed by a slow process (k_s) that limits the overall rate of translocation. (**B**)- Prediction of the discontinuous stepping model showing the expected decrease in the translocation kinetic step size as the ATP concentration is lowered. Each fast step is coupled to ATP binding and hydrolysis. Lower [ATP] results in slower ATP binding, thus the observed k_f will be slower. The discontinuous stepping model was simulated varying the rate constant for the rapid steps and the resulting translocation time courses analyzed with the n-step sequential model (eq. 6).

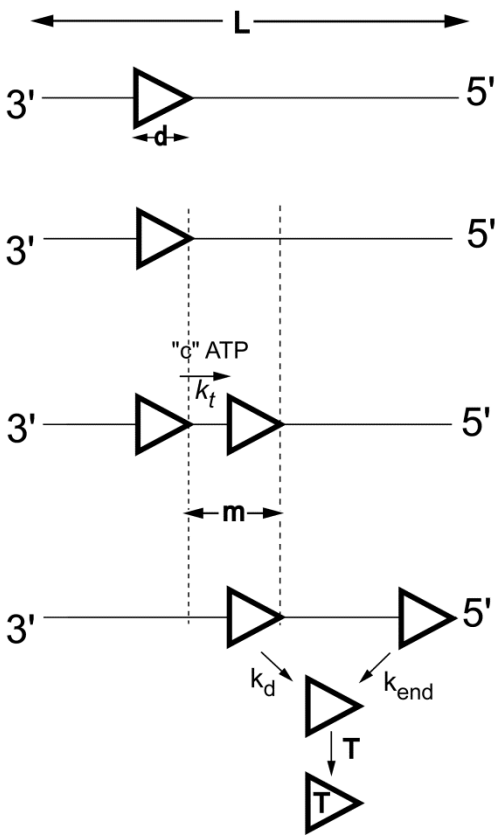


Figure S2. Cartoon depicting n-step sequential mechanism for ssDNA translocation. UvrD (triangle) binds randomly to ssDNA of L nucleotides, with contact size d nucleotides and translocates (3'-5') in steps of *m* nucleotides (kinetic step-size) with rate constant, k_t, hydrolyzing *c* ATP molecules. UvrD dissociates from internal ssDNA sites and the 5'-end with rate constants k_d and k_{end}, respectively. UvrD rebinding to the ssDNA is prevented by binding to excess trap (T) such as heparin.

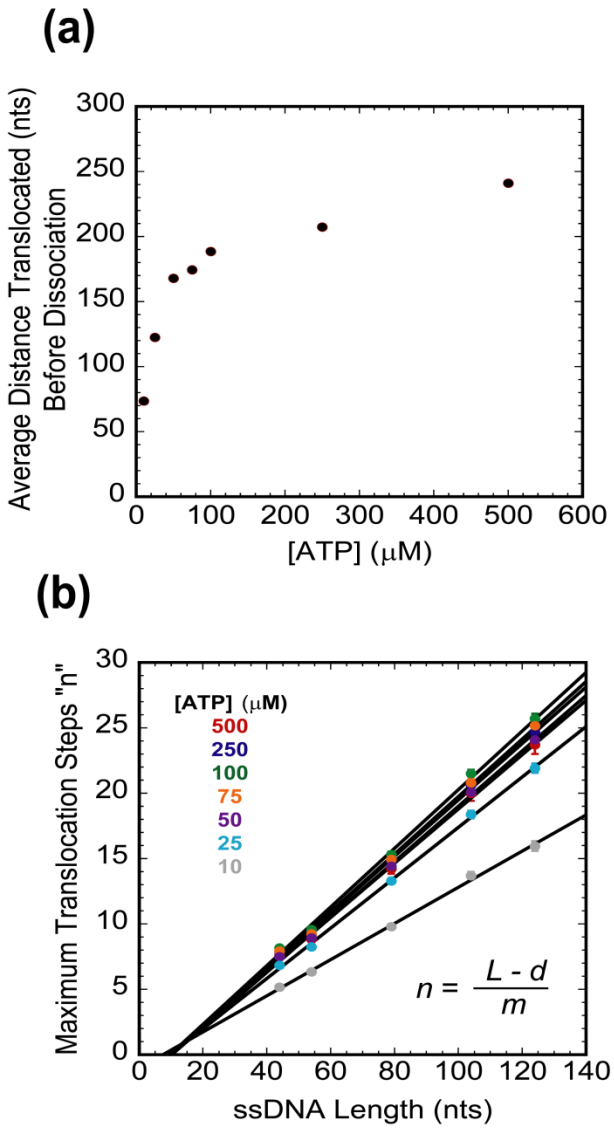
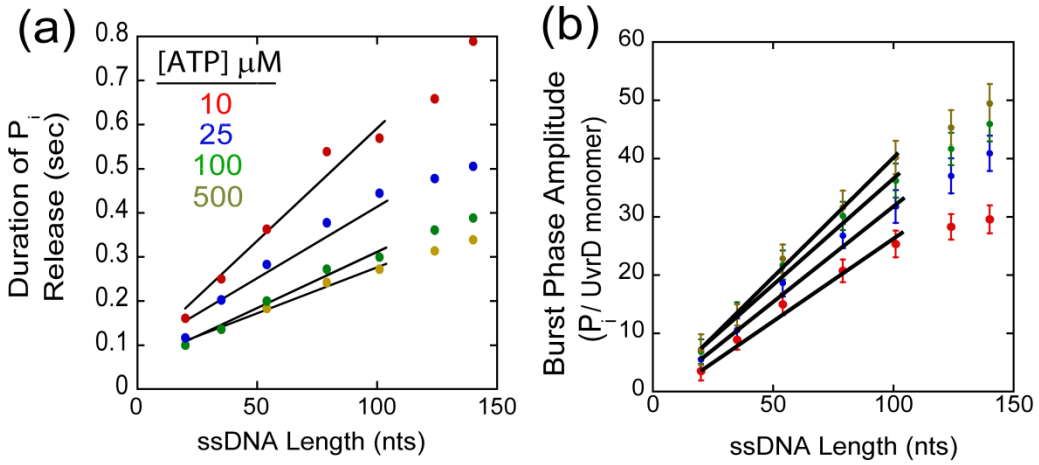


Figure S3. ATP concentration dependence of of UvrD translocation processivity and ATP concentration dependence of number of translocation steps as a function of ssDNA length. **A-** Average distance translocated before dissociation from the ssDNA (1/(1-P)) where processivity, P= $mk_t/(mk_t+k_d)$. **B-** At each ATP concentration the number of translocation steps is linearly dependent on the ssDNA length. The kinetic step size (m) and contact size (d) is determined from the inverse of the slope and x-intercept, respectively.



(c)

[ATP] μM	Semi-quantitative Duration Analysis		n-step Model Analysis	
	Translocation Rate (nts/sec)	ATP coupling stoichiometry (ATP/nt)	Translocation Rate, mk_t (nts/sec)	ATP coupling stoichiometry, c/m (ATP/nt)
10	78.5	0.43	49 ± 0.6	0.81 ± 0.04
25	114	0.63	91 ± 0.7	0.87 ± 0.04
100	168	0.68	160 ± 1	0.84 ± 0.04
500	208	0.71	199 ± 1	0.89 ± 0.06

Figure S4. Semi-quantitative analysis of UvrD ssDNA translocation P_i-release kinetics [1]. **A-** Duration of Pi-release as a function of ssDNA length at each ATP concentration. The rate of translocation is estimated from the slope, translocation rate = ½(slope) assuming random binding. **B-** Pi-release amplitude as a function of ssDNA length at each ATP concentration. The ATP coupling stoichiometry (ATP/nt) is determined from the slope, ATP coupling stoichiometry = 2(slope). A correction factor of 2 is applied assuming infinite processivity. **C-** Comparison of translocation rate and ATP coupling stoichiometry determined using semi-quantitative approach and sequential n-step analysis.

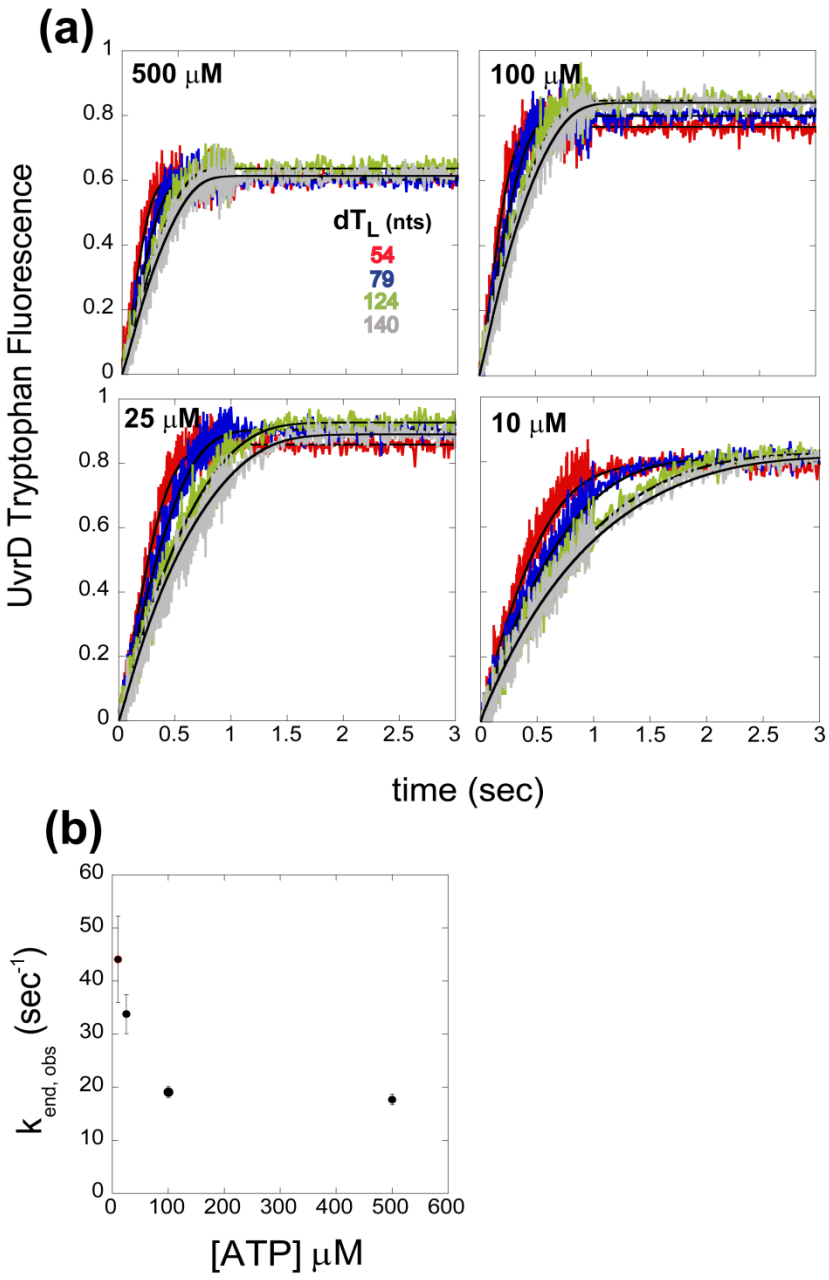


Figure S5. UvrD monomer dissociation from ssDNA during translocation along $(dT)_L$ as a function of ATP concentration. **(A)-** Time courses of UvrD monomer (25nM) dissociation from $(dT)_L$ (50nM) (L: 54, 79, 104, and 124 nts) in the presence of 10-500 mM ATP, 2 mM MgCl2, and 4 mg/ml heparin. The continuous lines are simulations based on eq. (5) and the best fit parameters determined from NLLS analysis of time courses for (dT)L with L: 54, 79, 84, 97, 101, 104, and 124 nts. The values of k_t, k_d, r, m and d were constrained (Table 1) and $_{kend}$ at each ATP concentration was determined from the NLLS fit. **(B)-** k_{end} as a function of ATP concentration.

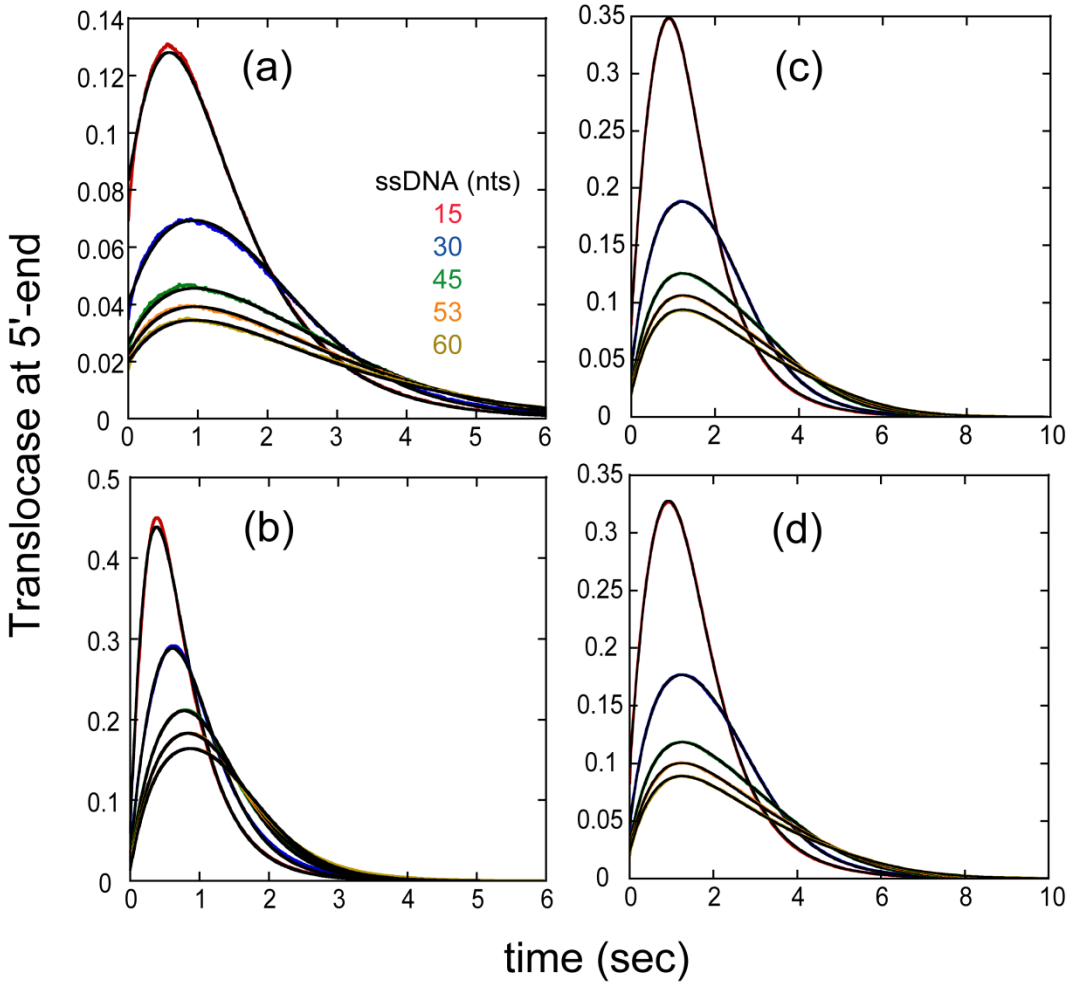


Figure S6. Simulated translocation time courses with backward slipping (a), static disorder in translocation rate (b), pausing with futile ATP hydrolysis (c), and ATP hydrolysis coupled recovery from pause (d). Black curves are the NLLS fit of n-step sequential model. The shown simulations correspond to the most extreme perturbations tested, showing that the n-step model can quantitatively describe the time courses under those conditions. Long lived pauses and excessive backward motion will result in time courses that cannot be quantitatively described by the n-step model in figure S2 (data not shown).

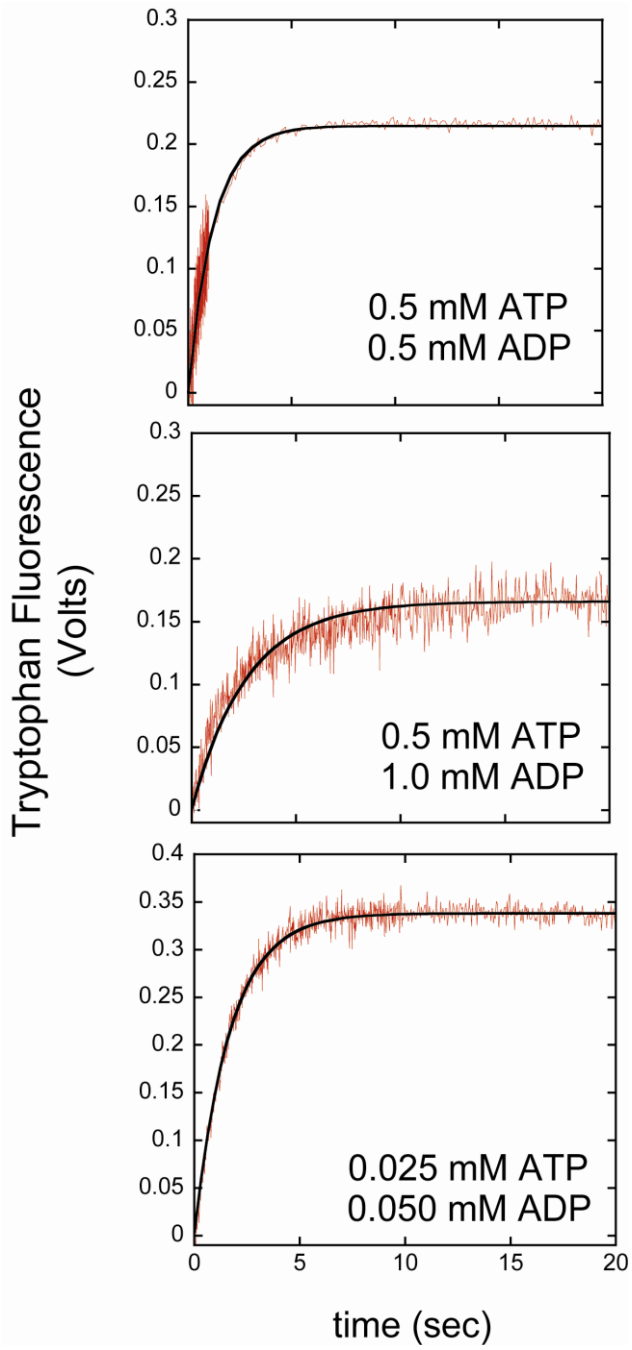


Figure S7. UvrD dissociation from internal sites of ssDNA at different ADP:ATP molar ratios. Time courses of UvrD monomer (25 nM, post mix) dissociation from poly(dT) (10 μM nts, post mix) (1.5 ± 0.05 kb), (buffer T_{20}, 25 or 500 μM ATP, 50, 500, or 1000 μM ADP, 2 mM $MgCl_2$, and 4 mg/ml heparin, 25°C) fit to a single exponential.

Supplemental Reference

1. Dillingham, M. S., Wigley, D. B. & Webb, M. R. (2000). Demonstration of unidirectional single-stranded DNA translocation by PcrA helicase: measurement of step size and translocation speed. *Biochemistry* **39**, 205-12.