Supporting Information

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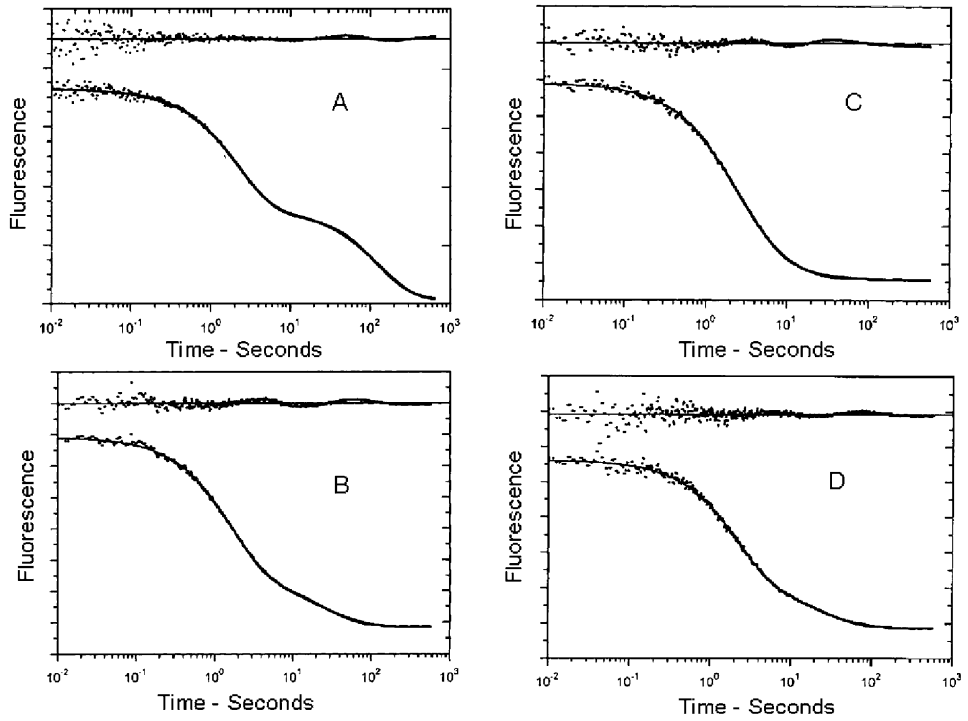

Fig. S1. Kinetics of ADP dissociation from various myosin 5a HMM species on actin. Myosin 5a-HMM (WT and IQ mutants) (0.25 μM) were mixed with 0.75 μM deac-aminoATP, held 20 s in a delay line, and then mixed with 20 μM actin and 2.0 mM ADP in a stopped-flow fluorimeter, as described in main text (*Methods*). The fit lines through the data are double exponential equations in which the total amplitudes are normalized to 1.0.: (*A*) (WT) $I(t) = 0.56e^{-0.49t} + 0.44e^{-0.015t} + C$; (*B*) (6IQ+2Ala) $I(t) = 0.72e^{-0.44t} + 0.28e^{-0.041t} + C$; (*C*) (4IQ) $I(t) = 0.76e^{-0.55t} + 0.24e^{-0.17t} + C$; (*D*) (8IQ) $I(t) = 0.73e^{-0.50t} + 0.27e^{-0.04t} + C$.

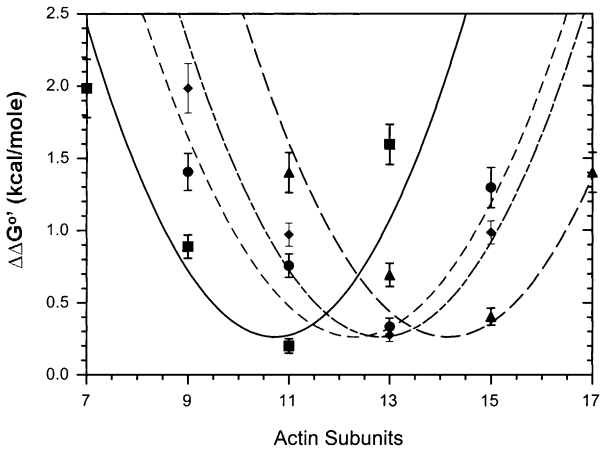

Fig. S2. Free energy analysis of stride lengths of myosin 5a and its mutants. $\Delta\Delta G^{o'}$ is the relative change in free energy of myosin 5 (WT and IQ mutants) binding to actin upon the distance between the heads, determined from the data in Fig. 2. $\Delta\Delta G^{o'} = -RT\ln(n_i)$, where n_i is the mole fraction of myosin molecules bound with a separation of i actin subunits on the genetic helix. Curves through the data are fits to the equation $\Delta\Delta G^{o'} = 0.5 \times k \times (i - B)^2 + C$, where i is the number of actin subunits between HMM heads bound to actin, k is a Hookean spring constant, B is the distance of minimum energy for each mutant, and C is a constant. Data for each IQ variant were fit to values for k (kcal/mole)/(actin subunit)^2 and B (actin subunit): 4IQ (filled squares, solid line), 0.31 ± 0.1, 10.7 ± 0.3; 6IQ+2Ala (filled circles, medium dash), 0.25 ± 0.07, 12.3 ± 0.4; 6IQ (filled diamonds, long and short dash), 0.28 ± 0.07, 12.8 ± 0.3; 8IQ (filled triangles, long dash), 0.27 ± 0.07, 14.2 ± 0.3, and the same value C = 0.26 kcal/mole for all. An almost identical fit could be obtained to a single value of k = 0.28 (kcal/mole)/(actin subunit)^2 to fit all of the IQ variants. A stiffness of 0.26 pN/nm per molecule is obtained by multiplication of the average value of k, 0.28 kcal/mole/(actin subunit)^2 by 4.18 kJ/kcal/6.02×10^{23} (molecules/mole)/(2.75 nm/actin subunit)^2, where 2.75 nm is the axial distance between successive actin subunits along the genetic helix.