# Supporting Material

**Czövek, Szöllősi, and Derényi**

**Table S1. Prescribed criteria for the optimization**

| observable | prescribed value | experimental conditions |
|---|---|---|
| velocity | $-6$ nm/s | $[\text{ATP}] = 10\ \mu$M, $\quad F = -15$ pN |
| velocity | $-23$ nm/s | $[\text{ATP}] = 1000\ \mu$M, $\quad F = -15$ pN |
| velocity | $140$ nm/s | $[\text{ATP}] = 10\ \mu$M, $\quad F = 0$ pN |
| velocity | $460$ nm/s | $[\text{ATP}] = 1000\ \mu$M, $\quad F = 0$ pN |
| velocity | $210$ nm/s | $[\text{ATP}] = 10\ \mu$M, $\quad F = 15$ pN |
| velocity | $520$ nm/s | $[\text{ATP}] = 1000\ \mu$M, $\quad F = 15$ pN |
| processivity | $110$ steps | $[\text{ATP}] = 1000\ \mu$M, $\quad F = 0$ pN |
| #ATP hydrolyzed/step | $1.35$ | $[\text{ATP}] = 1000\ \mu$M, $\quad F = 0$ pN |
| #fwd/#bwd steps | $400$ | $[\text{ATP}] = 1000\ \mu$M, $\quad F = 0$ pN |
| stall force | $-6.75$ pN | $[\text{ATP}] = 1000\ \mu$M |

The cost function for each of the first 9 observables has been set to $(X - X_0)^2/X_0^2$, where $X$ stands for the observable and $X_0$ is its prescribed value, whereas for the last observable it has been set to $(X - X_0)^2/(1\,\text{pN})^2$.

**Table S2. Effects of the parameter change on some of the observables**

| | velocity (nm/s) | #ATP/step | processivity | stall force (pN) | dwell time of fwd. steps at stall force (s) | F=15 pN (s) | F=15pN [ATP]=10 $\mu$M (s) | dwell time of bwd. steps at F=-15pN (s) | F=-15pN [ATP]=10 $\mu$M (s) | #fwd./#bwd. |
|---|---|---|---|---|---|---|---|---|---|---|
| Original model parameters | 457 | 1.36 | 106 | -6.75 | 0.20 | 0.016 | 0.039 | 0.35 | 1.27 | 308 |
| [ADP]=100 $\mu$M | 454 | 1.35 | 100 | -6.75 | 0.20 | 0.015 | 0.022 | 0.34 | **0.4** | 284 |
| [ADP]=1000 $\mu$M | 421 | 1.33 | 61 | -6.5 | 0.16 | 0.015 | **0.015** | 0.3 | **0.17** | **146** |
| [ADP]=10000 $\mu$M | **222** | 1.28 | **10** | -5.75 | 0.10 | 0.015 | **0.014** | 0.19 | **0.14** | **19** |
| $N = 6$ | 338 | 2.14 | **321** | -6.25 | 0.20 | 0.019 | 0.05 | 0.33 | 1.22 | **138** |
| $N = 7$ | **213** | **3.95** | **585** | -5.5 | 0.19 | 0.023 | 0.072 | 0.32 | 1.22 | **80** |
| $N = 8$ | **140** | **6.5** | **827** | -5.25 | 0.23 | 0.027 | **0.090** | 0.32 | 1.21 | **52** |
| $N_{\mathrm{d}} = 2$ | **68** | **13** | **3** | -3.75 | **0.47** | **0.042** | **0.14** | 0.46 | 1.36 | **51** |
| $N_{\mathrm{d}} = 3$ | 321 | 2.27 | **28** | -6 | 0.19 | 0.018 | 0.047 | 0.38 | 1.31 | 300 |
| $\alpha = 50°$ | 457 | 1.36 | 106 | -6.75 | 0.20 | 0.017 | 0.043 | 0.45 | 1.48 | 308 |
| $\alpha = 60°$ | 457 | 1.36 | 106 | -6.75 | 0.26 | 0.02 | 0.06 | **15.1** | **22.8** | 305 |
| $\alpha = 70°$ | 457 | 1.36 | 106 | -6.75 | **1.6** | **3.7** | **14** | N/A | N/A | 314 |
| $l_{\mathrm{p}} = 0.43$ nm | 357 | 1.93 | **26** | -6.75 | 0.22 | 0.017 | 0.044 | 0.42 | 1.55 | 359 |
| $l_{\mathrm{p}} = 0.45$ nm | 454 | 1.37 | 80 | -6.75 | 0.19 | 0.015 | 0.038 | 0.36 | 1.32 | 351 |
| $\Delta G_{\mathrm{T,T^*}} = 0\ k_{\mathrm{B}}T$ | 424 | 1.37 | 84 | **-3.25** | 0.21 | 0.015 | 0.039 | 0.35 | 1.28 | 196 |
| $\Delta G_{\mathrm{T,T^*}} = -2\ k_{\mathrm{B}}T$ | 453 | 1.35 | 103 | -4.25 | 0.20 | 0.015 | 0.039 | 0.35 | 1.28 | 287 |
| $\Delta G_{\mathrm{T,T^*}} = -4\ k_{\mathrm{B}}T$ | 457 | 1.35 | 106 | -5.25 | 0.20 | 0.015 | 0.039 | 0.35 | 1.28 | 306 |
| $\Delta G_{\mathrm{T,T^*}} = -10\ k_{\mathrm{B}}T$ | 444 | 1.47 | 106 | -8 | 0.16 | 0.016 | 0.040 | 0.34 | 1.25 | 306 |
| $\Delta G_{\mathrm{D,D^*}} = 0\ k_{\mathrm{B}}T$ | 408 | 1.28 | **223** | -4 | 0.18 | 0.014 | 0.032 | 0.32 | 1.20 | **57** |
| $\Delta G_{\mathrm{D,D^*}} = 2\ k_{\mathrm{B}}T$ | 418 | 1.28 | 194 | -5 | 0.18 | 0.014 | 0.03 | 0.32 | 1.20 | **66** |
| $\Delta G_{\mathrm{D,D^*}} = 4\ k_{\mathrm{B}}T$ | 445 | 1.29 | 132 | -6 | 0.19 | 0.014 | 0.035 | 0.33 | 1.23 | **121** |
| $\Delta G_{\mathrm{D,D^*}} = 7\ k_{\mathrm{B}}T$ | 416 | 1.64 | 99 | -7.25 | 0.20 | 0.019 | 0.051 | 0.40 | 1.32 | **845** |
| $\Delta G_{\mathrm{D,D^*}} = 10\ k_{\mathrm{B}}T$ | **133** | **7.1** | 93 | -7.75 | 0.28 | **0.068** | **0.25** | 0.52 | 1.41 | **990** |

[ATP] = 1000 $\mu$M and $F = 0$ throughout unless indicated otherwise. Values in bold indicate deviation by more than a factor of 2.

Table continued on next page.

| | velocity | #ATP/step | processivity | stall force | dwell time of fwd. steps at | | | dwell time of bwd. steps at | | #fwd./#bwd. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | stall force | F=15 pN | F=15pN [ATP]=10 $\mu$M | F=-15pN | F=-15pN [ATP]=10 $\mu$M | |
| | (nm/s) | | | (pN) | (s) | (s) | (s) | (s) | (s) | |
| $k_{0\to\mathrm{T}} = 1\ \mu\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ | 443 | 1.34 | 105 | -6.75 | 0.21 | 0.016 | **0.12** | 0.38 | **3.8** | 279 |
| $k_{0\to\mathrm{T}} = 2\ \mu\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ | 453 | 1.35 | 106 | -6.75 | 0.20 | 0.016 | 0.063 | 0.36 | 2.10 | 300 |
| $k_{0\to\mathrm{T}} = 5\ \mu\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ | 458 | 1.36 | 106 | -6.75 | 0.19 | 0.015 | 0.032 | 0.35 | 1.05 | 314 |
| $k_{0\to\mathrm{T}} = 10\ \mu\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ | 459 | 1.37 | 106 | -6.75 | 0.19 | 0.016 | 0.02 | 0.34 | 0.70 | 317 |
| $k_{0\to\mathrm{T}} = 30\ \mu\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ | 456 | 1.40 | 106 | -6.75 | 0.19 | 0.016 | 0.017 | 0.34 | **0.47** | 318 |
| $k_{\mathrm{T}\to 0} = 1\ \mathrm{s}^{-1}$ | 398 | 1.93 | 108 | -6.5 | 0.17 | 0.018 | 0.038 | 0.29 | **0.40** | 267 |
| $k_{\mathrm{T}\to 0} = 10\ \mathrm{s}^{-1}$ | 451 | 1.41 | 106 | -6.75 | 0.19 | 0.016 | 0.036 | 0.34 | **0.52** | 308 |
| $k_{\mathrm{T}\to 0} = 500\ \mathrm{s}^{-1}$ | 457 | 1.34 | 107 | -6.75 | 0.21 | 0.015 | 0.042 | 0.38 | **4.6** | 318 |
| $k_{\mathrm{T}^*\to 0} = 1\ \mathrm{s}^{-1}$ | 461 | 1.36 | 106 | -6.75 | 0.20 | 0.015 | 0.015 | 0.35 | 1.31 | 325 |
| $k_{\mathrm{T}^*\to 0} = 10\ \mathrm{s}^{-1}$ | 461 | 1.36 | 106 | -6.75 | 0.20 | 0.015 | 0.015 | 0.35 | 1.31 | 331 |
| $k_{\mathrm{T}^*\to 0} = 100\ \mathrm{s}^{-1}$ | 461 | 1.36 | 106 | -6.75 | 0.20 | 0.015 | 0.015 | 0.35 | 1.31 | 330 |
| $k_{0\to\mathrm{D}} = 0.1\ \mu\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ | 448 | 1.44 | 107 | -6.75 | 0.20 | 0.016 | 0.040 | 0.34 | 1.26 | 306 |
| $k_{0\to\mathrm{D}} = 100\ \mu\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ | 434 | 1.33 | 71 | -6.5 | 0.16 | 0.015 | 0.036 | 0.31 | 1.07 | 179 |
| $k_{\mathrm{D}\to 0} = 1\ \mathrm{s}^{-1}$ | **6.3** | 1.25 | **0.17** | **-0.25** | 0.12 | 0.014 | 0.015 | 0.15 | **0.18** | **1.8** |
| $k_{\mathrm{D}\to 0} = 10\ \mathrm{s}^{-1}$ | **60** | 1.25 | **1.8** | **-3.25** | **0.09** | 0.014 | 0.018 | 0.19 | **0.43** | **4** |
| $k_{\mathrm{D}\to 0} = 100\ \mathrm{s}^{-1}$ | 344 | 1.29 | **27** | -6.5 | 0.16 | 0.015 | 0.032 | 0.32 | 1.1 | **56** |
| $k_{\mathrm{D}\to 0} = 600\ \mathrm{s}^{-1}$ | 473 | 1.45 | 200 | -6.75 | 0.20 | 0.016 | 0.043 | 0.36 | 1.31 | **776** |
| $k_{\mathrm{D}\to 0} = 1000\ \mathrm{s}^{-1}$ | 467 | 1.56 | **272** | -6.75 | 0.21 | 0.017 | 0.047 | 0.36 | 1.31 | **1390** |
| $k_{\mathrm{D}^*\to 0} = 1\ \mathrm{s}^{-1}$ | 456 | 1.37 | 106 | -6.75 | 0.20 | 0.016 | 0.039 | 0.35 | 1.27 | 309 |
| $k_{\mathrm{D}^*\to 0} = 10\ \mathrm{s}^{-1}$ | 445 | 1.46 | 106 | -6.75 | 0.20 | 0.016 | 0.042 | 0.35 | 1.27 | 301 |
| $k_{\mathrm{D}^*\to 0} = 100\ \mathrm{s}^{-1}$ | 361 | 2.32 | 109 | -6.5 | 0.18 | 0.020 | 0.067 | 0.34 | 1.24 | 249 |

[ATP] = 1000 $\mu$M and $F = 0$ throughout unless indicated otherwise. Values in bold indicate deviation by more than a factor of 2.

Table continued on next page.

| | velocity | #ATP/step | processivity | stall force | dwell time of fwd. steps at | | | dwell time of bwd. steps at | | #fwd./#bwd. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | stall force | F=15 pN | F=15pN [ATP]=10 $\mu$M | F=-15pN | F=-15pN [ATP]=10 $\mu$M | |
| | (nm/s) | | | (pN) | (s) | (s) | (s) | (s) | (s) | |
| $k_{\widetilde{\mathrm{D}}\to\mathrm{D}} = 1\ \mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ | **197** | **4** | **7.8** | -6.5 | 0.28 | 0.025 | 0.077 | **0.92** | 2.51 | 204 |
| $k_{\widetilde{\mathrm{D}}\to\mathrm{D}} = 2\ \mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ | 282 | 2.63 | **15** | -6.5 | 0.22 | 0.020 | 0.057 | 0.62 | 1.87 | 290 |
| $k_{\widetilde{\mathrm{D}}\to\mathrm{D}} = 100\ \mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ | 470 | 1.25 | **315** | -6.75 | 0.19 | 0.015 | 0.037 | 0.33 | 1.22 | 173 |
| $k_{\widetilde{\mathrm{D}}\to\mathrm{D}} = 1000\ \mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ | 456 | 1.24 | **2099** | -6.75 | 0.19 | 0.014 | 0.037 | 0.32 | 1.21 | **108** |
| $k_{\mathrm{D}\to\widetilde{\mathrm{D}}} = 1\,\mathrm{s}^{-1}$ | 419 | 1.38 | **376** | -5.75 | 0.21 | 0.015 | 0.039 | 0.36 | 1.33 | **151** |
| $k_{\mathrm{D}\to\widetilde{\mathrm{D}}} = 30\,\mathrm{s}^{-1}$ | 462 | 1.38 | **28** | -7.25 | 0.17 | 0.016 | 0.040 | 0.35 | 1.18 | 462 |
| $k_{\mathrm{D}\to\widetilde{\mathrm{D}}} = 100\,\mathrm{s}^{-1}$ | 447 | 1.48 | **7** | -7.5 | 0.13 | 0.016 | 0.040 | 0.38 | 1.13 | **59** |
| $k_{\mathrm{D}^*\to\widetilde{\mathrm{D}}} = 1\,\mathrm{s}^{-1}$ | **10** | **17** | **12** | -6.5 | **0.55** | **0.76** | **0.97** | 0.42 | 1.35 | **29** |
| $k_{\mathrm{D}^*\to\widetilde{\mathrm{D}}} = 10\,\mathrm{s}^{-1}$ | **71** | **3.2** | 59 | -6.5 | 0.23 | **0.11** | **0.15** | 0.36 | 1.29 | 172 |
| $k_{\mathrm{D}^*\to\widetilde{\mathrm{D}}} = 1000\,\mathrm{s}^{-1}$ | **927** | 1.18 | 72 | -6.75 | 0.19 | **0.006** | 0.027 | 0.35 | 1.27 | **189** |
| $k_{\mathrm{T}\to\widetilde{\mathrm{D}}} = 0.1\,\mathrm{s}^{-1}$ | 460 | 1.35 | 155 | -7.75 | **1.42** | 0.016 | 0.039 | **3.1** | **11** | **736** |
| $k_{\mathrm{T}\to\widetilde{\mathrm{D}}} = 1\,\mathrm{s}^{-1}$ | 459 | 1.35 | 136 | -7.25 | **0.48** | 0.016 | 0.039 | **0.90** | **3.24** | 528 |
| $k_{\mathrm{T}\to\widetilde{\mathrm{D}}} = 10\,\mathrm{s}^{-1}$ | 451 | 1.38 | 58 | -6 | **0.07** | 0.015 | 0.039 | **0.11** | **0.43** | **114** |
| $k_{\mathrm{T}\to\widetilde{\mathrm{D}}} = 50\,\mathrm{s}^{-1}$ | 434 | 1.42 | **14** | -4.75 | **0.02** | 0.014 | 0.037 | **0.03** | **0.11** | **18** |
| $k_{\mathrm{T}\to\mathrm{D}} = 1\,\mathrm{s}^{-1}$ | 461 | 1.25 | 113 | -6.75 | 0.20 | 0.016 | 0.039 | 0.37 | 1.28 | 346 |
| $k_{\mathrm{T}\to\mathrm{D}} = 50\,\mathrm{s}^{-1}$ | 444 | 1.78 | 86 | -6.5 | 0.16 | 0.016 | 0.040 | 0.30 | 1.25 | 226 |
| $k_{\mathrm{T}\to\mathrm{D}} = 100\,\mathrm{s}^{-1}$ | 431 | 2.22 | 72 | -6.25 | 0.13 | 0.015 | 0.040 | 0.27 | 1.23 | 173 |
| $k_{\mathrm{T}^*\to\mathrm{D}^*} = 1\,\mathrm{s}^{-1}$ | **31** | 2.63 | 196 | -6 | 0.32 | **0.25** | **0.28** | 0.33 | 1.22 | **531** |
| $k_{\mathrm{T}^*\to\mathrm{D}^*} = 10\,\mathrm{s}^{-1}$ | **87** | 1.65 | 215 | -6.25 | 0.22 | **0.089** | **0.11** | 0.34 | 1.24 | **672** |
| $k_{\mathrm{T}^*\to\mathrm{D}^*} = 100\,\mathrm{s}^{-1}$ | 363 | 1.3 | 143 | -6.75 | 0.20 | 0.020 | 0.043 | 0.35 | 1.27 | 456 |
| $k_{\mathrm{T}^*\to\mathrm{D}^*} = 500\,\mathrm{s}^{-1}$ | 508 | 1.57 | 64 | -6.75 | 0.19 | 0.013 | 0.037 | 0.35 | 1.28 | 176 |
| $k_{\mathrm{T}^*\to\mathrm{D}^*} = 1000\,\mathrm{s}^{-1}$ | 478 | 1.94 | **41** | -6.5 | 0.16 | 0.012 | 0.038 | 0.35 | 1.28 | **106** |

[ATP] = 1000 $\mu$M and $F = 0$ throughout unless indicated otherwise. Values in bold indicate deviation by more than a factor of 2.

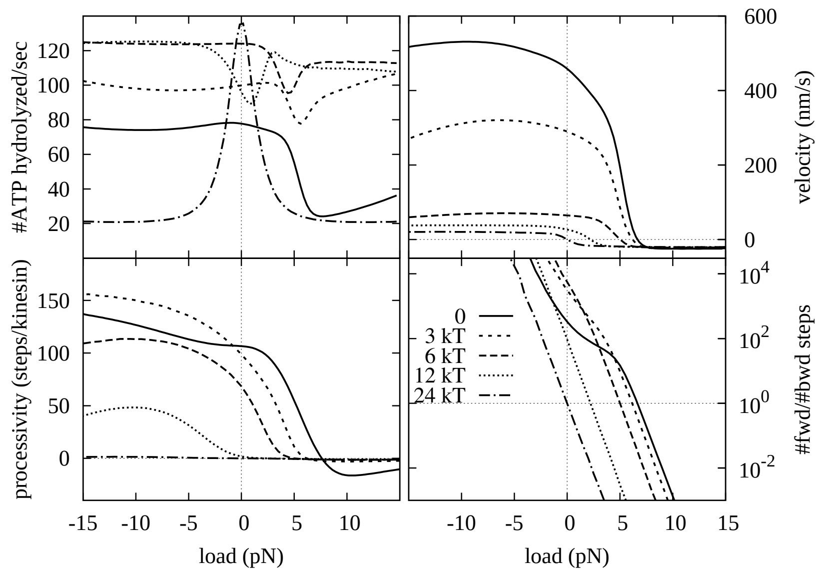



**Fig. S1.** Simulation results IV: Several observables for increased values (by $0, 3, 6, 12$, and $24\ k_{\mathrm{B}}T$) of the free energy changes of neck linker docking ($\Delta G_{\mathrm{T,T}^*}$ and $\Delta G_{\mathrm{D,D}^*}$) compared to the values of Table 1, at $1$ mM ATP as functions of the external load.

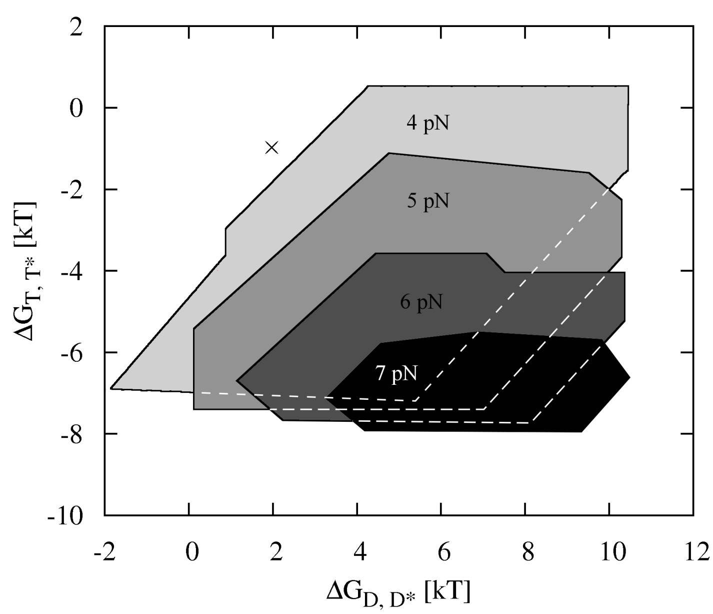
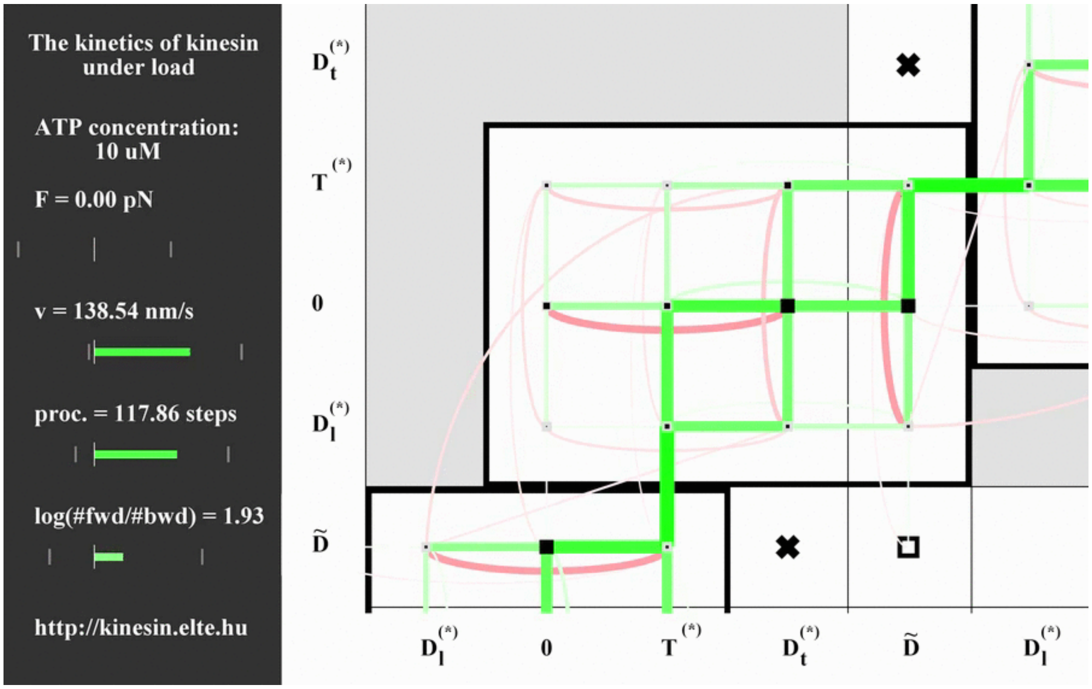
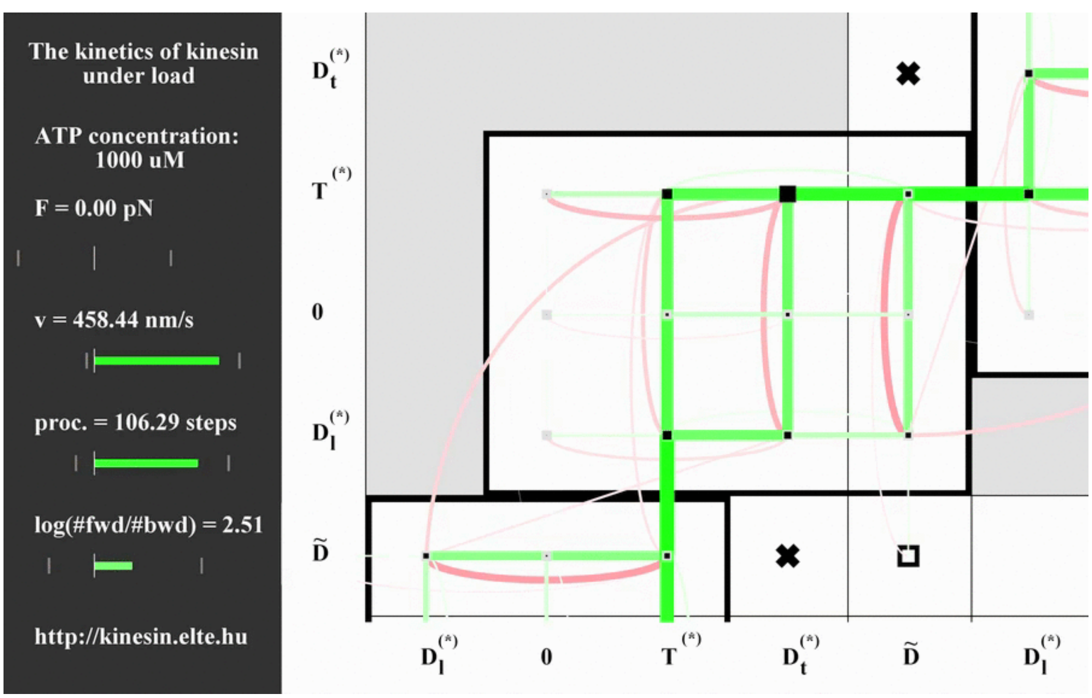



**Fig. S2.** Simulation results V: The gray areas indicate the parameter range along the $\Delta G_{\mathrm{T,T}^*} - \Delta G_{\mathrm{D,D}^*}$ plain, where the given stall loads (4, 5, 6, and 7 pN) at $[\mathrm{ATP}] = 1000\ \mu$M can be attained by our model, in such a way that all the first 9 prescribed conditions in Table S1 are also obeyed.




**Movie S1.** Flux distribution in the state space at low (10 $\mu$M) ATP concentration: The left panel (on a dark gray background) indicates how the velocity, the randomness, and the ratio of the numbers of forward and backward steps of kinesin change as the external force $F$ is varied from $-15$ pN to $15$ pN at low (10 $\mu$M) ATP concentration. Simultaneously, the right panel shows the steady-state occupancy distribution (with the areas of the black squares being proportional to the occupation probabilities of the corresponding kinetic states) and flux distribution (where positive fluxes are marked green and negative fluxes are marked red, with the width and saturation indicating the flux strength) in the two-dimensional state space (cf. Fig. 1). The flux for each transition is defined as the difference between the corresponding forward and backward transition rates.




**Movie S2.** Flux distribution in the state space at high (1000 $\mu$M) ATP concentration: The same as Movie S1, but at high (1000 $\mu$M) ATP concentration.