Supporting Information

1. Two well potential

The potential used in the two well model is in the following mathematical form[1]

$$U_{nat}(r_{ij})=\begin{cases}\varepsilon_1 Z(r_{ij})(Z(r_{ij})-a) & r<r_{1ij}\\ CY(r_{ij})^n\dfrac{\frac{Y(r_{ij})^n}{2}-(r_{bij}-r_{1ij})^{2n}}{2n}+\varepsilon_2 & r_{1ij}\le r_{ij}<r_{bij}\\ -B\dfrac{Y(r_{ij})-h_1}{Y(r_{ij})^m+h_2} & r_{bij}\le r_{ij}<r_{2ij}\\ \varepsilon_2\left[5\left(\dfrac{r_{2ij}}{r_{ij}}\right)^{12}-6\left(\dfrac{r_{2ij}}{r_{ij}}\right)^{10}\right] & r_{2ij}\le r_{ij}\end{cases} \quad (1)$$

where

$$Z(r)=\left(\frac{r_{1ij}}{r_{ij}}\right)^k$$

$$Y(r)=(r_{ij}-r_{bij})^2$$

$$C=\frac{4n(\varepsilon_1+\varepsilon_2)}{r_{2ij}-r_{bij}}$$

$$B=\varepsilon_1 m(r_{2ij}-r_{bij})^{2(m-1)}$$

$$h_1=\frac{\varepsilon_h(m-1)(r_{2ij}-r_{bij})^2}{m(\varepsilon_h+\varepsilon_2)}$$

$$h_2=\frac{\varepsilon_2(m-1)(r_{2ij}-r_{bij})^{2m}}{\varepsilon_h+\varepsilon_2} \quad (2)$$

U_{nat} gives the interactions among residues which are adjacent in either open or closed structures. It is designed to have the double-well potential. First, for $r_{ij}<r_{1ij}$ a Lennard-Jones type of interaction emerges, where ε_1 controls the depth of the first potential minimum. Second, for $r_{ij}>r_{bij}$, we set one boundary to be r_{bij} at which $U_{nat}(r_{bij})$ reaches a maximum ε_h. Afterwards, $U_{nat}(r_{ij})$ decreases toward the second minimum potential value at $r=r_{2ij}$ and depth is ε_2. At last, a tail is determined again by the Lennard-Jones potential. Here $m=5$, $k=8$ and $n=1$. We require that, at the junctions both force and potential are continuous.

Figure 9 in the main text shows the coarse grained microscopic interaction energy (at the residue level) between the two residues. (a) shows the case where the spatial native contacts r_{ij} between two specific residues for closed native conformation are the same as the open one i.e. $r_{ij}^{open}=r_{ij}^{closed}$. (b) shows the case where the spatial native contacts r_{ij} between two specific residues for closed native conformation are within a cut off distance with the open one i.e. $|r_{ij}^{open}-r_{ij}^{closed}|<2$ Å. The barrier height is

$|r_{ij}^{open} - r_{ij}^{closed}| \varepsilon_h/(2\text{A})$. Otherwise, (c) shows the case where the spatial native contacts r_{ij} between two specific residues for closed native conformation are at further distance with the open one i.e. $|r_{ij}^{open} - r_{ij}^{closed}|$> 2 Å. The corresponding barrier height is ε_h. The depth of the two well are ε_1 and ε_2 separately. The gap is the difference between them, i.e. $\varepsilon_2 - \varepsilon_1$.

2. Φ value analysis

In the main text, the ϕ value [3,4,5] for the closing direction at transition state 1, intermediate state and transition state 2 from the open state is calculated through the following equation:

$$\begin{aligned}
\phi_i^{tran1} &= \frac{< n_i >_{tran1} - < n_i >_{open}}{< n_i >_{closed} - < n_i >_{open}} \\
\phi_i^{int} &= \frac{< n_i >_{int} - < n_i >_{open}}{< n_i >_{closed} - < n_i >_{open}} \\
\phi_i^{tran2} &= \frac{< n_i >_{tran2} - < n_i >_{open}}{< n_i >_{closed} - < n_i >_{open}}
\end{aligned} \tag{3}$$

where $< n_i >$ is the thermal averaged value of the density of residue i over all the two-body contact interaction pairs, and "int" represents intermediate state, "open" represents open state, "closed" represents closed state, "tran1" represents the first transition state from open state, "tran2" represents the second transition state from the open state. These states are determined from the 2-D free-energy profile Fig.2(b) in the main text.

3. Contact map calculation

The contact probability ratio p_{ij} [5] in the closing direction for both LID-closing and NMP-closing pathways are calculated as follows:

$$\begin{aligned}
p_{ij}^{tran1} &= \frac{n_{ij}^{tran1}}{n^{tran1}} \\
p_{ij}^{int} &= \frac{n_{ij}^{int}}{n^{int}} \\
p_{ij}^{tran2} &= \frac{n_{ij}^{tran2}}{n^{tran2}}
\end{aligned} \tag{4}$$

where n^{tran1} is the total number of conformations with all contacts at the first transition state in the closing direction, and n_{ij}^{tran1} is the number of conformations at transition state 1 with specific contact between i and j. n^{tran2} is the total number of conformations with all contacts at the second transition state in the closing direction, and n_{ij}^{tran1} is the number of conformations at transition state 2 with specific contact between i and j. n^{tran1} is the total number of conformations with all contacts at the intermediate state in the closing direction, and n_{ij}^{tran1} is the number of conformations at intermediate state

with specific contact between i and j.
The first transition state, intermediate state and second transition state are determined from the 2-D free-energy profile Fig.2(b) in the main text.

4. Path weight at different temperatures

In Table 1, we show the pathway weight for both closing and opening directions at temperatures from 10^{o}C to 58^{o}C. There are no significant changes in the weights varying with temperatures; they remain almost constant compared to that at the room temperature.

5. Mutation studies

Table 2-7 show the contacts that have been deleted in the mutation simulations among the NMP-LID (in Table 2), LID-Core0 (in Table 3), LID-Core2 (in Table 4), NMP-Core0 (in Table 5), NMP-Core2 (in Table 6), NMP-NMP (in Table 7). The residue ranges for each domain are LID (residue 127-164), NMP (residue 31-60), and Core domains (Core0: 1-30, Core1: 61-126, Core2: 165-217). "dist1" represents the C_α distance between the two residues in X-ray closed structure 1ANK, and "dist2" is the C_α distance between the two residues in open structure 4AKE.

References

[1] Cheung, M. D., Garcia, A. E.& Onuchic, J. N. (2002) *Proc. Natl. Acad. Sci. USA* , 685–690.
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Table 1. The weights of two pathways, NMP-closing and LID-closing, from both opening and closing directions at different temperatures. "NMP" in the table stands for the NMP-closing pathway; "LID" in the table stands for the LID-closing pathway; "open" in the table stands for the opening direction for conformational switch; "close" in the table stands for the closing direction for conformational switch

Temp(^{o}C)	NMP open	LID open	NMP close	LID close
10	0.81	0.19	0.83	0.17
15	0.77	0.23	0.78	0.22
20	0.87	0.13	0.86	0.14
24	0.77	0.23	0.81	0.19
30	0.82	0.18	0.80	0.20
35	0.82	0.18	0.77	0.23
40	0.83	0.17	0.80	0.20
46	0.77	0.23	0.83	0.17

51	0.75	0.25	0.76	0.24
54	0.74	0.26	0.70	0.30
56	0.79	0.21	0.78	0.22
58	0.80	0.20	0.76	0.24

Table 2. In the mutation simulation, the following contacts interactions have been deleted between NMP and LID domains: LID(residue 127-164), Core0(residue 1-30), NMP(residue 31-60), Core1(residue 61-126),Core2(residue 165-217)

res1	res2	dist1	dist2
D33	A127	7.8613	26.1702
D33	S129	9.3554	25.2963
D33	G130	7.2825	22.8251
D33	R156	10.4039	24.2534
R36	R156	9.1277	25.9482
R36	K157	9.5760	25.7846
R36	D158	10.4455	23.6746
V39	K157	12.0825	30.5539
M53	K157	8.7234	24.9331
M53	D158	6.7752	23.4477
D54	K157	6.9968	26.2009
D54	D158	5.4675	25.2048
D54	Q160	9.6388	30.0985
D54	T163	7.6092	27.4231
G56	T163	5.1335	23.6117

Table 3. In the mutation simulation, the following contacts interactions have been deleted between LID and Core0 domains: LID(residue 127-164), Core0(residue 1-30)

res1	res2	dist1	dist2
P9	V164	7.8096	8.8898
G14	V132	7.3150	18.2887
T15	V132	6.2163	17.7928
T15	N138	9.7632	18.2668
Q18	S129	9.3355	26.3800
Q18	G130	8.0821	23.7201
Q18	R131	7.1890	23.5188
Q18	V132	8.9852	22.6857
M21	S129	11.8703	30.8022
Q28	S129	10.4103	28.4163
Q28	G130	9.7348	25.6269

Table 4. In the mutation simulation, the following contacts interactions have been deleted between LID and Core2 domains: LID(residue 127-164),Core2(residue 165-217)

res1	res2	dist1	dist2
F137	V202	8.5617	15.9505
N138	V202	7.6899	17.8427
R156	R167	13.8135	14.7328

D158	R167	9.4396	9.5197
D159	R167	10.0747	10.7649
E161	R165	6.4083	6.0302
E162	K166	6.5629	6.1992
T163	E167	6.2446	6.2663
V164	L168	6.2342	6.4308

Table 5. In the mutation simulation, the following contacts interactions have been deleted between NMP and Core0 domains: Core0(residue 1-30), NMP(residue 31-60)

res1	res2	dist1	dist2
Q28	D33	10.2947	9.4328
I29	D33	8.4023	6.9688
I29	M34	8.1996	6.7619
S30	M34	6.3134	6.1455
S30	R36	10.4140	10.1621

Table 6. In the mutation simulation, the following contacts interactions have been deleted between NMP and Core2 domains: NMP(residue 31-60),Core2(residue 165-217)

res1	res2	dist1	dist2
R36	R167	16.1892	24.0986
M53	R167	9.5222	23.0861
D54	K166	10.2918	28.2718
A55	K166	7.8532	27.9051
G56	K166	5.5788	24.1544
G56	R167	5.3468	22.1309
G56	E170	8.5521	22.3901
K57	K166	7.0736	23.9194
K57	E170	6.9814	21.2662
L58	E170	7.1720	18.4233
L58	Y171	8.6848	18.5931
L58	M174	9.0994	17.7530
L58	T175	9.9311	19.7699

Table 7. In the mutation simulation, the following contacts interactions have been deleted within NMP domain: NMP(residue 31-60)

res1	res2	dist1	dist2
T31	L35	6.0054	5.8857
G32	R36	6.0819	6.2701
G32	M53	8.7795	11.2905
D33	A37	6.3004	6.2160
M34	A38	6.3142	6.2952
L35	V39	6.4370	6.6315
L35	G46	5.4194	7.4124
L35	A49	6.4343	7.1324
L35	M53	8.8294	9.1986
L35	V59	9.3994	9.1765

R36	K40	6.1703	6.8502
R36	M53	8.7362	9.5842
A37	S41	5.9546	6.4164
A37	S43	7.9539	9.9062
A38	G42	5.5693	6.4739
A38	S43	4.5606	6.6256
A38	L45	6.7977	6.9054
A38	G46	5.2957	4.0160
A38	L47	5.0091	7.4232
A38	A49	8.3116	7.4039
V39	G46	6.6041	4.1596
V39	L47	4.1222	5.6671
V39	A49	7.0196	6.4524
V39	L50	6.0768	5.0498
V39	M53	10.344	9.4033
L40	L47	7.7290	8.4836
G42	L47	5.6221	5.5618
S43	L47	5.0623	6.0611
E44	Q48	7.8742	6.4522
L45	A49	9.0867	6.1045
G46	K50	8.2299	5.9057
L47	D51	9.4590	6.4022
Q48	I52	8.0501	6.6551
A49	M53	5.9003	6.2471
A49	V59	6.1792	6.8707
K50	D54	6.2765	5.9556
D51	A55	6.1404	5.9030
D51	K57	8.7105	8.3051
I52	G56	6.3057	6.2740
I52	K57	5.2216	4.9113
I52	L58	6.5863	6.4810
I52	V59	6.0765	6.4574
I52	T60	9.0193	9.2531
M53	K57	5.8118	5.5269
M53	V59	7.0100	7.2572