LIPIDS TRIGGER A CONFORMATIONAL SWITCH REGULATING SRP-MEDIATED PROTEIN TARGETING

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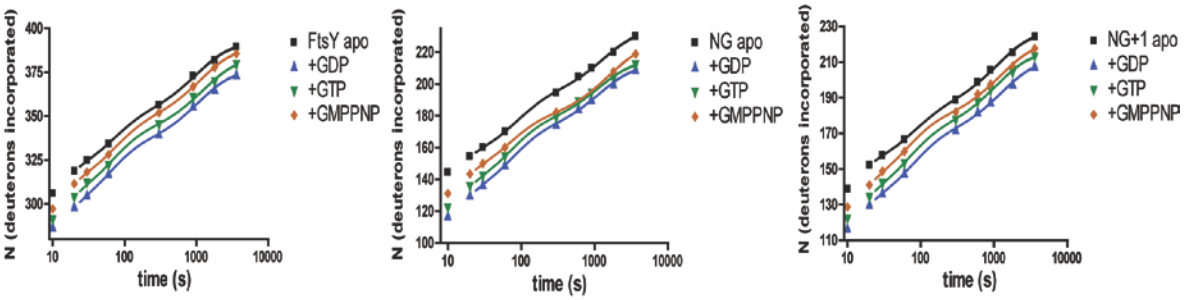
Supplemental Figures S1, S2 and S3

Supplemental Table S1

A



B

		N	A	k_A (10^{-3} s^{-1})	$t1/2_A$ (s)	B	k_B (10^{-3} s^{-1})	$t1/2_B$ (s)	C	k_C (10^{-3} s^{-1})	$t1/2_C$ (min)
FtsY	apo	386.3±1.2	301.7±1.7	413.8±23.4	1.7	46±1.7	16.1±1.3	43.0	38.6±1.1	0.8±0.1	15.1
	GDP	377.5±2.5	281.6±4	394.6±46.7	1.8	47.5±4	19.4±3.2	35.7	48.4±2.3	0.7±0.1	15.6
	GTP	384.7±2	286.5±3.4	405.2±43.4	1.7	51.1±3.4	18.6±2.5	37.3	47.1±2	0.8±0.1	14.4
	GMPPNP	383.8±0.5	297.9±1	358.3±8.2	1.9	46.5±1.1	17±0.8	40.8	39.4±0.7	0.9±0.0	12.6

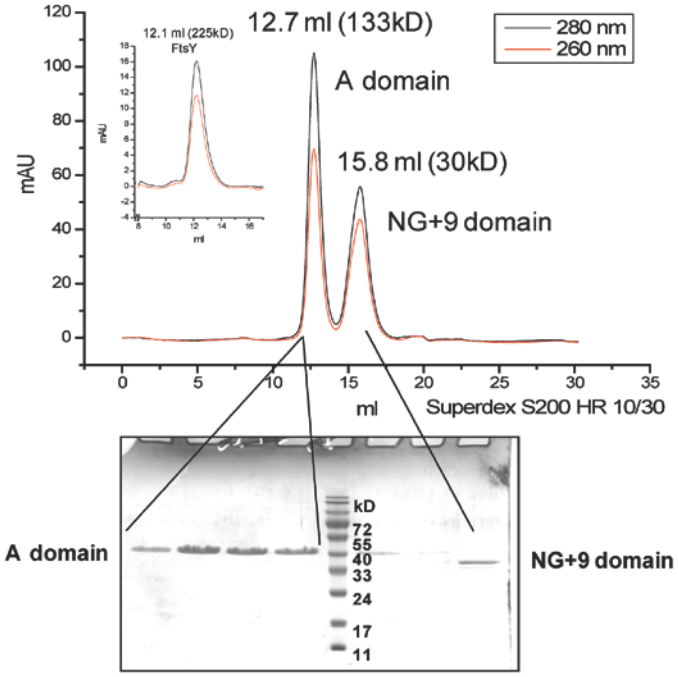
		N	A	k_A (10^{-3} s^{-1})	$t1/2_A$ (s)	B	k_B (10^{-3} s^{-1})	$t1/2_B$ (s)	C	k_C (10^{-3} s^{-1})	$t1/2_C$ (min)
NG (197-497)	apo	232.5±2	144.5±2.8	322.8±33.5	2.1	42.1±2.9	13.5±2.2	51.3	46±2	0.8±0.1	15.1
	GDP	210.6±1.5	116.9±2.8	271.4±23.7	2.6	48.4±2.9	15.9±2	43.6	45.3±1.8	0.9±0.1	13.4
	GTP	213.5±1.8	122.1±3.6	278±30.9	2.5	47.9±3.7	16.5±2.6	41.9	43.6±2.3	0.9±0.1	13.1
	GMPPNP	224.3±2.8	131.6±3	287.5±25.7	2.4	42.3±2.9	16.8±2.4	41.4	50.4±2.3	0.6±0.1	18.6

		N	A	k_A (10^{-3} s^{-1})	$t1/2_A$ (s)	B	k_B (10^{-3} s^{-1})	$t1/2_B$ (s)	C	k_C (10^{-3} s^{-1})	$t1/2_C$ (min)
NG+1 (196-497)	apo	225.7±1.4	143.3±2.7	267.7±18	2.6	35.3±2.8	14.9±2.6	46.6	47.2±1.9	0.9±0.1	12.8
	GDP	210±2	118.4±3.3	261.8±25.3	2.6	45.3±3.4	15.2±2.4	45.5	46.2±2.1	0.8±0.1	14.7
	GTP	214.5±1.7	120.9±3.6	288.8±34	2.4	46±3.7	17.7±2.8	39.2	47.5±2.2	0.9±0.1	12.6
	GMPPNP	219.7±2.3	127±4.3	298±41.3	2.3	46±4.3	18.7±3.3	37.1	46.7±2.4	0.8±0.1	14.3

$$y = N - (Ae^{-k_A t}) - (Be^{-k_B t}) - (Ce^{-k_C t})$$

Supplemental Figure S1 HX-MS analysis of the dynamic properties and nucleotide dependent conformation changes in FtsY. (**A**) The HX global kinetics of FtsY, NG and NG+1 show the nucleotide dependence of the numbers of deuterons incorporated over time. The solid lines and observed rate constants shown in (**B**) are derived from a nonlinear triple-exponential regression fit of the deuterons incorporated over time. Global exchange data were adjusted for deuteron loss during analysis.

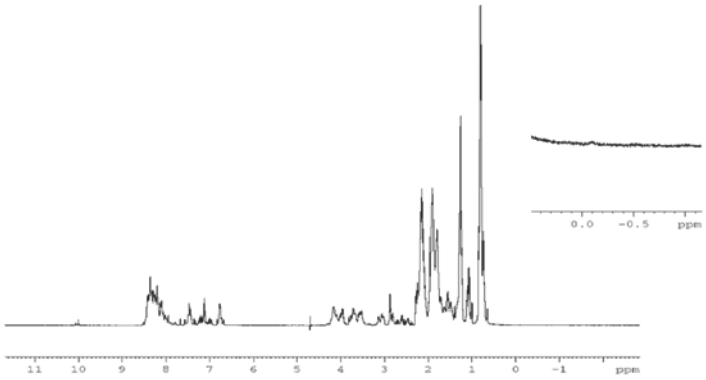
A



B

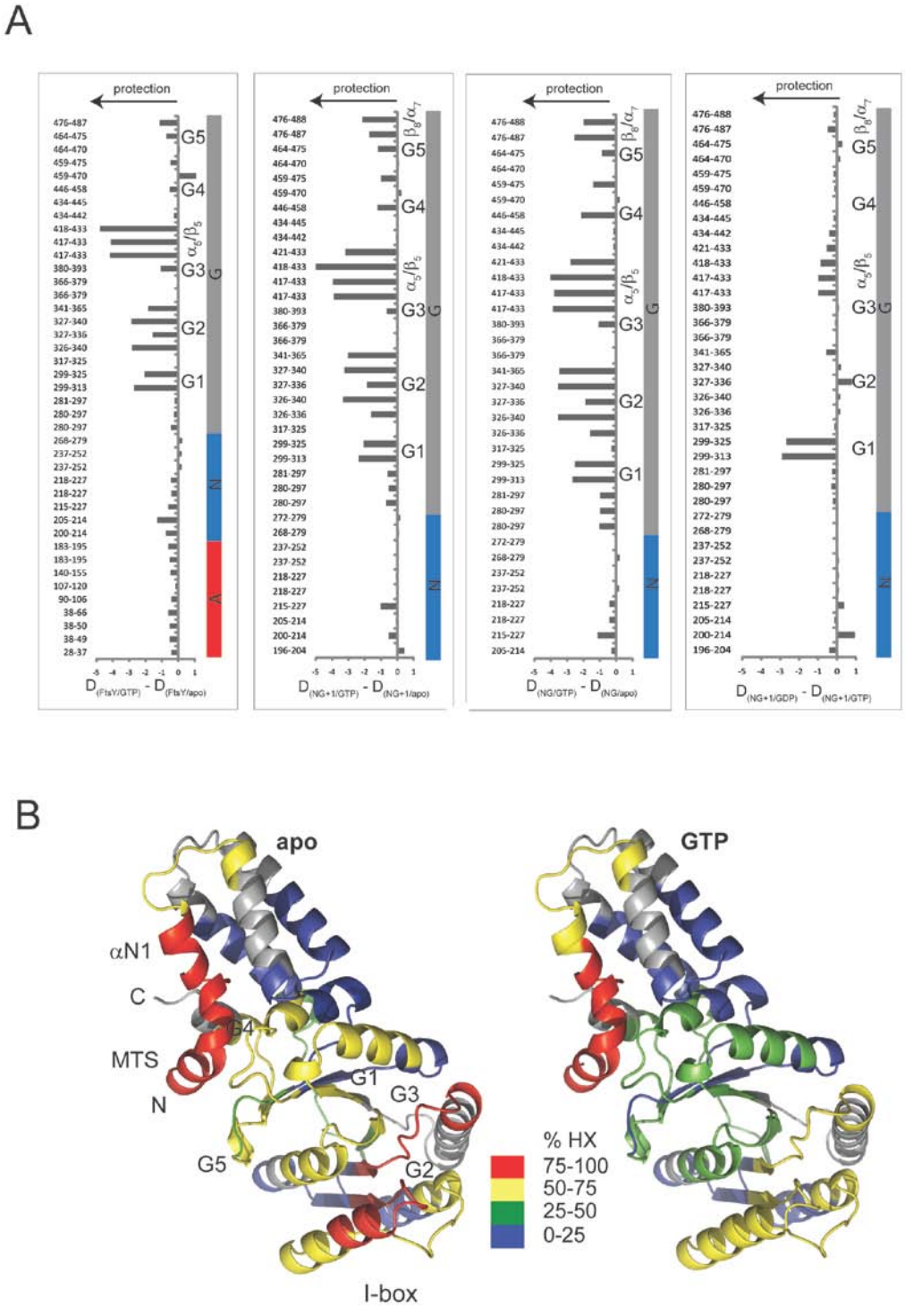
Construct	Aberrant MW	Absolute MW	Calculated MW
FtsY-Xa	225	56.7	55
A domain	130	20.8	21
NG+9	30	26.6	35

C



Supplemental Figure S2 Characterization of a proteolytically cleavable FtsY variant (FtsY-Xa). (**A**) FtsY-Xa was cleaved after purification and the fragments (A and NG+9 domains) analysed by size exclusion chromatography and SDS-PAGE. The A domain does not stably interact with the NG+9 domain. The unusually aberrant migration behavior of FtsY and the A domain indicates an elongated shape which is characteristic of unstructured proteins. (**B**) Hydrodynamic properties of FtsY, A and NG+9 domains. The aberrant molecular weight (MW) was determined by size-exclusion chromatography, the absolute MW by static light scattering and refractive index measurements. The MW is given in kDa. In size exclusion chromatography FtsY or the A domain migrate with aberrant

molecular weights. (**C**) One-dimensional ^{1}H NMR spectrum of the A domain. Details of the signals upfield of 0.5 p.p.m. are given in the inset. The one-dimensional ^{1}H NMR spectrum did not show any significant chemical shift dispersions indicating that the A domain is intrinsically disordered.



Supplemental Figure S3 HX-MS analysis of nucleotide dependent structural changes in FtsY. (**A**) Difference plots of deuteron incorporation into FtsY, NG or NG+1 in the presence of GTP minus deuterons incorporated in the absence of nucleotides (apo) after 10 s of incubation in D_2O. The

numbers corresponding to the protein segments are indicated on the left and domain structure of FtsY on the right. In the difference plot, bars to the left indicate nucleotide induced protection (less deuteron incorporation). The right panel gives the difference plot of deuteron incorporation into NG+1 in the presence of GDP minus deuterons incorporated in the presence of GTP. (**B**) Localization of fast and slow exchanging regions in the NG+1 variant (mapped on the crystal structure of NG+1 shown in ribbon representation). Segments corresponding to peptic fragments are colored according to the relative deuteron incorporation into the nucleotide-free protein after 10 s in D_2O.

Suplemental Table S1 Data collection and refinement statistics.

	FtsY
Data collection	
Space group	$P2_12_12$
Cell dimensions	
a, *b*, *c* (Å)	79.36, 108.41, 31.90
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	39.68-1.60
R_{merge}	0.102(0.59)*
$I / \sigma I$	12.8(2.7)
Completeness (%)	99.4(98.8)
Redundancy	6.1(5.9)
Refinement	
Resolution (Å)	39.679-1.6
No. reflections	36676
R_{work} / R_{free}	17.82/21.98
No. atoms	
Protein	2422
Ligand/ion	20
Water	214
B-factors	
Protein	16.575
Ligand/ion	23.141
Water	27.997
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	1.006

* Values in parentheses are for the highest resolution shell.