Supporting Information

Sun *et al.* 10.1073/pnas.0710867105

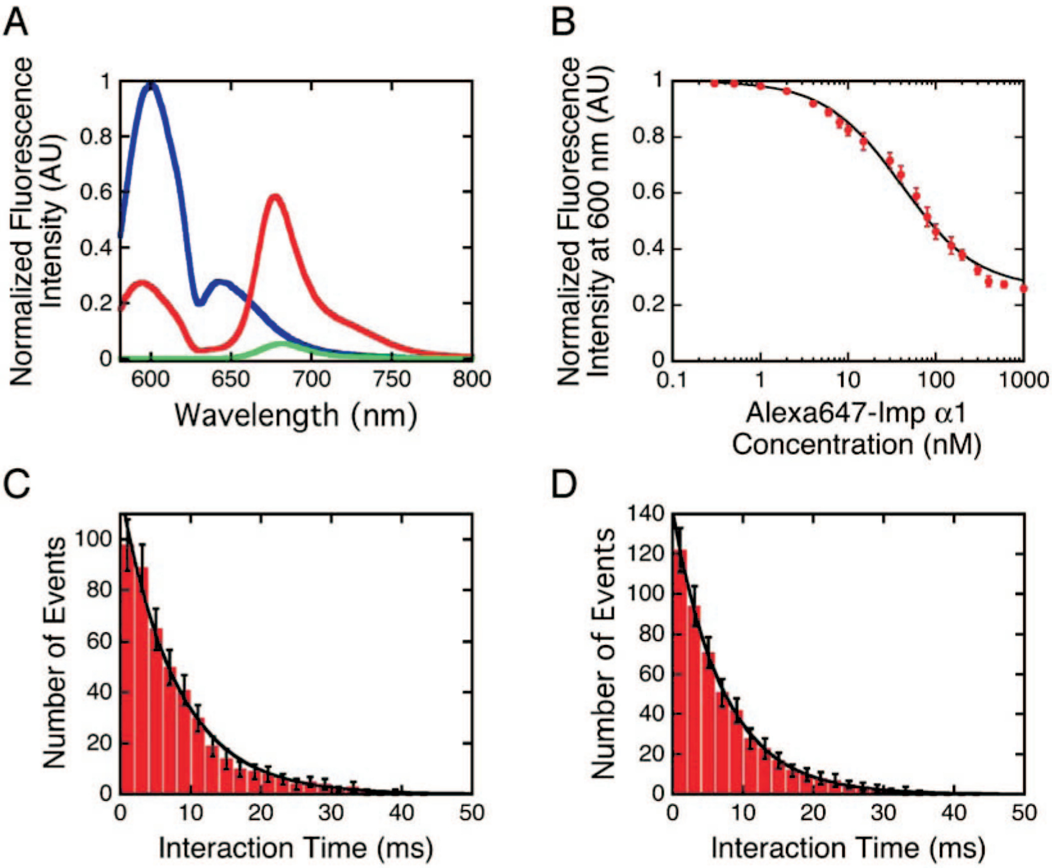


Fig. S1. Steady-state FRET and the effect of Imp α1 dye labeling on cargo interaction time. (*A*) FRET between Alexa Fluor 568–NLS–2xGFP(4C) (100 nM) and Alexa Fluor 647–Imp α1 (1 μM). Blue, NLS–2xGFP(4C) alone; red, NLS–2xGFP(4C) + Imp α1; green, Imp α1 alone. EX = 568 nm. (*B*) Donor emission quenching due to FRET ($K_D = 46 \pm 3$ nM). For comparison, the K_D is $\approx$40 nM in the absence of dyes (1). The data were fit to: normalized fluorescence intensity = $I_0 + (1 - I_0)(K_D/(K_D + [\text{Imp } \alpha 1]))$. [Alexa Fluor 568–NLS–2xGFP(4C)] = 100 nM. (*C* and *D*) NPC interaction time histograms for Alexa Fluor 647–NLS–2xGFP(4C) in the presence of (*C*) Alexa Fluor 568-Imp α1 (7.9 $\pm$ 0.3 ms; n = 459) and (*D*) Imp α1 (7.1 $\pm$ 0.1 ms; n = 502). Cargo interaction frequencies (2) for both C and D were 2.4 $\pm$ 0.3 events $s^{-1} \cdot \mu m^{-1}$. [NLS–2xGFP(4C)] = 0.1 nM; [Imp α1] = 250 nM; [Ran] = 2 μM; [GTP] = 1 mM; [Imp β1] = 0.5 μM; [NTF2] = 1 μM.

1. Catimel B, *et al.* (2001) Biophysical characterization of interactions involving importin-alpha during nuclear import. *J Biol Chem* 276:34189–34198.
2. Yang W, Musser SM (2006) Nuclear import time and transport efficiency depend on importin β concentration. *J Cell Biol* 174:951–961.

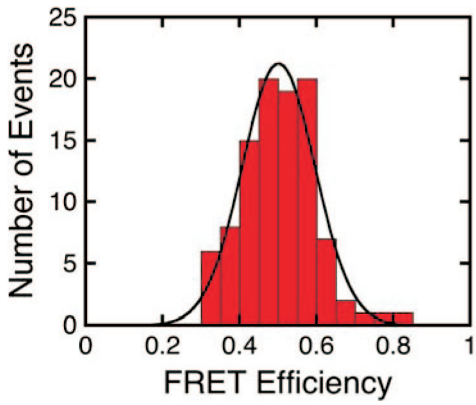


Fig. S2. Single molecule FRET efficiency of coverslip-adsorbed Alexa Fluor 568–Imp α1/Alexa Fluor 647–NLS–2xGFP(4C) complexes. For convenience, the FRET efficiency, *E*, was defined as $E = I_A/(I_A + I_D)$, where I_D and I_A denote the fluorescence emission intensities observed in the donor and acceptor channels, respectively [Ha T (2001) Single-molecule fluorescence resonance energy transfer. *Methods* 25: 78–86]. These values were corrected for background noise and the donor emission in the acceptor channel (cross-talk). No detectable acceptor emission was observed in the donor channel. As estimated from a Gaussian fit, the FRET efficiency was 50 $\pm$ 9% (n = 100).

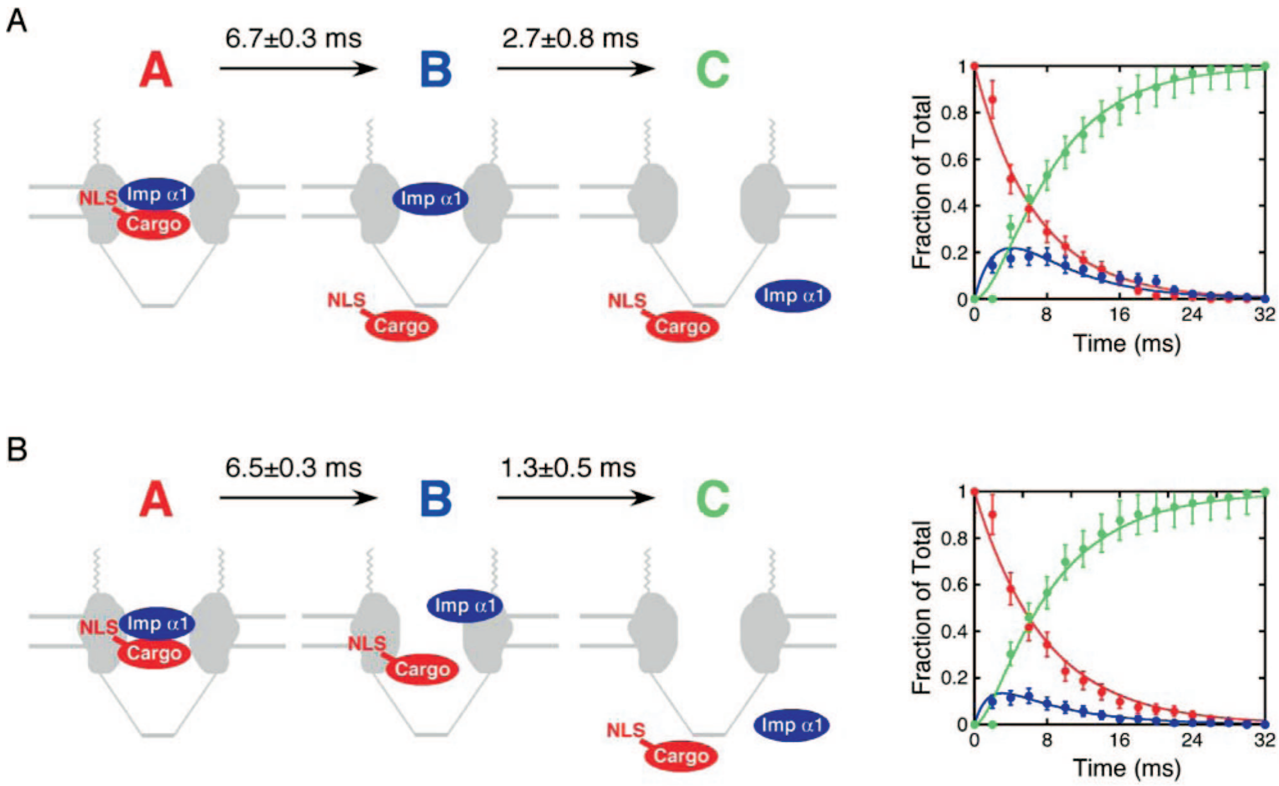
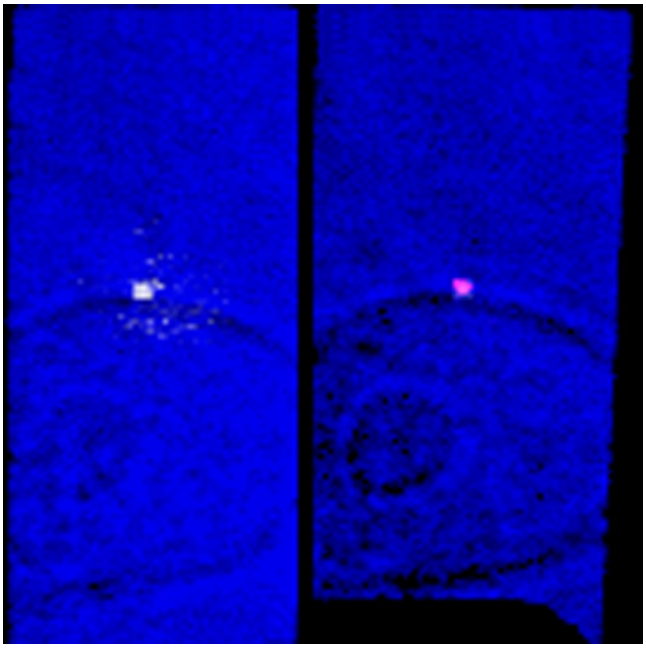


Fig. S3. Decomposition of Imp α1/cargo complexes (A → B → C). (*A*) Donor dyes on Imp α1. The definition of the three states (*A–C*) and the global kinetic fit when the donor dyes were on Imp α1 were described in the main text. The graph is reproduced from Fig. 1*D*. Global fitting yielded $\tau_1 = 6.7 \pm 0.3$ ms (A → B) and $\tau_2 = 2.7 \pm 0.8$ ms (B → C) ($n = 132$). [Alexa Fluor 568–Imp α1] = 0.1 nM; [Alexa Fluor 647–NLS–2xGFP(4C)] = 250 nM. (*B*) Donor dyes on the cargo. The identical experiment as in *A*, except that the donor and acceptor dye positions were switched, yielding $\tau_1 = 6.5 \pm 0.3$ ms and $\tau_2 = 1.3 \pm 0.5$ ms ($n = 122$). Comparing with *A*, note that the Imp α1/cargo complex dissociation times (τ_1) were identical (within error), as expected. However, the free cargo dissociated from the NPC faster than cargo-free Imp α1 (compare τ_2 values). Also note that $\tau_1 + \tau_2 = 7.8$ ms, consistent with the cargo interaction time reported in Fig. S1C. [Alexa Fluor 568–NLS–2xGFP(4C)] = 0.1 nM; [Alexa Fluor 647–Imp α1] = 250 nM (*A* and *B*) The position of the molecule with the acceptor dyes was unknown after Imp α1/cargo complex dissociation. Shown is one possible spatial arrangement for the B and C states. The B state could not be identified for ≈14% of the dissociation events in *A* and ≈21% in *B*. These data were not included in the analysis, but indicate that the τ_2 values are upper limits. Red, A; blue, B; green, C. [CAS] = 1.3 μM; [Ran] = 2 μM; [GTP] = 1 mM; [Imp β1] = 0.5 μM; [NTF2] = 1 μM.



Movie S1. Shows the transport event of Fig. 1*A*. Pixels are 240 nm^2, each frame was acquired in 2 ms, and the playback speed is 500× slower than real-time. Note: This QuickTime video was made from 16-bit CCD camera data converted to 8-bit. The QuickTime software on late-model Apple computers may destroy the pixelation for video display. If difficulties are encountered in attempts to observe the raw pixelated data, try using the RealPlayer software (Apple) or QuickTime on a PC.

Movie S1 (MOV)

Table S1. Effect of CAS concentration on dissociation and transport efficiencies

CAS, nM	Result	Total, *n*	Dissociation efficiency, %	Final destination		Transport efficiency, %
				Cytoplasm	Nucleoplasm	
+25% Glycerol						
0.1 nM Alexa Fluor 568-Imp α1 and 250 nM Alexa Fluor 647-NLS-2xGFP(4C)						
0	Did not dissociate at NPC	144	**0**	65 (45 ± 4%)	79 (55 ± 4%)	**55 ± 4**
	Did dissociate at NPC	0		0	0	
0.01	Did not dissociate at NPC	82	**0**	43 (52 ± 6%)	39 (48 ± 6%)	**48 ± 6**
	Did dissociate at NPC	0		0	0	
0.02	Did not dissociate at NPC	97	**1 ± 1**	44 (45 ± 5%)	53 (55 ± 5%)	**55 ± 5**
	Did dissociate at NPC	1		0	1	
0.08	Did not dissociate at NPC	103	**1 ± 1**	56 (54 ± 5%)	47 (46 ± 5%)	**46 ± 5**
	Did dissociate at NPC	1		0	1	
0.2	Did not dissociate at NPC	87	**0**	45 (52 ± 5%)	42 (48 ± 5%)	**48 ± 5**
	Did dissociate at NPC	0		0	0	
0.8	Did not dissociate at NPC	76	**1 ± 1**	39 (51 ± 5%)	37 (49 ± 5%)	**49 ± 6**
	Did dissociate at NPC	1		0	1	
1.6	Did not dissociate at NPC	92	**3 ± 2**	49 (53 ± 5%)	43 (47 ± 5%)	**46 ± 5**
	Did dissociate at NPC	3		3	1	
3.2	Did not dissociate at NPC	82	**8 ± 3**	47 (57 ± 5%)	35 (43 ± 5%)	**44 ± 5**
	Did dissociate at NPC	7		2	5	
8.0	Did not dissociate at NPC	65	**16 ± 4**	41 (63 ± 6%)	24 (37 ± 6%)	**55 ± 6**
	Did dissociate at NPC	12		1	11	
16	Did not dissociate at NPC	57	**28 ± 5**	31 (54 ± 6%)	26 (46 ± 6%)	**54 ± 6**
	Did dissociate at NPC	22		5	17	
32	Did not dissociate at NPC	53	**38 ± 5**	38 (72 ± 6%)	15 (28 ± 6%)	**48 ± 5**
	Did dissociate at NPC	32		6	26	
65	Did not dissociate at NPC	65	**51 ± 4**	54 (83 ± 5%)	11 (17 ± 5%)	**52 ± 4**
	Did dissociate at NPC	67		9 (13 ± 4%)	58 (87 ± 4%)	
100	Did not dissociate at NPC	58	**47 ± 5**	48 (83 ± 5%)	10 (17 ± 5%)	**46 ± 5**
	Did dissociate at NPC	52		11 (21 ± 6%)	41 (79 ± 6%)	
130	Did not dissociate at NPC	73	**53 ± 4**	65 (89 ± 4%)	8 (11 ± 4%)	**47 ± 4**
	Did dissociate at NPC	81		16 (20 ± 5%)	65 (80 ± 4%)	
160	Did not dissociate at NPC	43	**43 ± 6**	30 (70 ± 7%)	13 (30 ± 7%)	**51 ± 6**
	Did dissociate at NPC	33		4 (12 ± 6%)	29 (88 ± 6%)	
320	Did not dissociate at NPC	72	**48 ± 4**	65 (90 ± 4%)	7 (10 ± 4%)	**47 ± 4**
	Did dissociate at NPC	66		8 (12 ± 4%)	58 (88 ± 4%)	
1300	Did not dissociate at NPC	230	**55 ± 2**	189 (82 ± 2%)	41 (18 ± 2%)	**53 ± 2**
	Did dissociate at NPC	280		53 (19 ± 2%)	227 (81 ± 2%)	
+25% Glycerol						
0.1 nM Alexa Fluor 568-NLS-2xGFP(4C) and 250 nM Alexa Fluor 647-Imp α1						
0	Did not dissociate at NPC	128	**0**	59 (46 ± 4%)	69 (54 ± 4%)	**54 ± 4**
	Did dissociate at NPC	0		0	0	
5	Did not dissociate at NPC	177	**8 ± 2**	101 (57 ± 5%)	76 (43 ± 5%)	**45 ± 4**
	Did dissociate at NPC	16		5 (31 ± 11%)	11 (69 ± 11%)	
20	Did not dissociate at NPC	189	**35 ± 3**	134 (71 ± 3%)	55 (29 ± 3%)	**46 ± 4**
	Did dissociate at NPC	102		24 (24 ± 4%)	78 (76 ± 4%)	
80	Did not dissociate at NPC	112	**51 ± 3**	91 (81 ± 4%)	21 (19 ± 4%)	**49 ± 3**
	Did dissociate at NPC	117		25 (21 ± 4%)	92 (79 ± 4%)	
150	Did not dissociate at NPC	123	**59 ± 3**	96 (78 ± 4%)	27 (22 ± 4%)	**51 ± 3**
	Did dissociate at NPC	178		52 (29 ± 3%)	126 (71 ± 3%)	
1300	Did not dissociate at NPC	215	**61 ± 2**	178 (83 ± 3%)	37 (17 ± 3%)	**52 ± 2**
	Did dissociate at NPC	342		87 (25 ± 2%)	255 (75 ± 2%)	
–Glycerol						
0.1 nM Alexa Fluor 568-Imp α1 and 250 nM Alexa Fluor 647-NLS-2xGFP(4C)						
0.1	Did not dissociate at NPC	53	**4 ± 3**	31 (58 ± 7%)	22 (42 ± 5%)	**44 ± 7**
	Did dissociate at NPC	2		0	2	
1	Did not dissociate at NPC	62	**5 ± 3**	35 (54 ± 6%)	27 (46 ± 6%)	**46 ± 6**
	Did dissociate at NPC	3		0	3	
10	Did not dissociate at NPC	71	**13 ± 4**	38 (54 ± 5%)	33 (46 ± 5%)	**52 ± 6**
	Did dissociate at NPC	11		1	10	
50	Did not dissociate at NPC	57	**44 ± 5**	40 (70 ± 6%)	17 (30 ± 6%)	**49 ± 5**
	Did dissociate at NPC	44		12 (27 ± 7%)	32 (73 ± 7%)	
100	Did not dissociate at NPC	56	**53 ± 5**	51 (91 ± 4%)	5 (8 ± 4%)	**50 ± 5**
	Did dissociate at NPC	63		9 (14 ± 4%)	54 (86 ± 4%)	
500	Did not dissociate at NPC	43	**56 ± 5**	32 (74 ± 7%)	11 (26 ± 7%)	**53 ± 5**
	Did dissociate at NPC	55		14 (25 ± 6%)	41 (75 ± 6%)	

[Ran] = 2 μM; [GTP] = 1 mM; [Imp β1] = 0.5 μM; [NTF2] = 1 μM.

Table S2. Dissociation of Imp α1/cargo complexes in the absence of RanGTP and CAS

Destination	*n*	Interaction time, ms	Transport efficiency*	Number dissociated	Dissociation efficiency*
Nucleoplasm	87	27 ± 4	45 ± 4%	0	0%
Cytoplasm	107	28 ± 3		0	

[Alexa Fluor 568–NLS–2xGFP(4C)] = 0.1 nM; [Alexa Fluor 647–Imp α1] = 250 nM; [Imp β] = 0.5 μM; [NTF2] = 1 μM.
*Transport and dissociation efficiencies were calculated from the data in the nucleoplasm and cytoplasm rows.