Electronic Supplementary Material for

Substrate protein dependence of GroEL−GroES interaction cycle revealed by high-speed AFM imaging

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This PDF file includes:

Other Electronic Supplementary Materials for this manuscript include the followings:

Materials and Methods

Materials

We purchased following materials and used without further purification: tamavidin 2-LPI, Wako Pure Chemical (Osaka, Japan); GroES, Takara Bio (Shiga, Japan); rhodanese and polyethyleneglycol 8000 (average molecular weight: 7,000 – 9,000), Sigma (St. Louis, MO); glutaraldehyde (25% in water), nacalai tesque (Kyoto, Japan); biotin-$PEAC_5$-maleimide, Dojindo (Kumamoto, Japan). Zip competent *E. coli* cells BL21 (DE3) (BioDynamics Laboratory, Tokyo, Japan) were used for protein expression.

Protein expression and purification

GroEL D490C was expressed as a C-terminal hexahistidine-tagged protein [1] and purified by TALON metal affinity resin (Clontech, Mountain View, CA). The DNA encoding GroEL D490C was inserted into a pET-21a plasmid (Novagen, Madison, WI) at the NdeI and XhoI sites. Protein expression was induced by 0.5 mM isopropyl- β-D-thiogalactopyranoside (IPTG) at 37°C for 2.5 h. The *E. coli* cells were harvested by centrifugation and lysed by sonication in a buffer containing 50 mM Tris-HCl (pH 7.4), 500 mM NaCl, and 0.1 mM (*p*-amidinophenyl)methanesulfonyl fluoride (*p*-APMSF). After removal of insoluble cell debris by centrifugation, the supernatant was applied to TALON metal affinity resin. After washing the resin with a buffer containing 50 mM Tris-HCl (pH 7.4), 500 mM NaCl, and 5 mM imidazole, the protein was eluted by adding a buffer containing 50 mM Tris-HCl (pH 7.4), 500 mM NaCl, and 150 mM imidazole. The purity of GroEL D490C was confirmed by SDS-PAGE. Eluted GroEL D490C was concentrated in a buffer containing 50 mM HEPES-KOH pH 7.4, 50 mM KCl, 10 mM $MgCl_2$, and 2 mM tris(2-carboxyethyl)phosphine (TCEP) using a Vivaspin 6 (50 kDa MWCO) (GE Healthcare, Buckinghamshire, UK). The concentrated GroEL D490C was labeled with biotin by the reaction with biotin-$PEAC_5$-maleimide for 30 min at 25°C as previously described [2] and then stored at –80°C.

Double mutant (V8G/Y283D) maltose binding protein (DM-MBP) [3] was expressed as a protein with an N-terminal FLAG-tag, which was cleaved after purification. DNA encoding DM-MBP was inserted into an in-house modified pASG-IBA62 plasmid containing a TEV protease cleavage site downstream of an N-terminal FLAG tag using In-Fusion cloning kit (Takara Bio). Protein expression was induced by 0.2 μg/mL of anhydrotetracycline at 18°C overnight. The protein was purified by anti-FLAG M2 affinity gel (Sigma). After purification, DM-MBP containing an N-terminal FLAG-tag was concentrated by Vivaspin and incubated with TEV protease with a hexahistidine tag (Applied Biological Materials, Richmond, Canada) at 30°C for 3 h in a buffer containing 25 mM Tris-HCl (pH 8.0) and 1 mM DTT. After cleavage of FLAG-tag, TEV protease was removed by Ni-NTA agarose resin (Invitrogen, Carlsbad, CA). The purity of DM-MBP was confirmed by

SDS-PAGE. Then DM-MBP was concentrated using Vivaspin 500 (30 kDa MWCO) and denatured in 6 M guanidine-HCl and 5 mM DTT. Rhodanese was purchased from Sigma and denatured in 6 M guanidine-HCl and 5 mM DTT.

Mica-supported tamavidin 2-LPI 2D crystals

Tamavidin 2-LPI 2D crystals were prepared on a mica surface directly. 1 mg of tamavidin 2-LPI was dissolved in 0.5 mL water (2.0 mg/mL) and stored at 4°C. Before each observation, tamavidin 2-LPI solution was diluted to 0.12 mg/mL in a crystallization buffer (20 mM HEPES-NaOH pH 8.5, 400 mM NaCl, 200 mM $MgCl_2$, and 20% (w/v) polyethylene glycol 8000) and mixed gently and thoroughly. After incubation for 30−50 min at 21°C, a drop (2 μL) of the diluted tamavidin 2-LPI was deposited on a freshly cleaved mica surface and incubated for 5 min. After rinsing the mica surface with the crystallization buffer without polyethylene glycol, a drop (2 μL) of 2% glutaraldehyde diluted by 20 mM HEPES-NaOH pH 8.0 was deposited on mica for 5 min. Then the mica was rinsed with solution A (25 mM HEPES-KOH pH 7.4, 100 mM KCl, and 5 mM $MgCl_2$).

HS-AFM imaging

Observations were performed in the tapping mode using a lab-made HS-AFM apparatus [4]. The biotinylated GroEL D490C diluted to ~80 nM (as 14 mer) was deposited onto the tamavidin 2-LPI 2D crystal surface. After 5 min of incubation, unattached GroEL was washed out with solution A. HS-AFM imaging conditions were as follows: scan size, 64 × 80 nm^2; pixel size, 64 × 80 pixels; imaging rate, ~4.35 frames/sec (fps). For the observation of GroEL-GroES interaction in the absence of SP, imaging was performed in solution B (25 mM HEPES-KOH pH 7.4, 100 mM KCl, 5 mM $MgCl_2$, 2 mM ATP, and 1 μM GroES). For observation in the presence of DM-MBP, 80 μM denatured DM-MBP in 6 M guanindine-HCl and 5 mM DTT was diluted to 0.2 μM in solution B just before each observation. For the observation in the presence of rhodanese, 100 μM denatured rhodanese in 6 M guanindine-HCl and 5 mM DTT was diluted to 0.1 μM in solution B just before each observation.

Data fitting

The histograms of lifetime of intermediate species were fitted to single-exponential functions, except for Type II football. The histograms of lifetime of Type II football were fitted to a sequential two-step reaction model. The histograms of residence time of bound GroES were fitted to a sequential three-step reaction model, except for the case of GroES undergoing the main pathway, i.e., $F \rightarrow B^{\uparrow} \rightarrow F \rightarrow B^{\downarrow}$. The histograms of residence time of bound GroES undergoing the main pathway were fitted to a sequential four-step reaction model. The details of this fitting procedure are well described in the supplementary information of our previous work [5].

References

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2. Yamamoto D, Nagura N, Omote S, Taniguchi M, Ando T. 2009 Streptavidin 2D crystal substrates for visualizing biomolecular processes by atomic force microscopy. *Biophys. J.* **97**, 2358-2367.
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4. Uchihashi T, Kodera N, Ando T. 2012 Guide to video recording of structure dynamics and dynamic processes of proteins by high-speed atomic force microscopy. *Nat. Protoc.* **7**, 1193-1206.
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Movie S1. HS-AFM movie of GroEL−GroES interaction during steady-state ATPase reaction cycle in the presence of DM-MBP. D490C GroEL biotinylated at Cys490 was tethered onto a surface of tamavidin 2-LPI 2D crystals in a side-on orientation through a linker with length of ~2.5 nm, while GroES and denature DM-MBP were free in the bulk solution. The images were acquired at 4.35 fps for an area of 64 × 80 nm^2.

Movie S2. HS-AFM movie of GroEL−GroES interaction during steady-state ATPase reaction cycle in the presence of rhodanese. D490C GroEL biotinylated at Cys490 was tethered onto a surface of tamavidin 2-LPI 2D crystals in a side-on orientation through a linker with length of ~2.5 nm, while GroES and denature rhodanese were free in the bulk solution. The images were acquired at 4.35 fps for an area of 64 × 80 nm^2.

Movie S3. HS-AFM movie of GroEL−GroES interaction during steady-state ATPase reaction cycle in the absence of SP. D490C GroEL biotinylated at Cys490 was tethered onto a surface of tamavidin 2-LPI 2D crystals in a side-on orientation through a linker with length of ~2.5 nm, while GroES was free in the bulk solution. The images were acquired at 4.35 fps for an area of 64 × 80 nm^2.

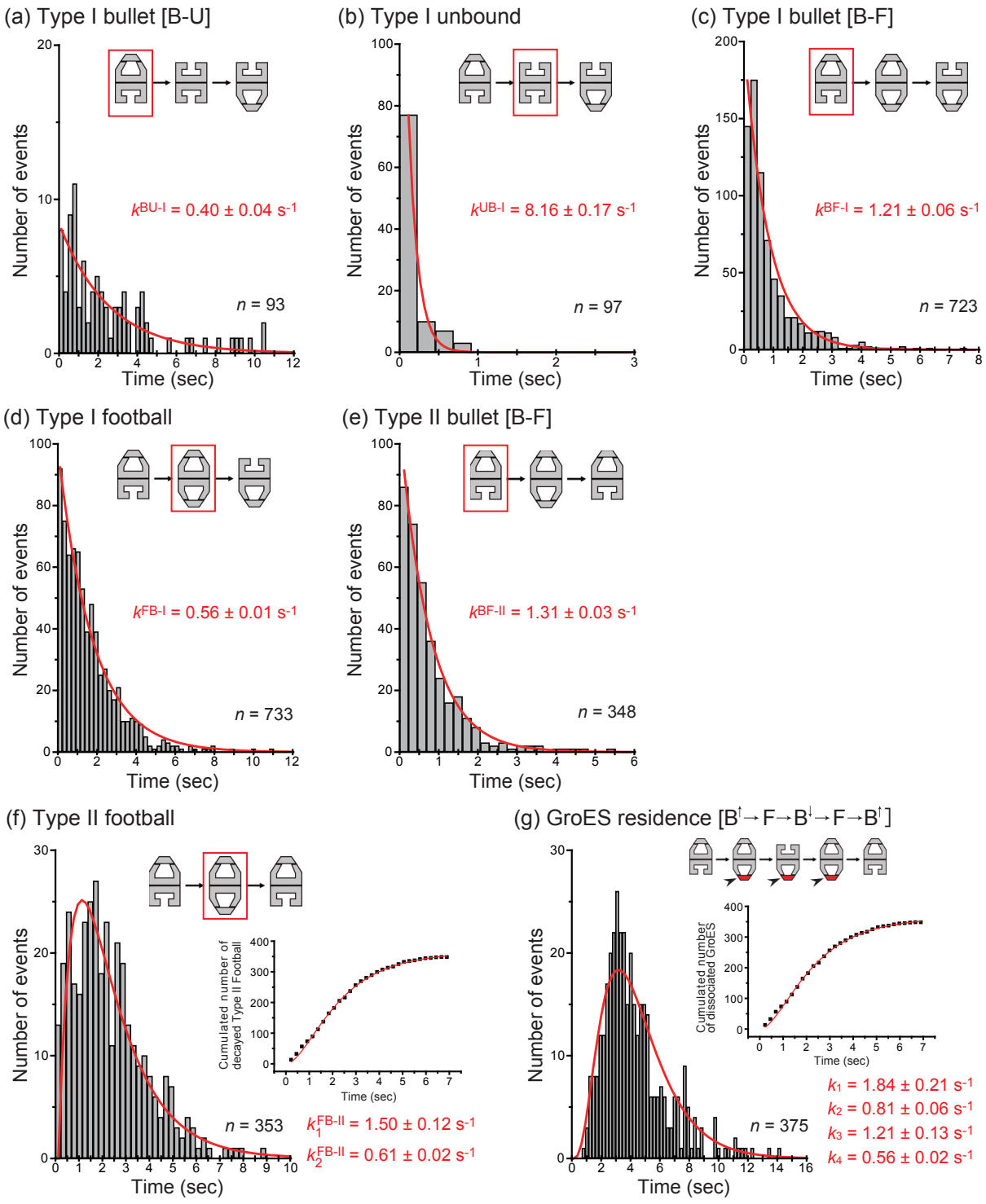

Figure S1. Histograms of lifetime of intermediate species and residence time of bound GroES observed in the absence of SP. The red curves overlaid on the histograms are those calculated using rate constants obtained by fitting. Each inset illustration in (a−f) shows a corresponding sequential pattern in which the intermediate concerned (surrounded with the red rectangle) appears. The inset illustration in (g) shows a sequential pattern, for which the residence time of the bound GroES (shown in red) is analyzed. "*n*" attached to each histogram indicates the total number of events used for the lifetime analysis. The graphs inserted in (f,g) show the cumulated numbers of corresponding events together with curves calculated using rate constants obtained by fitting of their histograms to corresponding models (f, sequential two-step reaction; g, four-step reaction).

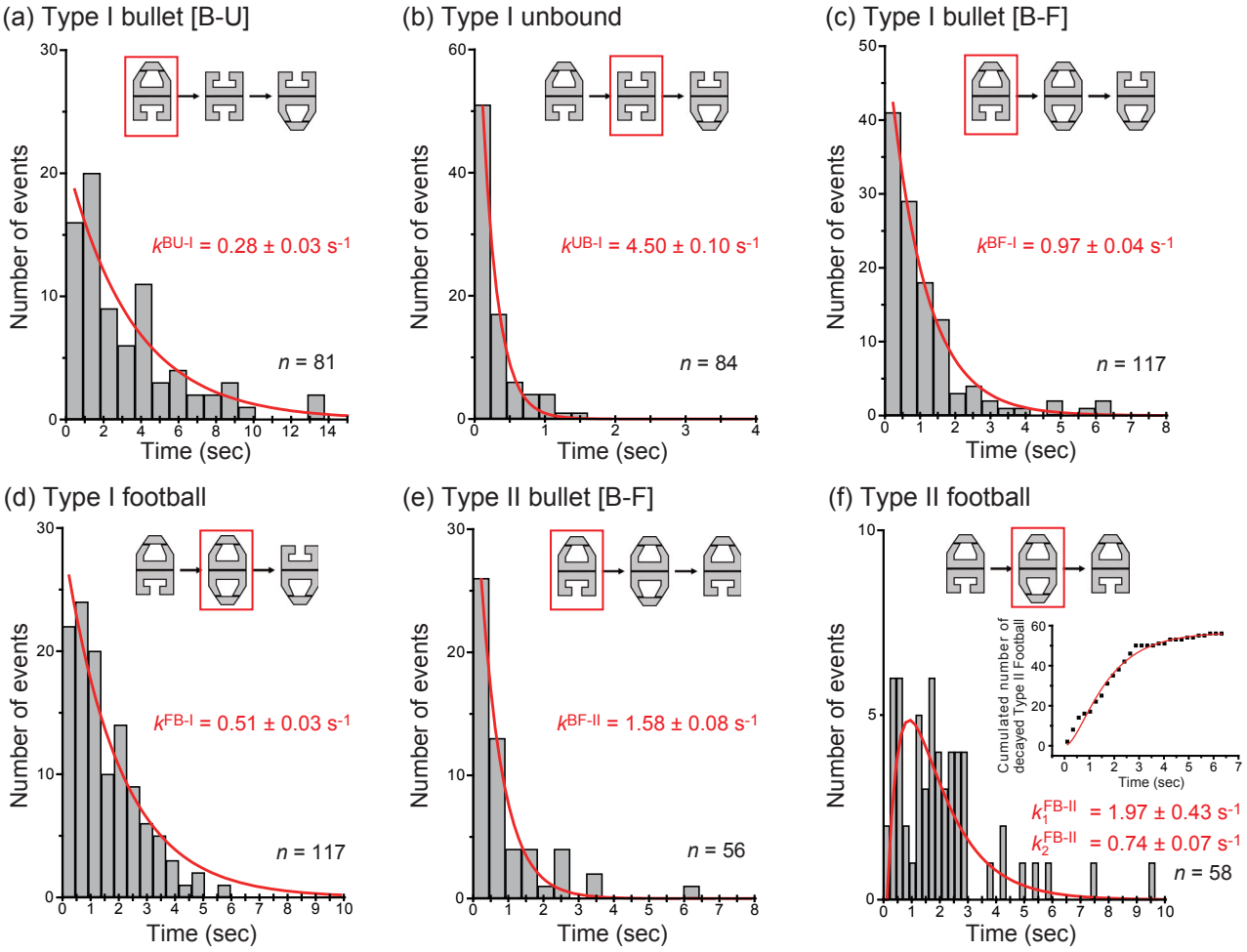

Figure S2. Histograms of lifetime of intermediate species observed in the presence of rhodanese. The curves overlaid on the histograms are those calculated using rate constants obtained by fitting. Each inset illustration in (a−f) shows a corresponding sequential pattern in which the intermediate concerned (surrounded with the red rectangle) appears. "*n*" attached to each histogram indicates the total number of events used for the lifetime analysis. The graph inserted in (f) show the cumulated number of events together with a curve calculated using rate constants obtained by fitting of the histogram to a sequential two-step reaction model.

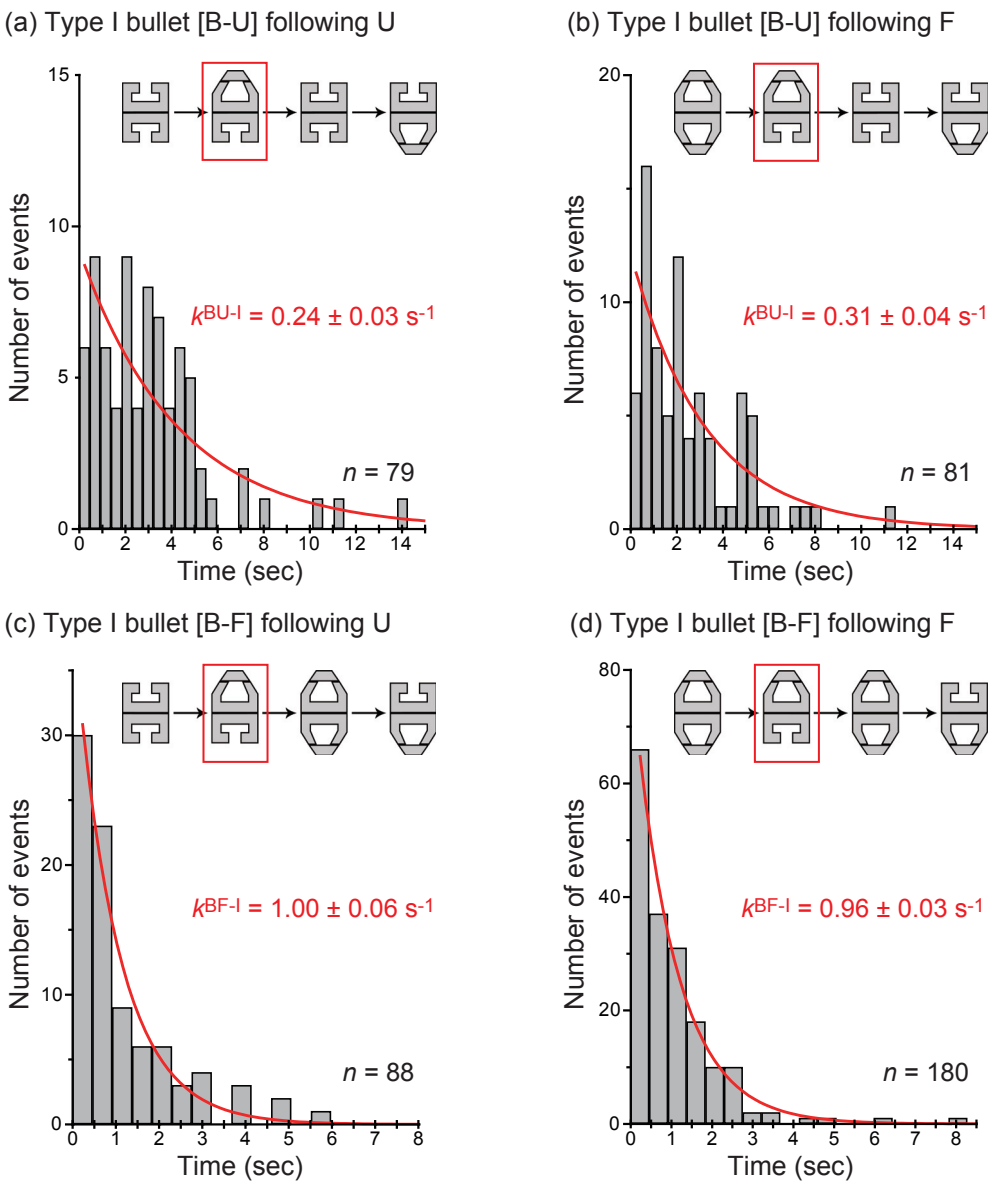

Figure S3. Dependence of lifetime of bullet complexes on the reaction pathways appeared in Type I process in the presence of DM-MBP. Bullet complexes are classified according to the pathways in which the bullet complexes concerned appeared: (a) U→B→U, (b) F→B→U, (c) U→B→F and (d) F→B→F (see the inset illustrations). The red curves overlaid on the histograms are those calculated using rate constants obtained by fitting.

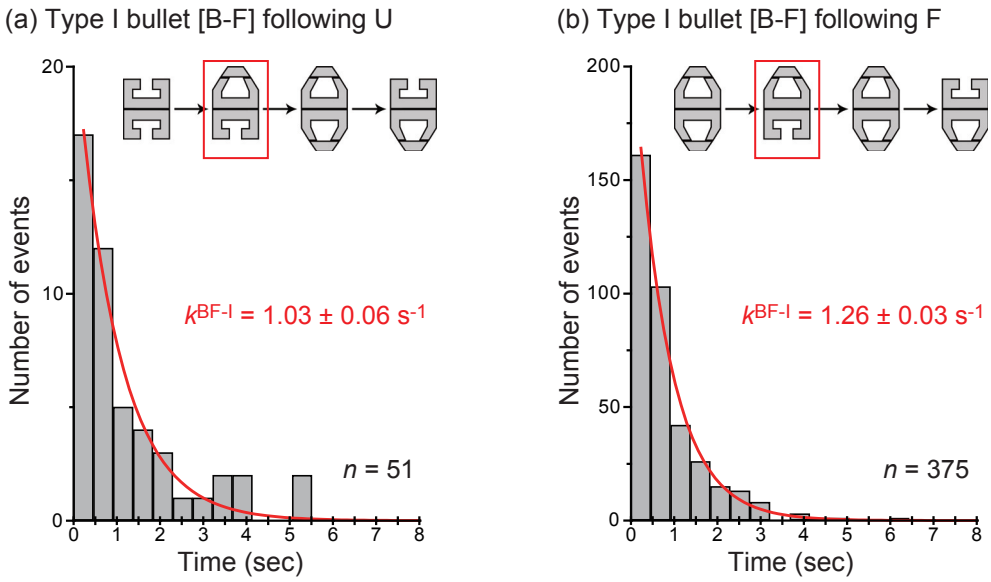

Figure S4. Dependence of lifetime of bullet complexes on the reaction pathways appeared in Type I process in the absence of SP. Bullet complexes are classified according to the pathways in which the bullet complexes concerned appeared: (a) U→B→F and (b) F→B→F (see the inset illustrations). The red curves overlaid on the histograms are those calculated using rate constants obtained by fitting. Although bullet complexes also appeared in the other pathways, U→B→U and F→B→U, the number of events observed were too small to be analyzed.

Table S1. The appearance frequency (%) of pathways in which bullet complexes appear in the presence of DM-MBP and in the absence of SP.

			Intermediates after $B^{\uparrow}$					
			Type I			Type II		
			U	F	$B^{\downarrow}$	U	F	*n*
no SP	intermediates before $B^{\uparrow}$	U	5.45	55.45	6.5	1.1	31.5	92
		F	7.3	54.4	14.2	1.2	22.9	689
		U or F	6.9	52.5	13.6	1.7	25.3	1,397
DM-MBP	intermediates before $B^{\uparrow}$	U	28.4	32.0	10.2	6.9	22.5	275
		F	19.1	41.4	14.6	8.9	16.0	425
		U or F	23.7	38.0	13.1	8.6	16.6	1,287

"*n*" indicates the total number of detected transition events that start from the corresponding intermediates appeared before bullet complexes.