Intact Transition Epitope Mapping – Force Interferences by Variable Extensions (ITEM-FIVE)

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Supplement

Supplemental Table S1: Ion intensities, charge states, and m/z values for the F-F-F homotrimer at various collision cell voltage difference settings. **1st determination** (15072021_Foldon_Pep1_MSMS_1849_02.raw)

ion	z	m/z	0V	2V	4V	6V	8V	10V	12V	15V	18V	22V	26V	30V	35V	40V	45V	50V	55V
F-F-F	**5+**	1848	92850	65322	111678	125873	132104	128171	139805	123661	77073	14291	378	164	44	42	41	9	/
F-F	**3+**	2053	204	196	182	321	461	635	1810	7754	22584	54422	52233	59050	29306	5965	1516	198	/
F	**2+**	1540	490	498	525	486	1427	3054	7721	31087	95942	238696	258080	357819	421986	441875	399284	585802	/

2nd determination (20072021_Foldon_FBTN_P1_MSMS_1848.raw)

ion	z	m/z	0V	2V	4V	6V	8V	10V	12V	15V	18V	22V	26V	30V	35V	40V	45V	50V	55V
F-F-F	**5+**	1848	/	/	160145	/	228438	/	223072	/	110400	724	304	/	141	/	115	/	100
F-F	**3+**	2053	/	/	334	/	1391	/	5149	/	43963	90997	64632	/	3088	/	80	/	130
F	**2+**	1540	/	/	501	/	4032	/	20916	/	172703	410203	577840	/	708641	/	682144	/	376570

Supplemental Table S2: Ion intensities, charge states, and m/z values for the F-F-bF heterotrimer at various collision cell voltage difference settings. **1st determination** (19072021_Foldon_P1BTN_MSMS_1952_01.raw)

ion	z	m/z	0V	2V	4V	6V	8V	10V	12V	15V	18V	22V	26V	30V	35V	40V	45V	50V	55V
F-F-bF	**5+**	1952	21377	34580	38452	31003	39316	39255	34383	30702	9567	1091	58	41	39	26	20	22	15
F-F	**3+**	2054	70	115	119	189	109	256	490	2123	3573	6544	6184	6523	2699	810	252	56	0
bF	**2+**	1798	0	218	195	271	259	486	1350	4737	8711	13307	12085	19956	20032	27843	29396	26371	21811
F-bF	**3+**	2225	0	0	78	77	87	303	692	1872	4091	5609	5480	5009	2794	978	119	0	0
F	**2+**	1541	102	149	251	256	601	1242	3833	14618	31826	47075	43997	65564	63684	78382	79474	81083	65318

2nd determination (20072021_Foldon_FFBTN_P1_MSMS_1951.raw)

ion	z	m/z	0V	2V	4V	6V	8V	10V	12V	15V	18V	22V	26V	30V	35V	40V	45V	50V	55V
F-F-bF	**5+**	1952	/	/	408760	/	384719	/	403319	/	319882	/	16584	/	266	/	99	/	95
F-F	**3+**	2054	/	/	398	/	522	/	1489	/	15830	/	91527	/	74599	/	11741	/	95
bF	**2+**	1798	/	/	537	/	1097	/	3474	/	37300	/	234565	/	265857	/	440975	/	477642
F-bF	**3+**	2225	/	/	244	/	562	/	1666	/	15678	/	86344	/	76233	/	16104	/	186
F	**2+**	1541	/	/	772	/	1644	/	8535	/	100301	/	609293	/	665798	/	913634	/	953036

Supplemental Table S3: Ion intensities, charge states, and m/z values for the F-bF-bF heterotrimer at various collision cell voltage difference settings. **1st determination** (20072021_Foldon_FBTN_P1_MSMS_2055.raw)

ion	z	m/z	0V	2V	4V	6V	8V	10V	12V	15V	18V	22V	26V	30V	35V	40V	45V	50V	55V
F-bF-bF	**5+**	2054	/	89313	/	/	84556	79738	75526	72486	65392	60604	51672	5215	2760	860	499	126	235
bF-bF	**3+**	2397	/	58	/	/	177	194	388	1050	2173	9035	18432	24319	25440	19185	18473	6062	1361
F	**2+**	1541	/	193	/	/	504	777	1308	2775	8512	26937	66681	90641	111032	105951	161977	132230	156167
F-bF	**3+**	2225	/	175	/	/	353	416	1094	2614	8423	35251	79991	98470	99147	62855	40741	7406	827
bF	**2+**	1798	/	201	/	/	350	711	1292	3444	9399	36182	91335	118962	139491	135419	226755	213665	252786

Supplemental Table S4: Ion intensities, charge states, and m/z values for the bF-bF-bF homotrimer at various collision cell voltage difference settings. **1st determination** (15072021_Foldon_BTN_MSMS_2159_01.raw)

ion	z	m/z	0V	2V	4V	6V	8V	10V	12V	15V	18V	22V	26V	30V	35V	40V	45V	50V	55V
bF-bF-bF	**5+**	2158	534373	1411979	1716272	2454268	2253328	2086819	2219942	2401570	1862383	809933	110522	7599	999	222	104	59	29
bF-bF	**3+**	2397	85	226	413	653	958	1936	2506	5583	15724	67215	697159	1186044	302551	282999	160255	54076	9182
bF	**2+**	1798	0	0	1074	1776	2702	6517	7694	22604	74606	347946	999319	1711191	2170672	2720714	3012266	3512346	3577762

2nd determination (19072021_Foldon_P1FBTN_MSMS_2158.raw)

ion	z	m/z	0V	2V	4V	6V	8V	10V	12V	15V	18V	22V	26V	30V	35V	40V	45V	50V	55V
bF-bF-bF	**5+**	2158	19333	58466	58057	55380	62102	74214	55149	52108	30501	6239	479	260	167	124	107	112	32
bF-bF	**3+**	2397	0	0	0	0	70	100	179	605	3766	6776	5675	5214	4317	2333	139	140	18
bF	**2+**	1798	87	78	133	305	367	580	897	3728	20079	38907	32294	31186	39621	52623	61767	64951	57852

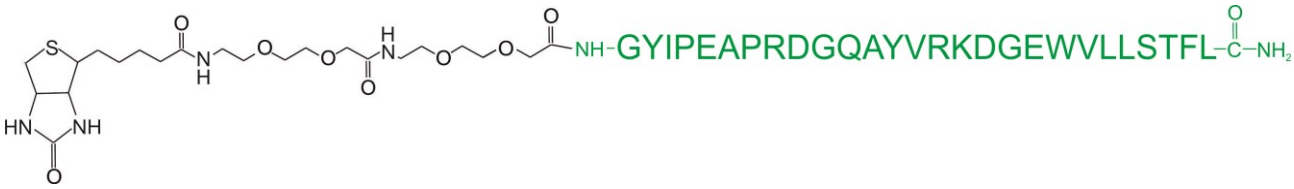
Supplemental Table S5: Biotin contacts with foldon monomer units in T4Ff trimers [a].

	F [b]	bF-bF-bF	F-bF-bF	F-F-bF	F-F-F
in touch with	+1	41.82	63.17	66.10	n.a.
in touch with	0	2.53	2.29	3.12	n.a.
in touch with	-1	2.26	1.00	0.67	n.a.
not in touch	/	62.97	40.12	37.69	n.a.

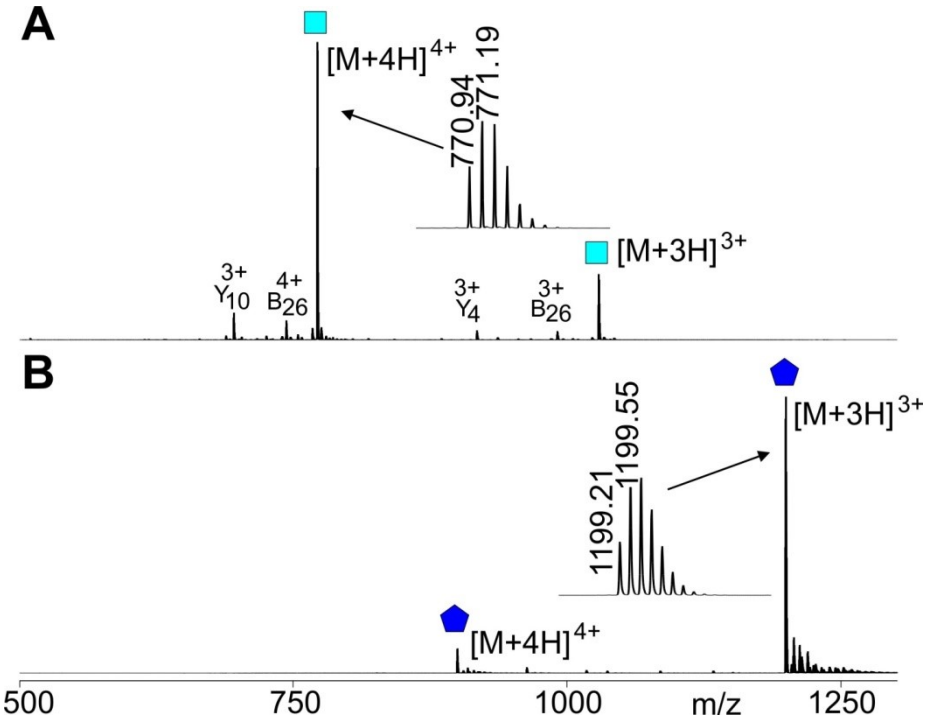
	F [b]	bF-bF-bF	F-bF-bF	F-F-bF	F-F-F
in touch with	+1	68.47	78.47	n.a.	n.a.
in touch with	0	5.91	6.78	n.a.	n.a.
in touch with	-1	0.50	0.23	n.a.	n.a.
not in touch	/	37.94	28.54	n.a.	n.a.

	F [b]	bF-bF-bF	F-bF-bF	F-F-bF	F-F-F
in touch with	+1	53.24	n.a.	n.a.	n.a.
in touch with	0	1.90	n.a.	n.a.	n.a.
in touch with	-1	0.70	n.a.	n.a.	n.a.
not in touch	/	49.36	n.a.	n.a.	n.a.

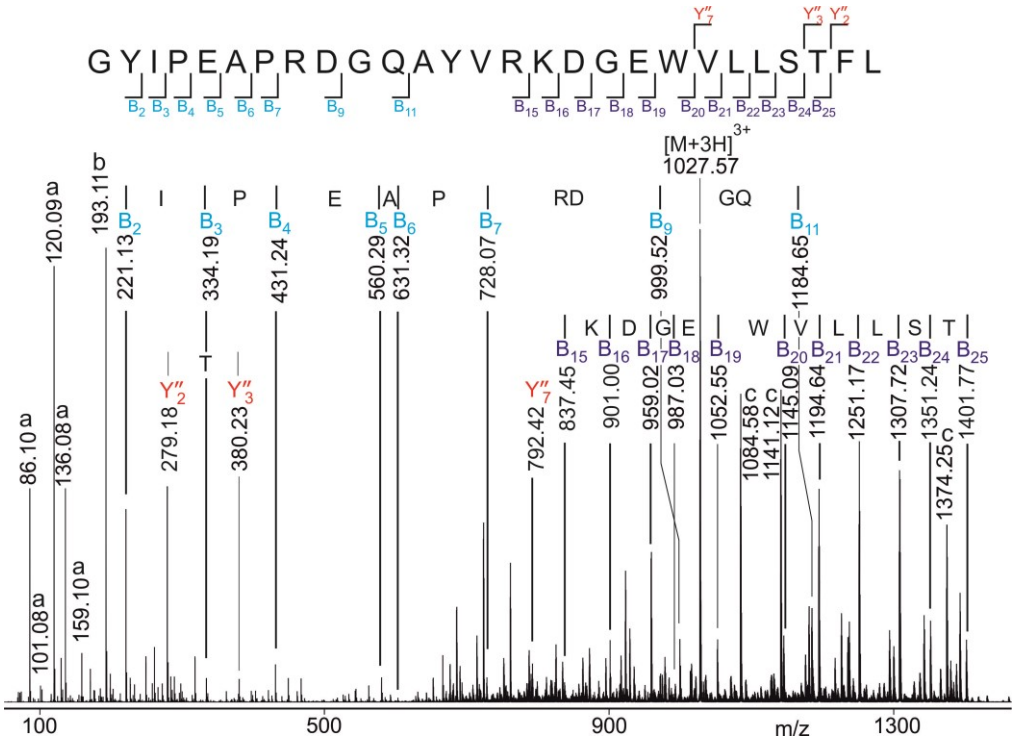
a) numbers in %. Color code as in figure 5.
b) foldon unit: 0: foldon unit to which the biotin moiety is bound to; +1: clock-wise oriented foldon unit to the one to which the biotin moiety is bound to; -1: counter-clock-wise oriented foldon unit to the one to which the biotin moiety is bound to
n.a. not applicable



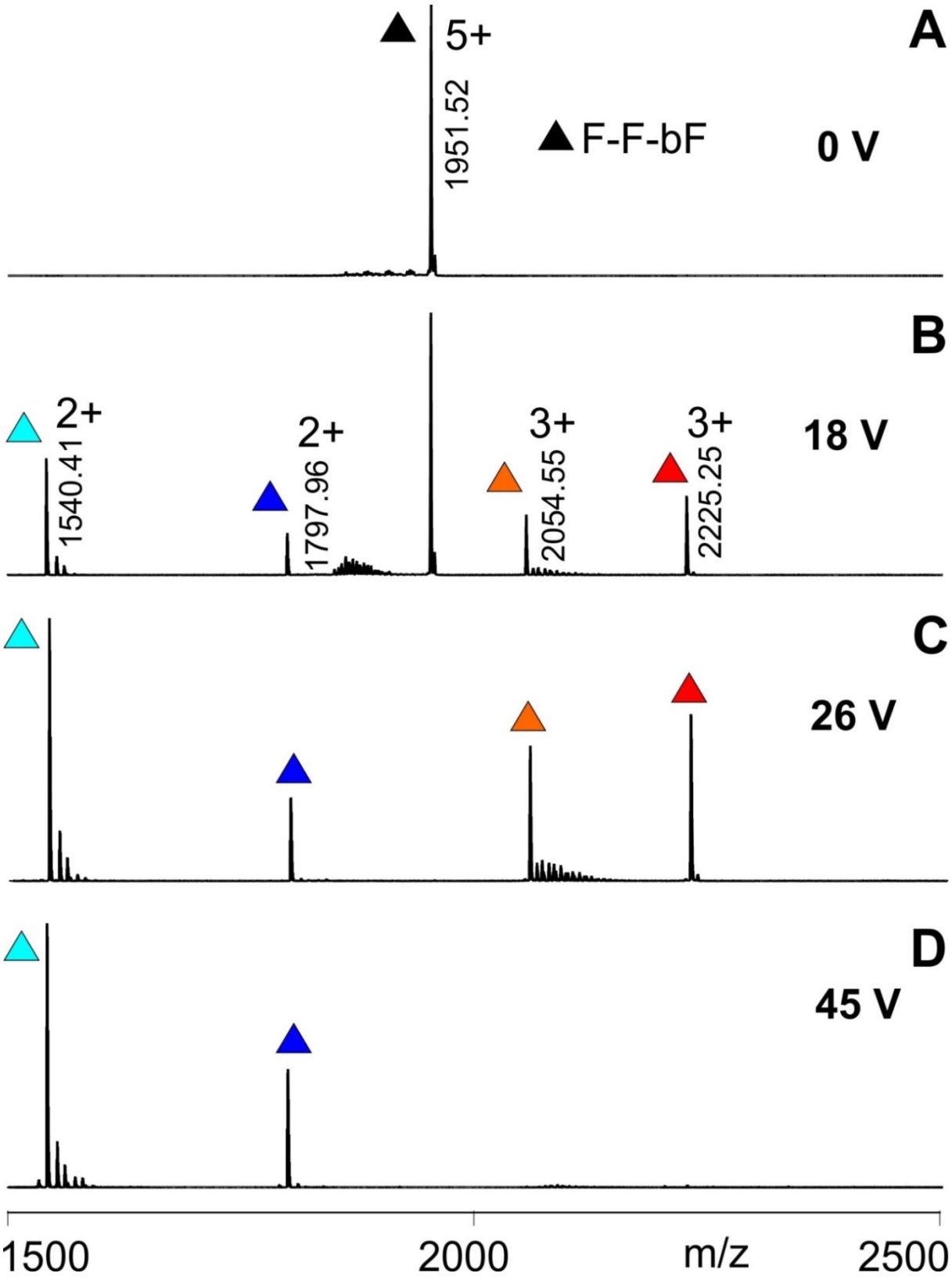
Supplemental Figure S1: Structure scheme of biotin anchored to T4Ff. Biotin is covalently attached to T4Ff's N-terminal amino group via two 8-amino-3,6-dioxa-octanoic acid spacers. T4Ff's amino acid sequence (green) is shown in single letter code and its C-terminal amido group is indicated.



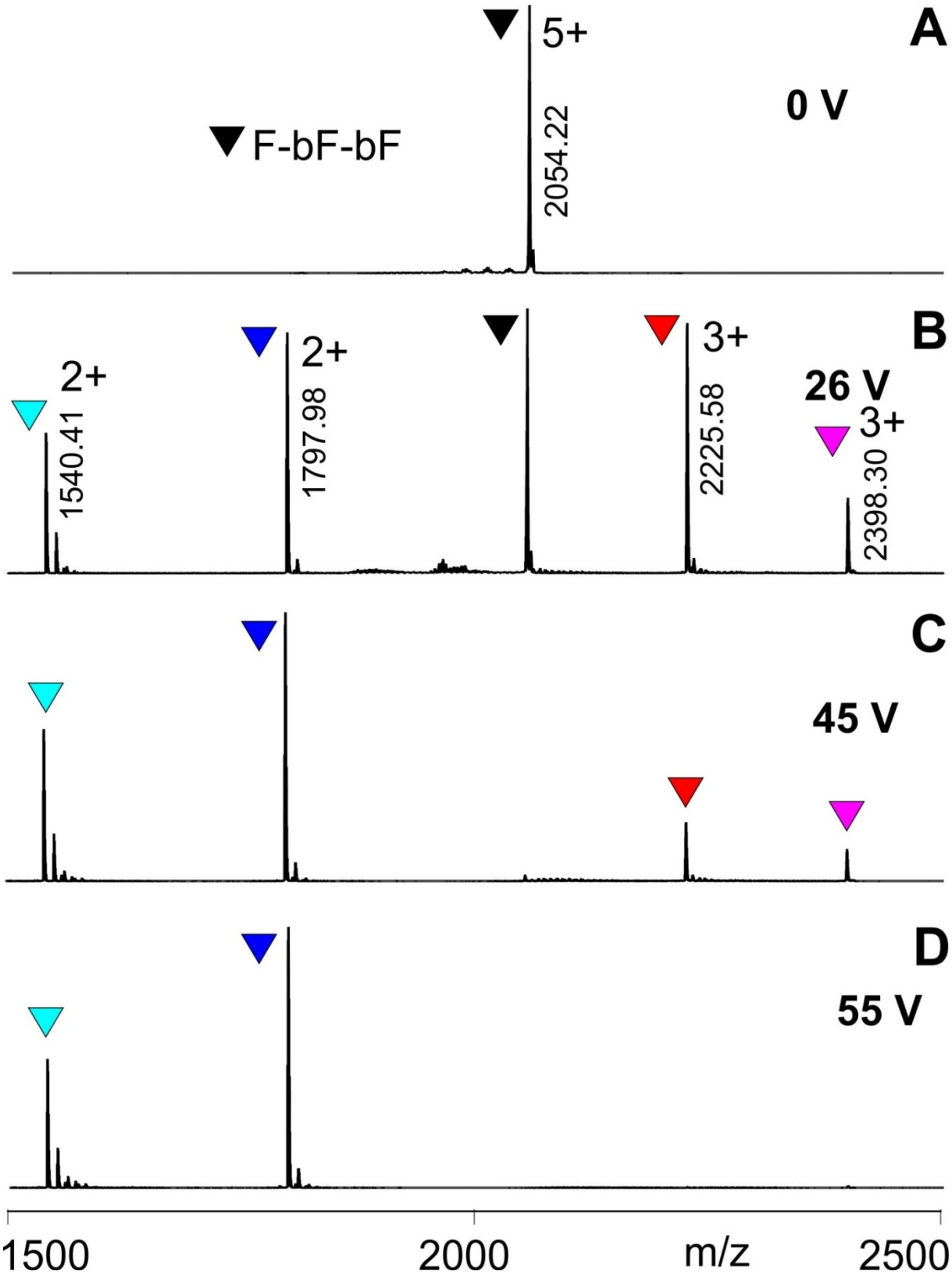
Supplemental Figure S2: Offline nanoESI mass spectra of foldon monomers. **A:** T4Ff (■). **B:** Biotinylated T4Ff (⬟). Protonation states of multiply charged ion signals are given. Zooms show isotope patterns of pseudo-molecular ions and m/z values are given for mono-isotopic ion signals and ion signals with peptides which contain one ^{13}C atom. Fragment ions are labeled. For symbol assignment see Table 1. Solvent: 10% acetic acid / methanol (9:1 v/v), pH 3.



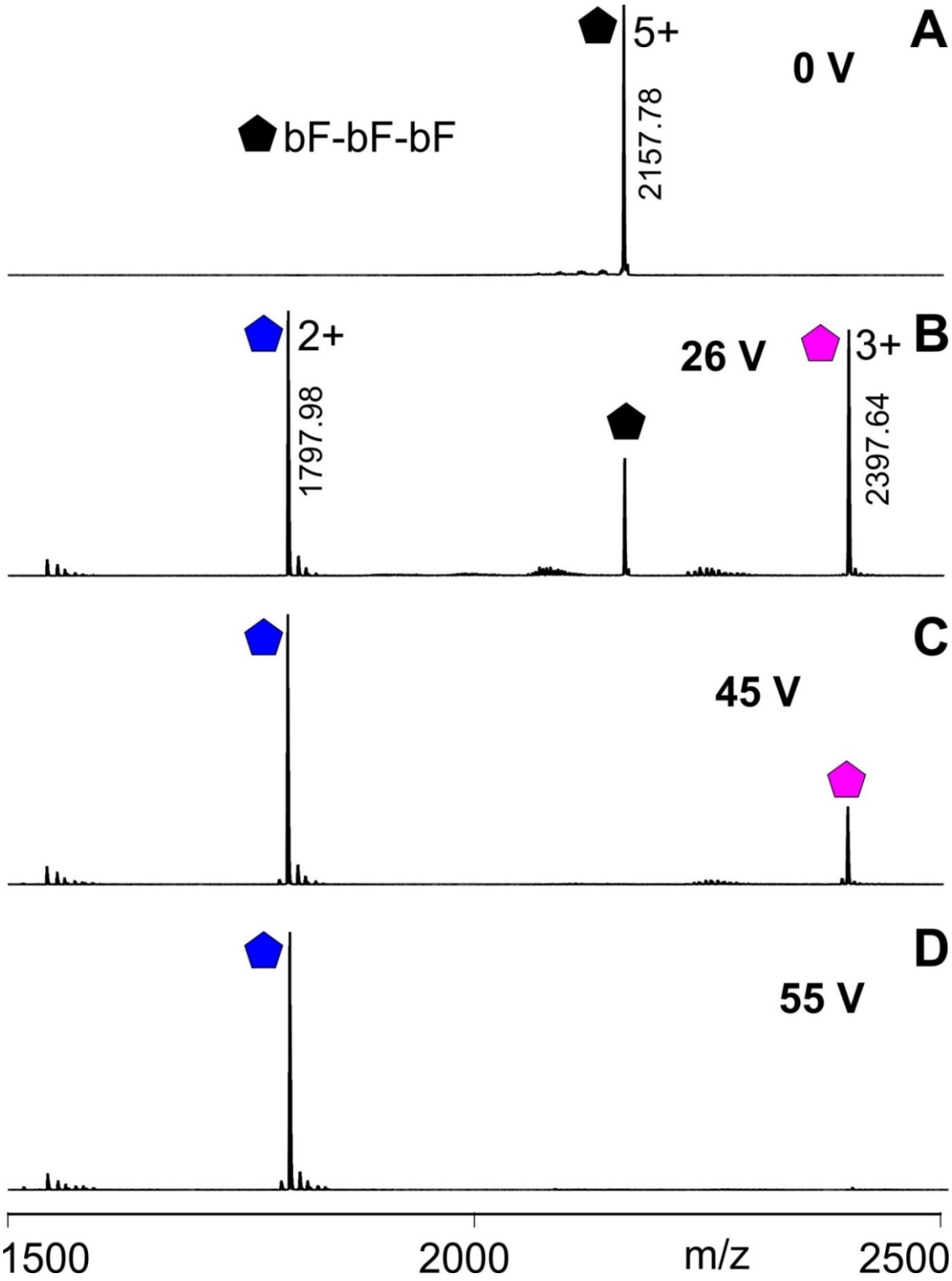
Supplemental Figure S3: Offline nanoESI tandem mass spectrum of foldon monomer. The triply-protonated pseudo-molecular ion of T4Ff served as parent ion. B and Y'' ion signals are labeled and m/z values of selected ion signals are given. Amino acid sequence is shown in single letter code. Solvent: 10% acetic acid / methanol (9:1 v/v), pH 3.



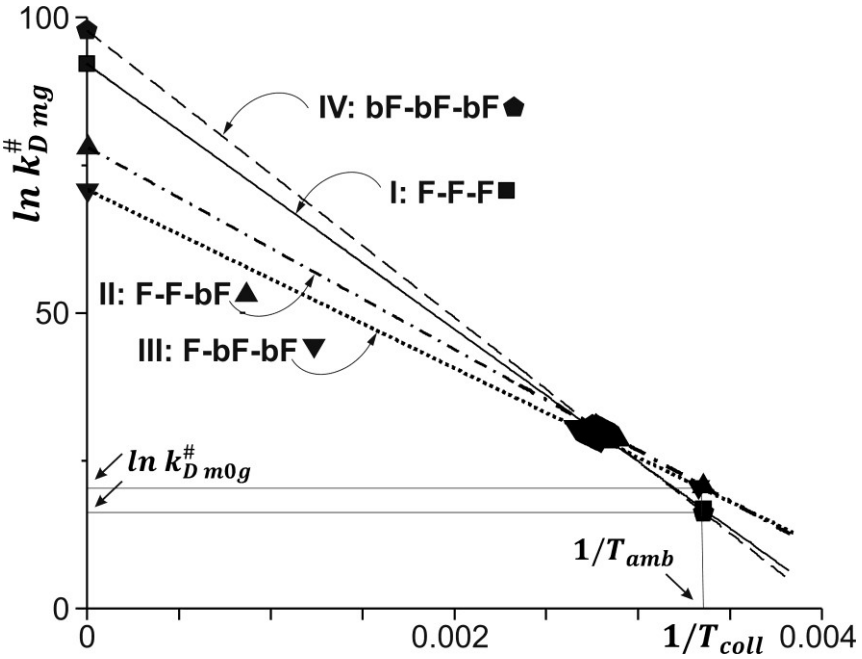
Supplemental Figure S4: ITEM-TWO analysis of quintuply protonated singly biotinylated T4Ff hetero-trimer (F-F-bF). Different collision cell voltage differences (ΔCV) were applied. **A:** 0 V. **B:** 18 V. **C:** 26 V. **D:** 45 V. Charge states and m/z values are given for the singly biotinylated T4Ff trimer ( ) (educts) as well as for the released T4Ff dimers (and) and monomers (and) (products). T4Ff concentration was 0.21 µg/µl. For intensities and m/z values of ion signals see Supplemental Table 2. For symbol assignments see Table 1 and Figure 2.



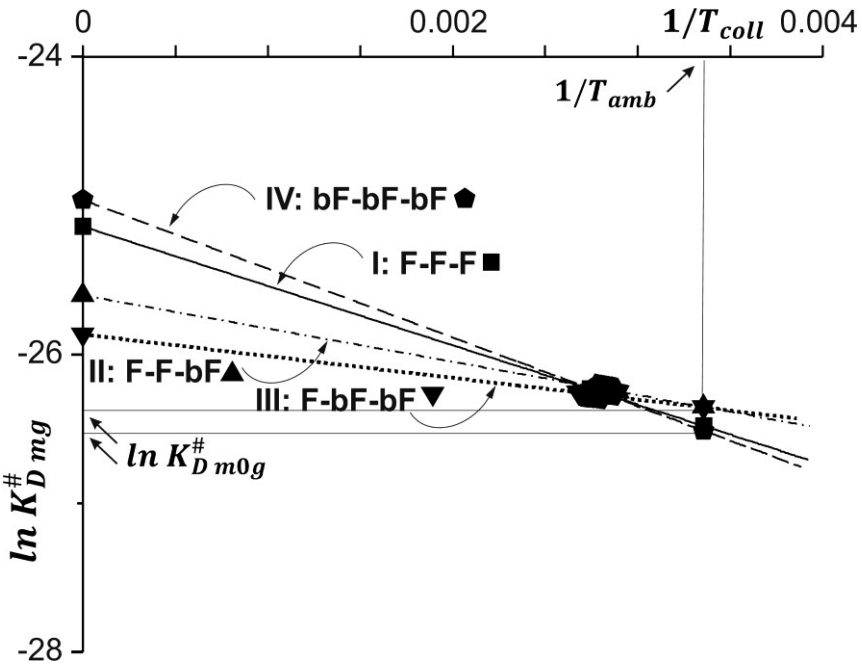
Supplemental Figure S5: ITEM-TWO analysis of quintuply protonated doubly biotinylated T4Ff hetero-trimer (F-bF-bF). Different collision cell voltage differences (ΔCV) were applied. **A:** 0 V. **B:** 26 V. **C:** 45 V. **D:** 55 V. Charge states and m/z values are given for the doubly biotinylated T4Ff trimers (▼) (educts) as well as for the released T4Ff dimers (▼ and ▼) and monomers (▽ and ▼) (products). T4Ff concentration was 0.21 µg/µl. For intensities and m/z values of ion signals see Supplemental Table 3. For symbol assignment see Table 1 and Figure 2.



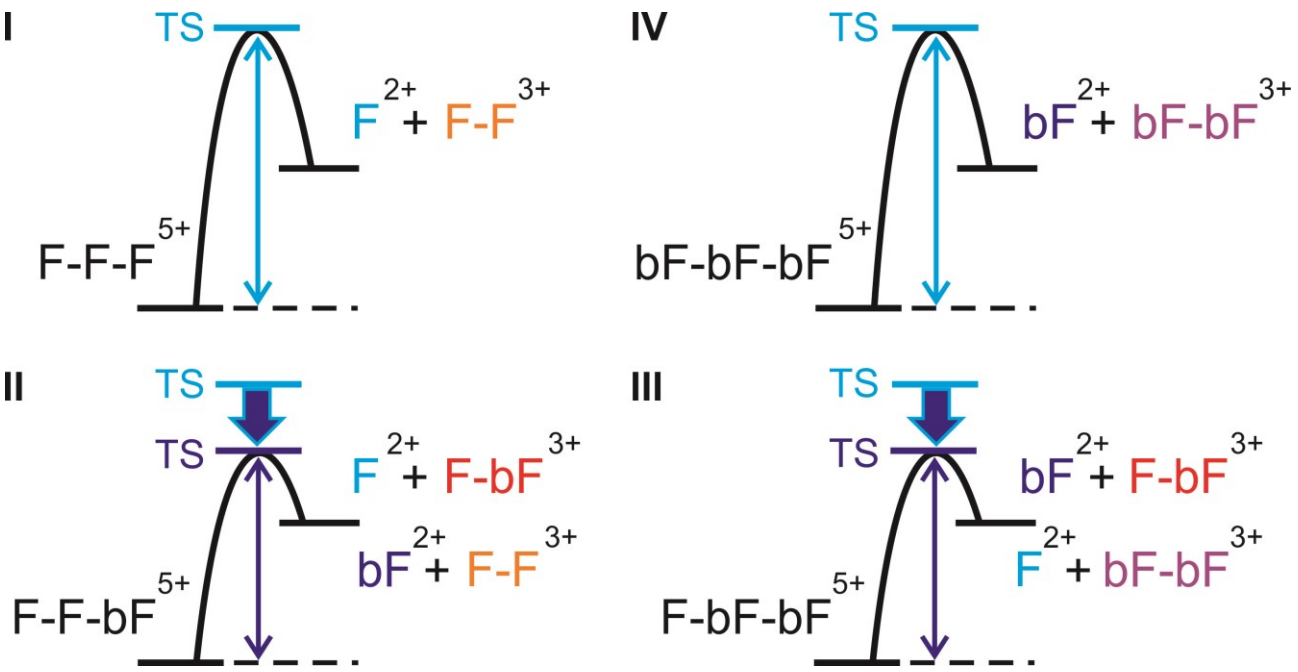
Supplemental Figure S6: ITEM-TWO analysis of quintuply protonated biotinylated T4Ff homo-trimers. Different collision cell voltage differences (ΔCV) were applied. **A:** 0 V. **B:** 26 V. **C:** 45 V. **D:** 55 V. Charge states and m/z values are given for the biotinylated T4Ff trimers (⬟) (educts) as well as for the released biotinylated T4Ff dimers (⬟) and biotinylated monomers (⬟) (products). Biotinylated T4Ff concentration was 0.21 µg/µl. For intensities and m/z values of ion signals see Supplemental Table 4. For symbol assignment see Table 1 and Figure 2.



Supplemental Figure S7: Arrhenius plot for (biotinylated) T4Ff trimer dissociation reactions in the gas phase. T4Ff homo-trimer: ■; singly biotinylated T4Ff hetero-trimer: ▲; doubly biotinylated T4Ff hetero-trimer: ▼; biotinylated T4Ff homo-trimer: ⬟. Each data point (thickened parts of the lines) has been obtained experimentally and corresponding lines have been linearly extrapolated. The values for $ln\ \mathrm{k}^{\#}_{\mathrm{D\ m0g}}$ are taken at $^{1}/_{\mathrm{T_{amb}}}$ (cf. Table 3).



Supplemental Figure S8: Gibbs-Helmholtz plot for (biotinylated) T4Ff trimer dissociation reactions in the gas phase. T4Ff homo-trimer: ■; singly biotinylated T4Ff hetero-trimer: ▲; doubly biotinylated T4Ff hetero-trimer: ▼; biotinylated T4Ff homo-trimer: ⬟. Each data point (thickened parts of the lines) has been obtained experimentally and corresponding lines have been linearly extrapolated. The values for $ln\ \mathrm{k}^{\#}_{\mathrm{D\ m0g}}$ are taken at $^{1}/_{\mathrm{T_{amb}}}$ (cf. Table 3).



Supplemental Figure S9: Energy diagrams showing the apparent enthalpies of activation required by multiply charged and accelerated (biotinylated) T4Ff trimers ($\Delta H^{\#}_{m0g}$) to reach the transition state (TS) before dissociating into products ions. **I:** T4Ff homo-trimer (F-F-F). **II:** singly biotinylated T4Ff hetero-trimer (F-F-bF). **III:** doubly biotinylated T4Ff hetero-trimer (F-bF-bF). **IV:** biotinylated T4Ff homo-trimer (bF-bF-bF). The dark blue arrow indicates lowering of the energy level of the transition states of the hetero-trimers with respect to those of the homo-trimers.