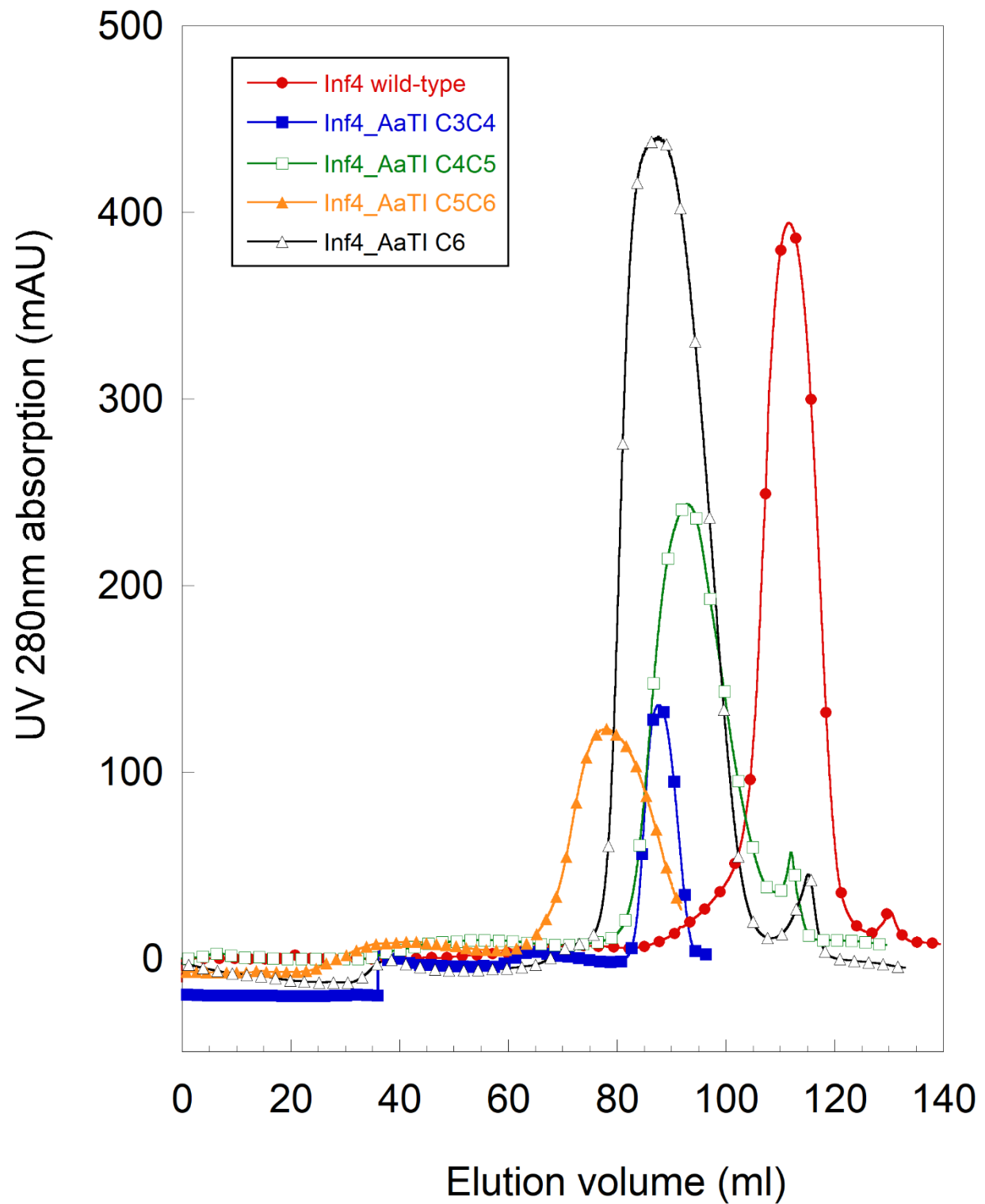


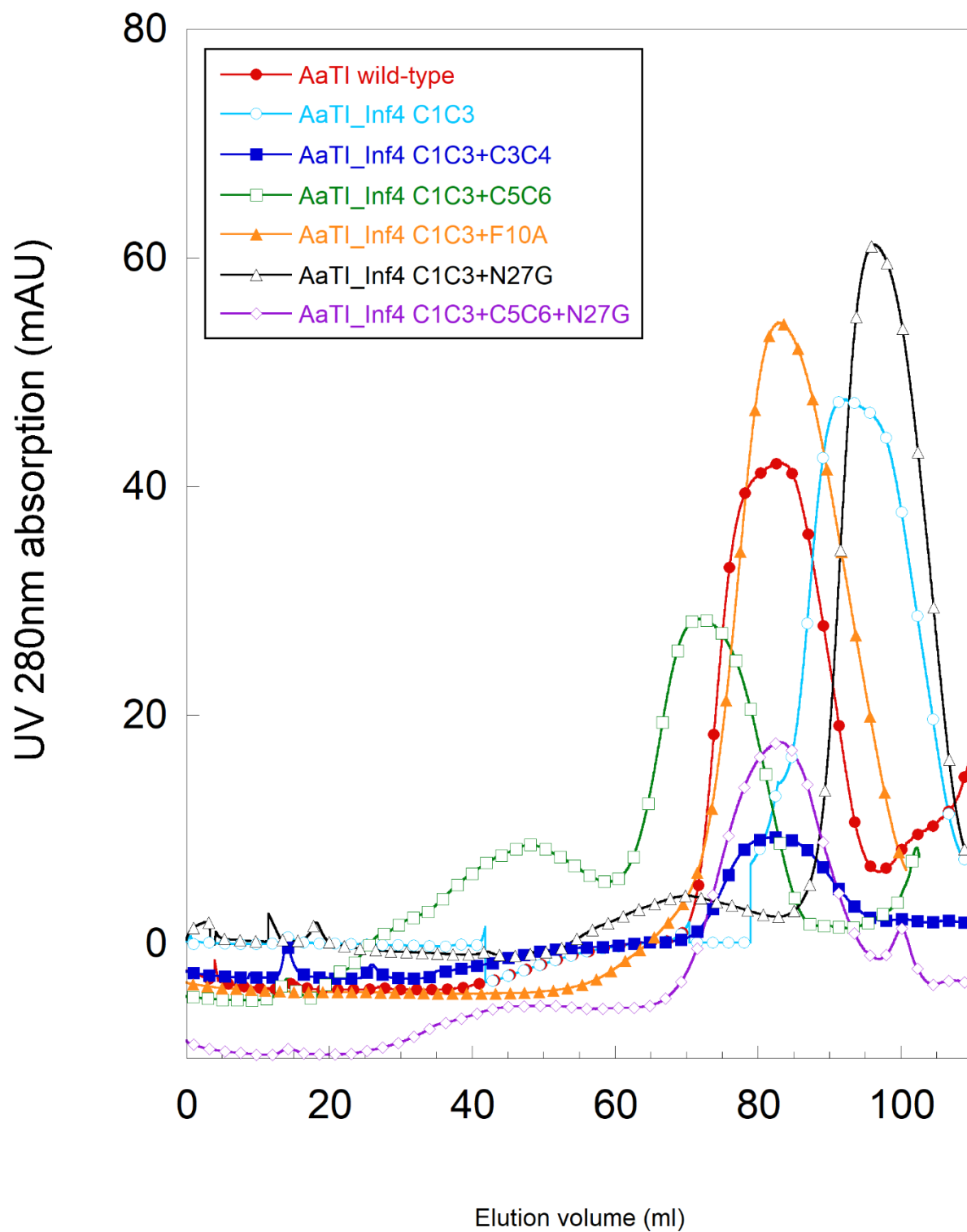
(A)

# Gel-filtration eluciton profiles of Inf4 chimeras



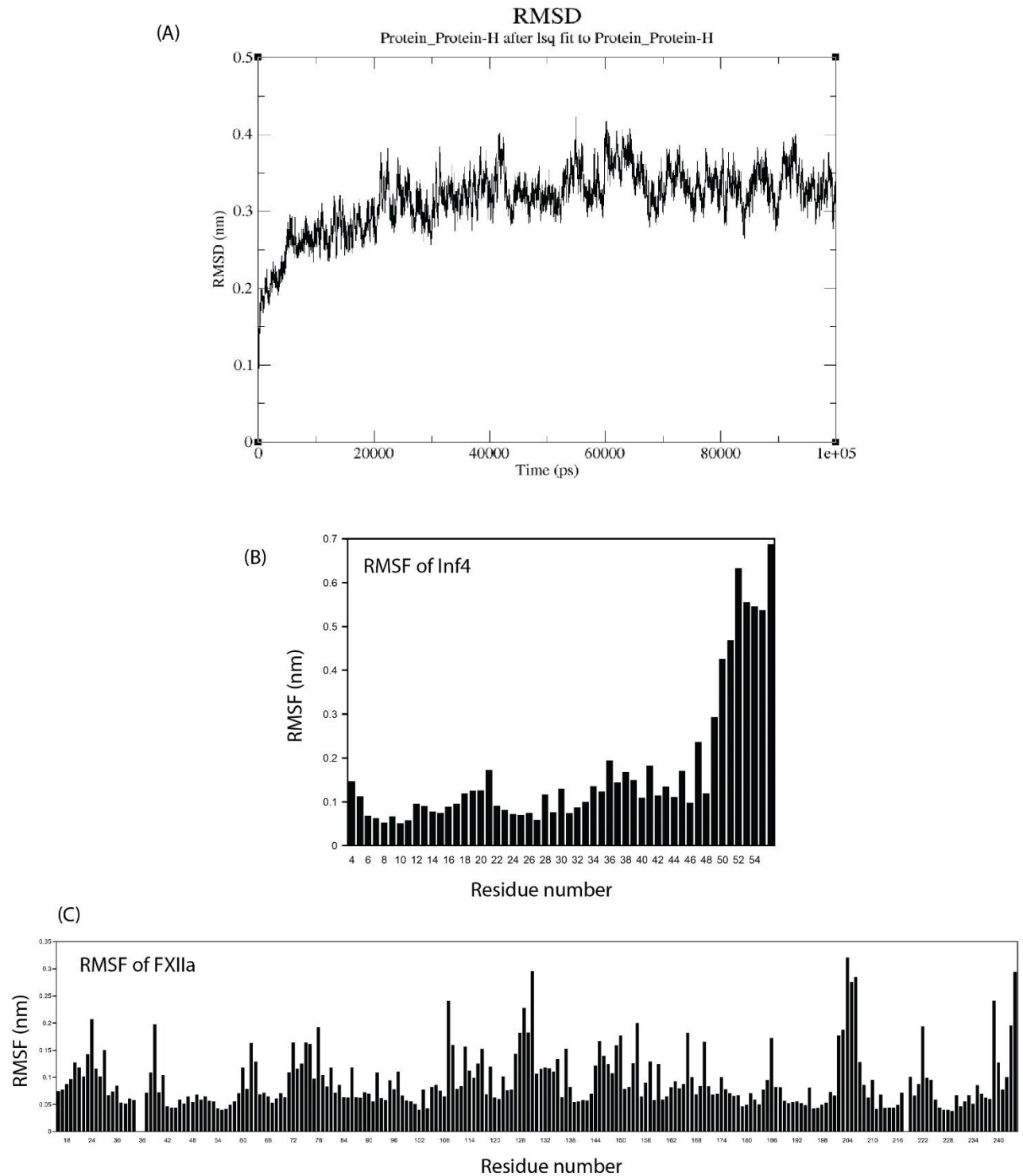
(B)

## Gel-filtration elution profiles of AaTI chimeras



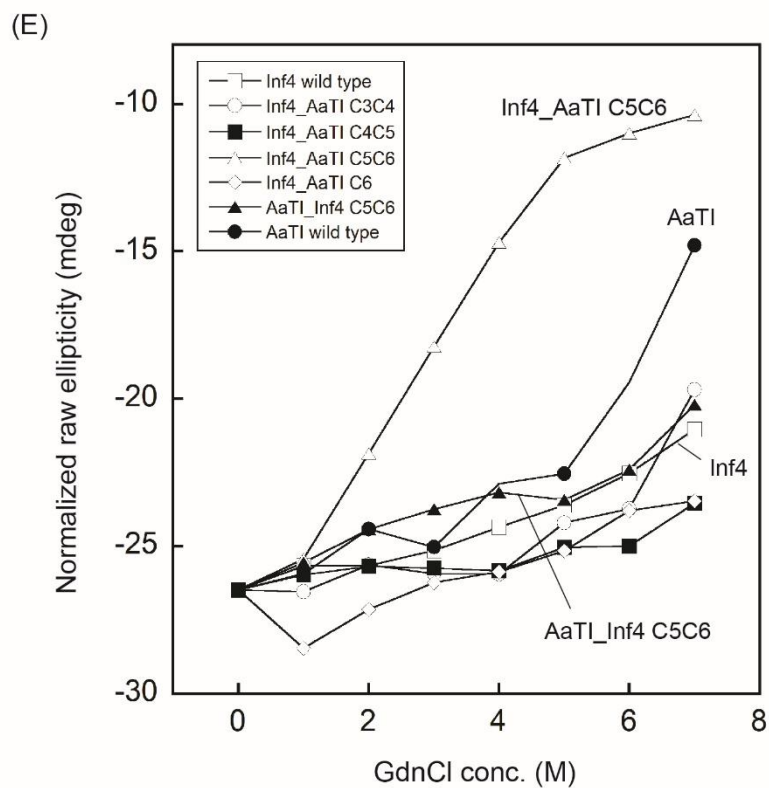
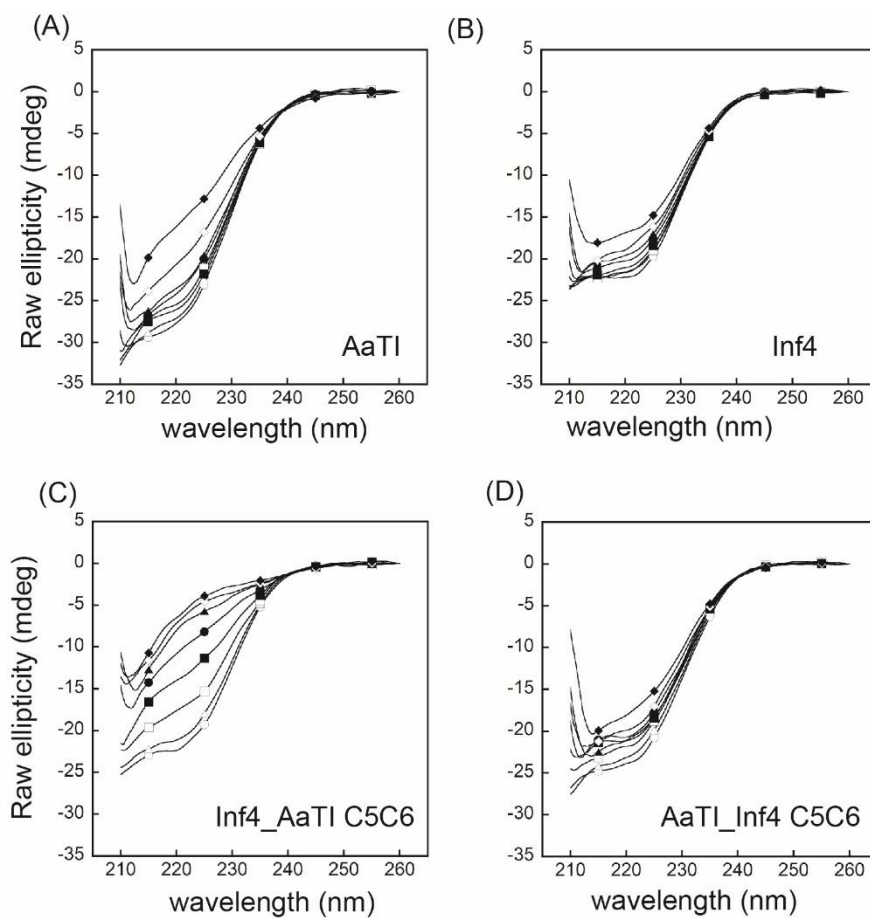
**Figure S1. Gel-filtration elution profiles of wild-type and chimeric Inf4 (A) and AaTI (B).**

(A) Gel-filtration elution profiles of wild-type and chimeric proteins of Inf4: wild-type Inf4 (red, closed circles), Inf4\_AaTI C3C4 (blue, closed squares), Inf4\_AaTI C4C5 (green, open squares), Inf4\_AaTI C5C6 (orange, closed triangles), and Inf4\_AaTI C6 (black, open triangles). (B) Gel-filtration elution profiles of wild-type and chimeric proteins of AaTI: wild-type AaTI (red, closed circles), AaTI\_Inf4 C1C3 (cyan, open circles), AaTI\_Inf4 C1C3+C3C4 (blue, closed squares), AaTI\_Inf4 C1C3+C5C6 (green, open squares), AaTI\_Inf4 C1C3+F10A (orange, closed triangles), AaTI\_Inf4 C1C3+N27G (black, open triangles), and AaTI\_Inf4 C1C3+C5C6+N27G (violet, open rhombuses). Gel-filtration was performed using HiLoad 16/600 Superdex 75 pg columns (16 × 600 mm).



**Figure S2. RMSD and RMSF plots of the Inf4:FXIIa complex from MD-simulation.**

(A) RMSD plot, (B) RMSF plot of Inf4, and (C) RMSF plot of FXIIa of the Inf4:FXIIa complex obtained from MD simulation.



**Figure S3. GdnCl denaturation (FarUV CD ellipticity at 222 nm) of Inf4 and AaTI chimera shows that the unstable scaffold of AaTI is caused by the C5C6 region.**

The protein scaffold of AaTI is less stable due to the elongated C5C6 region. The stability of AaTI can be enhanced significantly by replacing the C5C6 region with that of Inf4 (AaTI\_Inf4 C5C6). Alternatively, the stability of Inf4 can be reduced significantly by replacing the C5C6 region with that of AaTI (Inf4\_AaTI C5C6). Replacement of other regions of Inf4 with those of AaTI does not change the stability significantly. (A)-(D) Far-UV CD spectra of wild-type AaTI, wild-type Inf4, Inf4\_AaTI C5C6, and AaTI\_Inf4 C5C6 in the presence of 0 M GdnCl (open circles), 1 M GdnCl (open triangles), 2 M GdnCl (open squares), 3 M GdnCl (closed squares), 4 M GdnCl (closed circles), 5 M GdnCl (closed triangles), 6 M GdnCl (open rhombuses), and 7 M GdnCl (closed rhombuses). Data are based on raw ellipticity (mdeg) values, and normalized to zero at 260 nm. (E) Plots showing GdnCl denaturation curves for wild-type AaTI (closed circles), wild-type Inf4 (open squares), Inf4\_AaTI C5C6 (open triangles), AaTI\_Inf4 C5C6 (closed triangles), Inf4\_AaTI C3C4 (open circles), Inf4\_AaTI C4C5 (closed squares), and Inf4\_AaTI C6 (open rhombuses). Data are based on raw ellipticity (mdeg) values at 222 nm and normalized to wild-type AaTI at 0 M GdnCl.

		P14'	
AaTI	-----ERGVCACPRIYMPVCGSNLKT----	YNNDCLLRCEINSDLGR	38
Infestin4	-----EV-RNPCACFRNYVPVCGSDGKT----	YGNPCMLNCAAQTKVP-	38
Infestin1	-----LE-ENDCACPRVLHRVCGSDGNT----	YSNPCTLDCAKHEGKP-	38
LDTI	-----KKVCACPKILKPVCGSDGRT----	YANSCIARCNGVSIKSE	37
BdellinKL	-----IN-ADTECVCTKELLRVCGEDGVT----	YDNSCLATCHGTSVAHE	40
BdellinB3	-----DTECVCTKELHRVCGSDGVT----	YDNECLATCHGASVAHD	37
PSTI	-----DSLGREAKCYNELNGCTYEYRPVCGTDGDT----	YPNECVLCFENRKRQT-	46
OMSPV3	-----LAAVSVCSE-YPKPACTMEYRPLCGSDNKT----	YGNKCNFCNAVVESNG-	46
CmpI-II	-----AEDC-VGRKACTREWYPVCGSDGVT----	YSNPCNFSAQQEQCDP-	40
Rhodniin	-----EG-GEPCACPHALHRVCGSDGET----	YSNPCTLNCAKFNGKP-	38
Dipetalin	-----FQ-GNPCECPRALHRVCGSDGNT----	YSNPCMLTCAKHEGNP-	38
Greglin	SEDDGSASPESQEMSYTELPCPSICPLIYAPVCVEDSNQDFYLEFVNECEVRKCGCEAGFV	60	
AaTI	----ANNLRKIADQACDNLTDNVNDFIPOEY-	65	
Infestin4	-----GLKLVHEGRCQRSNVE---QF-----	56	
Infestin1	-----DLVQVHEGPCDPNDHD---F-----	55	
LDTI	-----GSCPTGI-----	44	
BdellinKL	-----HACEG-----	45	
BdellinB3	-----HACEGHEEHHVDEHGEDHD	56	
PSTI	-----SILIQKSGPC-----	56	
OMSPV3	-----TLTLSHFGKC-----	56	
CmpI-II	-----NITIAHMGEC-----	50	
Rhodniin	-----ELVKVHDGPCEPD-----	51	
Dipetalin	-----DLVQVHEGPCDEHDHD---F-----	55	
Greglin	YTFVPREMCKATTSLCPMQTKSS-----	83	

**Figure S4. Sequence alignment of Kazal-type inhibitors with AaTI showing residues at the P14' position.**

Sequence alignment of Kazal-type protease inhibitors showing the P14' residues (boxed in red) in the C3C4 region. Most inhibitors contains a small or branched residue with a short side-chain (e.g., Gly, Ala or Ser); however, AaTI contains an Asn residue at this position.

	140	150
FXIIa	GWGHQFEG-----AEE <b>Y</b> ASFL	
plasmin	GWGETQGT-----FGAGLL	
FXa	GFGRTHEK-----GRQSTRL	
Thrombin	GWGNRRETWTTTSVAEVQPSVL	
Trypsin	GWGNTASS-----GADYPDEL	
tPA	GYGKHEAL-----SPFYSERL	
Urokinase	GFGKENST-----DYLYPEQL	
	140-loop	

**Figure S5. Sequence alignment of the 140-loop in selected serine proteases.**

Sequence alignment of the 140-loop (boxed in red) from selected serine proteases using sequences, boundary of 140-loop based on Figure S1 in Dementiev *et al.*, 2018 [1]. The residue Y151 of FXIIa is bold-faced and in red.



## References

- 1 Dementiev, A., Silva, A., Yee, C., Li, Z., Flavin, M. T., Sham, H. and Partridge, J. R. (2018) Structure of human plasma  $\beta$ -factor XIIa cocrystallized with potent inhibitors. *Blood Advances*. **2**, 549-557