Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supporting Information

Fluorine-induced polarity increases inhibitory activity of BPTI towards chymotrypsin

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Content

Materials

Resins for solid phase peptide synthesis were purchased from CEM Corporation (Matthews, NC, USA). Canonical Fmoc- and side-chain protected amino acids came from ORPEGEN Peptide Chemicals GmbH (Heidelberg, Germany) and Carbolution Chemicals GmbH (St. Ingbert, Germany). Coupling reagent were obtained from CEM Corporation (Matthews, NC, USA), Iris Biotech GmbH (Marktredwitz, Germany), Carbolution Chemicals GmbH (St. Ingbert, Germany), Bachem AG (Bubendorf, Switzerland), Novabiochem (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Solvents for peptide synthesis were purchased as synthesis grade from Fisher Scientific (Schwerte, Germany), Acros Organics (Thermo Fisher Scientific, Geel, Belgium), VWR International GmbH (Darmstadt, Germany) and Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Solvents for peptide purification were procured as HPLC or spectroscopy grade from Fisher Scientific (Schwerte, Germany) or Merck Chemicals GmbH (Darmstadt, Germany).

Deionized water (Milli-Q® Reference Water Purification System, Merck KGaA, Darmstadt, Germany) was used for HPLC and to prepare buffers.

All other chemicals and solvents were purchased from abcr GmbH (Karlsruhe, Germany), Acros Organics (Thermo Fisher Scientific, Geel, Belgium), Alfa Aesar (Thermo Fisher Scientific, Geel, Belgium), Carl Roth GmbH & Co. KG (Karlsruhe, Germany), chemPUR Feinchemikalien und Forschungsbedarf GmbH (Karlsruhe, Germany), Deutero GmbH (Kastellaun, Germany), Fluorochem (Hadfield, UK), Iris Biotech GmbH (Marktredwitz, Germany), Merck Chemicals GmbH (Darmstadt, Germany), Novabiochem® (Merck KGaA, Darmstadt, Germany), PanReac AppliChem (AppliChem GmbH, Darmstadt, Germany), Roche Pharma AG (Grenzach-Wyhlen, Germany), Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), TCI Deutschland GmbH (Eschborn, Germany), VWR International GmbH (Darmstadt, Germany) and Worthington Biochemical Corp. (Lakewood, NJ, USA).

HPLC & ESI-ToF

Preparative HPLC

Purification of crude proteins was performed using a reversed-phase HPLC LaPrep∑ low-pressure HPLC system (VWR, Darmstadt, Germany). The system consists of a LaPrep∑ LP 1200 preparative solvent pump with a 100 mL titanium pump head, a ternary low-pressure gradient, a dynamic mixing chamber, a 6-port-3-channel injection valve with an automated preparative 20 mL sample loop, a LaPrep∑ LP 3101 1-channel UV-detector, a LaPrep∑ semi-preparative flow cell with a 0.5 mm path length and a LaPrep∑ LP 2016 17-port/1-channel fraction valve. As stationary phase a Kinetex® RP-C18 TMS end-capped LC column (5 µm, 100 Å, 250 × 21.2 mm, Phenomenex®, Torrance, CA, USA) was used. A SecurityGuard PREP Cartridge Holder (21.2 mm, Phenomenex, Torrance, CA, USA) equipped with a C18 cartridge (15 × 21.2 mm, Phenomenex®, Torrance, CA, USA) was used as precolumn. Both eluents, deionized water (solvent A) and acetonitrile (solvent B), contained 0.1% (v/v) TFA.

HPLC runs were performed as described in Table S 1 and Table S 2:

Table S 1. Gradient method used for preparative HPLC for purification of BPTI after full-length synthesis.

Time [min]	A [%]	B [%]	Flow rate [mL min^{-1}]
0	90	10	10
5	90	10	10
20	20	80	20
21	0	100	20
24	0	100	20
26	90	10	20
30	90	10	20

Table S 2. Gradient method used for preparative HPLC for purification of refolded BPTI.

Time [min]	A [%]	B [%]
0	90	10
18	20	80
20	0	100
23	0	100
24	90	10
27	90	10

Flow rate = 15 mL min^{-1}

Data analysis was performed with EZChrom Elite software (Version 3.3.2 SP2, Agilent Technologies, Santa Clara, CA, USA).

Analytical HPLC

Analytical HPLC was carried out on a VWR-Hitachi Chromaster HPLC 600 bar system (VWR International GmbH, Darmstadt, Germany) or a LaChrom ELITE®-HPLC system from VWR-Hitachi (VWR International GmbH, Darmstadt, Germany). The Chromaster system works with a low-pressure gradient and contains a 5160 pump with a 6-channel solvent degasser, an organizer, a 5260 autosampler with a 100 µL sample loop, a 5130 column oven, and a 5430 diode array detector (DAD) with a standard flow cell (10 mm optical path length). The LaChrom system comprises of two L-2130 HPLC pumps with solvent degasser, a high-pressure gradient mixer, an L-2200 autosampler with a 100 µL sample loop, and a L-2455 diode array flow detector. The column used was a Kinetex® RP-C18 (5 µM, 100 Å, 250 × 4.6 mm, Phenomenex®, Torrance, CA, USA). A SecurityGuardTM cartridge kit (Phenomenex®, Torrance, CA, USA) containing C18 cartridge (4.0 × 3.0 mm, Phenomenex®, Torrance, CA, USA) was used as precolumn. Deionized water (solvent A) and acetonitrile (solvent B), both containing 0.1 % TFA (v/v), were used as eluents. The flow rate was adjusted to 1.0 mL/min. In case of the Chromaster system, the column was heated to 24 °C. HPLC runs were performed according to Table S 3.

Table S 3. Gradient methods used for analytical HPLC.

Time [min]	A [%]	B [%]
0	90	10
18	20	80
20	0	100
23	0	100
24	90	10
27	90	10

The data was analyzed with EZChrom ELITE software (version 3.3.2, Agilent Technologies, Santa Clara, CA, USA).

Graphical visualization and data processing of the chromatograms occurred with the software OriginPro 2020b (version 9.7.5.184, OriginLab Corporation, Northhampton, MA, USA).

Mass Spectrometry

To determine the mass of the synthesized peptides, high resolution mass spectrometry (HRMS) was conducted on an Agilent ESI-ToF 6230 LC-MS spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). Parameters were optimized for the measurements: spray voltage was set to 3.5 kV, nebulizer to 20 psi, gas temperature to 325°C, fragmentor voltage to 250 V and drying gas flow rate to 5 L/min. All samples were dissolved in a mixture of water and acetonitrile (1/1, v/v) containing 0.1 % TFA (v/v). Infusion was carried out in a flow rate of 10 µL/min via a Harvard Apparatus 11 plus syringe pump (Harvard Apparatus, Holliston, MA, USA).

Solid-phase peptide synthesis

Protein synthesis was performed with a Liberty Blue automated microwave-assisted peptide synthesizer (CEM Corporation, Mathews, NC, USA) in an Fmoc-based SPPS approach. All proteins were synthesized as *C*-terminal acids on a Cl-MPA ProTide LL resin (0.18-0.22 mmol/g resin substitution). The exact conditions for loading, Fmoc deprotection, washing and amino acid coupling steps are described in Table S 4. Loading the first amino acid was performed according to the “Loading” cycle described in Table S 4, with KI and DIPEA as activation agents, followed by four washing cycles. Fmoc deprotection was carried out with 10 wt% piperazine in EtOH/NMP (1/9, v/v). All further amino acids were coupled with Oxyma/DIC as activators. Amino acids 2 to 19 were coupled with the “single coupling” and amino acids 20 to 58 and all arginines with “double coupling” cycle described in Table S 4. The non-canonical amino acids were introduced with the “special coupling” cycle. It used elongated microwave heating time, reduced equivalents of the amino acid employed and additional washing steps between Fmoc deprotection and coupling step. Total synthesis time of full-length BPTI variants was 11.5 h.

After synthesis, the resin was washed with CH_2Cl_2 and lyophilized overnight. Dried resin was treated with 20 mL of TFA/water/phenol/thioanisole/EDT (82.5/5/5/5/2.5, v/v) for 4 h at room temperature. Resin was washed thrice with TFA and CH_2Cl_2. Organic solvent was removed in vacuo, protein was precipitated in ice-cold Et_2O and centrifuged. Precipitate was washed thrice more with Et_2O and centrifuged. Supernatant was discarded, protein was dissolved in water and lyophilized overnight. Crude protein was obtained and purified according to Table S 1.

Table S 4. Cycles for automated microwave-assisted SPPS with a 0.1 mmol scale on Liberty Blue peptide synthesizer, if not indicated all reagents were dissolved in DMF.

Process step		Reagents			Reaction conditions		
		Name	Conc. [M]	Volume [mL]	Temp. [°C]	Power [W]	Time [s]
Loading	Activation & AA coupling	DIPEA	1	2	80	75	60
		KI	0.125		90	20	540
		AA	0.2	5			
	Washing (4x)	DMF	-	4	25	-	5
Single coupling	Fmoc deprotection	Piperazine	10 wt%	4*	75	155	15
		HOBt	0.1		90	30	60
	Washing (4x)	DMF	-	4	25	-	5
	AA coupling	AA	0.2	2.5			
		DIC	1	1	75	217	15
		Oxyma	1	0.5	90	43	225
		DIPEA	0.1				
	Washing	DMF	-	4	25	-	5
Double coupling	Fmoc deprotection	Piperazine	10 wt%	4*	75	155	15
		HOBt	0.1		90	30	60
	Washing (4x)	DMF	-	4	25	-	5
	AA coupling (2x)	AA	0.2	2.5			
		DIC	1	1	75	217	15
		Oxyma	1	0.5	90	43	225
		DIPEA	0.1				
	Washing	DMF	-	4	25	-	5
Special coupling	Fmoc deprotection	Piperazine	10 wt%	4*	75	155	15
		HOBt	0.1		90	30	60
	Washing (4x)	DMF	-	4	25	-	5
	AA coupling	AA	0.05	2.5			
		DIC	1	1	75	217	15
		Oxyma	1	0.5	90	43	585
		DIPEA	0.1				
	Washing	DMF	-	4	25	-	5

* EtOH/NMP, 1/9, v/v

Protein refolding

Purified BPTI variants were dissolved in buffer containing 6 M GdmCl, 0.1 M Na_2HPO_4, 20 mM TCEP at pH 7.0 with a final concentration of 1.5 mM. After shaking for 24 h at 25 °C, buffer exchange to 10 mM HCl was performed with a PD-10 desalting column (Sephadex G-25 M, GE Healthcare). Flow through was collected and lyophilized overnight. Lyophilized product was dissolved in 10 mM HCl and dropwise added to degassed oxidation buffer containing 0.1 M Tris, 0.2 M KCl, 1 mM EDTA, 1 M GdmCl, 150 µM GSSG and 300 µM GSH at pH 8.7 with a final peptide concentration of 30 µM. Mixture was shaken for 24 h at 37 °C. Solution was concentrated in Amicon® Ultra-15 3 kDa Centrifugal Filter Unit (Merck, Darmstadt, Germany), washed with 10 mM HCl and lyophilized. Crude refolded BPTI variants were purified with RP-HPLC according to gradient shown in Table S 2.

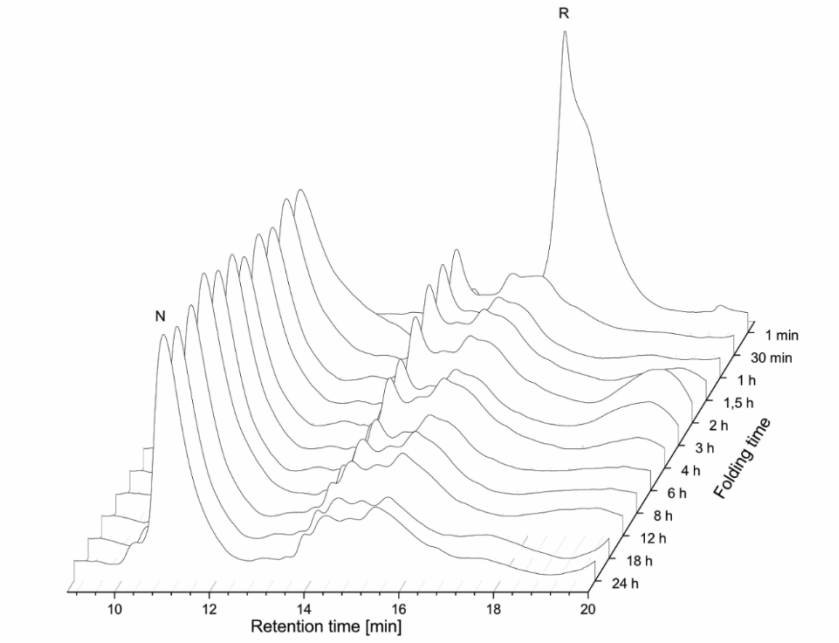

Figure S 1. HPLC profiles of oxidative refolding of BPTI K15MfeGly monitored over 24 h with denaturated and reduced (**R**) and refolded, native (**N**) BPTI.

CD spectroscopy

The ellipticity θ_{obs} was normalized for concentration c [mol L^{-1}], number of residues n and path length l (0.1 cm) using equation (1):

$$[\theta] = \frac{\theta_{obs}}{10^4 \cdot l \cdot c \cdot n} \quad (1)$$

Measured ellipticity θ_{obs} is given in mdeg and molar ellipticity per residue [θ] in 10^3 deg cm^2 $dmol^{-1}$ $residue^{-1}$.

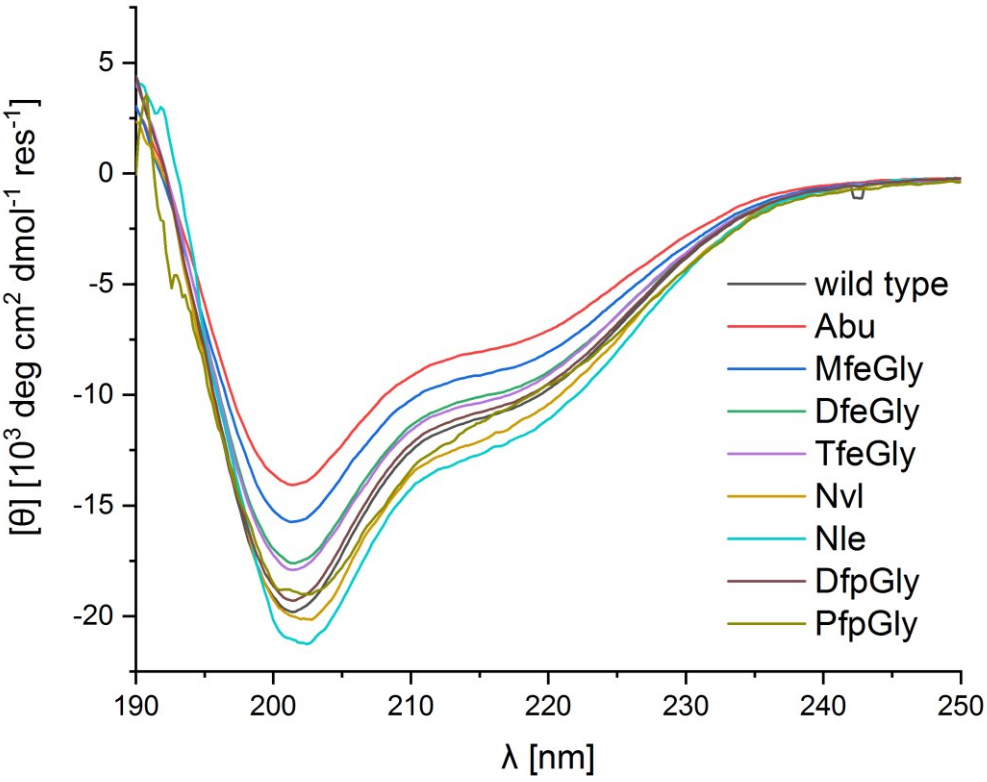

Figure S 2. CD spectra of BPTI variants containing Lys (wild type), Abu, MfeGly, DfeGly, TfeGly, Nvl, Nle, DfpGly, PfpGly. Measurements were performed I 10 mM phosphate buffer, pH 7.4 with an overall protein concentration of 20 µM. All spectra are normalized and represented the mean of three independent measurements.

Protein library

BPTI K15Abu

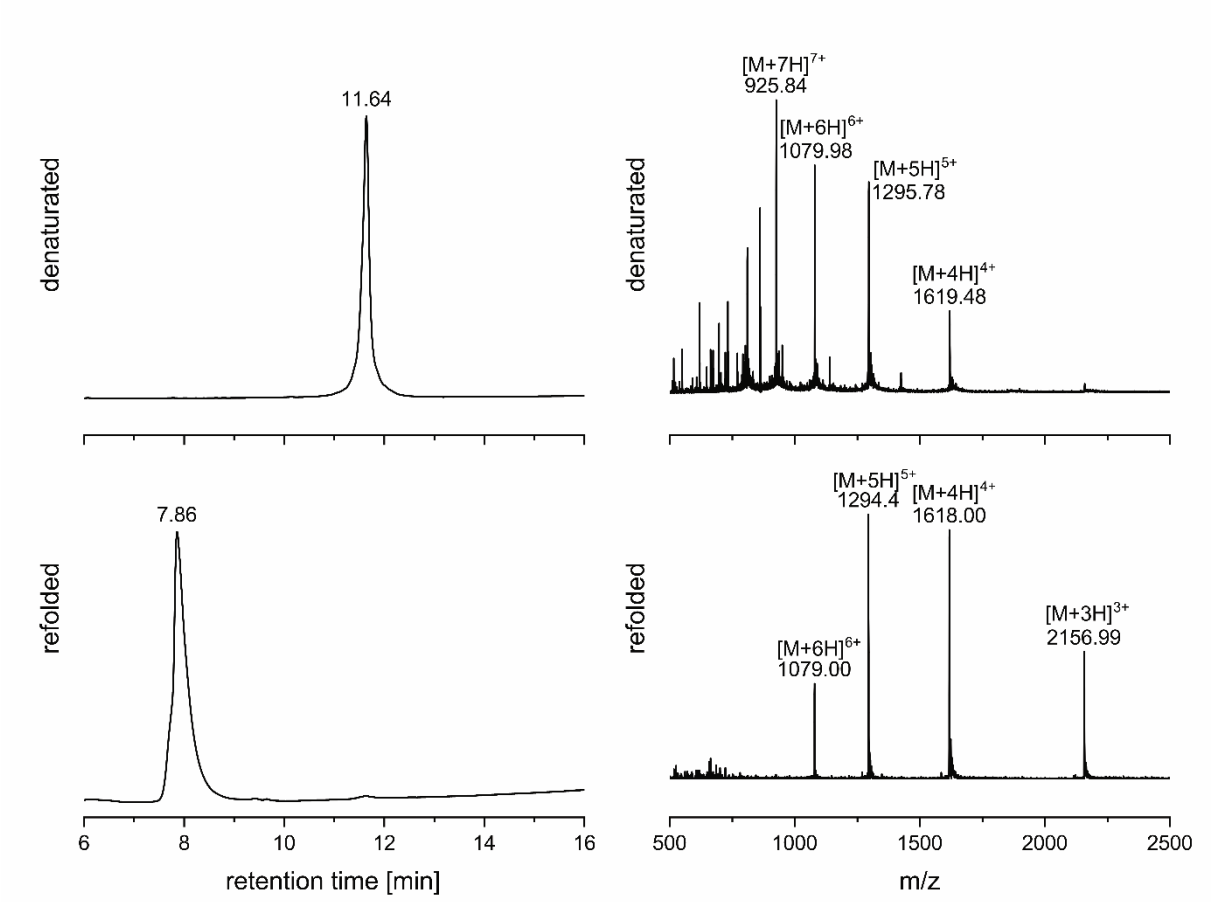

Figure S 3. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15Abu.

Table S 5. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15Abu

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1619.48	1619.52	1618.00	1618.01
$[M+5H]^{5+}$	1295.78	1295.81	1294.48	1294.61
$[M+6H]^{6+}$	1079.98	1080.02	1079.00	1079.00

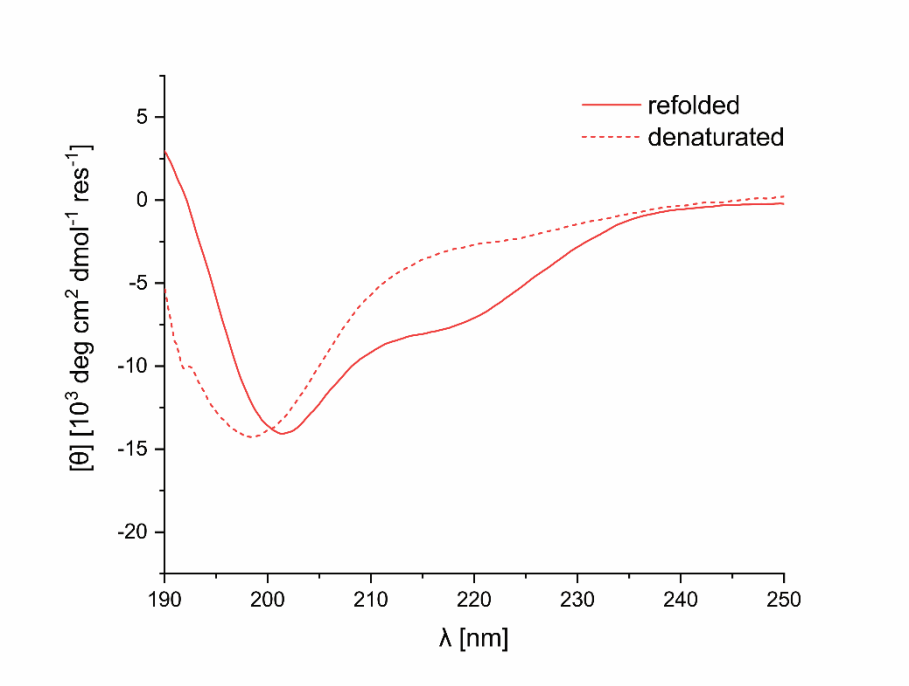

Figure S 4. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15Abu.

BPTI K15MfeGly

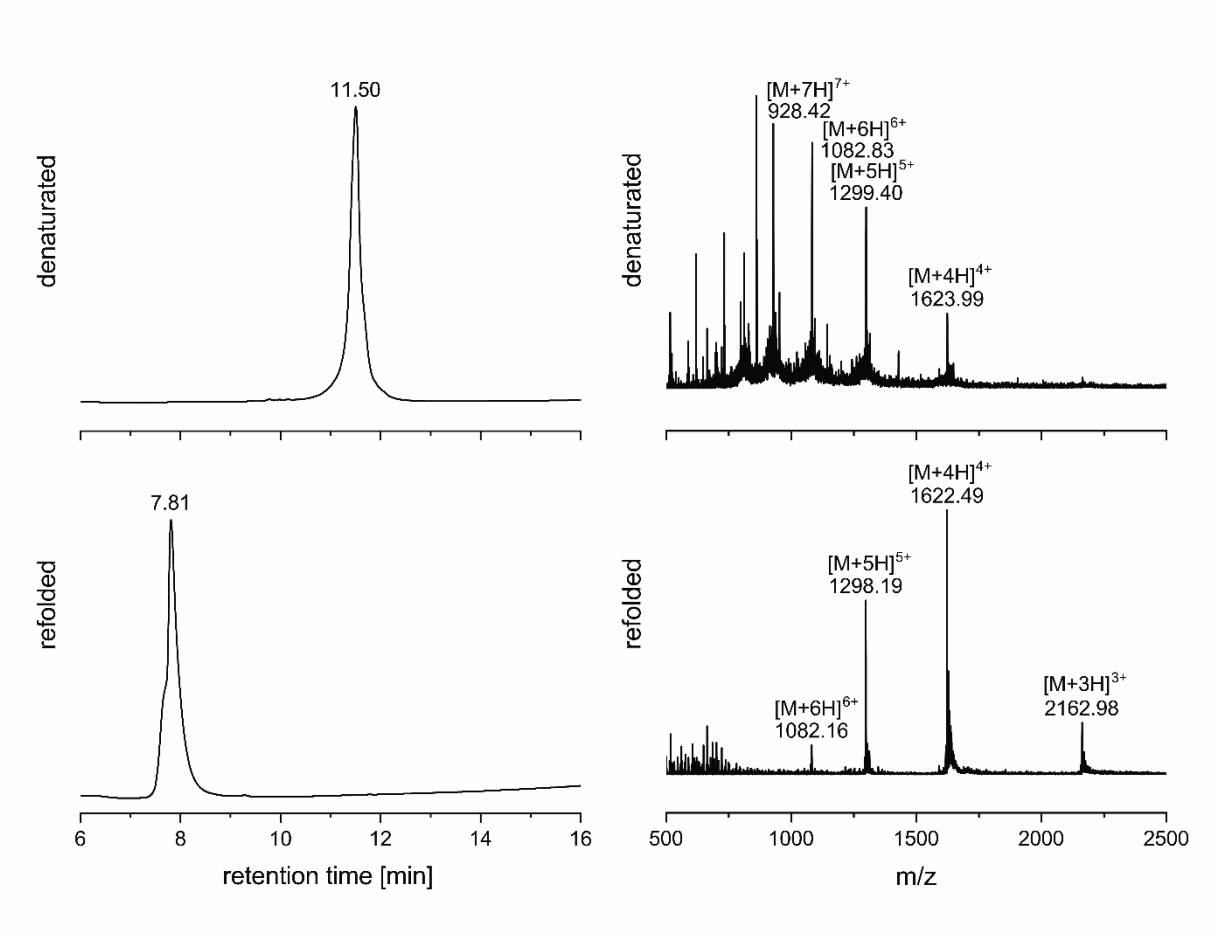

Figure S 5. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15MfeGly.

Table S 6. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15MfeGly

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1623.99	1624.02	1622.49	1622.51
$[M+5H]^{5+}$	1299.40	1299.42	1298.19	1298.21
$[M+6H]^{6+}$	1082.83	1083.01	1082.16	1082.01

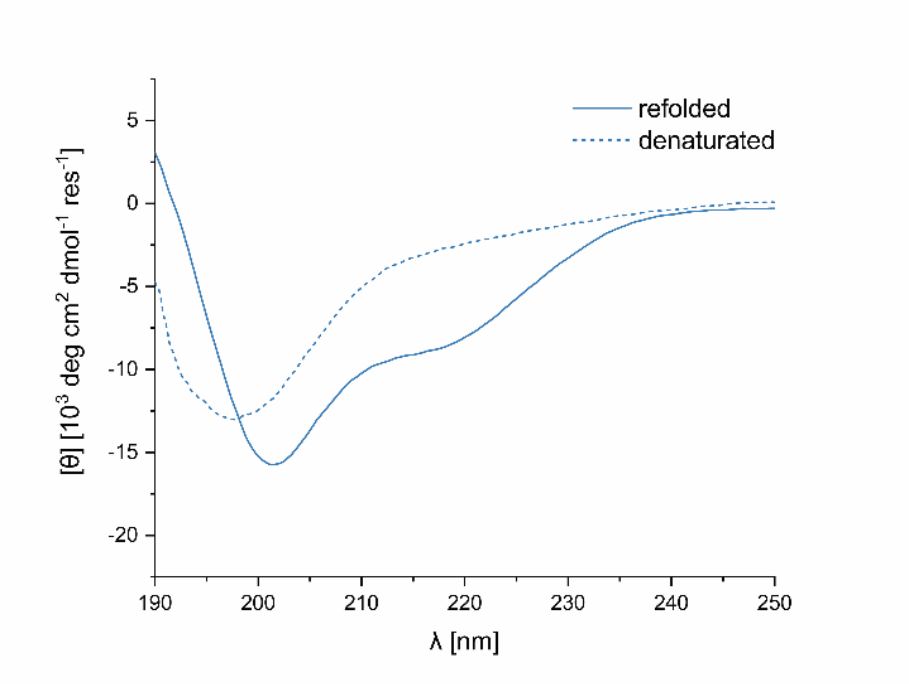

Figure S 6. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15MfeGly.

BPTI K15DfeGly

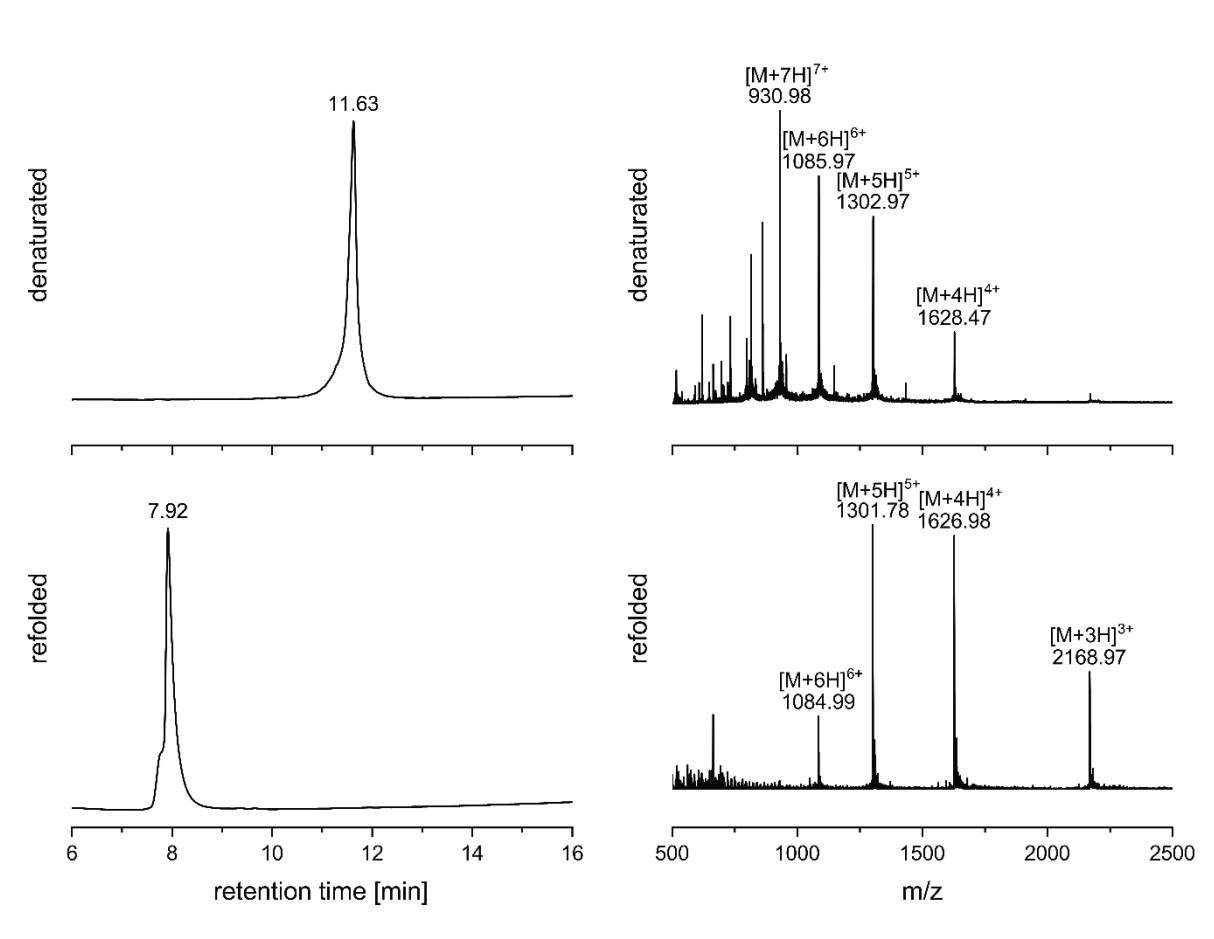

Figure S 7. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15DfeGly.

Table S 7. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15DfeGly

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1628.47	1628.52	1626.98	1627.00
$[M+5H]^{5+}$	1302.97	1303.01	1301.78	1301.81
$[M+6H]^{6+}$	1085.97	1086.01	1084.99	1085.01

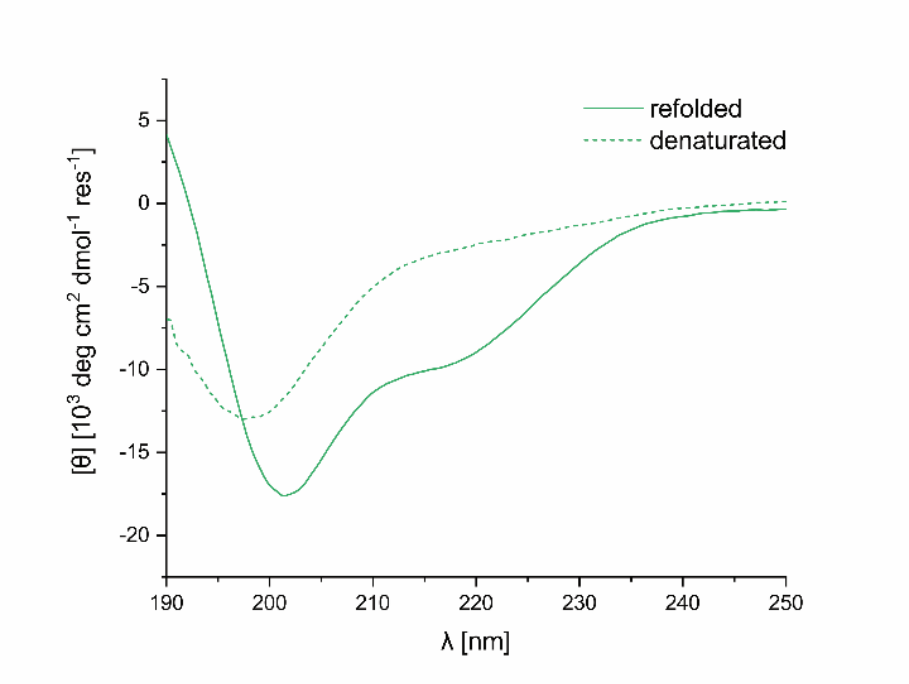

Figure S 8. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15DfeGly.

BPTI K15TfeGly

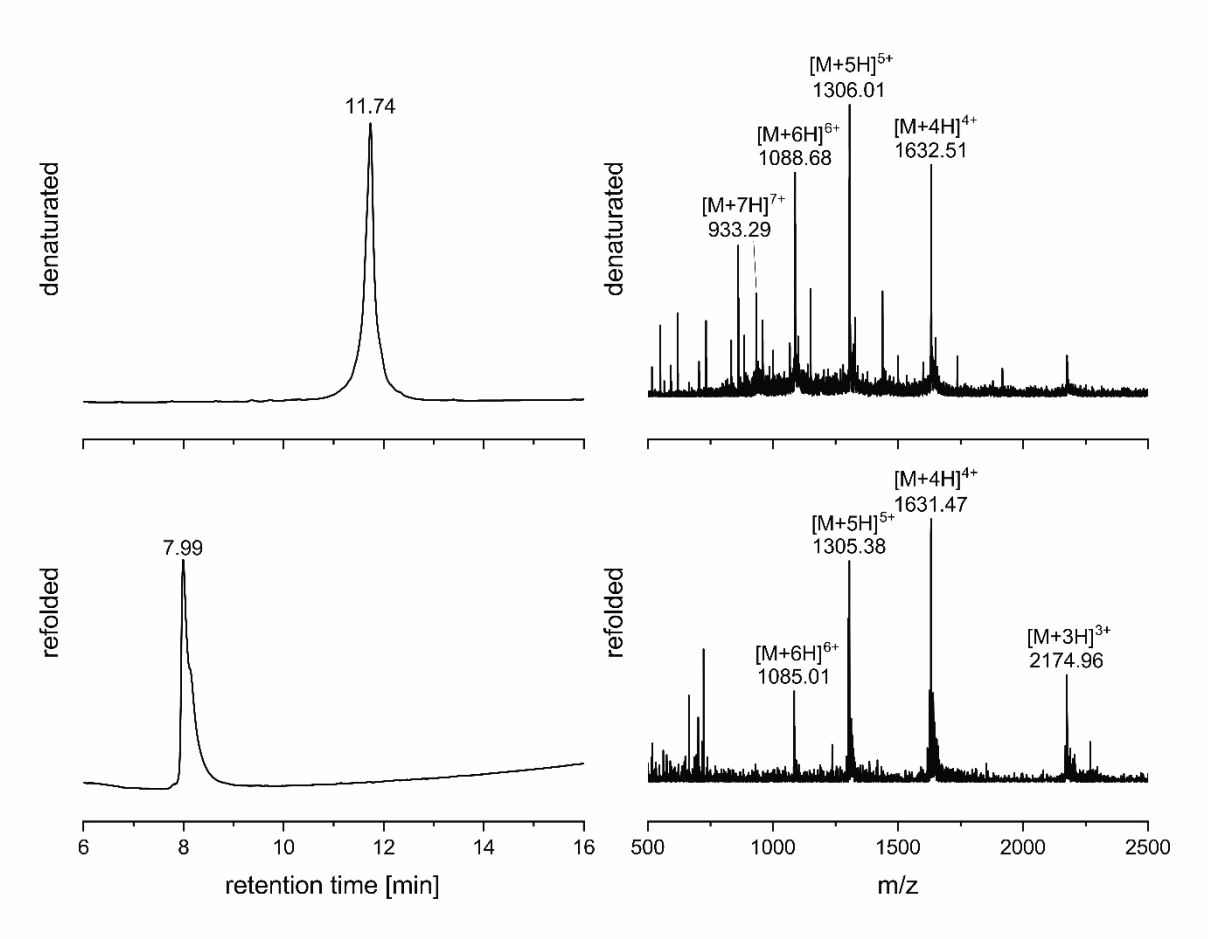

Figure S 9. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15TfeGly.

Table S 8. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15TfeGly

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1632.51	1633.01	1631.47	1631.50
$[M+5H]^{5+}$	1306.01	1306.61	1305.38	1305.40
$[M+6H]^{6+}$	1088.68	1089.01	1085.01	1088.00

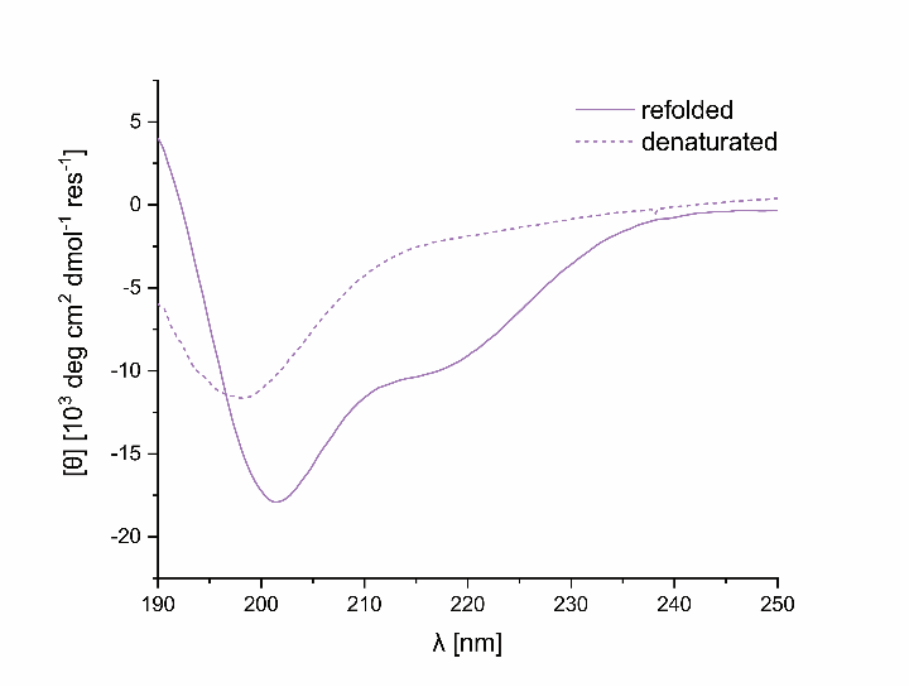

Figure S 10. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15TfeGly.

BPTI K15Nvl

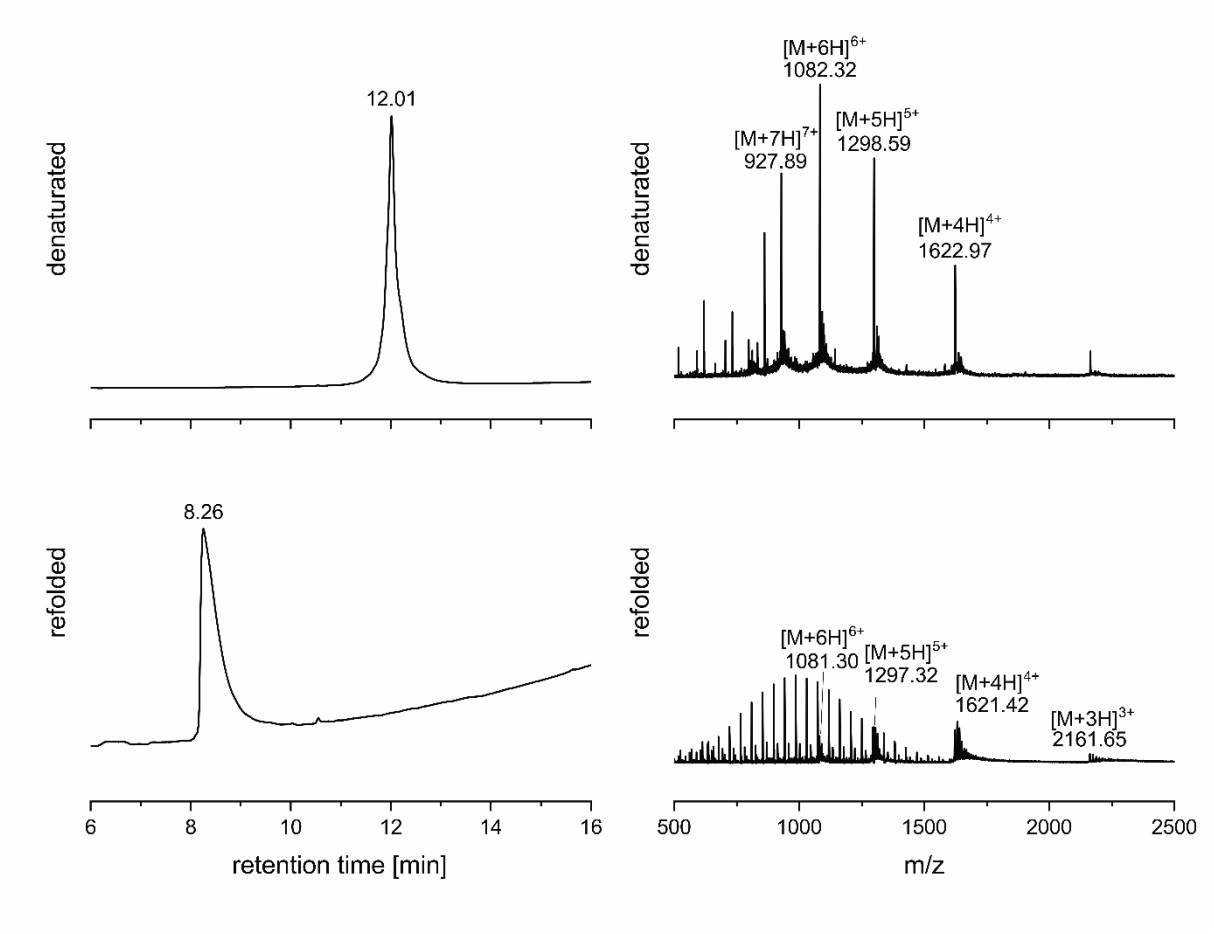

Figure S 11. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15Nvl.

Table S 9. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15Nvl

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1622.97	1623.02	1621.42	1621.51
$[M+5H]^{5+}$	1298.59	1298.62	1297.32	1297.41
$[M+6H]^{6+}$	1082.32	1082.35	1081.30	1081.34

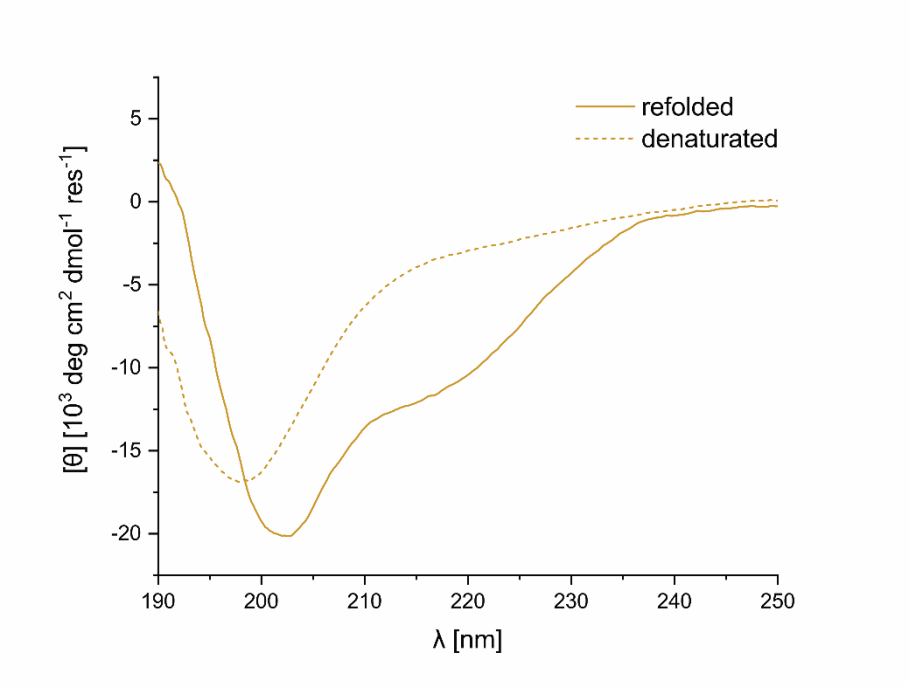

Figure S 12. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15Nvl.

BPTI K15Nle

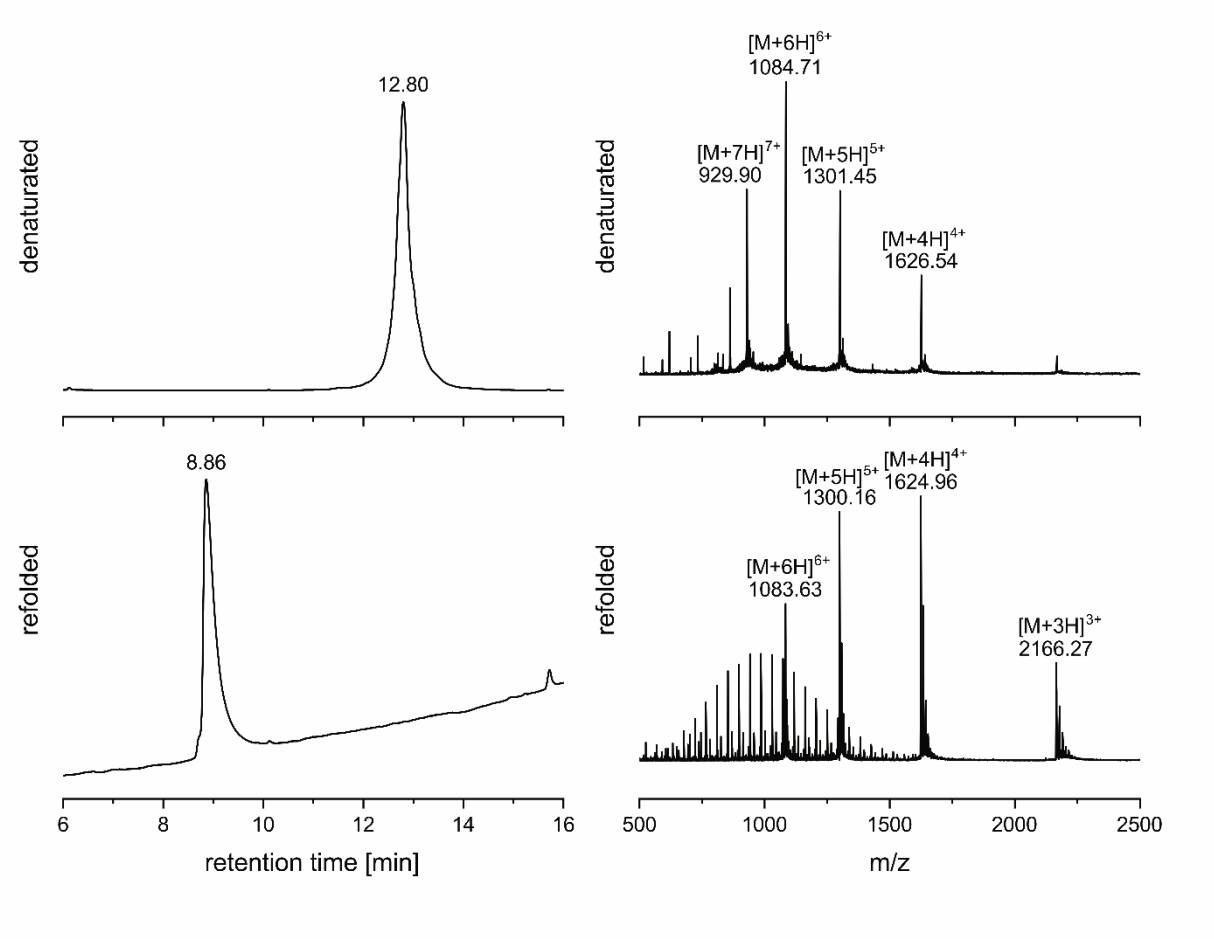

Figure S 13. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15Nle.

Table S 10. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15Nle

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1626.54	1626.53	1624.96	1625.02
$[M+5H]^{5+}$	1301.45	1301.42	1300.16	1300.22
$[M+6H]^{6+}$	1084.71	1084.69	1083.63	1083.68

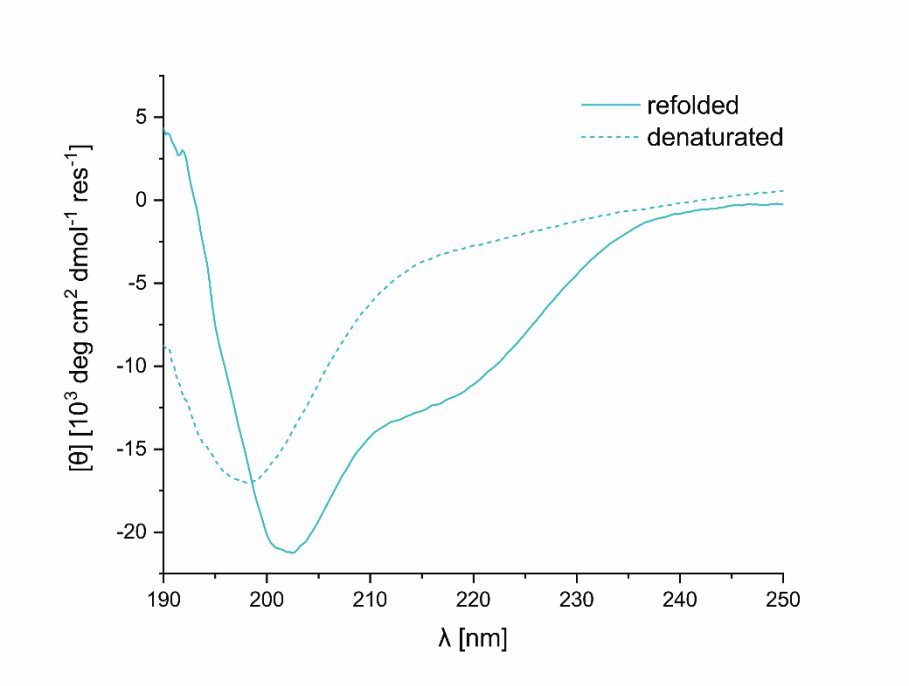

Figure S 14. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15Nle.

BPTI K15DfpGly

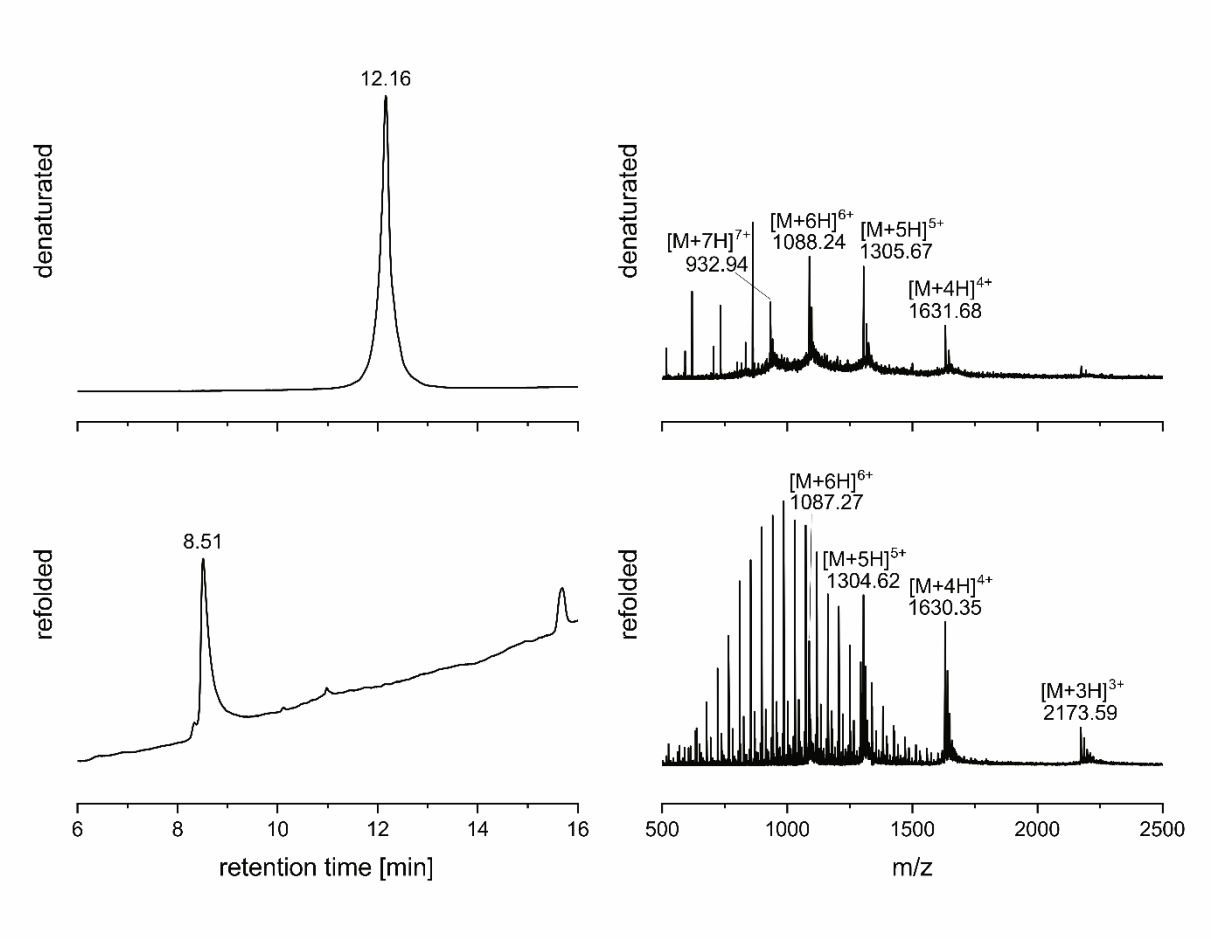

Figure S 15. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15DfpGly.

Table S 11. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15DfpGly

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1631.68	1632.02	1630.35	1630.51
$[M+5H]^{5+}$	1305.67	1305.82	1304.62	1304.61
$[M+6H]^{6+}$	1088.24	1088.35	1087.27	1087.34

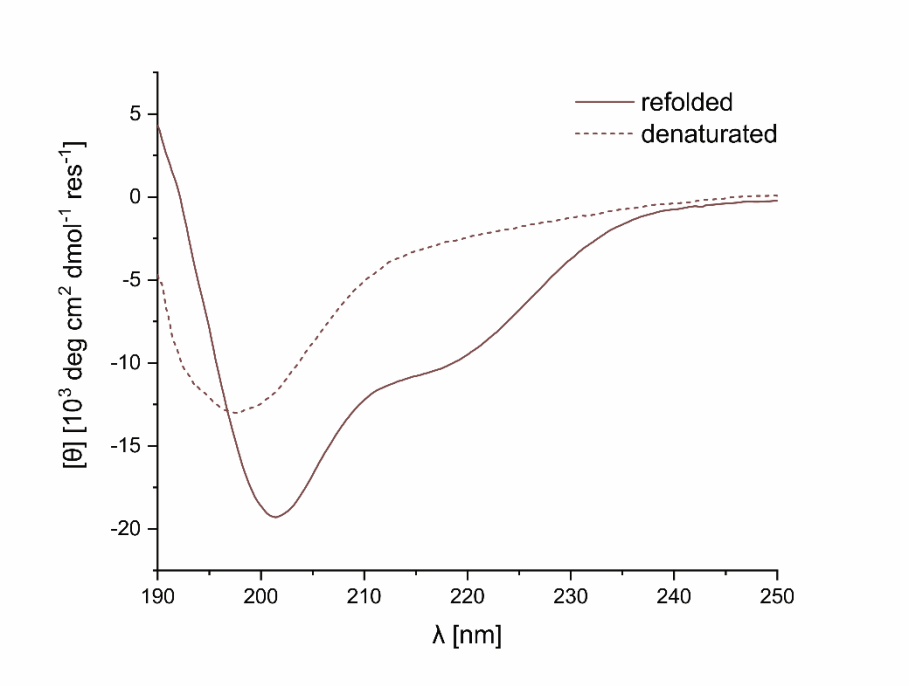

Figure S 16. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15DfpGly.

BPTI K15PfpGly

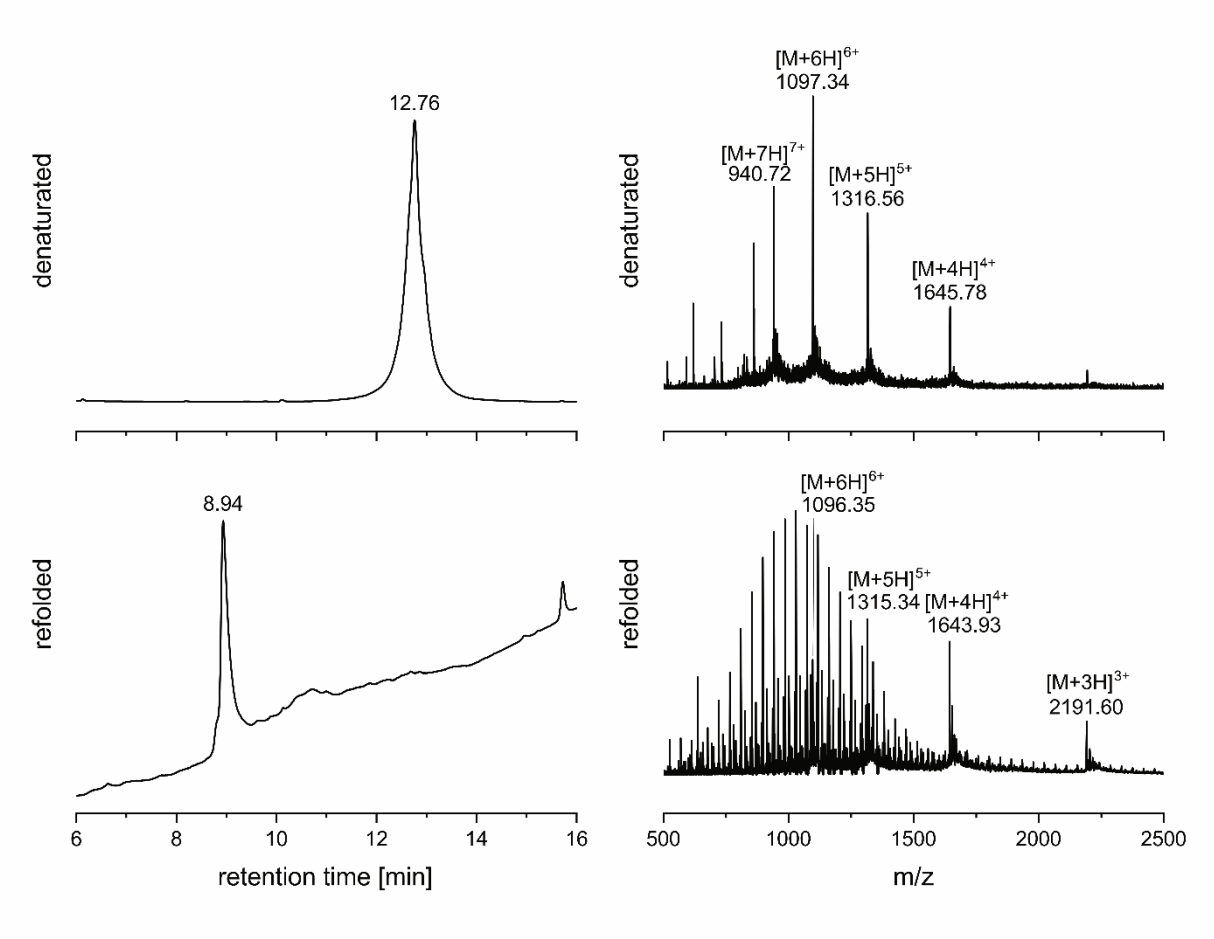

Figure S 17. HPLC chromatograms and ESI-ToF mass spectrum of denaturated (**top**) and refolded (**bottom**) BPTI K15PfpGly.

Table S 12. Observed and calculated m/z from ESI-ToF mass spectrometry for BPTI K15PfpGly

	denaturated		**refolded**	
Charge	**m/z observed**	**m/z calculated**	**m/z observed**	**m/z calculated**
$[M+4H]^{4+}$	1645.78	1645.51	1643.93	1644.00
$[M+5H]^{5+}$	1316.56	1316.61	1315.34	1315.40
$[M+6H]^{6+}$	1097.34	1097.34	1096.35	1096.34

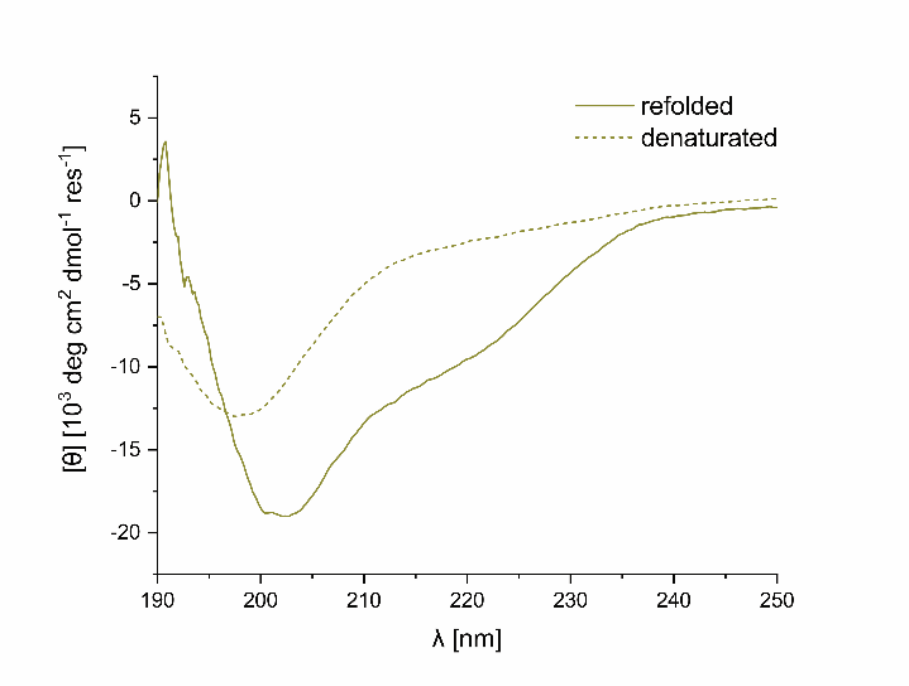

Figure S 18. CD spectra of denaturated (dashed) and refolded (solid) BPTI K15PfpGly.

Inhibitory activity assay

For inhibitory activity assays increasing concentrations of BPTI variants (0, 1, 5, 10, 25, 50, 75, 100, 250, 500, 750 nM, 1, 2.5, 5, 7.5, 10 µM) were incubated with 20 µL α-chymotrypsin (100 nM, Sigma-Aldrich) in 80 mM Tris-HCl, 20 mM $CaCl_2$, pH 7.8 in a 96-well plate for 1 h. 20 µL *N*α-Benzoyl-L-tyrosine ethyl ester (BTEE, Sigma-Aldrich) (1 mM) were added to 180 µL preincubated enzyme/inhibitor mixture. Hydrolysis of BTEE was monitored by measuring absorbance at 256 nm in an Infinite M200 microplate reader (Tecan Group AG, Männedorf, Switzerland) for 30 min at 25 °C. Initial velocities were determined by plotting absorbance against reaction time. IC_{50} values were determined by plotting residual enzyme activity against logarithmic inhibitor concentration using OriginPro 2020b (OriginLab Corporation, Northampton, MA, USA). K_i was determined according to Cheng and Pursoff,[1] with K_m = 1.48 mM:[2]

$$K_i = \frac{IC_{50}}{1 + {[S]}/{K_m}} \quad (1)$$

Table S 13. Determined IC50 values for BPTI variants

BPTI variant	IC50 [nM]
K15Abu	2300 ± 290
K15MfeGly	570 ± 120
K15DfeGly	114 ± 15
K15TfeGly	740 ± 200
K15Nvl	302 ± 67
K15Nle	218 ± 17
K15DfpGly	117 ± 34
K15PfpGly	464 ± 70

Protein crystallography

Table S 14. Crystallographic data collection, refinement, and validation statistics.

Dataset	Chymotrypsin/ BPTI Abu	Chymotrypsin/ BPTI MfeGly	Chymotrypsin/ BPTI DfeGly	Chymotrypsin/ BPTI TfeGly
PDB entry	7QIQ	7QIR	7QIS	7QIT
Wavelength [Å]	0.9184	0.9184	0.9184	0.9184
Temperature [K]	100	100	100	100
Space group	$P6_1$	$P6_1$	$P6_1$	$P6_1$
Unit Cell Parameters a, b, c [Å] α, β, γ [°]	 100.4, 100.4, 206.3 90.0, 90.0, 120.0	 100.1, 100.1, 206.2 90.0, 90.0, 120.0	 100.3, 100.3, 206.2 90.0, 90.0, 120.0	 100.0, 100.0, 206.5 90.0, 90.0, 120.0
Resolution [Å][a]	50.00 - 1.85 (1.96 - 1.85)	50.00 - 1.90 (2.01 - 1.90)	50.00 - 1.83 (1.94 - 1.83)	50.00 - 1.99 (2.11 - 1.99)
Reflections [a] Unique [a] Completeness [%][a] Multiplicity [a]	 100,371 (15,880) 99.6 (97.7) 11.7 (11.7)	 91,824 (14,757) 99.9 (99.5) 10.5 (10.7)	 103,236 (16,710) 99.7 (98.5) 10.5 (10.6)	 157,295 (25,322) 99.3 (98.6) 6.0 (6.1)
Data quality [a] Intensity [I/σ(I)] [a] R_{meas} [%][a,b] $CC_{1/2}$ [a,c] Wilson B value [Å^2]	 12.95 (1.03) 13.5 (216.6) 99.9 (67.1) 38.4	 11.35 (0.92) 16.7 (263.7) 99.8 (57.1) 38.8	 10.57 (0.96) 20.9 (306.9) 99.8 (52.7) 35.1	 9.57 (0.95) 13.0 (210.3) 99.8 (58.9) 44.4
Refinement				
Resolution [Å][a]	50.00 - 1.85 1.89 - (1.85)	50.00 - 1.90 (1.94 - 1.90)	50.00 – 1.83 (1.87 - 1.83)	50.00 - 1.99 (2.04 - 1.99)
Reflections [a] Number Test Set [%]	 100,263 2.1	 91,703 2.3	 102,990 2.0	 156,871 1.3
R_{work} [%][a] R_{free} [%][a]	16.1 (37.6) 18.8 (48.0)	16.1 (35.9) 18.9 (39.4)	16.3 (35.6) 18.9 (43.2)	17.0 (31.9) 19.1 (34.5)
Asymmetric Unit Protein: Residues, Atoms	 239 (A), 1,883 (A) 58 (B), 478 (B) 239 (C), 1,871 (C) 58 (D), 487 (D)	 239 (A), 1,864 (A) 58 (B), 479 (B) 240 (C), 1,899 (C) 58 (D), 479 (D)	 240 (A), 1,860 (A) 58 (B), 480 (B) 240 (C), 1,910 (C) 58 (D), 473 (D)	 239 (A), 1,884 (A) 58 (B), 481 (B) 239 (C), 1,860 (C) 58 (D), 474 (D)

Ligands: Molecules	15 (SO_4^{2-}), 29 (GOL)	17 (SO_4^{2-}), 28 (GOL)	15 (SO_4^{2-}), 29 (GOL)	20 (SO_4^{2-}), 30 (GOL)
Water Molecules	496	513	603	382
Mean Temperature factors [Å^2][b]				
All Atoms	40.1	41.4	36.9	52.0
Macromolecules	40.4 (A), 31.9 (B),	40.9 (A), 32.8 (B),	36.0 (A), 28.7 (B),	51.1 (A), 41.3 (B),
Ligands	39.2 (C), 32.4 (D), 46.5 (SO_4^{2-}), 67.5 (GOL)	42.1 (C), .32.6 (D), 46.5 (SO_4^{2-}), 68.1 (GOL)	36.7 (C), 29.0 (D), 44.7 (SO_4^{2-}), 61.9 (GOL)	54.9 (C), 40.5 (D), 57.9 (SO_4^{2-}), 78.4 (GOL)
Water Oxygens	46.8	47.5	44.8	54.6
RMSD from Target Geometry [d]				
Bond Lengths [Å]	0.014	0.013	0.014	0.014
Bond Angles [°]	1.136	1.152	1.165	1.130
Validation Statistics [f]				
Ramachandran Plot				
Residues in Allowed Regions [%]	0.4	0.4	0.4	0.4
Residues in Favored Regions [%]	98.3	98.3	98.3	97.7
Ramachandran plot *Z*-score, (r.m.s.d.) [e]				
whole	-0.13 (0.30)	-0.40 (0.30)	-0.20 (0.30)	-0.50 (0.31)
helix	0.36 (0.67)	0.46 (0.68)	0.46 (0.67)	-0.54 (0.58)
Sheet	-1.22 (0.32)	-1.63 (0.32)	-1.77 (0.31)	-1.75 (0.34)
loop	0.34 (0.28)	0.29 (0.28)	0.61 (0.30)	0.40 (0.29)
MOLPROBITY Clashscore [g]	2.74	3.45	3.55	2.87
MOLPROBITY score [f]	1.08	1.15	1.15	1.17

[a] data for the highest resolution shell in parenthesis

[b] $R_{meas}(I) = \sum_h [N/(N-1)]^{1/2} \sum_i | I_{ih} - \langle I_h \rangle | / \sum_h \sum_i I_{ih}$, in which $\langle I_h \rangle$ is the mean intensity of symmetry-equivalent reflections h, I_{ih} is the intensity of a particular observation of h and N is the number of redundant observations of reflection h. [3]

[c] $CC_{1/2} = (\langle I^2 \rangle - \langle I \rangle^2) / (\langle I^2 \rangle - \langle I \rangle^2) + \sigma^2_\varepsilon$, in which σ^2_ε is the mean error within a half-dataset.[4]

[d] RMSD – root mean square deviation

[e] calculated with PHENIX [5]

[f] calculated with MOLPROBITY [6]

[g] Clashscore is the number of serious steric overlaps (> 0.4) per 1,000 atoms.[6]

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