Supporting Information for:

Crystallographic analysis of counter-ion effects on subtilisin enzymatic action in acetonitrile

Michele Cianci^{1}, Bartłomiej Tomaszewski², John R. Helliwell³ and Peter J. Halling^{2*}*

¹European Molecular Biology Laboratory, Hamburg Outstation, c/o DESY, Building 25a,

Notkestraße 85, 22603 Hamburg, Germany; ²WestCHEM, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XL, United Kingdom.

³Department of Chemistry, University of Manchester, Manchester M13 9PL, United Kingdom;

Corresponding authors: m.cianci@embl-hamburg.de; p.j.halling@strath.ac.uk

Supplementary material 1. Data collection, data processing and refinement statistics for subtilisin in acetonitrile, with and without Cs soak.

	ACN	Cs-ACN
Crystallographic data		
Wavelength (Å)	2.167	2.167
Cell parameters	52.66,55.31,75.99	52.17,55.31,75.77
Space group	P2 ₁ 2 ₁ 2 ₁	
R _{merge}	0.13 (0.27)	0.201 (0.41)
Completeness	98.2 (95.9)	97.3 (94.5)
Redundancy	12.2 (9.9)	26.4 (22.4)
Resolution (outer shell)	44.72-2.23 (2.23-2.31)	44.68-2.24 (2.29-2.24)
I/σ(I)	16.6 (9.2)	14.1 (8.4)
Average B _{factor} from Wilson plot	35.1	23.7
Refinement results		
R _{factor} (R _{free})	0.18 (0.23)	0.16 (0.23)
bond lengths refined atoms (rms;	0.013 (0.022)	0.016 (0.022)
bond angles refined atoms (rms;	1.622 (1.943)	1.721 (1.943)
Cruickshank's DPI for coordinate	0.39	0.39
DPI based on free R factor (Å)	0.24	0.23
Number of ions		
Waters	125	113
Cesium	--	11
Chloride	--	8
ACN	15	15
Calcium	1	1
Sodium	2	1

Supplementary material 2. Cesium and chloride sites located with peak height in the anomalous difference Fourier maps (sigma level). Coordinating or interacting atoms are identified by standard PDB coding: O is a backbone carbonyl oxygen, N is a backbone amide nitrogen, other peptide atoms are in sidechains, “ring” indicates a number of the aromatic ring carbons of Tyr residues. Coordinating atoms from a symmetry related molecule in the crystal are marked by * after the residue number. In these cases the Cs site number is in italic. For waters (HOH) and other heteroatoms, assignment to a particular protein is debatable, so the presence of * is less significant. The distance to the ions is shown in brackets in Å. $v(\text{Cs})$ are monovalent cation specific valence values calculated using the WASP program, with the ligation distance set to 4.0 Å, and only oxygen, waters and non-protein atoms treated as ligating atoms.

Atom type	Atom no.	sigma level	occupancy	B factor	Target B factor	Coordination residues	$v(\text{Cs})$	(Potential) formal charge centres (within 7 Å)
Cs	1	39	0.6	22	21	O Ala-37 (2.9), O Ser-38 (4.0), O His-39 (3.0), O Leu-42 (3.0), HOH-40 (3.5)	0.594	Cl-8 (3.1), ND His-38 (6.8)
Cs	2	29	0.45	19	23	O Gly-193 (3.4), O Ala-194 (3.2), O Leu-196 (3.2), O Ser-260 (3.0), OG Ser-260 (3.7), HOH-34 (3.8), HOH-22 (4.3)	0.574	
Cs	3	12	0.25	20	28	O Gly-61 (3.3), O Ser-98 (3.2), HOH-98 (2.7), HOH-83 (3.7), HOH-110 (3.9), OG Ser-98 (3.2)	0.685	Cl-7 (6.6),
Cs	4	9.0	0.2	33	29	O Asn-183 (3.8), OD1 Asn-185 (3.2), ND2 Asn-185 (3.3), O Asn-77* (2.9), HOH-54* (3.6), HOH-77* (3.1)	0.592	Cl-1 (5.4), OD Asp-181 (6.4)
Cs	5	9.0	0.25	29	31	ring Tyr-256 (3.5-4.2), OH Tyr-256 (4.7), O Ala-52* (2.9), OE1 Glu-54* (4.3), CZ CCN-4* (3.6)	0.187	OE Glu-54* (4.3), Cl-2 (5.9), Cl-6 (6.1),
Cs	6	10	0.2	26	25	O Tyr-143 (3.0), ring Tyr-143 (~3.7),	0.514	

						OH-Tyr 143 (4.4), O Ala-144 (4.8), OG Ser-244 (3.6), HOH-113 (3.0)		
Cs	7	8.5	0.3	39	37	O Pro-239 (3.1), O Asn-240 (4.4), O Leu-241 (2.9), HOH-46 (3.1), HOH-62 (3.3),	0.767	OD Asp-120 (5.3), Cl-3 (6.8)
Cs	8	5.5	0.15	23	23	O Thr-253 (3.9), O Ala-254 (3.1), O Ser-101* (3.7), OG Ser-101* (4.1), O Gly-102* (4.1), HOH-90 (3.6), HOH-61 (3.9), HOH-104* (3.0), HOH-26 (3.4)	0.387	Cl-2 (4.7), NZ Lys-265 (5.9),
Cs	9	5.5	0.2	27	28	O Ala-1 (2.9), OG1 Thr-3 (2.9), O Gly-80 (3.2), OH Tyr-206 (4.5), HOH-53 (3.2), HOH-107* (2.1)	0.652	NH3 ⁺ Ala 1 (5.2),
Cs	10	5.0	0.2	42	40	O Ser-156 (3.2), O Asn-158 (3.2), Cl-1 (3.5),	0.181	Cl-5 (3.5) NH Arg-186 (6.8)
Cs	11	5.0	0.1	20	26	O Ser-182 (3.9), OG Ser-182 (3.6), OD1 Asn-183 (3.5), HOH-96 (3.8)	0.117	OD Asp-181 (7.2)
Cl	1	25	1.0	18	30	O Ser-184 (3.3), OD1 Asn-185 (3.1), O Leu-257 (3.7), OH Tyr-263 (3.2), O Gln-2* (2.9), HOH-54* (3.6)	---	Cs-4 (5.4),
Cl	2	9.0	0.55	30	30	O Ser-251 (3.7), O Ser-252 (4.2), O Ala-254 (3.3), O Lys-265 (3.2), OD1 Asn-97* (3.3), HOH-104* (4.3)	---	Cs-8 (4.7), NZ Lys-265 (5.6) Cs-5 (5.9)
Cl	3	6.0	0.45	38	35	O Asn-240 (3.2), N Ser-242 (3.9), OG Ser-242 (4.7), NE Gln-245 (2.9)	---	Cs-7 (6.8)
Cl	4	5.5	0.55	30	30	O Lys-170 (3.2), OE1 Glu-195 (4.7), HOH-3 (3.4), HOH-87 (3.8), HOH-64 (4.4),	---	OE Glu-195 (4.3), OD Asp-172 (5.1),
Cl	5	5	0.45	47	50	ND Asn-158 (2.8), O Asn-158 (3.3) HOH-72 (4.8)	---	Cs-10 (3.6), NH Arg-185 (6.7)
Cl	6	4.5	1	26	31	OH Tyr-256 (2.9)	---	Cs-5 (6.1)
Cl	7	4.5	0.4	31	33	O Ser-99 (3.0), ND2 Asn*-248 (3.0), OD1 Asn-248 (3.6)	---	Cs-3 (6.6)
Cl	8	4	0.25	21	21	Cs-1 (3.1), O Pro-40 (3.4), O Leu-42	---	Cs-1 (3.1), OD

						(3.4)		Asp-41 (6.9),
Ca	1	10.4	1.0	22.5	23	OD1 Asp-41 (2.4), OD2 Asp-41 (2.5), O Val-81 (2.4), OD1 Asn-77 (2.4), O Leu-75 (2.3), O Thr-79 (2.5)	--	
Na	2	--	1.0	20.4	19	O Val-174 (2.2), O Ala-169 (2.3), O Tyr-176 (2.1), OHO-47 (2.5), OHO-64 (2.4)	--	Cl-4 (6.4)

Supplementary material 3. Comparison of Ca²⁺ sites in the deposited PDB models with reported occupancy and B-factor.

Reference	Model	Site 1			Site 2			Site 3		
		Label	Occupancy	B-factor	Label	Occupancy	B-factor	Label	Occupancy	B-factor
	Native	Ca-3	1.00;	31.72;	Na-2	1.00;	27.51	Na-1	1.00;	43.40
	Cs-soak	Ca-1	1.00	23.01;	Na-2	1.00	20.12;	Cs-1	0.60;	22.64
51	1CSE	Ca-430	0.93	10.20	Ca-401	0.42	10.76	---	--;	--;
52	1C3L	Ca-282	1.00	13.84	Ca-284	1.00	39.35	---	--;	--;
25	1SCA	Ca-401	1.00	9.74	Na-402	1.00	16.08	Ca-403	1.00	47.05
25	1SCB	Ca-276	1.00	7.64	H2O-359	1.00	10.87	H2O-371	1.00	20.32
25	1SCD	Ca-401	1.00	11.34	Ca-403	1.00	32.82	H2O-598	1.00	36.8
54	1SCN	Ca-401	1.00	21.24	--	--	--	Ca-403	1.00	45.32
53	2SEC	Ca-1	1.00	7.89	Ca-2	0.91	9.88	Ca-3	0.88	12.96