Appendix

Table A.1 PISA analysis of interface areas and interactions of adjacent proteasome subunits in the murine cCP and iCP

Subunit		iCP			cCP			iCP compared to cCP		
		Interface			Interface			Interface		
1	2	[Å^2]	hb	sb	[Å^2]	hb	sb	[%]	Δhb	Δsb
α1	α2	1399.9	18	3	1403.3	17	3	99.8	+1	0
α1	α7	1323.6	19	4	1341.2	16	3	98.7	+3	+1
α2	α3	1459.1	16	5	1462.1	18	5	99.8	−2	0
α3	α4	1430.3	20	5	1500.0	18	6	95.4	+2	−1
α4	α5	1395.6	21	4	1423.8	25	9	98.0	−4	−5
α5	α6	1327.8	19	4	1336.7	18	4	99.3	+1	0
α6	α7	1367.0	19	1	1365.4	20	2	100.1	−1	−1
β1(i)	β1(i)′	750.8	8	6	945.7	8	8	79.4	0	−2
β1(i)	β2(i)	471.6	5	7	552.8	8	5	85.3	−3	+2
β1(i)	β7	717.4	11	8	715.7	13	8	100.2	−2	0
β1(i)′	β7	1110.7	16	3	1142.4	19	2	97.2	−3	+1
β2(i)	β3	1531.0	25	12	1601.7	25	11	95.6	0	+1
β2(i)	β7′	694.4	8	11	744.0	7	7	93.3	+1	+4
β3	β4	752.1	12	4	737.6	13	5	102.0	−1	−1
β3	β5(i)′	855.7	19	8	841.6	19	8	101.7	0	0
β4	β4′	734.0	14	0	761.7	11	0	96.4	+3	0
β4	β5(i)	589.4	3	5	590.0	4	4	99.9	−1	+1
β4′	β5(i)	597.0	8	7	654.3	7	10	91.2	+1	−3
β5(i)	β6	688.7	12	4	547.2	10	4	125.9	+2	0
β6	β7	970.8	13	1	974.3	13	3	99.6	0	−2
β6′	β2(i)	1130.6	15	4	1215.8	16	5	93.0	−1	−1
β6′	β3	657.6	8	5	673.8	10	6	97.6	−2	−1
β1(i)	α1	457.4	5	3	417.0	3	0	109.7	+2	+3
β1(i)	α7	457.8	7	3	440.7	2	1	103.9	+5	+2
β2(i)	α1	566.2	8	6	583.5	7	5	97.0	+1	+1
β2(i)	α2	441.8	3	0	463.3	5	0	95.4	−2	0
β3	α2	412.6	8	6	427.7	10	6	96.5	−2	0

The number of hydrogen bonds (hb) and salt bridges (sb) as well as their differences between cCP and iCP (Δ) are provided. Interface areas of iCP subunits are given as percentage of the corresponding values in the cCP

E. M. Huber, *Structural and Functional Characterization of the Immunoproteasome*, Springer Theses, DOI: 10.1007/978-3-319-01556-9,

Table A.2 X-ray data collection and refinement statistics of structures of the yCP mutant yβ5 Q53S

	yCP yβ5 Q53S	yCP yβ5 Q53S:bortezomib	yCP yβ5 Q53S:ONX 0914
Crystal parameters			
Space group	$P2_1$	$P2_1$	$P2_1$
Cell constants	a = 134.7 Å	a = 136.3 Å	a = 136.7 Å
	b = 301.9 Å	b = 301.4 Å	b = 301.5 Å
	c = 144.7 Å	c = 145.2 Å	c = 145.2 Å
	β = 112.9°	β = 113.1°	β = 112.8°
CPs / AU[a]	1	1	1
Data collection			
Beam line	X06SA, SLS	X06SA, SLS	X06SA, SLS
Wavelength (Å)	1.0	1.0	1.0
Resolution range (Å)[b]	49–2.9 (3.0–2.9)	49–3.0 (3.1–3.0)	49–3.4 (3.5–3.4)
No. observations	717696	653661	521471
No. unique reflections[c]	230488	209442	146379
Completeness (%)[b]	98.2 (98.6)	97.5 (99.1)	98.6 (99.0)
R_{merge} (%)[b, d]	7.9 (55.3)	8.4 (62.0)	13.6 (58.7)
I/σ (I)[b]	11.8 (2.6)	13.0 (3.2)	7.6 (2.3)
Refinement (REFMAC5)			
Resolution range (Å)	15–2.9	15–3.0	15–3.4
No. refl. working set	218963	308271	176276
No. refl. test set	10948	15413	8813
No. non hydrogen	50864	51032	51116
No. of ligand atoms	–	168	294
Water molecules	1322	1322	1322
R_{work}/R_{free} (%)[e]	15.1/21.1	14.7/20.6	14.2/21.7
r.m.s.d. bond (Å)/(°)[f]	0.011/1.599	0.012/1.637	0.011/1.591
Average B-factor (Å^2)	61.7	64.5	70.7
Ramachandran plot (%)[g]	94.3/4.7/1.0	94.4/4.9/0.7	92.3/6.6/1.1

[a]Asymmetric unit

[b]The values in parentheses of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell

[c]Friedel pairs were treated as identical reflections

[d]$R_{merge}(I) = \Sigma_{hkl}\Sigma_j \mid [I(hkl)_j - I(hkl)] \mid \Sigma_{hkl} I_{hkl}$, where $I(hkl)_j$ is the jth measurement of the intensity of reflection hkl and <I(hkl)> is the average intensity

[e]$R = \Sigma_{hkl} \mid |F_{obs}| - |F_{calc}| \mid / \Sigma_{hkl} |F_{obs}|$, where R_{free} is calculated for a randomly chosen 5 % of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections

[f]Deviations from ideal bond lengths/angles

[g]Number of residues in favoured, allowed or outlier region

Table A.3 X-ray data collection and refinement statistics of structures of the yCP mutant yβ5 A27S K32N A46S T57R G48C K71G V127T

	yCP	yCP	yCP
	yβ5 A27S K32N A46S T57R G48C K71G V127T	yβ5 A27S K32N A46S T57R G48C K71G V127T:bortezomib	yβ5 A27S K32N A46S T57R G48C K71G V127T:ONX 0914
Crystal parameters			
Space group	$P2_1$	$P2_1$	$P2_1$
Cell constants	a = 134.5 Å	a = 136.5 Å	a = 136.6 Å
	b = 301.0 Å	b = 300.4 Å	b = 300.3 Å
	c = 144.4 Å	c = 145.6 Å	c = 145.8 Å
	β = 113.0°	β = 113.1°	β = 113.2°
CPs / AU[a]	1	1	1
Data collection			
Beam line	X06SA, SLS	X06SA, SLS	X06SA, SLS
Wavelength (Å)	1.0	1.0	1.0
Resolution range (Å)[b]	49–2.5 (2.6–2.5)	49–2.8 (2.9–2.8)	49–2.6 (2.7–2.6)
No. observations	1048068	813713	1001216
No. unique reflections[c]	353449	260234	324245
Completeness (%)[b]	97.3 (98.4)	98.5 (99.0)	98.3 (99.4)
R_{merge} (%)[b, d]	7.5 (59.7)	7.5 (46.2)	7.6 (56.0)
I/σ (I)[b]	9.2 (2.0)	11.9 (3.4)	10.8 (2.7)
Refinement (REFMAC5)			
Resolution range (Å)	15–2.5	15–2.8	15–2.6
No. refl. working set	335776	247222	308031
No. refl. test set	16788	12361	15401
No. non hydrogen	50888	51047	51135
No. of ligand atoms	–	168	294
Water molecules	1340	1340	1340
R_{work}/R_{free} (%)[e]	19.0/23.6	15.5/21.0	16.9/21.5
r.m.s.d. bond (Å)/(°)[f]	0.012/1.652	0.011/1.551	0.012/1.649
Average B-factor (Å^2)	58.1	62.7	62.1
Ramachandran Plot (%)[g]	94.4/4.8/0.8	95.5/3.7/0.7	95.6/3.8/0.6

[a]Asymmetric unit

[b]The values in parentheses of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell

[c]Friedel pairs were treated as identical reflections

[d]$R_{merge}(I) = \Sigma_{hkl}\Sigma_j \mid [I(hkl)_j - I(hkl)] \mid \Sigma_{hkl} I_{hkl}$, where $I(hkl)_j$ is the jth measurement of the intensity of reflection hkl and <I(hkl)> is the average intensity

[e]$R = \Sigma_{hkl} \mid |F_{obs}| - |F_{calc}| \mid / \Sigma_{hkl} |F_{obs}|$, where R_{free} is calculated for a randomly chosen 5 % of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections

[f]Deviations from ideal bond lengths/angles

[g]Number of residues in favoured, allowed or outlier region

Table A.4 X-ray data collection and refinement statistics of structures of the yCP mutant yβ5 M45R

	yCP yβ5 M45R	yCP yβ5 M45R:bortezomib	yCP yβ5 M45R:ONX 0914
Crystal parameters			
Space group	$P2_1$	$P2_1$	$P2_1$
Cell constants	a = 134.6 Å	a = 136.0 Å	a = 135.9 Å
	b = 302.8 Å	b = 300.3 Å	b = 298.1 Å
	c = 145.2 Å	c = 145.1 Å	c = 144.4 Å
	β = 112.7°	β = 112.8°	β = 112.7°
CPs/AU[a]	1	1	1
Data collection			
Beam line	X06SA, SLS	X06SA, SLS	X06SA, SLS
Wavelength (Å)	1.0	1.0	1.0
Resolution range (Å)[b]	49–3.0 (3.1–3.0)	49–2.9 (3.0–2.9)	48–2.8 (2.9–2.8)
No. observations	640110	700484	747601
No. unique reflections[c]	208693	230999	248239
Completeness (%)[b]	97.7 (98.9)	97.7 (98.6)	95.6 (96.1)
R_{merge} (%)[b, d]	10.6 (49.4)	8.7 (55.5)	7.4 (50.6)
I/σ (I)[b]	8.7 (2.7)	10.1 (1.7)	11.1 (1.8)
Refinement (REFMAC5)			
Resolution range (Å)	15–3.0	15–2.9	15–2.8
No. refl. working set	198258	219448	235827
No. refl. test set	9912	10972	11791
No. non hydrogen	50894	51053	51137
No. of ligand atoms (ligand; MES)	–	168	318
Water molecules	1340	1340	1340
R_{work}/R_{free} (%)[e]	14.8/20.7	15.9/21.7	17.5/23.9
r.m.s.d. bond (Å)/(°)[f]	0.014/1.802	0.012/1.658	0.014/1.830
Average B-factor (Å^2)	59.2	61.0	64.1
Ramachandran Plot (%)[g]	94.2/5.1/0.8	95.1/4.2/0.8	93.7/5.4/0.8

[a]Asymmetric unit

[b]The values in parentheses of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell

[c]Friedel pairs were treated as identical reflections

[d]$R_{merge}(I) = \Sigma_{hkl}\Sigma_j \mid [I(hkl)_j - I(hkl)] \mid \Sigma_{hkl} I_{hkl}$, where $I(hkl)_j$ is the jth measurement of the intensity of reflection hkl and <I(hkl)> is the average intensity

[e]$R = \Sigma_{hkl} \mid |F_{obs}| - |F_{calc}| \mid / \Sigma_{hkl} |F_{obs}|$, where R_{free} is calculated for a randomly chosen 5 % of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections

[f]Deviations from ideal bond lengths/angles

[g]Number of residues in favoured, allowed or outlier region

Table A.5 X-ray data collection and refinement statistics of structures of the yCP mutant yβ5 I35T M45R

	yCP	yCP	yCP
	yβ5 I35T M45R	yβ5 I35T M45R:bortezomib	yβ5 I35T M45R:ONX 0914
Crystal parameters			
Space group	$P2_1$	$P2_1$	$P2_1$
Cell constants	a = 133.9 Å	a = 135.1 Å	a = 135.5 Å
	b = 300.6 Å	b=301.0 Å	b = 299.1 Å
	c = 144.2 Å	c = 146.1 Å	c = 145.7 Å
	β = 112.8°	β = 112.8°	β = 112.9°
CPs/AU[a]	1	1	1
Data collection			
Beam line	X06SA, SLS	X06SA, SLS	X06SA, SLS
Wavelength (Å)	1.0	1.0	1.0
Resolution range (Å)[b]	49–3.0 (3.1–3.0)	50–2.9 (3.0–2.9)	50–3.1 (3.2–3.1)
No. observations	621044	698969	578722
No. unique reflections[c]	205091	231926	186814
Completeness (%)[b]	97.9 (98.3)	97.7 (99.1)	96.8 (98.7)
R_{merge} (%)[b, d]	11.9 (52.2)	9.6 (50.3)	9.8 (46.8)
I/σ (I)[b]	8.6 (2.9)	8.6 (2.6)	9.8 (2.5)
Refinement (REFMAC5)			
Resolution range (Å)	15–3.0	15–2.9	15–3.1
No. refl. working set	194836	220329	177472
No. refl. test set	9741	11016	8873
No. non hydrogen	50891	51051	51161
No. of ligand atoms (ligand; MES)	–	168	318
Water molecules	1340	1340	1340
R_{work}/R_{free} (%)[e]	15.6/22.4	15.6/21.8	14.3/21.1
r.m.s.d. bond (Å)/(°)[f]	0.011/1.592	0.012/1.664	0.012/1.754
Average B-factor (Å^2)	55.7	62.8	61.3
Ramachandran Plot (%)[g]	94.7/4.4/0.9	94.5/4.6/0.9	93.7/5.3/1.0

[a]Asymmetric unit

[b]The values in parentheses of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell

[c]Friedel pairs were treated as identical reflections

[d]$R_{merge}(I) = \Sigma_{hkl}\Sigma_j \mid [I(hkl)_j - I(hkl)] \mid \Sigma_{hkl} I_{hkl}$, where $I(hkl)_j$ is the jth measurement of the intensity of reflection hkl and <I(hkl)> is the average intensity

[e]$R = \Sigma_{hkl} \mid |F_{obs}| - |F_{calc}| \mid / \Sigma_{hkl} |F_{obs}|$, where R_{free} is calculated for a randomly chosen 5 % of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections

[f]Deviations from ideal bond lengths/angles

[g]Number of residues in favoured, allowed or outlier region

Table A.6 X-ray data collection and refinement statistics of structures of the β5t-mimicking yCP mutant

	yCP	yCP
	yβ5 A20S, A22C, V31S, M45T, A46S, G48T	yβ5 A20S, A22C, V31S, M45T, A46S, G48T:ONX 0914
Crystal parameters		
Space group	$P2_1$	$P2_1$
Cell constants	a = 135.0 Å	a = 136.6 Å
	b = 302.5 Å	b = 299.9 Å
	c = 144.2 Å	c = 146.1 Å
	β = 112.8°	β = 113.2°
CPs/AU[a]	1	1
Data collection		
Beam line	X06SA, SLS	X06SA, SLS
Wavelength (Å)	1.0	1.0
Resolution range (Å)[b]	25–2.9 (3.0–2.9)	25–3.1 (3.2–3.1)
No. observations	724106	558889
No. unique reflections[c]	230082	185554
Completeness (%)[b]	99.4 (97.9)	95.0 (97.4)
R_{merge} (%)[b, d]	11.9 (53.3)	14.3 (54.0)
I/σ (I)[b]	7.5 (2.1)	6.3 (2.2)
Refinement (REFMAC5)		
Resolution range (Å)	15–2.9	15–3.1
No. refl. working set	218577	176276
No. refl. test set	10928	8813
No. non hydrogen	50876	51129
No. of ligand atoms	0	294
Water molecules	1322	1322
R_{work}/R_{free} (%)[e]	15.4/21.2	14.7/21.7
r.m.s.d. bond (Å)/(°)[f]	0.011/1.589	0.012/1.681
Average B-factor (Å^2)	54.9	66.5
Ramachandran Plot (%)[g]	95.7/3.7/0.6	92.9/6.0/1.1

[a]Asymmetric unit

[b]The values in parentheses of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell

[c]Friedel pairs were treated as identical reflections

[d]$R_{merge}(I) = \Sigma_{hkl}\Sigma_j \mid [I(hkl)_j - I(hkl)] \mid \Sigma_{hkl} I_{hkl}$, where $I(hkl)_j$ is the jth measurement of the intensity of reflection hkl and <I(hkl)> is the average intensity

[e]$R = \Sigma_{hkl} \mid |F_{obs}| - |F_{calc}| \mid / \Sigma_{hkl} |F_{obs}|$, where R_{free} is calculated for a randomly chosen 5 % of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections

[f]Deviations from ideal bond lengths/angles

[g]Number of residues in favoured, allowed or outlier region