Supplementary Information

for

DARPin-based Crystallization Chaperones exploit Molecular Geometry as a Screening Dimension in Protein Crystallography

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Supplementary Table ST1: Analysis of crystal contact interfaces and interactions in DB04_v3_off7

Construct	Interface	Interface area [Å^2]	H-bonds				Salt bridges			
				Chain A (x,y,z)	Dist. [Å]	Chain A (-x,-y+1,z)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x,-y+1,z)
	1	656.3	1	A:ARG 321[HH12]	1.96	A:GLU 327[OE2]	1	A:ARG 321[NH1]	3.7	A:GLU 327[OE1]
							2	A:ARG 321[NH1]	2.81	A:GLU 327[OE2]
							3	A:ARG 321[NH2]	3.37	A:GLU 327[OE2]
							4	A:GLU 327[OE1]	3.7	A:ARG 321[NH1]
							5	A:GLU 327[OE2]	2.81	A:ARG 321[NH1]
							6	A:GLU 327[OE2]	3.37	A:ARG 321[NH2]
	2	639.8		Chain B (x,y,z)	Dist. [Å]	Chain A (x,y,z-1)		Chain B (x,y,z)	Dist. [Å]	Chain A (x,y,z-1)
			2	B:LYS 122[HZ3]	1.77	A:ASP 13[OD2]	7	B:LYS 122[NZ]	2.42	A:ASP 13[OD2]
							8	B:ASP 77[OD2]	3.65	A:HIS 9[ND1]
DB04_v3_off7	3	633.1		Chain B (x,y,z)	Dist. [Å]	Chain A (x,y,z)		Chain B (x,y,z)	Dist. [Å]	Chain A (x,y,z)
			3	B:ARG 370[HE]	2.11	A:GLU 97[OE2]	9	B:ARG 195[NE]	3.53	A:GLU 193[OE1]
			4	B:GLN 397[HE22]	2.07	A:GLU 97[O]	10	B:ARG 195[NH2]	3.61	A:GLU 193[OE1]
			5	B:GLU 193[OE1]	2.32	A:ARG 195[H]	11	B:ARG 195[NH2]	3.8	A:GLU 193[OE2]
			6	B:GLU 194[OE2]	2.2	A:ARG 191[HH12]	12	B:ARG 370[NE]	3.16	A:GLU 97[OE1]
			7	B:ASN 305[OD1]	2.39	A:LYS 164[HZ3]	13	B:ARG 370[NE]	2.77	A:GLU 97[OE2]
							14	B:ARG 370[NH2]	3.75	A:GLU 97[OE1]
							15	B:GLU 194[OE2]	3.05	A:ARG 191[NH1]
	4	584.4		Chain A (x,y,z)	Dist. [Å]	Chain A (x-y,x,z-2/3)		Chain A (x,y,z)	Dist. [Å]	Chain A (x-y,x,z-2/3)
							16	A:LYS 241[NZ]	3.58	A:ASP 28[OD1]
							17	A:GLU 240[OE2]	3.16	A:ARG 31[NH1]
	5	574.3		Chain A (x,y,z)	Dist. [Å]	Chain B (x-y,x,z+1/3)		Chain A (x,y,z)	Dist. [Å]	Chain B (x-y,x,z+1/3)
			8	A:GLY 358[H]	2.13	B:SER 353[O]	18	A:GLU 405[OE1]	2.85	B:LYS 144[NZ]
	6	137.1		Chain B (x,y,z)	Dist. [Å]	Chain B (-y+1,x-y+1,z-1/3)		Chain B (x,y,z)	Dist. [Å]	Chain B (-y+1,x-y+1,z-1/3)
	7	97.8		Chain B (x,y,z)	Dist. [Å]	Chain B (-x,-y+1,z)		Chain B (x,y,z)	Dist. [Å]	Chain B (-x,-y+1,z)
	8	15.2		Chain A (x,y,z)	Dist. [Å]	Chain B (x-y,x,z-2/3)		Chain A (x,y,z)	Dist. [Å]	Chain B (x-y,x,z-2/3)
	9	10.3		Chain B (x,y,z)	Dist. [Å]	Chain A (x-y,x,z-2/3)		Chain B (x,y,z)	Dist. [Å]	Chain A (x-y,x,z-2/3)

All crystal contact interfaces were analyzed with PDBePISA for surface area and interactions including hydrogen bonds and salt bridges. The interface contacts, in which β-lactamase participates are highlighted in green (interface contacts), yellow (H-bonds) and blue (salt bridges), respectively.

Supplementary Table ST2: Analysis of crystal contact interfaces and interactions in DB04_v3_D12

Construct	Interface	Interface area [Å^2]	H-bonds				Salt bridges			
	1	856.3		Chain A (x,y,z)	Dist. [Å]	Chain B (-x-1/2,y-1/2,-z-1)		Chain A (x,y,z)	Dist. [Å]	Chain B (-x-1/2,y-1/2,-z-1)
			1	A:ARG 79[HH22]	1.89	B:TYR 89[O]	1	A:LYS 144[NZ]	3.03	B:GLU 416[OE1]
	2	490.7		Chain B (x,y,z)	Dist. [Å]	Chain A (x,y,z-1)				
			2	B:ARG 224[H]	1.82	A:GLN 218[OE1]				
	3	409.8		Chain A (x,y,z)	Dist. [Å]	Chain A (x,y,z-1)		Chain A (x,y,z)	Dist. [Å]	Chain A (x,y,z-1)
							2	A:GLY 61[H]	2	A:GLU 240[OE1]
	4	370.7		Chain B (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y+1/2,-z-1)		Chain B (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y+1/2,-z-1)
			3	B:ARG 370[H]	2.49	A:GLU 327[OE2]				
DB04_v3_D12	5	340.0		Chain A (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y+1/2,-z-1)		Chain A (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y+1/2,-z-1)
	6	246.6		Chain B (x,y,z)	Dist. [Å]	Chain A (x,y,z)		Chain B (x,y,z)	Dist. [Å]	Chain A (x,y,z)
	7	185.3		Chain B (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y+1/2,-z)		Chain B (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y+1/2,-z)
			4	B:ARG 31[HH21]	2.24	A:ASP 339[OD2]	3	B:ARG 31[NE]	3.95	A:ASP 339[OD2]
	8	182.3		Chain B (x,y,z)	Dist. [Å]	Chain B (-x-1,-y+1,z)	4	B:ARG 31[NH2]	3.04	A:ASP 339[OD2]
	9	55.2		Chain A (x,y,z)	Dist. [Å]	Chain B (-x-1/2,y-1/2,-z)		Chain A (x,y,z)	Dist. [Å]	Chain B (-x-1/2,y-1/2,-z)
	10	52.8		Chain A (x,y,z)	Dist. [Å]	Chain B (-x,-y,z)		Chain A (x,y,z)	Dist. [Å]	Chain B (-x,-y,z)
	11	14.0		Chain B (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y+1/2,-z-1)		Chain B (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y+1/2,-z-1)
	12	14.0		Chain A (x,y,z)	Dist. [Å]	Chain A (-x-1,-y,z)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x-1,-y,z)

All crystal contact interfaces were analyzed with PDBePISA for surface area and interactions including hydrogen bonds and salt bridges. The interface contacts, in which β-lactamase participates are highlighted in green (interface contacts), yellow (H-bonds) and blue (salt bridges), respectively.

Supplementary Table ST3: Analysis of crystal contact interfaces and interactions in DB12_v4_off7

Construct	Interface	Interface area [Å^2]	H-bonds				Salt bridges			
				Chain A (x,y,z)	Dist. [Å]	Chain A (-x,y-1/2,-z+1/2)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x,y-1/2,-z+1/2)
DB12_v4_off7	1	1010.9	1	A:ARG 221[HH12]	2.27	A:ASP 151[OD1]	1	A:ARG 221[NH1]	3.08	A:ASP 151[OD1]
			2	A:GLN 226[HE21]	2.25	A:ASP 151[OD2]	2	A:ARG 221[NH2]	3.25	A:ASP 151[OD1]
			3	A:LYS 284[HZ1]	2.29	A:GLU 159[OE2]	3	A:LYS 284[NZ]	3.97	A:GLU 159[OE1]
			4	A:LYS 284[HZ3]	2.04	A:GLU 159[OE2]	4	A:LYS 284[NZ]	2.5	A:GLU 159[OE2]
							5	A:HIS 296[ND1]	3.15	A:GLU 417[OE1]
							6	A:HIS 296[ND1]	3.87	A:GLU 417[OE2]
							7	A:GLU 29[OE1]	3.17	A:LYS 249[NZ]
							8	A:GLU 29[OE2]	3.51	A:LYS 249[NZ]
	2	647.1		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1,y,z)		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1,y,z)
			5	A:ASN 313[HD21]	2.24	A:GLN 344[OE1]				
			6	A:ASN 313[HD22]	2.15	A:ASP 347[OD2]				
			7	A:GLN 142[H]	2.04	A:ASP 390[OD2]				
	3	562.7		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y-1/2,-z+1)		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y-1/2,-z+1)
			8	A:GLY 11[N]	2.56	A:ASN 156[OD1]				
			9	A:SER 12[OG]	2.46	A:ASN 158[OD1]				
			10	A:LYS 17[HZ1]	2.49	A:GLY 124[O]				
			11	A:LYS 17[HZ2]	2.36	A:GLY 124[O]				
			12	A:ASN 45[HD21]	1.94	A:TYR 56[O]				
	4	311.3		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y-1/2,-z)				
	5	107.1		Chain A (x,y,z)	Dist. [Å]	Chain A (-x-1/2,-y,z-1/2)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x-1/2,-y,z-1/2)
							9	A:GLU 248[OE1]	3.81	A:LYS 144[NZ]
	6	105.3		Chain A (x,y,z)	Dist. [Å]	Chain A (-x-1,y-1/2,-z+1/2)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x-1,y-1/2,-z+1/2)
	7	77.7		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1/2,-y,z-1/2)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1/2,-y,z-1/2)
	8	11.4		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1,y-1/2,-z+1/2)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1,y-1/2,-z+1/2)

All crystal contact interfaces were analyzed with PDBePISA for surface area and interactions including hydrogen bonds and salt bridges. The interface contacts, in which β-lactamase participates are highlighted in green (interface contacts), yellow (H-bonds) and blue (salt bridges), respectively.

Supplementary Table ST4: Analysis of crystal contact interfaces and interactions in DB08_v4_off7:MBP

Construct	Interface	Interface area [Å^2]	H-bonds				Salt bridges			
				Chain C (x,y,z)	Dist. [Å]	Chain B (-x+1/2,-y,z-1/2)		Chain C (x,y,z)	Dist. [Å]	Chain B (-x+1/2,-y,z-1/2)
	1	449.3	1	C:ASN 36[HD21]	2.36	B:GLY 189[O]	1	C:ASP 13[OD2]	3.68	B:LYS 117[NZ]
							2	C:GLU 29[OE1]	3.24	B:ARG 113[NH2]
							3	C:GLU 29[OE2]	3.18	B:LYS 98[NZ]
							4	C:GLU 29[OE2]	3.19	B:ARG 113[NH2]
				Chain C (x,y,z)	Dist. [Å]	Chain A (x,y,z)		Chain C (x,y,z)	Dist. [Å]	Chain A (x,y,z)
	2	437.3	2	C:GLN 166[HE22]	2.43	A:ASN 288[O]	5	C:LYS 349[NZ]	3.56	A:GLU 61[OE2]
			3	C:PHE 145[O]	2.2	A:GLN 222[HE21]				
				Chain A (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y+1/2,-z)		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1/2,-y+1/2,-z)
	3	390.6	4	A:GLY 375[H]	2.34	A:THR 248[OG1]	6	A:GLU 238[OE1]	2.91	A:HIS 246[NE2]
							7	A:GLU 238[OE2]	3.22	A:HIS 246[NE2]
							8	A:GLU 244[OE1]	3.24	A:ARG 254[NH1]
							9	A:GLU 244[OE2]	3.05	A:ARG 254[NH1]
				Chain D (x,y,z)	Dist. [Å]	Chain C (x-1,y,z)		Chain D (x,y,z)	Dist. [Å]	Chain C (x-1,y,z)
	4	359.8	5	D:ASN 139[HD21]	1.93	C:ASN 45[O]	10	D:GLU 153[OE1]	3.88	C:HIS 9[ND1]
							11	D:GLU 153[OE1]	2.62	C:HIS 10[NE2]
							12	D:GLU 153[OE1]	3.93	C:HIS 9[NE2]
				Chain A (x,y,z)	Dist. [Å]	Chain B (x-1,y,z)		Chain A (x,y,z)	Dist. [Å]	Chain B (x-1,y,z)
	5	349.2	6	A:ARG 16[HH21]	2.08	B:ASP 135[O]	13	A:ARG 16[NH2]	3.95	B:ASP 135[OD1]
			7	A:GLY 11[H]	2.36	B:LEU 136[O]	14	A:HIS 9[ND1]	3.98	B:GLU 153[OE1]
							15	A:HIS 10[NE2]	2.93	B:GLU 153[OE1]
DB08_v4_off7:MBP							16	A:HIS 9[NE2]	3.81	B:GLU 153[OE1]
				Chain A (x,y,z)	Dist. [Å]	Chain A (x-1,y,z)		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1,y,z)
	6	297.2	8	A:ARG 374[HH12]	2.07	A:ASP 343[OD1]	17	A:ARG 374[NH1]	2.9	A:ASP 343[OD1]
			9	A:ARG 374[HH22]	1.91	A:ASP 343[OD2]	18	A:ARG 374[NH1]	3.12	A:ASP 343[OD2]
							19	A:ARG 374[NH2]	2.73	A:ASP 343[OD2]
				Chain C (x,y,z)	Dist. [Å]	Chain C (x-1,y,z)		Chain C (x,y,z)	Dist. [Å]	Chain C (x-1,y,z)
	7	295.0					20	C:ASP 343[OD2]	2.82	C:ARG 374[NH2]
				Chain C (x,y,z)	Dist. [Å]	Chain D (-x,y-1/2,-z-1/2)		Chain C (x,y,z)	Dist. [Å]	Chain D (-x,y-1/2,-z-1/2)
	8	258.5					21	C:HIS 292[NE2]	3.46	D:GLU 53[OE2]
				Chain A (x,y,z)	Dist. [Å]	Chain C (x-1,y,z)		Chain A (x,y,z)	Dist. [Å]	Chain C (x-1,y,z)
	9	257.8	10	A:LYS 68[HZ2]	2.02	C:GLU 373[OE1]	22	A:LYS 68[NZ]	2.84	C:GLU 373[OE1]
			11	A:HIS 69[NE2]	3.08	C:GLU 238[OE1]	23	A:LYS 68[NZ]	3.92	C:GLU 373[OE2]
							24	A:HIS 69[NE2]	3.08	C:GLU 238[OE1]
							25	A:HIS 69[NE2]	3.94	C:GLU 238[OE2]
	10	237.1		Chain B (x,y,z)	Dist. [Å]	Chain B (x-1,y,z)		Chain B (x,y,z)	Dist. [Å]	Chain B (x-1,y,z)
	11	222.6		Chain D (x,y,z)	Dist. [Å]	Chain D (x-1,y,z)		Chain D (x,y,z)	Dist. [Å]	Chain D (x-1,y,z)
	12	99.3		Chain B (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y-1/2,-z)		Chain B (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y-1/2,-z)
	13	90.2		Chain C (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y-1/2,-z)		Chain C (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y-1/2,-z)
	14	33.0		Chain B (x,y,z)	Dist. [Å]	Chain C (x,y,z)		Chain B (x,y,z)	Dist. [Å]	Chain C (x,y,z)
			12	B:ALA 156[O]	2.31	C:LYS 245[HZ2]				
	15	8.0		Chain A (x,y,z)	Dist. [Å]	Chain D (x-1/2,-y+1/2,-z)		Chain A (x,y,z)	Dist. [Å]	Chain D (x-1/2,-y+1/2,-z)
	16	7.9		Chain A (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y-1/2,-z)		Chain A (x,y,z)	Dist. [Å]	Chain B (x-1/2,-y-1/2,-z)

All crystal contact interfaces were analyzed with PDBePISA for surface area and interactions including hydrogen bonds and salt bridges. The interface contacts, in which β-lactamase participates are highlighted in green (interface contacts), yellow (H-bonds) and blue (salt bridges), respectively.

Supplementary Table ST5: Analysis of crystal contact interfaces and interactions in DB15_v4_3G61:GFP

Construct	Interface	Interface area [Å^2]	H-bonds				Salt bridges			
				Chain A (x,y,z)	Dist. [Å]	Chain B (-x+1/2,y-1/2,-z)		Chain A (x,y,z)	Dist. [Å]	Chain B (-x+1/2,y-1/2,-z)
	1	431.3	1	A:LYS 355[HZ2]	2.11	B:ASP 210[OD2]	1	A:LYS 355[NZ]	3.73	B:ASP 210[OD1]
							2	A:LYS 355[NZ]	2.87	B:ASP 210[OD2]
							3	A:GLU 261[OE2]	2.96	B:LYS 214[NZ]
	2	338.5		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+2,y,-z+1)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+2,y,-z+1)
			2	A:GLN 228[HE21]	2.46	A:THR 281[O]				
	3	277.8		Chain B (x,y,z)	Dist. [Å]	Chain B (-x,y,-z)		Chain B (x,y,z)	Dist. [Å]	Chain B (-x,y,-z)
			3	B:LYS 101[HZ3]	1.71	B:ASP 173[OD2]	4	B:LYS 101[NZ]	2.6	B:ASP 173[OD2]
			4	B:ARG 168[HH12]	2.02	B:ASP 173[O]	5	B:ASP 173[OD2]	2.6	B:LYS 101[NZ]
	4	251.5		Chain B (x,y,z)	Dist. [Å]	Chain A (x-1,y,z-1)		Chain B (x,y,z)	Dist. [Å]	Chain A (x-1,y,z-1)
DB15_v4_3G61:GFP	5	155.3		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1/2,y+1/2,z)		Chain A (x,y,z)	Dist. [Å]	Chain A (x-1/2,y+1/2,z)
							6	A:ASP 39[OD2]	3.67	A:LYS 394[NZ]
	6	106.0		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+3/2,y-1/2,-z+1)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+3/2,y-1/2,-z+1)
							7	A:ASP 255[OD1]	3.66	A:ARG 201[NH1]
							8	A:ASP 255[OD1]	2.63	A:ARG 201[NH2]
							9	A:ASP 255[OD2]	3.13	A:ARG 201[NH1]
							10	A:ASP 255[OD2]	3.61	A:ARG 201[NH2]
	7	66.9		Chain B (x,y,z)	Dist. [Å]	Chain A (-x,y,-z)		Chain B (x,y,z)	Dist. [Å]	Chain A (-x,y,-z)
	8	50.4		Chain B (x,y,z)	Dist. [Å]	Chain A (-x+1/2,y-1/2,-z)		Chain B (x,y,z)	Dist. [Å]	Chain A (-x+1/2,y-1/2,-z)
	9	49.2		Chain B (x,y,z)	Dist. [Å]	Chain A (-x+1,y,-z)		Chain B (x,y,z)	Dist. [Å]	Chain A (-x+1,y,-z)
	10	37.2		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1,y,-z+1)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1,y,-z+1)
	11	11.4		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1/2,y-1/2,-z)		Chain A (x,y,z)	Dist. [Å]	Chain A (-x+1/2,y-1/2,-z)

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Supplementary Table ST6.

Comparison of experimental structure to models or modeling templates:

Reference structure:		**DARPin 1SVX**		**β-Lactamase 3DTM**		**Model v1**[a]		**Model v4**	
		internal repeats 1-3		excluding terminal helices		full length		full length	
residues:		37-135		187-408		13-432		13-432	
		rmsd (Å)		**rmsd (Å)**		**rmsd (Å)**		**rmsd (Å)**	
DARPin 2XEE		0.521	99 atoms						
DB04_v3_D12	chain A	0.447	99 atoms	0.378	222 atoms	1.509	406 atoms	0.870	406 atoms
DB04_v3_D12	chain B	0.464	99 atoms	0.394	222 atoms	1.247	406 atoms	1.044	406 atoms
DB04_v3_off7	chain A	0.409	99 atoms	0.428	222 atoms	2.291	406 atoms	1.432	406 atoms
DB04_v3_off7	chain B	0.734	99 atoms	0.494	222 atoms	1.680	406 atoms	0.850	406 atoms
DB08_v4_off7:MBP	chain A+B	0.320	99 atoms	0.421	222 atoms	2.671	410 atoms	3.471	410 atoms
DB08_v4_off7:MBP	chain C+D	0.301	99 atoms	0.412	222 atoms	2.792	410 atoms	3.781	410 atoms
DB12_v4_off7	chain A	0.485	99 atoms	0.447	222 atoms	2.945	414 atoms	4.575	414 atoms
DB15_v4_3G61:GFP	chain A+B	0.757	97 atoms	0.321	222 atoms	1.683	391 atoms	0.801	391 atoms
β-Lactamase 1M40				0.526	222 atoms				

Comparison of two chains within the asymmetric unit of one crystal (full length, 13-433)

				rmsd (Å)	
DB04_v3_D12	chain A	**DB04_v3_D12**	chain B	1.077	406 atoms
DB04_v3_off7	chain A	**DB04_v3_off7**	chain B	1.105	406 atoms
DB08_v4_off7:MBP	chain A+B	**DB08_v4_off7:MBP**	chain C+D	0.587	410 atoms

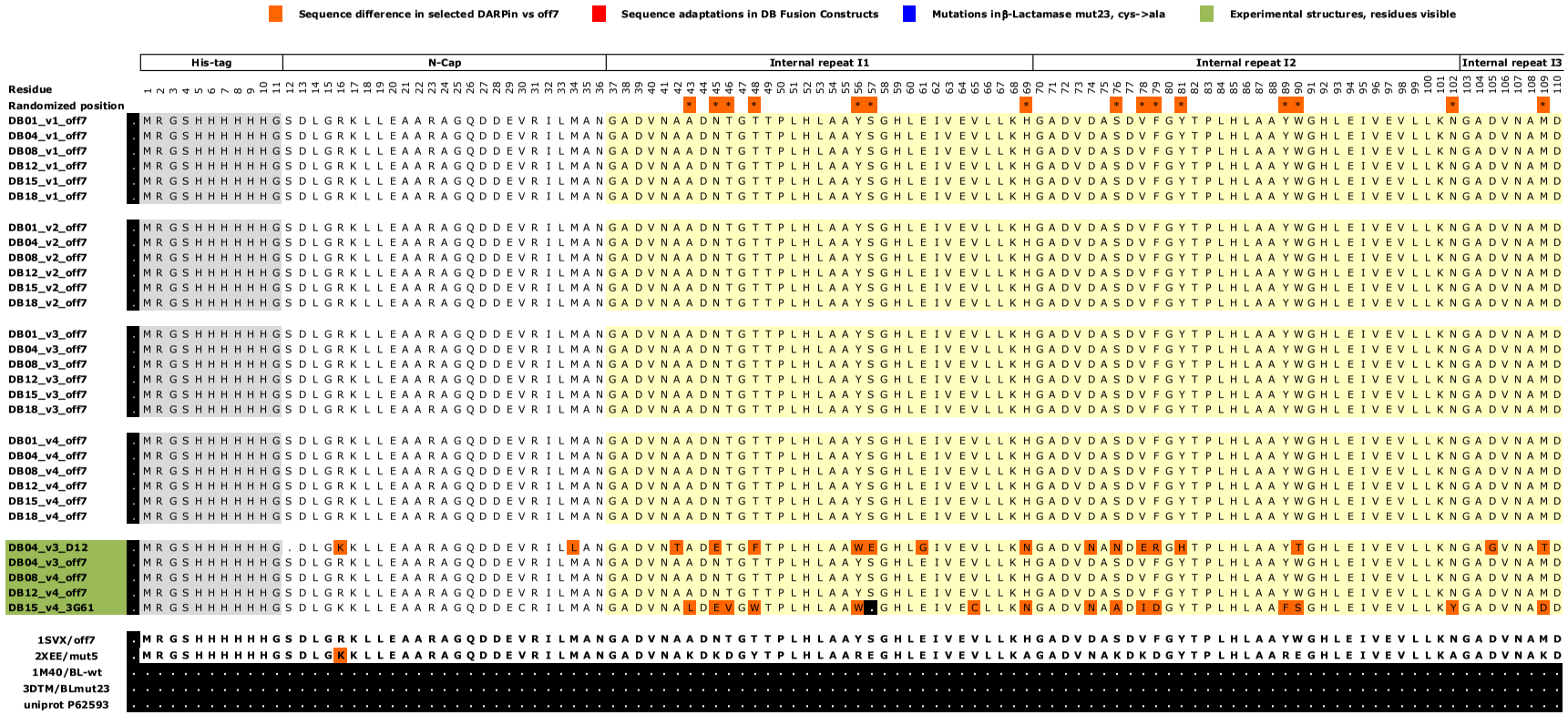
Comparison between two different structures (full length, 13-433)

				rmsd (Å)	
DB04_v3_D12	chain A	**DB04_v3_off7**	chain A	1.827	406 atoms
DB04_v3_D12	chain A	**DB04_v3_off7**	chain B	1.216	406 atoms
DB04_v3_D12	chain B	**DB04_v3_off7**	chain A	2.206	406 atoms
DB04_v3_D12	chain B	**DB04_v3_off7**	chain B	1.476	406 atoms

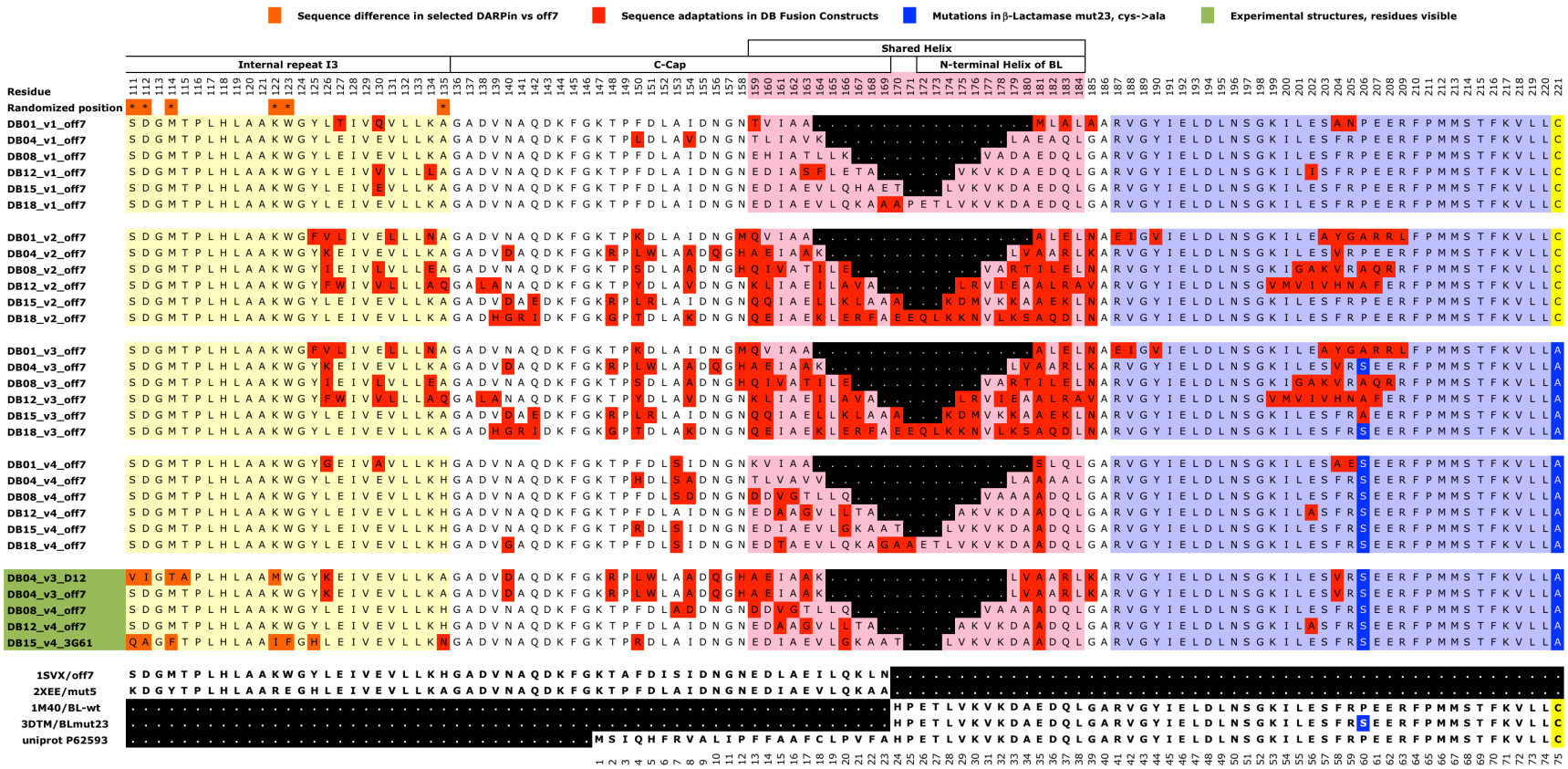
Ca rmsd fits were calculated using the PyMOL "fit" command without outlier rejection (cycles=0). Residue numbers are as indicated in Figure S1, DARPin internal repeats used for the superposition are highlighted in yellow, β-lactamase residues in blue in Figure S1.

[a] Model v1, which was submitted to extensive energy minimization, shows rmsds of 0.74±0.02 Å to the DARPin template and 0.88±0.03 Å to the β-lactamase templates. Model v4, where minimization was avoided, shows rmsds of 0.001 Å to either template domain.

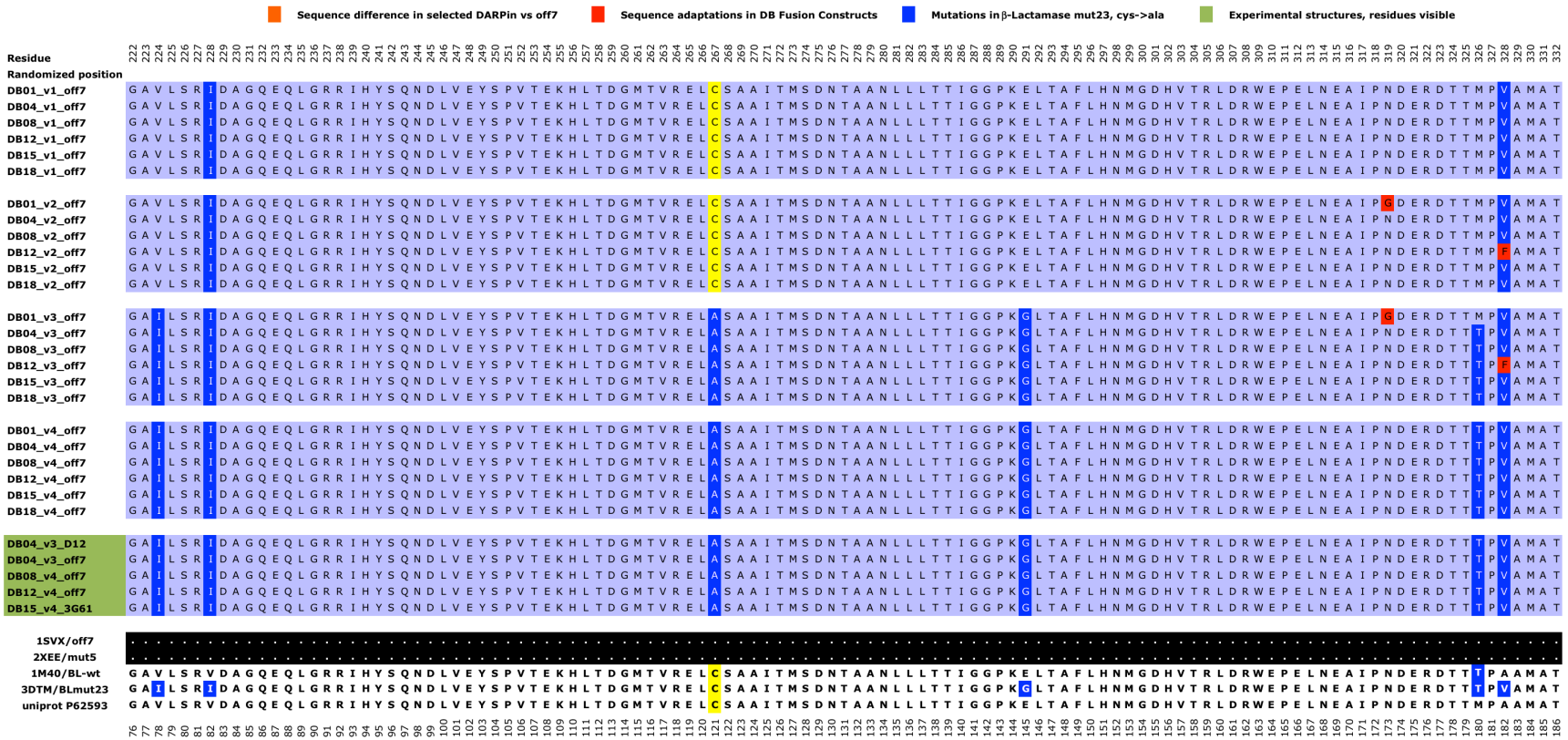
Supplementary Figure S1



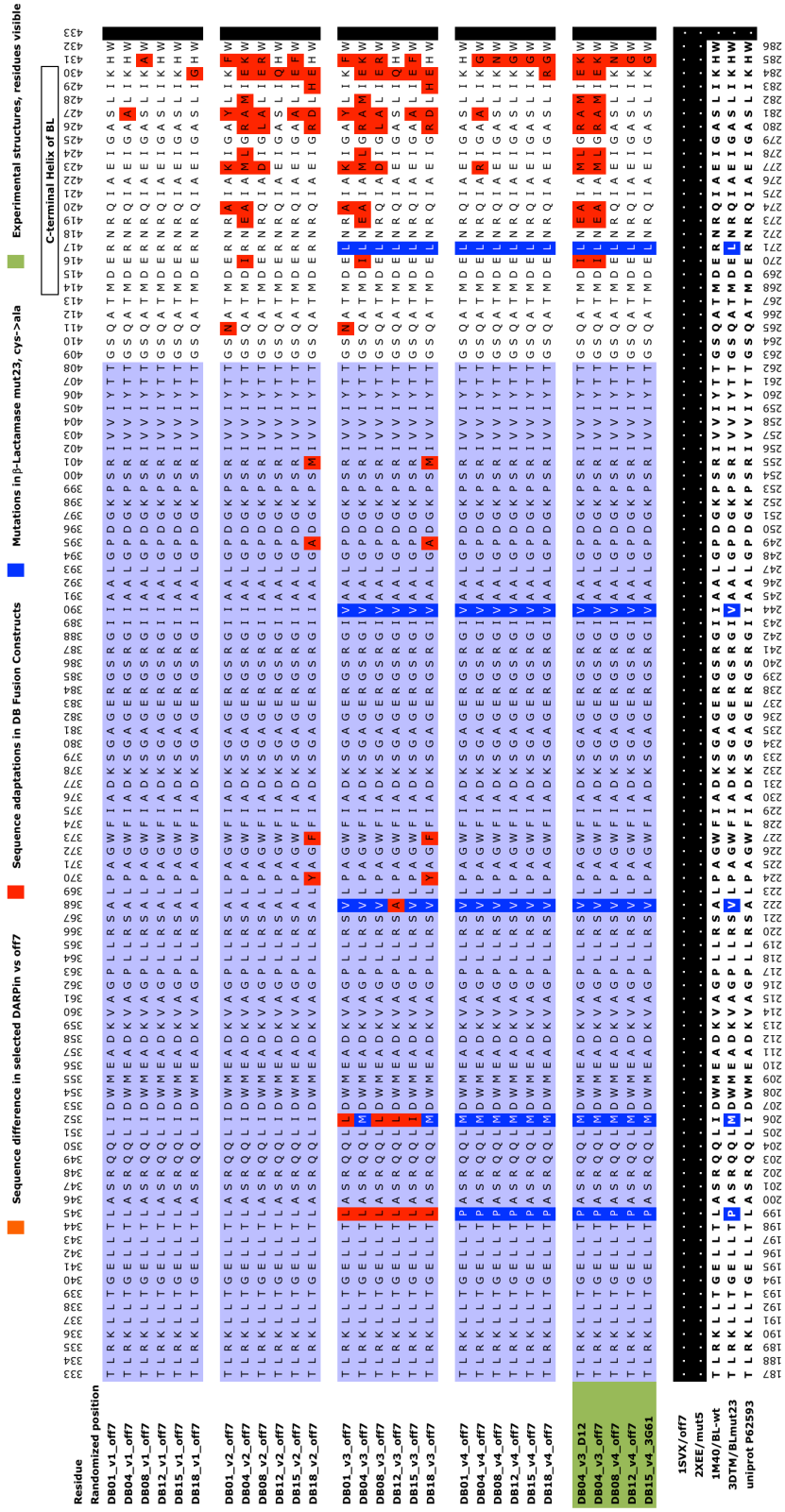
Supplementary Figure S1 (continued)



Supplementary Figure S1 (continued)

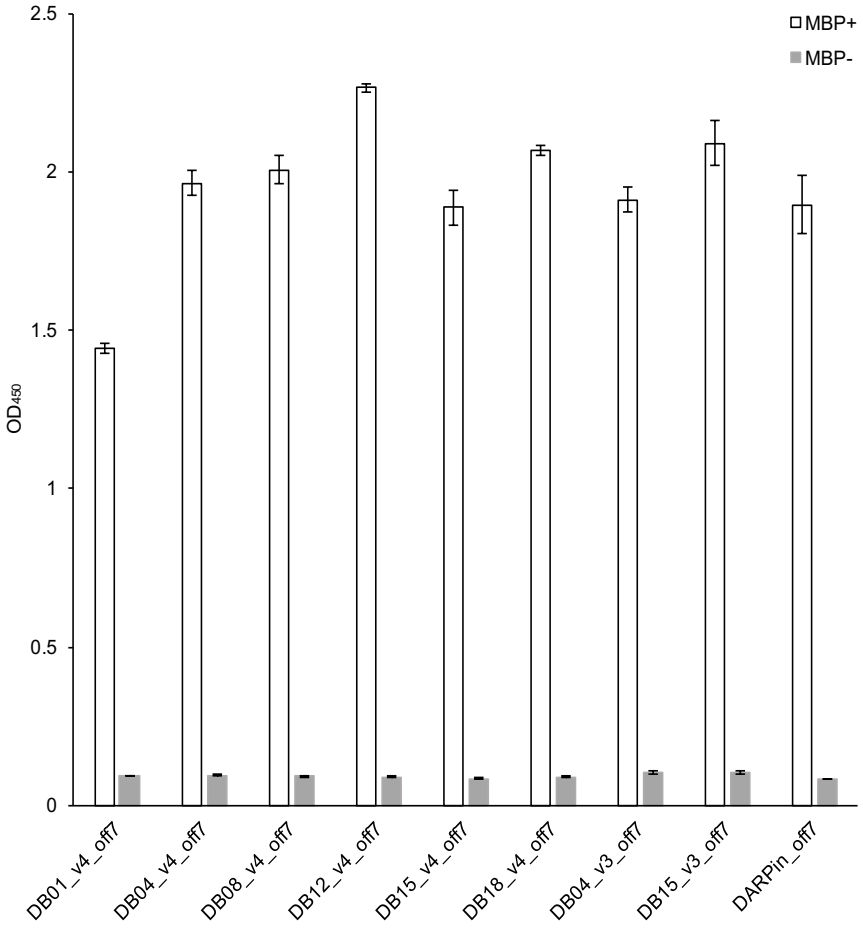


Supplementary Figure S1 (continued)



Supplementary Figure S1. Sequences of the shared helix construct with adaptations highlighted thsat were introduced to ensure folding and stability. *Orange*, sequence

differences in selected DARPin vs. off7; *red*, sequence adaptations in DB fusion constructs *blue*, mutations in beta-Lactamase mut23, cys->ala; *green labels*, experimental structures, only residues visible are plotted.



Supplementary Figure S2. Qualitative binding of DB fusion constructs to MBP measured in ELISA experiment. Eight purified DB constructs, plus MBP-binding DARPin off7 as a positive control, were analyzed for binding to MBP. Two out of eight constructs were from the third design version (DB04_v3_off7 and DB15_v3_off7) and the other six were from the forth design version. *White bars*, with MBP; *gray bars*, without MBP. The signals correspond to the average of triplicate measurements performed in parallel, and the error bars represent SDs.