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Supporting information for article:

MBP-binding DARPins facilitate the crystallization of an MBP fusion protein

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Table S1 Interactions between the DARPins and the DUSP1-CD molecules

Complex	**DARPin region (*symmetry*)**	**Residues involved in the interaction**	**Secondary structure element of the DUSP1-CD**	**Residues involved in the interaction**	**Interface area (Å²)**
MBP-DUSP1-CD with DARPin off7	N-terminal cap (*x, y, z*)	Leu14, Glu29, Ile32, Leu33, Ala35, Asn36§	β3-β4 loop	Glu215§, Gly216, His217, Tyr218§	290.4[a]
			strand β4	Gln219, Tyr220§	
MBP-DUSP1-CD with DARPin 16 (MBP open form)	C-terminal cap (*-x-3/2, y-1/2, -z+1/4*)	Ile121, Asp122§, Gly124, Glu126*, Glu130¶, Gln133	strand β2	Ser185	419.7
			helix α1	Tyr187, His188	
			β5-α5 loop	Gln259§, Ala260	
			α5-α6 loop	Arg292* Ser293¶, Ile294	
MBP-DUSP1-CD with DARPin 16	C-terminal cap	Phe117, Ile121,	helix α1	Tyr187, His188	358.2

(MBP closed form)	(*x-1/2, -y+1/2, -z*)	Asp122[§], Asn123, Gly124, Glu126[¶], Glu130, Gln133*	β5-α5 loop	Gln259[§], Ala260, Ile262	
			α5-α6 loop	Arg292[¶], Ile294*	
	N-terminal cap (*-x, y-1/2, -z-1/2*)	His8[§], Gly11, Ala35, Asn36, Gly37, Asp39, Asn41*	β4-α3 loop	His229, Lys230	363.7
			α5-α6 loop	Ser296, Pro297[§]	
			helix α6	Phe299, Gly303, Gln307*	

[§], [¶], * are the corresponding residues involved in hydrogen bonding.

A cut-off 3.3 Å for hydrogen bonding and 3.9 Å for hydrophobic interactions was applied.

[a] Average interface area in the heterodimer.

Table S2 Residues involved in crystal contacts mediated by the SER mutations of the MBP

A cut-off 4.4 Å is employed for the crystal contacts.

	Complex of the MBP-DUSP1-CD with DARPin			
SER mutation on MBP	**Off7**		**16**	**16**
	Chain A	**Chain C**	**(MBP open form)**	**(MBP closed form)**
Asp82Ala	Tyr242[a]	Glu131 [a]	Met34 [b] Ala35 [b] Gly37 [b]	Lys101 [b]
Lys83Ala		Asn124 [a] Pro126 [a] Glu131 [a] Leu135 [a]	Tyr69 [b]	
Glu172Ala				
Asn173Ala				
Lys239Ala	Ala84 [a]		Gln307 [c]	

[a] Residues from the symmetry related MBP.

[b] Residues from the symmetry related DARPin.

[c] Residues from the symmetry related DUSP1-CD.

Table S3 Hydrogen bonds and hydrophobic interactions

A cut-off 3.3 Å for hydrogen bonding and 3.9 Å for hydrophobic interactions was applied.

(*a*) List of the H-bonds and hydrophobic interactions between MBP and DARPin in the MBP-DUSP1-CD/off7 complex

DARPin off7 designed repeat module number	DARPin off7 (chain-B) interaction residue	H-bond (Å)	MBP interaction residue (chain-A)	DARPin off7 (chain-D) interaction residue	H-bond (Å)	MBP interaction residue (chain-C)
1						
	Tyr56	3.0	Lys140	Tyr56	2.8	Lys140
	Tyr56		Lys202	Tyr56		Lys202
2	Val78		Ser352	Val78		Ser352
	Val78		Gly353	Val78		Gly353
	Phe79		Val196	Phe79		Val196
	Phe79		Lys200	Phe79		Lys200
	Phe79		Ala351	Phe79		Ala351
	Phe79		Gly353	Phe79		Gly353
	Tyr81		Lys200			
	Tyr81	2.6	Asn201	Tyr81	2.4	Asn201
	Tyr89	2.8	Lys137	Tyr89	2.6	Lys137
	Tyr89		Asn201			

	Tyr89	2.6	His203	Tyr89	2.6	His203
	Trp90		Asp136	Trp90		Asp136
				Trp90		Lys137
	Trp90		Lys140			
	Trp90	2.9	Asn201	Trp90	3.0	Asn201
	Trp90		His203	Trp90		His203
3						
	Asp112		Lys200			
	Trp123		Lys137	Trp123		Lys137
	Tyr125	2.6	Lys137	Tyr125	2.6	Lys137

(*b*) List of the H-bonds and hydrophobic interactions between MBP and DARPin in the MBP-DUSP1-CD/DARPin 16 complexes

DARPin16 designed repeat module number	**DARPin 16 (open form of MBP) interaction residue**	**H-bond (Å)**	**MBP interaction residue**	**DARPin 16 (closed form of MBP) interaction residue**	**H-bond (Å)**	**MBP interaction residue**
	His23		Lys202	His23		Lys202
1	Asn45		Ser352	Asn45		Ser352
	Phe46		Val196	Phe46		Val196
	Phe46		Lys200	Phe46		Lys200

	Phe46		Ala350	Phe46		Ala350
	Phe46		Ala351	Phe46		Ala351
	Phe46		Gly353	Phe46		Gly353
	Val48		Lys200	Val48		Lys200
	Leu53		Lys202			
	Tyr56		Pro133	Tyr56		Pro133
				Tyr56		Asp136
	Tyr56	2.9	Lys137	Tyr56	2.6	Lys137
	Tyr56		Asn201	Tyr56		Asn201
	Tyr56	2.6	His203	Tyr56	2.6	His203
	Trp57		Asp136	Trp57		Asp136
	Trp57		Lys137			
	Trp57	3.0	Asn201	Trp57		Asn201
	Trp57		His203	Trp57		His203
2	Asp77	3.0	Lys200	Asp77		Lys200
	Thr79		Lys200	Thr79		Asp197
	Asp81	2.5	Lys200	Asp81	2.9	Lys200
	Trp90		Pro133			
	Trp90		Ala134	Trp90		Ala134
	Trp90		Lys137	Trp90		Lys137
	Tyr92	2.5	Lys137	Tyr92	2.6	Lys137
	Lys111		Thr193	Lys111		Thr193
	Lys111	3.2	Asp197	Lys111	2.4	Asp197

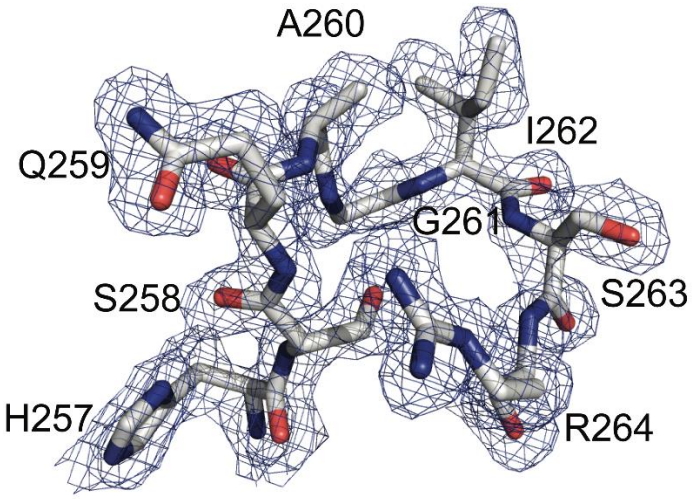

Figure S1 The residues forming a portion of the active site of DUSP1 from the MBP-DUSP1-CD complex with DARpin16 (MBP open form) superimposed onto the final $2F_o$-F_c electron density map (blue) contoured at 1σ level (2.22 Å resolution).