Supporting information

to

Purification of MBP fusion proteins using engineered DARPin affinity matrix

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Figure S1

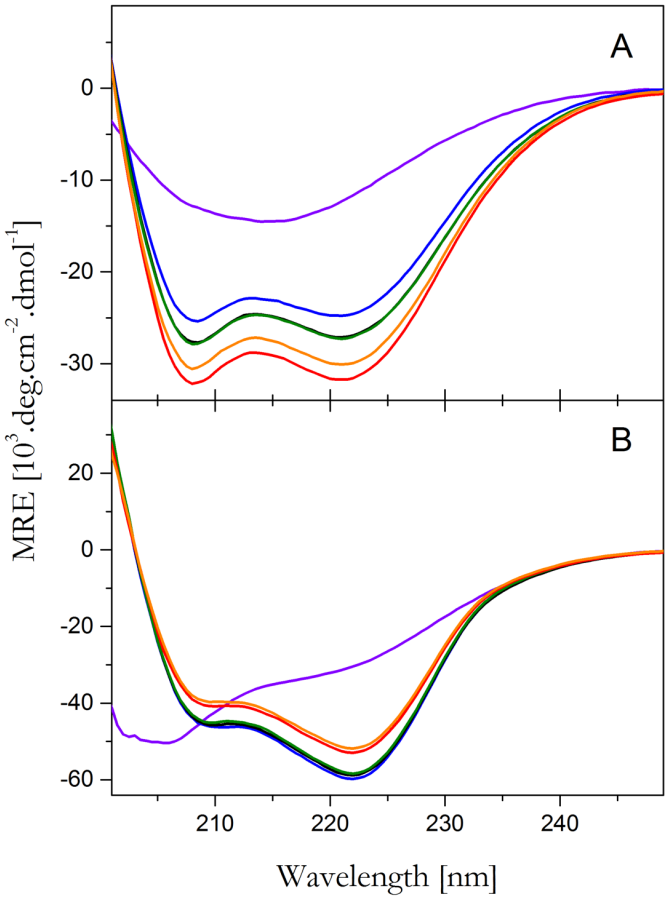

Figure S1. CD spectra in the far-UV spectral region of DARPin off7 (A) and MBP (B) at pH 2.7 (**purple**), pH 3.5 (**blue**), pH 7.0 (**black**), pH 8.0 (green), pH 9.0 (**red**) and pH 10.0 (**orange**).

Figure S2

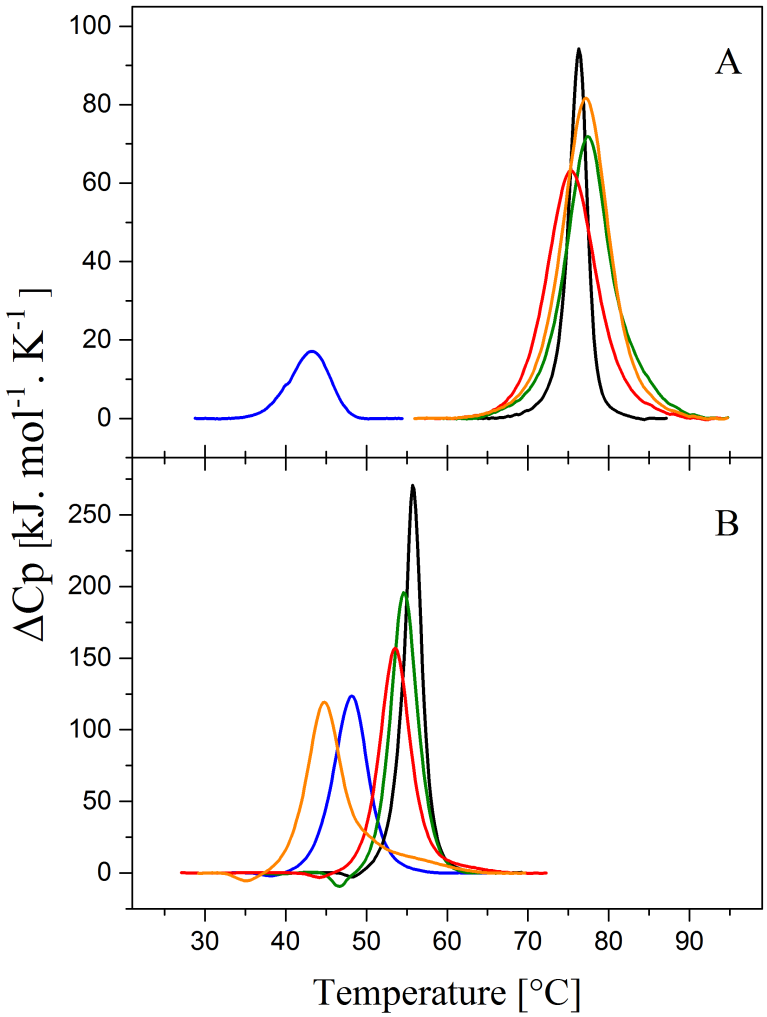

Figure S2. DSC thermograms of DARPin off7 (A) and MBP (B) at pH 3.5 (**blue**), pH 7.0 (**black**), pH 8.0 (green), pH 9.0 (**red**) and pH 10.0 (**orange**).

Figure S3

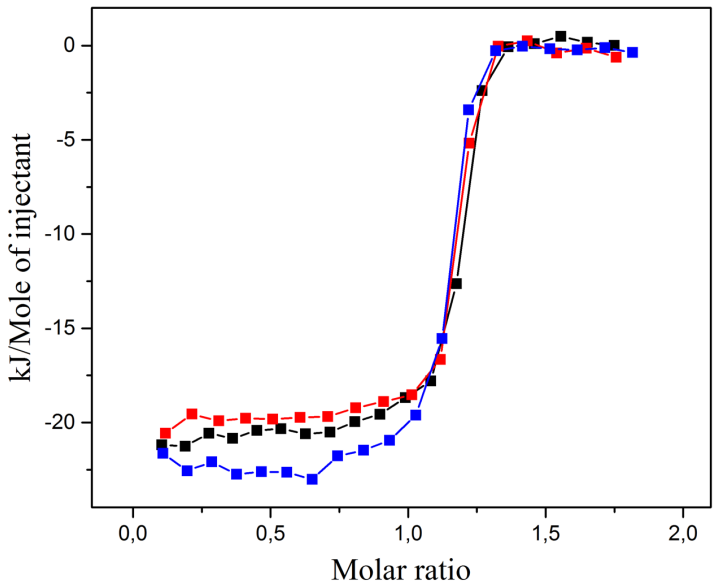

Figure S3. The influence of maltose on the DARPin off7/MBP interaction. The ITC experiment was performed with maltose concentrations of 0 mM (**black**), 250 mM (**red**) and 400 mM (**blue**) in PBS pH 7.4.

Figure S4

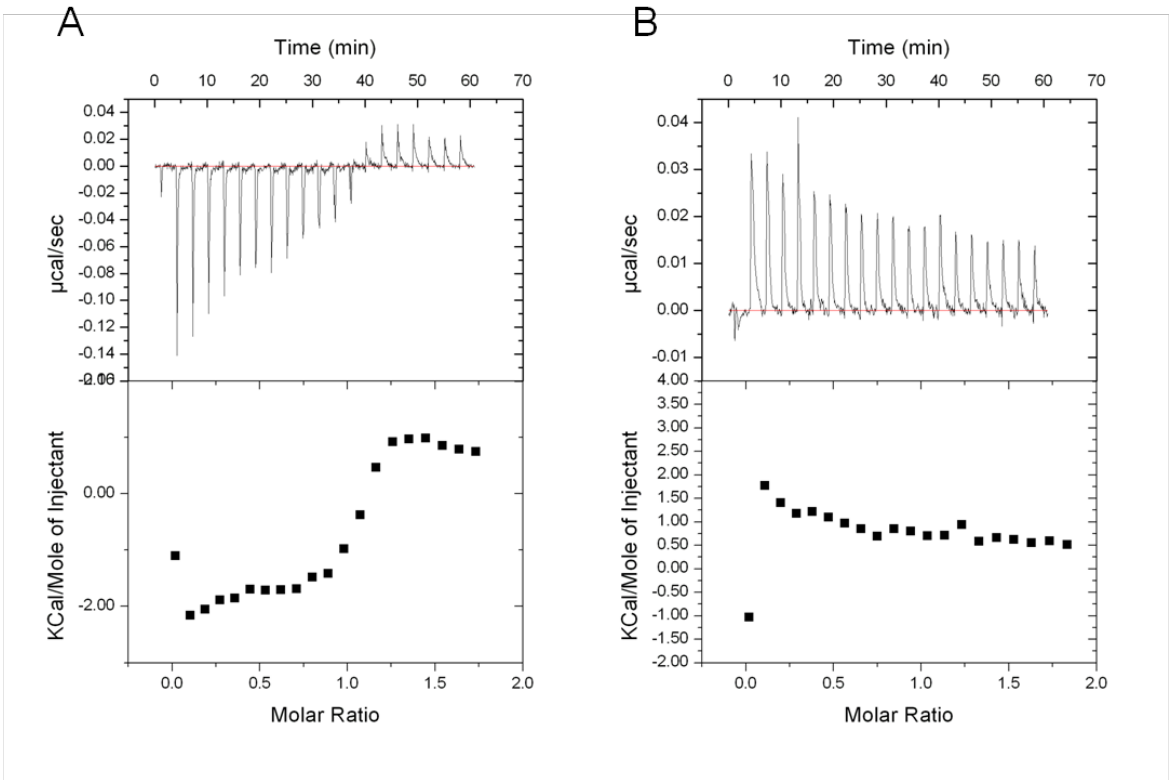

Figure S4. Raw ITC data of the interaction between DARPin off7 WT and MBP. (A) Titration of 500 µM solution of DARPin off7 WT into a 50 µM solution of MBP. (B) Baseline - titration of 500 µM solution of DARPin off7 WT into the buffer.

Figure S5

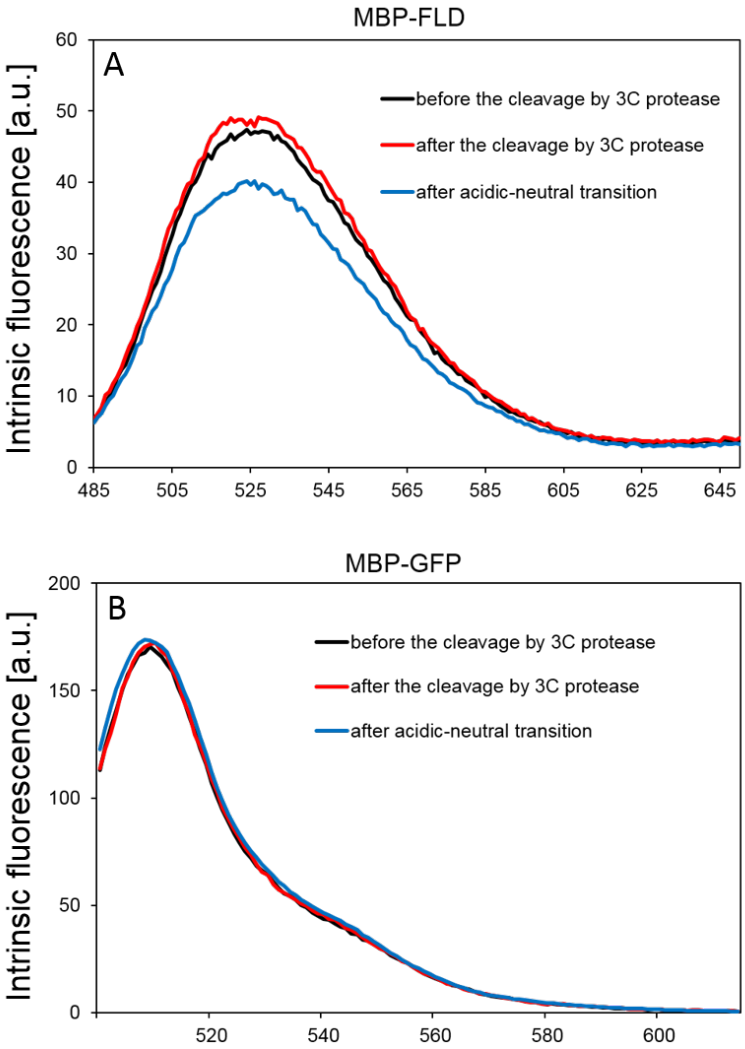

Figure S5. The influence of acidic pH (**blue**) and 3C protease cleavage (**red**) on the intrinsic fluorescence of MBP-FLD (A) and MBP-GFP (B). The intrinsic fluorescence of intact MBP fusion proteins is shown in **black**. Obtained results show that pH transition pH 7.0-pH 3.5-pH 7.0 slightly affects the fluorescence of FLD but not GFP suggesting a small pH-induced irreversible conformational change in FLD. Separation of POI from fusion protein by cleavage with 3C protease has apparently no effect on the tertiary structures of our studied proteins.

Figure S6

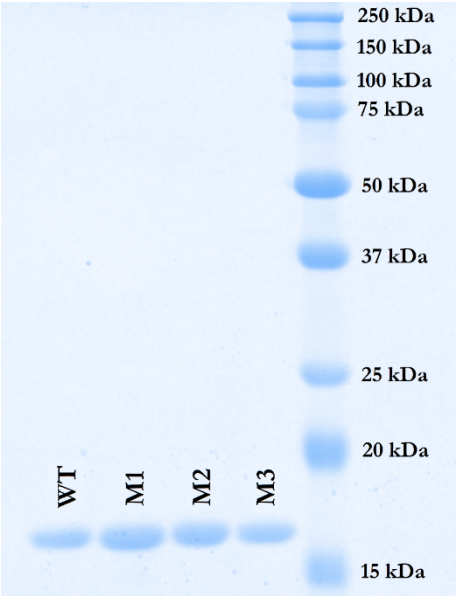

Figure S6. A comparison of the purification of the DARPin mutants.

Figure S7

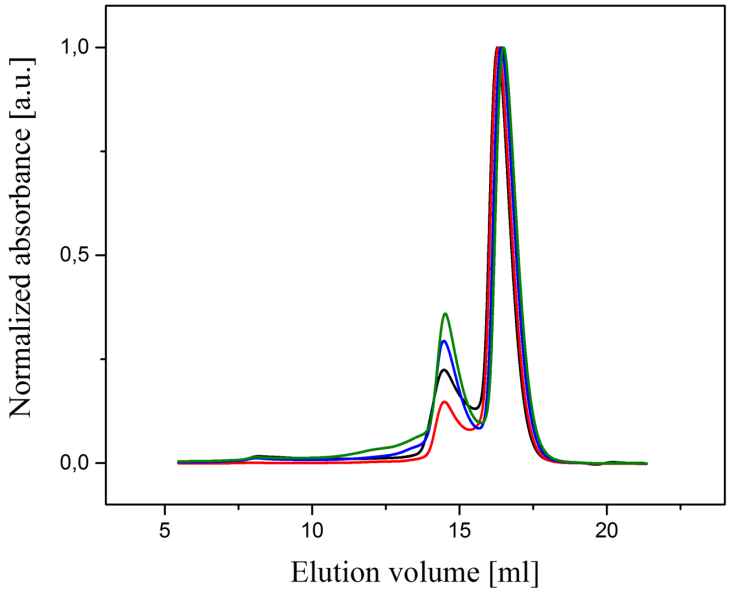

Figure S7. Size exclusion chromatography of the DARPin mutants: wild type (**black**), M1 (**red**), M2 (**blue**) and M3 (**green**). The experiment was performed in phosphate-buffered saline at pH 7.4.

Table S1. Thermodynamic parameters of the interaction between DARPin off7 and MBP in the presence of different maltose concentration.

Parameters	0 M maltose	250 mM maltose	400 mM maltose
Stoichiometric ratio	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
K_D [nM]	47 ± 3.0	27 ± 1.0	40 ± 2.0
ΔH [kJ/mol]	-28.4 ± 0.2	-19.6 ± 0.2	-22.2 ± 0.1
ΔS [J/mol/K]	43.9 ± 1.2	78.2 ± 1.3	66.5 ± 1.4

Table S2. Thermodynamic parameters of the interaction between DARPin off7 mutants and MBP.

Parameters	WT-MBP	M1-MBP	M2-MBP	M3-MBP
Stoichiometric ratio	1.04 ± 0.1	1.09 ± 0.1	1.16 ± 0.1	1.12 ± 0.1
K_D [nM]	47 ± 3.0	65 ± 4.0	114 ± 2.0	41 ± 2.0
ΔH [kJ/mol]	-28.4 ± 0.2	-24.4 ± 0.3	-16.7 ± 0.2	-20.1 ± 0.2
ΔS [J/mol/K]	43.9 ± 1.2	55.2 ± 2.5	76.7 ± 1.0	72.7 ± 0.8

Table S3. The capacity of 1 ml of the DARPin off7 matrix upon five consecutive purification cycles of MBP expressed in *E.coli*. The average error in the determination of the capacity is ±5-10%.

Purification cycle	1st	2nd	3rd	4th	5th
Capacity [mg]	5.3	4.8	4.5	4.5	4.4
Efficiency [%]	100	91	85	85	83

Table S4. The Pros and Cons of amylose resin and DARPin off7 matrix.

Characteristics	Amylose resin	DARPin off7 matrix
Affinity	$K_D \sim 3.5\mu M$ [37]	$K_D \sim 3.5nM$ [27]
Binding capacity for MBP	3.2 mg/ml	5.2 mg/ml
Number of purification steps	3	2
Regeneration	Up to five times	Multiple times

Nucleotide and amino acid sequences of full-length MBP-FLD and MBP-GFP

MBP-FLD

Nucleotide sequence:

ATGAAAACTGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAAGTCGGT
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Amino acid sequence:

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MBP-GFP

Nucleotide sequence:

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AACCATTACCTGTCGACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAG
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Amino acid sequence:

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