

Supplementary Figure 1

Sequences of designed AR proteins selected for MBP, JNK2 or p38 binding by ribosome display. The designed sequences for the N3C and the N2C libraries are given above the selected sequences (x represents a randomized potential interaction residue, where any amino acid was allowed except Cys, Gly or Pro; z represents a randomized framework residue where the three amino acids Asn, His or Tyr were allowed¹). This restriction of allowed amino acids was achieved by mixed trinucleotides in the synthetic oligonucleotides used for AR assembly². The residue numbers are given above the designed sequence. Only differences to the designed sequences are indicated. The names of the clones and the number of selection rounds are given on the left side of the respective sequence. The N3C sequences of the MBP binders are divided in three groups according to their sequences, and the respective group consensus sequences are shown below the sequences (in blue). The N2C sequences of the MBP binders as well as the JNK2 and p38 binders, respectively, are represented in two separate blocks. The five clones analyzed in more detail are highlighted in red font. The residues of off7 interacting with MBP are additionally highlighted in green. Note that in this representation, the N-terminal MRGSHHHHHH-tag (residues 1 to 10) has been removed.

References

1. Binz, H.K., Stumpp, M.T., Forrer, P., Amstutz, P. & Plückthun, A. Designing Repeat Proteins: B. Well-Expressed, Soluble and Stable Proteins from Combinatorial Libraries of Consensus Ankyrin Repeat Proteins. *J. Mol. Biol. in press*.
2. Virnekäs, B. *et al.* Trinucleotide phosphoramidites: ideal reagents for the synthesis of mixed oligonucleotides for random mutagenesis. *Nucleic Acids Res.* **22**, 5600-5607 (1994).

Supplementary Fig. 1.

Selected Groups:

N3C:	11	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	
Designed	GSDLGKKLL	EAARAGQ	DDVEVRI	LMMANGA	DVNAXDxxGxT	PLHLAAxxGHLEI	VEVLLKz	GADVNAx	DxxGxT	PLHLAAxxGHLEI	VEVLLKz	GADVNAx	DxxGxT	PLHLAAxxGHLEI	VEVLLKz	GADVNAQDKFGKTA	FDISIDNGNEDLAEILQKLN
Group1:																	
5 mbp3-10	T.T.....	IT.....	D.TE.N.....	V.VH.....	D..Y.....	H.VW.Q.....	YY.....	H.....	H.TW.T.....	YD.....	Y.G.....	C.....	A.....			
5 mbp3-20	VH.....	VH.....	VH.....	G.....	Y.....	H.VW.Q.....	YY.....	H.....	I.H.TW.T.....	YDS.....	Y.....	T.....				
5 mbp3-5	D.TE.N.....	V.VH.....	Y.....	H.VW.Q.....	YYD.....	Y.....	D.DT.I.....	RW.....	Y.....							
4 mbp44	I.....	N.....	D.TE.N.....	TVH.....	Y.V.....	H.VW.Q.....	YY.....	H.....	H.TW.T.....	YD.....	Y.V.....					
4 mbp48	D.TE.N.....	VH.....	Y.....	H.VW.Q.....	YY.....	H.....	D.DD.D.....	TD.....	M.....	N.....	H.....	C.....				
5 mbpoff13	D.TE.N.....	VH.....	Y.....	H.VW.Q.....	MT.YY.....	L.....	C.....	H.TW.T.....	YD.....	Y.....						
Consensus	D.TE.N.....	VH.....	Y.....	H.VW.Q.....	YY.....	H.....	H.TW.T.....	YD.....	Y.....							
Group2:																	
5 mbp3-11	I.TA.S.....	YL.....	MG.....	Y.....	I.VW.Y.....	T.YW.....	H.....	A.VT.L.....	HW.....	Y.....						
5 mbp3-8	E.....	I.TA.S.....	YL.....	D..Y.....	I.VW.Y.....	YW.....	N.....	M.SD.M.....	EW.....	H.V.....						
4 mbp417	V.....	I.TA.S.....	YL.....	Y.....	I.VW.Y.....	YWD.....	N.....	M.SD.MA.....	EW.....	Y.....						
5 mbpoff10	I.TA.S.....	YL.....	Y.....	I.VW.Y.....	YW.....	N.....	M.SD.M.....	EW.....	P.H.V.....	N.....						
5 mbpoff12	I.TA.S.....	YL.....	Y.....	I.VW.Y.....	YW.....	N.....	M.SD.D.....	T.ADD.P.....	RH.....							
5 mbpoff5	I.TA.S.....	M.YS.....	H.....	S.VF.Y.....	YW.....	Y.....	W.LT.MA.....	EN.D.....	N.....							
Consensus	I.TA.S.....	YL.....	Y.....	I.VW.Y.....	YW.....	N.....	M.SD.M.....	EW.....	H.....							
Group3:																	
5 mbp3-19	S.....	W.WY.R.....	IS.....	N.....	K.VE.N.....	M.....	YW.Y.....	RN.....	N.QD.T.....	RN.....	Y.....	S.....				
4 mbp45	DT.....	R.YV.S.....	TES.....	H.....	S.VF.Y.....	YW.....	Y.....	W.LT.M.....	EN.D.....	N.....						
4 mbp49	R.....	I.WT.S.....	VH.....	Y.T.....	SV.LI.E.....	KW.....	H.....	H.NT.AN.....	YN.....	N.....	T.....					
5 mbpoff2	A.NT.....	T.....	YS.....	H.....	S.VF.Y.....	YW.....	Y.....	W.LT.M.....	EN.D.....	N.....						
5 mbpoff7	R.....	A.NT.....	H.....	S.....	D.S.....	Y.....	N.....	M.S.....	K.....	K.....	H.....					
5 mbpoff8	K.....	E.FQ.T.....	Q.TN.....	YD.....	I.VW.Y.....	YW.....	F.....	N.....	M.SD.M.....	T.EW.....	H.....	T.....	A.....			
Consensus	-..T.T.....	YS.....	H.....	S.VF.Y.....	YW.....	N.....	N.....	T.M.....	EN.....	N.....						
N2C:	11	20	30	40	50	60	70	80	90	100	110	120	130				
Designed	GSDLGKKLL	EAARAGQ	DDVEVRI	LMMANGA	DVNAXDxxGxT	PLHLAAxxGHLEI	VEVLLKz	GADVNAx	DxxGxT	PLHLAAxxGHLEI	VEVLLKz	GADVNAQDKFGKTA	FDISIDNGNEDLAEILQKLN				
5 mbp2-10	S.IT.T.A.....	VVM.....	H.....	D.AW.T.....	YY.....	H.....	G.....	T.....								
5 mbp2-16	S.IT.T.A.....	VM.....	H.....	SD.AW.T.....	Y.....	YY.....	H.....									
5 mbp2-8	S.IT.T.A.....	VM.....	H.....	D.AW.T.....	YYD.....	H.....										
5 mbp3-16	H.....	M.NF.V.....	YW.....	F.....	Y.....	S.AT.D.....	KW.Y.G.....	Y.....								
5 mbpoff30	Q.WA.F.....	SY.....	Y.....	L.EF.D.....	KN.....	K.....	I.....									
N2C:	11	20	30	40	50	60	70	80	90	100	110	120	130				