Supplementary Material for

Structure based optimization of designed Armadillo-repeat proteins.

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Table S1. Hydrogen bonding interactions between the His_6-tag and the shallow concave groove on $Y_{II}M_4A_{II}$.

chains A and D (N-terminal His_6 tag residues)	**Hydrogen bonding**		**chains B and C**
	Atom	**Atom**	
His4	ND1	OE1	Glu114
	ND1	OE2	Glu114
His5*	NE2	OE1	Gln152
	NE2	NE2	Gln152
His6	N	OE2	Glu156
	NE2	OD1	Asn121
His7	O	NE1	Trp159
His8	NE2	NE2	Gln197
His9	N	OE2	Glu198
	NE2	NE1	Trp159
	NE2	ND2	Asn163

*His5 interacts only in chains A/B but not in chains C/D.

Table S2. Super-helical parameters of importin-α and designed ArmRPs as defined by Forwood et al.[1]

Structure*	Repeats [#]	Curvature[†]	Twist[†]	Lateral bending[†]
$Y_{II}M_4A_{II}$	M1/M2	16.25 ± 2.95	-22.32 ± 1.65	-14.68 ± 0.98
	M2/M3	15.18 ± 2.95	-23.63 ± 0.74	-9.66 ± 0.70
	M3/M4	15.38 ± 1.07	-23.16 ± 0.50	-9.10 ± 0.52
	M4/A5	20.91 ± 3.04	-28.18 ± 3.43	-7.60 ± 1.06
	average	16.92	-24.32	-10.26
$Y_{II}M_3A_{II}$	M1/M2	14.48 ± 0.33	-22.39 ± 0.79	-13.52 ± 0.97
	M2/M3	13.93 ± 0.60	-22.98 ± 0.71	-10.47 ± 0.21
	M3/A4	20.41 ± 1.22	-26.09 ± 2.75	-7.80 ± 0.62
	average	16.27	-24.07	-10.60
$Y_{III}M_3A_{III}$	M1/M2	14.06 ± 0.72	-21.47 ± 0.17	-7.93 ± 0.07
	M2/M3	16.34 ± 0.32	-20.43 ± 0.26	-6.29 ± 0.09
	M3/A4	21.84 ± 1.61	-28.32 ± 1.24	-8.34 ± 0.67
	average	17.42	-23.41	-7.52
$Y_{III}M_3A_{II}$	M1/M2	16.17	-25.94	-11.70
	M2/M3	15.08	-24.64	-10.91
	M3/A4	18.76	-23.25	-5.06
	average	16.67	-24.61	-9.22
importin-α (1bk5)	R6/R7	13.28 ± 0.68	-23.96 ± 0.01	-13.58 ± 0.46
	R7/R8	19.10 ± 0.47	-22.87 ± 0.48	-13.83 ± 0.22
	R8/R9	27.43 ± 0.42	-27.68 ± 0.16	-13.49 ± 0.23
	average	19.94	-24.84	-13.63
importin-α: NLS complex (1bk6)	R6/R7	13.11 ± 0.19	-24.64 ± 0.01	-12.76 ± 0.37
	R7/R8	18.66 ± 0.13	-22.15 ± 0.09	-12.82 ± 0.02
	R8/R9	27.76 ± 0.16	-27.60 ± 0.03	-13.04 ± 0.13
	average	19.84	-24.80	-12.95

* The PDB IDs are given in brackets.

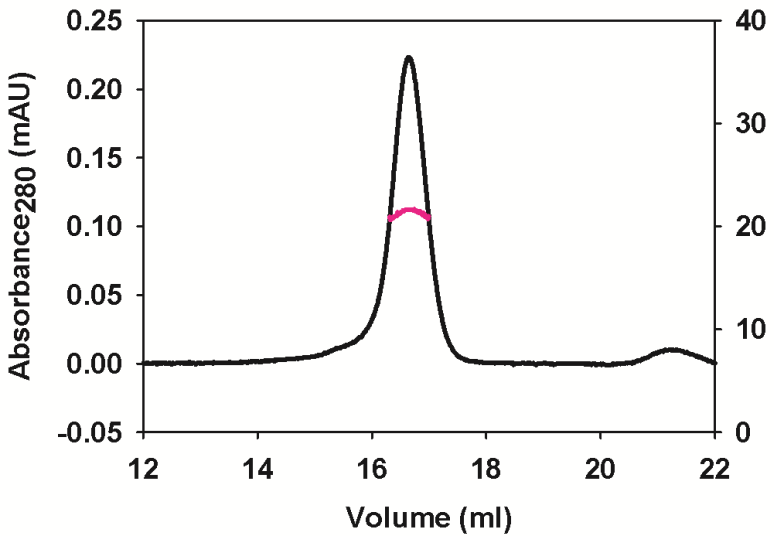
[#] Given are the repeats that define the indicated super-helical parameters. The designed ArmRP internal repeats (M-type), C-caps (A_{II} or A_{III}-type) and importin-α NLS binding repeats are defined by the following residue ranges: 43-84 (M1), 85-126 (M2), 127-168 (M3), 169-210 (M4), 169-206 (A4), 211-248 (A5), 289-330 (R6), 331-372 (R7), 373-414 (R8), and 415-456 (R9).

[†] Values are given in degrees and were calculated between the indicated repeat pairs. For designed ArmRPs that crystallize with multiple copies in the AU, the values were averaged across individual copies and errors estimates are given. Since $Y_{III}M_3A_{II}$ crystallized with one molecule in the AU no error estimates are given.

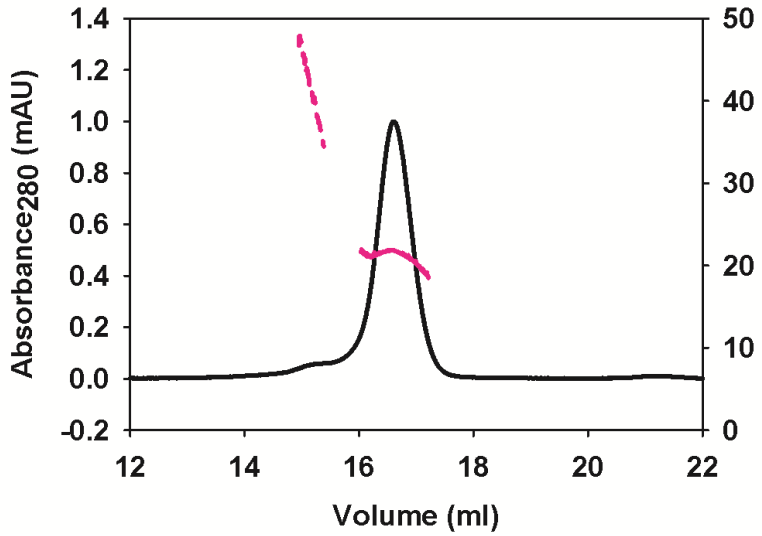
Table S3. Oligonucleotides used for the assembly and cloning of designed armadillo repeat protein genes

Name	Sequence 5'-3' direction	Description (for=forward, rev=reverse)
pQE_f_1	CGGATAACAATTTCACACAG	forward primer for pQE vectors
pQE_r_1	GTTCTGAGGTCATTACTG	revers primer for pQE vectors
Y_{II}1F	CCAGGCATCCGAACTGCCGCAGATGAC CCAGCAGCTGAACTCTG	for assembly Y_{II} module and amplification
Y_{II}2R	CGGTAGCAGACAGCTGTTCCTGCATGTCG TCAGAGTTCAGCTGCTGGG	rev assembly Y_{II} module
Y_{II}3F	GAACAGCTGTCTGCTACCCGTAAATTCTC TCAGATCCTGTCTGATGG	for assembly Y_{II} module
Y_{II}4R	TTCCTGGTACCCTAAGGTCTCAACCATCA GACAGGATCTGAGAG	rev assembly Y_{II} module and amplification
Y_{III}1F	CCAGGGATCCGAACTGCCGCAGATGGTTC AGCAGCTGAACTCTC	for assembly Y_{III} module and amplification
Y_{III}2R	GCAGAGCAGACTGCAGTTCCTGCTGGTCC GGAGAGTTCAGCTGCTGAACC	rev assembly Y_{III} module
Y_{III}3F	GAACTGCAGTCTGCTCTGCGTAAACTGTC TCAGATCGCTTCTGGAGG	for assembly Y_{III} module
Y_{III}4R	TTCCTGGTACCCTAAGGTCTCAACCTCCA GAAGCGATCTGAG	rev assembly Y_{III} module and amplification
A_{II}1F	CCAGGGATCCTAGGAAGACCTTGGTAACG AACAGAAACAGGC	for assembly A_{II} module and amplification
A_{II}2R	GTTTCTCCAGAGCACCAGCTTCTTTAACA GCCTGTTTCTGTTCGTTACC	rev assembly A_{II} module
A_{II}3F	GCTGGTGCTCTGGAGAAACTGGAACAGCT GCAGTCCCACGAG	for assembly A_{II} module
A_{II}4R	CCTGAGCTTCTTTCTGGATCTTCTCGTTC TCGTGGGACTGCAGC	rev assembly A_{II} module
A_{II}5F	GATCGAGAAAGAAGCTCAGGAAGCTCTGG AGAAGCTGCAGTCCC	for assembly A_{II} module
A_{II}6R	TTCCTGGTACCTCATTAGTGGGACTGCAG CTTCTCCAG	rev assembly A_{II} module and amplification
A_{III}1F	CCAGGGATCCTAGGAAGACCTTGGTAACG AACAGAAACAGGC	for assembly A_{III} module and amplification
A_{III}2R	GAGCCGGTTCAGCACCAGCTTCTTTAACA GCCTGTTTCTGTTCGTTACC	rev assembly A_{III} module
A_{III}3F	GCTGGTGCTGAACCGGCTCTGGAACAGCT GCAGTCCTCCCCG	for assembly A_{III} module
A_{III}4R	CCTGAGCTTCTTTCTGGATCTTCTCGTTC GGGGAGGACTGCAGC	rev assembly A_{III} module
A_{III}5F	GATCCAGAAAGAAGCTCAGGAAGCTCTGG AGAAGATCCAGTCCC	for assembly A_{III} module
A_{III}6R	TTCCTGGTACCTCATTAGTGGGACTGGAT CTTCTCCAG	rev assembly A_{III} module and amplification

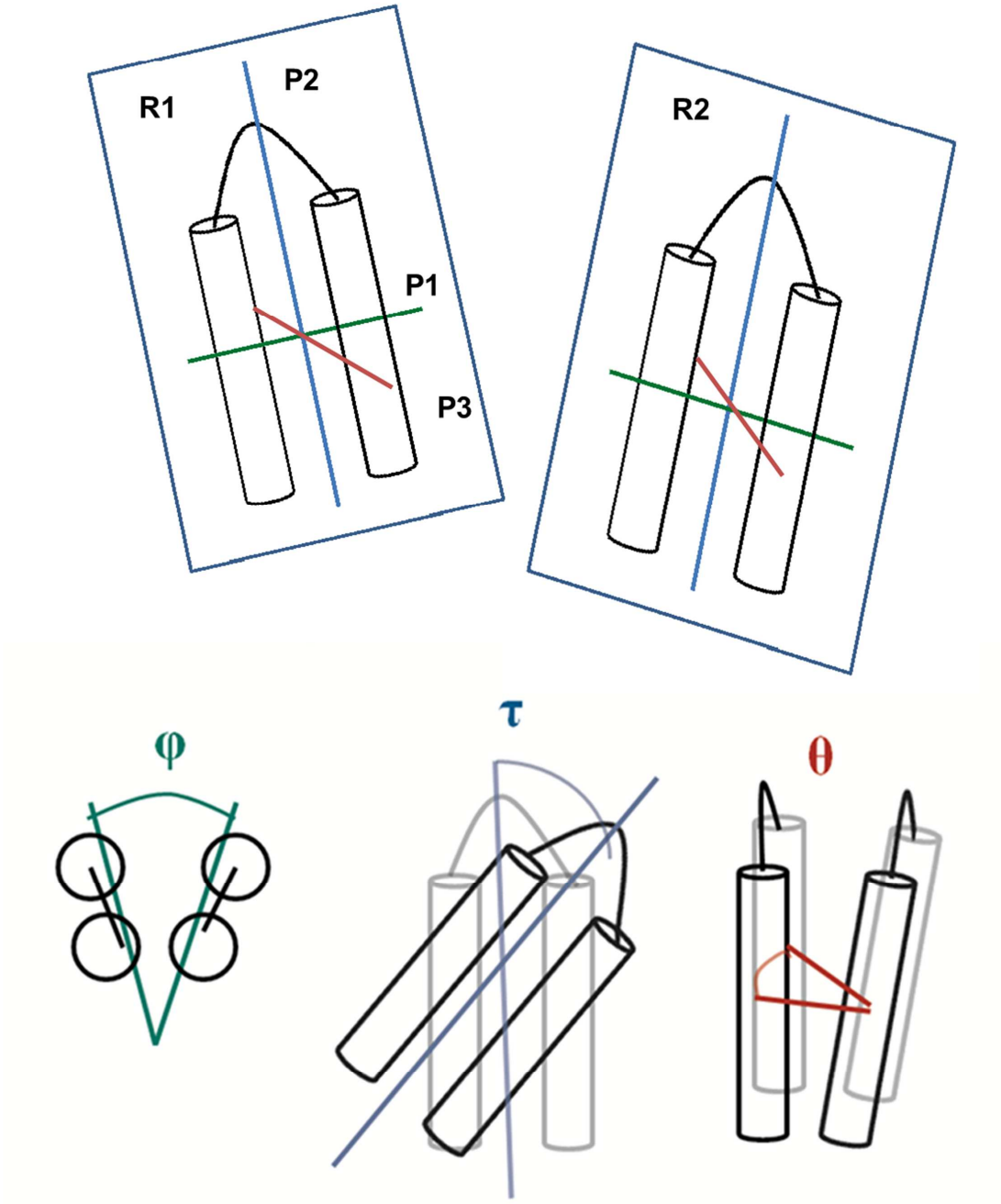
(a)



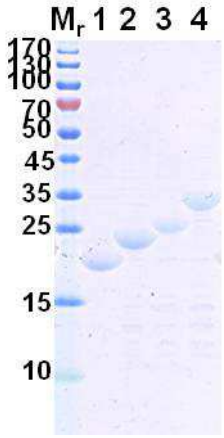
(b)



Supplementary Figure S1. Size-exclusion chromatography combined with multi-angle light scattering (MALS). The absorption at 280 nm and the molecular mass are shown as black and magenta curves, respectively. $Y_{II}M_3A_{II}$ was analysed at 5 mg/ml (a) and 18 mg/ml (b) concentration.



Supplementary Figure S2. Sketch illustrating the definitions of curvature, twist, and lateral bending (adapted from Forwood et al. [1]). R1 and R2 indicate two neighboring repeats of the ArmRP. The three principal axes are: the P1 axis is in the plane of the helices but perpendicular to them (green), the P2 axis is parallel to the helices (cyan) and the P3 axis is perpendicular to axes P1 and P2 (red). Curvature (φ) is the angle between two P1 axes of adjacent repeats, twist (τ) is the angle between two P2 axes of adjacent repeats and the lateral bending angle (θ) is the angle between two P3 axes of adjacent repeats.



Supplementary Figure S3. SDS-PAGE after single-step IMAC purification of designed ArmRPs. Constructs with 3 to 6 internal repeats. Lane 1, $Y_{II}M_3A_{II}$ (23 kDa); lane 2, $Y_{II}M_4A_{II}$ (27 kDa); lane 3, $Y_{II}M_5A_{II}$ (31 kDa); lane 4, $Y_{II}M_6A_{II}$ (35 kDa).

References

1. Forwood JK, Lange A, Zachariae U, Marfori M, Preast C, Grubmuller H, Stewart M, Corbett AH, Kobe B (2010) Quantitative structural analysis of importin-beta flexibility: paradigm for solenoid protein structures. Structure **18**:1171-1183.