Folding and Unfolding Mechanism of Highly Stable Full Consensus Ankyrin Repeat Proteins

Supplementary Material

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Sequence alignment of the different ankyrin consensus repeat modules. The full consensus repeat from Mosavi *et al.*¹⁷ and Wetzel *et al.* as well as the library module from Binz *et al.*¹⁵ are shown with their respective numbering. For analyzing molecular dynamics data, Interlandi *et al.* used a different numbering.¹⁸ Variable residues are highlighted in red, the fixed positions in the full consensus design are shown in blue, while the differences to the repeat of Mosavi *et al.*¹⁷ are highlighted in yellow. Secondary structure elements are symbolized by a and t.

tt	28 29 30 31 32 33	<pre>< N A K D K</pre>	<pre>< v v v v v v v v v v v v v v v v v v v</pre>		30 31 32 33		
a a	23 24 25 26 27	E A G A D	K Z G A D	K A G	25 26 27 28 29	31 32 33	a a
a a a a	18 19 20 21 22	V K L L	V E V L L	V E V L L	20 21 22 23 24	26 27 28 29 30	a a a a a
a a a	3 14 15 16 17	H L E	H L E	H L E	5 16 17 18 19 3	1 22 23 24 25 3	a a a
a a a	9 10 11 12 13	a a r <mark>n</mark> g	A X X A A	A A R E G	11 12 13 14 15	17 18 19 20 21	a a a
a a a	4 5 6 7 8	ГРГНГ	ГРГНГ	ГРСНГ	5 7 8 9 10	2 13 14 15 16	a a a a
t t t	123	ר ש ע	ר א ש א א	ר ש ס ע	2 3 4 5 6	8 9 10 11 1	t t t t
			۵	A D V N A K D	28 29 30 31 32 33 1	1 2 3 4 5 6 7	t
	Mosavi Nr.	Mosavi	Binz	Wetzel	Binz/Wetzel Nr.	Interlandi Nr.	

Supplementary Material Figure 2:

Representative kinetic traces for refolding (a),(b) and unfolding (c),(d) of NI₂C and NI₃C at 20°C followed by CD stopped-flow CD. The refolding kinetics (a),(b) are fitted to a single exponential equation, whereas the unfolding kinetics (c),(d) were fitted to a double exponential equation. The final protein concentration was 18 μ M. The final GdnHCl concentrations were 1.7 M for refolding of NI₂C (a) and 1.5 M for refolding of NI₃C (b); 4.4 M for unfolding of NI₂C (c) and 6.8 M for unfolding of NI₃C (d). The fit to a single exponential is shown in red, the fit to a double exponential in blue.

