

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

Supplementary Material

Svava Wetzel¹, Giovanni Settanni², Manca Kenig^{1,‡}, H. Kaspar Binz^{1,‡}, and Andreas Plückthun^{1,*}

¹ Department of Biochemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

² MRC Centre for Protein Engineering, Hills Road, CB2 0QH Cambridge, UK

* Corresponding author

‡ present addresses: M. K. Novartis Lek Pharmaceuticals, Kolodvorska 27, S1-1234 Menges, Slovenia; H. K. B., Molecular Partners AG, Grabenstrasse 11a, CH-8952 Zürich-Schlieren, Switzerland

Correspondence address:

Prof. Dr. Andreas Plückthun, Department of Biochemistry, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland, Tel +41 44 635 5570, Fax +41 44 635 5712, plueckthun@bioc.uzh.ch

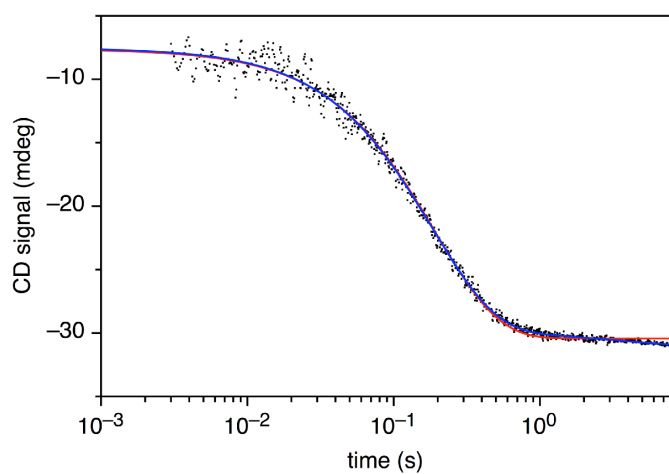
Supplementary Material Figure 1:

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.

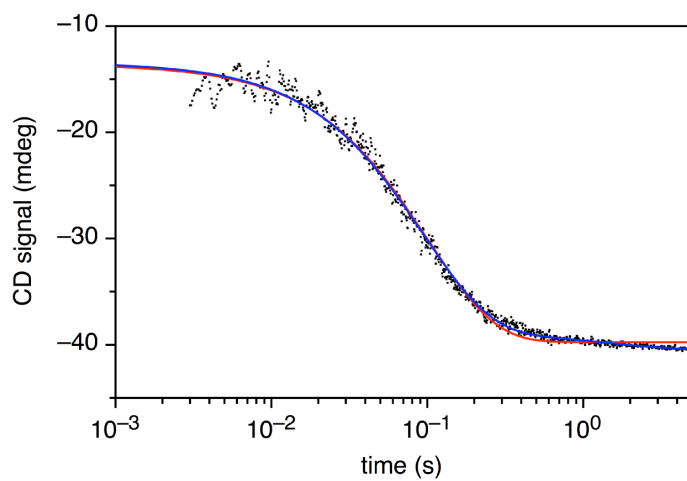
Supplementary Material Figure 2:

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 μM. 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.

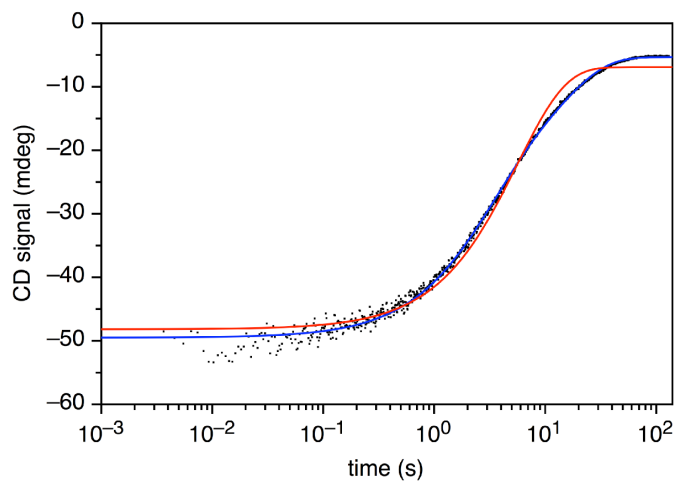
(a)



(b)



(c)



(d)

