Supporting Material for Slow, reversible, coupled folding and binding of the spectrin tetramerization domain

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Table S1 Collated equilibrium and association rate constants for protein systems where one or more partner is at least partially disordered.

Complex	***k*+** ($M^{-1}s^{-1}$)	K_d (M)	**Method***	**Reference**
SH2/Y751	3.34E+06	4.20E-08	SPR	(1)
SH2/pYHmT	2.00E+05	1.00E-06	SPR	(2)
SH2/pY531	2.00E+05	5.00E-06	SPR	(2)
SH2/ITAM	4.17E+06	2.34E-09	Scintillation proximity-based assay	(3)
Grb2-mSos1/EGFR	6.30E+06	3.10E-08	SPR	(4)
Grb2-mSos1/IR-PEP	1.05E+06	2.96E-07	SPR	(4)
BRCA1-BRCT/bBACH1-P	4.00E+06	5.00E-09	SPR	(5)
Cdc42/WASP	1.90E+05	6.30E-08	SPR	(6)
SUA/Ecto-Tva	5.60E+04	1.49E-07	RMB	(7)
CaM/CKII	1.30E+08	2.00E-09	SF	(8)
TolA/ColN	5.00E+04	8.30E-07	SPR	(9)
TolA/ColN	1.10E+05	1.00E-07	SF	(10)
TCR/pMHC	3.72E+04	5.90E-06	SPR	(11)
S-peptide/S-protein	1.80E+07	6.00E-12	SF	(12)
Sky/β_1	1.90E+04	7.10E-08	SPR	(13)
Sky/β_2	3.80E+04	3.80E-08	SPR	(13)
Sky/β_3	4.80E+04	2.40E-08	SPR	(13)
HIF/TAZ1	1.29E+09	1.43E-07	NMR	(14)
GCN4 D7A	8.00E+06	1.90E-10	SF	(15)
UbF45W	3.10E+03	2.20E-07	SF	(15)
GCN4	3.00E+08	2.40E-10	SF	(16)
GCN4-p1	2.75E+06	5.60E-08	SF	(17)
GCN4	6.63E+05	2.60E-07	SF	(18)
Leucine zipper	4.00E+06	2.50E-06	SF	(19)
KIX/pKID	1.30E+02	1.08E-05	QCM	(20)
KIX/KID	1.20E+02	2.08E-04	QCM	(20)
MICA/NKG2D	7.50E+03	1.70E-06	SPR	(21)
AR-AF1/SRC-1	2.40E+02	1.40E-05	SPR	(22)
AR-AF1/RAP74-NTD	4.60E+03	6.30E-07	SPR	(22)
E6/GST-E6AP	7.13E+04	2.59E-06	SPR	(23)
E6/MBP-E6AP	5.42E+04	5.26E-06	SPR	(23)
MEM-265/peptide	2.74E+05	2.10E-09	SPR	(24)
MDM2/p53	9.20E+06	2.20E-07	SF	(24)
Impα/NLS	2.00E+04	1.55E-08	SPR	(25)
D-KQTSV/PSD-95PDZ3	7.90E+06	8.00E-07	SF	(26)
TolB/Pal	7.60E+04	5.00E-08	SF	(27)
TolB/TBEp	1.75E+05	6.20E-06	SF	(27)
NOS PDZ/PSD-95 PDZ 2	4.00E+05	4.00E-06	SF	(28)
S-peptide/S-protein	4.40E+05	2.80E-07	SF	(29)
PEP-19/apo-CaM	1.00E+06	1.30E-05	SF	(30)
HIF-OH/TAZ1	1.29E+09	1.43E-07	NMR	(14)
E-cadherin/β-catenin	3.56E+05	4.60E-08	SPR	(31)
pE-cadherin/β-catenin	4.49E+06	5.20E-11	SPR	(31)
HPV16 E716-31/ RbAB	3.44E+07	5.10E-09	SF	(32)
MICA/NKG2D	7.50E+03	1.73E-06	SPR	(21)
TAZ2/p53 AD2	1.70E+10	3.20E-08	NMR	(34)

GCN4-p1	4.20E+05	3.30E-08	CD SF	(35)
ROP	1.80E+06	4.02E-13	CD	(36)
hFoB	8.10E+03	6.59E-05	SF	(37)
hPyA1	9.00E+05	1.55E-12	SF	(37)
Arc repressor	9.00E+06	7.88E-09	SF	(38)
CopG (Y39W)	ND	1.48E-10	CD	(39)
ORF56h	7.00E+07	3.00E-15	SF	(40)
Gene V protein	1.10E+07	1.11E-12	CD manual mixing	(41)
HIV-1 protease	ND	3.84E-11	equilibrium denaturation	(42)
SIV-1 protease	ND	1.76E-10	equilibrium denaturation	(42)

*SPR: surface plasmon resonance, RMB: resonant mirror biosensor, SF: stopped-flow technique, QCM: quartz crystal microbalance, NMR: nuclear magnetic resonance, CD: circular dichroism, ND: not determined.

Table S2 Collated equilibrium and association rate constants for protein systems where both partners are structured prior to binding

Complex	***k*+** ($M^{-1}s^{-1}$)	**K_d** (M)	**Method***	**Reference**
IgG/anti-IgG	5.25E+06	1.50E-08	FCS	(43)
BPTI/trypsin	9.90E+05	5.00E-14	Enzymatic studies	(44)
BPTI/chymotrypsin	1.70E+05	1.10E-08	Enzymatic studies	(44)
E225/D1.3	1.00E+03	3.60E-07	SPR	(45)
FVIIa/sTF	3.40E+05	6.30E-09	SPR	(46)
hIL5/shIL5Rα-Fc	4.90E+05	7.60E-09	SPR	(47)
hGH/hGPbp	3.00E+05	9.00E-10	SPR	(48)
sCD4/gp120	8.30E+04	1.90E-08	SPR	(49)
CD4/gp120	6.72E+04	2.20E-08	SPR	(50)
g5p/Trx-(SH)2	4.70E+04	2.20E-09	SPR	(51)
Cytochrome *c*/2B5	6.50E+05	1.20E-10	SF	(52)
Cytochrome *c*/5F8	1.50E+06	6.70E-11	SF	(52)
SUA-rIgG/Ecto-Tva	2.76E+05	1.64E-08	RMB	(7)
IαI/trypsin (human)	1.10E+07	6.40E-08	Enzymatic studies	(53)
IαI/chymotrypsin (bovine)	2.20E+05	2.40E-09	Enzymatic studies	(53)
AMY2/BASI	1.19E+05	1.70E-09	SPR	(54)
Myosin/CaM	4.60E+07	2.20E-08	SF	(55)
HEL/VL::VH-MalE	7.84E+04	8.46E-09	SPR	(56)
HEL/VH::VL-MalE	8.66E+04	8.96E-09	SPR	(56)
Barstar/barnase	6.00E+08	1.30E-14	SF	(57)
B-CheZ/CheY	5.64E+06	7.10E-09	SF	(58)
HyHEL/BWQL	1.80E+07	5.30E-08	SF	(59)
PI3-K/IGF-1R	4.83E+05	4.40E-09	SPR	(60)
GroEL/GroES	8.00E+05	1.40E-08	SPR	(61)
smGN/CaM	1.24E+06	4.42E-09	SPR	(62)
BoNT/scFv	2.09E+06	4.51E-11	SPR	(63)
Palivizumab/Fab	1.26E+05	5.25E-09	SPR	(64)
Palivizumab/IgG	1.27E+05	3.39E-09	SPR	(64)
Fyn SH3/PRD1	7.70E+04	5.20E-06	SPR	(65)
AChR/Fyn SH2	4.20E+03	1.90E-09	RBA	(66)
AChR/Fyk SH2	6.20E+03	1.30E-09	RBA	(66)
IgG/protein A/G	4.26E+05	1.13E-10	SPR	(67)
AR-AF1/RAP74	2.00E+04	1.70E-07	SPR	(22)
p53/NPM	4.30E+03	3.14E-07	SPR	(68)
Ras/Raf-RBD	3.55E+07	5.00E-08	SF	(69)
Stathmin/tubulin	8.90E+03	5.60E-07	SPR	(70)
IL5/sIL5Rα	2.40E+06	3.10E-09	SPR	(71)
IgG/CGRPα (human)	1.16E+05	5.78E-09	SPR	(72)
hPRLr-ECD/hPRL	1.40E+05	4.40E-09	SPR	(73)
hPRLr-ECD/hGH	3.58E+04	6.70E-09	SPR	(73)
IGF-II/IGF2R	6.62E+05	1.19E-07	SPR	(74)
BLIP/TEM-1	2.40E+05	7.00E-10	SPR	(75)
AF6 RA1/Ras	6.40E+06	2.40E-06	SF	(76)
CD81LEL-GST/HCV	8.90E+03	5.20E-08	SPR	(77)
CopY/*cop* promoter	4.30E+04	1.70E-10	SPR	(78)
actin gelsolin	2.50E+04	ND	Fluorescence	(79)

MMP1/TIMP1ΔC	5.20E+04	2.11E-09	SPR	(80)
casein kinase II b chain/ a chain	6.65E+04	5.41E-09	SPR	(81)
ACE2 receptor/SARS spike protein	7.12E+04	1.62E-08	SPR	(82)
Ad12/coxsackie and adenovirus receptor	7.31E+04	1.50E-08	SPR	(83)
shark IgNAR/lysozyme	9.00E+04	1.00E-09	SPR	(84)
CR2/ Staphylococcus enterotoxin B	1.00E+05	1.40E-04	SPR	(85)
anthrax protective antigen/CMG2	1.10E+05	7.80E-10	SF FRET	(86)
Fab/ flu virus hemagglutinin	1.10E+05	1.00E-09	SPR for k+, ELISA for K_d	(87)
HPrK/P/HPR	1.30E+05	4.50E-08	SPR	(88)
ICAM-1/integrin aL domain	1.33E+05	3.00E-06	SPR	(89)
MHC class1 HLA-A2/CD8	1.40E+05	1.26E-04	SPR	(90)
proMMP2/TIMP-2	1.40E+05	5.20E-09	SPR	(91)
TIMP-1/MMP3	2.00E+05	6.30E-10	SPR	(80)
camel VHH/pancreatic a-amylase	2.40E+05	3.50E-09	SPR	(92)
Fab/HIV-1 capsid protein p24	3.50E+05	2.90E-08	SPR	(93)
xylanase/inhibitor	3.60E+05	1.07E-09	SPR	(94)
CD2/CD58	4.00E+05	1.55E-05	SPR	(95)
IgG1 Fc/FcgRIII	5.40E+05	1.80E-06	SPR	(96)
HIV-1 integrase/ p75	4.80E+05	1.09E-08	FRET	(97)
albumin/albumin-binding protein	5.50E+05	1.20E-09	SPR	(98)
TGFb/TGFb receptor	7.40E+05	7.30E-08	SPR	(99)
a-chymotrypsin/LCMI II	8.00E+05	2.00E-10	Enzymatic studies	(100)
chymotrypsin/ecotin	8.90E+05	3.00E-12	Enzymatic studies	(101)
Fab D3H44/tissue factor	9.80E+05	1.00E-10	SPR	(102)
Mlc transcription regulator/ EIICB	9.95E+05	4.14E-09	SPR	(103)
streptogrisin B/OMTKY3	1.20E+06	ND	Enzymatic studies	(104)
Fv D1.3 /HEW lysozyme	1.40E+06	3.70E-09	SF for k+, fluorescence titration for K_d	(105)
Ga$_{i1}$/RGS4	1.70E+06	ND	SF	(106)
elastase/elafin	3.60E+06	1.70E-10	Enzymatic studies	(107)
b actin/profilin	6.60E+06	4.80E-07	SF	(108)
AchE/fasciculin-II	1.50E+07	ND	Enzymatic studies	(109)
streptokinase/plasmin	5.40E+07	5.00E-11	SF	(110)
CheY/CheA	5.60E+06	4.00E-06	SF	(111)
cytochrome f/plastocynin	1.80E+08	ND	SF	(112)
λ Cro (F58W)	1.9E+04	4.92E-09	FRET manual mixing	(113)
CcdB	ND	4.4E-17	Equilibrium denaturation	(114)
Tctex-1	ND	3E-15	Equilibrium denaturation	(115)

* FCS: fluorescence correlation spectroscopy, SPR: surface plasmon resonance, SF: stopped-flow technique, RMB: resonant mirror biosensor, RBA: radioligand binding assay, FRET: fluorescence resonance energy transfer, ELISA: enzyme-linked immunosorbent assay, ND: not determined.0

Outline of the kinetic schemes used for analysis of protein-protein interactions:

Considering the two-state bimolecular reaction, $A + B \underset{k-}{\overset{k+}{\leftrightarrow}} AB$ (scheme 2 in main text), where the association rate constant is $k+$ (in $M^{-1}s^{-1}$), the dissociation rate constant is $k-$ (in s^{-1}) and the dissociation constant K_d is identified as $k-/k+$ (in M).

(A) Irreversible schemes

(i) Equimolar association and dissociation

In some cases, appropriate conditions may be found so that the K_d is very low compared with the protein concentration, making a 1:1 molar association reaction essentially irreversible. Such cases are described by a kinetic scheme $A + B \overset{k+}{\rightarrow} AB$ (scheme 1 in main text), which allows kinetic traces to be fit to Eq. 2 in Materials and Methods (57, 116, 117). Similarly, conditions, such as high denaturant concentration, may be found so that the equilibrium constant is much higher than the employed protein concentration, and therefore the dissociation reaction is essentially irreversible, allowing kinetic traces to be fit to Eq. 7. For example, protein solutions may be rapidly mixed with a series of denaturant solutions at a variety of concentrations. The empirical linear variation of ln(*k*-) with denaturant can then be used to estimate *k*- in appropriate solvent conditions (12). Inherently the irreversible approach is likely to result in 'gaps' where neither association nor dissociation can be modelled as irreversible, such as intermediate denaturant concentrations (118), where the dominant process switches from folding/association to unfolding/dissociation. An elegant method to estimate dissociation rate constants under conditions where association is strongly favoured, is to dilute (radio- or dye-) labelled complexed subunits into an excess of unlabelled subunits so that dissociation of labelled complex is nonetheless irreversible, and identify the 'off' rate constant as the dissociation rate constant (12, 57). As well as being practically challenging, extraction of the equilibrium properties in this manner assumes that the presence of the label does not alter the rate constants, and therefore complex stability.

(ii) Pseudo-first order studies

The most common approach for obtaining association rate constants is the creation of pseudo-first order conditions by providing an excess (typically described as above five-fold) of one or other of the components (12, 119-121). In this case the association constant may be extracted by simply dividing the observed rate constant by the concentration of the excess species, assumed to be constant. This approach is arguably the most accurate way to estimate association rate constants, as accurate concentration determination of only one reactant is required and, where measurements are

made for a number of protein ratios the estimate is averaged, reducing errors. Other notable consequences of having one species in excess are that the equilibrium is shifted towards the bound state, assisting in the creation of essentially irreversible association reactions, and, lower overall concentrations are required to reach non-diffusion limited regimes, where mechanistic information about conformational changes may be inferred (122).

In principle the pseudo first order method is also capable of taking into account a significant back reaction during association kinetics and give estimates of the dissociation rate constant. Since $k_{obs} = k_+[A] + k_-$, where [A] is the concentration of the excess reagent, if measurements are performed for various [A], a straight line fit can be used to extrapolate to [A]=0, where $k_{obs}=k-$. However, typically $k-$ is small relative to $k+$, so that the large required extrapolation provides a very poor estimate of the off rate (29). For this reason, $k-$ is commonly measured using an alternative technique, such as the out-competition of bound protein using an excess of unlabelled protein (12), or, by assuming that complex formation is a two-state reaction,, calculate $k-$ given a pre-determined K_d (29).

(B) Reversible schemes

(i) Concentration dependence of apparent rate constants

Recently, equations describing the expected concentration dependence of apparent rate constants in the reversible regime (123) have been used to extract both association and dissociation rate constants for PDZ domain ligand binding (26, 124). This method can be used even if kinetic data are fairly poorly sampled, where only a single exponential fit can be justified. An inherently similar approach is used in relaxation rate analysis, where only small changes are made to the equilibrium, so that the reaction is well described by a single exponential with an apparent rate constant that has a known dependence on the concentration of reactants (125).

(ii) Direct fitting of kinetic traces

It is possible to fit individual kinetic traces by simple numerical integration of rate equations, and this approach has been used previously (35). However, this provides little insight into the process itself, and is more computationally expensive than an analytical approach, especially where data sampling is extensive enough to provide accurate estimates. An analytical solution to the reversible scheme has already been reported and successfully used for the study of a reversible homodimer association by Milla et al. (126). A similar equation has been used by Wendt et al (127) to describe the association of leucine zippers, a heterodimeric system, following 1:1 mixing of the two subunits.

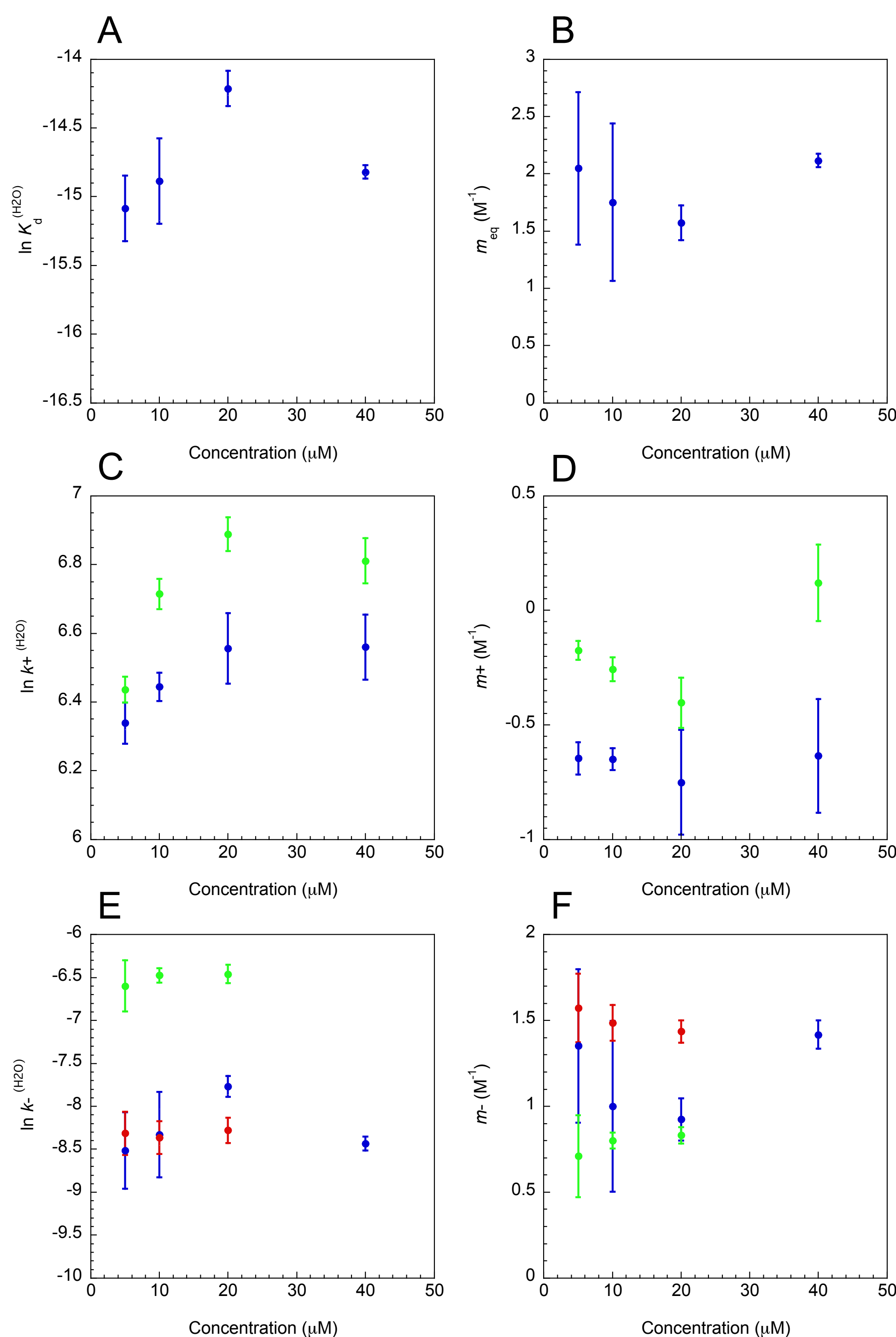

Figure S1 Concentration dependence of fitted rate and equilibrium constants, and their dependence upon denaturant concentration. Values for $K_d^{(H2O)}$ (*A*) and k+(H_2O) (*C*) were estimated using the y-axis intercepts and m_{eq} (*B*) and $m+$ (*D*) using the slopes, of the linear fits plotted in Fig. 5 *B* and Fig. 5 *A*, respectively (*solid blue circles*) or their equivalents for the irreversible model fits (*solid green circles*). Values for *k*- (H_2O) (*E*) and *m*- (*F*) were obtained using the y-axis intercepts and gradients, respectively, of the linear fits plotted in Fig 5 *C* for folding (*solid blue circles*) and unfolding (*solid red circles*), and in Fig. 5 *D* for unfolding traces fit with the irreversible model (*solid green circles*).

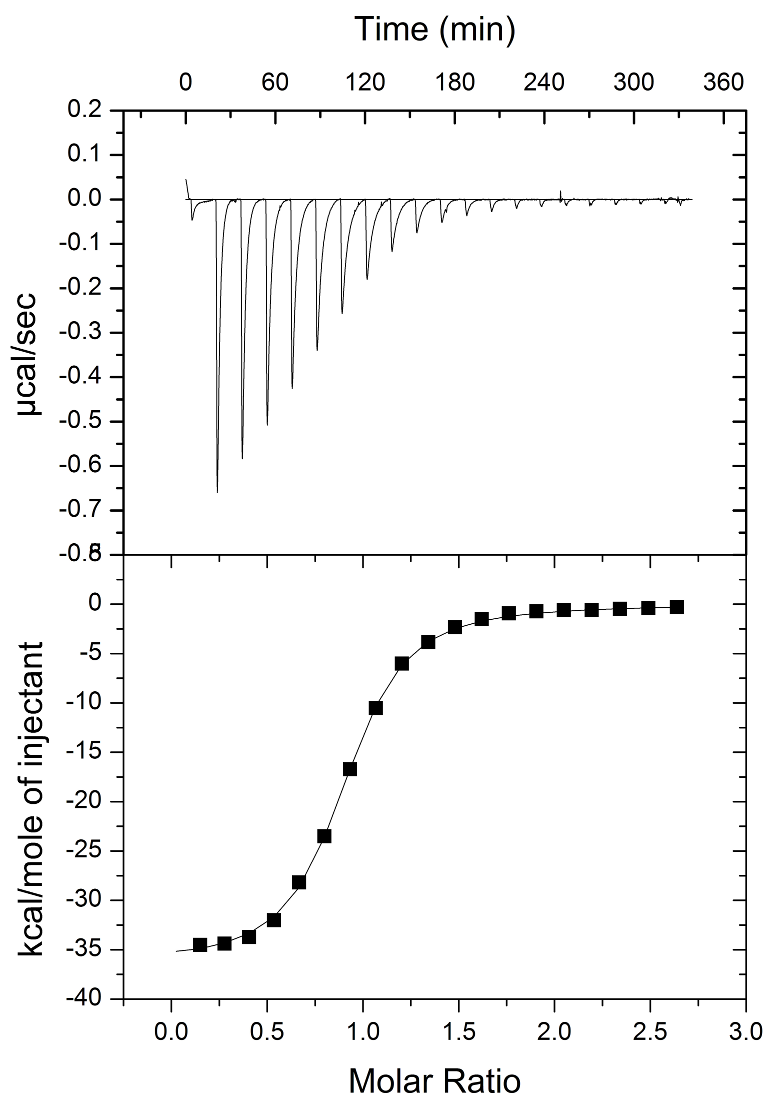

Figure S2 Representative ITC measurement for the titration of α0α1 (193 μM) into β16β17 (16.3 μM) at 25 °C. (Upper) Injection profile (Lower) Calorimetric binding isotherm. The curve through the points represents the best fit to the data using the independent binding model (MicroCal software.)

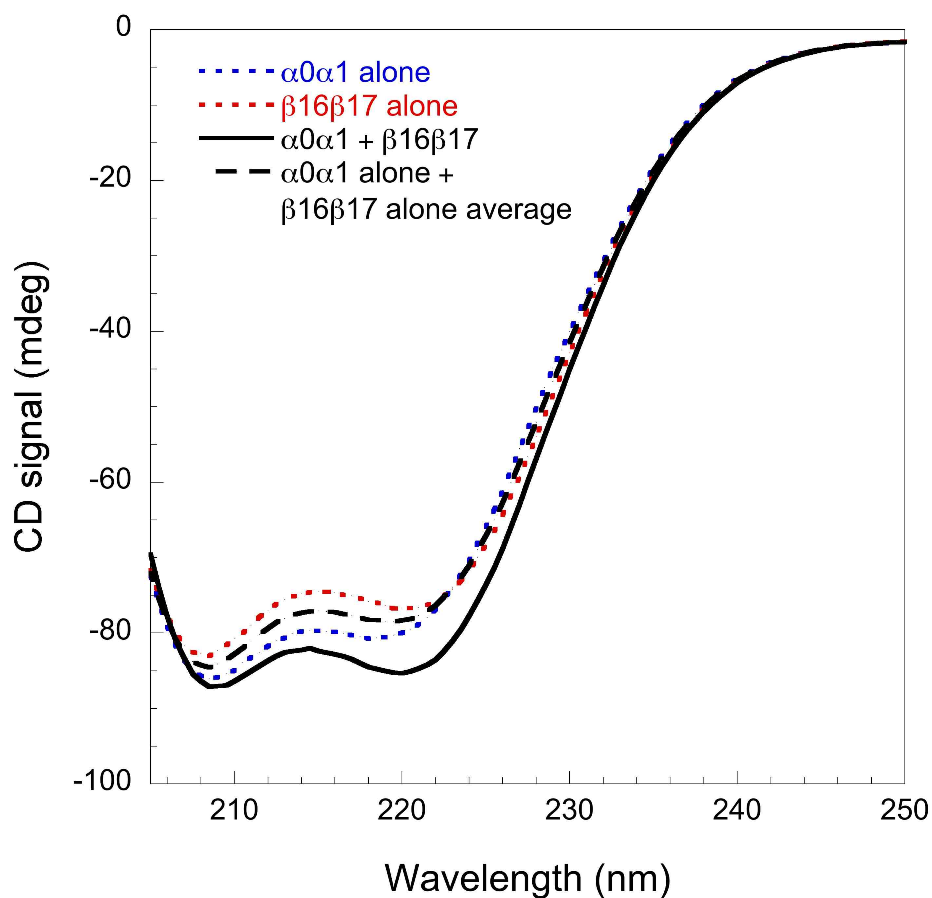

Figure S3 CD spectra demonstrate an increase in helicity on association: Far UV circular dichroism spectra of constructs α0α1 alone, β16β17 alone, and α0α1 + β16β17 together. The average of the two scans of the constructs alone is shown for comparison. This represents the expected spectrum for the mixture in the absence of an interaction. An increase in CD signal is observed due to the formation of the 'tetramerization domain'. Note that the tetramerization domain forms in the presence of two fully folded spectrin domains (α1 and β16) whose helical CD spectrum presumably do not change upon association.

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