Supporting information for

Transient opening of fibronectin type III (FNIII) domains: the interaction of the third FNIII domain of FN with anastellin

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Running title: FNIII3 domain stability and anastellin binding

Table S1. Tryptophan emission peaks of III3 mutants and ANS emission intensities with III3 mutants in the presence or absence of anastellin

III3 mutants	Positions of mutations	Tryptophan emission peak (nm)	ANS intensity with anastellin[a]	ANS intensity without anastellin[a]
Wild-type	-	318	54% (53%)[b]	5% (5%)[b]
V788D	A strand	318	37%	7%
I796D	B strand	347	55%	11%
V798D	B strand	350	52%	12%
W800D	B strand	-	55%	10%
I807D	B-C loop	347	31%	13%
Y810D	C strand	335	31%	9%
I812D	C strand	346	43%	22%
Y814D	C strand	348	35%	15%
L825D	C' strand	332	33%	10%
L827D	C' strand	344	31%	15%
A831D	C'-E loop	318	45%	8%
V834D	E strand	347	55%	45%
L836D	E strand	345	54%	45%
D838G	E-F loop	318	65%	7%
I847D	F strand	325	58%	18%
I849D	F strand	348	35%	9%
V861D	G strand	328	51%	24%
I863D	G strand	327	38%	9%
III3SS	B and E strands	320 (322) [b]	98% (60%)[b]	7% (8%)[b]
None	-	-	100%[c]	4%[d]

[a]The emission intensity was normalized by that of ANS plus anastellin alone at 470 nm.
[b]The emission intensity in the presence of DTT is shown in parentheses.
[c]The emission intensity of ANS plus anastellin alone.
[d]The emission intensity of ANS alone.

Table S2. FRET signals in urea

	Emission ratio[a]	Emission ratio[a] in 2 M urea	Emission ratio[a] in 6 M urea
III3-FRET	1.04 (0.49)[b]	0.90	0.65
III3-FRET + Anastellin	1.43	-	-
III3(V798D)-FRET	1.19	0.80	0.65
III3(V798D)-FRET + Anastellin	1.4	-	-
III3(V834D)-FRET	1.22	0.70	0.65
III3(V834D)-FRET + Anastellin	1.41	-	-
III3(D838G)-FRET	1.09	0.92	0.65
III3(D838G)-FRET + Anastellin	1.37	-	-
III3SS-FRET	1.65	1.32	1.00
III3SS-FRET + Anastellin	1.62	-	-

[a]Emission ratio is calculated by dividing the acceptor intensity at 528 nm by the donor intensity at 475 nm.
[b]The emission ratio of trypsin-treated III3-FRET is shown in parentheses.

Supplementary results for FRET

We used mild (2 M) urea treatment to study the relation between the conformations of the FRET constructs and the FRET signals in this assay. 2 M urea slightly decreased the FRET signal from wild-type III3 and the V838G mutant, while the FRET signals from V798D, V834D and the disulfide mutant were significantly reduced by 2 M urea (Table S2). Since this concentration of urea does not denature wild-type III3 or the disulfide mutant, as shown in Fig. 2, it seems likely that a weak interaction between the GFPs and the III3 domain favors a compact conformation, which enhances the FRET signals, and this interaction is disrupted by urea. The degree of interaction appears to depend on the specific mutations, although it is not clear why some mutants interact with GFPs more than the others. The FRET signals for V798D and V834D were higher than wild-type in the absence of urea, while in the presence of 2 M urea they were

lower, so it seems that mild urea treatment reveals the true stability of the mutants. This is consistent with the pronounced shift in the peak of tryptophan fluorescence for these two mutants (Table S1). In comparison, the disulfide mutant, which is more stable than wild-type, always showed higher FRET signals than wild-type.

In the presence of 6 M urea, the FRET signals from all constructs were dramatically decreased, although they did not lose the FRET signals completely, as can be seen by comparing the values for the FRET constructs with that of the trypsin-treated sample (Table S2). Only the III3SS-FRET construct showed any substantial FRET signal in the presence of 6 M urea, indicating that this mutant was not as extended as the rest of the constructs were. This is consistent with the result from tryptophan fluorescence in which 6 M urea did not denature III3SS. The observed reduction of the FRET signal for III3SS in the presence of urea is probably due to an increase in the stiffness of unstructured regions of the construct in urea, as reported previously (1).

1. Ohashi, T., Galiacy, S. D., Briscoe, G., and Erickson, H. P. (2007) An experimental study of GFP-based FRET, with application to intrinsically unstructured proteins, *Protein Sci 16*, 1429-1438.