

Supporting Information

The Interaction between the Third Type III Domain from Fibronectin and Anastellin Involves β -Strand Exchange

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Table S1. Sequences of recombinant proteins used in this study

Protein	Residues from human fibronectin (P02751)	Sequence ^a
anastellin	631-705	mrgsNAPQ...TSTPgsrshhhhhh
3FN3	808-905 ^b	gshmgTTAP...PRSDgt
AB	806-834 ^b	gSQTT...WSRP
CG	835-907	gQAPI...SDTV

^a Extraneous residues preceding or following the fibronectin sequences are shown in lower case.

^b The main entry in Uniprot for human fibronectin (P02751) contains a threonine at position 817 and lists proline as a natural variant (rs2577301). Threonine corresponds to the minor allele (T; frequency 0.02% in the 1000 Genomes Project Phase 3) and proline to the major allele (G). Both 3FN3 and AB contain a proline at this position.

Table S2. NMR experiments acquired for the AB:anastellin complex

Sample	Experiments
¹³ C- and ¹⁵ N-labeled AB + unlabeled anastellin	2D ¹ H- ¹⁵ N HSQC; 2D ¹ H- ¹³ C HSQC (aliphatic region); 2D ¹ H- ¹³ C HSQC (aromatic region); 3D HNCACB; 3D C(CO)NH; 3D H(CCO)NH; 3D HNCO; 3D HN(CA)CO; 3D CCH-TOCSY; 3D HCCH-TOCSY; 3D aliphatic ¹³ C-edited NOESY (without sensitivity enhancement); 3D aliphatic ¹³ C-edited NOESY (with sensitivity enhancement); 3D ¹³ C- and ¹⁵ N-filtered, aliphatic ¹³ C-edited NOESY; 2D ¹³ C- and ¹⁵ N-filtered, aliphatic ¹³ C-edited NOESY; 3D ¹³ C- and ¹⁵ N-filtered, ¹⁵ N-edited NOESY; 2D ¹³ C- and ¹⁵ N-filtered, ¹⁵ N-edited NOESY
¹⁵ N-labeled AB + unlabeled anastellin	2D ¹ H- ¹⁵ N HSQC; 3D ¹⁵ N-edited TOCSY; 3D ¹⁵ N-edited NOESY; 2D double-quantum-filtered COSY; 2D TOCSY; 2D ¹ H- ¹⁵ N NOE
¹³ C- and ¹⁵ N-labeled anastellin + unlabeled AB	2D ¹ H- ¹⁵ N HSQC; 2D ¹ H- ¹³ C HSQC (aliphatic region); 2D ¹ H- ¹³ C HSQC (aromatic region); 3D HNCACB; 3D CBCA(CO)NH; 3D C(CO)NH; 3D H(CCO)NH; 3D HNCO; 3D HN(CA)CO; 3D CCH-TOCSY; 3D HCCH-TOCSY; 3D aliphatic ¹³ C-edited NOESY (without sensitivity enhancement); 3D aliphatic ¹³ C-edited NOESY (with sensitivity enhancement); 3D aromatic ¹³ C-edited NOESY; 2D HBCB(CGCD)HD; 3D ¹³ C- and ¹⁵ N-filtered, aliphatic ¹³ C-edited NOESY; 2D ¹³ C- and ¹⁵ N-filtered, aliphatic ¹³ C-edited NOESY; 3D ¹³ C- and ¹⁵ N-filtered, aromatic ¹³ C-edited NOESY; 2D ¹³ C- and ¹⁵ N-filtered, aromatic ¹³ C-edited NOESY; 3D ¹³ C- and ¹⁵ N-filtered, ¹⁵ N-edited NOESY; 2D ¹³ C- and ¹⁵ N-filtered, ¹⁵ N-edited NOESY
¹⁵ N-labeled anastellin + unlabeled AB	2D ¹ H- ¹⁵ N HSQC; 3D ¹⁵ N-edited TOCSY; 3D ¹⁵ N-edited NOESY; 2D ¹ H- ¹⁵ N NOE

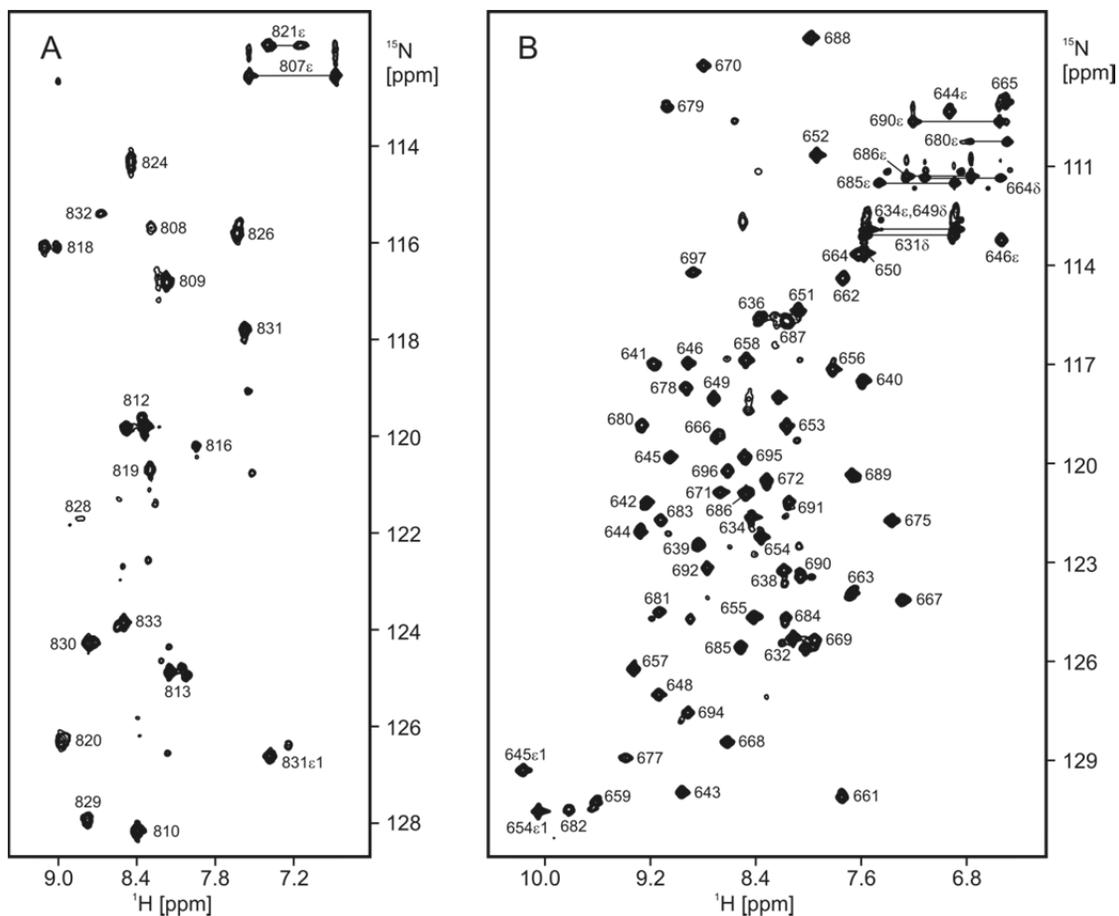


Figure S1. 2D ^1H - ^{15}N HSQC spectra of (A) ^{15}N -labeled AB bound to unlabeled anastellin and (B) of ^{15}N -labeled anastellin bound to unlabeled AB. The assignments are indicated. Signals from T825 and V828 in AB are very weak, and the former is not observable at this contour level. Signals from G661, R644 ϵ and R646 ϵ in anastellin are aliased.

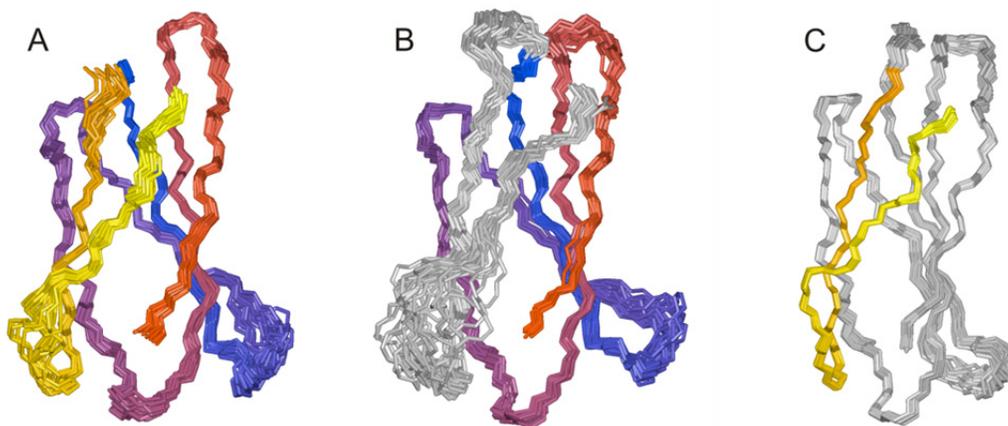


Figure S2. Comparison of the AB:anastellin complex with 1FN3 and 3FN3. (A-C) Superimposed backbone traces of the AB:anastellin complex (residues 813-834 of AB and 638-697 of anastellin) (panel A), 1FN3 (residues 610-697) (panel B), and 3FN3 (residues 813-896) (panel C). The color scheme for the AB:anastellin complex is the same as in Fig. 4, i.e. the color of AB changes smoothly from yellow at the N-terminus to orange at the C-terminus, and the color of anastellin changes from blue at the N-terminus to red at the C-terminus. Residues 638-697 in 1FN3 (panel B) and 813-834 in 3FN3 (panel C) are colored as in panel A to facilitate their comparison with anastellin and AB, respectively. The rest of 1FN3 and 3FN3 is colored gray.