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Supporting information for article:

Crystal structure of the second fibronectin type III (FN3) domain from human collagen α1 type XX

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S1. Large-Scale Cell-Free Protein Synthesis Reaction protocol (Kigawa, 2010, Methods Mol Biol. 607,101-111.)

(i). Prepare 9 mL of the reaction solution and 90 mL of the feeding solution at 277 *K*. The reaction solution contains 2 μg/mL template plasmid, 66.7 μg/mL T7 RNA polymerase, 30 % (v/v) S30 extract, 0.175 mg/mL tRNA, 1.5 mM each of 19 amino acids without L-tyrosine, 37.3 % (v/v) LMCPY mixture, 0.25 mg/mL creatine kinase, 10 mM magnesium acetate, and 0.05 % (w/v) sodium azide. The feeding solution contains 30 % (v/v) S30 buffer, 1.5 mM each of 19 amino acids without L-tyrosine, 37.3 % (v/v) LMCPY mixture, and 10 mM magnesium acetate.

To prepare 90 mL of the feeding solution, gently shake the tube containing the LMCPY mixture, to make it homogeneous. Combine the LMCPY mixture (33.6 mL), the amino acid mixture (6.75 mL), the magnesium acetate solution (0.522 mL), the S30 buffer (27 mL), and the sodium azide solution (0.9 mL). Bring the volume of the mixture solution to 90 mL with water. Place the mixture in a 100-mL centrifuge tube, and mix it thoroughly by turning the tube upside down gently several times.

To prepare 9 mL of the reaction solution, gently shake the tube containing the LMCPY mixture to make it homogeneous. Combine the LMCPY mixture (3.36 mL), the amino acid mixture (0.675 mL), the magnesium acetate solution (0.052 mL), the sodium azide solution (0.9 mL), and the tRNA solution (0.09 mL). To this solution, sequentially add the creatine kinase solution (0.6 mL), the T7 RNA polymerase solution (0.06 mL), and the plasmid DNA templates. Bring the volume of the mixture to 9 mL with water. Place the reaction solution in

a 50-mL centrifuge tube and mix it thoroughly by turning the tube upside down gently several times.

(ii)Place 90 mL of the feeding solution in a 400-mL square-shaped polystyrene case.

(iii)Seal one end of the dialysis tube (Spectra/Por 7, MWCO = 15,000) with a closure (Spectrum). Place the reaction solution (9 mL) in the dialysis tube, remove as much air from the tube as possible, and seal the open end of the tube with a closure.

(iv) Submerge the dialysis tube in the feeding solution in the polystyrene case. Wrap the case with plastic wrap.

(v) Shake the case reciprocally with one incubator shaker (50-mm amplitude, 50 rpm) at 373 *K* for 4 h.

(vi) Transfer the reaction solution to two fresh 50-mL centrifuge tubes. The reaction solution was centrifuged at 16,000 *g* at 277 K for 20 min. The supernatant loaded onto a HisTrap column (5 ml) for protein purification.

Table S1 Macromolecule production information

Source organism	*Homo sapiens*
DNA source	cDNA
Forward primer	5′-CCGAACATATG CAGGGCCCAGCAGC- 3′[#]
Reverse primer	5′-CGGATCC *TTA*GGCCTGGTGACCACAG-3′[#]
Expression vector	pEHISTEV
Expression host	*E. coli* (codon-plus RIL strain) cell-free system
Complete amino acid sequence of the construct produced	*G*APTSLVLSQVTSSSIRLSWTPAPRHPLKYLIVWRASRGGTPREVVV EGPAASTELHNLASRTEYLVSVFPIYEGGVGEGLRGLVTTAP[$]

[#] *Nde*I restriction site and *Bam*HI restriction site are underlined. The stop codons are shown in italics.

[$]Additional residue after His-tag cleavage with TEV protease is shown in italics.

Table S2 Amino acid sequence identities between the conversed structural domain of human collagen α1(XX) and that of chick collagen α1(XX).

Domain	Amino acid residues		Sequence identities (%)
	Human	*Gallus*	
FN#1	32 - 103	30 - 102	74
VWFA	169 - 358	231 - 420	61
FN#2	378 - 456	447 - 526	54
FN#3	468 - 554	537 - 612	39
FN#4	559 - 644	627 - 702	49
FN#5	650 - 731	717 - 795	51
FN#6	742 - 826	810 - 888	42
Tsp	830 - 958	910 - 1154	64
Col 2	959 - 1192	1155 -1309	60
NC2	1193 - 1231	1310-1355	61
Col 1	1232 - 1287	1356-1458	45
NC1	Not conserved	1459-1473	-

Table S3 Amino acid sequence identities (%) between any two FN3 domains from human collagen α1(XX).

	FN#1	FN#2	FN#3	FN#4	FN#5
FN#2	18				
FN#3	26	39			
FN#4	45	35	30		
FN#5	26	29	-*	28	
FN#6	36	33	35	28	38

* No significant similarity between FN#3 and FN#5.