Supporting Information

Baldock et al. 10.1073/pnas.1014280108

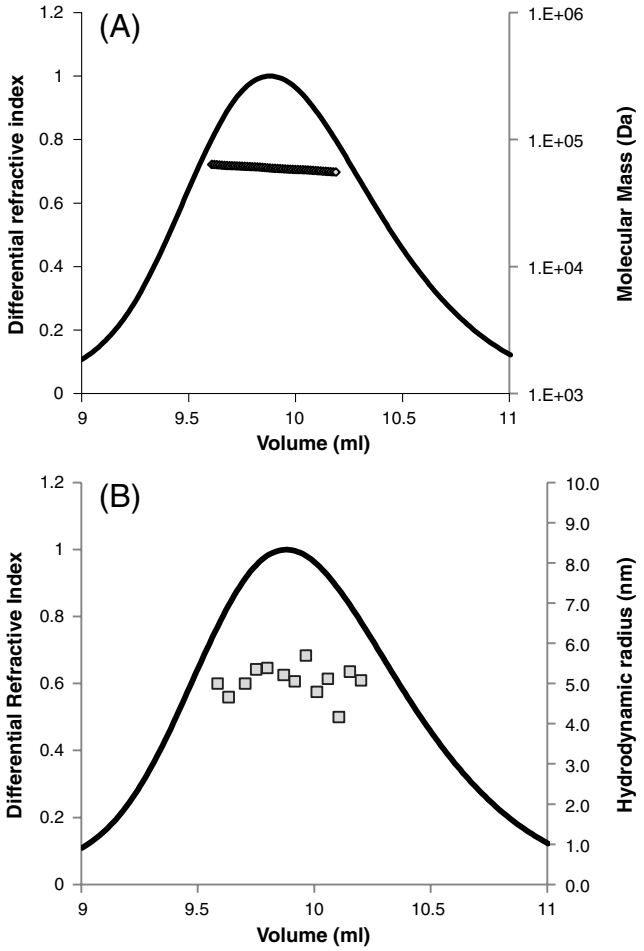

Fig. S1. The trace for the refractive index from MALLS of full-length tropoelastin is shown. Samples (0.5 mL) were separated according to size in PBS on a Superdex-200 24/30 gel filtration column (Amersham Pharmacia Biotech) on a Dionex BioLC HPLC at 0.71 mL/min. The eluate was passed through a Wyatt EOS 18-angle laser photometer with the 13th detector replaced with a Wyatt QELS detector for the simultaneous measurement of hydrodynamic radius. This system was coupled to a Wyatt Optilab rEX refractive index detector and the hydrodynamic radius, molecular weight moments, and concentration of the resulting peaks was analyzed using Astra 5.3.2 (Wyatt Technologies UK Ltd). The single peak was selected and used for molecular weight and hydrodynamic radius calculations. (*A*) Light scattered intensity and refractive index were analyzed to give a weight-averaged M_r of 59,520 Da +/− 0.5%. (*B*) Results also yielded a hydrodynamic radius of 5.09 +/− 0.4 nm.

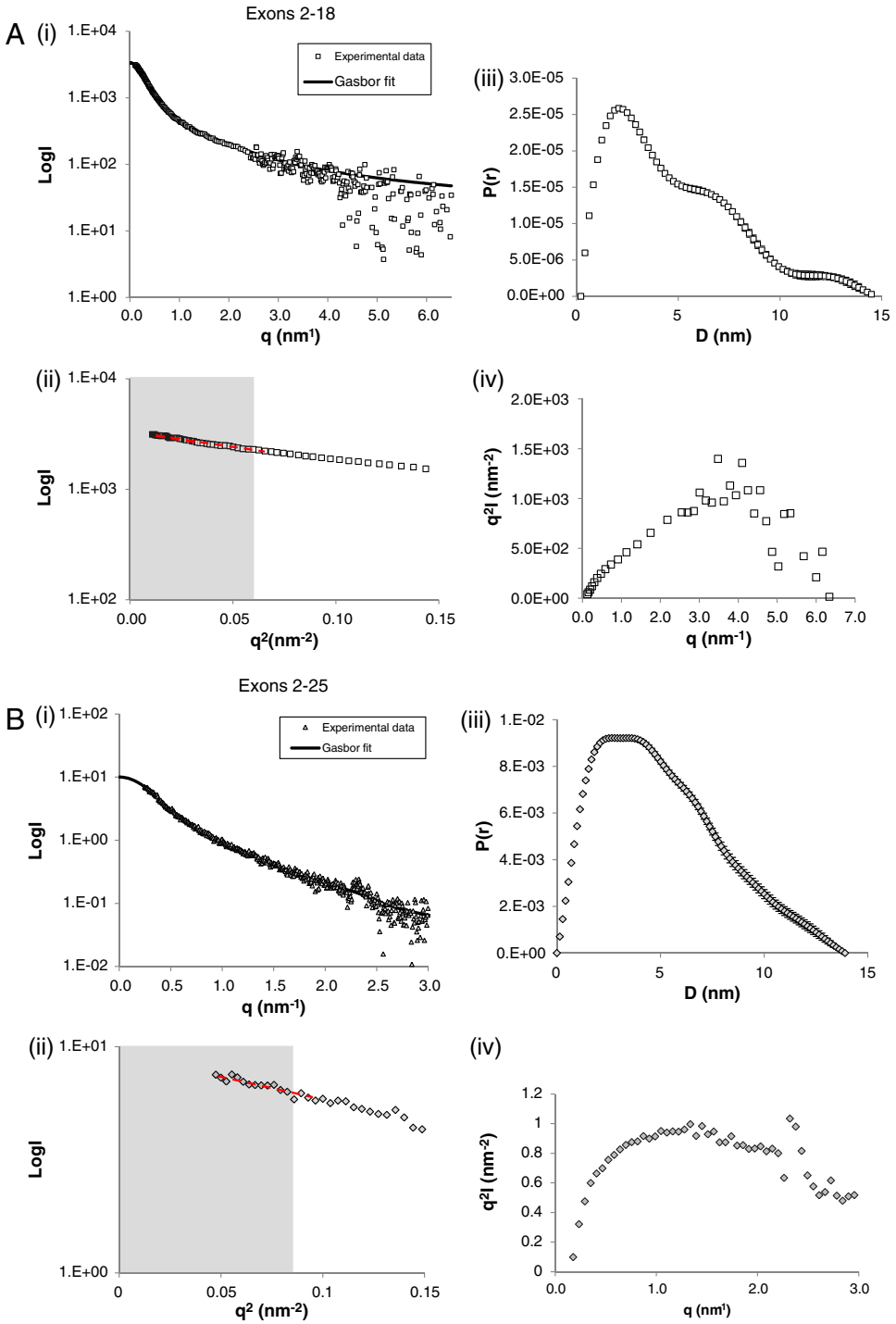

Fig. S2. (*A*, *i*) The experimental SAXS data for exons 2–18 are plotted as a function of q, and compared with a typical theoretical fit obtained with GASBOR (solid line). (*ii*) The low angle regions of the X-ray scattering data were analyzed in the form of Guinier plots ($\log I$ vs. q^2), from which the radius of gyration (Rg) can be extracted from the slope ($Rg^2/3$) of the straight line. The slope demonstrates the expected linearity for the values $q \leq 1/Rg$ (shaded region). (*iii*) Pair distribution function calculated for the SAXS dataset. The curve shows with error bars the distribution of interatomic spacings, with maxima at 14.5 nm. (*iv*) A Kratky plot is shown for the SAXS data. (*B*, *i*) The experimental SAXS data for exons 2–25 are plotted as a function of q, and compared with a typical theoretical fit obtained with GASBOR (solid line). (*ii*) The low angle regions of the X-ray scattering data were analyzed in the form of Guinier plots ($\log I$ vs. q^2), from which the radius of gyration (Rg) can be extracted from the slope ($Rg^2/3$) of the straight line. The slope demonstrates the expected linearity for the values $q \leq 1/Rg$ (shaded region). (*iii*) Pair distribution function calculated for the SAXS dataset. The curve shows with error bars the distribution of interatomic spacings, with maxima at 14.0 nm. (*iv*) A Kratky plot is shown for the SAXS data.

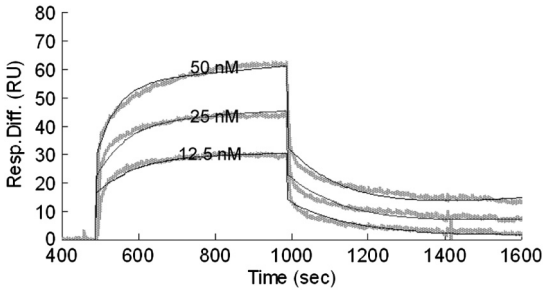

Fig. S3. Kinetic study of tropoelastin monomer binding to fixed tropoelastin monomer using methods developed for other elastic fibre and extracellular matrix proteins (1–3). In each case, the gray curve represents the measured data while the black curve displays a bimolecular fit to these data. The concentrations of analyte are displayed above each curve. Tropoelastin was prepared in 0.01 M Hepes, 0.2 M NaCl, 0.005% Tween 20, pH 7 at various concentrations from 0.195 nM to 50 nM, with a control containing no tropoelastin. The samples were injected over fixed tropoelastin at 32 °C for 8.33 min. at a flow rate of 30 μL/ min, followed by 30 min. dissociation. After dissociation, chip surfaces were regenerated with two 1 min. injections of 1 M NaCl, 0.05% NaOH. Rate constants were obtained by globally fitting to a 1 : 1 binding model with drifting baseline, with kinetic and affinity data (ka) of $1.71 \pm 0.31 \times 10^5$ $M s^{-1}$ and a kd of $3.8 \pm 0.22 \times 10^{-3}$ s^{-1} resulting in a KD of $2.28 \pm 0.29 \times 10^{-8}$ M at a χ^2 of 1.18.

1 Choudhury R, et al. (2009) Differential regulation of elastic fiber formation by fibulin-4 and -5. *J Biol Chem* 284:24553–24567.
2 Keane FM, Clarke AW, Foster TJ, Weiss AS (2007) The N-terminal A domain of *Staphylococcus aureus* fibronectin-binding protein A binds to tropoelastin. *Biochemistry* 46:7226–7232.
3 Rock MJ, et al. (2004) Molecular basis of elastic fiber formation. Critical interactions and a tropoelastin-fibrillin-1 cross-link. *J Biol Chem* 279:23748–23758.

Table S1. Comparison of theoretical sedimentation parameters for the ab initio model of full-length tropoelastin, calculated using the program Hydropro (1), with experimentally derived values (2).

Parameter	Shell modelling	Sedimentation velocity AUC
Stokes radius (hydrodynamic radius) (nm)	5.53	5.6
Sedimentation coefficient (Svedburg)	2.24	2.3 ± 0.2
Frictional ratio	2.25	2.2
Translational diffusion coefficient (10^{-11} m^2/s)	3.80	4.0 ± 0.3

1 Garcia De La Torre J, Huertas ML, Carrasco B (2000) Calculation of hydrodynamic properties of globular proteins from their atomic-level structure. *Biophys J* 78:719–730.
2 Toonkool P, Regan DG, Kuchel PW, Morris MB, Weiss AS (2001) Thermodynamic and hydrodynamic properties of human tropoelastin. Analytical ultracentrifuge and pulsed field-gradient spin-echo NMR studies. *J Biol Chem* 276:28042–28050.