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Supporting information for article:

Structural consequences on transforming growth factor beta-1 activation from near-therapeutic X-ray doses

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Table S1 Experimentally determined parameters from SAXS analysis of LTGFB-1 and LAP

(a) Sample Details

Sample	LTGFβ-1	LAP
Organism	*Homo sapiens*	*Homo sapiens*
Source	HEK293	HEK293
UniProt sequence ID (residues in construct)	P01137 (1-390)	P01137(1-278)
Extinction coefficient [A_{280}, 0.1% (w/v)]	1.338	0.977
M from chemical composition (Da)	~95000	~70000
Average *C* in combined frames (mg ml^{-1})	1.0	4.5

(b) SAXS data-collection parameters

Instrument/data processing	Advanced Light Source SIBYLS SAXS beamline with 2M detector (Dyer *et al.*, 2014)
Wavelength (Å)	1.127
Camera length (m)	2
q measurement (Å^{-1})	0.0114-0.40156
Monitoring for radiation damage	X-ray dose maintained below 250 Gy, data frame-by frame comparison
Exposure time	Continuous 0.1 s data-frame measurements of static sample
Sample configuration	Static sample cell
Sample temperature (˚C)	10

(c) Software employed for SAXS data reduction, analysis, and interpretation

SAXS data reduction	Solvent subtraction using PRIMUSqt (ATSAS 2.8.3 (Franke *et al.*, 2017)
Extinction coefficient	ProtParam (Gasteiger *et al.*, 2005)
Basic analysies: Guinier, *P(r)*, V_P	PRIMUSqt (ATSAS 2.8.3 (Franke *et al.*, 2017)
Atomic structure modelling	CRYSOL from PRIMUSqt in ATSAS 2.8.3 , EOM 2.1 (Tria *et al.*, 2015)
Missing sequence modelling	MODELLER via Chimera (Yang *et al.*, 2012)
Three-dimensional graphic model representation	Chimera v.1.11.2 MacOS

(d) Sample Details

Sample	LTGFB-1	LAP
Guinier Analysis		
I(0) (arbitrary units)	6.72 +/- 0.017	27.96 +/- 0.087
Rg (Å)	38.3 +/- 0.53	40.71 +/- 0.09
q min ($Å^{-1}$)	0.012	0.0114
qRg max	1.29	1.3
P(r) analysis		
I(0) (arbitrary units)	6.80 +/- 0.027	28.63 +/- 0.097
Rg (Å)	40.60 +/- 0.363	43.60 +/- 0.271
D_{max} (Å)	175	175
q range ($Å^{-1}$)	0.0120-0.4016	0.0114-0.401
Porod volume ($Å^{-3}$)	206000	178000

(e) Atomistic modelling

Sample	LTGFβ-1	LAP
Crystal structure	PDB: 3RJR	PDB: 3RJR (LAP domain)
q range for all modelling	0.0120-0.4016	0.0114-0.401
CRYSOL (with default parameters)		
χ^2 (unglycosylated, glycosylated)	95.0, 3.46	147.35, 17.41
Predicted Rg (Å) (unglycosylated, glycosylated)	29.58, 35.23	30.94, 35.41
Vol (Å), Ra (Å), Dro ($e\ Å^{-3}$)	10349, 1.800, 0.020	74723, 1.800, 0.020
Multistate/ensemble models (EOM, default parameters, 30,000 models in initial pool, native-like, constant subtraction allowed)		
χ^2		6.79
Starting crystal structure		PDB: 3RJR (LAP domain)
Flexible residues		1-45
Number of states		4
Volume fraction of each state		0.222, 0.111, 0.222, 0.444
R_g value of each state		36.17, 38.04, 39.88, 38.26
Overall R_g		38.13
Overall D_{max}		144.96
GLYCOSYLATION		
Total number of glycans	6	6
Amino acids with glycans	N53, N107, N147	N53, N107, N147
Type of glycans	complex	complex
Total mass of glycans	11217.8	11217.8

Table S2 Parameters for X-ray diffraction weighted dose calculations using RADDOSE-3D

Flux	1.2 $\times 10^{12}$ ph/s
Sample container	20 μm mica window
Attenuation by sample container (%)	10
Beam type	Top-hat
Beam size	1 mm × 2 mm
Energy	11 keV

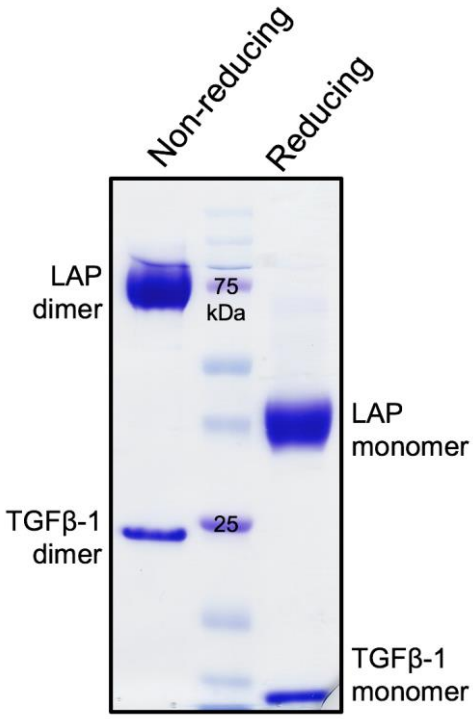

Figure S1 SDS PAGE of recombinant LTGFβ-1 shows high purity and migration of dimers (non-reducing) and monomers (reducing) close to the theoretical molecular weight.

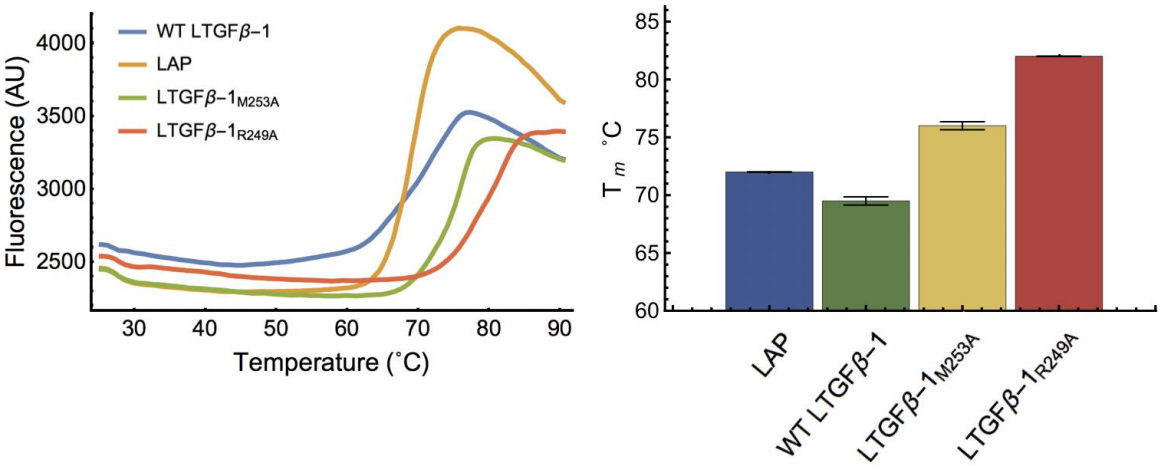

Figure S2 Thermal shift assay shows that all recombinant proteins are well-folded (a) and that LAP has a similar melting temperature when bound (LTGFβ-1) and unbound to TGFβ-1 (b).

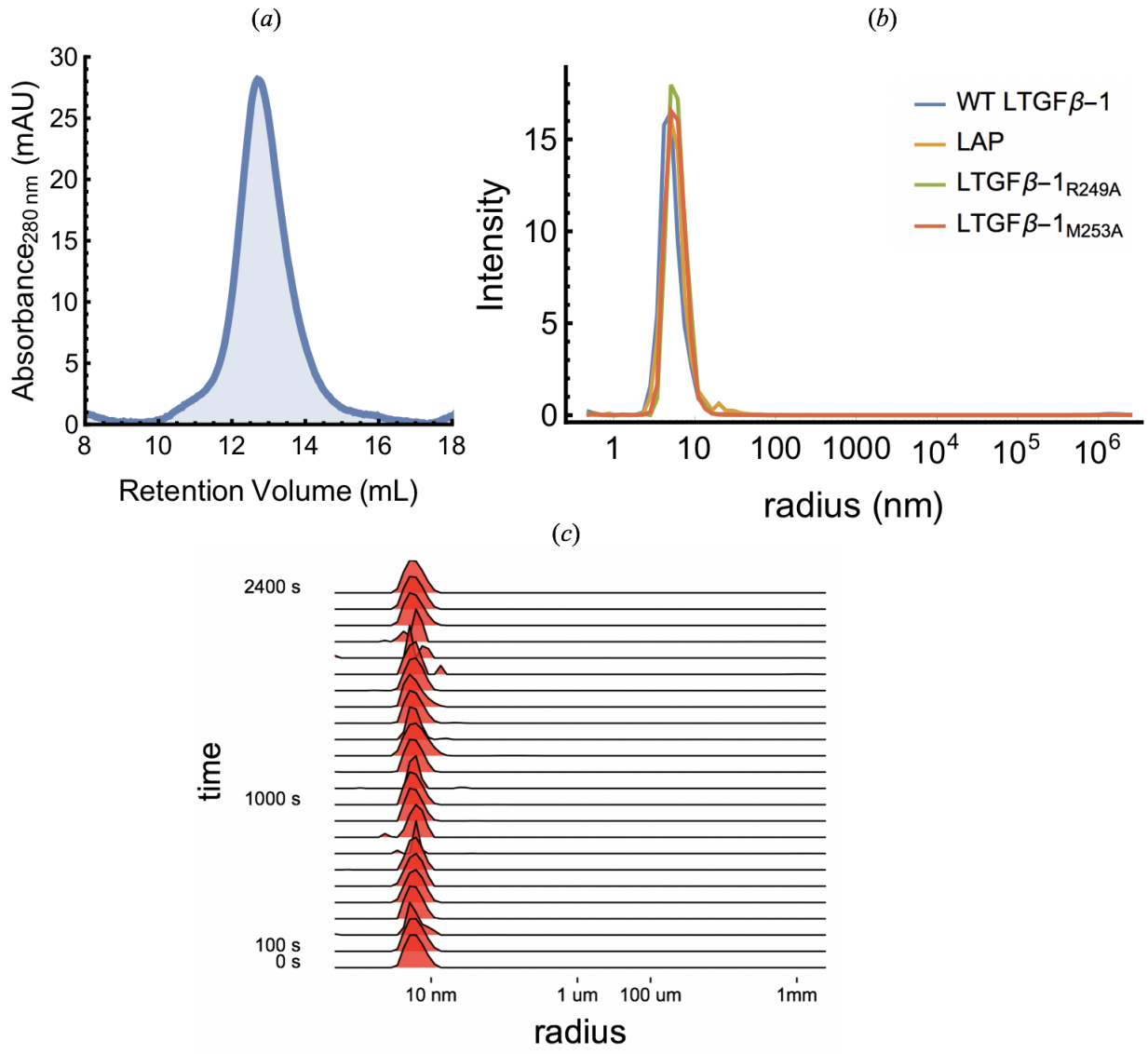

Figure S3 SEC (*a*) and DLS (*b* and *c*) measurements show a uniform distribution, indicating a monodisperse and aggregate free sample. (a) The protein was purified using a Superdex-200 10/300 with a void volume (V_0) of 8 mL and and total column volume (V_t) of 24 mL. (b) DLS measured approximate hydrodynamic radii (R_H) of 4.6, 5.8, 5.3, and 4.7 nm for LTGFβ-1, LTGFβ-1_{R249A}, LTGFβ-1_{M253A}, and LAP, respectively. (c) Measurements of the size distribution of LTGFβ-1 show that the protein complex is stable on a time frame much larger than the total exposure time of the SAXS experiments.

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