Neat Protein Single-Chain Nanoparticles from Partially Denatured BSA

Paula Malo de Molina,[1,2] Thu Phuong Le[1], Amaia Iturrospe,[1] Urs Gasser,[3] Arantxa Arbe,[1] Juan Colmenero,[1,4,5] and José A. Pomposo[1,2,4]*

[1] Materials Physics Center (MPC), Centro de Física de Materiales (CFM) (CSIC-UPV/EHU), Paseo Manuel de Lardizabal 5, E-20018 Donostia, Spain

[2] IKERBASQUE—Basque Foundation for Science, Plaza Euskadi 5, E-48009 Bilbao, Spain

[3] Laboratory for Neutron Scattering and Imaging, Paul Scherrer Institut, CH-5232 Villigen, Switzerland

[4] Departamento de Polímeros y Materiales Avanzados: Física, Química y Tecnología, University of the Basque Country (UPV/EHU) PO Box 1072, E-20018 Donostia, Spain

[5] Donostia International Physics Center, Paseo Manuel de Lardizabal 4, E-20018 Donostia, Spain

AUTHOR INFORMATION

Corresponding Author

*p.malodemolina@ehu.eus

Supplementary Information

1. ^{1}H-NMR: Estimation of the apparent lysine conversion and peak assignment

To aid with the peak assignment in the ^{1}H-NMR spectra we measured poly-L-lysine (Sigma Aldrich, poly-L-lysine hydrobromide, M_w: 150,000-300,000 g/mol) cross-linked with 20 mol % DSS before and after purification compared to the pure poly-lysine in D_2O. Also, by comparison of the curves, we assigned the peaks corresponding to the cross-linker spacer at 2.10 (**b**), 1.42 (**c**) and 1.18 (**d**) ppms. The peaks in the unpurified sample at 2.6-2.7 ppm correspond to the succinimide pendant group.

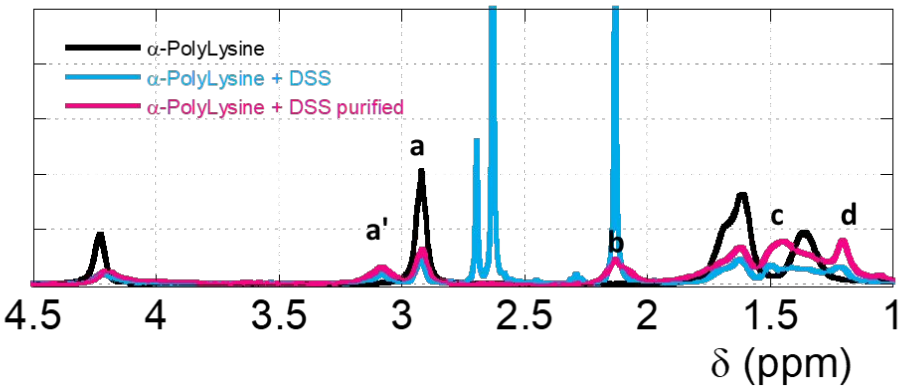

Figure S1. ^{1}H-NMR spectra of poly-L-lysine cross-linked with DSS unpurified and purified compared to the uncross-linked polymer.

Similar to the case of the **a** and **a'** peaks in BSA, the degree of lysine conversion was estimated by integrating the peaks and resulted to be 38 %, almost the 40% expected from adding 20% bifunctional cross-linker.

Apparent lysine conversion in BSA.

In order to estimate the apparent lysine conversion, we performed a decomposition of the peaks **a** and **a'**. To do so, we first normalized the spectra to the total spectrum area. Then we subtracted a baseline to the region between 3.4 and 2.7 ppm and then fitted the ^{1}H-NMR signal to a sum of two Lorentzians. Then we estimated the area of each peak. Figure S1 shows the baseline-subtracted data along with the total fit and each peak´s contribution for all measured samples.

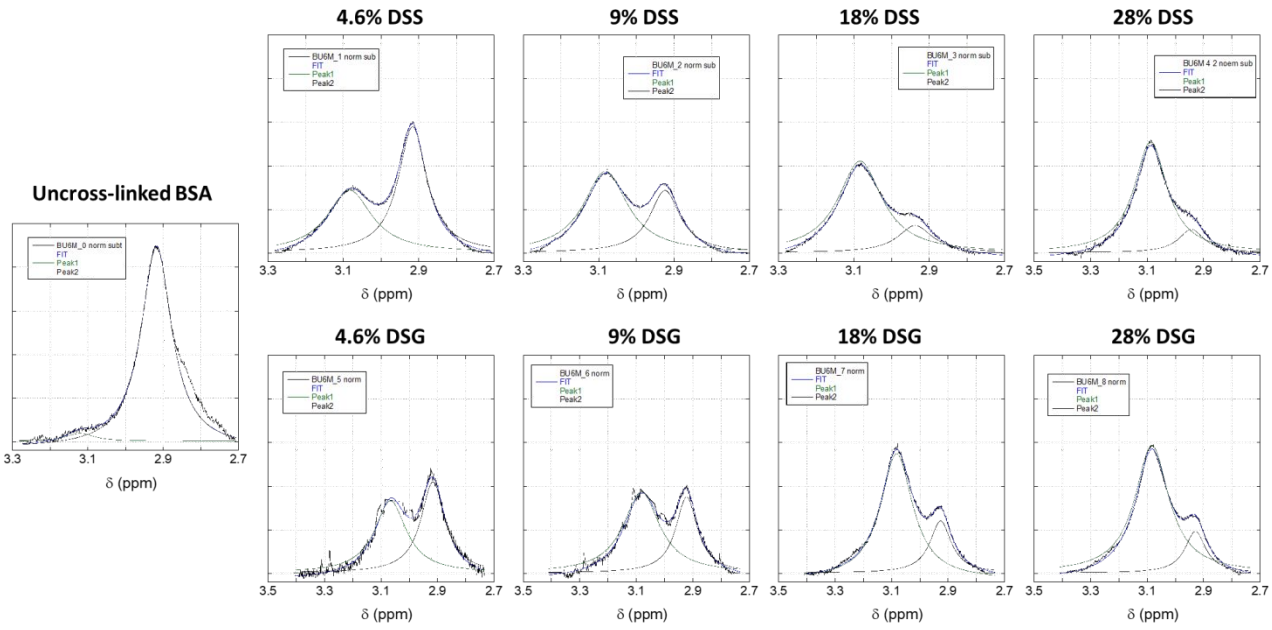

Figure S2. Decomposition of **a** and **a'** peaks. Background subtracted ^{1}H-NMR spectra. The lines correspond to the fit of the sum of two Lorentzian functions and the individual contributions of each peak.

2. Small Angle Neutron Scattering (SANS)

Table S1. Molecular properties and scattering length densities of all compounds.

Compound	*Elemental composition*	M_W *(g/mol)*	*d (g/mL)*	*Neutron SLD,* ρ_N *(cm⁻²)*	*X-ray SLD,* ρ_N *(cm⁻²)*
BSA	$C_{2932}H_{4614}N_{780}O_{898}S_{39}$	66372	1.1	1.482	10.011
DSG	$C_{13}H_{14}N_2O_8$	326.26	~1.1	2.014	9.769
DSS	$C_{16}H_{20}N_2O_8$	368.18	~1.1	1.739	9.872
D_2O	D_2O	20.02	1.1056	6.367	9.417
H_2O	H_2O	18.02	0.997	-0.559	9.441
Urea	CH_4N_2O	60.06	1.32	2.146	11.987
Urea d4	CD_4N_2O	64.08	1.41	7.657	11.983
NaCl	NaCl	58.44	2.16	2.954	17.962
DMSO	C_2H_6OS	78.13	1.1	-0.042	10.136
d-DMSO	C_2D_6OS	84.17	1.19	5.278	10.179

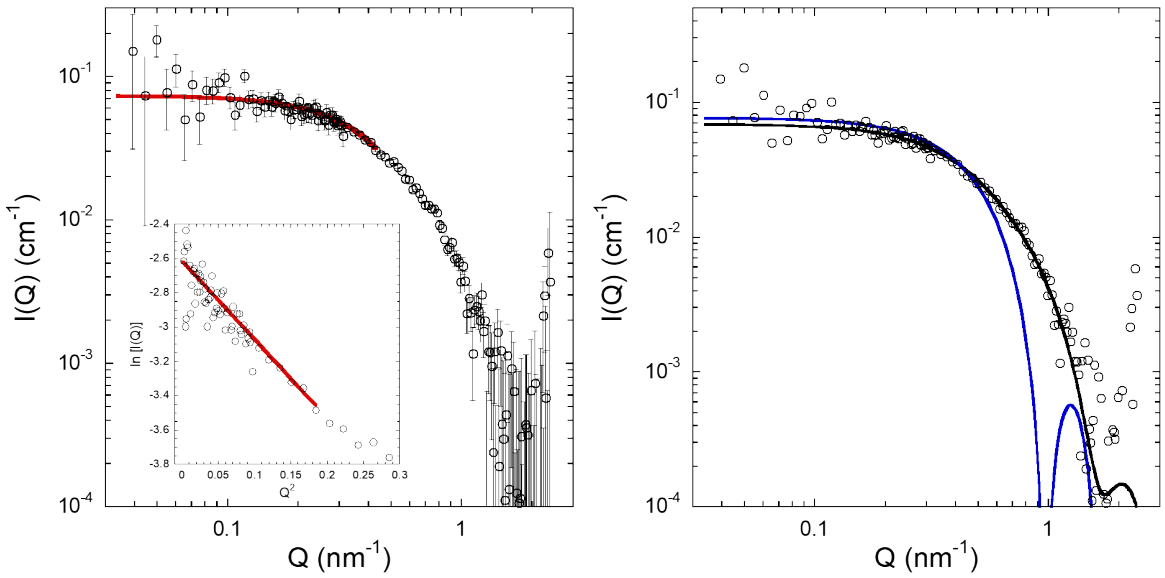

Figure S3. SANS patterns of BSA in PBS buffer at 1mg/mL. (Left) Solid red line corresponds to the Guinier approximation fit with $R_g = 3.7$ nm. The inset figure is the linearized Guinier plot of the same data. (Right) Comparison of the fit with ellipsoid model described in the main text with a fit to a rigid sphere with $R = 4.64$ nm, assuming $R = \sqrt{5/3}R_g$.

3. Small Angle X-Ray Scattering (SAXS)

BSA in salt and urea solutions has very low contrast in SAXS. Therefore, in-house SAXS measurements were performed with the aim of narrowing down the experimental parameters in preparation of the SANS measurements.

SAXS experiments were performed at 25˚C on a Rigaku 3-pinhole PSAXS-L instrument operating at 45% kV and 0.88 mA. The MicroMax-002 + X-ray Generator Systems is composed by a microfocus sealed tube source module and an integrated X-ray generator unit that produces Cu-Kα transition photons of wavelength λ = 1.54 Å. The flight path and the sample chamber were under vacuum. The scattered X-rays were detected on a two-dimensional multi-wire X-ray detector (Gabriel design, 2D-2000×). The radially averaged scattered intensities were obtained as a function of scattering vector Q. Reciprocal space calibration was done using silver behenate as standard. The solutions were filling boron-rich capillaries with an outside diameter of 2% mm and a wall thickness of around 0.01% mm. The contribution from the corresponding buffer (measured on the same capillary) was subtracted by applying the proper factors obtained from transmission measurements. The sample-detector distance was 2 %m, allowing the coverage of a Q-range from 0.008 to 0.2 $Å^{-1}$.

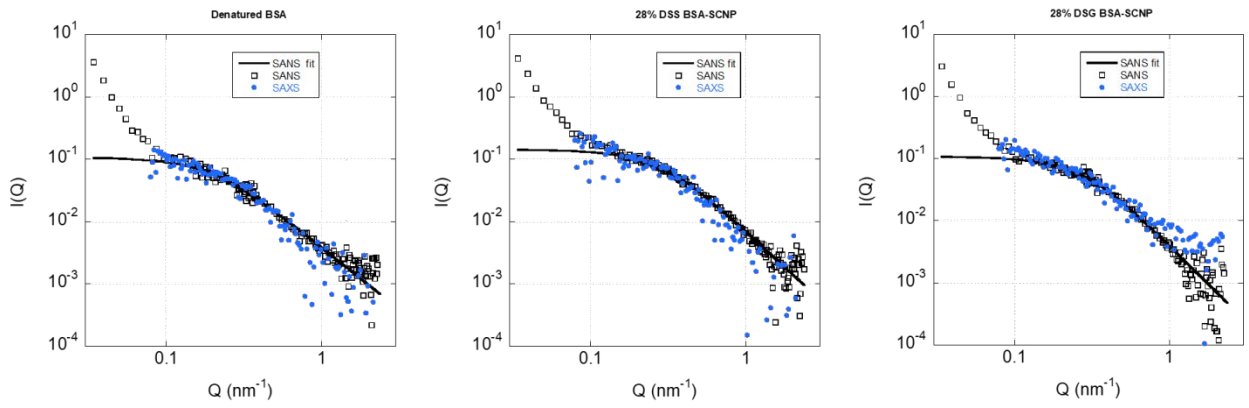

Figure S4. Comparison of SAXS and SANS measurements of denatured BSA and BSA SCNPs prepared with 28% DSS and DSG all at 1 mg/mL. The SANS scattering patterns are in absolute scale and the SAXS measurements are rescaled to overlap the SANS data.

Figure S4 shows the comparison of SAXS and SANS measurements of denatured BSA and BSA SCNPs prepared with 28% DSS and DSG all at 1mg/mL. The fits obtained from the SANS patterns agree well with the SAXS patterns within the experimental uncertainties. In addition, we obtained very good reproducibility between different batches.