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Supporting information for article:

DATASW, a tool for HPLC–SAXS data analysis

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S1. Sample preparation, SAXS measurements and processing

Synchrotron X-ray scattering data were collected at the BM29 beamline (ESRF, Grenoble, France) and SWING beamline (Synchrotron Soleil, Saint-Aubin, France), both equipped with an inline HPLC system. The used samples were >95% pure based on Coomassie-stained SDS-PAGE. Immediately prior to the experiment, the samples were centrifuged at 14,200 g at 4°C for 10 min. 100 µl of each sample (~10 mg/ml) was injected into a Shodex KW404-4F (4.8 mL) column pre-equilibrated in sample buffer (20mM Tris.HCl, 150mM NaCl, pH7.5 and 2.5 mM DTT) with a flow-rate of 0.3 ml/min at 4°C (BM29) and a flow-rate of 0.2 ml/min at 10°C (SWING). During each HPLC run at BM29, 750 frames were collected every 2 *s*, corresponding to approximately 1.5 column volumes (CV). During each HPLC run at SWING, 250 frames were collected after 16 min (~0.67 CV) using 1.5 *s* per frame and 0.5 *s* dead time. After that sample frames were radially averaged and buffer-subtracted using FOXTROT. At BM29, the buffer-subtracted frames were calculated by an automatic pipeline. The excluded volume was calculated from 15 DAMMIN reconstructions generated with P1 symmetry constraint from regularized with GNOM experimental scattering data (Petoukhov *et al.*, 2012).