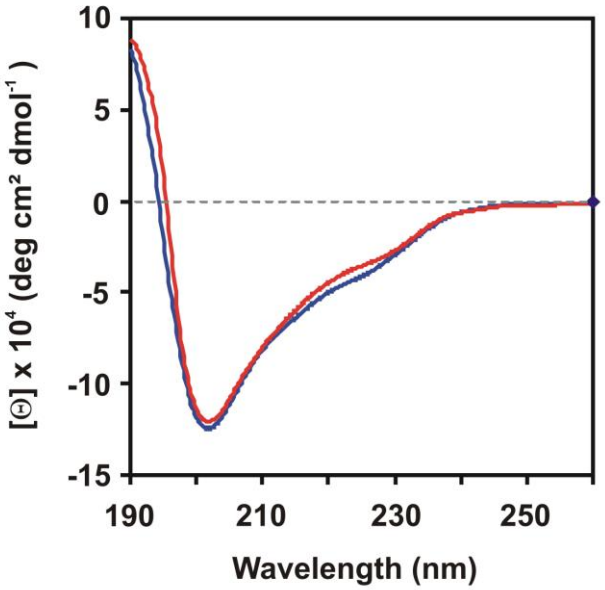
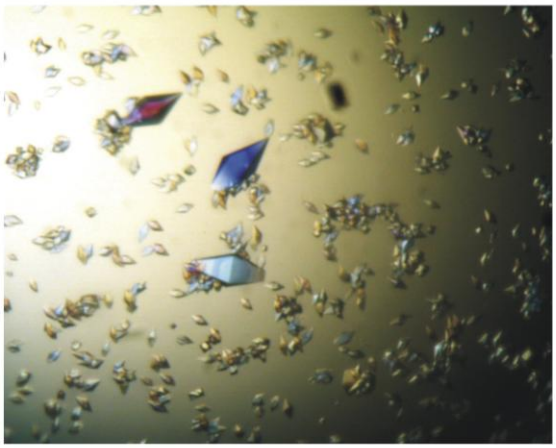
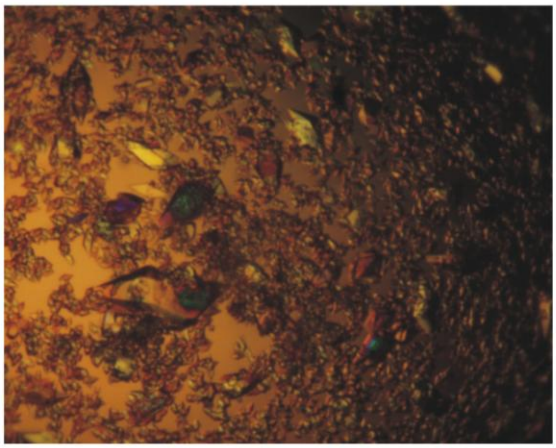
Supplementary Material



Supplementary Figure S1. Far UV CD (190-260 nm) spectra of *r*ShPI-1A in solution after concentration by lyophilization (sample A, *red line*) or by ultrafiltration (sample B, *blue line*) were recorded at 20°C and a protein concentration of 9.0 µM. Ten individual scans were averaged in each experiment, the background spectra were subtracted, and the data were finally transformed into molar ellipticities [Θ]. The almost superimposable spectra consistently displayed the curve progression expected for *r*ShPI-1A (Gil *et al*., 2011). Thus, neither the different aggregation state of *r*ShPI-1A within sample A and B nor the lyophilization process itself irreversibly affected the native secondary structure of this inhibitor.

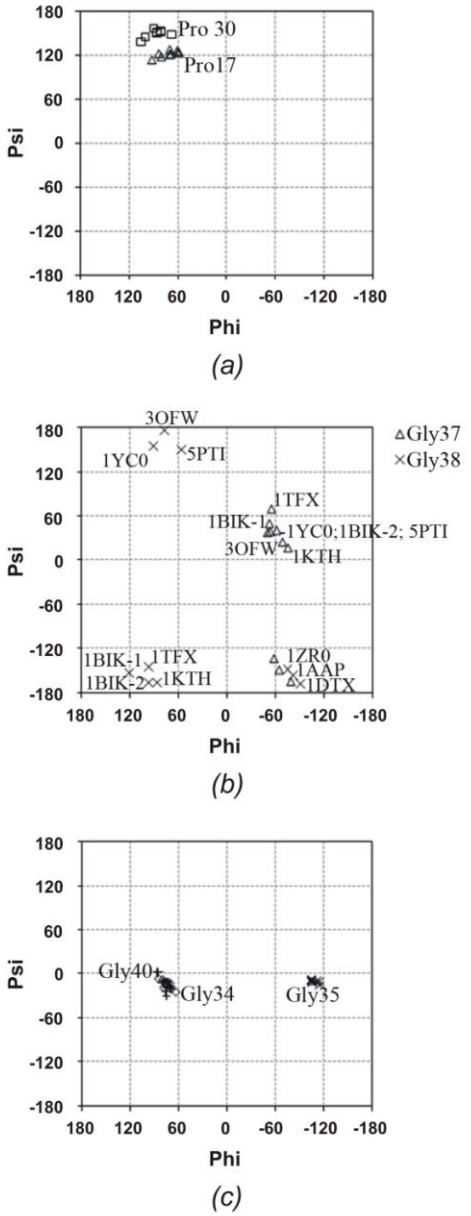


(a)



(b)

Supplementary Figure S2. Crystals formed in setups containing the trypsin complex of lyophilized *r*ShPI-1A sample A in a monomeric state (*a*) or of highly heterogeneous ultrafiltered *r*ShPI-1A sample B (*b*). Both reservoir solutions contained 1.7 *M* $(NH_4)_2SO_4$ and 0.1 *M* Tris-HCl pH 8.5, and 6% (*w/v*) glycerol. Crystals consistently grew independent of the aggregation state. However, the single crystals grown in (*a*) appeared to be optimized regarding size and quality. Thus, a single crystal from setup (*a*) was used for diffraction data collection.



Supplementary Figure S3. Ramachandran plots of selected proline and glycine residues of *r*ShPI-1A and other BPTI-Kunitz domains. Residues are numbered according to the *r*ShPI-1A sequence. BPTI-Kunitz domains are identified according to their PDB code (see Fig. 3a). (*a*) The phi angles of Pro17 (×) / Pro30 (□) in *r*ShPI-1A and equivalent residues in all so far known structures of BPTI-Kunitz inhibitors are clustered from -58.7° to -105.5° independent of the type of amino acid, different from standard values usually reported for ß-sheet structures (-139° ± 20°). (*b)* Within the glycine-rich remote fragment, the highly conserved residues Gly^{34} and Gly^{35} as well as residues equivalent to non-conserved Gly^{40} (*r*ShPI-1A numbering) adopt similar

phi/psi values in all BPTI-Kunitz domains. *(c)* In contrast, angles of residues equivalent to Gly^{37} and Gly^{38} are clustered in two and three different groups, respectively. 1BUN, 1TAP and 1TOC are not included in this comparison.