Report for MX2508

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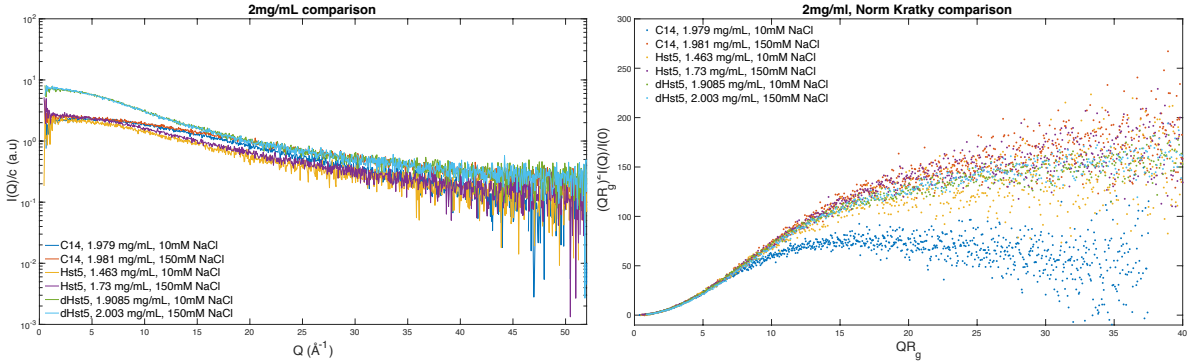
Histatin 5 and variants

The antimicrobial peptide Histatin 5 (Hst 5) has previously been determined to have a flexible structure. In this project, two variants (one with a shorter chain called C14 with 12 aa, and one with double the sequence called dHst5 with 48 aa) of Hst 5 (24 aa) have been investigated using SAXS to determine the form factor of the two variants. As shown in the top left figure in Figure 1 below, and there seem to be an effect of the chain length on the conformational ensemble. The difference in low Q, especially for dHst5 compared to the others, is indicative of different interparticle interactions, hence, that there is more aggregation in these samples. There is no clear effect from the salt concentration. The peptides seem to be unaffected by this. In the normalized Kratky plot shown below (top right), all peptides display mainly a flexible structure, but the shorter peptide C14 in 10mM NaCl is slightly more compact than the others. In the bottom Figure is shown the distance distribution, and as expected the maximum length of the peptide is largest for dHst5, followed by Hst5, and lastly C14. This trend also holds for the peak, which is the most populated size. The results shown below are obtained at a concentration of ~2mg/mL. Shown in Table 1 below are some parameters obtained from fitting. From the MW we can assume that all peptides (except Hst5 in 10mM NaCl) is slightly aggregated since they display a larger MW than the theoretical one.



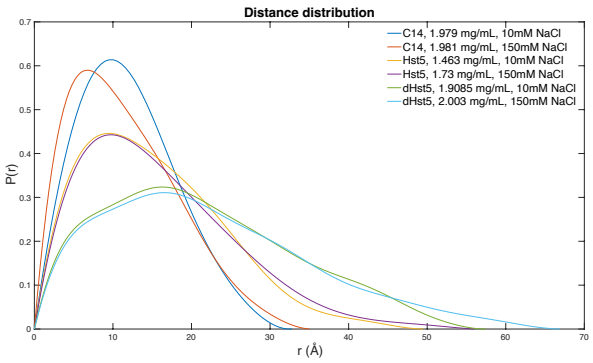


Figure 1. Intensity curves, normalized with concentration (top left), and Kratky plot (top right) of Hst 5 and variants at 10mM, and 150mM NaCl.

Table 1. Properties of the peptides obtained from SAXS analysis.

Sample	Concentration (mg/mL)	Guinier Rg (Å)	MW (Da) (theoretical)
C14 (10mM)	1.979	7.2	2639 (1847)
C14 (150mM)	1.981	9.4	2921 (1847)
Hst5 (10mM)	1.463	11.3	2656 (3036)
Hst5 (150mM)	1.730	12.6	3078 (3036)
dHst5 (10mM)	1.909	16.3	8305 (6055)
dHst5 (150mM)	2.003	17.2	8635 (6055)

BvPgb1.2

This project focuses on Phytoglobin from sugar beet (BvPgb1.2) wild-type (WT) and a mutant (C86A), where a cysteine is exchanged for an alanine. Both proteins were measured (Figure 2A) at several concentrations and in presence and absence of 150 mM NaCl (all in 10 mM Tris pH 8.5). Both proteins are well folded globular proteins, as seen from Kratky plots (Figure 2B). Both WT and C86A mainly assume a dimeric structure as supported by the dimensions of the pair-distance distribution functions (Figure 2C) and the molecular mass calculated from I(0), with some monomeric structure present at low concentrations. With higher concentrations a larger proportion of both proteins exist in larger oligomeric structures (presumably tetramers, hexamers and octamers). The effect of salt on the association numbers was insignificant for both protein structures, however the mutation of cysteine 86 to alanine resulted in a larger association number of the protein (Figure 2D). This difference can be attributed to solvent-exposure of hydrophobic parts of BvPgb, when the otherwise buried cysteine 86 is exchanged with alanine.

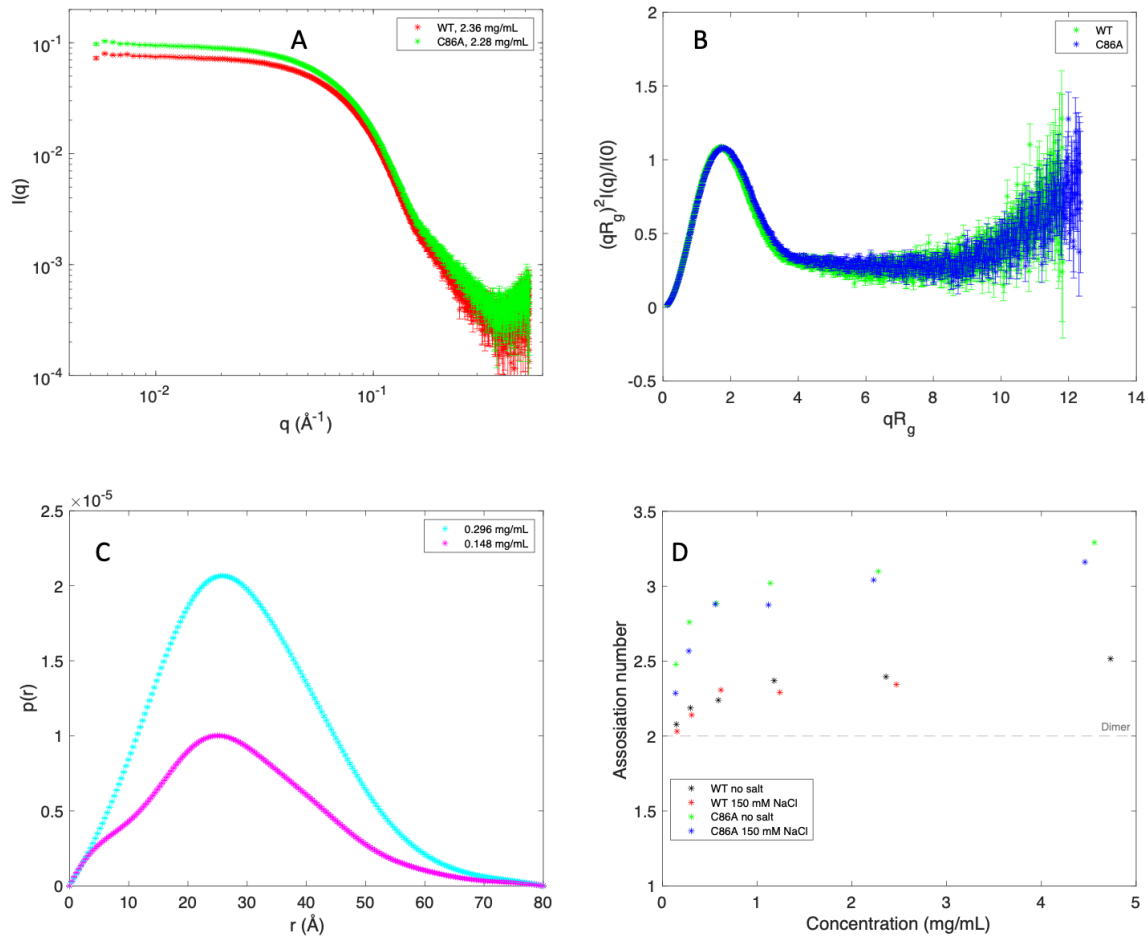


Figure 2. (**A**) Intensity curves for BvPgb1.2 WT and C86A at comparable concentrations, show larger structures for C86A. (**B**) The bell-shaped Kratky plots for WT and C86A suggest equally well folded proteins. (**C**) p(r) for the WT has dimensions consistent with a primarily dimeric structure, however at the lowest concentration measured, some monomer is likely also present, as seen by the extra bump at small distances. (**D**) Association number plotted against concentration for the WT and C86A with and without 150 mM NaCl, show negligible salt effect, but significant higher association numbers for C86A compared to WT.