



	Experiment title: A BioSAXS study of the self-assembly of alpha-helical peptides	Experiment number: MX 1620
Beamline: BM29	Date of experiment: from: 3/7/14 to: 4/7/14	Date of report: 26/11/14
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Report:

We examined the self-assembled structure of toll-like receptor agonist lipopeptides containing the CSK4 peptide sequence in aqueous solution. Cryo-transmission electron microscopy (cryo-TEM) results shown a remarkable dependence of morphology on the number of attached hexadecyl lipid chains, with spherical micelle structures for mono-lipidated (PAMCSK4) and di-lipidated (PAM2CSK4) structures, but flexible wormlike micelles for the homologue containing three lipid chains (PAM3CSK4).¹

SAXS provides support to the findings from cryo-TEM regarding self-assembled nanostructure, and it enables more detail to be obtained on the average dimensions and internal structure of the micellar assemblies. As shown in Fig. 1, the SAXS profiles measured for the three lipopeptides in dilute solution can be fitted to model form factors for spherical micelles in the case of PAMCSK4 (mono-lipidated) and PAM2CSK4 (di-lipidated) and for a bilayer structure in the case of PAM3CSK4 (three-lipidated).

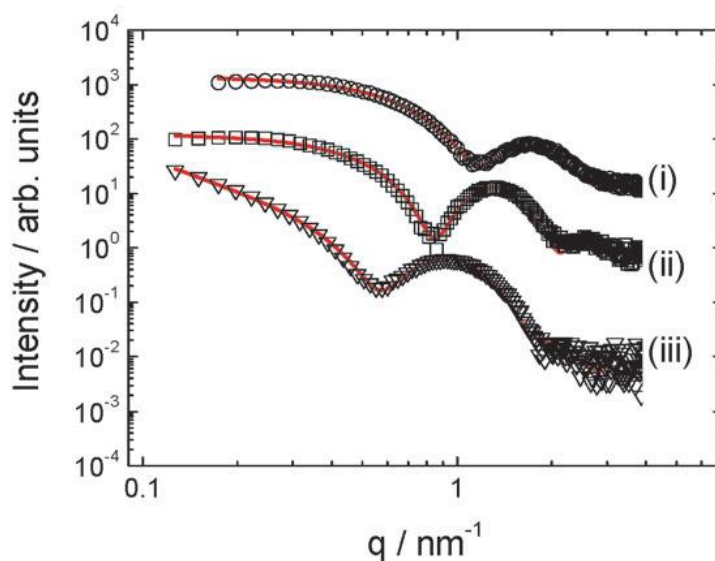


Fig. 1. SAXS data with form factor models described in text for (i) PAMCSK4, (ii) PAM2CSK4 and (iii) PAM3CSK4. The open symbols are the experimental data, the solid red lines are the model form factor fits described in the text, using the software Sasfit². Curves are offset for convenience, and only every 5th data point is shown.

SAXS experiments were also performed on systems containing mixtures of a designed bioactive lipopeptide C₁₆-GGGRGDS, comprising a hexadecyl lipid chain attached to a functional heptapeptide, with the lipid-free apolipoprotein, Apo-AI.³ Fig. 2 shows the SAXS data obtained for a solution containing only Apo-AI or mixtures of Apo-AI with C₁₆-GGGRGDS at different molar ratios $Mr = [C_{16}\text{-GGGRGDS}]/[\text{Apo-AI}]$.

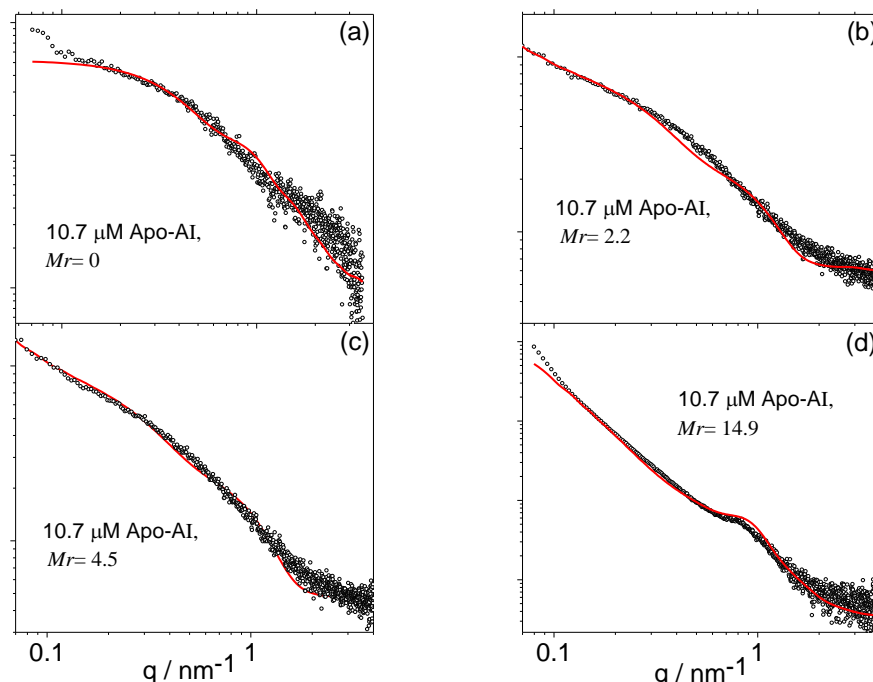


Fig. 2. SAXS data (symbols) along with calculated form factor profiles (full lines) according to the models described in the text. (a) $Mr = 0$, (b) $Mr = 2.2$, (c) $Mr = 4.5$ and (d) $Mr = 14.9$. Binary samples were made by mixing weighed solutions of Apo-AI and C₁₆-GGGRGDS at different molar ratios $Mr = [C_{16}\text{-GGGRGDS}]/[\text{Apo-AI}]$.

The form factor of Apo-AI (Fig. 6a) was computed from the crystallographic structure published for dimers of the C-terminal truncated lipoprotein,⁴ using the software Crysol (Version 2.8 © ATLAS team 1995-2011).⁵ Cryo-TEM results show a persistence of fibrillar structures due to self-assembly of C₁₆-GGGRGDS together with a small fraction of “nanodisc” structures, in mixtures with Apo-AI for $Mr = 2.2\text{-}14.9$.³ The SAXS data $Mr = 2.2\text{-}14.9$ Fig. 2 (b-d) was modelled as a co-existence of flat cylinders with nanotape-like structures, using the software Sasfit², which accounts for the mixture of extended fibrils and disk-like objects measured by cryo-TEM.

References

1. Hamley, I. W.; Kirkham, S.; Dehsorkhi, A.; Castelletto, V.; Reza, M.; Ruokolainen, J., Toll-like receptor agonist lipopeptides self-assemble into distinct nanostructures. *Chemical Communications* **2014**, *50*, 15948-15951.
2. Kohlbrecher, J.; Bressler, I. *Software package SASfit for fitting small-angle scattering curves*, 2011.
3. Castelletto, V.; Hamley, I. W.; Reza, M.; Ruokolainen, J., Interactions between Lipid-Free Apolipoprotein AI and a Lipopeptide Incorporating the RGDS Cell Adhesion Motif. *Nanoscale* **2014**, Advanced Article.
4. Mei, X.; Atkinson, D., Crystal Structure of C-Terminal Truncated Apolipoprotein A-I Reveals the Assembly of High Density Lipoprotein (HDL) by Dimerization. *Journal of Biological Chemistry* **2011**, *286*, 38570-38582.
5. (a) ATLAS, t. *Crysol - Version 2.8 1995-2011*; (b) Svergun, D.; Barberato, C.; Koch, M. H. J., CRYSOLO - A program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates *Journal of Applied Crystallography* **1995**, *28*, 768-773