Poster Presentation

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Withdrawn - Small-angle X-ray scattering of BAMLET protein-oleic acid complexes at pH 12

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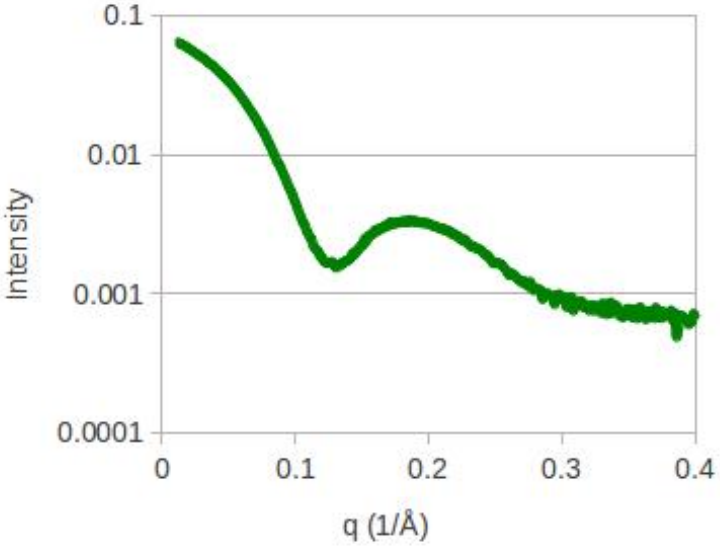
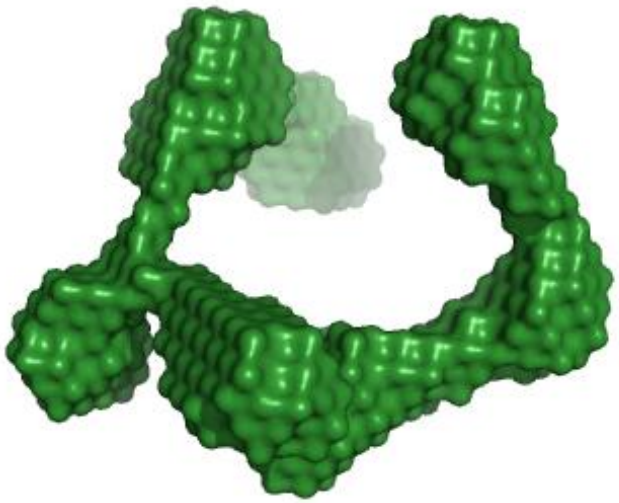
BAMLET and HAMLET represent a new class of compound having unrealised potential for treating a broad spectrum of cancers and some multi-drug resistant bacterial infections (Brinkmann et al. 2013; Marks et al. 2013). These compounds are composed of protein (14 to 84 kDa) and oleic acid (282 Da), the latter being the main active component. Hypothesised molten-globularity makes structural determination by NMR and X-ray crystallography very challenging. We carried out small angle X-ray scattering (SAXS) on BAMLET at pH 12 (Rath et al. 2014), the pH at which the complex can be prepared. SAXS showed that the protein component was an ensemble of extended, irregular, partially-unfolded conformations that varies with the amount of oleic acid incorporated into the complex. Increases in oleic acid concentration (from 1 to 20 molecules of oleate per protein molecule) correlate with increasing radius of gyration (from 21 to 29 Ang) without an increase in maximum particle dimension, indicating decreasing protein density. Three-dimensional models were generated that satisfy the probability distribution function that was derived by indirect Fourier transform of the SAXS data (Figure A). Models for the highest oleic acid content BAMLET (Figure B) indicate a partially unfolded conformation with the majority of the protein mass distributed around the periphery of the complex. Our results suggest that oleic acid inhibits the folding or collapse of the protein component of BAMLET to the globular form. SAXS was not able to identify the structure of the oleic acid component due to the very weak X-ray scattering contrast. However, the results support a model in which BAMLET retains oleic acid by non-specific association in the core of the partially unfolded protein. This represents a new type of lipid-binding protein structure. The structure of BAMLET will guide efforts to incorporate BAMLET into a delivery vehicle with the aim of realising the significant clinical potential of BAMLET.

[1] *Brinkmann et al. (2013) FEBS J. 280, 1733.,* ***[2]*** *Marks et al. (2013) PLoS One 8, e63158.,* ***[3]*** *Rath et al. (2014) Proteins: Struct., Funct., Bioinf. doi: 10.1002/prot.24508.*

A. SAXS curve for BAMLET



B. SAXS-derived model for BAMLET



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