	Experiment title: A BioSAXS Study of the Interaction between lipid membranes and antimicrobial peptides	**Experiment number**: SC-4981
Beamline:	**Date of experiment**: from: 11/9/21 to:13/9/21	**Date of report**: 11/01/22
Shifts:	**Local contact(s)**: Thomas Zinn, Narayanan Theyencheri	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Ian W. Hamley, Dr Valeria Castelletto

Report:

Due to COVID-19 restrictions, the experiments were carried out with no users on site by Dr Thomas Zinn and Dr Narayanan Theyencheri. The samples were mounted in capillaries.

Due to changed laboratory arrangements caused by COVID-19 restrictions, the proposed samples were not run. Instead the self-assembly of a designed surfactant-like peptide was examined, using a combination of cryogenic-transmission electron microscopy and SAXS. It was to have remarkable pH-dependent self-assembly properties. Peptide Arg_3-Leu_{12} (R_3L_{12}) forms a network of peptide nanotubes at pH 9 and below. These are associated with α-helical conformation in a "cross-α" nanotube structure, in which peptide dimers lie perpendicular to the nanotube axis, with arginine coated inner and outer nanotube walls. In contrast, this peptide forms decorated vesicular aggregates at higher pH values, close to the pKa of the arginine residues. These structures are associated with a loss of α-helical order as detected through x-ray scattering, circular dichroism and FTIR spectroscopy. The observation of the self-assembly of a simple surfactant-like peptide into these types of nanostructure is remarkable, and peptide R_3L_{12} shows unique pH-dependent morphological and conformational behaviour, with the potential for a range of future applications.

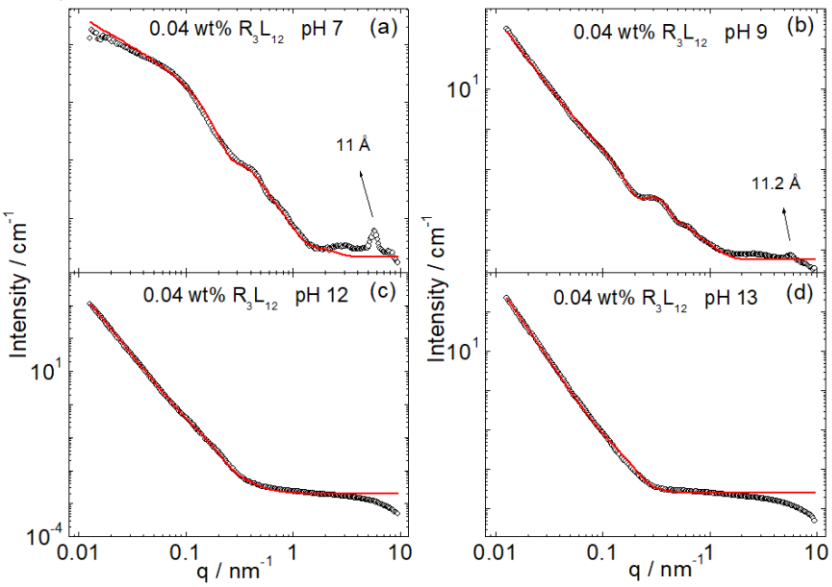

Figure 1. Measured SAXS intensity profiles (open symbols) fitted with form factors (solid lines) corresponding to nanotubes at pH 7 and pH 9, and spherical shells at pH 12 and pH 13 with a fractal-like background scattering term at pH 9, 12 and 13 only.

SAXS curves measured at pH 9, 12 and 13 for 0.04 and R_3L_{12} are displayed in Figure 1. The SAXS data provide form factors which yield information on the self-assembled nanostructure *in situ* in solution. The data at pH 9 show well-defined oscillations, which result from interference scattering from the nanotube walls, and these features were fitted to a model of a hollow cylinder (long cylindrical shell) consistent with a nanotube structure (also revealed by cryo-TEM). The nanotube scattering oscillations are superposed on a background which is due to scattering from small clusters in the cryo-TEM images and/or unassociated peptides. The SAXS data for the samples at pH 12 and pH 13 show distinct features, with a power-law scattering at low q, which is due to the presence of fractal-like structures, also revealed by cryo-TEM and TEM. The SAXS data was measured out to high q, in order to probe possible peptide secondary structure. A peak corresponding to a spacing d = 11.2 Å is apparent in the data in Fig.1 for the peptide at pH 9 (and at d = 11.0 Å at pH 7). This is consistent with the expected diameter of a α-helix.[1, 2] This peak is lost at higher pH, consistent with the loss of α-helix secondary structure revealed in the CD spectra in Fig.1 and also via FTIR spectra, to be discussed shortly.

Structural parameters obtained from the SAXS fitting for 0.04 wt% peptide solution at pH 9 provide a nanotube diameter equal to 2x[(5.3+3.7) ±2.8] = 18 ± 5.6 nm, which compares very well with 16.4±1.7 nm calculated from the average of the nanotube diameters from the cryo-TEM images. As previously discussed by us, the nanotube wall thickness corresponds closely to the length of R_3L_{12} estimated using average residue spacings,[2] l = (0.15n)+(0.34p)= 2.82 nm (~3 nm) where n = 12 (number of L residues in the α-helix) and p= 3 (number of R residues). Our results are in agreement with a model for the nanotubes that have walls built from a single layer of α-helical antiparallel peptide dimers.

This work has been published.[3]

5. References

1. D. Voet and J. G. Voet, *Biochemistry*, John Wiley, New York, 1995.
2. T. E. Creighton, *Proteins. Structures and Molecular Properties*, W.H.Freeman, New York, 1993.
3. V. Castelletto, J. Seitsonen, J. Ruokolainen and I. W. Hamley, *Soft Matter*, 2020, **17**, 3096-3104.