Experimental report

07/05/2022

Proposal: 8-03-1038 **Council:** 10/2020

Title: DNA structuration by a bacterial amyloid

Research area: Biology

This proposal is a new proposal

Main proposer: veronique ARLUISON

Experimental team: Xiaoli QU
Judith PETERS
Frank WIEN
veronique ARLUISON
Tatsuhito MATSUO

Local contacts: Sylvain PREVOST
Anne MARTEL

Samples: Hfq CTR peptide
DNA dAdT59

Instrument	Requested days	Allocated days	From	To
D33	2	0		
D11	2	0		
D22	2	2	31/05/2021	02/06/2021

Abstract:

Expected breakthroughs of the proposal consist in the investigation of a self-assembled amyloid nucleoprotein nanostructure. Small angle neutron scattering will be applied to follow the effect of an amyloid structure formed by Hfq, a key protein involved in the control of bacterial virulence, on DNA. Precisely, a peptide issued from Hfq results in a specific helicoidal fibrillar structure where DNA is a part of the complex. Nevertheless, the precise organization of the complex and whether DNA is located inside the fibre is not known. Solvent contrast matching will allow to match out the signal from Hfq peptide conserving however its influence on the DNA structure. In this way, only the structure of DNA under the influence of the amyloidogenic peptide will be seen. It should thus be possible to accurately determine the Hfq amyloid fibers shape and the conformation of the DNA inside the fiber. As Hfq is a virulence factor, the expected long-term benefits for this project will be opportunities for the development of new antibiotics.

Report on the proposal: 8-03-1038
DNA structuration by a bacterial amyloid

Biological material used:
Protein: C-terminal region (CTR) of Hfq; DNA: $(dA:dT)_{59}$

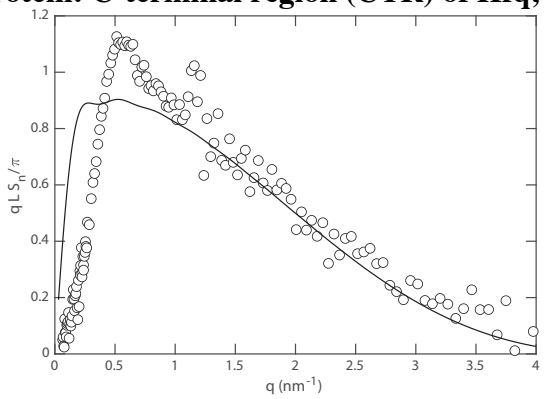

FIG. 1. Normalized DNA structure factor $qL\,S_{\rm n}/\pi$ versus momentum transfer q. The solid line denotes the form factor of a rigid rod with a length of 20 nm and a radius of 0.8 nm.

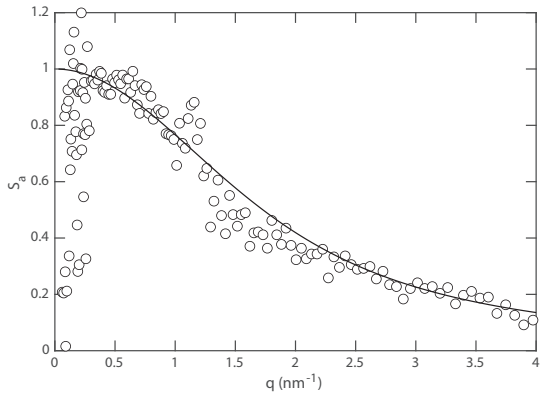

FIG. 2. CTR structure factor $S_{\rm a}$ versus momentum transfer q. The solid line denotes the form factor of a Gaussian coil (Debye function) with an optimized radius of gyration $R_g = 0.9$ nm.

Contrast variation. SANS experiments were done with the D22 diffractometer. The total counting times for all detector settings was approximately 2 h per sample. The sample temperature was 298 K. Contrast variation in the solvent was used to resolve the contributions to the scattering from DNA and CTR peptide (structure factors). Scattering length contrasts pertaining to the different H_2O/D_2O solvent compositions were calculated based on partial molar volumes and scattering lengths of the molecular components.

DNA and CTR reference solutions. The DNA and CTR structure factors were obtained from the solutions in H_2O. As shown in Fig. 1, the high q limiting behavior of the DNA structure factor agrees with a rod-like molecule of length 20 nm (59 bps) and radius of 0.8 nm (cross-sectional radius of gyration of 0.6 nm). Deviations for lower values of momentum transfer are related to intermolecular interference. The CTR structure factor is shown in Fig. 2. Despite the noisy data, the structure factor agrees with the form factor of a Gaussian coil with a radius of gyration of 0.9 nm. Again, deviations observed at lower values of momentum transfer are due to intermolecular interference.

Fiber structure factors. The DNA and CTR structure factors pertaining to the amyloid fiber are obtained from the scattered intensities of samples with solvent contrast variation. Figure (3) displays the intensities of the fiber solutions. Note the significant variation in intensity with solvent composition. All samples show an upturn in intensity at low values of momentum transfer $q < 0.1$ nm^{-1}. The low-q upturn is plausibly due to clustering (secondary aggregation) of fibers.

With a least-squares procedure, the structure factors were fitted to the data. The fitted intensities are given by the solid curves in Fig. (3). In the low-q (upturn) region, the standard deviation of the fit diverges, and the intensities do not comply with solvent composition

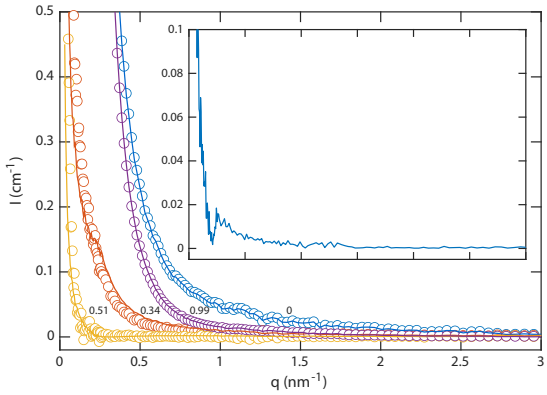

FIG. 3. SANS intensity versus momentum transfer from the CTR-DNA fiber solution. The D_2O volume fractions are indicated. The curves represent a fit in which the structure factors are optimized. The inset displays the standard deviation.

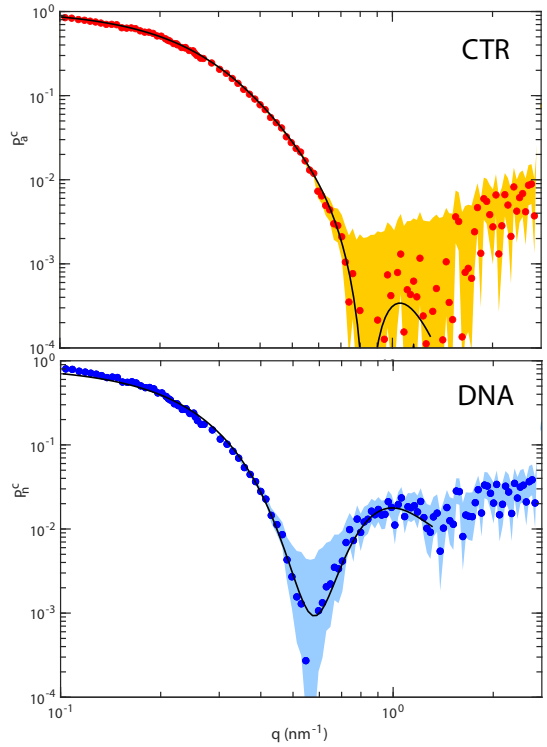

FIG. 4. CTR (top) and DNA (bottom) cross-sectional form factors $P_{\rm a}^c$ and $P_{\rm n}^c$, respectively, versus momentum transfer q. The colored patches denote uncertainty margins as derived from error propagation. The solid curves represent the optimized factors corresponding to the radial density profiles in the top panel of Fig. (5).

independent structure factors [see inset of Fig. (3)]. This shows that the samples differ in secondary aggregation, despite the fact that they have been prepared in the same way. For higher q values, the standard deviation levels off and the fitted curves agree with the data. Accordingly, it is assumed that for q exceeding, say, 0.1 nm^{-1} the data are not influenced by any residual clustering of fibers. The fitted structure factors, multiplied with momentum transfer q and normalized to unity at $q = 0$, are displayed in Fig. (4). The results represent the cross-sectional form factors $P_{\rm n}^c(q)$ and $P_{\rm a}^c(q)$ pertaining to the radial distribution of DNA and CTR, respectively. From the ratio of the normalization factors follows a CTR:DNA molecular ratio of 13:1. The samples were prepared with a ratio of 15:1, which implies that about 10% of the CTR molecules do not form part of the fiber (the scattering contribution of free CTR is negligible).

Fiber cross-sectional analysis. Information regarding the radial density of DNA and CTR is obtained from analysis of the cross-sectional form factors. We have verified that form

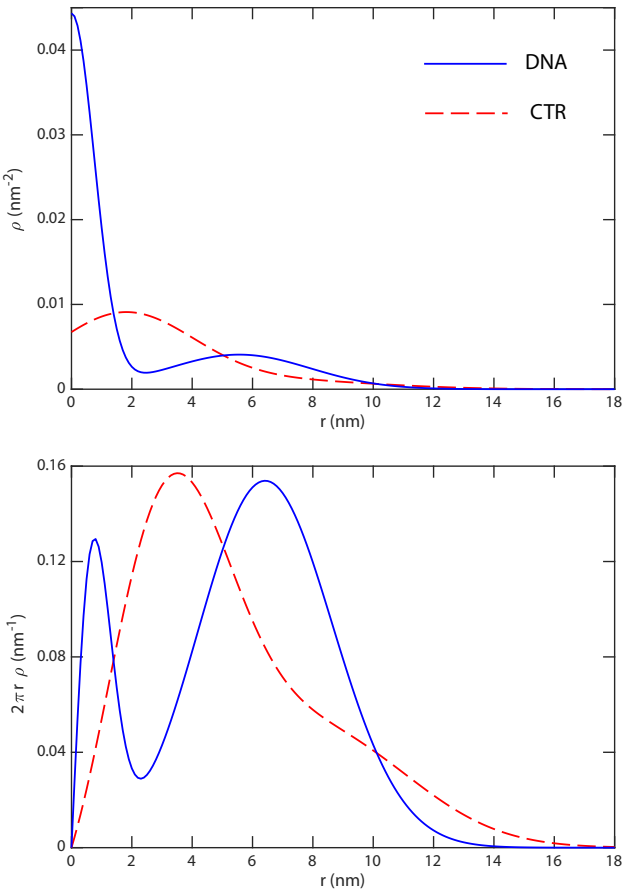

FIG. 5. (Top) Radial density profile for DNA (solid, blue) and CTR (dashed, red) according to the fit parameters in Table **??**. (Bottom) As in top panel but for the fractional number of molecules.

factors pertaining to a single Gaussian distribution do not agree with the data. Accordingly, the analysis calls for at least a two-layered structure with an inner and outer layer for both DNA and CTR. For each layer, we assume a Gaussian distribution, so that the radial profiles take the form of a sum of two Gaussians. The radial profiles were optimized with a simplex procedure, in such a way that the calculated cross-sectional form factors agree with the data for both DNA and CTR. As shown in Fig. (4), perfect agreement is observed. The fitted radial density $\rho_{\rm i}(r)$ and fractional number $2\pi r\rho_{\rm i}(r)$ profiles are displayed in Fig. (5).

The radial profiles show an overall diameter of the fiber of about 30 nm . Furthermore, a layered structure is observed. The DNA distribution peaks at the core of the fiber with a width of 1.1 nm. For a single DNA molecule, the cross-sectional radius of gyration is 0.6 nm. Accordingly, the spine of the fiber must be composed of a few partially parallel DNAs. These central molecules are surrounded by an outer layer at a most probable distance of 5.5 nm. The fraction of molecules in the outer layer is 84%, which implies that the central DNA molecules are surrounded by 5–10 DNA molecules. Notice that the diameter of the duplex is about 2 nm, which leaves a space between the central and outer DNA layers of 2.5 nm. As shown by the CTR density profiles, 66% of the CTR is distributed in the space between the inner and outer DNA layers and between the DNA molecules in the azimuthal direction. The remaining 34% is at the outer edge of the fiber. We continue interpreting the radial profiles with different models such as hexagonal arrangements. Furthermore complementary cryoTEM analysis will be performed at CNB Madrid (Instruct-ERIC proposal).