Experiment title: Investigation on the structural properties of cationic amphiphilic cyclodextrin vesicles interacting with DNA	**Experiment number**: SC-3002

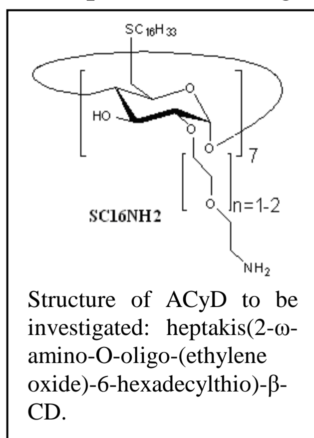
Beamline: ID02	**Date of experiment**: from: 28/01/2011 to: 01/02/2011	**Date of report**: 28/07/2011
Shifts: 12	**Local contact(s)**: Manuel Fernandez	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*** VILLARI Valentina, CNR - Istituto Processi Chimico-Fisici, Viale F. Stagno d'Alcontres 37, 98158 Messina, Italy**
*** MICALI Norberto, CNR - Istituto Processi Chimico-Fisici, Viale F. Stagno d'Alcontres 37, 98158 Messina, Italy**
*** MAZZAGLIA Antonino, CNR - Istituto per lo Studio dei Materiali Nanostrutturati, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy**

Report:

The aim of the experiment was the study of the structural features of amphiphilic cyclodextrin (ACyDs) aggregates interacting with DNA. These ACyDs present specific features of interest as molecules for the development of new gene vectors. Indeed, they were reported to be nonhemolytic and noncytotoxic and, in the recent past, we demonstrated that β-CyDs grafted with oligoethylene glycol moieties have amphiphilic properties and are capable of forming aggregates of potentially low immunogenicity according to the hydrophobic–hydrophilic balance.[1-3] For application in gene delivery, however, the knowledge of the interaction character between ACyDs vesicle and DNA and the structure of the resulting complex is of fundamental importance.



Structure of ACyD to be investigated: heptakis(2-ω-amino-O-oligo-(ethylene oxide)-6-hexadecylthio)-β-CD.

The measured scattered intensity of the cationic SC16NH2 aggregates (see fig.1) indicates that a vesicle structure is formed. The fit was performed by using a model of unilamellar vesicle with a symmetric scattering length density profile constituted by two Gaussians for the outer and inner hydrophilic shells and one for the hydrophobic central shell, according to the law:[4]

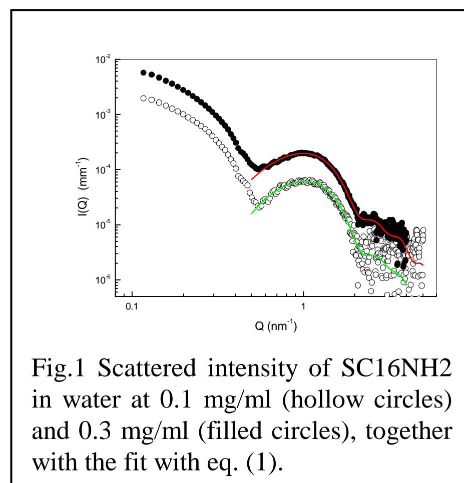


Fig.1 Scattered intensity of SC16NH2 in water at 0.1 mg/ml (hollow circles) and 0.3 mg/ml (filled circles), together with the fit with eq. (1).

$$P(Q) \propto 2\sigma_H \exp\left(-\sigma_H{}^2 Q^2/2\right)\cos\left(Qz_H\right) - \sigma_C \rho_r \exp\left(-\sigma_C{}^2 Q^2/2\right) \qquad (1)$$

where σ_H and σ_C are the Gaussian widths of the hydrophylic and hydrophobic region, respectively, $\pm z_H$ the center of the hydrophilic region and ρ_r the ratio between the excess length density of the hydrophobic and hydrophilic region. In fig.2 the fit result is reported for the two concentration values investigated: the vesicle thickness is about 5 nm independently of concentration. On considering that the molecule length is about 3.5 nm, the value of the thickness indicates a partial interdigitation of the hydrophobic chains.

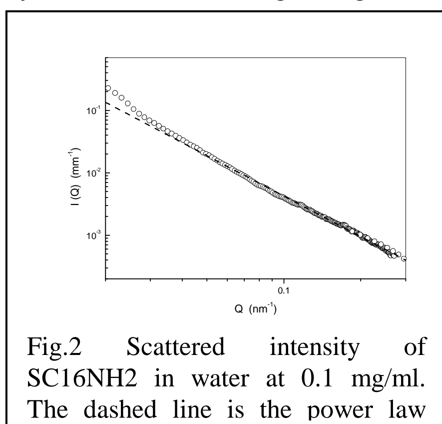


Fig.2 Scattered intensity of SC16NH2 in water at 0.1 mg/ml. The dashed line is the power law

The investigated Q region is too high to allows for extracting the vesicle radiusof gyration; however, within the Q values $0.02<Q<0.2$ nm^{-1}, the scattered intensity obeys the power law $I(Q) \propto Q^{-2}$, according to the scattering from a surface (see fig.2).

In the presence of calf thymus DNA, it is evident the progressive disappearance of the unilamellar vesicle on increasing the molar ratio DNA/SC16NH2 from 1:18 to 1:4 and the apppearance of a peak at Q~0.9 nm^{-1} (fig.3). At the molar ratio 1:4 the scattering profile of vesicle has completely disappeared, putting in evidence the presence of a second peak at Q~1.7 nm^{-1}. These Bragg peaks suggest the formation of multilamellar structures with spacing of about 7 nm. Such a transition, which is independent of SC16NH2 concentration, is caused by the electrolitic condensation between the hydrophilic cationic region (the NH_2 terminus of the hydrophilic chains is protonated in water, giving to the molecule positive charge) and the anionic DNA. The latter is situated in the aqueous region between two adjacent lamellae. Dynamic Light Scattering (DLS) measurements showed that the vesicle hydrodynamic radius increases in the presence of calf thymus DNA, consistently with the occurrence of a uni-to-multilamellar vesicle structural transition.

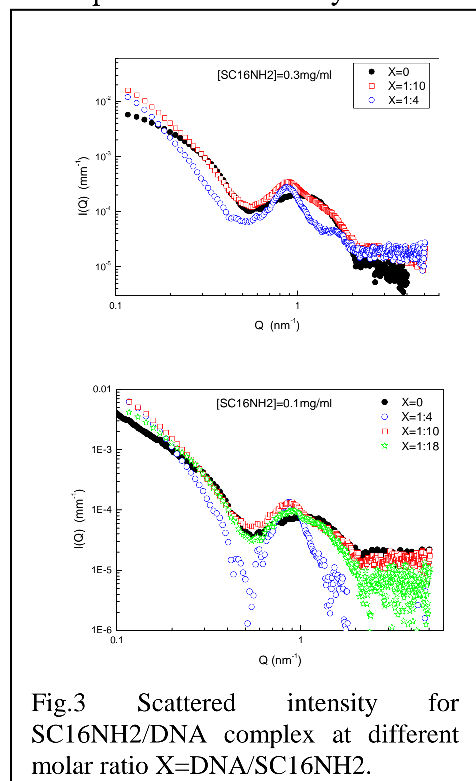


Fig.3 Scattered intensity for SC16NH2/DNA complex at different molar ratio X=DNA/SC16NH2.

Unlike SC16NH2, the SC16OH moiety which possesses a OH group instead of NH_2, does not seem to interact with DNA and the Q-dependence of the scattered intensity is unaffected. Also DLS and electrophoretic mobility measurements do not show any change upon the addition of DNA.

Concerning with the water solution of SC12NH2, differing from SC16NH2 for shorter hydrophobic chains, SAXS results seem to indicate that the vesicle structure is not affected by the presence of calf thymus DNA (see fig.4). The Q-dependence of the scattered intensity slightly changes only at smaller Q values, suggesting the presence of an aggregation phenomenon. An interaction between calf thymus DNA and SC12NH2, however, occurs as confirmed by the hydrodynamic radius increase measured by DLS and by the change of the electrophoretic mobility of SC12NH2 aggeregate. In particular, on increasing the DNA/SC12NH2 molar ratio to 1:4, the electrophoretic mobility displays a reversed sign with respect to SC12NH2. In view of these results, it is possible that, for SC12NH2, calf thymus DNA has the role of shielding the repulsive interaction between the charged SC12NH2 with consequent aggregation of vesicles, without affecting the membrane features.

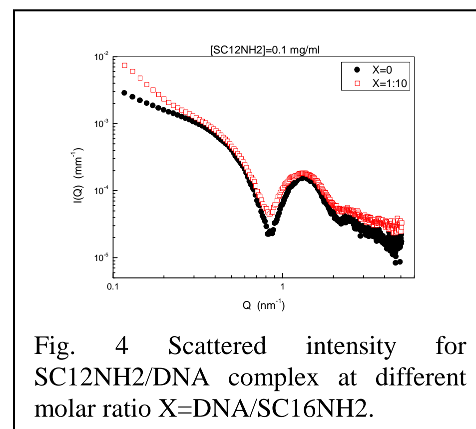


Fig. 4 Scattered intensity for SC12NH2/DNA complex at different molar ratio X=DNA/SC16NH2.

It is worthnoting that plasmid DNA, unlike the calf thymus DNA, does not cause the structural change of SC16NH2 from uni- to multilamellar vesicle. However, a strong electrostatic interaction of plasmid with the vesicle surface is present as indicated by a slight increase of the vesicle hydrodynamic radius and the decrease of the vesicle net surface charge.

Finally, SAXS data shows that a temperature increase to T=40°C, despite causing a slight broadening of the vesicle thickness interference peak, leaves the interaction feature with calf thymus DNA unaffected; this temperature stability can play an important role for future perspective in application of these system for gene delivery.

[1] A. Mazzaglia, N. Angelini, R. Darcy, R. Donohue, D. Lombardo, N. Micali, M. T. Sciortino, V. Villari, L. Scolaro, *Chem. Eur. J.* **9**, 5762-5769 (2003); A. Mazzaglia, A. Valerio, V. Villari, A. Rencurosi, L. Lay, S. Spadaro, L. Monsù Scolaro and N. Micali, *New J. Chem.* **30**, 1662–1668 (2006).

[2] N. Micali, V. Villari, A. Mazzaglia, L. Monsù Scolaro, A. Valerio, A. Rencurosi, L. Lay, *Nanotechnology* **17,** 3239–3244 (2006).

[3] F. Quaglia, L. Ostacolo, A. Mazzaglia, V. Villari, D. Zaccaria, M. T. Sciortino, *Biomaterials* **30**, 374-382 (2009).

[4] G. Pabst, R. Koschuch, B. Pozo-Navas, M. Rappolt, K. Lohner and P. Laggner, *Journal of Applied Crystallography* **36**, 1378-1388 (2003).