

### NON-VIRAL VECTORS FOR GENE THERAPY BASED ON CHOLESTERIC LIQUID-CRYSTAL POLYMERS. STRUCTURE ANALYSIS BY SAXS.

### Mercedes Pérez Méndez\* 1, Daniel Rodriguez Martinez 2 and José Fayos 3.

<sup>1</sup>Institute of Polymer Science and Technology, CSIC, C/ Juan de la Cierva, 3. 28006 Madrid. <sup>2</sup>Retinal degeneration: from genetics to therapy Laboratory, Centro Andaluz de Biología Molecular y Medicina Regenerativa Edif. CABIMER Avda. Américo Vespucio, s/n Parque Científico y Tecnológico Cartuja 93. 41092 Sevilla, Spain <sup>3</sup>Instituto de Química Física Rocasolano, CSIC, C/ Serrano 119. 28006 Madrid.

\*Email address: perezmendez@ictp.csic.es

#### HISTORICAL INTRODUCTION

At the end of autumn of 1944, Erwin Schrödinger, one of the founders of the Quantum Mechanics completed in Dublin a small book: What is life? The physical aspect of the living cell [1].

Referring to the nature of the chromosome material, he said: "In Physics we have tried, up to now, with periodic crystals (...). Organic Chemistry (...) has approached much more to the 'aperiodic crystal' that, in my opinion, is the material carrier of life...





Photo51-1951 according to [2a]

The concept of "aperiodic crystal" was surprisingly premonitory, 9 years before the crystallographic x-ray diffraction patterns proved the long range order in DNA fibres (main constituent of chromosome material), explained in terms of a helical structure by Franklin [2a] and Wilkins [2b], opening the way to the double helix of Watson and Crick [2c], in Fig. 2, with periodic order of the sugar-phosphate backbone, every 34Å, along the helix and with aleatory distribution of the complementary bases pairs.

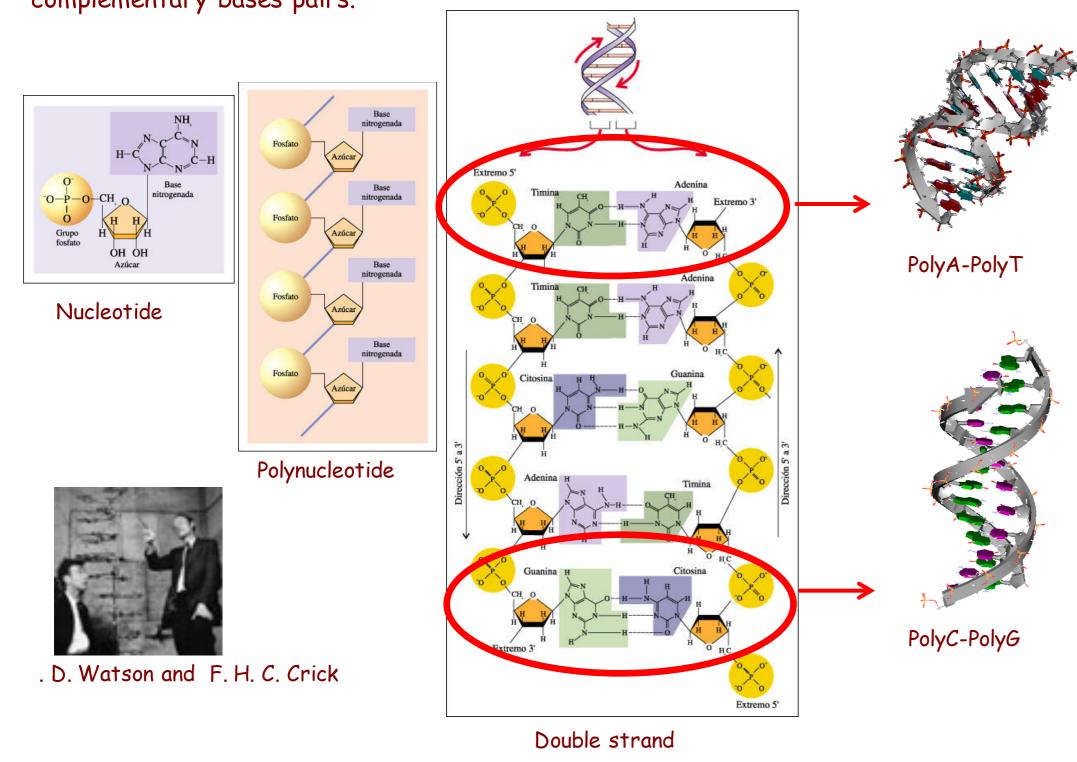


Figure 2. DNA structure according to [2c]

- In 1984 a new ordering of matter appeared, no crystalline but with discrete diffraction patterns of extraordinary quality, quasiperiodic crystal [3]
- In 1988 Ringsdorf established a parallelism between the behavior of liquid crystals in materials science and lipids in life science, due to their liquidcrystalline nature, both being self-organizing systems [4].
- In 1992 the International Union of Crystallography re-defines the concept of crystal as: "Any solid which has a diffraction pattern essentially discrete" (Fourier space) [5].

Since then CRYSTAL FAMILY is accepted to be composed by:

-Periodic crystals and -Aperiodic crystals



The parallel orientation of Liquid-Crystals longitudinal molecular axes is common to all mesophases (long-range orientational order).

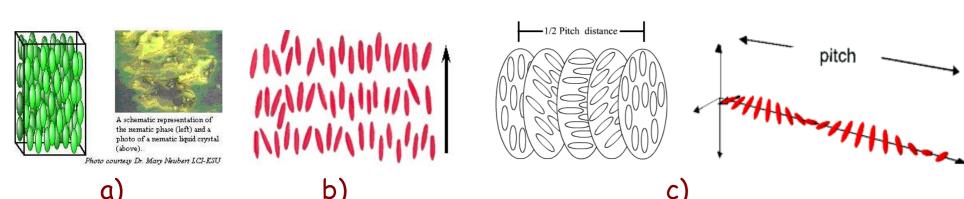


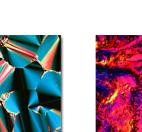
Figure 3. Liquid-crystal mesophases: a) Nematic, b) Smectic A and c) Cholesteric., helical pitch.

In Fig. 4, the cholesteric nature of Liquid -Crystal DNA is shown.









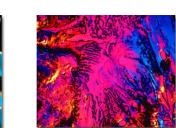


Figure 4. Cholesteric Morphology of Liquid Crystal DNA

## SYNTHETIC CHOLESTERIC LIQUID CRYSTAL POLYMERS

Cholesteric Liquid Crystal Polymers (ChLCP), have been synthetized in our laboratory through a stereoselective polycondensation reaction [6] as optically active materials, being extensively characterized by NMR, Raman spectroscopy, steady-state fluorescence, molecular modeling, and SAXS/WAXS [7].

These ChLCP behave both as thermotropic and lyotropic, confering interesting macromolecular properties indicative of potential application on the biomedical and engineering field.

They have shown to be biocompatible against macrophages and fibroblasts cellular lines, and able to interact with biomacromolecules such as lipids (both neutral and cationic) - Fig. 5- and nucleic acids, the structures of the complexes being identified by synchrotron radiation source [8, 9, 10].

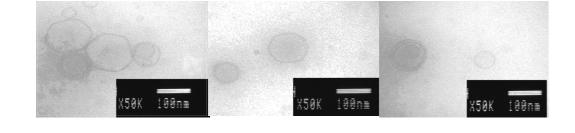


Figure 5. TEM images of L- $\alpha$ -DMPC liposomes interacting with PTOBDME

Cationic liposomial/surfactant systems based on our CLCP were developed which entrapped DNA plasmids, acting as NON VIRAL CATIONIC VECTORS for GENE THERAPY, which successfully transfected in several tumor cell lines [11, 12].

## OBJECTIVE

New cationic functionalized ChLCP, dispersed in TAE are directly complexed with single stranded polynucleotides: [Poly-A]; [Poly-C]; [Poly-G]; [PolydT]; with double stranded [PolyC-PolyG]; [PolyA-PolydT]; and with nucleic acids: Calf-Thymus DNA and Plasmid PBR322.

Tree different proportions ChLCP:DNA are prepared: (1:2), (1:1), and (2:1) respectively by mixing and digesting in a swinging shaker.

Contrast Variation Neutron Scattering experiment was successfully performed at NIST [13] for cholesteric PTOBEE-Ammonium (1.5- 1.9  $\times$  10<sup>10</sup>/cm<sup>2</sup>) directly complexed with polynucleotide [PolyC-PolyG]  $(3.32 \times 10^{10} / \text{cm}^2)$ .

The structure of the new cationic complexes are studied by SAXS.

The different polyplexes size and shape are estimated.

### NEW NON VIRAL VECTORS FOR GENE THERAPHY EXPERIMENTAL MATERIALS:

#### 1. Commercial Polynucleotides and Nucleic Acids

|   | Molecular Weight            | Structure                                    |
|---|-----------------------------|--|
| PolyA                                   | 200,000 to<br>700,000       | Single Strand                                |
| PolyC                                   | Not determined              | Single Strand                                |
| PolyG                                   | 80,000 to<br>500,000        | Single Strand                                |
| PolydT                                  | Not determined              | Single Strand                                |
| PolyA-PolydT <sub>10</sub>              | Not determined              | Double Strand                                |
| PolyG-PolyC                             | 300,000                     | Double Strand                                |
| Deoxyribonucleic Acid<br>Plasmid PBR322 | 2,900,000                   | Double Strand                                |
| Deoxyribonucleic Acid<br>Calf Thymus    | 10,000,000 to<br>15,000,000 | Double<br>(predominant)<br>and Single Strand |

#### 2. NEW FUNCTIONALIZED CHLCP from PTOBDME, and PTOBEE:

- **✓PTOBEE-Ammonium**
- ✓PTOBDME-Ammonium
- ✓PTOBEE-coline
- ✓PTOBDME-coline
- **✓PNOBDME**

\*All of them have been characterized by NMR and DSC as ChLCP

#### 3. COMPLEXES CHLCP/DNA FORMATION

- > The CLCP were dispersed in TAE (0,04M TRIS; 0,001M EDTA) > The CLC polymers were complexed directly with commercial DNA
- by mixing and digesting for 12h in a swinging shaker

### STRUCTURAL CARACTERIZATION BY SAXS.

A monochromatized beam at  $\lambda$ = 0,9795 Å was used at BM16 Beamline at ESRF.

Two-dimensional data recorded by an image-plate detector was placed at 5975 cm from the sample. The program Fit2D was employed to evaluate the beam centre position and to generate a mask file. Binary data are normalized by the detector response and pixels are radially averaged into 1D. Silver behenate (d= 58.3 Å) was used to calibrate the angular axis. The net scattering intensity I(n) was obtained through the standard equation.

$$I(n) = \frac{1}{c \operatorname{Det}(n)} \left[ \frac{I_{s}(n)}{I_{0s} T_{s}} - \frac{I_{m}(n)}{I_{0m} T_{m}} - \frac{I_{e}(n)}{I_{0e}} \left( \frac{1}{T_{s}} - \frac{1}{T_{m}} \right) \right],$$

Where s, m and e correspond to sample, matrix and empty cell; T transmission and c sample concentration.

❖Information about size [Rg (Guinier)] and shape could be estimated based on Ln I(q) versus Ln q slope.

Structural analysis of the cationic complexes can be complemented with the program: GNOM [ATSAS] [14].

## **RESULTS**

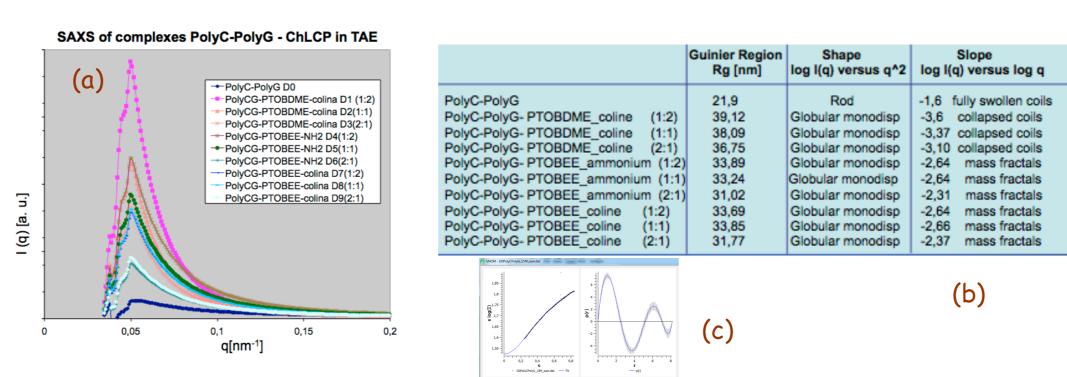


Figure 7. (a) SAXS of Complexes between PolyC-PolyG and cationic ChLCPs; (b) Rg and shape estimations and (c) Fitting PolyC-PolyG to a rod model with ATSAS.

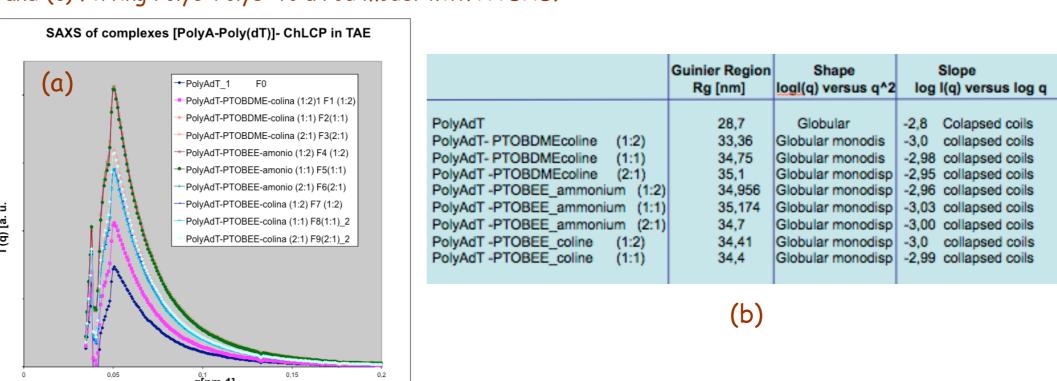


Figure 8. (a) SAXS of Complexes between PolyA-PolyT and cationic ChLCPs; (b) Rg and shape estimations

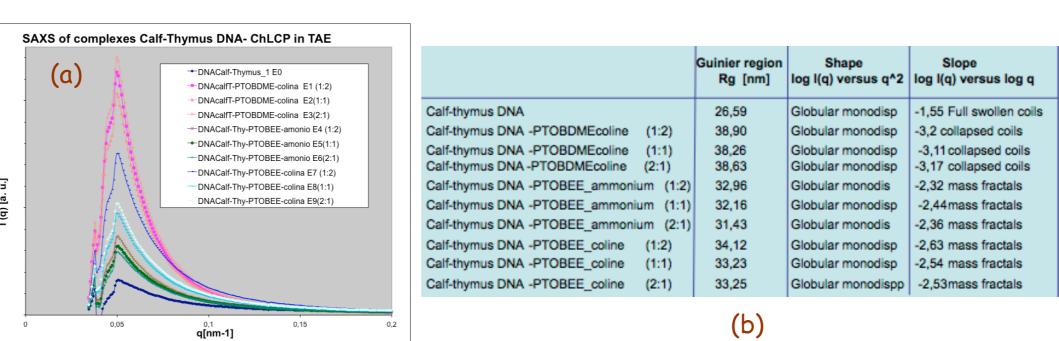


Figure 9. (a) SAXS of Complexes between Calf-Thymus DNA and cationic ChLCPs; (b) Rg and shape.

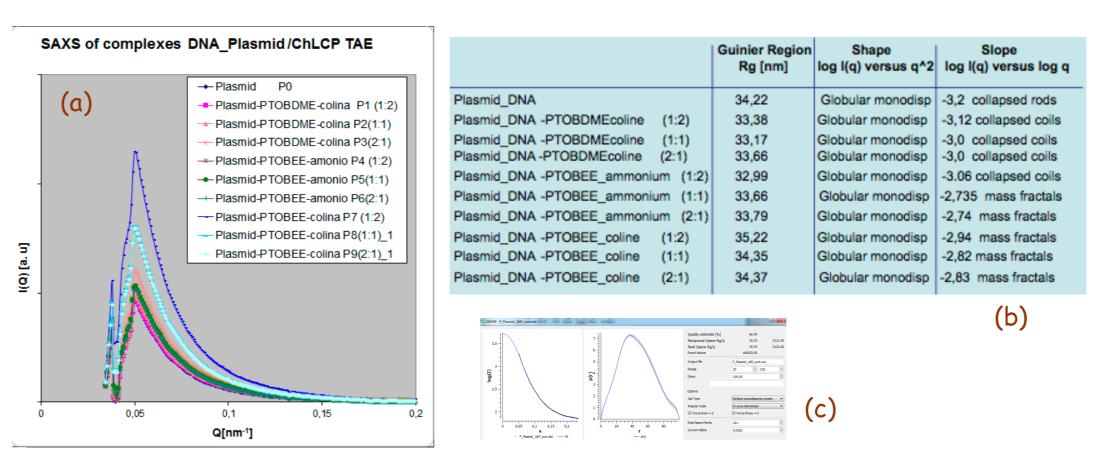


Figure 10. (a) SAXS of Complexes between plasmid DNA and cationic ChLCPs; (b) Rg and shape estimations and (c) Fitting the plasmid to a globular monodispersed system model with GNOM (ATSAS).

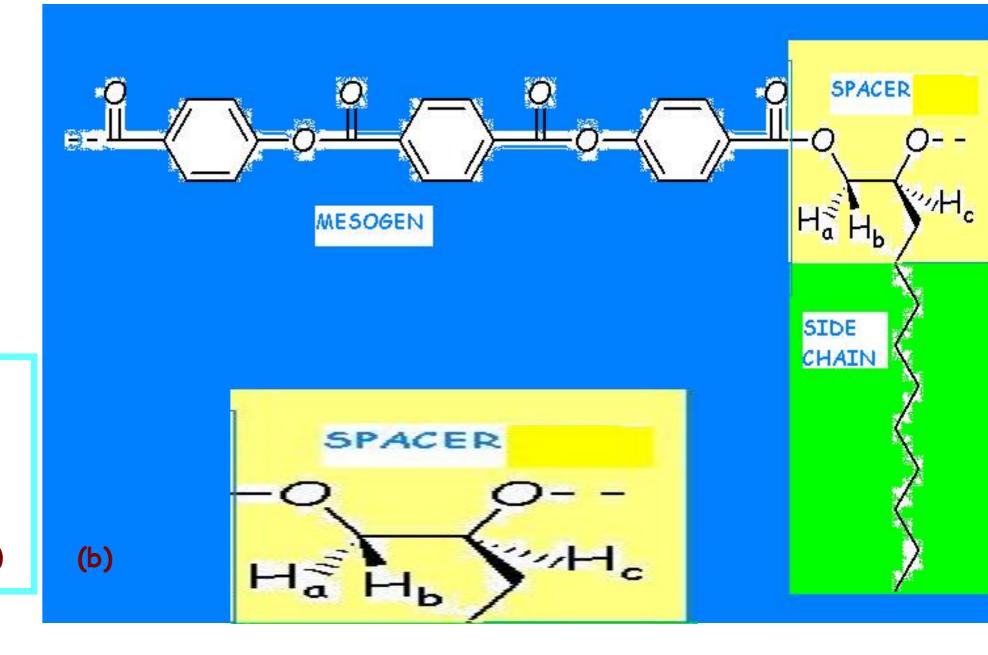
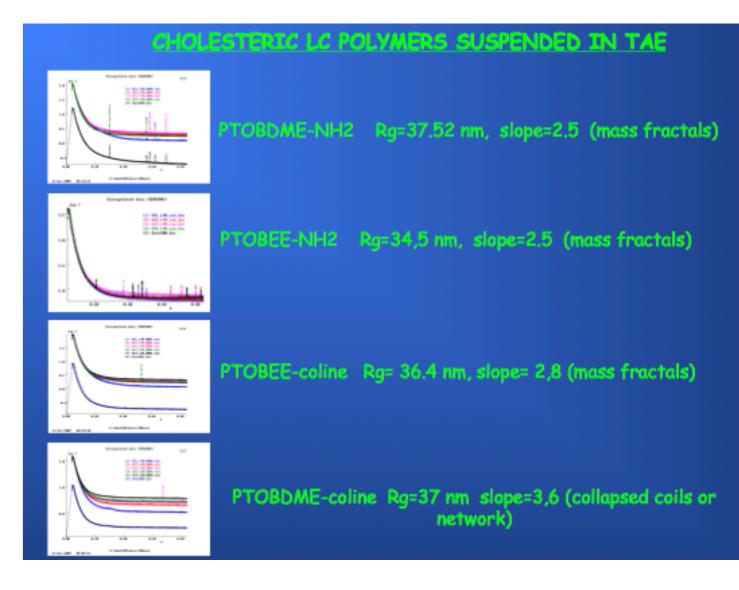


Figure 1. a) Molecular model of helical PTOBDME, b) Schematic formula of PTOBDME with the division in mesogen, spacer and side chain of cholesteric liquid crystal polymers



**Figure 6**. SAXS curves of ChLCP suspended in TAE. Evaluation of  $R_q$  at zero concentration

#### DISCUSION

The radii of gyration  $(R_a)$  have been calculated from the slope of the corresponding Guinier plots - log (I) vs q2 - (slope =  $R_q^2/3$ ). The Guinier region corresponds to the range  $QR_a < \sqrt{3}$ .

This is obtained when the probed range is larger than the particle size [15]. The shapes were adjusted with the help of GNOM in this range.

The Porod plot [Ln (I) vs Ln (q)] for scattering objects with smooth interfaces, yields an exponent from the slope. The Porod slope n is the inverse of the excluded volume paramete v (n = 1/v).

A slope n = 2 is a signature of Gaussian chains in a dilute environment, a slope n = 5/3 is for *fully swollen coils* and a slope n = 3 is for *collapsed polymer* coils. A slope between 2 and 3 is for "mass fractals" such as branched systems (gels) or networks.

# CONCLUSIONS

1. The polynucleotide PolyC-PolyG and Calf-Thymus DNA, dispersed in TAE, show gradients corresponding to fully swollen coils (1,6), while PolyA-PolyT seems to be present as globular monodispersed -mass fractals (2,87). The gradient= -3,2 of the plasmid DNA is interpreted as due to collapsed coils with globular particles shape.

2. The cholesteric liquid crystalline dispersed in TAE exhibit R<sub>a</sub> values higher than those of the nucleic acids studied, and their slopes seems to confirm mass fractals and network systems.

3. The cationic ChLCP directly get complexed with negatively charged DNA and polynucleotides in TAE suspension presenting monodispersed globular particles.

4. The Porod slope n values of the studied complexes seems to correspond to mass fractals and collapsed coils systems, in agreement with previous SANS results [15, 16].

5. Hence the new cationic cholesteric liquid-crystal, would act as carrier for the genetic material, with potencial application in Gene Therapy.

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