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Phage-formed liquid crystal fiber biotemplates for material synthesis

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INTRODUCTION & AIM

Liquid crystals (LCs) is a state of matter that exhibit both liquid and crystal properties at the same time. Molecules (mesogens) in LCs are able to flow, but they are prone to stay in an orienteted position. Mesogens are self-assembled in several orientation in function of the concentration and the temperature producing smetic, nematic and cholesteric phases, mainly. [1]

Biological molecules such as proteins, nucleic acids and virus are able to self-assembly in ordered structures. In particular, family of filamentous phages, such as M13 behave as mesogens organizing it as LCs.

M13 phage consist of a single DNA molecule covered by a coat protein, whose major small helical protein (2700 subunits) called p8 is involved in the structural stability of the phage. [3,4]. Figure 1.

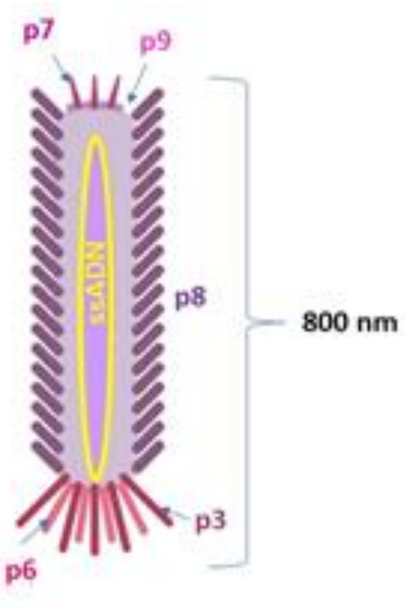

Figure 1. Structure of M13 bacteriophage, showing the positions of all proteins of the capsid. Created in Biorender.com

Recently, it has been observed that changes in the sequence of the helical protein p8 are associated with the formation of different LCs phases. These changes are related mainly with the amino acids from hydrophobic region.

In this work we are interested in change the identity of amino acids of the region hydrophobic by introducing thiol moiety and observe how this mutation affect the formation of LCs phases. In adittion, the introduction of SH groups it will allow to use them as platform for the synthesis nanoparticles in the LCs.

Objetive

- To introduce puntual mutations in the hydrophobic region and the N-teminal end. In particular, mutations in the hydrophobic region it will be to introduce the cysteine amino acid.
- To Prepare LCs.
- To synthesize gold and silver nanoparticles on M13 mutants with the thiol functionality

METHOD

Genetical modification

Puntual mutations were introduced by site directed mutagenesis using the Agilent Stratagene. Primers were designed to change the Val in position 31 by Cys (V31C) and to change Tyr in position 24 by Ala (Y24A). Once the mutants were plated, amplified, purified and DNA sequenced (according to manufacturer´s protocols) we obtained the followings results.

	Mutant	Position
1	V31C	31
2	Y24A	24

Synthesis of liquid crystals

The preparation of phage-based liquid crystals was carried out primarily in capillary tubes by introducing 50 µL of wild-type and mutant phages. The samples were allowed to stand for seven days and incubated at 37 °C. At both the beginning and the end of the incubation period, the samples were examined using polarized optical microscopy (POM) to assess structural changes.

Figure 2. Deposition of phage in capillary tubes and examination under polarized optical microscopy (POM). Created in Biorender.com

Synthesis of gold and silver nanoparticles on the surface of M13 phage.

To 20 µL of phage (wt), we added 100 µL of a solution 5 mM of $HAuCl_4.3H_2O$, and aged for 16 hours at least. After this time 100 µL of a solution 5 mM $NaBH_4$ fresh were added to original mixture. This procedure was repeated with mutants. For silver nanoparticle we used a solution of $AgNO_3$ (5 mM).

RESULTS & DISCUSSION

Mutants were successfully identified through sequencing results. Once the desired mutants had been confirmed, liquid crystals were synthesized in capillaries and analyzed using polarized optical microscopy (POM). Changes in birefringence, attributable to the molecular arrangement in different phases, were observed by adjusting the angle of the polarizers.

Nanoparticle synthesis was successfully achieved using the V31C mutant, as demonstrated by SEM images that revealed their formation and organization within the phage body

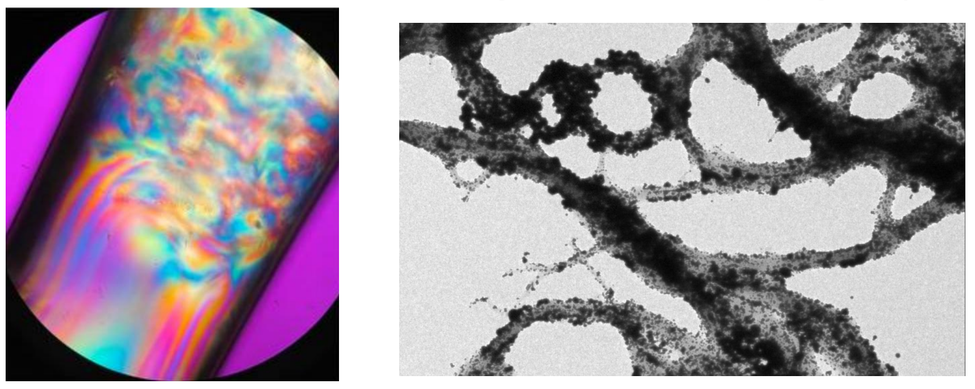

Figure 3. Images taken with polarized light and SEM images nanoparticle synthesis.

CONCLUSION

In conclusion, the mutated phage samples demonstrated satisfactory performance, enabling the synthesis of liquid crystals in which birefringence was attributed to the distinct phases induced by the mutants. As a future direction, phase transitions of these liquid crystals will be investigated under varying temperature conditions to further elucidate their structural and dynamic properties

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[1] D. Andrienko. *J. Mol. Liq.* (2018) 1-22., **[2]** A. Kornberg. DNA Replication. *W.H Freeman and Company. ,*[3] Park, J. P. ACS applied materials & interfaces *6 (21)* (2014). , **[4]** L.I. Vera-Robles. *Adv. Eng. Mat. 15* (2013) 954., **[5]** N. C. K. Yun & A. G Fredickson . *J. Mol. Crys. And Liq. Crys. 24* (1973) 69.