Small-angle X-ray (SAXS) and Raman spectroscopy studies of biot-CMG(2)-DOPE quasicrystalline phases

A M Maslennikov1,2, A V Zalygin1, E V Shtykova4, N V Bovin1, V A Oleinikov1,3,https://orcid.org/0000-0003-4623-4915
1Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences 16/10 Miklukho-Maklaya str. Moscow 117997 Russian Federation.
2D. Mendeleev University of Chemical Technology of Russia 9 Miusskaya square Moscow 125047 Russian Federation.
3National Research Nuclear University MEPhI (Moscow Engineering Physics Institute) Kashirskoe shosse 31 Moscow 115409 Russian Federation.
4Shubnikov Institute of Crystallography of Federal Scientific Research Centre 'Crystallography and Photonics' of Russian Academy of Sciences Moscow 119333 Russian Federation.
E-mail: voleinik@mail.ru

Abstract. Neoglycolipids due to their amphiphilic properties exhibit self-assembly in aqueous phases. In high concentrations the liquid crystalline or gel phases may form. So-called soft-material are a subject of interest of many scientists especially as biosensors and wound healing materials. In this study we examine the structure of a quasicrystalline phase of biot-CMG(2)-DOPE obtained at the concentration of 150 mg/ml (13wt.%) in PBS. The structural data such as interplanar spacing, order parameter and long-range order were obtained by SAXS, while the changes in chemical structure were studied by Raman spectroscopy. It was also in our interest to examine a correlation between the ionic strength and the self-assembly, so we also studied a similar quasicrystalline phase of the same compound but in a buffer containing CaCl2 at the concentration of 4wt.%. According to SAXS data, FSL-biotin construct formed a complex ordered phase consisting of overlapping lattices of different kind. The addition of CaCl2 into PBS resulted in obtaining a more structured system demonstrating cubic-like crystal lattice. Change in peak intensities on Raman spectrums of -C-H- and -C-C- bonds vibrations explained the change in phase properties.

1. Introduction
Soft materials also known as complex or structured fluids are becoming an object of interest for many scientists due to their unique properties, the main property being intermediate flow properties between those of a crystalline solid and a liquid [1]. Usually soft matter is also characterised by self-assembly, shape-memory, luminescence, self-oscillation, etc. Soft functional materials are mainly used in nanotechnology as soft robotics, biosensors [2], bioelectronic devices [3] and in biomedicine as self-healing materials [4], biomedical devices, gellers, encapsulators and substrates for drug delivery [5].

The most studied soft materials are various gels. Gels are semi-solid materials that exhibit liquid-like behaviour when put under external forces. Depending on the filler gels can be classified into hydrogels (water), organogels (organic solvent) and aerogels (air) [6]. Amphiphilic molecules are prone to self-assembly in aqueous phases into various structures, [7, 8] the formation of isotropic (3D structure that consists of entangled fibers) or anisotropic (lamellar 2D structure) gels is both possible, but the latter is observed much less frequently [9]. In the last 20 years the new kind of gels was introduced - molecular
gel obtained by the self-assembly of Low Molecular Weight Gelators (LMWG) into a Self-Assembled Fibrillar Network (SAFiN). The self-assembly of such compounds is reversible due to their stimuliresponsiveness (towards pH [10], temperature [11], ionic strength, light exposure [12], mechanical stimuli [10], etc), so the gelation process can be controlled better than in most polymer-based hydrogels [13].

Glycolipids also exhibit gelation properties. Carbohydrate based gelators have several advantages such as biocompatibility and biodegradability compared to other classes of gelators. The most important quality to carbohydrates is the presence of multiple functional groups that can form hydrogen bonds necessary for forming supramolecular structure [14].

The present work aims to characterize structure of lamellar-gel-like quasicrystalline system of Function-Spacer-Lipid Kode constructs with biotin functional group (FSL-biotin) [15, 16] by an in situ small angle X-ray scattering (SAXS) and Raman spectroscopy. It was also in our interest to investigate the effect of ionic strength variations by adding low molecular weight electrolyte, therefore exploring the micellar-to-lamellar phase transition and how the change in the supramolecular structure can be detected using spectral methods. These data can provide not only general information on hydrogel’s structure, but also on the possibility of altering the quasicrystalline structure by changing the ionic strength of a filler.

2. Materials and methods

2.1. Chemicals
Function-Spacer-Lipid Kode constructs with biotin functional group (biotin-CMG(2)-DOPE) consists of biotin as a functional group (“head”) N-(carboxymethyl)-N-dimethyl-glycine (CMG(2)) as a spacer and DOPE as lipid “tail” [16, 17].

The first quasicrystalline system was obtained by concentrating a solution of FSL-biotin in phosphate-buffered saline (PBS). The concentration of the construct in the resulting gel-like system was 150 mg/ml (13wt.%). Mixing was achieved by vortexing for approximately 30-40 seconds and the sample was allowed to stand still with gelation taking place at room temperature.

The second quasicrystalline system was obtained by concentration in phosphate-buffered saline where calcium chloride (CaCl₂) was dissolved at a concentration of 4 wt.% in advance. The mixing was also done by vortexing for approximately 30-40 seconds and the sample was left to stand still at room temperature.

2.2. Small angle X-ray scattering (SAXS)
Synchrotron SAXS measurements were performed at the European Molecular Biology Laboratory (EMBL) on the EMBL-P12 BioSAXS beam line at the PETRAIII storage ring (DESY, Hamburg) equipped with a robotic sample changer and a 2Dphoton counting pixel X-ray detector Pilatus 2M (DECTRIS, Switzerland). The scattering intensity, I(s), was recorded in the range of the momentum transfer 0.027<s<3.0 nm⁻¹, where s = (4πsinθ)/λ, 2θ is the scattering angle, and λ = 0.124 nm, the X-ray wavelength. The measurements were carried out at 20°C using continuous sample flow operation over a total exposure time of 1 second, collected as 20 x 50 microsecond individual frames. The data were corrected for the solvent scattering and processed using standard procedures and a program suit ATSAS.

2.3. Raman spectroscopy
Raman spectroscopy measurements were performed at Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (IBCh RAS) on the Renishaw InVia confocal Raman microscope equipped with Leica N Plan 50x/0.75na Objective at an excitation wavelength of 633 nm and a sample power of 4.78 mV with the accumulation time of 30 s. 20 spectra with the same parameters were averaged.

The data were corrected for the saline spectrum and processed using standard procedures and programs GRAMS Suite and WiRE.

3. Results and discussions
3.1. Structure of the FSL-biotin construct probed by SAXS

The position of the minimum on the scattering curve (see Figure 1) of the first quasicrystalline system matches with the position of the minimum on the scattering curve of the solution of the FSL-biotin construct in phosphate-buffered saline with a construct content of 4.5 mg/ml. The peaks of the quasicrystalline sample themselves are located at the maximum of the scattering profile of the original substance, i.e., the scattering by the ordered quasicrystalline structure contains the scattering from the form factor of the FSL-biotin construct. The degree of disorder (Δd), quasicrystalline structure size (L), interplanar spacing (d₁), and Bragg spacing (s₀) were obtained using the PEAK program.

The main interplanar distance of 11.4 nm, corresponding to the size of the micelles, indicates that the micelles in this system are organized into ordered structures. The size of quasicrystalline structures is average and does not carry any distinguishable features of this system.

![Graph](image)

**Figure 1.** SAXS profile of original quasicrystalline system of FSL-biotin construct in concentration 150 mg/ml (13 wt.%) (blue) and micellar solution of FSL-biotin in concentration 4.5 mg/ml (0.5 wt.%) (red)

Although the distances between the Bragg peaks are not in a ratio that allows to certainly determine the crystal lattice, but it is highly possible that the structure is formed by an overlay of several types of crystal lattice: lamellar and cubic. Also, the relatively low degree of disorder suggests the formation of an ordered, quasicrystalline structure.

The SAXS data from the second quasicrystalline system (see Figure 2) indicate that the resulting structure shows signs of greater structuring compared to the initial quasicrystalline system. The shift of the minimum to the small angle region corresponds to the formation of a more compact structure.

This is also confirmed by the interplanar distance data. The new interplanar distance of 9.4 nm corresponds to the length of the FSL-biotin construct molecule. In addition to the decrease in the interplanar distance, the compactization and structuring of the system is indicated by the decrease in the degree of disordering. However, the size of quasicrystalline sites remains quite large.

From the ratio of the Bragg peaks distances and the period of ordered motifs the formation of a cubic quasicrystal structure can be assumed.
Figure 2. SAXS profile of quasicrystalline system of FSL-biotin construct (13wt.%) with additional CaCl₂ in the saline (green) and original quasicrystalline system of FSL-biotin construct (13wt.%) (blue)

It is worth noting that to find the interplanar distance and degree of disorder, the Atsas Data Plotting program was also used, the data of which confirmed the PEAK program data.

3.2. Raman spectroscopy of FSL-biotin construct
FSL-biotin construct consists of biotin, which is a water-soluble vitamin containing 2-oxohexahydroimidazole ring and tetrahydrothiophene ring, CMG(2) spacer that is a polypeptide chain and DOPE aminophospholipid.

Figure 3. Chemical structure of FSL-biotin construct

The bands attributed to imidazole ring of the biotin are observed in the regions of 840 cm⁻¹ (C4-H and C5-H bonds), 1180 cm⁻¹ (N1-C2 and N3-C4 bonds), 1230 cm⁻¹ (C4-N3 and C5-N bonds), 1490 cm⁻¹ (C-N bonds vibration lines around the N3 atom) and 1580 cm⁻¹ (C-N bonds vibration lines around the N1 atom) [18, 19]. The band attributed to the bond vibrational lines -CH2-S-CH2- in the tetrahydrothiophene ring of biotin is present at 683 cm⁻¹ [20].
In the vibrational spectra of polypeptides one can distinguish vibrations of the amide group of polypeptides, whose characteristic bands lie in the spectral regions near 1650, 1540, and 1240 cm\(^{-1}\), and the forms of vibrations are determined by changes in the C=O peptide bond length, changes in the CH angle and the CN bond length. These vibrations are commonly referred to as Amide I, Amide II, and Amide III vibrations, respectively [21]. The positions of Amide I and Amide III peaks in the Raman spectra can be used for determining the secondary structure of the polypeptide chain. For the alpha helix structure, Amide I and Amide III are located between 1645-1655 cm\(^{-1}\) and 1260-1310 cm\(^{-1}\), respectively; for the beta-fold structure, between 1665-1680 cm\(^{-1}\) and 1230-1245 cm\(^{-1}\); for irregular or disordered structures, between 1655-1665 cm\(^{-1}\) and 1245-1270 cm\(^{-1}\) [22]. The bands in the region of 1230-1280 cm\(^{-1}\) correspond to the imide groups of type Amide III, vibrations of which are mainly contributed to by the vibrations of N-H bonds [21]. These groups are part of the CMG spacer. This arrangement of the peaks indicates that the spacer is in a conformation similar to the alpha helix. The peak at 1652 cm\(^{-1}\) corresponds to imide group O=C-NH- type Amide I and, therefore, to the polypeptide chain in the CMG molecule. The peak near 1425 cm\(^{-1}\) corresponds to the vibrations of the side carboxyl groups –COO, that are part of the CMG. The carboxyl group is in a dissociated state because there is no peak near 1700-1750 cm\(^{-1}\) corresponding to the undissociated carboxyl group –COOH [23].

Bands attributed to amino group of DOPE are hard to distinguish from the band of imidazole ring, and the band of phosphate group is located in the region near 1084 cm\(^{-1}\), but due to low intensities of picks in that area are hardly detectable. [24] The most important are bands attributed to alkyl chains, because the phase transitions can be monitored by analyzing them.

In the region of 1000-1150 cm\(^{-1}\) the lines of oscillations at which alternating carbon atoms move along the chain in opposite directions lie. The line at 1085 cm\(^{-1}\) correlates to vibrations of the C-C bonds of the carbon skeleton in lipids in disordered conformation, observed in liquid and liquid crystalline phases [25]. Based on the low intensity of this peak, we can talk about the formation of the gel phase. The line at 1439 cm\(^{-1}\) corresponds to vibrations of CH\(_2\) methylene groups (scissoring mode). Their intensity along with the 2800-2900 cm\(^{-1}\) lines (stretching Mode) depends on the packaging of the DOPE.
hydrocarbon chain. The intensity of these peaks correlates with the strength of lateral interactions between lipid tails depending on their packing [24]. The line near 1655 cm\(^{-1}\) (1652 cm\(^{-1}\)) corresponds to vibrations of the C=\(\text{C}\) double bond, specifically for the cis-isomer [25]. This peak is also superimposed on the Amide I type oscillations.

In the region of 2800-3000 cm\(^{-1}\) (see Figure 5.) lie the bands attributed to vibrations of C-H bonds of methylene and methyl groups of the lipid fragment. The 2851 cm\(^{-1}\) - methylene CH\(_2\) groups, 2851 cm\(^{-1}\) - symmetric and shoulder 2880 cm\(^{-1}\) - asymmetric vibrations of methylene groups; 2938 cm\(^{-1}\) - methyl CH\(_3\) groups 2938 cm\(^{-1}\) symmetric vibrations and peak in the shoulder near 3000 cm\(^{-1}\) - asymmetric vibrations of the methyl group [26].

![Raman spectra](image.png)

**Figure 5.** Raman spectra of quasicrystalline system of FSL-biotin construct (13wt.\%) with additional CaCl\(_2\) in the saline (red); original quasicrystalline system of FSL-biotin construct (13wt.\%) (blue) and their difference spectrum (green) in the region of 2000-3000 cm\(^{-1}\).

Comparative analysis was focused on the bands corresponding to vibrations in the methylene group at 1439 cm\(^{-1}\) and 2851 cm\(^{-1}\). A slight decrease in peak intensity at 1439 cm\(^{-1}\) is observed in molecular phospholipids upon cooling, but the specific reason for the decrease in intensity remains unexplored. At the same time an increase in peak intensity at 2851 cm\(^{-1}\) was observed, a similar phenomenon was observed in phospholipid bilayers during the so-called triclinic splitting of the hexagonal lattice, which consisted in that the triclinically packed phospholipid domains within the hexagonal lattice increased in size or the free dispersed phospholipid molecules began to orient themselves into the triclinic lattice with decreasing temperature [26].

4. Conclusions
This work shows that in the Function-Spacer-Lipid Kode constructs with biotin functional group (FSL-biotin), an analogue of neoglycolipids, micelles are formed and packed into a quasicrystalline structure. This system has a complex structure and represents an overlay of several types of crystal lattices. A system from the same construct but formed in a buffer where a low molecular weight electrolyte CaCl\(_2\) was added exhibited a form factor of molecules and a more structured, most likely cubic packing.

At high concentration, the Raman spectrum of FSL-biotin turned out to be well defined, with a large number of characteristic peaks. Even the normally weak biotin spectrum was seen on the spectrum. It
was necessary to figure out which peaks correlate directly with the phase changes in quasicrystalline system. From the comparative analysis, it was concluded that the bands at 1439 cm\(^{-1}\) and 2851 cm\(^{-1}\) corresponding to CH\(_2\) groups' vibrations in scissoring mode and valent vibrations accordingly are the most characteristic of the phase structure defining, as they directly correlate to the amount of lateral interactions between the carbohydrate chains of DOPE lipid tail.

5. Acknowledgments
The study was supported by the grant of the Ministry of Science and Higher Education of the Russian Federation (agreement No. 075-15-2020-773).

6. References
[1] Hamley IW and Castellotto V 2007 Biological soft materials Angew Chem Int Ed Engl. 46(24) 4442-55
[2] Lim HR, Kim HS, Qazi R, Kwon YT, Jeong JW and Yeo WH 2020 Advanced Soft Materials, Sensor Integrations, and Applications of Wearable Flexible Hybrid Electronics in Healthcare, Energy, and Environment Adv Mater. 32(15) e1901924.
[3] Jia M and Rolandi M 2020 Soft and Ion-Conducting Materials in Bioelectronics: From Conducting Polymers to Hydrogels Adv Healthc Mater. 9(5) e1901372.
[4] Thangavel G, Tan MWM and Lee PS. 2019 Advances in self-healing supramolecular soft materials and nanocomposites Nano Converg. 6(1) 29.
[5] Li J, Wong WY and Tao XM 2020 Recent advances in soft functional materials: preparation, functions and applications Nanoscale. 12(3) 1281-1306.
[6] Jones CD and Steed JW 2016 Gels with sense: supramolecular materials that respond to heat, light and sound Chem Soc Rev. 45(23) 6546-6596.
[7] Wang G, Yang H, Cheuk S and Coleman S 2011 Synthesis and self-assembly of 1-deoxyglucose derivatives as low molecular weight organogelators Beilstein J Org Chem. 7 234-42.
[8] Tuzikov AB, Chinarev AA, Gambaryan AS, Oleinikov VA, Klinov DV, Matsuo NB, Kadykov VA, Ermishov MA, Demin IV, Demin VV, Rye PD, Bovin NV 2003 Polyglycine II Nanosheets: Supramolecular Antivirals? - ChemBioChem. 4 147-154
[9] Warriner HE, Idziak SH, Slack NL, Davidson P and Safinya CR. 1996 Lamellar biogels: fluid-membrane-based hydrogels containing polymer lipids. Science. 271(5251) 969-73.
[10] Ben Messaoud G, Le Griel P, Hermida-Merino D, Baccile N 2020 Effects of pH, temperature and shear on the structure-property relationship of lamellar hydrogels from microbial glucolipids probed by in situ rho-SAXS. Soft Matter. 16(10) 2540-2551.
[11] Kameta N, Matsuzawa T, Yaoi K, Fukuda J and Masuda M 2017 Glycolipid-based nanostructures with thermal-phase transition behavior functioning as solubilizers and refolding accelerators for protein aggregates Soft Matter. 13(17) 3084-3090.
[12] Matsumoto S, Yamaguchi S, Ueno S, Komatsu H, Ikeda M, Ishizuka K, Iko Y, Tabata KV, Aoki H, Ito S, Noji H and Hamachi I. 2008 Photo gel-sol/sol-gel transition and its patterning of a supramolecular hydrogel as stimuli-responsive biomaterials Chemistry 14(13) 3977-86.
[13] Baccile N, Van Renterghem L, Le Griel P, Ducouret G, Brennich M, Cristiglio V, Roelants SLKW, Soetaert W 2018 Bio-based glyco-bolaamphiphile forms a temperature-responsive hydrogel with tunable elastic properties Soft Matter. 14(38) 7859-7872.
[14] Morris J, Kozlowski P and Wang G 2019 Synthesis and Characterization of Hybrid Glycolipids as Functional Organogelators and Hydrogelators Langmuir. 35(45) 14639-14650.
[15] Williams E, Barr K, Korchagina E, Tuzikov A, Henry S, Bovin N 2016 Ultra-Fast Glyco-Coating of Non-Biological Surfaces Int J Mol Sci. 17 118.
[16] Zalygin A, Solovyeva D, Vaskan I, Henry S, Schaefer M, Volynsky P, Tuzikov A, Korchagina E, Ryzhov I, Nizovtsev A, Mochalov K, Efremov R, Shytkova E, Oleinikov V, Bovin N 2020 Structure of Supramers Formed by the Amphiphile Biotin-CMG-DOPE ChemistryOpen. 9(6) 641–648
[17] Vaskan IS, Solovyeva DO, Chistyakov AA, Efremov RG, Volynsky PE, Shtykova EV, Korchagina EYu, Mochalov KE, Bovin NV, Oleinikov VA 2018 Neoglycolipids Micelle-like Structures as a Basis for Drug Delivery Systems KnE Energy & Physics 3(2) 519-527
[18] Markham LM., Mayne LC, Hudson BS, and Zgierski MZ 1993 Resonance Raman studies of imidazole, imidazolium, and their derivatives: the effect of deuterium substitution The Journal of Physical Chemistry 97 (40) 10319-10325
[19] Cuicui Fu, Chengxu Hu, Yu Liu, Shuping Xua and Weiqing Xu 2012 Bioidentification of biotin/avidin using surface plasmon resonance and surface-enhanced Raman scattering (SPR-SERS) spectroscopy Anal. Methods, 4 3107-3110
[20] Daimay Lin-Vien, Colthup NB, Fateley WG and Jeanette G. Grasselli 1991 The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules
[21] Chen C, Liu W, Tian S and Hong T 2019 Novel Surface-Enhanced Raman Spectroscopy Techniques for DNA, Protein and Drug Detection. Sensors (Basel). 19(7) 1712.
[22] Miura T and Thomas GJ Jr. 1995 Raman spectroscopy of proteins and their assemblies. Subcell Biochem. 24 55-99
[23] Mochalov KE, Ustinova OA, Strel'tsov SA, Grokhovskii SL, Zhuze AL, Nabiev IR, Sukhanova AV, Oleinikov VA 2002 Raman Spectroscopy of Topotecan, Inhibitor of DNA Topoisomerase I Optics and Spectroscopy 93(4) 493-500.
[24] Gaber BP, Yager P and Peticolas WL 1978 Interpretation of biomembrane structure by Raman difference spectroscopy. Nature of the endothermic transitions in phosphatidylcholines. Biophys J, 21(2) 161-76
[25] Palla-Papavlu A, Paraico I and Shaw-Stewart J 2011 Liposome micropatterning based on laser-induced forward transfer. Appl. Phys. A 102 651–659
[26] Oleinikov VA, Fleury F, Ianoul A, Zaitsev S, Nabiev I 2006 P-glycoprotein effect on the properties of its natural lipid environment probed by Raman spectroscopy and Langmuir-Blodgett technique FEBS Letters 580 4953-4958.