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Laser-Assisted Top-Down Analysis of Biogenic and Biosynthetic Nanoparticles or Supramolecular Assemblies for Chemical Evolution Analysis: From Viral Particles to Self-Assembly of Biomolecular and Prebiotic Nanostructures

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Abstract

Conventional flow virometry methods are limited by their reliance on fluorescent labeling and molecular-biological specificity, making them unsuitable for universal detection of morphologically diverse virus particles across their entire size range (20–300 nm with some representatives reaching 1.4 μm). We propose an alternative approach based on laser aerosol spectrometry and optoelectronic aerosol counting, which enables size-based discrimination and morphological characterization of viral particles without requiring genetic modification or staining. This method exploits the Stokes radius as a fundamental descriptor to differentiate viruses by their hydrodynamic properties and geometric parameters. Using aerosol spectrometers, we demonstrate that viral particles and their aggregates can be detected and classified through multimodal size distribution analysis. We discuss the theoretical foundations for extending this methodology to macromolecular levels, connecting viral particle detection to fundamental concepts in molecular evolution, including the divergent evolution of semantides and episemantides. Based on the contemporary molecular genetics concepts, which propose a divergent origin of enzymatic function evolution, typically coupled with genome expression (beginning with proto-ribozymes combining RNA and enzyme functions), it is appropriate to pose the question of whether viral functions and the corresponding biochemical agent forms can be separated using laser-assisted analysis of the particle size/dispersive parameters, which provided transcription and translation processes. In this connection, fundamental significance is possessed by the separation of viruses and native genetic code carriers as semantides, on the one hand, and episemantides as small molecules and individual enzymes and allosteric regulators, on the other hand, in accordance with their dimensions. A minimal cell size (which can be measured by different laser methods and belongs to the submicron range) is determined by the compact localization of the cell components providing genome expression functions. It is impossible to further reduce it, except for the possibility of the function combination in a single structure. Thus, laser-assisted measurement techniques can be used for solving the fundamental problems of protobiology and synthetic biology, in particular, for the sufficiency validation of various protocells and artificial cells. The existence of a minimum size of a functional cellular structure with expressible genetic material can be interpreted only from the standpoint of evolutionary cytometry. The problem of detection of self-assembly processes in model prebiological environment / prebiotic media also can be addressed by laser optical biophysical techniques within the framework of coevolution of semantides, asemanties, and episemantides from nanosized to microsized assemblies and condensates due to self-assembly processes and non-covalent interactions.

Keywords: Aerosol spectrometry; Flow virometry; Viral detection; Size distribution; Stokes radius; Optoelectronic counters

Optical Virometry Techniques

In recent years, alongside optical cytometry based on the analysis of fluorescent (or cell-scattered [1,2]) light signals, a complex of virometric methods has gained considerable efficacy, notably flow virometry [3,4], analogous to flow cytometry (FACS, FCM). This suite of virometric technologies is currently positioned as a unique means of analyzing infectious profiles [5], antigen spectra [6], and kinetic analysis of viral infection, as well as conformational kinetics and comparative analysis of

response chemistry relative to normal reaction parameters [7]. Consequently, emphasis has been placed predominantly on the molecular-biological aspect rather than on ultra morphological and dimensional (dispersive) distinctions among viruses.

Furthermore, in accordance with current policy at the United States National Institutes of Health, emphasis has been placed upon viral particle enumeration, particularly concerning HIV-1, the retrovirus responsible for the AIDS pandemic and belonging to the subfamily Lentiviridae [8]. This represents an extremely taxonomically restricted subset of relevant analytes and samples, with correspondingly limited nomenclature of viral variations [9-11]. Consequently, one cannot reasonably speak of universality of this method in diagnostic virology. A universal method cannot be founded upon selective, molecular-biologically specific carriers, particularly for supramolecular systems exhibiting high specificity of complementary binding, such as viral genetic mechanisms. It is in principle impossible to create a system equivalently recognizing all viral types—whether DNA or RNA viruses; possessing single-stranded or double-stranded DNA genomes, or single-stranded or double-stranded RNA genomes; accounting for all capsomer morphologies (protein subunits/protomers of viral capsids); considering the probability of detecting both unencapsulated viruses and those bearing xenogenic lipid supercapsids in samples—without contradicting the phyletic diversity of viral molecular taxonomy.

It should be noted that selective fluorescent methods of flow virometry likewise cannot serve as a universal means of detecting geometrically distinct viruses, owing to the indistinguishability of their signals at sub-wavelength viral dimensions, excepting PALM/STORM-type microfluorimetric methods. Viruses with non-identical scattering diagrams according to geometric criteria—that is, possessing different morphological, or more precisely, geometric capsid types (helical, icosahedral, elongated, and complex)—can be distinguished only within the framework of diffraction fingerprinting approximation, concordant with the corresponding viral geometry, calibrated using test particles adequate in both geometry and metrology (dimensions) to the virus studied. Ideally, such particles should possess automorphism to the virus (for instance, a virus of identical morphology but inactivated or non-pathogenic), or be homeomorphic to it. A morphism failing to establish bijective correspondence between the structure of the investigated and calibration virus/particle, for "morpho-physical" reasons, is insufficiently adequate for identification. Yet even within the framework of category morphism correspondence, whilst maintaining identical symmetry groups in calibration and flow virometry-measured particles, the possibility of dual approximation of individual structures may, in principle, constitute a source of artefacts. A classical example: rotavirus, possessing icosahedral symmetry yet effectively approximated by a spherical pattern, owing to its large number of capsomers (pentons and hexons) tiling the surface (pertailing [12]). The "transition of quantity into quality" upon introducing additional capsomers into the system typically affects the diffraction pattern also because of the objective increase in particle dimensions relative to the wavelength employed, since, with invariant capsomer dimensions determined by protein particle size, increasing the number of capsomers on the surface necessarily entails approximation of form towards greater smoothness (analogous to approximations employed in mesh methods in scientific visualization and three-dimensional graphics).

It is well established that viral dimensions vary by orders of magnitude across different taxonomic groups: from 20 to 300 nm (although certain representatives of the family Filoviridae with negative-sense single-stranded RNA reach lengths of 1.4 μm with diameters of 0.08 μm, whilst *Pandoravirus sp.* and *Pithovirus sp.* exhibit genome compaction levels that physically permit extension far beyond micron boundaries—1.0–0.5 μm and 1.5–0.5 μm, respectively). This necessitates qualitatively distinct metrology and analytical principles for these qualitatively different groups. Notwithstanding the widespread simplification involving extrapolation of Mie light-scattering theory interpretations across virtually the entire particle size range, including Fraunhofer diffraction as a special case, it is well established that Mie theory is inapplicable at small diffraction parameters. Computational capabilities afforded by Mie theory permit application of a single (universally interpretable, within the aforementioned extrapolating misconception) method/calculation algorithm for additive dispersity spectra—that is, particle sizes. In fact, however, this means that a portion of the sample will be miscalculated, since it is scarcely possible (unless dealing with monodisperse calibration media—for instance, specialized latexes lacking heteroscedastic distribution)—to anticipate in advance the concentrations of individual particles and the contribution of dispersive fractions (or sizes of ultrastructural and microstructural biomaterial units, differing both in static geometry and in dynamic morphometric parameters, which under normal reaction conditions represent non-stationary correlates of general protoplasmic reactivity and excitation propagation therein [13,14]). The qualimetric weighting criterion in such problems is difficult to compute, owing to differences in particle geometries ("form factors"). One might assert that "if all particles in a sample exceed the light wavelength, the Fraunhofer portion of Mie theory dominates in Mie theory calculations of particle sizes"; yet, considering anisometric particles (including viruses—such as Filoviridae with extraordinary elongation coefficients: up to 1.4 to 0.08), one cannot objectively express the numerical magnitude of the above dominance (particularly without accounting for orientation) in most cases. Evidently, since machine-solvable abstract models applicable to distribution calculations are developed upon the assumption of spherical, that is, simplest, particle form, the particle size distribution obtained from analysis actually represents a distribution of "equivalent

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spherical particles" rather than actual analyte particles (a classical electrodynamics problem solved in 1908 by Gustav Mie through expansion of the electromagnetic field in spherical harmonics).

For viruses differing in morphology and genetic or evolutionary position, geometric deviations from sphericity will be distinct; consequently, equivalent measurements will prove differentially effective for systematically different viruses. One cannot therefore speak about a universal "functional virometry" technology, since the dimensions of even the most prevalent or clinically relevant viral nomenclature (and thus diffraction limits and applicability boundaries of various mathematical approaches, including those differing from Mie theory) vary extremely, whilst functional activity of virulently distinct forms, as a rule, correlates comparatively weakly with the averaged morphology computed during data processing from the flow detector (even accepting that viral measurements are conducted not on dispersive substrates but in an abstract empty medium devoid of adsorbing agents [15]), and cannot be revealed in this manner. Regarding insufficient information for functional identification, one should also note that Mie theory requires knowledge of the sample's refractive and absorption coefficients (obviously non-identical for different potential viruses) and of the "dispersive medium," which in the native case likewise exhibits certain uncertainty, since samples must be analyzed from various natural environments.

Evidently, in the active medium analysis, the possibilities for staining viruses—particularly inactivated ones (for instance, by preliminary heating to 80–90 °C for SyBR green-I staining [16,17]), not fixed upon dispersive particles (nanogold, etc.) or in agaroze immobilizing beads [18], not genetically modified for fluorescent protein expression following cellular penetration [19,20], and so forth—are typically substantially limited. Particularly challenging is identifying, analyzing, and measuring in the natural infection transmission environment infectious viral agents transmitted via aerosol/airborne-droplet routes. Among the most well-known, widespread, and readily differentiable diseases (easily attributable to specific viruses) with pronounced etiology, this encompasses an extremely significant stratum of infections, beginning with upper respiratory tract infections (URTI), encompassing respiratory syncytial viral infection, rhinoviral and adenoviral infections requiring pathognomonic identification via virometry, and extending to influenza/parainfluenza, measles, mumps, adenoviral infection, and so forth. Viral determination without cultivation can be realized only in the abiogenic transmission environment. Therefore, for infections transmitted via airborne-droplet (aerosol) routes, implementation of a method enabling analysis with particle capture in the natural atmosphere is necessary. Simultaneously, in physiological aerosols, droplet size may substantially exceed viral dimensions themselves; however, standard virometry recognizes viral aggregation effects, through which "viral aggregometry" is realized on standard flow cytometers (FACS), enabling detection of HSV-1 and certain other specifically or "physiochemically" aggregating viral particles with aggregate sizes exceeding the 300–500 nm threshold applicable to most cytometers with non-super-resolution optics and factory software settings. Consequently, were there a sufficiently broad-range measurement method with calibration to specific geometric prototypes, providing size determination discreteness sufficient for determining viral particle types, aggregation, and other particles within statistical deviation limits attributable to anisotropic particle orientation variation during "gasometry" atmosphere passage through the optical flow volume, followed by partial viral deposition upon selective "sieves" for subsequent size determination validation, then the technique enabling such aerosol viral dispersive-morphological analysis would, in principle, address the majority of typical infectious epidemic/pandemic disease cases transmitted via airborne-droplet routes.

Diffusion Aerosol Spectrometers and Their Applications Proximal to Virometry

Industrially manufactured virometry systems of this designation do not exist. Owing to the sub-wavelength dimensions of most viruses, biomedical engineers typically do not emphasize such problems, regarding them as inherently intractable or prohibitively expensive, though this does not necessarily reflect the reality of functional devices. (For instance, the so-called "super-resolution microscope" of Skrynnik, positioned in 2015 as a means of achieving such visualization of sub-wavelength bio objects without dyes [21], despite extreme financing by Russian standards at the level of Russian Science Foundation and "Skolkovo" grants amounting to approximately 3 million rubles for optical installation components alone, has to date failed to deliver ultrastructural or cytometric super-resolution.) However, this does not imply that the very ideology of such measurements lacks legitimacy. Already in the 1970s, the USSR developed aerosol spectrometry systems for laser-resonator particle measurements [22], including the capability to calculate their hydrodynamic radius [23] (Stokes-Einstein radius; in spherical approximation; though similar calculations are possible for non-spherical particles according to Kunkel [24]), multiplexed and probabilistic-statistical analytical studies [25,26]. Laser aerosol and hydrosol counters were applied to analyze condensation nucleus formation, comparable in size [27] to the aforementioned viral aggregates, and formation of submicron aerosols [28,29] in natural environments, likewise comparable in dispersity to large viruses and viral aggregates. The simplicity of calibrating photoelectric, essentially photoelectronic counters (regulated by photomultiplier power supply) [30] and the possibility of investigating typical hygienic problems (for instance, measuring filter bypass coefficients—the ratio of air contamination at filter outlet to inlet contamination; or analogous filter coefficients determining the ratio of air dustiness at outlet to inlet [31]), for physical and ergonomic reasons, render rational the use of such systems as prototypes for a distinct class of virometers.

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The Diffusion Aerosol Spectrometer (DAS Model 2702) can operate in monitoring mode, covering the size range from 3 to 200 nm, implementing not only measurement of total nanoparticle concentration and size distribution or "ultrafine particles" in this range, but also environmental parameters (air or carrier gas temperature, pressure, and humidity). Consequently, given that most viral sizes range from 20 nm to 300 nm, no fundamental restrictions exist for measurement across a broad range of most prevalent viruses. Regarding residuals beyond the 200 nm boundary, as well as extreme cases of micron aggregates (Pandoravirus sp., Pithovirus sp., Filoviridae, etc.), the model 2702-M includes the capability to be equipped with a submicron particle measurement module covering the range from 0.2 to 10 μm. Moreover, for any physically permissible particles, particle enlargement is realizable (via an enlargement module to optically active size). The applicability potential of aerosol and hydrosol optical counting systems, which has generated substantial resonance since the 1970s [32], can be implemented in submicron-range virometry under any physically descriptive environmental conditions favoring or inhibiting viral material proliferation: submicron particles from arid zones and aerosol parameter variations in high-altitude conditions (with corresponding atmospheric conditions, including distinct pressure and insolation—that is, natural source energy irradiance)—were measured on such equipment in the 1980s [33,34].

Moreover, laser aerosol spectrometry is likewise applicable to the problem of viral genetic material survival in cosmic conditions, as well as to the related problems of space biology/astrobiology and so-called "cosmic abiogenesis" based on particles [35,36], or virus-mediated panspermia (or astrosol transport [37,38]) and other problems of gas-phase exobiology [39,40] (typically based on gas-phase and aerosol organic chemistry reaction principles [41,42]). Thus, in the 1980s, Zhulanov aerosol counters operated on Venus aboard the Soviet spacecraft VEGA-1 and VEGA-2 [43], through which the cloud layer structure and size distribution of Venusian atmospheric aerosols [44,45] therein were studied, and the mechanism of cloud layer formation [46] was investigated. From an engineering perspective, this task encompassed creation of a specialized photoelectric converter and signal transmitter [47]. Therefore, analysis of dispersity and size spectra of organic aerosols, to which, by additive chemistry (consisting predominantly of organogenic elements), bioaerosols [48,49] belong (including those containing nucleic acids—as do viruses [50]), using laser aerosol spectrometers is self-evident both in native (terrestrial) and in xenobiotic/exobiological monitoring conditions. At present, it is evident that laser radiation utilization may prove useful in this method not solely as a source of metrological information regarding particle/viral Stokes radius, but also as a source of desorption-ionization for laser-mediated (particularly LAMMA-like, MALDI/ELDI-like, etc.) mass spectrometry of such particles, effectively replacing pyrolytic viral infection measurement methods previously applied at larger sample scales [51]. Thus, from the perspective of verifying viral concepts of abiogenesis and evolution (corresponding member of the Russian Academy of Sciences V.I. Agol [52] and others), laser aerosol spectrometry likewise constitutes an excellent instrumental tool.

Expanded Possibilities of Optical Laser Biochemical / Biophysical Techniques Based upon Evolutionarily-Divergent Analysis of Semantides and Episemantides

Based on the contemporary molecular genetics concepts, which propose a divergent origin of enzymatic function evolution, typically coupled with genome expression (beginning with proto-ribozymes combining RNA and enzyme functions), it is appropriate to pose the question of separating the functions and the corresponding biochemical agents using laser-assisted analysis of the particle size/dispersive parameters, which provided transcription and translation processes [53,54]. In this connection, fundamental significance is possessed by the separation of viruses (incapable of autonomous replication, yet not constituting "cellular machinery" / episemantides for servicing replication of other elements – the so-called primary and secondary "semantides" [55]; because "Episemantides are molecules, precursors of which often are taken up from the environment and modified, built up, or combined, through enzymatic action, into other molecular units. Semantides are informational macromolecules" [56]) and the native genetic code carriers—"semantides," on the one hand, and semantides and episemantides as small molecules and individual enzymes and allosteric regulators, on the other hand, in accordance with their dimensions. There is an effective minimum cell size, lying in the submicron range [57,58], based upon the compact localization of the minimum components providing genome expression functions. It is impossible to reduce this size further, excepting the possibilities of the function combination in a single structure (such as peptide nucleic acid combining "semantide"/nucleic acid and "episemantide"/peptide structural elements [59]).

The existence of a minimum size of a functional cellular structure with expressible genetic material can be interpreted only from the standpoint of evolutionary cytometry, providing separation of certain semantides and episemantides (by Stokes radius and ellipticity descriptors). Considering the approximately half-century debates [60,61] regarding the genetic code origin and evolution from non-genetic precursors, as well as an intensive development of the "viral world" or "virus world" [62,63] concept, representing an important step in this evolution, occurring not as "autonomous viral genome evolution," but as coevolution of self-assembling capsids, viruses proper, or capsidless elements [64], it is logical to analyze, using optical virometry methods (considering not only DNA and RNA viruses, but also their possible analogues/precursors from xenonucleic acids [65], existing in the "XNA world" [66] period preceding the "RNA world"), structures participating in the divergent evolution of the organized elements

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with different sizes and semantide/episemantide natures and sterically linked thereto (such as PNA-like viral particle analogues, capable of cooperative self-assembly [67]).

The appropriateness of employing the Stokes radius descriptor for characterizing episemantides and tertiary semantides—proteins—directly follows from dynamic light scattering principles and enumeration of various episemantide and tertiary semantide classes whose Stokes radii have been measured and calculated since the 1960s:

a) Enzymes: glutamate dehydrogenases (L-glutamate: NAD oxidoreductase 1.4.1.2) [68], grape catechol oxidases [69], etc.

b) Hormones: pituitary hormones [70], radioiodinated iodothyronines [71]

c) Vitamins: for instance, vitamin B_{12} versus transcobalamin [72], including radiocobalt-labelled variants for parallel gel filtration on sephadexes [73,74]

d) Globular proteins—spheroproteins (for instance, milk proteins) [75,76]

e) Antibodies/immunoglobulins [77]

f) Secreted proteins, plasma membrane transmembrane proteins, and lysosomal proteins maturing in the Golgi apparatus [78]

g) Ion channels: for instance, GABAA receptor—ligand-dependent channels, also known as benzodiazepine receptors [79]

h) Neuroenzymes (for instance, acetylcholinesterase [80]) and neurotransmitter receptors (for instance, muscarinic receptor—a serpentine receptor effecting signal transmission through heterotrimeric G-proteins [81])

i) Supramolecular biocoordination metalorganic chemistry or bioinorganic chemistry and metallomics agents: for instance, chelators—metallothioneins [82], the highly conserved calcium-binding protein calmodulin [83].

Viral dimensions have obviously likewise been measured. Qualitative correspondence was observed between the data obtained through dynamic light scattering [84] and the data obtained using chromatography (employing an agarose gel) [85]. Strictly speaking, from the cross-validation perspective, for most cases of obtaining ambiguous data of this type, independent methods—that is, operating on different physical principles—for measuring hydrodynamic radius should be employed. In this regard, it is appropriate to indicate the existence of such measurement methods for this descriptor as capillary electrophoresis, time-dependent gradient electrophoresis [86,87], related methods based upon isoelectric point (isoelectric focusing and analogous methods) [88]; gel filtration [89] on inorganic and organic supports (from conventional sephadexes to silicon-containing porous gels [90,91]) or size-exclusion chromatography [92]; time-of-flight methods (predominantly for nanostructures such as fullerenes [93,94]); diverse dialysis variants [95], including under external field action. The only problem is that most of the above methods do not permit assessment of the molecular asymmetry at the measurement stage [96]. This can prove critical: an elongated molecule, from a hydrodynamic perspective, will behave as a spherical molecule of larger radius and correspondingly greater mass. Friction force depends upon the moving particle's surface area; therefore, upon increasing the surface area, friction force as the physical criterion of the above methods will not remain constant upon compression of a given molecule (for instance, in certain methods employing porous supports). Yet without fulfilling the equivalence condition of molecular form and density, mass-based interpolation cannot replace the radius metric fixed through gel filtration. Additionally, since conformation contribution is fixed also from the environment—solvent (for instance, molecular hydration may occur, which is true for virtually all biorelevant episemantide ions not smaller than 5–6 *ångströms*), comparing optoelectronic hydrodynamic radius measurement data with chromatography and electrochemical method data is actually impossible without observing equivalences regarding the medium properties (and in thin-layer methods—the substrate properties). Polarization per se can likewise affect symmetry/asymmetry.

Environmental Effects and Self-Organization of Prebiotic Synthesis Products as Features for Laser-Assisted Measurements

Owing to the reactivity of biomacromolecule parameters (whether primary or secondary, or tertiary semantides, as well as episemantides) towards the environmental factors, the experimental method with programmable environmental conditions can likewise be given the character of an experiment modeling analogous environmental changes in protobiological or early chemical-evolutionary conditions. Therefore, it is appropriate, in this connection, to note that for variable environmental conditions, it is more important to employ the monitoring methods (not static point measurement) of hydrodynamic radius of a series of biorelevant or model/biomimetic molecules not for the structures, but for the processes. For instance, from the perspective of analyzing prebiological particle self-organization (particularly underlying self-organizing viral structure or particle formation, as well as magnetic hybrid "metalorganic" viral particles [97-99]), investigation of the particle aggregation and dissociation, characterized through hydrodynamic radius measurement methods [100,101], is appropriate. From the membrane-mimetic perspective, particularly evolutionary membrane-mimetics and evolutionary membrane biomimetics, one can consider the data on bioavailable particle transport processes through membranes and membrane models. Whilst work in this direction has proceeded exclusively in biomedical and veterinary applications, employing dialyzable sheep erythrocytes

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[102,103] and Bruch's membranes (vitreous plates of eyes) [104], the biochemical and cytological reasons for investigating arbitrary membranes using various labels introducing minimal contribution to the measurement results [105] appear evident, at least since the early 1980s. It may also be appropriate to employ supramolecular self-assembly principles as methods for demarcating the target agents and that portion of the signal which, not constituting noise in the strict sense, predominates over those target signals identifiable with viruses. For instance, in nanotechnological synthesis of artificial viruses [106,107], self-assembly principles are frequently employed [108]. Self-assembly of supramolecular polymeric viral mimetics (for example, of tobacco mosaic virus mimetic [109]) is known, and self-assembly of synthetic viral capsid structures based on a 24-mer peptide is also possible [110]. The founder of such work is considered to be a corresponding member of the USSR Academy of Sciences B.F. Poglazov, who conducted relevant work on the artificial viral particle reconstruction at the Institute of Molecular Biology of the USSR Academy of Sciences, at the Department of Biochemistry of the Biological-Soil Faculty of Moscow State University, and at the Interfaculty Problem Research Laboratory of Molecular Biology and Bioorganic Chemistry of Moscow State University (now known as the A.N. Belozersky Research Institute of Physical-Chemical Biology of Moscow State University). However, as a consequence of self-organization, self-assembling viral particles, beginning at the nanosize level, frequently exhibit structural polymorphism [111], similar to that observed for calibration particles in laser-aerosol-spectrometric investigations. However, owing to the identity of self-organization mechanisms, it is substantially less than the scatter of mechanically obtained particles (for example, latex particles or other calibration particles).

Most likely, these phenomena and artifacts, as well as ordinary viruses, will be studied not only by cryo-electron microscopy and AFM, but also by super-resolution optical and fluorescence microscopy [112-123].

Conclusion

The very statistical proximity of the self-assembling structure sizes may in certain cases be a descriptor of their biological, biogenic, abiogenic or biomimetic origin – and a descriptor of the chemical evolution stages for prebiotic synthesis and self-assembly. Residuals or heteroscedasticity indicators of the sample, based upon the optical signal registration data, also may constitute criteria for distinction from the reference standard biomaterial (or model abiogenic / prebiotic or biomimetic / synthetic biological material).

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