The great basic question of science: Membrane compartment or non-membrane phase compartment is a physical basis for origin of life?

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Full text of the paper in Russian: http://www.bioparadigma.spb.ru/files/Matveev-2016-Protophysiology.Rus.pdf

Comments for slides

Slide 1.

The main idea of my presentation is the following: Phase approach, based on sorption properties of proteins, is able to explain a most initial stage of formation of a protocell, and is in full accordance with available experimental data. The membrane approach has no experimental support.

Slide 2.

What does it mean that the cell is a phase? This means that most part of intracellular water and potassium are bound. They are bound by proteins. On the other hand, from the viewpoint of membrane approach, the physical state of water and potassium in cell and outside the cell is no different.

Slide 3.

Both approaches, the phase approach and membrane one, are designed to explain the four fundamental physical properties of the living cell. First, the cell is semi-permeable, which is expressed in the fact that the larger solute molecule, the worse it gets into the cell. The second, the cell is able to accumulate selectively some solutes, such as potassium, and excludes other solutes, for example, sodium. Third, why the cell is osmotically stable. And finally, the fourth property is the ability of the cell to generate electric potentials. Our understanding of the physical nature of these properties dramatically affects our understanding of cellular function and our ideas about how life could have arisen.

Slide 4.

From the viewpoint of the standard cell model (membrane one), the plasma membrane is the carrier of all four physical properties of the cell. The structural basis of the membrane is a lipid bilayer which includes numerous proteins with different roles: specific ion channels, pumps, transporters and others. From the standpoint of such model, the origin of life is reduced essentially to the simultaneous origins of the "living" membrane and a mechanism of its energy supply.

Slide 5.

From the standpoint of the bulk phase model of the cell, proteins are carriers of the fundamental physical properties. With this approach, the origin of life is reduced to a single key event, to spontaneous formation of polypeptides. Once they are formed, they inevitably exercise their sorption properties: bind water and potassium, associate with each other to form a compartment with special physical conditions inside. Since bound water is a poor solvent (this subject to be discussed later), all of solutes, if they are not adsorbed by proteins, will be excluded out of this compartment or protocell, including sodium. Thus, we see that with this approach, bulk phase approach, there is no need for a complicated structure called plasma membrane to separate internal environment of protocell from outside medium.

Slide 6.

The question arises, why proteins are able to bind water and ions, in particular, potassium? The fact is that despite all the variety of proteins, they all have exactly the same polypeptide backbone which represents a geometrically regular alternation of positive and negative charges (dipoles). A water molecule is also a dipole, and it inevitably interacts with dipoles of a polypeptide chain. Acid carboxyl groups of dicarboxylic amino acid residues are the acceptors, or binding sites, for cations, especially for potassium.

Slide 7.

The dipole moment of water is not a constant value. In a transition from gaseous to a liquid phase, its dipole moment is increased by 60%. This is the result of dipole-dipole interactions between water molecules in the liquid phase. During these interactions, water molecules are polarized more than in the gas phase, and thus their dipole moment increases. If a water molecule will interact with a stronger dipole than itself, it will lead to even greater polarization of water molecule and to an even greater increase in its dipole moment. Peptide bonds contain such stronger dipoles: dipole moment of the carbonyl group is 46% more than own dipole moment of water; dipole moment of a peptide bond is nearly twice more than own dipole moment of water. These factors contribute to water binding by protein peptide backbone with the formation of adsorption layer on the molecular surface of a protein. This adsorption layer will be stabilized by two factors: by stronger than water dipoles of peptide bond functional groups, and, a second point, by stronger hydrogen bonds between water molecules, included in the adsorption layer. Last bonds will be stronger than the normal one because the adsorption layer is formed by water molecules with stronger dipole moments. The first adsorption layer will serve as a matrix for adsorption of the second, the second for the third, etc. As a result, adsorption water phase is formed on a polypeptide backbone of a protein with stronger hydrogen bonds than in adjacent bulk water.

Slide 8.

This slide shows that the adsorbed water phase can be formed only on those regions of a protein that are free from secondary structures. In the secondary structures, peptide functional groups involved in intramolecular linkages and are not available for interaction with water. Therefore, these structures have a hydrophobic character. It should be noted that according to current data, 40% of the proteome have no secondary structures. For this reason, these extended proteins and extended protein regions are available for water and so that is the needed condition to form the phase of adsorbed water.

Slide 9.

After all is said, we get the following structure, the protein-adsorbed water complex or biophasosome. Below we see a polypeptide chain, the adsorbent on which surface the adsorbed aqueous phase is formed with stronger hydrogen bonds between water molecules. This phase is poor solvent in compare with bulk water. Since bulk water has a comparatively good solubility, all solutes are excluded from the shell of adsorbed phase into the bulk phase and the faster, the more size of particles (in this case sodium is shown). Only solutes adsorbed by protein remain inside biophasosome (in this case potassium ions are shown).

Slide 10.

Now let's ask ourselves how we could experimentally verify the presence of non-solvent phase of water in a system. For this, they use the simplest experimental method - equilibrium dialysis. The dialysis bag with a semipermeable membrane is filled with a polymer solution (the membrane is impermeable for a polymer). Then they add an electrically neutral solute in bathing solution (bag membrane should be permeable for a tested solute). After that diffusion fluxes begin in the dialysis system. After some time, the diffusion fluxes reach the end and the diffusion equilibrium establishes. Now, if we calculate the ratio of a solute concentration in the bag water to its concentration in the bathing solution, we get a distribution coefficient of a solute between bag and medium. This distribution coefficient is an indicator for us showing single-phase or two-phase system is the dialysis system. In the other words, does water have different dissolving ability depending on its location in the bag and in the bathing solution? If the coefficient is equal to one, no difference in a solvent power of the two water fractions. If the coefficient is less than one, then we are dealing with a two-phase system. It means that dialysis bag contains water with reduced solvency.

Slide 11.

This slide provides a summary table of data that I collected from various sources, but a more visual representation of the data we will get on the next slide.

Slide 12.

This figure shows the dependence of the solute distribution coefficients on their molecular weight in different systems. There are polymer solutions in dialysis bags, coacervates, living cells (frog muscle fibers) and granules of ion exchange resin Dowex here. These data shed light on two important topics that I have mentioned.

The first subject is the role of protein conformation for determining its sorption properties in relation to water. The upper dashed line indicates that native hemoglobin, even in high concentration (39%), placed in a dialysis bag, does not change the properties of water in the bag. We see that the distribution coefficients for all solutes studied are closed to one in the case of native hemoglobin. Another case, when hemoglobin has been placed in the alkaline environment due to sodium hydroxide. We see that in the alkaline solution hemoglobin's ability to affect the water properties sharply increases and we see here a clear dependence of the distribution coefficients on the molecular mass of the investigated solutes. Why?

I talked about the fact that secondary structures of a protein are hydrophobic and are not able to interact with water. This is the case of native hemoglobin. 80% of the amino acid residues of its four subunits are included in alpha-helixes. In the alkaline environment, the alpha-helixes are destroyed and hemoglobin polypeptide backbone becomes available for the solvent, water is adsorbed, the adsorption builds non-solvent aqueous phase and as a result, the line got the slope.

The second subject is the fundamental physical properties of the living cell, how to explain them? On this slide, we see a clear example of the manifestation of the first of these properties, the semi-permeability. Let’s look at the frog muscles. We see that the higher molecular weight of a solute, the worse it gets into the living muscle cells. Habitually, we attribute this property of semi-permeability to special devices of the plasma membrane: to pores of a different size that membrane contains. However, the comparison of the living muscle with coacervates, Dowex resin, dialysis bags filled with polyethylene oxide, gelatin, and denatured hemoglobin shows that in all these cases the same physical factor acts and this factor is not the membrane with its special properties, because there are no biomembrane-like structures in coacervates or in Dowex granules, or in the case of dialysis bags. Therefore, the explanation of the fundamental property of semi-permeability with help of plasma membrane pores of different sizes is absolutely untenable in the light of the comparison of the properties of the living cells with the similar properties of the model systems. It is clear that in these systems a single common factor operates, and this factor is the phase of non-solvent water or aqueous adsorption phase. Moreover, the fact that coacervates and polymer solutions possess semi-permeability simply indicates that first proteineous protocells, that gave rise to life, possessed semi-permeability as well. In other words, mechanism of semi-permeability, due to sorption properties of proteins, did not change during the entire evolution of life on Earth.

Slide 13.

At last decade, it was obtained obvious facts proving the formation of aqueous phases as a result of water adsorption by hydrophilic surfaces. These micrographs are presented fibres from hydrophilic materials which have formed adsorption aqueous phase which thickness are indicated by red arrows. For visibility, colloidal particles were added into the systems. We see that the particles are forced out of this phases and the turbid regions delineate the borders of the phases. In the case of Nafion, we see that the adsorption phase is not formed instantly and it takes some time. Of particular interest to us is the living muscle, the surface of which forms the phase of adsorbed water too.

Slide 14.

Basing on the data reviewed, we arrive at the following representation of biophasosome or nano-cell, the structure of initial level, which provides the necessary physical conditions for the origin of life. This, above all, a polypeptide with extended conformation which peptide bonds are available for water. It makes possible to adsorb water on molecular surface of a polypeptide to form a shell of multilayer adsorbed water. This adsorbed water excludes from its volume all unnecessary solutes of the ocean of prebiotic era, for us it is primarily sodium, but potassium is adsorbed. As a result, necessary for life ratio of potassium to sodium, which we know, forms inside of the biophase. Biophase structure is under the control of cardinal adsorbates, ATP or its predecessors. If cardinal adsorbate is adsorbed by a protein, a protein conformation becomes extended and nano-cell passes in the resting state with all discussed attributes necessary for the resting living cell: semi-permeability, the ability to bind potassium (which Fox's microspheres demonstrate), and osmotic stability. I will tell about the electrical properties later. I do not speak on Fox's microspheres ability to accumulate potassium due to lack of time.

As I said, the nano-cell structure is controlled by cardinal adsorbates. Once ATP is split, polypeptide changes its conformation and secondary structures appear (it is the active state). After that polypeptide loses its ability to adsorb water. The phase of adsorbed water collapses, an influx of sodium ions reaches protein and displaces potassium from its binding sites. Adsorption of a new molecule of cardinal adsorbate restores nano-cell resting structure completely. In these cycles of binding and dissociation of the cardinal adsorbate, energy, accumulated at the resting state, releases at active state for making biological work occurs. However, the important feature of biophasosome is that it does not require a continuous supply of energy to support its native structure. Nano-cell can keep its phase state until a cardinal adsorbate is bound by polypeptide. For example, Fox's microspheres are remarkably stable in a wide range of environmental conditions. It means that biophasosome is able to keep internal physical conditions needed for the origin of life during a long-time period. Biophasosome is called a nano-cell because biophasosomes have a native ability to associate with each other to form multiprotein complex — protocell.

Slide 15.

Fox's microspheres are a well-studied example of a protocell. There are two action potentials on the left: the top is recorded with a live neuron, the lower - with a microsphere. The differences between these records are insignificant, and it is surprising because according to the Hodgkin-Huxley model, generation of the action potential is needed in a lipid membrane, highly specific sodium and potassium ion channels and sodium-potassium pump. None of these structures are in microspheres, but, nevertheless, they are able to generate an action potential.

On the right, we see a record of spontaneous changes of Fox's microsphere conductivity with use of intracellular, as we would say, microelectrode recording. When it comes to living cells, they explain such activity by the presence of the ionic channels that open and close spontaneously. However, Fox's microspheres have no lipid membranes and specific ion channels. Nevertheless, the protocells have channel-like activity. Comparison of Fox's microspheres with living cells shows, firstly, that the microspheres can, however, generate electric potentials without a fully functional membrane; thus they have one of the fundamental physical properties I spoke about.

Secondly, the microspheres are clearly the phase systems, and this suggests that the phase nature of protocell does not preclude the generation of electrical potentials. It means that in the case of the microspheres ion-exchange processes at the surface of the microspheres take place. With respect to living cells, theories were published on the formation of the action potentials based on sorption and ion exchange properties of the living cell surface, but scientific community pays no attention to them. From the point of view of the sorption theory of action potential, Fox's microsphere is an appropriate model of the living cell.

Slide 16.

To disprove the bulk phase approach, it is necessary to give another explanation for the non-uniform distribution of solutes between the considered phase systems and the environment. If this is not adsorbed water, which explanation is possible without water.

Slide 17.

The available experimental data suggests that since the origin of the first protocell and up today the physical mechanism of the equilibrium distribution of solutes between the cell and its environment has remained unchanged.

Slide 18.

Let us now compare the membrane and bulk phase approaches in terms of their compliance with available experimental data.

Despite 60 years of experimental work aimed at to solve the mystery of the origin of life, no one has yet demonstrated a spontaneous formation of the sodium-potassium pump. The pump is critically essential to the creation of the specific intracellular environment necessary for vital processes and, therefore, for evolution (so the pump must originate before evolution, not in result of it). Also, there was not shown the spontaneous formation of specific ion channels which are essential for proper functioning of the membrane according to membrane approach. Spontaneous self-assembly of the sophisticated complex - membrane-channels-pumps has not been shown yet. It remains a mystery the simultaneous formation of the pumps and a system of supply of them by energy.

With regard to the bulk phased approach, the spontaneous formation of all the necessary elements has been shown experimentally. It was demonstrated a spontaneous formation of amino acids and polypeptides, an ability of polypeptides to associate up to the formation of protocell (remember the dialysis bags), multilayer adsorption of water by polypeptides. Also proven the ability Fox's microspheres (as protocells) to accumulate potassium (I have no time to talk about it). Maintaining the integrity of protocell does not require a permanent flow of energy from the perspective of the bulk phase approach. Spontaneous formation of ATP precursors, pyrophosphate or polyphosphates, are also proved.

Slide 19.

Main conclusions.

The sorption properties of phase-making proteins are able to explain the earliest, most initial steps of the origin of life. Due to the properties, it is possible to form bulk phase compartments (biophases) with physical conditions needed for life processes, which is the start point for evolution.

The fundamental physical properties of the cell remain qualitatively unchanged throughout all stages of evolution, from the origin of life to the present.

The minimal cell is the minimal structure on the basis of a single protein molecule which has all four fundamental physical properties of living cells mentioned at the beginning of my presentation.

Slide 20.

The biophase, due to the simplicity of its physical nature, is the best candidate for the role of the physical basis of life.