The Biophysical Society of Japan General Incorporated Association

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In cells, biological macromolecules are contained at a volume fraction of 20-30 vol %. Recently, this situation has much drawn attention as a crucial factor of the crowding effect of the cytosol. To construct cell models, giant vesicles (GVs) confining macromolecules, microspheres, or both have been adopted. However, in the conventional methods such as the natural swelling method, the inner state (concentration, number density and so on) of macromolecules or microspheres is scarcely controlled and non-uniform among the GVs. To form microsphere-containing GVs as desired, we employed the water-in-oil (W/O) emulsion centrifugation method reported by Puot et al. (Langmuir 2003, 19, 2870), because this method is based on W/O emulsion droplet as a template of GV. By using polystyrene microspheres (diameter = 1 μm) dispersions of 2.0-25 vol % to form W/O emulsions in liquid paraffin, we obtained GVs that encapsulate microspheres with volume fractions in the range of 0-50 vol % and their diameters were in range of 4-40 μm. Although the distribution of the volume fraction of GVs was broad, it should be noted that the highest volume fraction of GV was higher than that of W/O emulsion droplets. It is the advantage that we can achieve GVs confining microspheres at high number density rather than that of microspheres dispersion we prepared initially. This technique can be applied to the GV encapsulation of other microspheres or macromolecules that exhibit entropy-driven effects, such as the crowding effect.

3G1046 オイルフリー-GUV に取り入れた分子演算システム RTRACS
Molecular computing system RTRACS encapsulated in oil-free giant unilamellar vesicle
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RTRACS (Reverse-transcription-and-RT-transcription-based Autonomous Computing System) is a molecular computing system in which RNA molecules work as input and output.¹,² RTRACS is a reaction network system which consists of modularized molecular logic gates containing the Boolean gate such as AND, OR, etc. We intend to construct a giant unilamellar vesicle (GUV) encapsulating RTRACS as a minimal model of living cell. At present, the best preparation method of GUV is the w/o emulsion centrifuge method.³ However the GUV prepared with the centrifuge method potentially contains the oil which surrounded the water droplet in the w/o emulsion. The oil changes the membrane properties, such as thickness, permeability, dynamics, etc. Hence we developed an oil-free GUV preparation method based on the lipid film gentle hydration method in the presence of mono- and di- valent metal cations. The inner water pool of GUV is stained with TMRA-dextran (M.W. 10,000) as a volume marker. The FAM-labeled molecular beacon probe which hybridizes with the output RNA molecule of RTRACS was used as a reaction marker. The dual-labeled GUV in which RTRACS runs was analyzed by optical microscopy and flow cytometry.


3G1058 低いpHが誘起するDOPS/MO 膜の液晶相からキュビック相への相転移の初期過程
Initial Step of Low pH-Induced Lamellar to Bicontinuous Cubic Phase Transition in Dioleoylphosphatidylserine/Monoolein
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The modulation of electrostatic interactions due to surface charges of lipidic membrane induces transitions between the Lα phase and the inverse bicontinuous cubic (Qh) phase [1]. Using time-resolved small angle X-ray scattering (TR-SAXS) and a homemade rapid mixing method, we investigated the kinetics of low pH-induced Lα to Qh⁰ phase transition in MLVs of dioleoylphosphatidylserine (DOPS)/monoolein (MO) in the presence of PEG-6K, and found that the H2 phase appeared after the pH change (the initial step), and then the H2 phase slowly converted into the Qh⁰ phase (the second step) [2]. However, we could not follow the initial step due to the limited time-resolution of the method. In this report, we investigated the initial step of the low pH-induced Lα to Qh⁰ phase transition in DOPS/MO in the absence of PEG-6K using TR-SAXS with a stopped flow apparatus. We succeeded in following structural changes in the membranes after 100 ms of the mixing of DOPS/MO-MLV in a neutral buffer with a low pH buffer with a time-resolution of 100 ms. We observed that at the initial step the peak intensity of the Lα phase gradually decreased but at the same time that of the H2 phase gradually increased, indicating that the Lα phase directly converts into the H2 phase without formation of any intermediates. The rate constant of the initial step greatly depended on final pH and DOPS concentrations in DOPS/MO membranes.

2003.09.16


3G1110 X線及び電子線図析法を用いた皮膚角層の構造解析
Breakthrough for Unresolved Structural Problems in Skin Function by Combined Use of X-ray and Electron Diffraction Methods
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The outermost layer of the human skin, stratum corneum (SC), consists of terminally differentiated keratinocytes and highly ordered intercellular lipid layers. The SC serves as a physicochemical barrier between the inner body and the outer external environment. Notwithstanding the extensive studies on structure of intercellular lipids which plays a crucial role for the skin barrier properties, owing to lack of the structural evidence under functioning lot of unresolved problem are left open. Using X-ray and Electron diffraction methods we deepened understanding the healthy skin state and developing the percutaneous drugs.

Both synchronon X-ray diffraction (XD) and electron diffraction (ED) methods are the very powerful tool to analyze the intercellular lipid organization in SC. The XD has higher resolution than the ED, but requires invasive operation because a large amount of skin sample is necessary to obtain reliable data. On the other hand, the ED makes it possible to analyze the structures of the SC noninvasively, but radiation damage by electron beam must be suppressed. By best use of these methods we will report here a fundamental structural change of SC in the skin permeation of chemical agents, the effect of temperature in the skin permeation, and the regional structural differences on the body. We will present these results and future perspective for the SC structural study.

3H0900 GTP 結合状態とGDP 結合状態の親水性分子構造における大きな構造変化
Large Conformational Changes in Tubulin in the GTP- and GDP-States Microtubules Observed by Cryo Electron Microscopy
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Microtubules are dynamic polymers that stochastically switch between growing and shrinking phases and their dynamics is regulated by GTP hydrolysis by β-tubulin, but its mechanism remains elusive because high-resolution microtubule structures have only been revealed for the GTP-bound state. Here we solved the cryo-EM structure of microtubule stabilized with a GTP analogue guanylyl 5'-di, β-methylenediphosphonate (GMPCPP) at 8.8 Å resolution by developing a novel cryo-EM image reconstruction algorithm. In contrast to the crystal structures of GTP-bound tubulin relatives such as γ-tubulin and bacterial tubulins, significant changes were detected between GTP- and GDP-bound states at the contacts between tubulins both along the protofilament and between neighboring protofilaments, contributing to the stability of the microtubule lattice. These findings are consistent with the structural plasticity or lattice model, and suggest the structural basis not only for the regulatory mechanism of microtubule dynamics, but also for the recognition of the nucleotide state of microtubule by several microtubule-binding proteins, such as EB1 or kinesin.

3H0912 細胞膜は一方の形から他方の構造変化に変遷することで対称形状を維持する
Miticotic spindles maintain the symmetrical shape by propagating structural changes to the opposite side
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A microtubule-based spindle is designed for proper chromosome segregation. Recent studies have demonstrated that bipolar spindle formation requires the force balance sustained by molecular motors. However, it remains unclear