論 文 の 内 容 の 要 旨

論文題目　Study of Molecular Transport in Bio-mimetic Extended Nanospace
（疑似細胞拡張ナノ空間における分子輸送に関する研究）

氏　名　李　麗瀟

In inter/intra cellular spaces with 10-100 nm size e.g., synapse and cellular tubes, molecular transport plays significant role in cell-cell communication and other interactions, which are critical for neuron activities and immune functions. Previous *in vivo* studies has speculatively suggested specific fluid property like higher viscosity and is dominant in the molecular transport. However, further understanding is difficult due to lack of experimental tools because of space sizes.

On the other hand, our group has recently focused on 10-1000 nm space, i.e., extended-nanospace, and revealed various specific liquid properties. From the results, we suggested the presence of a proton transfer phase of loosely coupled water molecules by hydrogen bonding in the vicinity of the wall within 50 nm, which is much thicker than well-known adsorbed water layer of several-molecules thickness. The size of extended space is similar to inter/intra cellular space. In addition, it is suggested that there are similar liquid properties between the extended-nano space and inter/intra cellular space.

Therefore, we are inspired to study molecular transport in bio-mimetic extended nanospace which has similar size to inter/intra cellular space. To realize this, firstly bio-mimetic extended nanospace modified by lipid bilayer with accurately controlled space size should be created. Furthermore, there is no method to explore the fluidic property in extended nanochannel.

The study is divided into three parts, (1) development of measurement method of fluid viscosity in nanospace. (2) construction of bio-mimetic extended nanospace and evaluation of fluid viscosity in bio-mimetic extended nanospace and (3) investigation of molecular transport in bio-mimetic extended nanospace.

(1) Development of measurement method of fluid viscosity in nanospace.

Previous work of our group revealed various specific liquid properties in fused silica nanochannel, and we proposed the presence of the proton transfer phase published in Angew.

Chem. 2007, 119, 1199-1202. For the fluidic property, spontaneous capillary filling into a nanochannel showed a specific viscosity. However, since spontaneous capillary filling depends on both fluidic and interfacial properties, accurate property could not be obtained. Hence precise understanding is still insufficient even in fused-silica extended nanospace.

This study successfully developed a method to simultaneously measure the fluid viscosity and interfacial property in nanospace by controlling meniscus motion using an MPa order external pressure. By using the method, the water viscosity and wetting property in fused silica nanochannels were measured for various sizes and confinement dimension for the first time.

Results showed that water viscosity increases with deceasing the channel size, especially for square nanochannel (nanoscale width and depth by two dimensional nanoconfinement), compared with plate nanochannel (microscale width and nanoscale depth by one dimensional nanoconfinement), while the wetting property was almost constant. These results suggest specificity of liquid two dimensionally confined in extended nanospace, which has high viscosity by a viscous boundary water phase of similar scale to the proton transfer phase.

(2) Construction of bio-mimetic extended nanospace and evaluation of fluid viscosity in bio-mimetic extended nanospace.

In inter/intra cellular space, specific fluidic property like higher viscosity was suggested. However, further investigation is difficult due to space size.

Here, bio-mimetic extended nanospace was successfully developed by lipid bilayer modified to fused-silica extended nanochannel with vesicle fusion method. The principle is that sphere vesicles in aqueous rupture and form lipid bilayer by interacting with glass substrate. In this *in vitro* approach, space size and materials can be accurately controlled. The thickness of lipid bilayer was evaluated by fluidic AFM and modification result was validated by XPS analysis.

Afterwards, fluidic property in bio-mimetic extended nanospace was investigated. An increase of viscosity in bio-mimetic extended nanospace, which has similar tendency to fused silica nanospace, was revealed for the first time. This result suggests that contribution of the near-field liquid structure to the fluidic property is also significant in inter/intra cellular spaces. The lipid bilayer has a strong interaction with the water molecules by hydration, while the ions included in the bio space may reduce the interaction by ion hydration. This study suggests that the fluidic property in the bio space will be determined by effects of cellular space size and geometry, and liquid components.

(3) Investigation of molecular transport in bio-mimetic extended nanospace.

Molecular transport in inter/intra cellular space is very important for cell-cell communication and cellular function. It is very difficult for *in vivo* work to exclude the effect of cells and

macromolecules, therefore exploration of molecular transport by fluidic property is complicated. In this part, we measured the diffusion coefficient of Green Fluorescent Protein (GFP) in artificial biological solution in bio-mimetic nanospace. The result suggests that the diffusion coefficient of GFP decreased in bio-mimetic extended nanospace. In addition, the decreased diffusion coefficient is consistent with increased viscosity, which indicates that the specific molecular transport is induced by unique fluid viscosity.

Conclusively, measurement method of fluidic property in nanosapce was successfully developed, and fluidic property in bio-mimetic extended nanospace was revealed for the first time, finally specific molecular transport was clarified in bio-mimetic extended nanospace. This work will not only provide a general model of the cellular functions derived from inter/intra cellular spaces based on the microscopic liquid model, but also propose a new concept of importance of morphology of 10-100 nm inter/intracellular space, which have not been understood in conventional biology.