2P249**Inhibition of *Mycoplasma mobile* gliding proteins by monoclonal antibodies isolated from phage display library of human scFv**○Shuhei Yoshii¹, Makoto Miyata^{1,2}¹Dept. of Biology, Graduate School of sci. Osaka City Univ., ²PRESTO, JST

M. mobile isolated from freshwater fish glides smoothly and continuously on glass surface at an average speed of 2.0-4.5 $\mu\text{m/s}$, exerting a force up to 27 pN. Recently, four proteins, Gli123, Gli349, Gli521 and P42 were found to be involved in the gliding mechanism. Here, we isolated 11 monoclonal antibodies against Gli521 from a phage display library. These recombinant antibodies, single-chain Fvs (scFv) are composed of the VH and VL immunoglobulin domains connected by a flexible linker. The addition of these antibodies reduced the gliding speed of *M. mobile* to 8-86% of the initial, and removed cells from glass surface to be 22-99.4% of bound cell ratio to the initial. DNA sequencing showed that these antibodies have different structures of the binding domain. Analyses by surface plasmon resonance (SPR) showed binding kinetic parameters of these antibodies to Gli521 protein. The association rates, dissociation rates and association constants ranged $1.6 \times 10^2 - 1.6 \times 10^4 / \text{Ms}$, $5.5 \times 10^{-6} - 1.0 \times 10^{-2} / \text{s}$, and $5.8 \times 10^3 - 1.2 \times 10^8 / \text{M}$, respectively. The efficiency of inhibiting gliding motility did not depend on any of these kinetic parameters, suggesting that antibody should bind to actively moving position of the target molecule to inhibit gliding.

2P251**The effects of cytokine concentration on the flow around a suspended chemotactic neutrophile in liquid**○Masaaki Tamagawa¹, Yuta Kubomoto¹, Kusuhiro Mukai², Yoshinori Furukawa³¹Grad. Sch. of Life Sci. and Sys. Eng., Kyushu Inst. of Tech., ²Kurosaki Harima,³Institute of Low Temperature Science, Hokkaido University

Inflammation reaction is very important role as immune response in human body. But the driving force/mechanism of the chemotactic motion of neutrophile in liquid is not elucidated yet. In the previous investigations, the motion of neutrophile has been considered to be amebic swimming in liquids as same as amebic motion on solids. Generally, the micro particle in liquid on the gradient of concentration has the force by Marangoni effects. So the neutrophile has the driving force of the motion in liquids by both amebic motion and Marangoni effects force. This paper describes the effects of gradient of cytokine concentration on chemotaxis of neutrophile by observing the motion on the microscope with changing cytokine concentration. The aim of this investigation is to distinguish the driving force by Marangoni effects from amebic motion. In the experiments, the motion of neutrophile in the physiological salt is measured by using PTV. The average velocity of neutrophile is obtained with changing the cytokine concentration. From the experiments, it is found that there is a peak velocity between 1 (ng/ml) and 1000 (ng/ml) concentration. Comparing with inorganic materials, the membrane of the neutrophile has high efficient sensing mechanism for the cytokine concentration and interfacial tension distribution. The model of membrane for sensing is also discussed by comparing with inorganic particle model in water. It is concluded that there are optimum value of gradient of cytokine concentration for maximum neutrophile velocity.

2P250**Elevation of intracellular cAMP concentration at tips of filopodia alters actin organization in neuronal growth cones.**○Makoto Goda¹, Ayumu Inutsuka², Yoshinori Fujiyoshi²¹RIKEN Harima Institute, ²Kyoto University., Science, Biophysics

Recent biochemical and genetic studies in invertebrates and vertebrates led to identification of various guidance cues which navigate neurite outgrowth toward the target cells during development of nerve system. The guidance cues activate receptors at growth cone surface to elicit localized intracellular signaling events. The second messenger cyclic AMP (cAMP) has been identified as a key intracellular signaling molecule of the growth cone navigation. It has furthermore been shown by cAMP imaging with optical microscopy that cAMP gradients occur in cytoplasm of neuronal cells under physiological conditions. In the present study, using membrane-permeable caged cAMP analogues as well as the new polarized microscope system (LC-PolScope), we investigated where react sensitively within growth cones to elevation of intracellular cAMP concentration which induce the organization of actin filaments. By photolysing caged cAMP with a fine focus UV laser beam, we clarified that limited regions at tips of filopodia were sensitive to the elevation of intracellular cAMP concentration to change length of actin bundles in growth cones, while the other parts showed less sensitive. These observations support that a sensor for cAMP is localized at filopodia of neuronal growth cones to change morphological features of growth cones and therefore steers the axon in developing nervous systems.

2P252**Simultaneous Recording of Traction Force and Molecular Dynamics in a Migrating *Dictyostelium* Cell**

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Freely migrating *Dictyostelium* cells protrude pseudopods not only at the leading edge but also at the lateral regions frequently. When the protrusion at the lateral region is more dominant than at the leading edge, the cell changes the direction of migration to the new main pseudopod.

To address how the direction of migration is decided, we combined the recording of molecular dynamics by TIRFM and measurement of traction force on elastic substratum. *Dictyostelium* cells, transfected with a construct of several kinds of GFP fusion proteins (GFP-myosin II, -CH domain from 120k actin binding protein, and so on), were placed on elastic substratum embedded with red fluorescent marker beads. Fluorescence images of GFP and marker beads were recorded simultaneously under TIRFM. Evanescent field was created above the surface of the elastic substratum because of its high reflective index. The traction force exerted by the cell was calculated from the displacement of the marker beads using finite element method and its distribution was visualized by pseudocolor.

Once the protrusion of filopodia was anchored on the elastic substratum, the cell pulled it exerting intense traction force toward the cell body at the tip of it. The amplitude of the traction force observed in the lateral filopodia was larger than that in the leading edge. Based on these observations, we proposed a hypothesis that filopodia act as mechano-sensors to decide the direction of migration. The relationship between molecular dynamics and traction force is discussed.