**S1.14**

**Arrayed lipid bilayer chambers for single-molecule transporter analysis**

Hiroyuki Noji  
*The University of Tokyo, Japan*  
**E-mail address:** hnoji@appchem.t.u-tokyo.ac.jp

Nano/micron-sized reaction chamber arrays (femtolitre-chamber arrays) enable highly sensitive and quantitative biological assays, such as single-molecule enzymatic assays [1, 2], digital PCR [3, 4], and digital ELISA [5]. However, the versatility of femtolitre-chamber arrays has been limited to reactions in aqueous solutions. In this presentation, I will introduce an arrayed lipid bilayer chamber system (ALBiC) that displays a sub-million femtolitre volume, each sealed with a stable 4-μm diameter lipid bilayer membrane with extremely high efficiency (yield: ~99%). When reconstituted with a limiting amount of the α-hemolysin or FoF1-ATP synthase, the chambers of the ALBiC exhibited stochastic and quantized transporting activities, demonstrating that the single molecule analysis of passive and active membrane transports is achievable with the ALBiC system. Thus, this new platform has vastly extended the versatility of femtolitre chamber arrays and could contribute to the understanding of the working mechanism of membrane proteins as well as to further analytical and pharmacological applications. If time allows, I would like to talk about new versions of the ALBiC that we recently developed.


**doi:**10.1016/j.bbabio.2014.05.191

**S1.01**

**Channel formation by yeast F-ATP synthase and the role of dimerization in the mitochondrial permeability transition**

Michela Carraro, Valentina Giorgio, Justina Šlejkošová, Geppo Sartori, Michael Forte, Giovanna Lippe, Mario Zoratti, Ildikó Szabó, Paolo Bernardi  
*Department of Biomedical Sciences, University of Padova, Italy*  
*Vollum Institute, Oregon Health and Sciences University, Portland, OR, USA*  
*Department of Food Science, University of Udine, Italy*  
*Consiglio Nazionale delle Ricerche Neuroscience Institute, Department of Biomedical Sciences, University of Udine, Italy*  
*Department of Biology, University of Padova, Italy*  
**E-mail address:** carraro.miche@gmail.com

Purified F-ATP synthase dimers of yeast mitochondria display Ca2+-dependent channel activity with properties resembling those of the permeability transition pore (PTP) of mammals [1]. After treatment with the Ca2+ ionophore ETH129, which allows electrophoretic Ca2+ uptake, isolated yeast mitochondria undergo inner membrane permeabilization due to PTP opening [2]. Yeast mutant strains ΔTIM11 and ΔATP20 (lacking the e and g F-ATP synthase subunits, respectively, which are necessary for dimer formation [3]) display a striking resistance to PTP opening. These results show that the yeast PTP originates from F-ATP synthase, and indicate that dimerization is required for pore formation in situ.


**doi:**10.1016/j.bbabio.2014.05.192

**S1.02**

**Mechanism of the F0-stepping motor revealed by single-molecule experiments**

Wayne D. Frasch, Jennifer Hudson, Tassilo Hornung, James Martin  
*Arizona State University, Tempe, AZ, USA*  
**E-mail address:** frasch@asu.edu

Single-molecule experiments of the *Escherichia coli* F0F1 ATP synthase reveal for the first time the existence of an F0-dependent power stroke that can rotate the c-ring up to a maximum of ~36°, the equivalent of one c-subunit, in the ATP synthase direction against the force of F0 ATPase-driven rotation. Evidence supports a grab-and-push mechanism in which subunit-a grabs one subunit-c near the membrane–cytoplasm interface, then pushes the c-ring as the result of a protonation-dependent conformational change of subunit-a. The location at which subunit-a grabs was identified by mutations that eliminated charged residues. These mutations decreased the ability of subunit-a to grab the c-ring, and adversely affected ATP synthesis and proton translocation in the ATP synthase direction indicating the participation of these residues in a gating mechanism for ATP synthesis-dependent proton translocation.

**doi:**10.1016/j.bbabio.2014.05.193

**S1.03**

**Modulation of F-ATP synthase by pH: Role of His112 protonation of OSCP**

Manuela Antoniel, Barbara Spolaore, Valentina Giorgio, Federico Fogolari, Valeria Petronilli, Paolo Bernardi, Giovanna Lippe  
*Department of Biological Sciences, University of Padova, Italy*  
*CRIBI Biotechnology Centre, University of Padova, Italy*  
*Department of Biomedical Sciences, University of Padova, Italy*  
*Department of Biomedical Sciences, University of Udine, Italy*  
*Department of Food Science, University of Udine, Italy*  
**E-mail address:** manuela.antoniel@studenti.unipd.it

The mitochondrial FO1F0-ATP synthase forms long rows of dimers in the inner membrane cristae and is composed of the catalytic F1 and the membranous FO sectors linked by central and peripheral