

3145-Pos Board B837**Efficiency Studies in Supercritical Fluid Chromatography: Importance of Thermal Diffusivity Near the Critical Point**

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Fast efficient chromatography is important in order to resolve the individual components of complex biological mixtures. Classically, thermal effects that decrease chromatographic efficiency accompany both HPLC and SFC. In HPLC, viscous flow causes the mobile phase temperature to increase from the inlet to the outlet of steel analytical scale (2mm-5mm ID) columns. In SFC, a pressure drop is accompanied by isenthalpic expansion and cooling of the mobile phase. As a result, radial temperature gradients form across the column due to heat exchange with the column surroundings. These dynamic radial gradients form a radial distribution of retention properties and excess chromatographic band spreading. Thermal effects can be minimized in HPLC at the cost of narrow bore capillary columns which help to dissipate the heat formed at high flow rates. In SFC however, commercially available instruments are capable of handling the increased flow rate due to the high diffusivity of supercritical CO₂. In addition, retention near the critical point is sensitive to variations in temperature and pressure. This allows chemists the ability to tune retention properties without changing mobile phase composition. Recent research has shown that efficiency in this region can be significantly improved by operating the SFC column under near-adiabatic conditions. The results presented here suggest that the thermal diffusivity of the mobile phase near the column outlet may be an important factor in controlling chromatographic efficiency near the critical point. In order for chromatographers to be able to take full advantage of the speed and "tunability" of operating near the critical point, a better understanding of the factors causing excess efficiency loss in this region is needed.

3146-Pos Board B838**A Kinesin Driven Enzyme Linked Immunosorbant Assay (ELISA) for Ultra Low Protein Detection Applications**

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Detecting low levels of protein in serum samples is critical for early disease detection. In clinical settings ELISA is the most common method of protein detection. Typical ELISA requires long incubation times, significant handling procedures, and has a detection limit that is often too high for early disease detection. Our goal is to develop a kinesin-driven ELISA platform that reduces typical ELISA incubation times, eliminates laborious rinse steps, and challenges the standard ELISA detection limit. To achieve this goal we incorporate antibody-functionalized microtubule shuttles into our previous microfluidic, kinesin-driven concentrator device (*NanoLett.*8:1041). These functionalized microtubules bind high densities of antibody on the microtubule surface (~100 antibodies per μm of microtubule) while retaining motility characteristics. Theoretically, these microtubules allow for the adsorption of small amounts of analyte on the microtubule surface in small (nL) sample volumes, ideal for low detection limits in ELISA. Concentrating the fluorescently-tagged protein of interest in a $625\mu\text{m}^{-2}$ area increases the signal to noise ratio of the fluorescent signal leading to lower detection limits that are not restricted by fluorescent background. By incorporating these microtubules into the concentrator device, we collected data to determine the detection limit and optimize the assay conditions to create a novel ELISA. Our data suggests that integrating the developed antibody-functionalized microtubules into the concentrator device creates an ELISA platform that significantly reduces the assay time, improves the ease of use, and challenges detection limits of standard ELISA. In conclusion, by successfully powering this concentrator device via kinesin driven solely by chemical energy and integrating appropriately functionalized microtubules, we created a novel ELISA platform that does not require external pumps, power supplies, or excessive handling procedures. Furthermore, this kinesin-driven technology rivals the detection capabilities of the standard ELISA platform.

3147-Pos Board B839**Mimicking Apoptosis using Asymmetric Liposomes: A Therapeutic Approach Against Hiv-1 Infection**

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Macrophages are an important cellular target of HIV-1. Interestingly, they are believed to play also a potential role counteracting the infection. However, HIV-1 is known to impair macrophage's immune functions, such as antibody-mediated phagocytosis.

Here, we present liposomes that can bind HIV-1 virus-like particles (HIV-VLPs) while being specifically phagocytosed by macrophages. This, in turn, results into co-internalization of HIV-VLPs and their delivery to lysosomes.

The investigated liposomes are decorated with anti-Env antibodies and contain phosphatidylserine (PS). PS causes internalization by macrophages (e.g. during apoptosis) via a mechanism supposedly not affected by HIV-1. Hence, PS-liposomes are internalized into the phagocytes due to specific recognition, carrying the previously bound HIV-VLPs.

However, if PS were present right away in the outer leaflet of the liposomes, these would be immediately internalized by phagocytes before binding to the virus in the infected organism could be completed. For this reason, we engineered asymmetric vesicles containing PS only in the inner leaflet of the vesicles, where the lipid is "hidden" from macrophages. Spontaneous flipping of PS towards the external milieu is a slow process. During the time required for significant PS exposure (~several hours), liposomes can bind the virus without being cleared out by the immune system. Eventually, PS becomes fully exposed and the virus-liposome complexes can be internalized by phagocytic cells.

With a combination of FACS, spectroscopy, confocal live-cell imaging and electron microscopy we demonstrate that the PS-liposomes presented here are able to elicit efficient HIV-VLPs phagocytosis by macrophages in a time-controlled manner and might represent a new nanotechnological approach to enhance HIV-1 antigen-presentation and reduce ongoing inflammation processes.

3148-Pos Board B840**Novel Method for High throughput Formation of Lipid Membrane Arrays**Naoki Soga¹, Rikiya Watanabe^{1,2}, Hiroyuki Noji¹.¹Department of Applied Chemistry, The University of Tokyo, Tokyo, Japan,²JST, PRESTO, Tokyo, Japan.

The artificial lipid membrane systems, such as liposome and black membrane, have been useful to study the function of membrane proteins. These lipid membrane systems, however, have some drawbacks such as low productive efficiency and complicated procedures. Here, we show the novel method to form the lipid bilayer membrane arrays (ALBiC) with simplicity and high throughput, using a micro-fluidics device. In this method, we fabricated the flow channel with the millions of cylindrical micro pores ($\phi \approx 4\mu\text{m}$), which were used as retainers of the lipid membrane. Then, we alternatively infused two kinds of liquids, aqueous solution and organic solvent containing lipid, into the flow channel. In this process, the lipid membrane was formed on the inlet of each pore with high efficiency (99% ~), resulting in the formation of millions of the lipid membrane arrays. Thus, the problems as mentioned above can be solved with this method. In addition to the technical advantage, the lipid membrane formed with this method had high compatibility to the membrane proteins, which allowed us to measure the molecular transporting activities of transporters, α -hemolysin and F₀F₁-ATP synthase. Accordingly, we are convinced that this novel method would be the de facto standard for the study of membrane proteins.

3149-Pos Board B841**Formation of a Gel-Supported Lipid Membrane Array on a Micropatterned Substrate**

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An artificial lipid membrane is a suitable platform for the optical and electrophysiological *in vitro analysis* of membrane peptides and proteins. Suspended lipid membranes are intrinsically fragile and require a water permeable mechanical and functional supports. Here, we describe the preparation of long-lived lipid membranes gently supported on gels, which provide a water permeable support.

The microwell array was fabricated on a Si substrate using a conventional photolithographic and etching technique. The gel was prepared by photo-initiating free radical polymerization from gel-precursor solution. The gels were only formed on the substrate where light was irradiated. The lipid membrane on the gel-confined microwell was prepared by rupturing negatively charged small unilamellar vesicles (SUVs) with a smaller diameter than the microwells. The lipid membrane formation on the gel-confined microwell array was observed by fluorescence microscopy.

When positively charged gels were confined in the microwells, gel-supported lipid membranes were formed. Lipid membrane formation was not observed at the microwells either without positive gels or with neutral gels. The gel-supported lipid membrane sealed the microwells with gigaohm resistance and the seal was maintained for at least three weeks. These results indicate that function of gels confined in the microwells mechanically stabilizes lipid