

can be combined with other techniques, such as fluorescence and polarization microscopy.

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Ellagic Acid Nanotubular and Poly-Cationic Conjugates as Nano-Carriers for Delivery into Mammalian Cells

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Ellagic acid (EA) is a naturally occurring plant polyphenol formed by the hydrolysis of ellagitannins, which are primarily found in grapes, nuts and fruits. EA has been known to have potent anticarcinogenic activities, however, its insolubility under physiological conditions limits its potential applications. In this work, we have prepared complexes of ellagic acid with peptide nanotubes and polycations such as low molecular weight polyethylenimine (PEI), polyarginine and polylysine to enhance its properties for drug delivery. In particular, polycations such as PEI are well known non-viral vectors. Briefly, EA nanofibers were grown by self-assembly and were complexed with peptide nanotubes or poly cations at varying temperature and pH. The formation of the nanocomplexes was confirmed by zeta-potential analysis. The morphologies of the complexes were examined by electron microscopy. Because of the rigid core of EA that offers shape consistence, and the poly-cation shells that passivate the surfaces, core-shell nanoconjugates whose average diameters were dependent upon the concentrations and pH were formed. In the formed complexes, the charged amine groups of the polycations most likely interact with the partially deprotonated carboxylate and hydroxyl groups of EA. In some cases, the EA was coupled with rhodamine to examine the effect of bound versus unbound nanocomplexes formed using confocal microscopy. The interactions of the complexes with mammalian cells were examined by live-cell imaging in the presence of normal rat kidney cells. The anticarcinogenic effects of the nanoprobes was explored using HeLa cells. Finally, the ability of the nanocomplexes for drug release was examined at varying pH and concentrations. Such nanocomplexes may have potential applications not only for anticarcinogenic activities but may also help probe mechanisms involved with EA based biodegradable polycationic-based delivery and cellular attachment towards use in varying therapeutic applications.

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Single-Cell Oxygen Consumption Measurements in Bacteria Using a Microobservation Chamber

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Respiration can be a powerful parameter for assessing the physiological state of cells or aiding in determining metabolic functions of cells in a natural sample. Both of these approaches benefit from single cell methodology to ensure that the analysis targets individual cells as opposed to the population average. Using a Respiration Detection System: Microobservation Chamber (RDS:MC), we are able to isolate single cells and measure their consumption of oxygen in real time. The cells are seeded on a chip comprised of an array of microwells containing Pt-porphyrin embedded microspheres. A lid actuator allows for sealing of the microwells for single cell oxygen consumption measurements, as well as opening the microwells for reoxygenation. The phosphorescence lifetime of the Pt-porphyrin varies based on the oxygen concentration, so the decreasing oxygen level in the well after sealing can be monitored by optimized rapid lifetime determination (ORLD). This platform can be coupled with other microscopy techniques, such as fluorescence microscopy or NanoSIMS.

Single cell oxygen consumption measurements give an indication a cell's physiological state and provide an additional tool for assessing cell-to-cell variation. For environmental samples, the RDS:MC allows for the detection of microbes carrying a specific biochemical transformations by monitoring respiration before and after the addition of a substrate. Thus RDS:MC holds the potential to determine the functional role of yet uncultivable microbes by monitoring respiration before and after the addition of a substrate. Cells that show a response to the substrate can be selected for further genomic analysis.

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Simultaneous Real-Time Measurements of 10,000 Antibody-Antigen Reactions Using a Label-Free Scanning Ellipsometric Microscope

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Phage display and similar techniques produce large combinatorial libraries of antibodies. The libraries must be screened by high-throughput technologies to select antibodies with desired binding specificities and physicochemical properties (e.g. on-rates, off-rates, binding affinities) for applications from basic research to human disease therapy. Microarrays can contain tens of thousands of antigens or antibodies on a 1"x3" chemically functionalized glass slide, matching the required throughput. However, traditional fluorescence-based microarray detection doesn't directly give kinetic information, only reac-

tion endpoints. Furthermore, modification of an antibody (or other proteins) with a fluorophore can alter binding kinetics and affinity [Sun et al., *Langmuir* **24** (2008), 13399]. We have developed ellipsometry-based scanning optical microscopes for *high-throughput* and *label-free* readout of microarrays. Our microscopes sense the mass density of proteins or other biomolecules on a substrate through the disproportionate change of s- and p-polarized Fresnel coefficients of obliquely reflected light. Our scanning microscopes can operate in a "high spatial-resolution" scanning mode, yielding end-point images of a 2x4 cm² microarray installed in a flow cell. This area can bear at least 10,000 printed biomolecular spots. Moreover, the microscopes can operate in a "high-speed" (coarse resolution) scanning mode, allowing the binding kinetics of all 10,000 microarray spots to be measured *simultaneously*. We demonstrate this capability by measuring the on-rates, off-rates, and equilibrium dissociation constants of antibodies exposed to a ~10,000 spot antigen microarray. For high-affinity binders with small off-rates, the long dissociation time needed can reduce the overall throughput and may not be known beforehand. In such cases, we present a method for estimating an upper bound for the off-rate when dissociation is measured for a limited time. This maintains the overall throughput of early-stage assays while more detailed measurements, if needed, can be performed later.

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Seeing Theranostic Nano-Carriers by Freeze-Fracture Electron Microscopy

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The potency of nano- and micro-particles, loaded with therapeutic and/or diagnostics is frequently depending upon their morphology adopted in biological relevant environments. Freeze-fracture transmission electron microscopy (ff-TEM) as a cryo-fixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, antimicrobial- and imaging molecules[1]. At a 2 nm resolution limit we are able to study structural modifications of such carriers related to their pay load, application milieu, and during cell interaction.

Using ff-TEM we studied the morphology of a wide variety of nano- and micro particles suitable as carriers for diagnostics as well as therapeutics including quantum dots (coupled to drug-loaded immunoliposomes)[2], gold nano-particles, superparamagnetic iron oxide nano-particles loaded in polymeric immunomicelles[3], micelles (spherical-, disc-, and worm-type micelles) [4,5], small unilamellar liposome[6], multilamellar liposome, niosomes, cationic liposome/DNA complexes, integrin-targeted lipopolyplexes[7], polymer-, lipid- or surfactant-stabilized gas bubbles[8], cochleate cylinder, depofeam particles, and drug crystals. Recently we explored liposome-, viro-some-, and virus-based vaccines, including measles vaccine powders, by ff-TEM. Furthermore, we explored structural modifications within bilayers such as domain-formation [1] but also transformations to non-bilayer structures such as hexagonal and cubic phases.

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Sizing Nanomatter in Biological Fluids by Fluorescence Single Particle Tracking

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One of the major applications of nanotechnology in the life sciences is the development of functional nanoparticles. Accurate nanoparticle characterisation in biological media is a prerequisite to make the transition from the laboratory to a successful product. For many applications, such as nanomedicines for targeted drug delivery, it is essential to monitor the size of nanoparticles in the blood circulation as this directly influences their biodistribution and processing by target cells. However, due to a lack of suitable sizing methods, systematic studies are missing and hamper development of improved nanoparticulate systems. Here we show that fluorescence single particle tracking (fSPT) with maximum entropy analysis is the first technique suited for accurate sizing of nanoparticles dispersed in undiluted biofluids. The fSPT sizing method was