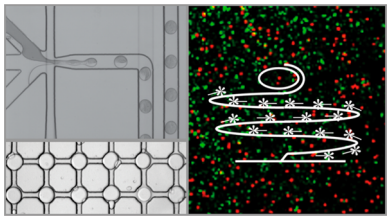
A New "REEAD-Out" for Malaria Parasite Detection

Enzymatic signal enhancement can provide a useful way to detect biomarkers for pathogenic organisms. Techniques based on polymerase chain reaction (PCR) are currently the most sensitive way to detect such biomolecules, but these techniques require specialized equipment and processing methods that are not available to most physicians, especially those physicians in resource-poor settings. Isothermal nucleotide amplification, such as rolling-circle amplification (RCA), can provide a viable alternative. However, to date, most published RCA methods have involved extensive sample preparation and have resulted in few detectable products per target molecule.

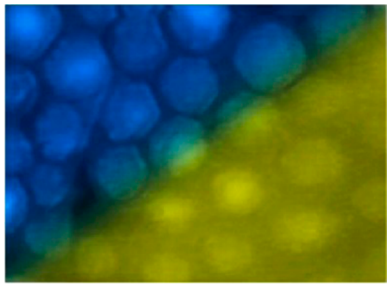
Seeking a better way to detect pathogens, Juul *et al.* (DOI: 10.1021/nn3038594) developed a new protocol that combines the rolling-circle-enhanced enzyme activity detection (REEAD) system with a droplet microfluidics lab-on-a-chip platform. The researchers used the malaria-causing genus, *Plasmodium*, as a model to test their system, using the essential DNA-cleaving enzyme topoisomerase I (pTopI) to circularize a RCA substrate. After testing five different substrates, the researchers selected the one that worked most efficiently for pTopI and did not interact with the human form of the enzyme. They incorporated this REEAD substrate into a droplet microfluidics lab-on-a-chip platform, then tested its ability to detect *Plasmodium* infection in very small volumes of blood or saliva. Results show that this technique could detect infection with limits below one parasite/μL of blood in all species that cause human malaria. The researchers showed that parasite detection in saliva was also possible, opening the door to noninvasive testing. They suggest that this technique may eventually offer a way to detect low-level infection of *Plasmodium* and other pathogens in environments that do not allow for extensive equipment and processing.



Proteins Behave Differently on Curves, Straightaways

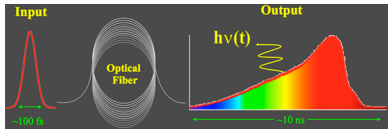
Several studies have demonstrated that the nanoscale geometry of substrates can affect the behavior of some proteins, a property that might be exploitable for a variety of biological and clinical applications such as biosensing, drug delivery, and creating new classes of biomaterials. For example, some studies have suggested that proteins adhered onto nanoparticles can change their structure and function in response to the nanoparticles' diameters. Other studies using curved substrates, including liposomes and single-walled carbon nanotubes, address the effects of catalytic enzymes and suggest that these surfaces stabilize proteins better by suppressing unfavorable lateral protein–protein interactions.

In a new study, Kurylowicz *et al.* (DOI: 10.1021/nn302948d) used a variation of atomic force microscopy, called single-molecule force spectroscopy (SMFS), to investigate whether surface curvature also affects favorable protein–protein interactions. As a model protein, the researchers used β-lactoglobulin (β-LG), a bovine milk protein that acts as an emulsifier at the oil–water interface. This protein is known to form a dimer in solution but dissociates into monomers below pH 3. Creating substrates with patches of polystyrene nanoparticles of diameters ranging from 25 to 190 nm interspersed with flat polystyrene films, the researchers found that the smaller nanoparticles resulted in fewer protein dimers. They propose that this effect results from each adsorbed protein having more space in the highly curved geometry of the smallest nanoparticles, leaving them less likely to interact with neighboring proteins. The authors suggest that eventually researchers may be able to take advantage of this effect to manipulate proteins' functional states.



New Spectroscopy Method Takes Flight

Researchers have become increasingly interested in understanding the optical properties of individual small objects. Consequently, numerous recent studies have focused on probing the emission spectra of many different objects on the nanoscale, including carbon nanotubes, metallic particles, biological molecules, and hybrid systems that combine molecules and particles. Several of these studies have worked toward developing correlated measurements of different optical properties on a single particle. Yet, these collective examinations are often missing analyses of the emission spectra, typically because single, nanosized particles emit intrinsically weak signals. Researchers have been lacking a method that allows spectral analyses during the course of gathering other spectroscopic data.



Seeking to fill this gap, Loumaigne *et al.* (DOI: 10.1021/nn304842c) developed a new spectroscopic technique based on photon time-of-flight that takes advantage of the wavelength dependence of the time-of-flight of a photon in a dispersive medium. Using an optical fiber as a spectrometer, the researchers collected data on photons' absolute times of arrival and their colors, providing spectral and temporal fluctuations of nanosized objects' emission from a single measurement. To demonstrate the effectiveness of this technique, dubbed time-of-flight photon spectroscopy (TOFPS), the scientists showed its ability to provide spectroscopic data on fluorescence of rhodamine B molecules in solution, Raman spectra of DNA-wrapped single-wall carbon nanotubes dispersed in water, and the luminescence spectra of gold nanorods. The researchers suggest that the ability to collect spectral and temporal information simultaneously could be used to sort particles of different sizes and shapes inside the same sample, in addition to a variety of other spectroscopic applications.

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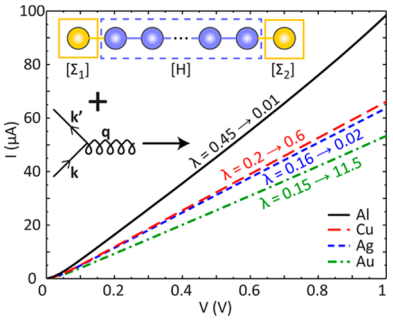
Aluminum a Better Conductor than Copper?

The properties of a given material are often vastly different at the nanoscale than they are in the bulk. Consequently, scientists are increasingly interested in studying quantum confinement of the thinnest of metallic nanowires, linear chains of metallic atoms. Both experimentally and theoretically, findings indicate that this extreme confinement causes oscillation in a material's conductance that correlates with wire length. Previous studies have suggested that, per atom, Al nanocontacts are better conductors than monovalent metals, and that Al could have lower resistivity than Cu because of the effects of surface and grain boundary scattering. However, there have been no definitive studies on which metal is the better conductor at such a small scale.

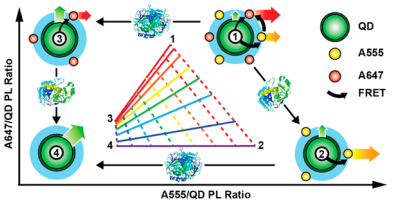
To help answer this question, Simbeck *et al.* (DOI: 10.1021/nn303950b) used first-principles density functional methods to study the electronic structure, electron–phonon coupling, and quantum transport properties of Ag, Al, Au, and Cu arranged in nanowires composed of 3–10 atoms attached to Au contacts. Through various calculations, the researchers found that the current through the Al atomic wire was higher than that of the other metals. Further analysis suggests that the reason for this surprising finding is that the number of current-carrying channels is higher in Al than for the three other metals studied. Additionally, the electron–phonon coupling constant is the lowest among these metals, with the value in the atomic nanowires reduced by a factor of 50 compared to bulk Al. The authors suggest that because atomic nanowires are the ultimate limit for conductors this finding could have important implications for miniaturizing electronics.



A Novel Way to FRET: Pass It on

Semiconductor quantum dots (QDs) have found use in a variety of biological research applications, including Förster resonance energy transfer (FRET). Investigators have used this technique as a molecular ruler to characterize QD bioconjugates, as a way to turn QD luminescence on and off for biosensing, or as a signal to release theranostic cargos from QD vectors. Typically, FRET configurations consist of a QD donor with an array of fluorescent dye or protein acceptors clustered around the central nanocrystal. Energy transfer takes place in a single step from the donor to the acceptor. Recently, some studies have explored the idea of FRET relays, which pass energy down a chain of acceptors.

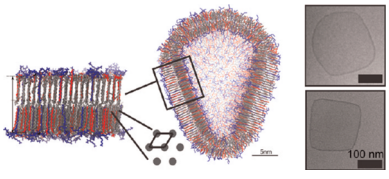
Taking this concept a step further, Algar *et al.* (DOI: 10.1021/nn304736j) designed a "concentric" FRET relay in which energy transfers from the QD to an acceptor, less efficiently to another receptor, and from the first receptor to a second. At the center of this system is a green-emitting CdSe/ZnS quantum dot, surrounded by multiple copies of two peptides. Each of these peptides is labeled with a different fluorescent dye, either A555 or A647, which were designed with recognition sites for one of two serine proteases, trypsin or chymotrypsin, respectively. Through a series of experiments, the researchers showed that this system could be used as a sensor for individual or multiplexed proteolytic activity, including the simultaneous activation of two different proteases and the activation of a zymogen to an active protease. The authors suggest that this system could be used in a variety of FRET applications that require multiplexed detection in a single vector.



pH Gives Membrane Its Shape

Many naturally occurring closed membranes, including those of viral capsids, bacterial microcompartments, and the envelopes that enclose halophilic organisms, have complex shapes such as icosahedra and other polyhedral geometries. Membranes displaying these multifaceted configurations are likely to have internal crystalline ordering. While it might be useful to copy these natural geometries for applications including catalysis, gene therapy, and targeted drug delivery, researchers still are not sure how the crystal structure within a membrane translates into a membrane's overall morphology.

In a new study, Leung *et al.* (DOI: 10.1021/nn304321w) explore this question by looking at the effects of electrostatic

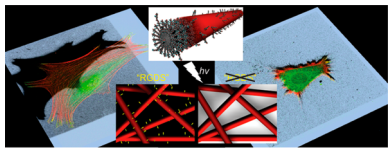


interactions between membrane components. The researchers used two oppositely charged amphiphiles with dissimilar headgroups but identical hydrophobic tails: the anionic fatty acid C_{16}–COOH and the cationic C_{16}–K_3. A series of experiments show that these components can be assembled together at different pH values to create membranes with varying, controllable shapes. At low and high pH values, the researchers found that the resulting membranes took on closed, faceted morphologies with two-dimensional hexagonal molecular arrangements, confirmed by transmission electron microscopy, small-angle X-ray scattering, and wide-angle X-ray scattering. At intermediate pH values, the membranes took on a ribbon-like morphology with rectangular-C packing. Molecular dynamics simulations add further insight to these configurations, suggesting that pH is controlling the membranes' morphology by changing the local crystalline order of the amphiphiles' hydrophilic tails. The authors suggest that these findings could add insight to how natural membranes form and eventually be used to design novel and useful nanoscale containers.

Nanofiber Matrix Sees the Light

The extracellular matrix not only supports cells but also plays a crucial role in cell development, maturation, and repair by sending biochemical and biophysical cues. These signals are highly dynamic throughout an organism's life, changing to account for needs as diverse as normal cellular operations to regeneration of whole organs or limbs in amphibian species. To mimic the extracellular matrix, researchers have used self-assembling peptides, including peptide amphiphiles (PAs), a broad family of materials consisting of peptide sequences covalently attached to hydrophobic segments such as alkyl tails. These PAs can spontaneously form filamentous assemblies in aqueous media that resemble the fibrous structures in the extracellular matrix. However, these PA matrices are missing the dynamic signaling native to the natural extracellular matrix.

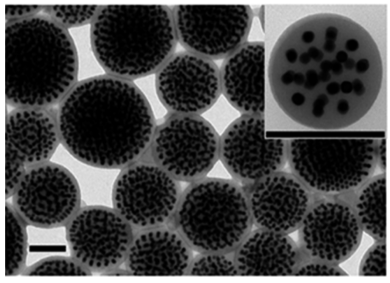
To include this feature, Sur *et al.* (DOI: 10.1021/nn304101x) incorporated a photocleavable nitrobenzyl ester group into the PAs' peptide backbone. They created several different variations of this material, some of which incorporated an epitope that has been well-studied for its role in cell adhesion. Fibroblasts cultured on PA nanofibers containing this epitope showed increased cell spreading and more mature focal adhesions compared to those without it, an effect demonstrated by immunostaining and cell morphological analyses. Further tests showed that when matrices containing this epitope were exposed to light, the vast majority of cells remained viable, but this fibroblast spreading arrested. In contrast, matrices in which the epitope was incorporated by a light-insensitive linker did not show this effect. The authors suggest that this method could be used to synthesize PAs incorporating other epitopes to control different types of cellular behavior dynamically.



For Au Nanoparticles, Hydrophobicity Breeds Togetherness

Creating new structures from nanosized building blocks requires a way to organize these components precisely in three dimensions, necessitating knowledge and tools to control their spatial distributions. One area in which such knowledge is not yet well-developed is that needed to control the size and shape of metallic nanoparticle clusters, their sensitivity toward environmental factors, and the precise distances between the nanoparticle building blocks. Although solvent-mediated self-assembly of these clusters has been intuitively associated with hydrophobic effects, exactly how these effects act on individual particles and using these forces to tailor the resulting structures remain challenges. Gaining a better understanding of these forces could lead to new strategies to fabricate nanoscale devices.

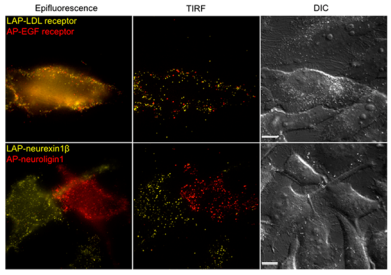
Sánchez-Inglesias *et al.* (DOI: 10.1021/nn3047605) add to the knowledge base by studying the solvent-induced, reversible self-assembly of gold nanoparticles into nanoclusters with controllable sizes, a phenomenon dominated by hydrophobic interactions. By adding water to a tetrahydrofuran (THF) solution containing Au nanoparticles stabilized with hydrophobic polystyrene chains, the researchers induce the nanoparticles to form clusters up to 904 nm in diameter. Theoretical examinations suggest that the main attractive force forming the clusters is hydrophobicity. In pure THF, brush repulsions between the polystyrene chains overcome van der Waals attractions between the nanoparticles. However, when water is added to the solution, the hydrophobic polymer chains compress and attract each other, expelling solvent and water into the bulk. By adding a stabilizing amphiphilic diblock copolymer, the researchers were able to control the sizes of the resulting nanoclusters. The authors suggest that harnessing hydrophobicity represents a new methodology for preparing Au nanoparticle clusters.



Lighting Up Proteins in a New Way

Quantum dots (QDs) are often used to visualize individual proteins on cells, using conjugated antibodies to home in on proteins of interest. However, this method is limited by the low affinity and/or specificity of many antibodies, as well as many antibodies' inability to recognize the extracellular portions of some proteins. To get around these problems, researchers recently developed an alternative way to target QDs to proteins based on biotin ligase and streptavidin. Although this method has been used to image numerous cellular proteins, having an orthogonal way to label proteins with quantum dots would make it possible to image multiple proteins in the same cell or in neighboring cells.

In a new study, the same research team of Liu *et al.* (DOI: 10.1021/nn304793z) detail a



new method for attaching quantum dots to proteins of interest. Their technique uses *E. coli* lipoic acid ligase to site-specifically attach 10-bromodecanoic acid onto an engineered 13-amino acid peptide, which can be fused onto the protein. By attaching a modified haloalkane dehalogenase called HaloTag to QDs, the researchers reacted this combination with the ligated bromodecanoic acid to covalently attach the QDs and protein. Experiments showed that this method could label a variety of proteins and could be used in combination with the formerly developed method for two-color quantum dot imaging of proteins on the same cell. They also demonstrate that both methods could track single molecules of a neural adhesion protein with its trans-synaptic adhesion partner. The authors suggest that this method could have wide-ranging applications beyond QD visualization.