Trapping single biomolecules in free solution

Adam Cohen and W. E. Moerner report on the successful suppression of Brownian motion of individual biomolecules in free solution. The authors used an anti-Brownian electrokinetic (ABEL) trap, which monitors the Brownian motion of a nanoparticle through fluorescence microscopy and applies a feedback voltage to a microfluidic cell so that the resulting electrophoretic and electroosmotic forces produce a drift that cancels the Brownian motion of the particle. The ABEL technology was used to trap individual particles of fluorescently labeled tobacco mosaic virus (TMV), measuring ~300 nm in length and 15 nm in diameter. Cohen and Moerner were also able to trap individual ~100-nm-diameter vesicles of egg-phosphatidylcholine that had been doped with one to two fluorescent lipid molecules to create a low fluorescent intensity. To trap objects <20 nm in diameter, a higher-viscosity trapping medium was used. By using a solution of 50% glycerol, single molecules of large proteins (GroEL and B-phycoerythrin) and single fluorescent CdSe nanocrystals were trapped. The ABEL technology may enable advanced biophysical measurements, such as providing a nonperturbative way to study the folding of single proteins in solution. — R.N.

“Suppressing Brownian motion of individual biomolecules in solution” by Adam E. Cohen and W. E. Moerner (see pages 4362–4365)

Interconnection of tropospheric ozone and sulfate

Nadine Unger et al. show that ozone (O3) precursor emissions can dramatically affect both air quality and climate forcing by increasing the levels of tropospheric sulfate (SO4). Ozone and sulfate are strongly coupled through their photochemistry; sulfate is generated by the oxidation of sulfur dioxide (SO2) by the hydroxyl radical (OH) or hydrogen peroxide (H2O2), both of which can be derived from O3. Likewise, O3 production requires the presence of nitrogen oxides, which sulfate can remove by conversion to nitric acid. Using an atmospheric composition–climate model, Unger et al. investigated how this interaction might affect future climate change. They found that, in 2030, increases in ozone precursor emissions will raise surface sulfate levels in several regions, including a 20% increase over the Indian subcontinent. In the model, ozone precursor emissions contribute 20% of the negative sulfate forcing in this region, which is more than twice the direct positive forcing of ozone itself. In contrast, changes in sulfate precursor emissions do not significantly affect future ozone levels. This interconnection between ozone and sulfate can complicate environmental efforts, because a reduction in ozone precursors would improve surface air quality but would also impose additional positive forcing through sulfate reduction. The authors suggest that future regulations should address ozone and sulfate simultaneously. — N.Z.

“Cross influences of ozone and sulfate precursor emissions changes on air quality and climate” by Nadine Unger, Drew T. Shindell, Dorothy M. Koch, and David G. Streets (see pages 4377–4380)
Resolution measure and single-molecule microscopy

Sripad Ram et al. developed a resolution measure that overcomes limitations encountered by applying Rayleigh’s criterion to single-molecule microscopy, providing a quantitative tool for designing and evaluating experiments probing biomolecular interactions. It is commonly held that Rayleigh’s criterion imposes a limit on the study of molecular interactions at distances of <200 nm. Yet, this criterion was formulated in a deterministic setting that neglects the stochastic nature of the photon detection process and thus fails to consider the total number of photons in the acquired data. Ram et al. worked within a stochastic framework and developed an alternative resolution measure, which is given in terms of quantities such as the expected number of detected photons, numerical aperture of the objective lens, and wavelength of the detected photons. This measure, unlike Rayleigh’s criterion, predicts that the resolution of a microscope can be improved by increasing the number of photons collected from the point sources. The authors presented experimental results in which distances from images of closely spaced single molecules were estimated. These results indicated that distances well below Rayleigh’s resolution limit could be determined with an accuracy as specified by the new resolution measure. — R.N.

“Beyond Rayleigh’s criterion: A resolution measure with application to single-molecule microscopy” by Sripad Ram, E. Sally Ward, and Raimund J. Ober (see pages 4457–4462)

Non-Watson–Crick mispairs in tRNA promote translation

William McClain reports that non-Watson–Crick mispairs in tRNA actually enhance aminoacylation and translation. Previous research has shown that non-Watson–Crick mispairs, such as G-U and A-C, are infrequent yet fundamental units of RNA secondary structure found in nearly every class of RNA. However, the functional importance of these mispair sites has not been well understood. McClain characterized six mutant G-U and A-C mispairs in Escherichia coli tRNA<sub>Gly</sub> and determined the in vivo aminoacylation specificity and translational property of the mutants. The mispairs boosted aminoacylation and translation primarily because they activated tRNA through their conformational flexibility. Conversion of the G-U mispair to the Watson–Crick G-C pair was shown to inactivate tRNA function. The author suggests that, because the six mispair sites are preserved across tRNA<sub>Gly</sub> in many organisms, a basic structure–function signature exists within tRNA<sub>Gly</sub>, with analogous functions in other RNAs. — F.A.

“Surprising contribution to aminoacylation and translation of non-Watson–Crick pairs in tRNA” by William H. McClain (see pages 4570–4575)

Alfalfa nodule bacterium illuminates vitamin B<sub>12</sub> synthesis

An unusually fluorescent bacterial colony has yielded insight into a previously unresolved step in the synthesis of vitamin B<sub>12</sub>, Gordon Campbell et al. report. Cobalamin, or vitamin B<sub>12</sub>, is synthesized only by certain bacterial species, such as Sinorhizobium meliloti, a bacterium that lives symbiotically within the roots of the alfalfa plant. Although most aspects of cobalamin synthesis are known, the source of cobalamin’s lower ligand, 5,6-dimethylbenzimidazole (DMB), is not well understood. In a genetic screen of S. meliloti mutants, Campbell et al. identified a highly fluorescent mutant with a severe symbiotic defect: it was unable to fix nitrogen when inoculated into alfalfa plants. The gene responsible for the mutant phenotype resembled the bluB gene from Rhodobacter capsulatus, which is involved in cobalamin synthesis. Treatment with vitamin B<sub>12</sub> restored the bacterium’s ability to establish symbiosis with alfalfa. The mutant produced cobalamin only when supplemented with DMB, suggesting that BluB is involved in the synthesis of DMB. The S. meliloti bluB gene’s role in DMB biosynthesis thus provides insight into this previously unsolved aspect of cobalamin synthesis. — M.M.

“Sinorhizobium meliloti bluB is necessary for production of 5,6-dimethylbenzimidazole, the lower ligand of B<sub>12</sub>” by Gordon R. O. Campbell, Michiko E. Taga, Kavita Mistry, Javier Lloret, Peter J. Anderson, John R. Roth, and Graham C. Walker (see pages 4634–4639)