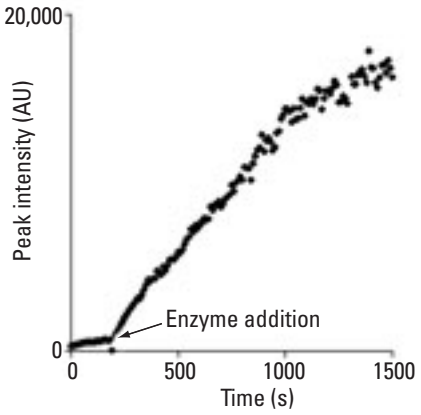
Masked substrates for enzyme detection by SERRS

Barry Moore, Duncan Graham, and their colleagues at the University of Strathclyde and the University of Edinburgh (both in the U.K.) have developed an enzyme activity assay based on surface-enhanced resonance Raman scattering (SERRS). The researchers estimate that the method can detect activity from as few as 500 enzyme molecules. This degree of sensitivity may enable scientists to detect in vivo levels of enzymatic activity within single cells.

The key to the new assay's high sensitivity is in the substrates, which contain an enzyme recognition site and a benzotriazole azo dye. The recognition site and dye are linked by an enzyme-cleavable spacer. Once an enzyme binds to the substrate and cleaves the spacer, the dye is released and adsorbs to silver nanoparticles, which results in a SERRS signal. Because the dye is initially masked, or blocked, the substrate does not produce a signal prior to enzyme cleavage.

The researchers tested lipases, proteases, and esterases from several fungal and bacterial organisms using the new SERRS assay. The signal varied, depending on the enzyme. This is the first time that SERRS has been demonstrated as a way to screen enzyme activities. The enzymes often displayed a selectivity for the chirality of the substrate. Many of the 14 enzymes hydrolyzed the *S*-enantiomer of a particular substrate at a faster rate than the *R*-enantiomer.

Comparing SERRS data with those from other methods is difficult, but enzyme activity ratios for *S*- and *R*-enantiomers were similar with SERRS and HPLC techniques. The new assay is also fast, and the researchers predict that the activity of all 14 enzymes can be tested by this method within 30 s if only one time point is taken. (*Nat. Biotechnol.* **2004**, *22*, 1139–1145)

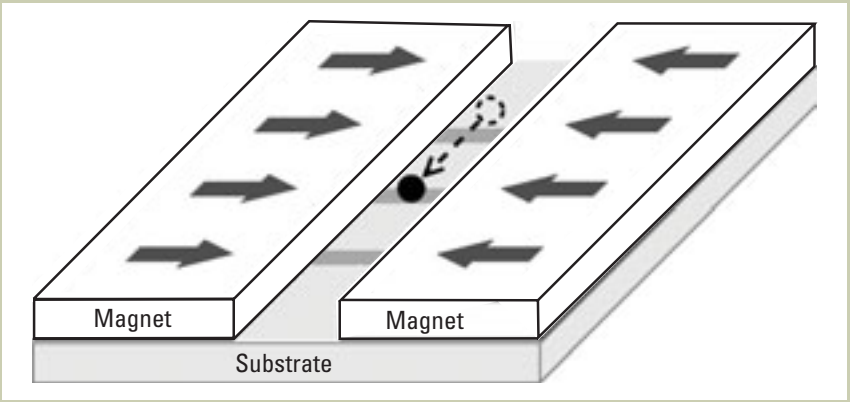


The SERRS signal intensity increases over time when an enzyme is added. (Adapted with permission. Copyright 2004 Macmillan Magazines Ltd.)

Levitating tiny particles and droplets

Igor Lyuksyutov and colleagues at Texas A&M University have developed a magnetic micromanipulation chip that can levitate pico- to femtoliter volumes of fluid and particles. The levitated particles and droplets can be merged, rotated, assembled, and translated on the chip. Such controlled manipulations of levitated particles and droplets could lead to advances in nanotechnology, crystal growth, and microfluidics.

Lyuksyutov and colleagues built the chip using micrometer-sized permanent magnets, which produced a magnetic field of 0.5 T. The droplets and particles had a small, induced magnetic moment opposite to the magnetic field. The force from the interaction between the small magnetic moment and the magnetic field caused the droplets and particles to levitate.



A magnetic microchip can levitate and manipulate small droplets and particles. (Adapted with permission. Copyright 2004 American Institute of Physics.)

The investigators levitated and manipulated a variety of substances on the chip, including alcohol solutions, oils, polystyrene microspheres, and red blood cells. Because the chip has planar, open surfaces, problems encountered in conventional microfluidic devices with solution and reagent flow in closed channels can be avoided. (*Appl. Phys. Lett.* **2004**, *85*, 1817–1819)