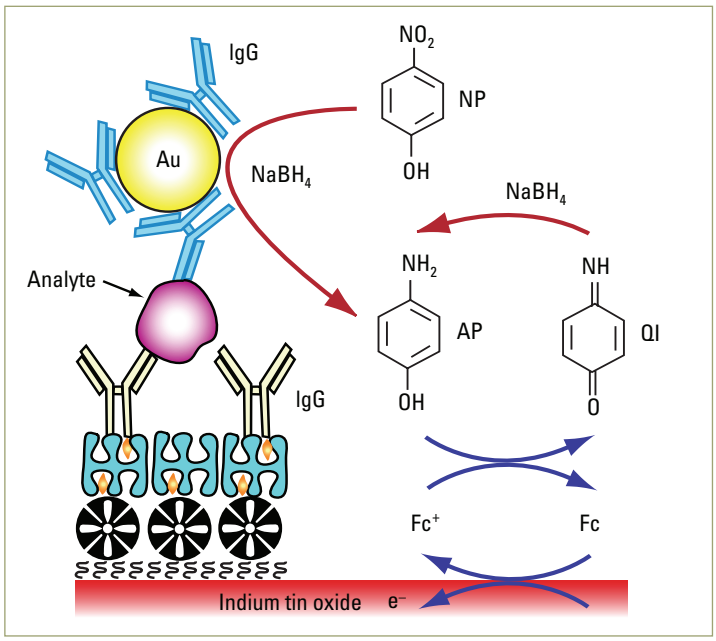
An ultrasensitive nanocatalyst assay for disease proteins

DNA-based amplification methods have subfemtomole sensitivity, but their practical use in clinical settings is limited by the complexity of detection procedures and conjugation chemistries. To avoid these issues, Haesik Yang and colleagues at Pusan National University in Korea have developed a simple, ultrasensitive electrochemical detection technique that uses gold-nanocatalyst labels.

The new assay is based on catalytic reduction of ρ-nitrophenol to ρ-aminophenol (AP) by gold nanoparticles, and the electrochemical signal is amplified by the chemical reduction of ρ-quinone imine to AP. Yang and colleagues prepared an immunosensing layer of biotinylated immunoglobulin G (IgG) immobilized to an indium tin oxide electrode that was modified with ferrocenyl-tethered dendrimer to help reduce the noise; this permitted slow electrooxidation of $NaBH_4$ and fast electrooxidation of AP. The investigators also made nanocatalyst particles by directly adsorbing IgG onto 10-nm gold nanoparticles, and a target protein bridged the IgG–nanocatalyst conjugate to the immunosensing layer, allowing detection.

Yang and colleagues tested the system with mouse IgG and prostate-specific antigen and found strong dependence of the anodic peak current on target protein concentration. The investigators determined a detection limit of 1 fg/mL for both analytes; this compared favorably with DNA-based ultrasensitive biomarker assays. (*J. Am. Chem. Soc.* **2006**, *128*, 16,022–16,023)



Schematic showing the electrochemical protein detection system, including modified indium tin oxide immunosensing layer, gold-nanoparticle–IgG conjugate, and signal-amplification reactions. NP, ρ-nitrophenol; QI, ρ-quinone imine.

Fast movements watched by 2D IR spectroscopy

Molecules in solution change their conformations very rapidly, making it very difficult to watch the process. Now Peter Hamm, Christoph Kolano, and colleagues at the University of Zurich and Ruhr-Universität Bochum (Germany) have demonstrated that 2D IR spectroscopy can track changes in conformations of a molecule over a few hundred picoseconds.

Spectroscopy in 1D measures the primary properties of vibrating groups in molecules, such as oscillation frequencies and dipole strengths. But when a biomolecule folds and unfolds, the interactions between different oscillators, known as secondary properties, are more interesting to observe. However, these effects can be a thousand times smaller than the primary properties and are difficult to measure by conventional spectroscopic methods.

Secondary properties can be measured by 2D optical spectroscopy using a process called differencing—one spectrum is subtracted from another taken under different conditions to correlate differences with secondary properties. Hamm, Kolano, and colleagues used a double differencing technique to study a cyclic peptide of four amino acids. The peptide had a weak disulfide bridge and an intramolecular hydrogen bond to hold the peptide in a rigid conformation with a β-turn.

The investigators initiated the peptide's unfolding by applying a picosecond UV pulse to break the disulfide bridge. As the molecule started to unfold, the investigators introduced increasingly longer delays before recording the 2D IR spectra so that they could continuously watch the peptide unravel.

Because the 2D IR spectra contained data from both unexcited and excited peptides, Hamm and colleagues simultaneously recorded two sets of spectra. The UV light was on in one and off in the other. When one set of spectra was subtracted from the other, the results were the transient 2D IR spectra that showed the changes in the peptide over a picosecond timescale. Thus, Hamm and colleagues could watch the weakening of the hydrogen bond and the opening of the β-turn. They determined that the rate of the unfolding process was 2 orders of magnitude faster than the "folding speed limit" established for contact between protein side chains. (*Nature* **2006**, *444*, 469–472)