# Advanced Immunochemical Biosensors and Assays: From Label-Free to Single-Molecule Detection

**Zdeněk Farka**
Department of Biochemistry, Faculty of Science, Masaryk University

The ability of rapid detection of low concentrations of analytes, in particular low-abundance biomarkers, pathogenic bacteria, and viruses, is of fundamental importance for early-stage disease diagnosis. Among many analytical techniques, immunoassays and immunosensors are gaining attention due to the high specificity provided by antibodies and excellent sensitivity provided by various readout techniques.

We have employed immunosensors for the detection of *Salmonella* Typhimurium, which belongs to leading agents of gastrointestinal diseases. The point-of-care sensor was based on electrochemical impedance spectroscopy and screen-printed electrodes modified by a specific antibody. We have further developed a signal enhancement by enzymatic precipitation for the highly sensitive laboratory-based detection of *Salmonella* using surface plasmon resonance. Alternatively, catalytic nanoparticles can be used for signal generation. We have introduced the method for the conjugation of catalytic Prussian blue nanoparticles with antibodies and demonstrated the universal applicability of the label by the development of sandwich nanozyme-linked immunosorbent assays for human serum albumin and *Salmonella*.

Photon-upconversion nanoparticles (UCNPs) are lanthanide-doped nanocrystals, which convert near-infrared light to light of shorter wavelengths. We have exploited UCNPs as a label in an upconversion-linked immunoassay for the detection of various analytes, including pharmaceutical diclofenac and honeybee pathogen *Melissococcus plutonius*. The low background and high photostability make UCNPs a powerful tool for the detection of single molecules. We have developed an optical approach for visualizing individual UCNPs and applied it for the sensitive detection of the cancer biomarker prostate-specific antigen. Furthermore, the unique optical properties make UCNPs suitable for cell imaging. We have employed the UCNPs for the labeling of HER2 biomarker on breast cancer cells. The minimum optical background and low non-specific binding provided a superior signal-to-background ratio compared to conventional fluorescent labeling.

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