# Plasmonic Single-Molecule Affinity Detection at $10^{-20}$ Molar

**Eleonora Macchia[1], Cinzia Di Franco[2], Cecilia Scandurra[3], Lucia Sarcina[3], Matteo Piscitelli[4], Michele Catacchio[1], Mariapia Caputo[1], Paolo Bollella[3], Gaetano Scamarcio[4,5], Luisa Torsi[3,†]**

[1]Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari Aldo Moro, Bari, 70125 Italy
[2]CNR IFN, Bari, 70125 Italy
[3]Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Bari, 70125 Italy
[4]Dipartimento Interateneo di Fisica, Università degli Studi di Bari Aldo Moro, Bari, 70125 Italy
[5]NEST, Istituto Nanoscienze – CNR and Scuola Normale Superiore, Pisa, I-56127 Italy
† corresponding author's email: luisa.torsi@uniba.it

DNA can be readily amplified through replication, enabling the detection of a single-target copy. A comparable performance for proteins in immunoassays has yet to be fully assessed. Surface-plasmon-resonance (SPR) serves as a probe capable of performing assays at concentrations typically around $10^{-9}$ molar. In this study, plasmonic single-molecule assays for both proteins and DNA are demonstrated, achieving limits-of-detections (LODs) as low as $10^{-20}$ molar (1 ± 1 molecule in 0.1 mL), even in human serum, in 1 h. This represents an improvement in typical SPR LODs by eleven orders-of-magnitude. The single-molecule SPR assay is achieved with a millimeter-wide surface functionalized with a physisorbed biolayer comprising trillions of recognition-elements (antibodies or protein–probe complexes) which undergo an acidic or alkaline pH-conditioning [1]. Potentiometric and surface-probing imaging experiments reveal the phenomenon underlying this extraordinary performance enhancement. The data suggest an unexplored amplification process within the biomaterial, where pH-conditioning, driving the biolayer in a metastable state, induces a self-propagating aggregation of partially misfolded proteins, following single-affinity binding. This process triggers an electrostatic rearrangement, resulting in the displacement of a charge equivalent to 1.5e per $10^2$ recognition elements. Such findings open new opportunities for reliable SPR-based biosensing at the physical detection limits, with promising applications in point-of-care plasmonic systems.

## References

[1] E. Macchia, C. Di Franco, C. Scandurra, L. Sarcina, M. Piscitelli, et al., Advance Materials, 2025, vol.37, 2418610.