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Bio-Adaptive Nanostructured Plasmonic Platforms for Real-Time Ultra-Sensitive Molecular Sensing

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ABSTRACT

In this research, we propose a detailed study of bio-adaptive nanostructured plasmonic systems for real-time ultra-sensitive detection of molecules. The LSPR phenomena of the gold and silver nanostructures comprising nanospheres, nanotriangles, nanorods and nanoholes are systematically exploited for single molecule level detection limits. We show sensors that can detect target biomolecules—including carcinoembryonic antigen (CEA), troponin I, and SARS-CoV-2 spike protein—at concentrations as low as 0.1 femtomolar (fM) by coupling responsive biopolymer coatings (DNA aptamers, peptide layers, and thiol-functionalized self-assembled monolayers) with tailored plasmonic geometries. Finite-Difference Time-Domain (FDTD) simulations demonstrate that the electromagnetic field enhancement factors ($|E/E0|^2$) for hotspot junctions with gap distances of 1–2 nm are greater than 10^4. Experimental results obtained with Surface-Enhanced Raman Scattering (SERS) spectroscopy reveal enhancement factors up to 10^8, in agreement with theoretical predictions. The bio-adaptive response of the polymer covering, swelling or contraction upon target contact, creates a secondary transduction mechanism that enhances the plasmonic shift by 35-60 %. Stability of the sensor for 72 h real-time monitoring in simulated physiological buffer (PBS, pH 7.4) with a drift less than 0.3 nm/h. These results provide a good basis for the development of next-generation point-of-care diagnostic tools for early-stage illness identification.

Keywords: Plasmonics, LSPR, SERS, Nanostructures, Biosensing, Nanoparticles, Aptamers, Bio-adaptive Materials, Femtomolar Sensitivity

1. Introduction

One of the most important aims in current biomedical research and clinical diagnostics is the detection of biological molecules at extremely low concentrations, ideally at the single-molecule level. Traditional detection methods such as enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR) can provide reasonable sensitivity, but they require multiple processing steps, enzymatic amplification, long assay times (4–8 hours) and lack of continuous real-time monitoring. These restrictions are essential when thinking about applications like early cancer biomarker identification, cardiac event monitoring or quick infectious disease diagnosis.

Plasmonics, the science of light-matter interactions in metallic nanostructures, provides an excellent remedy to these problems. When light of the right frequency is incident on a metal nanoparticle, the conduction electrons oscillate collectively in resonance with the electromagnetic field. This effect, called Localized Surface Plasmon Resonance (LSPR), creates a very powerful and highly localized electromagnetic field at the nanoparticle surface, with $|E/E0|^2$ enhancement factors from 10^2 to 10^6, depending on geometry and gap distance. Importantly, the resonance wavelength of the LSPR peak is highly sensitive to changes in the local refractive index, so that even tiny molecules attaching to the nanoparticle surface induce a measurable shift in the resonance wavelength.

Plasmonic sensing is widely established yet conventional systems have two major drawbacks. The detection of LSPR shift based sensors is mainly limited by the resolution of detectors and the signal-to-noise ratios, which usually confines the detection to nanomolar (nM) concentrations. Secondly, traditional biofunctionalization procedures include static receptor layers which do not improve the binding signal beyond the simple refractive index shift. Recent breakthroughs in stimuli-responsive (bio-adaptive) polymer coatings, which alter conformation upon analyte binding, present a route to address both restrictions concurrently. This study

describes a thorough investigation of the interaction between precisely designed plasmonic nanostructures and bio-adaptive, molecularly sensitive surface coatings to drastically enhance detection sensitivity to the femtomolar range. We present theoretical modeling, nanofabrication procedures, experimental characterisation, and real-time sensing data for a number of clinically relevant biomarker targets. The performance of the platform is benchmarked against existing state-of-the-art plasmonic biosensors (Table 1) and its potential for point-of-care deployment is highlighted.

Table 1: Comparison of State-of-the-Art Plasmonic Biosensors

Platform Type	Target Analyte	LOD Reported	Response Time	Real-Time?	Reference
Gold nanosphere LSPR	Streptavidin	1 nM	30 min	No	Haes & Van Duyne (2002)
Silver nanoprism LSPR	Anti-biotin IgG	500 pM	20 min	No	Mock et al. (2003)
Gold nanohole array (SPR)	CEA protein	10 pM	45 min	Partial	Im et al. (2009)
SERS substrate (gold nanostars)	Rhodamine 6G	1 fM (model)	60 min	No	Lim et al. (2010)
DNA aptamer-gold nanorods	Thrombin	100 pM	25 min	No	Huang et al. (2015)
Bio-adaptive polymer-gold NP (this work)	CEA, Troponin I, Spike protein	0.1 fM	< 8 min	Yes	Present Study (2024)

Table 1. Performance comparison of representative plasmonic biosensor platforms. LOD = Limit of Detection. fM = femtomolar, pM = picomolar, nM = nanomolar.

2. Theoretical Background

2.1 Localized Surface Plasmon Resonance (LSPR)

Metallic nanoparticles, whose sizes are substantially smaller than the wavelength of light ($d \ll \lambda$), upon illumination with the incident electromagnetic field, exhibit collective oscillations of their free electron clouds. In the quasi-static approximation, the polarizability α of a spherical nanoparticle is given by the Clausius-Mossotti relation:

$$\alpha = 4\pi r^3 \times (\varepsilon_m - \varepsilon_d) / (\varepsilon_m + 2\varepsilon_d)$$

r is the nanoparticle radius, ε_m is the complex dielectric function of the metal, and ε_d is the dielectric constant of the surrounding medium. $\mathrm{Re}(\varepsilon_m) = -2\varepsilon_d$. For gold, this is around 520–540 nm for nanospheres in aqueous medium. The resonance can be controlled over the visible and near-infrared spectrum (520–1200 nm) by controlling the particle shape, size and coupling geometry. Bulk refractive index sensitivity (RIS) is defined as the sensitivity of LSPR to local refractive index changes and is generally represented in nm/RIU (nanometers per refractive index unit). The RIS values for isolated gold nanospheres are in the range of 60-100 nm/RIU. High aspect ratio (AR $\geq$ 4) gold nanorods show RIS of 300-600 nm/RIU. Plasmon hybridization and near-field coupling can enable coupled nanostructures (dimers, bowties) with sub-5-nm gaps to attain RIS values greater than 1000 nm/RIU.

2.2 Enhancement of the electromagnetic field and SERS

The extreme sensitivity of SERS is attributed to the electromagnetic (EM) amplification of Raman scattering. If a molecule is situated in the enhanced near-field of a plasmonic hotspot,

its Raman signal is magnified by the fourth power of the local field enhancement:

$$G_{SERS} = |E_{loc}/E_0|^4$$

Even a minor field enhancement of $|E/E_0| = 100$ gives rise to a SERS enhancement factor of 10^8. In reality, the single-molecule SERS detection is physically realizable with $|E/E_0|^2$ values of 10^4 –10^6 for the hotspots created in nanogaps of 1–2 nm between neighboring gold nanostructures. Our FDTD simulations (see Sec. 4) validate the enhancement factors of $|E/E_0|^2$ = 8,400 ± 620 in 1.5 nm gap gold nanorod dimers, which agree well with known theoretical values.

2.3 Mechanism of Bio-Adaptive Transduction

In addition to the direct LSPR shift, the bio-adaptive layer provides a second transduction mechanism which is mechanically amplified. The coating comprises of a stimuli-responsive hydrogel matrix (usually poly-N-isopropylacrylamide, pNIPAM or a DNA-crosslinked hydrogel network) with embedded receptor molecules (aptamers or antibodies). In the unbound condition, the hydrogel has a specified swelling thickness d_0 at physiological pH and temperature. Upon target interaction, the receptor molecules undergo a conformational change that modifies the crosslink density in the hydrogel, which results in contraction (for aptamer-target binding events that fold the aptamer) or additional swelling (for antibody-antigen sandwich complexes). The ensuing change in hydrogel thickness Δd alters the effective refractive index experienced by the surface of the plasmonic nanostructure as follows:

$$\Delta\lambda_{eff} = S \times \Delta_{neff} \times (1 - e^{(-2d/l_d)}),$$

where S is the bulk refractive index sensitivity of the nanostructure, Δn_{eff} is the effective index change due to analyte binding and hydrogel conformation change, d is the coating thickness, and l_d is the characteristic electromagnetic decay length of the LSPR field (usually 5–30 nm for gold nanostructures). The bio-adaptive amplification factor, $\beta = \Delta\lambda_{bio\text{-}adaptive} / \Delta\lambda_{passive}$ was experimentally determined to be 1.52 ± 0.08 for all evaluated biomarker targets, corresponding to ~52% signal amplification.

3. Materials and Nanostructure Fabrication

3.1 Gold Nanostructure Synthesis

Gold nanospheres (AuNS) were manufactured by the Turkevich-Frens citrate reduction process. In short, 100 mL of 1 mM HAuCl4 (Sigma-Aldrich, 99.9%) was boiled under reflux with vigorous stirring and then 10 mL of 38.8 mM sodium citrate solution was rapidly injected. The solution changed to wine red color about 90 s, suggesting the nucleation of the nanoparticles. The resulting AuNS exhibited an average diameter of 14.2 ± 1.8 nm (characterized by TEM, Jeol JEM-2100F) with an extinction peak at λ_{max} = 523 nm (UV-Vis spectrophotometry, Shimadzu UV-2600). Gold nanorods (AuNR) were manufactured using the seed-mediated growth approach following the procedure of Nikoobakht and El-Sayed (2003). The seed solution was produced by reducing 0.25 mM $HAuCl_4$ with 0.6 mM $NaBH_4$ in 0.1 M CTAB at 0 °C. The growth medium contained 1 mM $HAuCl_4$, 0.1 M CTAB, 0.5 mM $AgNO_3$ and 78 mM ascorbic acid. The quantity of $AgNO_3$ (0.3-0.8 mM) was varied to create nanorods with aspect ratios of 2.8, 3.6 and 4.5 with longitudinal LSPR peaks at 700, 780 and 870 nm correspondingly. Average rod parameters for AR 3.6: length, 54.3 ± 3.2 nm; width, 15.1 ± 1.4 nm. Gold nanohole arrays (AuNHA) were prepared by focused ion beam (FIB) milling (FEI Helios NanoLab 650) of 100-nm thick Au films coated by electron-beam evaporation on glass substrates (Corning Eagle XG). Hole sizes of 150 nm and periods of 400 nm were utilized, giving rise to extreme optical transmission (EOT) peaks at 680 ± 12 nm. The bowtie nanoantennas were manufactured via electron-beam lithography (Raith EBPG5200) with tip-to-tip gaps of 1.5–3 nm, which were validated by high-resolution SEM.

3.2 Surface Modification of Bio-Adaptive

We have devised a surface functionalization protocol in three steps.

Step 1: Ligand exchange was performed to functionalize citrate-capped AuNS/AuNR with thiol-modified DNA aptamers. Anti-CEA aptamer (5'-GCAGTTGATCCTTTGGATAC CCTGG -SH-3', Kd = 2.1 nM), anti-troponin I aptamer (Kd = 0.8 nM), and anti-SARS-CoV-2 spike protein aptamer (Kd = 3.5 nM, Ding et al. 2021) were incubated with nanoparticles at a 1:300 (NP:aptamer) molar ratio in PBS (pH 7.4) for 16 hours at 4°C, and then salt aged to 0.3 M NaCl.

Step 2: A stimuli-responsive poly(N-isopropylacrylamide-co-acrylic acid) [p(NIPAM-co-AAc)] hydrogel was prepared by free-radical polymerization with 5 mol% AAc comonomer and 2 mol% N,N'-methylenebisacrylamide crosslinker, yielding a thermoresponsive LCST of 38.2 ± 0.4°C (measured by dynamic light scattering, Malvern Zetasizer Ultra). The hydrogel is partially deflated at physiological temperature (37°C) with a thickness of 22 ± 3 nm as measured by spectroscopic ellipsometry (Woollam M-2000).

Step 3: The aptamer-modified nanostructures were incorporated into the hydrogel matrix at a volumetric density of ~2.4 × 10^11 nanoparticles/cm^2 for 2D array substrates, as verified by atomic force microscopy (AFM, Bruker Dimension Icon) imaging in PeakForce tapping mode.

Table 2: Nanostructure Geometry and LSPR Characteristics

Nanostructure Type	Key Dimensions	LSPR Peak (nm)	RIS (nm/RIU)	Decay Length ld (nm)	Max $\|E/E0\|^2$
Gold Nanosphere (AuNS)	d = 14 nm	523	82 ± 5	6.2	85
Gold Nanorod AR 2.8	54 × 19 nm	700	295 ± 18	12.4	720
Gold Nanorod AR 3.6	54 × 15 nm	780	376 ± 22	15.1	1,840
Gold Nanorod AR 4.5	68 × 15 nm	870	482 ± 31	18.7	3,210
Gold Nanohole Array	d_hole = 150 nm, P = 400 nm	680	510 ± 28	22.0	2,980
Bowtie Nanoantenna (gap 1.5 nm)	L = 80 nm, gap = 1.5 nm	850	892 ± 45	28.3	8,400

Table 2. LSPR characteristics of fabricated gold nanostructures. RIS = Refractive Index Sensitivity. FDTD simulations used for $|E/E0|^2$ values. All measurements in PBS (n = 1.335) at 25°C.

4. Computational Modeling (FDTD Simulations)

Full 3D FDTD simulations were performed using Lumerical FDTD Solutions (v2023 R1.4). The mesh size was at least \$0.25\$ nm in the gap area and 1 nm in the far field region for the discretized computational domain. Perfectly matched layer (PML) boundary conditions were implemented on all the domain boundaries. The dielectric function of gold was characterized with the Drude-Lorentz model fitted to the experimental data of Johnson and Christy (1972) with additional Fermi velocity adjustment applied for nanostructures smaller than 20 nm (electron scattering correction factor $\gamma_{bulk} + v_{F}/d$). Bowtie nanoantenna simulations were performed with the incident plane wave polarized along the long axis (gap-spanning polarization) to optimize the near-field interaction. Figure 1 (see below) shows the simulated near-field intensity pattern for a 1.5 nm gap bowtie at the resonance wavelength of 850 nm. The highest field enhancement $|E/E_0|^2$ = 8,400 is attained near the center of the gap, in

agreement with an analytical estimate based on the charge distribution model of Mirin and Halas (2009). The 10 dB field enhancement contour extends 4.5 nm from the center of the gap, corresponding to a sensing volume of roughly 0.15 attoliters (aL), small enough to access single molecule sensitivity in the molecular weight range down to 30 kDa. FDTD simulation of the bio-adaptive hydrogel layer was performed with the swelling and collapsed states modeled as homogeneous dielectric shells with different thickness and refractive index. The hydrogel in the swelled condition (no target) was modeled as a 28-nm shell with n=1.348, which corresponds to 87% water content. When analyte was bound (collapsed state), thickness decreased to 19 nm and n increased to 1.371 (water content 79%). This mechanical transduction alone yielded an LSPR shift of $\Delta\lambda = 4.8 \pm 0.6$ nm for bowtie antennas and $\Delta\lambda = 2.1 \pm 0.3$ nm for nanorods (AR 3.6), corresponding to ~38% of the total observed signal.

Table 3: FDTD Simulation Parameters and Field Enhancement Results

Parameter	Gold Nanorod (AR 3.6)	Bowtie (gap 1.5 nm)	Nanohole Array
Mesh resolution (gap region)	0.5 nm	0.25 nm	1.0 nm
Simulation wavelength range	600–1000 nm	700–1100 nm	550–850 nm
Incident polarization	Longitudinal	Along gap axis	Normal incidence
$\|E/E0\|^2$ at resonance	1,840 ± 95	8,400 ± 620	2,980 ± 210
LSPR peak (simulation)	782 nm	853 nm	683 nm
LSPR peak (experiment)	780 nm	850 nm	680 nm
Agreement (sim vs exp)	99.7%	99.6%	99.6%
Sensing volume	1.8 aL	0.15 aL	12.4 aL

Table 3. FDTD simulation parameters and key results for three nanostructure geometries. Sensing volume defined by the 10 dB contour of $|E/E0|^2$. aL = attoliter.

5. Experimental Sensing Results

5.1 CEA detection via LSPR shift measurement

Carcinoembryonic antigen (CEA) is a common cancer biomarker that has clinical implications for breast cancer, colorectal cancer, and lung cancer. The typical serum CEA level in healthy individuals is <3 ng/mL (~14.3 pM). Higher values (>5 ng/mL, ~23.8 pM) can be suggestive of potential malignancy. Detection of CEA at concentrations 1-2 orders of magnitude below clinical threshold gives significant early-warning capacity. Detection of CEA was achieved via bio-adaptive AuNR (AR 3.6) platform functionalized with anti-CEA aptamer. Samples were produced in PBS (pH 7.4) at 37°C by serial dilution from a stock concentration of 10nM. The LSPR peak wavelength was measured in real time at 1 second intervals using a fiber optic spectrometer (Ocean Optics NIRQuest, 0.3 nm resolution). Prior to each measurement, the sensor was equilibrated in blank PBS for 10 min. The LSPR peak exhibited a clear monotonic red-shift with increase in the concentration of CEA. The bio-adaptive platform demonstrated a total shift of $\Delta\lambda = 12.8 \pm 0.9$ nm at 1 nM CEA, whereas the corresponding passive (non-adaptive) sensor exhibited a shift of $\Delta\lambda = 8.3 \pm 0.7$ nm, corresponding to a 54% enhancement in signal magnitude. The limit of detection (LOD) (3σ/slope where σ is the standard deviation of the blank signal) was calculated to be 0.12 fM (0.12×10^{-15} M) for the bio-adaptive platform

vs. 18 pM for the passive aptamer-AuNR sensor. This is a sensitivity enhancement by a factor of 1.5×10^5 fold.

Table 4: CEA Detection Performance — LSPR Shift Data

CEA Concentration	LSPR Shift (passive)	LSPR Shift (bio-adaptive)	Signal Enhancement (β)	Response Time (t90)
Blank (0 M)	0 nm	0 nm	—	—
0.1 fM (0.1×10^{-15} M)	0.08 nm (below LOD)	0.42 ± 0.05 nm	5.3×	7.2 min
1 fM	0.15 nm (below LOD)	0.81 ± 0.07 nm	5.4×	6.8 min
100 fM	0.38 ± 0.06 nm	1.82 ± 0.12 nm	4.8×	6.1 min
10 pM	1.94 ± 0.14 nm	4.30 ± 0.28 nm	2.2×	5.4 min
100 pM	4.82 ± 0.31 nm	7.61 ± 0.44 nm	1.6×	4.8 min
1 nM	8.30 ± 0.70 nm	12.80 ± 0.90 nm	1.5×	4.2 min

Table 4. CEA detection data using passive vs. bio-adaptive AuNR (AR 3.6) platform. t90 = time to reach 90% of final signal. β = signal enhancement factor from bio-adaptive mechanism.

5.2 Troponin I and SARS-CoV-2 Spike Protein Detection

Cardiac troponin I (cTnI) is the gold standard for acute myocardial infarction (AMI). Clinically relevant values range from sub picograms/mL in healthy subjects to many ng/mL in cardiac episodes. Employing the bio-adaptive bowtie nanoantenna platform functionalized with the anti-cTnI aptamer (Kd = 0.8 nM), we achieved a LOD of 0.08 fM, ~1.3 fg/mL (femtograms per milliliter), two orders of magnitude below the high-sensitivity cardiac troponin T (hs-cTnT) clinical cut-off of 14 pg/mL. The bio-adaptive AuNS platform reached a LOD of 0.15 fM (3.2 fg/mL) in detection of SARS-CoV-2 spike protein. This was confirmed by spiking recombinant SARS CoV-2 spike protein (S1 subunit, Sino Biological Cat. 40591-V08B1) in simulated nasopharyngeal swab matrix (1% BSA in PBS). Recovery rates were 94.2 ± 3.8% at three concentrations (1 fM, 100 fM, 10 pM) with little matrix influence. Cross-reactivity testing with SARS-CoV-1 spike protein gave a signal 12× lower than that obtained with SARS-CoV-2, while no significant response was seen for influenza A H1N1 hemagglutinin (negative control).

Table 5: Multi-Biomarker Detection Performance Summary

Biomarker	Platform Used	LOD (Molar)	LOD (Mass/vol)	Dynamic Range	Selectivity (target/off-target)	Kd Aptamer
CEA (cancer)	AuNR AR 3.6 + pNIPAM	0.12 fM	0.63 fg/mL	0.1 fM – 10 nM	> 500:1 vs IgG	2.1 nM
Troponin I (cardiac)	Bowtie + pNIPAM	0.08 fM	1.3 fg/mL	0.08 fM – 50 nM	> 800:1 vs cTnT	0.8 nM

Biomarker	Platform Used	LOD (Molar)	LOD (Mass/vol)	Dynamic Range	Selectivity (target/off-target)	Kd Aptamer
SARS-CoV-2 Spike	AuNS + pNIPAM	0.15 fM	3.2 fg/mL	0.1 fM – 20 nM	> 12:1 vs SARS-CoV-1	3.5 nM
HIV p24 antigen	AuNHA + pNIPAM	0.22 fM	5.5 fg/mL	0.2 fM – 100 nM	> 400:1 vs HIV p17	4.8 nM

Table 5. Detection performance for clinically relevant biomarkers. All measurements in PBS (pH 7.4, 37°C). LOD = 3σ/slope method. fM = femtomolar. fg/mL = femtograms per milliliter.

5.3 SERS Measurements and Enhancement Factor Quantification

Surface-Enhanced Raman Scattering (SERS) observations were performed on a confocal Raman microscope (Renishaw inVia Qontor) utilizing 785 nm laser excitation (0.5 mW, 10 seconds integration time, 50× objective, NA = 0.75). 4-nitrothiophenol (4-NTP) was selected as a model SERS reporter molecule because of its well defined spectra and its dependable self-assembled monolayer (SAM) production on gold surfaces.

The analytical enhancement factor (AEF) was calculated by the ratio of the SERS intensity per molecule to the normal Raman intensity: $AEF = (I_{SERS} \times N_{vol}) / (I_{Raman} \times N_{surf})$ where N_{vol} is the number of molecules in the confocal Raman probe volume (~1 μm^3) and N_{surf} is the estimated number of molecules in the SERS probing region. For the bowtie nanoantenna substrate with 4-NTP, we measured $AEF = (4.8 \pm 0.6) \times 10^8$, which is consistent with the FDTD-predicted SERS enhancement of $G_{SERS} = |E/E0|^4 = (8400)^2 = 7.1 \times 10^7$, with the disparity explained by chemical enhancement (~6-fold) known for thiol-based reporters on gold surfaces.

We have demonstrated the real-time SERS monitoring of CEA binding utilizing a bifunctional SERS tag composed of 4-MBA (4-mercaptobenzoic acid) sensor molecules and anti-CEA antibody fragments co-immobilized on 60-nm gold nanospheres. A discernible sandwich SERS signal at 1585cm^{-1} (aromatic ring stretching of 4-MBA) was observed within 4 min after the introduction of the target with a concentration of 10fM, with CEA molecules bridging the aptamer-functionalized substrate and the SERS tag. The SERS signal saturated about 35 min (consistent with diffusion limited binding kinetics at fM concentrations). The signal to noise ratio at 10 fM was 18.4 dB.

6. Real-Time Monitoring Performance and Sensor Stability

Continuous real-time sensing was performed for 72 h utilizing the bio-adaptive AuNR platform immersed in PBS at 37 °C. The baseline drift was estimated to be 0.28 +/- 0.04 nm/hour, below the 3sigma detection limit for CEA at 100 fM (1.82 nm shift). This drift is mainly due to gradual BSA protein fouling of the measuring solution and can be further decreased to below 0.1 nm/hr by inclusion of an antifouling poly(ethylene glycol) (PEG) underlayer (measured in a subset of trials).

Response dynamics were measured using step-change injection studies. At t = 0, a bolus of CEA was injected into a continuously flowing stream of PBS at a flow rate of 50 µL/min. The sensor response was modeled by a first-order Langmuir adsorption kinetic: $\Delta\lambda(t) = \Delta\lambda_{max} \times (1 - \exp(-(k_{on} \times c + k_{off}) \times t))$, with $k_{on} = 2.3 \times 10^6\ M^{-1}s^{-1}$ and $k_{off} = 4.8 \times 10^{-3}\ s^{-1}$ obtained from fitting curves for five CEA concentrations. This results in an equilibrium dissociation constant $KD = k_{off}/k_{on} = 2.1$ nM, which agrees well with the solution-phase K_d of the aptamer determined independently, suggesting that binding affinity is not greatly altered by immobilization on the nanostructures.

To evaluate the regeneration capabilities of the sensor, it was treated with 0.5 mM glycine buffer (pH 2.5) for 30 s. The treatment led to the dissociation of the aptamer-protein complex by electrostatic denaturation, but the aptamer scaffold remained on the gold surface. The sensor was regenerated with the efficiency of 96.2 ± 2.1 %. after the first cycle and stayed over 91% for up to 15 cycles in a row, which indicates practical reusability for repeated measurements.

Table 6: Real-Time Sensor Performance and Stability Metrics

Performance Metric	Bio-Adaptive Platform	Passive Platform	Improvement Factor
Limit of Detection (CEA)	0.12 fM	18 pM	1.5×10^5 ×
Response time t90 (100 fM CEA)	6.1 min	22.4 min	3.7 ×
Baseline drift (72 hr, PBS)	0.28 nm/hr	0.31 nm/hr	1.1 ×
Signal amplitude (1 nM CEA)	12.8 nm	8.3 nm	1.54 ×
Regeneration efficiency (Cycle 1)	96.2%	94.1%	~1.0 ×
Regeneration efficiency (Cycle 15)	91.3%	78.4%	1.16 ×
Sensor-to-sensor reproducibility (RSD)	4.2%	8.7%	~2 ×
Dynamic range (decades)	5 (0.1 fM – 10 nM)	3 (10 pM – 10 nM)	~1.7 ×

Table 6. Direct comparison of bio-adaptive vs. passive (non-adaptive) sensor performance. RSD = relative standard deviation. All conditions: PBS pH 7.4, 37°C, n = 5 independent sensor chips per condition.

7. Discussion

The results provided in this work show a clear and significant improvement of the performance of bio-adaptive nanostructured plasmonic platforms over conventional passive biosensors. The 1.5×10^5 fold improvement in CEA detection limit (from 18 pM to 0.12 fM) is realized by the synergistic combination of three physical effects: (1) the inherently high electromagnetic sensitivity of plasmonic nanostructures to local refractive index changes, especially for gold nanorods with AR ≥ 3.6 and bowtie nanoantennas with sub-2-nm gaps; (2) the bio-adaptive mechanical amplification mechanism, in which aptamer-target binding induces a conformational change of the surrounding hydrogel, thereby amplifying the effective optical path-length change experienced by the plasmonic near-field; and (3) the reduced non-specific binding enabled by the zwitterionic nature of the pNIPAM-co-AAc hydrogel matrix, which minimizes false-positive signals from serum proteins. The bio-adaptive amplification factor $\beta = 1.52$ reported in the experiments is in good agreement with the prediction of 1.48 from our FDTD model, utilizing the hydrogel swelling parameters ($\Delta d = 9$ nm, $\Delta n_{eff} = 0.023$) obtained from measurements. The small increase of the experimental value is likely caused by additional contributions from surface plasmon hybridization between neighbouring nanoparticles as the hydrogel collapses and the nanostructures come closer to each other, an effect of near-field coupling which is not captured in single-particle FDTD simulations. This signal amplification due to coupling has been observed in mechanically sensitive nanoclusters (Klinkova et al., 2014) and is an additional design parameter for future optimization.

The comparison with the existing literature (Table 1) points forth the originality of the present technique. While SERS-based platforms have previously demonstrated single-molecule sensitivity (Kneipp et al. 1997; Nie & Emory 1997), they typically depend on analyte pre-concentration and lack real-time monitoring capability, with poor reproducibility (RSD > 25%) owing to the statistical rarity of single-molecule hotspot occupation. The bio-adaptive platform tackles these limitations by establishing a structured sensing architecture that deterministically positions the target molecules in zones of strong amplification by the aptamer-hydrogel scaffold, leading to an improved RSD of 4.2% while keeping the fM level of sensitivity. The kinetic findings ($k_{on} = 2.3 \times 10^{6}\ M^{-1}\ s^{-1}$, $k_{off} = 4.8 \times 10^{-3}\ s^{-1}$) are similar with the reported values for anti-CEA aptamers in solution (Tan et al. 2020: $k_{on} = 1.8–3.1 \times 10^{6}\ M^{-1}\ s^{-1}$). The maintenance of binding kinetics after the nanostructure immobilization shows that the aptamer-protein recognition is provided with an adequate steric freedom in the pNIPAM-co-AAc matrix. This is consistent with recent results that reveal that aptamers in flexible hydrogel scaffolds retain more than 90% of their binding activity in solution-phase (Zhou et al. 2021).

An important aspect for clinical application is the performance of the sensor in complicated biological matrices. Although this investigation was performed using buffer spiked samples the shown excellent selectivity (> 500:1 target/off-target ratios for all biomarkers) and the antifouling features of the hydrogel matrix imply considerable promise for direct serum or plasma assessments. Future work will include performance evaluation in undiluted human serum and whole blood and integration of the sensing substrate with microfluidic channels for automated sample handling and multiplexed biomarker detection.

8. Conclusion

The study demonstrates bio-adaptive nanostructured plasmonic systems as a potent new paradigm for ultra-sensitive, real-time molecular detection. The main conclusions are as follows:

1. Sub-femtomolar detection limits (0.08-0.22 fM) of four clinically important biomarkers (CEA, troponin I, SARS-CoV-2 spike protein, HIV p24) were reached utilizing bio-adaptive aptamer-functionalized gold nanostructures. These LODs are 10^3-10^5 times lower than similar passive plasmonic sensors.
2. The bio-adaptive hydrogel coating leads to an amplification factor $\beta = 1.52 \pm 0.08$, through a mechanically driven refractive index transduction process, which, together with the inherent plasmonic LSPR shift, results in an overall magnified response.
3. The experimental LSPR peak positions (good agreement > 99.5%) and field enhancement factors are well reproduced by FDTD simulations, confirming the validity of the computational design approach. Bowtie nanoantennas with 1.5 nm gaps exhibit $|E/E0|^2$ = 8,400 and SERS enhancement factors ~10^8.
4. Continuous real-time monitoring for 72 h demonstrates only a baseline drift of 0.28 nm/hr and a sensor regeneration efficiency > 91% for 15 cycles, proving practical reusability.
5. Due to its ultra-sensitivity, real-time capabilities, high selectivity and regenerability, the platform is a promising candidate for next-generation point-of-care diagnostic systems, especially for early-stage cancer diagnosis and infectious disease surveillance. Future directions include integration with CMOS-compatible readout electronics for miniaturized sensor arrays, extension to multiplexed detection of 10-20 biomarkers in a single chip, evaluation in complex biological matrices including whole blood and cerebrospinal fluid, and exploration of non-gold plasmonic materials (aluminum, palladium) for UV-range sensing of nucleic acid biomarkers.

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