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Imprinted Au-Nanoparticle Composites for the Ultrasensitive Surface Plasmon Resonance Detection of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

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The analysis of explosives attracts recent research efforts due to homeland security needs and the broad demand for the clearance of minefields. While numerous studies have addressed the development of sensing platforms for nitroaromatic explosives, and specifically trinitrotoluene (TNT), the detection of more hazardous explosives, such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) or pentaerythritol tetranitrate (PETN), is less developed and needs further efforts, particularly the improvement of the sensitivities associated with the analyses of these substrates.[1,2] Different optical, electrochemical, or microgravimetric sensors or biosensors for TNT were reported. Fluorescent organic polymers, which are quenched by nitroaromatic explosives,[3,4] luminescent polysilole nanoparticles (NPs),[5,6] or fluorescent silicon NPs quenched by nitroaromatic vapors enabled the development of optical sensors. The electrochemical activity of the nitro groups of TNT provided the basis for developing voltammetric sensors for this explosive,[7,8] and recently, a composite of Au NPs linked to electrodes enabled a sensitive electrochemical detection of TNT.[9] Also, different sensing matrices, such as cyclodextrin polymers,[10] carbowax,[11] or silicon polymers,[12] were used for TNT analysis by surface acoustic wave devices, and the aggregation of functionalized Au NPs in the presence of TNT was used to develop an optical sensor for the explosive.[13] Similarly, antibody-based optical[14–16] or microgravimetric quartz-crystal-microbalance[17] biosensors for TNT were developed. Different optical[18,19] or voltammetric[20] sensors for RDX were also reported. These included the fluorescence detection of RDX with an acridinium dye,[18] or the application of NADH-functionalized quantum dots.[21] Also, a competitive fluorescence immunoassay for the detection of RDX was reported.[22] The sensitivities accomplished by these methods are, however, unsatisfactory for analyzing trace amounts of the RDX explosive.

Surface plasmon resonance (SPR) is a versatile method for probing changes in the refractive index occurring on thin metal films as a result of recognition events or chemical reactions.[23,24] Numerous SPR sensors and biosensors were developed,[25–27] and metal NPs were implemented to enhance the SPR response and to amplify SPR-based sensors.[28,29] The electronic coupling between the localized plasmon of the metallic NPs (e.g., Au NPs) and the surface plasmon wave enhances the SPR response and, thus, the labeling of recognition complexes with metallic NPs amplifies the sensing events. Different biosensing processes, such as DNA hybridization,[30] formation of immunocomplexes,[31] and the probing of biocatalytic transformations,[32] used Au NPs as labels for amplified SPR analyses. Recently, composites of bisaniline-crosslinked Au NPs were electropolymerized on Au electrodes, and the resulting matrices were used for the ultrasensitive SPR detection of TNT.[33] The formation of π-donor–acceptor complexes between TNT and the π-donor bisaniline bridging units altered the dielectric properties of the Au-NP composites. This affected the coupling between the localized plasmon of the NPs and the surface plasmon wave, resulting in a shift in the surface resonance spectrum (reflectance changes), that enabled the optical readout for analyzing TNT. Theoretical modeling of the SPR shifts indicated that the charge-transfer complexes between TNT and the bisaniline bridging units altered the dielectric functions of the Au-NP composite, and this enabled the highly sensitive detection of TNT.[33]

Here, we report on the ultrasensitive SPR detection of RDX by the composites of bisaniline-crosslinked Au NPs associated with a Au surface (detection limit 12 fM). Specifically, we demonstrate that electropolymerization of the Au NPs in the presence of Kemp's acid yields an imprinted composite with high binding affinities for RDX. This imprinting leads to the selective and sensitive detection of this explosive by SPR.

Au NPs, 3.5 nm, were functionalized with a capping mixed monolayer consisting of thioaniline electropolymerizable units and mercaptoethane sulfonic acid to enhance the solubility of the NPs in an aqueous medium. The functionalized Au NPs were electropolymerized onto a thioaniline-monolayer-modified Au electrode, to yield the matrix of bisaniline-crosslinked Au NPs (Fig. 1A). Ellipsometry and coulometric analyses of the matrix of bisaniline-crosslinked Au NPs, generated by the application of ten electropolymerization cycles, indicated that the thickness of the matrix corresponded to ca. 10 nm and that ca. 4×10^{14} bisaniline units cm^{-2} were electropolymerized on the electrode. Knowing the size of the Au NPs and the thickness of the composite, we estimate that approximately three random densely packed Au-NP layers compose the matrix. Complementary AFM measurements indicated that the height of the Au-NP composite is ca. 12 ± 1 nm.

The π-donor bisaniline units bridging the Au NPs could, then, associate RDX (that includes π-acceptor nitro groups), via

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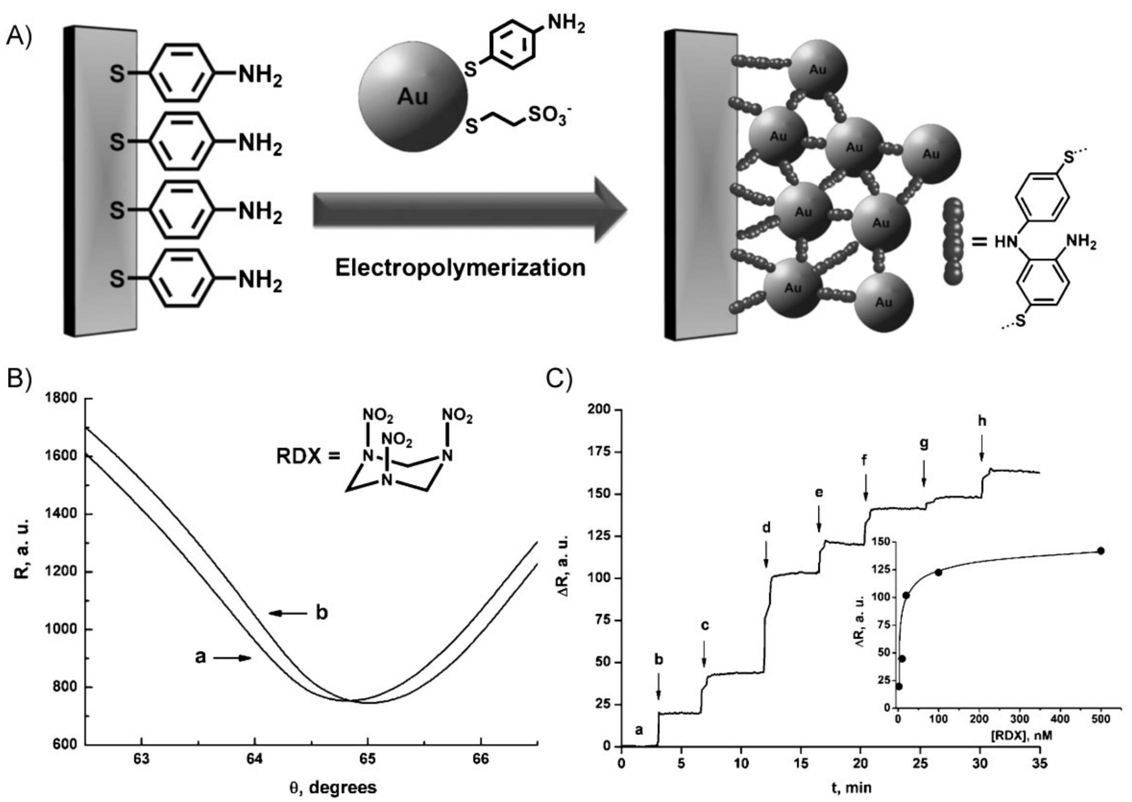
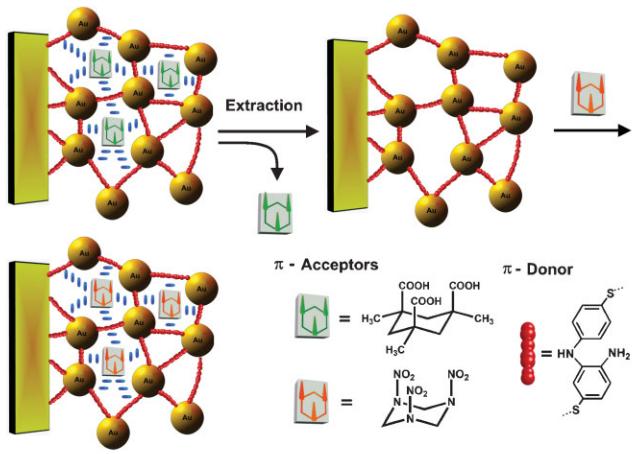


Figure 1. A) Schematic presentation for the electropolymerization of a composite of bisaniline-crosslinked Au NPs for the sensing of RDX by π-donor–acceptor interactions. B) SPR curves corresponding to the bisaniline–crosslinked Au-NP composite: (a) before the addition of RDX and (b) after the addition of 20 nM RDX. C) Sensogram corresponding to the changes in the reflectance intensities, at a constant angle $\theta = 63.3^\circ$, upon addition of variable concentrations of RDX: (a) 0 nM, (b) 4 nM, (c) 10 nM, (d) 20 nM, (e) 100 nM, (f) 500 nM, (g) 2 μM, and (h) 10 μM. Inset: calibration curve relating the reflectance changes to the concentrations of RDX. All measurements were performed in a 0.1 M HEPES buffer solution of pH 7.2.

π-donor–acceptor interactions. The resulting charge-transfer complexes are anticipated to alter the dielectric properties in the Au-NP matrix, resulting in an amplified shift in the SPR spectrum due to the coupling between the localized NPs plasmons and the surface plasmon wave. Figure 1B shows the SPR curves of the modified surface of Au-NP-crosslinked composites before (curve a) and after (curve b) treatment with RDX, 20 nM. The SPR curve is shifted, suggesting that the association of RDX to the matrix can be monitored by the reflectance changes of the SPR spectrum. Figure 1C shows the sensogram corresponding to the reflectance changes of the modified surface upon treatment with variable concentrations of RDX and the resulting calibration curve (Fig. 1C, inset). The reflectance changes increase upon elevating the concentration of RDX and level off to a saturation value at a RDX concentration corresponding to ca. 100 nM. This result is consistent with the fact that saturation of the π-donor sites with RDX leads to a constant reflectance value. Using the Langmuir binding model, the association constant of RDX to the bisaniline bridging units was estimated to be $K_a^{NI} = 3.4 \times 10^7\ \mathrm{M}^{-1}$ (see the Supporting Information Fig. S1). The detection limit for analyzing RDX was 4 nM. In a control experiment, we constructed a two-layer structure of Au NPs that lacked the π-donor sites on the Au surface. For this, we assembled a layer of thiopropionic acid-capped Au NPs on a cystamine-modified Au surface.[34] Subsequently, a second layer of the thiopropionic acid-capped Au NPs was linked using 1,4-butane dithiol as bridging units. The resulting two-layer assembly showed a minute response only at elevated concentrations of RDX (>10 μM), implying that the π-donor–acceptor interactions between RDX and the bisaniline units are, indeed, essential to concentrate the explosive at the surface.

The imprinting of molecular recognition sites in organic or inorganic polymer matrices is a common practice to generate selective binding sites for the imprinted substrates,[35,36] and imprinted polymers for sensing were extensively used.[37,38] Thus, a possible approach to improve the sensitivity of the Au-NP composite toward the sensing of RDX involves the imprint of specific recognition sites for this explosive in the sensing composite. The crystallographic structure of RDX reveals that this molecule adopts a chair-like configuration, with the 1,3,5-trinitro substituents in a quasi-axial position stabilized by intramolecular dipole–dipole interactions.[39–41] While it is impossible to use RDX as an imprint molecule, due to its low solubility in the aqueous electropolymerization solution, we selected Kemp's acid (**1**), as an analog for RDX. The three carboxylic acid substituents



Scheme 1. Imprinting of Kemp's acid molecular recognition sites into the composite of bisaniline-crosslinked Au NPs associated with a Au electrode, for RDX analysis.

in (**1**) exhibit comparable dimensions to the nitro groups. The carboxylic acid substituents adopt axial positions due to intramolecular hydrogen bonds and as a result of the occupation of the equatorial positions by the bulky methyl groups. Accordingly, the electropolymerization of the Au-NP composite was conducted in the presence of a high concentration of (**1**). The electrostatic association of Kemp's acid to the bisaniline bridging units suggests that electropolymerization of the Au-NP matrix in the presence of (**1**) will yield, after the subsequent removal of the Kemp's acid, imprinted cavities of π-donor bisaniline units with molecular contours that preferably accommodate RDX (Scheme 1). Thus, the resulting Au-NP matrix is anticipated to reveal enhanced affinity for RDX due to the synergistic co-association of the explosive to the imprinted sites by π-donor–acceptor interactions and cooperative geometrical constraints dictated by the imprinting process. Figure 2A shows the SPR curves corresponding to the imprinted composite of bisaniline-crosslinked Au NPs before, curve (a), and after, curve (b), the addition of RDX, 300 fM. Figure 2B depicts the sensogram corresponding to the reflectance changes of the (**1**)-imprinted composite of bisaniline-crosslinked Au NPs upon sensing variable concentrations of RDX and the resulting calibration curve. As the concentrations of RDX increase, the reflectance changes are intensified, and they level off at a concentration corresponding to ca. 1 pM. The detection limit for analyzing RDX by the (**1**)-imprinted Au-NP matrix is 12 fM. We realize that the imprinted composite reveals a 4×10^5-fold lower detection limit for RDX as compared to the nonimprinted sensing matrix. From the calibration curve we extracted the association constant of RDX to the imprinted sites, $K_a^{I} = 1.9 \times 10^{12}\ \mathrm{M}^{-1}$. Thus, the enhanced sensitivity of the imprinted composite is attributed to the improved association of RDX to the imprinted sites, resulting from the steric confinement of the explosive molecules to the imprinted molecular contours consisting of the π-donor bridging units.

The (**1**)-imprinted Au-NP composite also demonstrates selectivity toward the analysis of RDX. We find that the nonimprinted Au-NP array reveals a lower detection limit for sensing TNT as compared to RDX (10 pM vs. 4 nM, respectively). This is attributed to the fact that TNT is a substantially better electron acceptor as compared to RDX ($K_a^{NI}(\mathrm{TNT}) = 3.9 \times 10^9\ \mathrm{M}^{-1}$ versus $K_a^{NI}(\mathrm{RDX}) = 3.4 \times 10^7\ \mathrm{M}^{-1}$). The (**1**)-imprinted matrix demonstrates impressive selectivity for detecting RDX and provides quantitative SPR responses for analyzing RDX up to a concentration of 1 pM, with a detection limit of 12 fM. Furthermore, we observed that the (**1**)-imprinted composite only responds to TNT concentrations that are higher than 30 pM. We also find that the Kemp's acid-imprinted Au-NP matrix is highly selective. Nitroaromatic substrates of weaker acceptor properties such as 2,4-dinitrotoluene or 4-nitrotoluene exhibit small reflectance changes at concentrations higher than 10 μM. Other aromatic substances that lack π-acceptor features, such as 4-hydroxy toluene, aniline, or salicylic acid, did not affect the SPR response of the composite even at high concentrations, 1×10^{-4} M. Also, different anions, such as PO_4^{3-}, NO_3^{-}, or SO_4^{2-}, did not affect the SPR responses upon analyzing RDX.

The successful selection of Kemp's acid as a molecular imprint analog for RDX was highlighted by an additional experiment, where *cis*-1,3,5-hexane tricarboxylic acid (**2**), was used as the imprint molecule. This latter compound lacks the three equatorial methyl groups and exhibits conformational flexibility. As a result, the population of the imprinted sites is anticipated to be lower, and the structural fits of the sites (the molecular contours) toward RDX are expected to be less effective. Figure 2C depicts the calibration curves corresponding to the analysis of RDX by the (**1**)-imprinted and the (**2**)-imprinted Au-NP composites. Evidently, the analysis of RDX by the (**2**)-imprinted composite reveals a less effective detection limit, 500 fM, and a substantially lower saturation value of the reflectance changes. These results are consistent with the fact that (**2**) exhibits conformational flexibility and, thus, the imprinting procedure yields imprinted sites of lower quality molecular contours (higher detection limit), and lower saturation reflectance values. For further examples of inefficient imprinting of the composite with *cis*-1,3-cyclohexane-dicarboxylic acid, see Supporting Information and Figure S3.

A further support that the concentration of RDX at the Au surface originates from π-donor–acceptor interactions of the explosive with the bisaniline bridging units was obtained by probing the effect of the applied potential on the SPR response of the modified surface in the absence and presence of RDX. The bisaniline units crosslinking the Au NPs exhibit a quasireversible redox wave at $E \sim 0.1$ V versus a Ag quasireference electrode (QRE), pH 7.2, in accord with the redox process depicted in Figure 3, inset. Thus, at $E < 0.1$ V the bisaniline bridges exist in their reduced π-donor state, whereas at $E > 0.1$ V, the bridges are present in a quinoid-type π-acceptor configuration. Accordingly, the analysis of RDX is expected to be controlled by the applied potential, provided that π-donor–acceptor interactions play a role in the concentration of the explosive at the surface. Figure 3, curve (a), depicts the reflectance changes observed for the (**1**)-imprinted matrix under different bias potentials. Only minute reflectance changes are observed upon transferring the oxidized quinoid-bridged state (at $E > 0.1$ V) to the reduced bisaniline state ($E < 0.1$ V) in the pure electrolyte solution; Figure 3, curve (b), shows the effect of the applied potential on the reflectance values of the modified surface in the presence of RDX, 120 fM. At

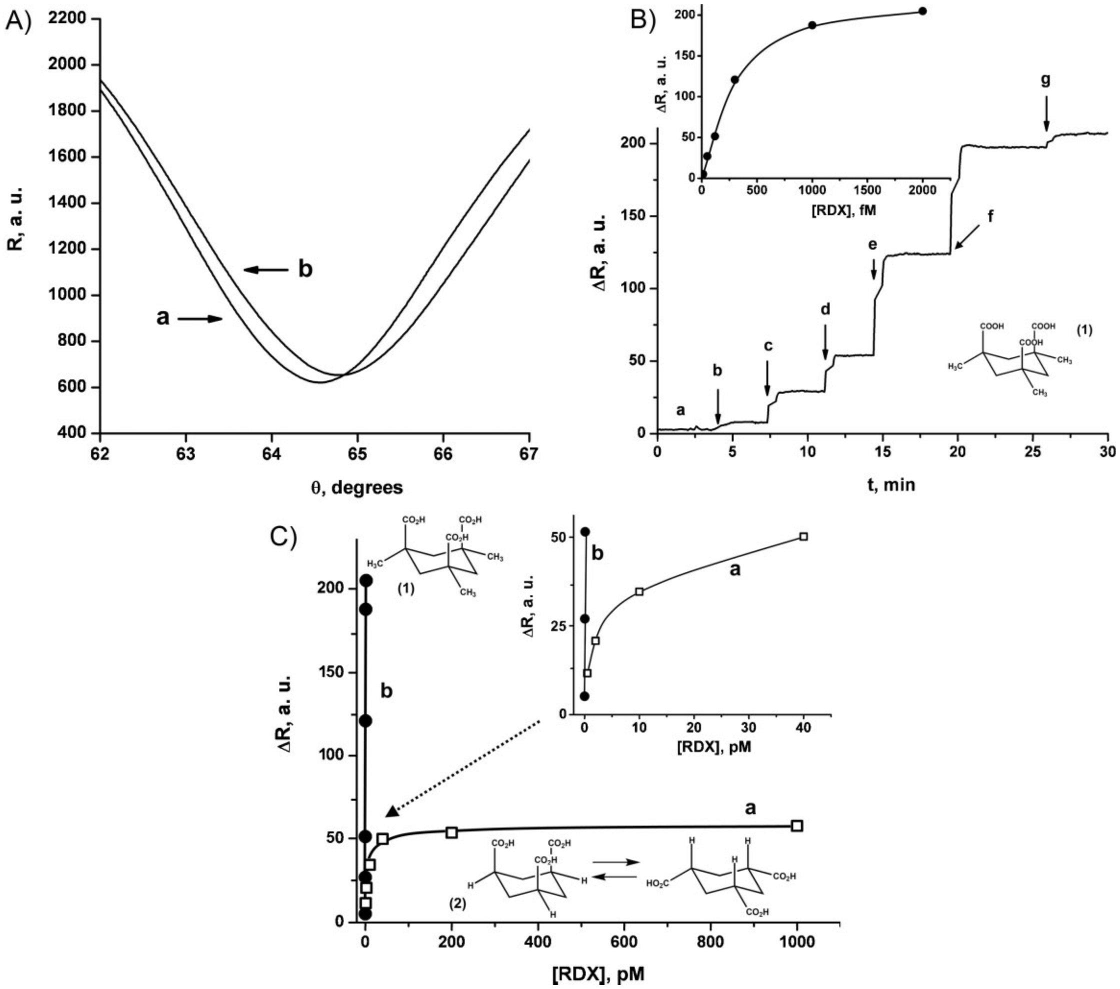


Figure 2. A) SPR curves corresponding to the Kemp's acid-imprinted bisaniline–crosslinked Au-NP composite: (a) before the addition of RDX and (b) after the addition of RDX, 300 fM. B) Sensogram corresponding to changes in the reflectance intensities at $\theta = 63.6^\circ$, upon addition of variable concentrations of RDX: (a) 0 fM, (b) 12 fM, (c) 50 fM, (d) 120 fM, (e) 300 fM, (f) 1 pM, and (g) 2 pM. Inset: calibration curve relating the reflectance changes to the concentrations of RDX. C) Calibration curves corresponding to the analysis of RDX on (a) Kemp's acid-imprinted bisaniline–crosslinked Au-NP composite and (b) cyclohexane tricarboxylic acid-imprinted composite of bisaniline-crosslinked Au NPs. The inset shows the low concentration region of the calibration curve.

potentials negative to $E = 0.1$ V, the reflectance changes exhibit high values, while low values are obtained as the applied potential is shifted more positively. These results are consistent with the fact that at $E < 0.1$ V, the bisaniline units bind the RDX to the matrix, whereas at $E > 0.1$ V, the affinity interactions of RDX with the composite are depleted, eliminating the ability to sense the explosive.

In conclusion, we have developed an imprinted composite of bisaniline-crosslinked Au NPs for the ultrasensitive detection of RDX. The major advancement of the present study is the identification of Kemp's acid (**1**) as a rigid, nonplanar, imprinting substrate for generating imprinted sites for RDX. We revealed that the changes in the dielectric properties of the Au-NP composite, as a result of the formation of the π-donor–acceptor complex, are sufficient to perturb the coupling between the localized plasmon of the NPs and the surface plasmon wave to the extent that measurable reflectance changes in the SPR spectrum could be observed. This enabled the analysis of RDX at a bulk concentration corresponding to 12 fM. As far as we are aware, this is the most sensitive sensor device for the detection of RDX.

Experimental

The Au NPs were prepared according to a previously reported method [9,33].

The modification of the Au-coated glass slides is provided as Supporting Information.

Instrumentation and Measurements: A SPR Kretschmann-type spectrometer NanoSPR 321 (NanoSPR devices, USA), with a LED light source, $\lambda = 650$ nm, and a prism refraction index $n = 1.61$, was used. The SPR measurements were performed using a home-built cell. Electropolymerization and SPR measurements were performed using a Pt wire (diameter 0.5 mm) counter electrode and a Ag wire QRE ($d = 0.5$ mm), which were installed in the cell (volume 0.5 mL, working electrode area 0.2 cm^2). A PC-controlled (Autolab GPES software)

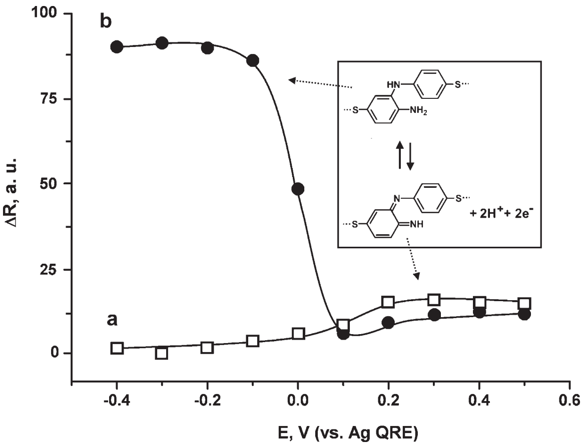


Figure 3. Effect of applied potential on the reflectance changes, at $\theta = 63.6^{\circ}$, of the Kemp's acid-imprinted composite of bisaniline-crosslinked Au NPs: (a) The imprinted composite in a pure 0.1 M HEPES buffer solution at pH 7.2 and (b) The imprinted composite in a 0.1 M HEPES buffer solution at pH 7.2 that included 300 fM RDX. Inset: the redox states of the bisaniline bridges.

electrochemical analyzer potentiostat/galvanostat (μAutolab, type III) was employed. During the recording of the RDX-concentration-dependent sensogram experiments, ascending concentrations of RDX in HEPES buffer, 0.1 M, pH 7.2, were injected into the SPR cell. Prior to each injection, a removal and washing of the previous sample with pure HEPES buffer was carried out, and the SPR response was re-equilibrated to the buffer baseline level.

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