



Sensitive sandwich-type voltammetric immunosensor for breast cancer biomarker HER2 detection based on gold nanoparticles decorated Cu-MOF and Cu₂ZnSnS₄ NPs/Pt/g-C₃N₄ composite

Mehmet Lütfi Yola¹

Received: 17 December 2020 / Accepted: 27 January 2021

© The Author(s), under exclusive licence to Springer-Verlag GmbH, AT part of Springer Nature 2021

Abstract

A sandwich-type sensitive voltammetric immunosensor for breast cancer biomarker human epidermal growth factor receptor 2 (HER2) detection was prepared. The electrochemical immunosensor was developed based on gold nanoparticles decorated copper-organic framework (AuNPs/Cu-MOF) and quaternary chalcogenide with platinum-doped graphitic carbon nitride (g-C₃N₄). Cu₂ZnSnS₄ nanoparticle (CZTS NP) quaternary chalcogenide with platinum (Pt)-doped g-C₃N₄ composite (Pt/g-C₃N₄) was tagged as CZTS NPs/Pt/g-C₃N₄. AuNPs/Cu-MOF composite was successfully synthesized by amidation reaction between AuNPs functionalized with amino group and Cu-MOFs containing carboxylic acid. After the conjugations of primer HER2 antibody and antigen HER2 protein to AuNPs/Cu-MOF as sensor platform, CZTS NPs/Pt/g-C₃N₄ composite was prepared by one-pot hydrothermal method. After immune reaction of 30 min, the prepared HER2 immunosensor was characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), x-ray diffraction (XRD) method, x-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The developed immunosensor showed high sensitivity with a detection limit of 3.00 fg mL⁻¹. Additional properties of the voltammetric immunosensor are high selectivity, stability, reproducibility, and reusability.

Keywords Breast cancer biomarker · Immunosensor · Voltammetry · Quaternary chalcogenide · MOF

Introduction

HER2 can facilitate excessive breast cancer cell growth and is one of the most significant diagnostic biomarkers. The development of about 35% of breast cancer tumors is mainly corresponded to HER2's overexpression [1]. These HER2-positive breast cancers are responsible for about 35% of breast cancers. Hence, HER2's fast analysis improves the effectiveness of treatment [2, 3]. According to literature, while HER2 content in the blood of healthy people is between 2.0 and 15.0 ng mL⁻¹, the amount of HER2 increases up to 75.0 ng mL⁻¹ in breast cancer patients [4, 5]. Lately, the fluorescent-based detection methods including in situ hybridization (FISH) have been utilized for HER2 gene expression to determine the presence of HER2 protein on the cell.

Nonetheless, the biopsy tissue exemplification and expensive instruments can be needed in immunohistochemistry (IHC) and FISH methods, causing a limited application for HER2 recognition [6, 7]. Hence, the simple and sensitive analysis techniques are of great importance for HER2 recognition. Lately, the new-type immunosensors based on voltammetry started to attract great attention in the terms of high selectivity, sensitivity, and low cost [8–15]. Even, some voltammetric biosensors for HER2 recognition have presented by using different nanoparticle systems [16, 17]. There are two-type voltammetric immunosensors including sandwich type containing label and label-free. To obtain sensitive biosensor signals, the sandwich-type immunosensors are especially used and applied to the complex samples [18]. In the preparation of sandwich-type immunosensors, the primer (capture) antibody is firstly immobilized to any sensor platform such as electrochemical surface. Then, the analyte (target) antigen sandwiched between the primer and detection (second) antibodies. However, the type of nanomaterial/composite conjugated capture and second antibodies is very important to observe the sensitive sensor signals [9]. For example, label-

✉ Mehmet Lütfi Yola
mlutfi.yola@hku.edu.tr

¹ Faculty of Health Sciences, Department of Nutrition and Dietetics, Hasan Kalyoncu University, Gaziantep, Turkey

free electrochemical immunosensor based on Fe_3O_4 nanoparticles into an amino-functionalized MOF ($\text{Fe}_3\text{O}_4@$ TMU-21) and multi-walled carbon nanotubes was prepared for HER2 detection. Thus, a linear relation of $1.00\text{--}100.00\text{ ng mL}^{-1}$ and detection limit (LOD) of 0.30 pg mL^{-1} were recorded [3]. Secondly, cerium oxide-monoclonal antibody bioconjugate-based electrochemical immunosensing was performed for HER2. After the immobilization of this bioconjugate on a carbon-AuNPs electrode, the prepared immunosensor was applied to human serum plasma, and LOD of 34.90 pg mL^{-1} was obtained [19]. Then, lead sulfide quantum dots-conjugated secondary HER2 antibody ($\text{Ab}_2\text{-PbS QDs}$) was designed and used for electrochemical immunosensing of HER2. The developed immunosensor demonstrated a linear relation of $1.00\text{--}100.00\text{ ng mL}^{-1}$ and LOD of 0.28 ng mL^{-1} [20]. In other study, DNA self-assembly amplification generating electric current was developed for HER2 detection. In this study, a sandwich-type aptamer and a DNA primer could form a long one-dimensional DNA which was treated with molybdate for generation of electrochemical current. According to the obtained results, the prepared aptasensor for HER2 detection in serum was highly correlated with ELISA measurements [21]. Finally, an aptamer-based colorimetric technique based on AuNPs was developed for HER2 detection. The aptamers were conjugated on AuNPs by adsorption-desorption colorimetric approach and HER2 selectively connected to the aptamers conjugated with AuNPs. Hence, LOD of 20.00 nM was obtained for HER2 detection [22].

Metal-organic frameworks (MOFs) are constructed by unification of metal ions with organic ligands having highly ordered structures. Due to specific surface area, low density, and porosity structure, MOFs have started to attract great attention [23]. They are used as a sensor amplification especially in drug delivery, electrochemistry applications, and energy storage [24–26]. In addition, MOFs have been currently utilized for significant substances' analyses [27]. Nonetheless, the incompatibility between metal nanoparticles such as AuNPs and MOFs occasionally result in sensor application problems. The incorporation of functional groups such as $-\text{NH}_2$ on metal nanoparticles can enhance the compatibility, providing a stable nanoparticles on MOFs. Hence, the materials including MOFs suggest a chance to prepare enzyme-free voltammetric sensors [28].

Metal chalcogenide as an inorganic molecule is composed of electropositive metal cation/cations and one chalcogen such as sulfides, selenides, and tellurides. Currently, due to the physico-chemical and electrochemical properties, metal chalcogenides have attracted attention. Generally, alkali/alkaline-earth metals and transition metals can form unique chalcogenides. Especially, empty d-orbitals in transition metals result in chalcogenide formation with different structures and composites such as binary (MoSe_2), ternary (MoSSe), and quaternary ($\text{Cu}_2\text{ZnSnS}_4$). In addition, the chalcogenides have perfect

properties such as high conductivity and electrocatalytic activity, exclusive mechanical properties [29, 30]. Especially, $\text{Cu}_2\text{ZnSnS}_4$ has important interest because of its catalytic activity and large optical properties. Hence, $\text{Cu}_2\text{ZnSnS}_4$ has been currently used as a potential candidate in sensor applications [31].

$g\text{-C}_3\text{N}_4$ as a carbon-nitrogen based nanomaterial has currently attracted important interest owing to biocompatibility, specific surface area, low cytotoxicity, and chemical inertness. In addition, $g\text{-C}_3\text{N}_4$'s modification with metal nanoparticles such as gold and platinum can significantly enhance catalytic performance and electron transfer in comparison with pristine $g\text{-C}_3\text{N}_4$ [32–34].

In this study in comparison with previous reports, HER2 detection has been performed for the first time with a higher precision and sensitivity using gold nanoparticles decorated Cu-MOF and $\text{Cu}_2\text{ZnSnS}_4$ NPs/Pt/ $g\text{-C}_3\text{N}_4$ composite. Currently, metal nanoparticles and MOFs are embedded to enhance biosensors/sensors' performances. The traditional preparation techniques of metal nanoparticles/MOF composites are in situ preparation of nanoparticles into MOFs or nanoparticles' direct coatings on MOFs. We performed AuNPs coatings on MOFs via the stable hydrogen bonds between AuNPs and Cu-MOF, providing the high stability of AuNPs/Cu-MOF composite in comparison with the other metal nanoparticles/MOFs [35, 36]. In preparation of CZTS NPs/Pt/ $g\text{-C}_3\text{N}_4$, one-pot hydrothermal method was carried out with minimal waste generation and high reaction efficiency. Hence, an environmentally friendly immunosensor was constructed for HER2 detection. Finally, the prepared immunosensor can provide a chance in the terms of early detection of breast cancer via this study.

Experimental section

Materials

Antigen HER2, capture antibody1-HER2 (primer, anti-HER2- Ab_1), detection antibody2-HER2 (second, anti-HER2- Ab_2), bovine serum albumin (BSA), Mucin 1 (MUC1), hepatitis B surface antigen (HBS), carcinoembryonic antigen (CEA), human immunoglobulin (IgG), melamine, chloroplatinic acid (H_2PtCl_6), thiourea ($\text{CH}_4\text{N}_2\text{S}$), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, L-cysteine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1,3,5-benzenetricarboxylic acid (BTCA) were purchased from Sigma-Aldrich. Phosphate-buffered saline (PBS, 0.1 M , $\text{pH } 7.0$) as supporting electrolyte and dilution buffer were also utilized.

Instrumentation

SEM and XRD images were obtained by using ZEISS EVO 50 SEM analytical microscopy and Rigaku X-ray diffractometer, respectively. JEOL 2100 TEM was utilized for obtaining TEM images. FTIR measurements were obtained by Bruker Tensor 27 FT-IR with DTGS detector. XPS analysis was carried out by using PHI 5000 Versa Probe. In addition, differential pulse voltammetry (DPV), CV, and EIS measurements were performed by using reference 600 workstation (Gamry, USA).

Preparation of Cu-MOF and AuNPs/Cu-MOF composite

CuSO₄·5H₂O solution (0.01 mmol), NH₃·H₂O (1.0 mL), and BTCA solution (0.01 mmol) were mixed at 25.0 °C under strong stirring for 45 min. Then, the prepared solution was transferred into Teflon autoclave at 100 °C during 12 h. After cooling treatment, the centrifugation at 10,000 rpm created a blue precipitation (**Cu-MOFs**). Cu-MOFs was washed with methanol and dried at 30.0 °C for 12 h [37].

AuNPs having the mean diameters of 20–25 nm were prepared by using Na₃C₆H₅O₇·2H₂O as reducing agent and HAuCl₄·3H₂O as precursor agent in harmony with literature [38]. Then, L-cysteine solution (0.1 mM, 250.0 μL) was added into AuNP solution under strong stirring during 6 h. After centrifugation treatment, **-NH₂-AuNPs** was collected and washed with methanol.

After the preparation of Cu-MOF (0.25 g) dispersion in ethanol (25.0 mL), NHS/EDC mixture (1:1, v/v) was added into the above dispersion, and the obtained solution was mixed for 30 min. Finally, **AuNPs/Cu-MOF** composite was formed with incorporation of **-NH₂-AuNPs** on Cu-MOFs. The preparation steps of AuNPs/Cu-MOF are shown on Scheme 1.

AuNPs/Cu-MOF composite as electrochemical sensor platform with anti-HER2-Ab₁ and antigen HER2 immobilizations

After dropping of AuNPs/Cu-MOF composite (25.0 μL) on clean glassy carbon electrode (GCE), the solvent on electrode surface was removed by IR lamp and AuNPs/Cu-MOF-modified GCE was developed (AuNPs/Cu-MOF/GCE). The immobilization process of primer antibody was carried out by amino gold affinity between anti-HER2-Ab₁ and AuNPs/Cu-MOF/GCE. For this aim, anti-HER2-Ab₁ dispersion (25.0 μL, 25.0 μg mL⁻¹) was dropped on AuNPs/Cu-MOF/GCE and left at 37.0 °C for 30 min [39] and tagged as **anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE**. Then, anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE was washed with pH 7.0, 0.1 M PBS to remove non-interacting proteins at 37.0 °C for 60 min. In

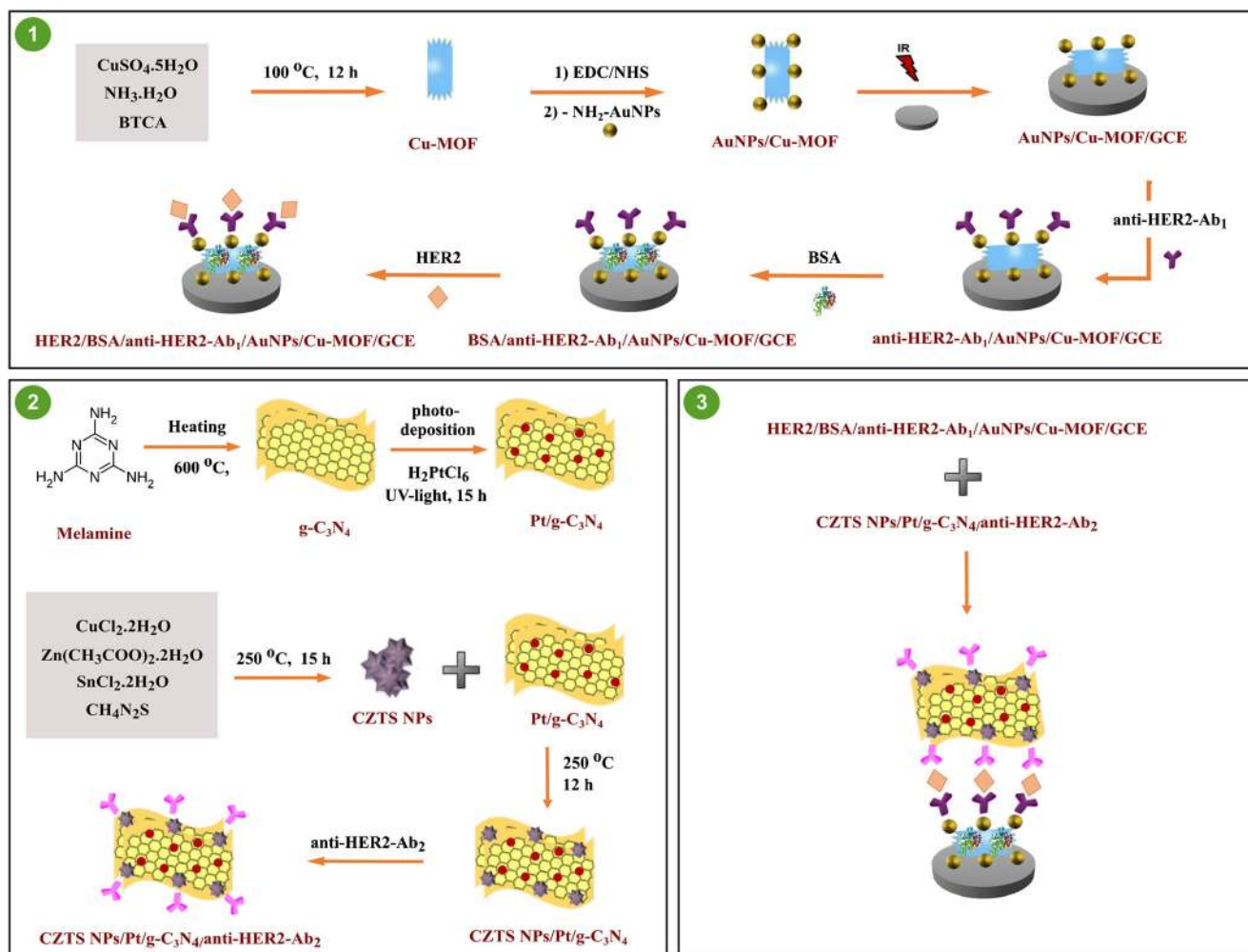
order to eliminate non-specific interactions, BSA (2.0% w/v) solution was incubated on anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE at 37.0 °C for 30 min. After washing with pH 7.0, 0.1 M PBS and distinct antigen HER2 proteins having different concentration were immobilized on anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE for 30 min at 37.0 °C and tagged as **HER2/anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE**. In order to remove non-interacting proteins, HER2/anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE was washed with 0.1 M PBS (pH 7.0).

Preparation of g-C₃N₄, Pt/g-C₃N₄ composite, CZTS NPs, and CZTS NPs/Pt/g-C₃N₄

After the transfer of melamine (5.0 g) into an alumina crucible, the annealing treatment was applied at 600 °C during 3 h (rising rate of 3 °C min⁻¹). Then, the obtained bulk form was cooled to 25 °C and grounded in a mortar. Lastly, a faint-yellow polymeric powder (**g-C₃N₄**) was kept in a closed environment [40]. Pt/g-C₃N₄ composites including distinct Pt amounts were prepared by photo-deposition method, and H₂PtCl₆ was utilized as platinum source. After preparation of g-C₃N₄ solution (1.0 mg mL⁻¹) in the mixture of ultra-pure water/methanol (1:1), g-C₃N₄ solution was slowly added into H₂PtCl₆ dispersion (0.20 mg mL⁻¹) under ultrasonication treatment for 30 min. After that, photo-deposition treatment was applied to g-C₃N₄/H₂PtCl₆ dispersion for 15 h. The obtained product was washed with ultra-pure water for three times and dried at 70 °C. Finally, the obtained powder was calcined at 550 °C for 1 h, providing **5.0 wt% Pt/g-C₃N₄** composite. The same procedure was applied to Pt/g-C₃N₄ composites with different Pt ratios, denoting as xPt/g-C₃N₄ where x was 5.0, 2.5, and 1.0 wt%.

In typical hydrothermal technique, CuCl₂·2H₂O (4.0 mmol), Zn(CH₃COO)₂·2H₂O (2.0 mmol), SnCl₂·2H₂O (2.0 mmol), and CH₄N₂S (2.0 mmol) were dissolved in the mixture of ultra-pure water/ethanol (1:1) under strong stirring for 2 h at 70 °C. After transferring of the dispersion into a Teflon-autoclave, the heating treatment was applied at 250 °C for 15 h. After cooling process at 25 °C, the precipitates (**CZTS NPs**) were washed with ultra-pure water and stored in a closed environment [41].

The one-pot hydrothermal method was also utilized for the preparation of CZTS NPs/Pt/g-C₃N₄. Firstly, 5.0 wt% Pt/g-C₃N₄ (1.0 g) and CZTS NPs (10.0 g) were mixed in ultra-pure water/ethanol (1:1) under ultra-sonication for 45 min. After the suspension was transferred into a Teflon-autoclave, the heating treatment at 250 °C for 12 h was performed. Then, the product was cleaned with ultra-pure water and dried at 80 °C. Finally, the composite was calcined at 450 °C for 1 h, and the obtained composite was named as **CZTS NPs/5.0 wt% Pt/g-C₃N₄**. The preparation steps of CZTS NPs/Pt/g-C₃N₄ are shown on Scheme 1.



Scheme 1 Preparation procedure of electrochemical sensor platform, signal amplification and voltammetric immunosensor

CZTS NPs/Pt/g-C₃N₄ as signal amplification with anti-HER2-Ab₂ conjugation

After preparation of anti-HER2-Ab₂ (25.0 μL , 25.0 $\mu\text{g mL}^{-1}$), the second antibody was added into CZTS NPs/5.0 wt% Pt/g-C₃N₄ (25.0 μL , 30.0 mg mL^{-1}) dispersion under magnetic stirring at 37.0 $^{\circ}\text{C}$ for 30 min. Centrifugation process was carried out at 15,000 rpm during 45 min, and CZTS NPs/5.0 wt% Pt/g-C₃N₄ conjugated to anti-HER2-Ab₂ (CZTS NPs/5.0 wt% Pt/g-C₃N₄/anti-HER2-Ab₂) was preserved in pH 7.0, 0.1 M PBS.

Electrochemical measurements

Finally, electrochemical measurements were performed by the developed sandwich-type voltammetric immunosensor occurring the antibody-antigen interactions between CZTS NPs/5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂ and HER2/anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE. For the immune reaction, CZTS NPs/5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂

dispersion (25.0 μL , 30 mg mL^{-1}) was dropped on sensor platform for 30 min. Lastly, the developed immunosensor (CZTS NPs/5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂/HER2/anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE) was preserved in 0.1 M PBS. 0.1 M PBS (pH 7.0, 2.0 mL) as supporting electrolyte containing 1.0 mM H₂O₂ solution was prepared for electrochemical measurements. After working potential was applied in the range of 0.0/+0.6 V for DPV measurements, the voltammograms were recorded at +0.40 V. Before electrochemical measurements, argon gas (99.999%) was passed to remove dissolved oxygen for 15 min. In addition, the used parameters for DPV were frequency of 50 Hz, pulse amplitude of 20 mV and scan increment of 3 mV.

Sample preparation

HER2 free plasma samples were supplied from Blood Bank in TURKEY. Sample preparation protocol was explained in detail on Supplementary Data [42].

Results and discussion

Principle of voltammetric HER2 immunosensor based on AuNPs/Cu-MOF and CZTS NPs/Pt/g-C₃N₄

In the preparation of gold nanoparticles decorated Cu-MOF, benzene-1,3,5-tricarboxylate (BTC³⁻) ligands serve as μ_4 -bis-monodentate and bidentate coordination style to five-coordinated Cu(II) centers for trigonal bipyramidal formation. Four BTC³⁻ ligands also link to four Cu(II) centers into a 28-membered ring and Cl⁻ bridges two neighboring Cu(II) centers to create small 6-membered ring into 28-membered ring. Hence, 28-membered rings' growth via edge-sharing results in two-dimensional sheet along *ab*-plane. The amination reaction of AuNPs with L-cysteine forms -NH₂ groups on AuNPs. These -NH₂ groups create the stable hydrogen bonds between -NH₂ groups and -COO groups of Cu-MOF, providing the stabilization of the prepared AuNPs/Cu-MOF composite. In addition, -COO groups of Cu-MOF are activated by NHS/EDC mixture, and this activation provides the formation of AuNPs/Cu-MOF composite via amide bond. Finally, AuNPs/Cu-MOF serves as capture antibody1-HER2's immobilization via amino-gold affinity [43] and conductivity enhancement on sensor platform.

CZTS NPs/Pt/g-C₃N₄ composite as signal amplification is prepared by one-pot hydrothermal method with high efficiency between Pt/g-C₃N₄ and CZTS NPs. CZTS NPs/Pt/g-C₃N₄ composite is developed by the co-catalyst behavior of CZTS NPs and Pt NPs on g-C₃N₄ sheets, providing a high surface area and efficient charge separation. The synergistic effect between quaternary chalcogenide's electropositive metal cations and functional groups of detection antibody2-HER2 provided a stable conjugation by strong electrostatic interactions.

H₂O₂ as a redox probe was chosen because of its usage in biomolecules' medical diagnosis [44]. The continuous oxidation of H₂O₂ into O₂ at about +0.40 V was monitored by the prepared HER2 voltammetric immunosensor including antigen HER2 proteins with different concentrations.

Characterizations of gold nanoparticles decorated Cu-MOF (AuNPs/Cu-MOF) as sensor platform

XRD spectra were firstly obtained for characterizations of Cu-MOF and AuNPs/Cu-MOF (Fig. 1a). The characteristic peaks corresponding to Cu-MOF were observed in harmony with literature [45], confirming the high purity of the synthesized Cu-MOF. AuNPs/Cu-MOF also demonstrated an analogous XRD pattern, and the diffraction peaks located at 37.92° and 44.73° were attributed to (111) and (200) planes of cubic AuNPs [46, 47]. Thus, the similar and different XRD peaks showed AuNP presence and the structural stability of Cu-MOF in AuNPs/Cu-MOF. FTIR spectra of Cu-MOF and AuNPs/Cu-MOF showed that the absorption peaks belonging

to BTC³⁻ ligand verified the presence of Cu-MOF (Fig. 1b). The peak at 1620 cm⁻¹ attributing to OH-C=O stretching vibration and the absorption bands at 1500–1200 cm⁻¹ corresponding to C=O of carboxyl groups indicated the incorporation of BTC³⁻ ligand on Cu-MOF and AuNPs/Cu-MOF composite [37]. The absorption bands attributing to C=C stretching of benzene structure were emerged at 1559 cm⁻¹ and the absorption bands at 950–550 cm⁻¹ were owing to C-H bending of benzene structure [48]. Finally, the band at 1650 cm⁻¹ confirmed the presence of -NH₂ groups on AuNPs/Cu-MOF composite [49]. The morphological properties of Cu-MOF and AuNPs/Cu-MOF composite were also investigated by SEM measurements. According to Fig. 1c, Cu-MOF had column-type shape with average size of 120–410 nm. Lastly, many granular reliefs on Cu-MOF surface confirmed AuNPs' expansion on Cu-MOF (Fig. 1d).

Characterizations of g-C₃N₄, CZTS NPs, Pt/g-C₃N₄, and CZTS NPs/Pt/g-C₃N₄ composites as signal amplification

The morphological features of all samples were firstly investigated by using SEM measurements. Figure 2a demonstrated irregular two-dimensional nanosheet structure belonging to g-C₃N₄'s bulk form. Nonetheless, CZTS NPs, having flower-like agglomerates, were comprised of many nanoplates with an average size of 120–140 nm (Fig. 2bs). SEM images (Figs. 2c, d, and e) of Pt/g-C₃N₄ composites showed curly, bulky, and two-dimensional nanosheet structures with an average size of 5–10 nm and Pt/g-C₃N₄ composites' structures were distinct in comparison with g-C₃N₄ including solid agglomerates. The well dispersion of Pt NPs on g-C₃N₄ surfaces was also seen, and no significant distinctions could be observed on morphological features of Pt/g-C₃N₄ composites with the increase of Pt amounts. According to SEM image (Fig. 2f) of CZTS NPs/5.0 wt% Pt/g-C₃N₄, the composite was composed of irregular agglomerates having spherical particles. Moreover, SEM image of CZTS NPs/5.0 wt% Pt/g-C₃N₄ showed the obvious incorporation and coating of flower-like CZTS NPs on 5.0 wt% Pt/g-C₃N₄ composite. After the conjugation of anti-HER2-Ab₂ on CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite, the observed cloudy clusters indicated the specific interaction between seconder antibody and the final composite (Fig. 2g). According to EDS image (Fig. 2h), the presence of elements such as C, N, Pt, Cu, Zn, Sn and S was confirmed on composite structure.

Secondly, TEM measurements were carried out to investigate the morphological properties of all samples. The thread and porous structure was shown for g-C₃N₄ on Fig. S1a. According to Fig. S1b, the well dispersion of Pt NPs on g-C₃N₄ surface was demonstrated, and the lattice space of 0.21 nm belonging to (111) plane of Pt NPs was obtained.

Fig. 1 (a) XRD patterns and (b) FTIR spectra of Cu-MOF and AuNPs/Cu-MOF composite, SEM images of (c) Cu-MOF and (d) AuNPs/Cu-MOF composite

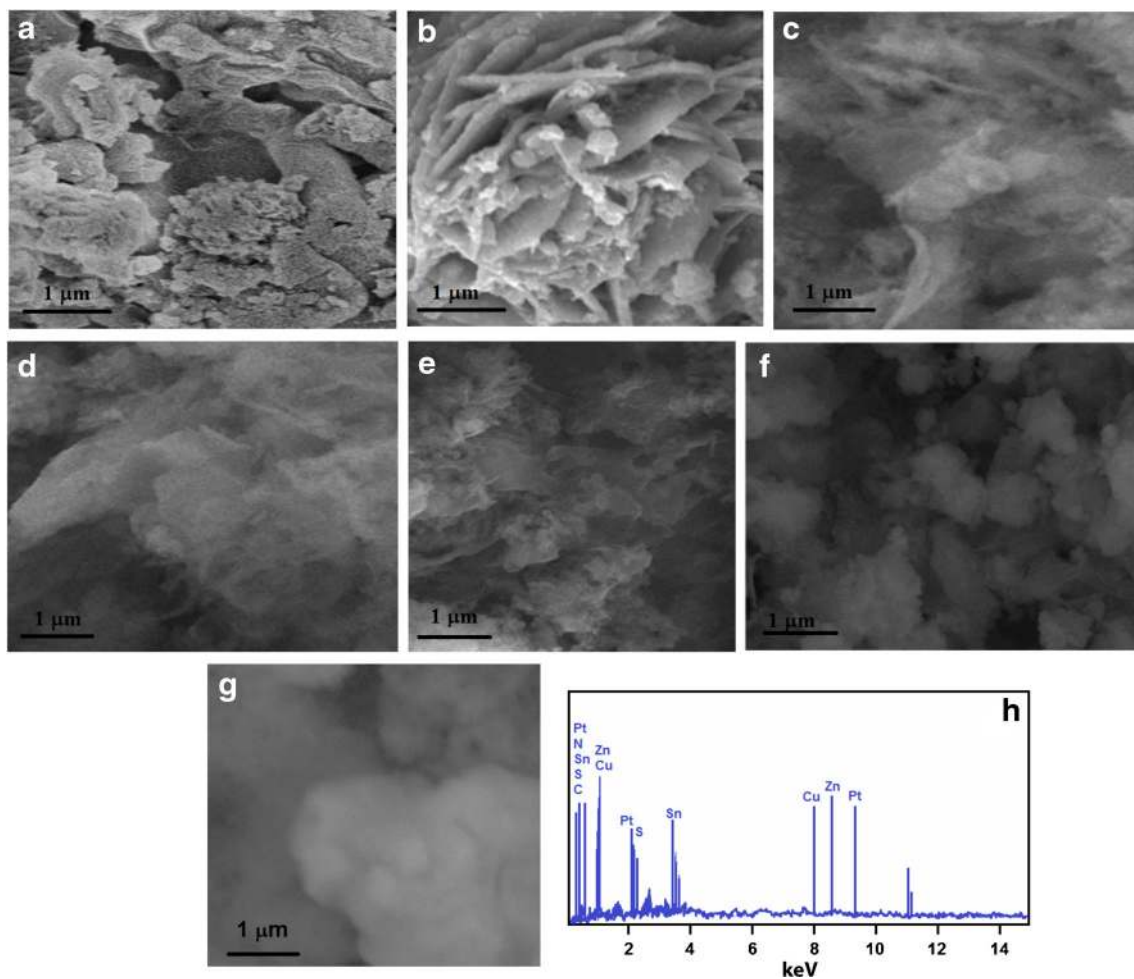
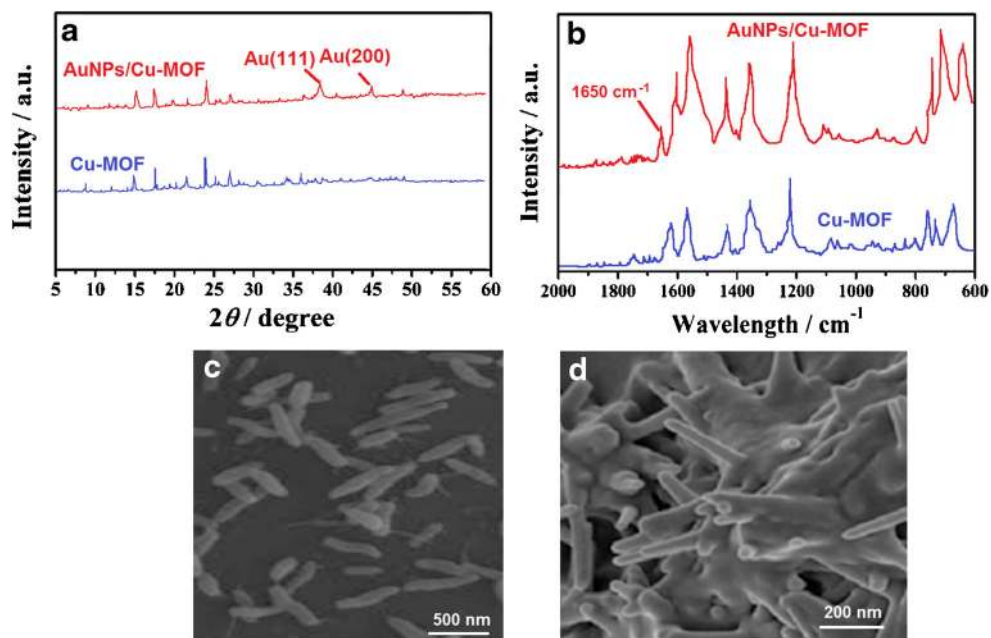


Fig. 2 SEM images of (a) g-C₃N₄, (b) CZTS NPs, (c) 1.0 wt% Pt/g-C₃N₄, (d) 2.5 wt% Pt/g-C₃N₄, (e) 5.0 wt% Pt/g-C₃N₄, (f) CZTS NPs/5.0 wt% Pt/g-C₃N₄, (g) CZTS NPs/5.0 wt% Pt/g-C₃N₄/anti-HER2-Ab₂, and (h) EDS spectra of CZTS NPs/5.0 wt% Pt/g-C₃N₄

Figure S1C verified flower-like agglomerates of CZTS NPs. Figure S1D shows that g-C₃N₄ having thread and porous structure acted as substrate for Pt NPs and CZTS NPs. The dark areas on the layered structure were also corresponded to the agglomerated CZTS NPs. Moreover, Pt NPs' distribution with an average size of 4–9 nm verified the decoration on g-C₃N₄ sheets. In addition, the close interface (demonstrated by white area line) between 5.0 wt% Pt/g-C₃N₄ and CZTS NPs resulted in effective charge transfer, and thus, the catalytic performance could be obviously improved.

FTIR spectra were obtained to investigate the structural properties relating to inter- and intra-bonds in g-C₃N₄, xPt/g-C₃N₄, and CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite (Fig. S2A). Absorption bands related to amine groups and –OH stretching of adsorbed water were observed in the range of 3000–3400 cm⁻¹ [50, 51]. Absorption bands in range of 1100–1650 cm⁻¹ were corresponded to sp³ C–N breathing and sp² C=N stretching modes [52]. In addition, the bands at 810–815 cm⁻¹ and 890 cm⁻¹ were attributed to triazine ring's breathing and N–H deformation modes, respectively [53]. Furthermore, the absorption bands at 441, 1088, and 1731 cm⁻¹ were corresponded to stretching vibrations of Zn–N, C=S, and C=O, respectively, on CZTS NPs of composite [54, 55]. Thus, it is concluded that the structure of graphitic carbon nitride could not significantly change with the incorporation of CZTS NPs and PtNPs. In addition, the structural properties of g-C₃N₄, 5.0 wt% Pt/g-C₃N₄, and CZTS NPs/5.0 wt% Pt/g-C₃N₄ were highlighted by XRD (Fig. S2b). The intensive peak at 27.30° was attributed to (002) plane of heptazine units of g-C₃N₄ [51]. Besides the peak located at 27.30°, two additional peaks at 39.70° and 45.80° were attributed to (111) and (200) planes of platinum, respectively, indicating Pt NPs' presence on g-C₃N₄. The peaks at 29.40°, 33.60°, 49.12°, and 55.22°, attributing to (112), (200), (220), and (312) planes, confirmed CZTS NP presence on composite.

XPS analysis was used to determine the chemical and electronic states of CZTS NPs/5.0 wt% Pt/g-C₃N₄ (Fig. 3). The survey spectra (Fig. 3A) demonstrated that 5.0 wt% Pt/g-C₃N₄ composite was composed of Zn2p, Sn3d, S2p, Cu2p, Pt4f, C1s, and N1s. Figure 3b showed Cu2p spectra including two peaks at 931.2 eV and 951.1 eV for Cu2p_{3/2} and Cu2p_{1/2}, respectively. The oxidation state of Zn²⁺ was confirmed by two peaks at 1022.1 eV (Zn2p_{3/2}) and 1045.2 eV (Zn2p_{1/2}) (Fig. 3c), and the oxidation state of Sn⁴⁺ was verified by two characteristic peaks at 487.1 eV (Sn3d_{5/2}) and 494.9 eV (Sn3d_{3/2}) (Fig. 3d). Two obvious peaks at 160.5 eV and 162.1 eV were attributed to S2p_{3/2} and S2p_{1/2}, respectively, indicating the presence of S²⁻ oxidation state (Fig. 3e). For C1s spectra, three peaks located at 284.8 eV, 286.2 eV, and 287.8 eV were corresponded to –C–NH₂, graphitic C–C, and –N–C=N bonds, respectively (Fig. 3f). The peaks at 398.5 eV, 399.5 eV, and 401.1 eV attributing to

aromatic ring with –C–N=C, sp³ bonded N–[C]₃ and –NH₂ groups were shown on N1s spectra (Fig. 3g) [56]. Finally, the peaks corresponding to 70.4 eV and 75.1 eV indicated Pt4f_{7/2} and Pt4f_{5/2}, confirming Pt (II) state [57] (Fig. 3h). The binding energy of Pt4f_{7/2} attributing to Pt²⁺ is generally located at 72.4 eV. Hence, the binding energy of Pt4f_{7/2} containing in CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite shifted to the lower energy, indicating electron transfer from g-C₃N₄ to Pt NPs [58]. Figure S3 demonstrated N₂ adsorption-desorption isotherms of g-C₃N₄, 5.0 wt% Pt/g-C₃N₄, and CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite. According to Fig. S3, the whole samples had type IV isotherms, confirming mesoporous structures of samples. The specific surface areas (BET) were obtained as 9.12, 12.43, and 37.81 m² g⁻¹ for g-C₃N₄, 5.0 wt% Pt/g-C₃N₄, and CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite, respectively. About three times enhancement on BET surface area belonging to 5.0 wt% Pt/g-C₃N₄ and CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite was owing to the incorporation of CZTS NPs. In addition, the pore volumes were calculated as 0.052, 0.098, and 0.177 cm³ g⁻¹ for g-C₃N₄, 5.0 wt% Pt/g-C₃N₄, and CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite, respectively. Hence, the electrochemical activity of the prepared composite may be increased by these large surface areas [59]. In literature, many catalysts such as Zn-MOF [60] and curcumin-MOF [61] were prepared according to their usage areas as photocatalysis and bioprocess. The surface areas of these catalysts can be higher and lower in comparison with CZTS NPs/5.0 wt% Pt/g-C₃N₄ composite. However, the one of basic aims of this study was to demonstrate the electrochemical biosensor usability and the textural properties of the prepared signal amplification (CZTS NPs/5.0 wt% Pt/g-C₃N₄). Especially, a perfect sensitivity with detection limit of 3.00 fg mL⁻¹ for HER2 detection was obtained using AuNPs/Cu-MOF as sensor platform and CZTS NPs/5.0 wt% Pt/g-C₃N₄ as signal amplification in this study. Hence, this developed immunosensor may show a new way in real clinical cases.

Electrochemical characterizations of voltammetric immunosensor

Firstly, electrochemical characterizations of CZTS NPs/Pt/g-C₃N₄ composites containing different Pt/g-C₃N₄ amounts were performed in 1.0 mM [Fe(CN)₆]³⁻ containing 0.1 M KCl by CV (Fig. S4A). Curve a of Fig. S4A showed a small anodic and cathodic peak signals by using bare GCE. Nonetheless, CZTS NPs/Pt/g-C₃N₄/GCE demonstrated the enhanced anodic and cathodic peak signals in 1.0 mM [Fe(CN)₆]³⁻. Even CZTS NPs/5.0wt%Pt/g-C₃N₄ showed the highest electrocatalytic effect in comparison with composites including 2.5wt%Pt/g-C₃N₄ and 1.0wt%Pt/g-C₃N₄. Thus, we chose signal amplification including 5.0wt%Pt/g-C₃N₄ for

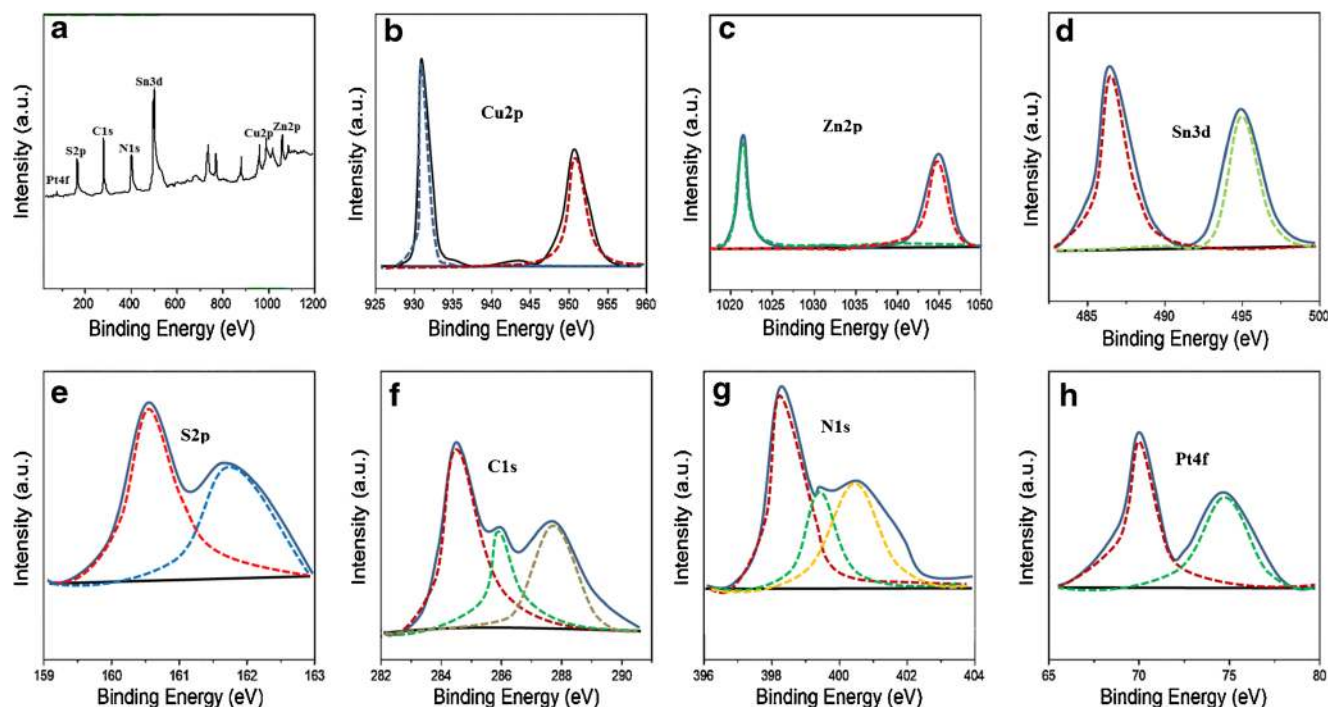


Fig. 3 (a) XPS survey spectra of 5.0 wt% Pt/g-C₃N₄ composite, High-resolution spectra of (b) Cu2p, (c) Zn2p, (d) Sn3d, (e) S2p, (f) C1s, (g) N1s, and (h) Pt4f

voltammetric immunosensor development and subsequent applications.

Secondly, electrochemical characterizations of the developed sensor platform were progressively carried out (Fig. S4b). Curve a of Fig. S4B showed a small anodic and cathodic peak signals by using bare GCE. The more pronounced anodic and cathodic peak signals were observed by Cu-MOF-modified GCE (Cu-MOFs/GCE) (curve b of Fig. S4B) owing to high surface area and more electrocatalytic tunable structure of Cu-MOFs [24, 62, 63]. Then, the increased anodic and cathodic peak signals were observed at AuNPs/Cu-MOF/GCE (curve c of Fig. S4B) because of the enhanced conductivity on electrode surface [64]. After anti-HER2-Ab₁'s immobilization on AuNPs/Cu-MOF/GCE, an important decrease on signals of anodic and cathodic peaks was observed due to electron transfer prevention ability of capture antibody (curve d of Fig. S4B). Then, BSA's blocking effect caused the further decreases on the peak signals (curve e of Fig. S4B). Lastly, antigen HER2's immobilization on anti-HER2-Ab₁/AuNPs/Cu-MOF/GCE (curve f of Fig. S4B) made electron transfer more difficult in comparison with curve e of Fig. S4B. Hence, according to CV measurements, the incorporation of proteins such as primer, seconder, antigen, and BSA was successfully realized on sensor platform.

According to EIS measurements in 1.0 mM [Fe(CN)₆]³⁻ containing 0.1 M KCl, the electrode conductivity on bare GCE surface (curve a of Fig. S4C) was gradually increased

by Cu-MOFs/GCE (curve b of Fig. S4C) and AuNPs/Cu-MOF/GCE (curve c of Fig. S4C). Owing to primer anti-HER2-Ab₁ immobilization, the electrode resistance increased in harmony of CV results (curve d of Fig. S4C). Finally, electrochemical conductivity on electrode surface progressively decreased due to incorporation of BSA, antigen, and seconder (curve e-f of Fig. S4C). Hence, CV and EIS measurements showed that the accomplished sensor platform based on AuNPs/Cu-MOF composite was developed for novel HER2 detection.

Finally, various immunosensors including different signal amplifications such as 5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂ with CZTS NPs and without CZTS NPs were prepared to observe the effect of signal amplification (Fig. S5). Firstly, the previous prepared sensor platforms (AuNPs/Cu-MOF/GCE) including primer antibody and antigen HER2 were put on an immune reaction of 30 min with CZTS NPs/5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂, 5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂, and anti-HER2-Ab₂, respectively. The whole immunosensors were tested in 1.0 mM H₂O₂ in pH 7.0, 0.1 M PBS, and in the absence of H₂O₂. The signal amplification containing only anti-HER2-Ab₂ without any 5.0wt%Pt/g-C₃N₄ composite and CZTS NPs demonstrated a small signal for 1.0 mM H₂O₂ at about +0.40 V (curve b of Fig. S5). However, the signal amplifications containing 5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂ (curve c of Fig. S5) and CZTS NPs/5.0wt%Pt/g-C₃N₄/anti-HER2-Ab₂ (curve d of Fig. S5) showed the peak signals corresponding to 6.0 μA and

1.0 μA , respectively, at about +0.40 V. Because of the introduction of CZTS NPs, it caused a significant increase in electrochemical catalytic activity as a result of higher surface area. This synergistic effect relating to the interactions of CZTS NPs and Pt/g- C_3N_4 produced a combined effect greater than their separate effects, making the developed voltammetric immunosensor a potential candidate in clinical applications. In order to determine this synergistic effect, the specific surface areas of developed modified electrodes were calculated as $0.170 \pm 0.04 \text{ cm}^2$ for bare GCE, $0.508 \pm 0.02 \text{ cm}^2$ for 5.0wt%Pt/g- C_3N_4 /GCE, and $0.938 \pm 0.03 \text{ cm}^2$ for CZTS NPs/5.0wt%Pt/g- C_3N_4 /GCE in the presence of 1.0 mM $[\text{Fe}(\text{CN})_6]^{3-}$ solution by the equation (Randles–Sevcik) of $i_p = 2.69 \times 10^5 A n^{3/2} D^{1/2} C v^{1/2}$, where i_p was current, C (mol cm^{-3}) was $[\text{Fe}(\text{CN})_6]^{3-}$ concentration, v was scan rate (10–500 mV s^{-1}), and A was surface area (cm^2) ($n = 1$, $D = 7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for $[\text{Fe}(\text{CN})_6]^{3-}$) [65]. According to the obtained results by the whole prepared signal amplifications, we preferred CZTS NPs/5.0wt%Pt/g- C_3N_4 /anti-HER2-Ab₂ for subsequent immunosensor application.

Optimization for voltammetric measurements

The effects of pH, immune reaction time, H_2O_2 , and CZTS NPs/5.0wt%Pt/g- C_3N_4 /anti-HER2-Ab₂ solution concentrations were evaluated in detail on Fig. S6.

Linearity range

The obtained voltammograms at +0.4 V by developed immunosensor were shown on Fig. 4, and the inset of Fig. 4 demonstrated the obtained calibration equation as “ $y (\mu\text{A}) = 98.994x (\text{pg mL}^{-1}) + 1.095$ ” based on linearity relation between immunosensor signals and HER2 concentrations. The quantification limit (LOQ) and LOD were calculated as 0.01 pg mL^{-1} and 3.00 fg mL^{-1} , respectively, by equations below:

$$\text{LOQ} = 10.0 S/m \quad (1)$$

$$\text{LOD} = 3.3 S/m \quad (2)$$

where S is the standard deviation of intercept and m is the slope of calibration equation. Table 1 showed the comparative studies in terms of sensitivity. Because of the synergistic effect between Pt NPs and CZTS NPs on g- C_3N_4 surface and high specific surface, the developed voltammetric immunosensor demonstrated the sensitive current signals, indicating a LOD of 3.00 fg mL^{-1} . We have also demonstrated the important advantages of HER2 voltammetric immunosensor in this study. Firstly, the prepared immunosensor with high selectivity was presented. Hence, the developed immunosensor had the potential to be used in place of conventional methods currently used. The immunosensor was also prepared under

one-pot hydrothermal conditions in this study. During the preparation processes of sensor platform and signal amplification, little waste generation was also observed. Hence, cheaper, eco-friendly, and environmentally friendly immunosensor was prepared in this study. Moreover, the analysis process was performed in the shorter time in comparison with the other methods.

Recovery

Table S1 showed the recovery of HER2 in presence of pH 7.0, 0.1 M PBS containing 1.0 mM H_2O_2 . The recovery was calculated by the eq. (3) below:

$$\text{Recovery} = \text{Found HER2} (\text{pg mL}^{-1}) / \text{Real HER2} (\text{pg mL}^{-1}) \quad (3)$$

Table S1 indicated recovery values close to 100.00%, confirming HER2 voltammetric immunosensor's high accuracy in complex protein medium. Furthermore, standard addition method was applied to plasma samples to investigate high selectivity properties of HER2 voltammetric immunosensor. Calibration equation of standard addition method was found as $y (\mu\text{A}) = 98.981x (\text{pg mL}^{-1}) + 10.189$. Hence, the close slopes between direct calibration and standard addition methods demonstrated that HER2 voltammetric immunosensor had perfect selectivity in complex plasma samples.

Selectivity, stability, reproducibility, and reusability

Six different protein solution mixtures including in HER2, HER2 + MUC1, HER2 + HBS, HER2 + CEA, HER2 + IgG, and HER2 + BSA were prepared to show the selectivity test of voltammetric immunosensor. Six different immunosensors were separately prepared by using above protein mixtures. After that, these 6 different immunosensors were applied to 1.0 mM H_2O_2 solution including in pH 7.0 and 0.1 M PBS (2.0 mL). According to Fig. S7A, 0.29% of relative standard deviation (RSD) verified high selectivity of prepared HER2 immunosensor.

The prepared HER2 immunosensor were preserved at 4.0 °C for 7 weeks and applied to 1.0 mM H_2O_2 solution for 7 weeks. According to Fig. S7B, the continuous acquired current signals during 7 weeks were about 99.02% of first current signal. In addition, the same stability tests were repeated for the prepared HER2 immunosensor at 25.0 °C for 7 weeks and 0.13% of RSD for immunosensor signals was obtained during 7 weeks at 25.0 °C. Hence, high stability at 4.0 and 25 °C showed that the prepared HER2 immunosensor was suitable for clinical applications. Twenty different HER2 immunosensors containing 0.100 pg mL^{-1} antigen HER2 were separately also prepared to demonstrate the

Table 1 Comparison of voltammetric HER2 immunosensor with other techniques

Material/method	Linear range	LOD	Ref.
Fe ₃ O ₄ @TMU-21	1.00–100.00 ng mL ⁻¹	0.30 pg mL ⁻¹	[3]
CdSe@ZnS	10.00–150.00 ng mL ⁻¹	2.10 ng mL ⁻¹	[66]
Streptavidin-alkaline phosphatase	7.50–50.00 ng mL ⁻¹	0.16 ng mL ⁻¹	[67]
Ab ₂ -PbS QDs	1.00–100.00 ng mL ⁻¹	0.28 ng mL ⁻¹	[68]
Label-free	10.00–70.00 ng mL ⁻¹	1.60 ng mL ⁻¹	[69]
Label-free	10.00–100.00 ng mL ⁻¹	10.00 ng mL ⁻¹	[70]
CdSe@ZnS quantum dots	0.50–50.00 ng mL ⁻¹	0.29 ng mL ⁻¹	[71]
Potentiometric	0.10–100.00 ng mL ⁻¹	0.08 ng mL ⁻¹	[72]
EIS	0.001–10.00 ng mL ⁻¹	1.00 pg mL ⁻¹	[73]
PbS QDs	1.00–100.00 ng mL ⁻¹	0.28 ng mL ⁻¹	[20]
CZTS NPs/5.0wt%Pt/g-C₃N₄	0.01–1.00 pg mL⁻¹	3.00 fg mL⁻¹	This study

reproducibility of HER2 immunosensor and utilized for 1.0 mM H₂O₂ solution. 0.73% of RSD showed the high reproducibility of the developed immunosensor. Finally, the reusability of HER2 immunosensor was presented in 1.0 mM H₂O₂ solution (Fig. S7C). One developed HER2 immunosensor was used at least 50 times and 0.92% of RSD for immunosensor signals was obtained. Thus, we successfully prepared HER2 immunosensor having high reusability in this study. DPV responses of the proposed HER2 immunosensor for investigations of selectivity, stability, reproducibility, and reusability were recorded at device

parameters such as frequency of 50 Hz, pulse amplitude of 20 mV, and scan increment of 3 mV.

Conclusions

In this report, a breast cancer biomarker HER2 detection based on gold nanoparticles decorated Cu-MOF as sensor platform and Cu₂ZnSnS₄ quaternary chalcogenide with platinum-doped g-C₃N₄ composite as signal amplification was successfully realized by the prepared voltammetric immunosensor. Especially, HER2 voltammetric immunosensor demonstrated a satisfactory selectivity, stability, and reproducibility. The obtained calibration curve was established between 0.01 and 1.00 pg mL⁻¹ and LOD of 3.00 fg mL⁻¹ was observed. Moreover, due to easy, low-cost, and efficient preparation procedure for sensor platform and signal amplification, an environmentally friendly immunosensor was developed. In the same way, an alternative detection method was presented for clinical diagnosis by using breast cancer biomarker HER2 immunosensor. Nonetheless, the additional experiments using real blood samples (not HER2 free plasma samples) from healthy and breast cancer patients should be performed to show the prepared immunosensor's utility in real clinical cases.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00604-021-04735-y>.

Acknowledgements Mehmet Lütfi YOLA would like to thank Turkish Academy of Sciences (TUBA- GEBIP) for their invaluable support.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interests.

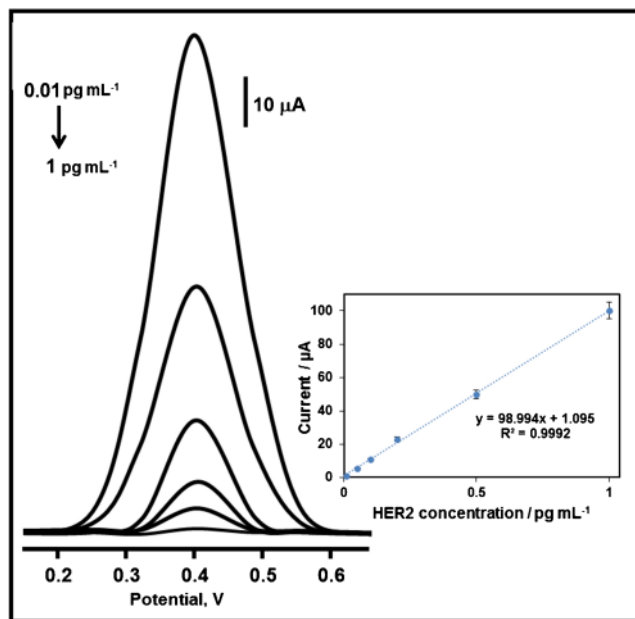


Fig. 4 Concentration effect (from 0.01 to 1.0 pg mL⁻¹ HER2) on immunosensor signals, Inset: Calibration curve for voltammetric HER2 immunosensor (Potential range is 0.0/±0.6 V; Parameters are frequency of 50 Hz, pulse amplitude of 20 mV and scan increment of 3 mV)

Conflict of interest The authors declare that they have no competing of interests.

References

- Dennis J, Slamon PN, Chia S, Fasching PA, De Laurentiis M, Im S-A, Petrakova K, Bianchi GV, Esteve FJ, Martini M, Nusch A, Sonke GS, De la Cruz-Merino L, Beck JT, Pivrot X, Vidam G, Wang Y, Lorenc KR, Miller M, Taran T, Jerusalem G (2018) Phase III randomized study of Ribociclib and Fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast Cancer: MONALEESA-3. *J Clin Oncol* 36(24):2465–2472. <https://doi.org/10.1200/JCO.2018.78.9909>
- Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, Di Cosimo S, Matias-Guiu X, Ramon y Cajal S, Arribas J, Baselga J (2007) Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast Cancer. *J Natl Cancer Inst* 99(8):628–638. <https://doi.org/10.1093/jnci/djk134>
- Ehzari H, Samimi M, Safari M, Gholivand MB (2020) Label-free electrochemical immunosensor for sensitive HER2 biomarker detection using the core-shell magnetic metal-organic frameworks. *J Electroanal Chem* 877:114722. <https://doi.org/10.1016/j.jelechem.2020.114722>
- Chun L, Kim S-E, Cho M, W-s C, Nam J, Lee DW, Lee Y (2013) Electrochemical detection of HER2 using single stranded DNA aptamer modified gold nanoparticles electrode. *Sens Actuators B Chem* 186:446–450. <https://doi.org/10.1016/j.snb.2013.06.046>
- Capobianco JA, Shih WY, Yuan QA, Adams GP, Shih WH (2008) Label-free, all-electrical, in situ human epidermal growth receptor 2 detection. *Rev Sci Instrum* 79(7):076101. <https://doi.org/10.1063/1.2949831>
- Agersborg S, Mixon C, Nguyen T, Aithal S, Sudarsanam S, Blocker F, Weiss L, Gasparini R, Jiang S, Chen W, Hess G, Albitar M (2018) Immunohistochemistry and alternative FISH testing in breast cancer with HER2 equivocal amplification. *Breast Cancer Res Treat* 170(2):321–328. <https://doi.org/10.1007/s10549-018-4755-5>
- Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JMS, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF (2013) Recommendations for human epidermal growth factor receptor 2 testing in breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *Arch Pathol Lab Med* 138(2):241–256. <https://doi.org/10.5858/arpa.2013-0953-SA>
- Wang M, Hu M, Hu B, Guo C, Song Y, Jia Q, He L, Zhang Z, Fang S (2019) Bimetallic cerium and ferric oxides nanoparticles embedded within mesoporous carbon matrix: electrochemical immunosensor for sensitive detection of carbohydrate antigen 19-9. *Biosens Bioelectron* 135:22–29. <https://doi.org/10.1016/j.bios.2019.04.018>
- Yola ML, Atar N (2020) Amperometric galectin-3 immunosensor-based gold nanoparticle-functionalized graphitic carbon nitride nanosheets and core-shell Ti-MOF@COFs composites. *Nanoscale* 12(38):19824–19832. <https://doi.org/10.1039/D0NR05614F>
- Medetalibeyoglu H, Kotan G, Atar N, Yola ML (2020) A novel and ultrasensitive sandwich-type electrochemical immunosensor based on delaminated MXene@AuNPs as signal amplification for prostate specific antigen (PSA) detection and immunosensor validation. *Talanta* 220:121403. <https://doi.org/10.1016/j.talanta.2020.121403>
- Karimi-Maleh H, Arotiba OA (2020) Simultaneous determination of cholesterol, ascorbic acid and uric acid as three essential biological compounds at a carbon paste electrode modified with copper oxide decorated reduced graphene oxide nanocomposite and ionic liquid. *J Colloid Interface Sci* 560:208–212. <https://doi.org/10.1016/j.jcis.2019.10.007>
- Karimi-Maleh H, Karimi F, Alizadeh M, Sanati AL (2020) Electrochemical sensors, a bright future in the fabrication of portable kits in analytical systems. *Chem Rec* 20(7):682–692. <https://doi.org/10.1002/tcr.201900092>
- Miraki M, Karimi-Maleh H, Taher MA, Cheraghi S, Karimi F, Agarwal S, Gupta VK (2019) Voltammetric amplified platform based on ionic liquid/NiO nanocomposite for determination of benserazide and levodopa. *J Mol Liq* 278:672–676. <https://doi.org/10.1016/j.molliq.2019.01.081>
- Tahernejad-Javazmi F, Shabani-Nooshabadi M, Karimi-Maleh H (2019) 3D reduced graphene oxide/FeNi₃-ionic liquid nanocomposite modified sensor; an electrical synergic effect for development of tert-butylhydroquinone and folic acid sensor. *Compos B Eng* 172:666–670. <https://doi.org/10.1016/j.compositesb.2019.05.065>
- Khodadadi A, Faghhi-Mirzaei E, Karimi-Maleh H, Abbaspourad A, Agarwal S, Gupta VK (2019) A new epirubicin biosensor based on amplifying DNA interactions with polypyrrole and nitrogen-doped reduced graphene: experimental and docking theoretical investigations. *Sens Actuators B Chem* 284:568–574. <https://doi.org/10.1016/j.snb.2018.12.164>
- Patris S, De Pauw P, Vandepuit M, Huet J, Van Antwerpen P, Muylderms S, Kauffmann J-M (2014) Nanoimmunoassay onto a screen printed electrode for HER2 breast cancer biomarker determination. *Talanta* 130:164–170. <https://doi.org/10.1016/j.talanta.2014.06.069>
- Al-Khafaji QAM, Harris M, Tombelli S, Laschi S, Turner APF, Mascini M, Marrazza G (2012) An electrochemical immunoassay for HER2 detection. *Electroanalysis* 24(4):735–742. <https://doi.org/10.1002/elan.201100501>
- Medetalibeyoglu H, Beytur M, Akyıldırım O, Atar N, Yola ML (2020) Validated electrochemical immunosensor for ultrasensitive procalcitonin detection: carbon electrode modified with gold nanoparticles functionalized sulfur doped MXene as sensor platform and carboxylated graphitic carbon nitride as signal amplification. *Sens Actuators B Chem* 319:128195. <https://doi.org/10.1016/j.snb.2020.128195>
- Hartati YW, Letelay LK, Gaffar S, Wyantuti S, Bahti HH (2020) Cerium oxide-monoclonal antibody bioconjugate for electrochemical immunosensing of HER2 as a breast cancer biomarker. *Sens Biosensing Res* 27:100316. <https://doi.org/10.1016/j.sbsr.2019.100316>
- Lah ZMANH, Ahmad SAA, Zaini MS, Kamarudin MA (2019) An electrochemical Sandwich Immunosensor for the detection of HER2 using antibody-conjugated PbS quantum dot as a label. *J Pharm Biomed* 174:608–617. <https://doi.org/10.1016/j.jpba.2019.06.024>
- Shen C, Zeng K, Luo J, Li X, Yang M, Rasooly A (2017) Self-assembled DNA generated electric current biosensor for HER2 analysis. *Anal Chem* 89(19):10264–10269. <https://doi.org/10.1021/acs.analchem.7b01747>
- Ranganathan V, Srinivasan S, Singh A, DeRosa MC (2020) An aptamer-based colorimetric lateral flow assay for the detection of human epidermal growth factor receptor 2 (HER2). *Anal Biochem* 588:113471. <https://doi.org/10.1016/j.ab.2019.113471>
- Liao X, Fu H, Yan T, Lei J (2019) Electroactive metal-organic framework composites: design and biosensing application.

- Biosens Bioelectron 146:111743. <https://doi.org/10.1016/j.bios.2019.111743>
24. Furukawa H, Cordova KE, O'Keefe M, Yaghi OM (2013) The chemistry and applications of metal-organic frameworks. *Science* 341(6149):1230444. <https://doi.org/10.1126/science.1230444>
 25. Zhou H, Zheng M, Tang H, Xu B, Tang Y, Pang H (2020) Amorphous intermediate Derivative from ZIF-67 and its outstanding Electrocatalytic activity. *Small* 16(2):1904252. <https://doi.org/10.1002/smll.201904252>
 26. Abuzalat O, Wong D, Park SS, Kim S (2020) Highly selective and sensitive fluorescent zeolitic imidazole frameworks sensor for nitroaromatic explosive detection. *Nanoscale* 12(25):13523–13530. <https://doi.org/10.1039/D0NR01653E>
 27. Chen J, Xu Q, Shu Y, Hu X (2018) Synthesis of a novel au nanoparticles decorated Ni-MOF/Ni/NiO nanocomposite and electrocatalytic performance for the detection of glucose in human serum. *Talanta* 184:136–142. <https://doi.org/10.1016/j.talanta.2018.02.057>
 28. Chen Y-Z, Zhang R, Jiao L, Jiang H-L (2018) Metal-organic framework-derived porous materials for catalysis. *Coord Chem Rev* 362:1–23. <https://doi.org/10.1016/j.ccr.2018.02.008>
 29. W-c P, Chen Y, X-y L (2016) MoS₂/reduced graphene oxide hybrid with CdS nanoparticles as a visible light-driven photocatalyst for the reduction of 4-nitrophenol. *J Hazard Mater* 309:173–179. <https://doi.org/10.1016/j.jhazmat.2016.02.021>
 30. J-w S, Y-w J, S-w P, Nah H, Moon T, Park B, Kim J-G, Kim YJ, Cheon J (2007) Two-dimensional Nanosheet crystals. *Angew Chem Int Ed* 46(46):8828–8831. <https://doi.org/10.1002/anie.200703175>
 31. Badkoobehzavah AM, Abdizadeh H, Golobostanfard MR (2018) Electrophoretic behavior of solvothermal synthesized anion replaced Cu₂ZnSn(SxSe_{1-x})₄ films for photoelectrochemical water splitting. *Int J Hydrog Energy* 43(27):11990–12001. <https://doi.org/10.1016/j.ijhydene.2018.04.140>
 32. Devaraji P, Gopinath CS (2018) Pt – g-C₃N₄ – (au/TiO₂): electronically integrated nanocomposite for solar hydrogen generation. *Int J Hydrog Energy* 43(2):601–613. <https://doi.org/10.1016/j.ijhydene.2017.11.057>
 33. Ong W-J, Tan L-L, Ng YH, Yong S-T, Chai S-P (2016) Graphitic carbon nitride (g-C₃N₄)-based photocatalysts for artificial photosynthesis and environmental remediation: are we a step closer to achieving sustainability? *Chem Rev* 116(12):7159–7329. <https://doi.org/10.1021/acs.chemrev.6b00075>
 34. Zhang Y, Chai C, Zhang X, Liu J, Duan D, Fan C, Wang Y (2019) Construction of Pt-decorated g-C₃N₄/Bi₂WO₆ Z-scheme composite with superior solar photocatalytic activity toward rhodamine B degradation. *Inorg Chem Commun* 100:81–91. <https://doi.org/10.1016/j.inoche.2018.12.019>
 35. Li C, Wu R, Zou J, Zhang T, Zhang S, Zhang Z, Hu X, Yan Y, Ling X (2018) MNPs@anionic MOFs/ERGO with the size selectivity for the electrochemical determination of H₂O₂ released from living cells. *Biosens Bioelectron* 116:81–88. <https://doi.org/10.1016/j.bios.2018.05.045>
 36. Liu CS, Sun CX, Tian JY, Wang ZW, Ji HF, Song YP, Zhang S, Zhang ZH, He LH, Du M (2017) Highly stable aluminum-based metal-organic frameworks as biosensing platforms for assessment of food safety. *Biosens Bioelectron* 91:804–810. <https://doi.org/10.1016/j.bios.2017.01.059>
 37. Xu Q-Q, Liu B, Xu L, Jiao H (2017) Ionothermal synthesis and structural transformation targeted by ion exchange in metal-1,3,5-benzenetricarboxylate compounds. *J Solid State Chem* 247:1–7. <https://doi.org/10.1016/j.jssc.2016.12.006>
 38. Yola ML, Atar N (2014) A novel voltammetric sensor based on gold nanoparticles involved in p-aminothiophenol functionalized multi-walled carbon nanotubes: application to the simultaneous determination of quercetin and rutin. *Electrochim Acta* 119:24–31. <https://doi.org/10.1016/j.electacta.2013.12.028>
 39. La Belle JT, Demirok UK, Patel DR, Cook CB (2011) Development of a novel single sensor multiplexed marker assay. *Analyst* 136(7):1496–1501. <https://doi.org/10.1039/c0an00923g>
 40. Yola ML, Eren T, Atar N (2016) A molecular imprinted voltammetric sensor based on carbon nitride nanotubes: application to determination of melamine. *J Electrochem Soc* 163(13):B588–B593. <https://doi.org/10.1149/2.0311613jes>
 41. Yu X, Shavel A, An X, Luo Z, Ibáñez M, Cabot A (2014) Cu₂ZnSnS₄-Pt and Cu₂ZnSnS₄-au heterostructured nanoparticles for photocatalytic water splitting and pollutant degradation. *J Am Chem Soc* 136(26):9236–9239. <https://doi.org/10.1021/ja502076b>
 42. Yola ML, Atar N (2019) Development of cardiac troponin-I biosensor based on boron nitride quantum dots including molecularly imprinted polymer. *Biosens Bioelectron* 126:418–424. <https://doi.org/10.1016/j.bios.2018.11.016>
 43. Glišić BĐ, Rychlewska U, Djuran MI (2012) Reactions and structural characterization of 33(iii) complexes with amino acids, peptides and proteins. *Dalton Trans* 41(23):6887–6901. <https://doi.org/10.1039/C2DT30169E>
 44. Stanković V, Đurđić S, Ognjanović M, Mutić J, Kalcher K, Stanković DM (2020) A novel nonenzymatic hydrogen peroxide amperometric sensor based on AgNp@GNR nanocomposites modified screen-printed carbon electrode. *J Electroanal Chem* 876:114487. <https://doi.org/10.1016/j.jelechem.2020.114487>
 45. Sheberla D, Sun L, Blood-Forsythe MA, Er S, Wade CR, Brozek CK, Aspuru-Guzik A, Dincă M (2014) High electrical conductivity in Ni₃(2,3,6,7,10,11-hexamino triphenylene)₂, a semiconducting metal-organic Graphene analogue. *J Am Chem Soc* 136(25):8859–8862. <https://doi.org/10.1021/ja502765n>
 46. Zhan W, Shu Y, Sheng Y, Zhu H, Guo Y, Wang L, Guo Y, Zhang J, Lu G, Dai S (2017) Surfactant-assisted stabilization of au colloids on solids for heterogeneous catalysis. *Angew Chem Int Ed* 56(16):4494–4498. <https://doi.org/10.1002/anie.201701191>
 47. Maji SK, Sreejith S, Mandal AK, Ma X, Zhao Y (2014) Immobilizing gold nanoparticles in Mesoporous silica covered reduced graphene oxide: a hybrid material for cancer cell detection through hydrogen peroxide sensing. *ACS Appl Mater Interfaces* 6(16):13648–13656. <https://doi.org/10.1021/am503110s>
 48. Abbasi AR, Karimi M, Daasbjerg K (2017) Efficient removal of crystal violet and methylene blue from wastewater by ultrasound nanoparticles cu-MOF in comparison with mechanochemical method. *Ultrason Sonochem* 37:182–191. <https://doi.org/10.1016/j.ultsonch.2017.01.007>
 49. Feng Y, Jiang H, Li S, Wang J, Jing X, Wang Y, Chen M (2013) Metal-organic frameworks HKUST-1 for liquid-phase adsorption of uranium. *Colloids Surf A Physicochem Eng Asp* 431:87–92. <https://doi.org/10.1016/j.colsurfa.2013.04.032>
 50. Yu J, Wang K, Xiao W, Cheng B (2014) Photocatalytic reduction of CO₂ into hydrocarbon solar fuels over g-C₃N₄-Pt nanocomposite photocatalysts. *Phys Chem Chem Phys* 16(23):11492–11501. <https://doi.org/10.1039/C4CP00133H>
 51. Raza A, Shen H, Haidry AA, Cui S (2019) Hydrothermal synthesis of Fe₃O₄/TiO₂/g-C₃N₄: advanced photocatalytic application. *Appl Surf Sci* 488:887–895. <https://doi.org/10.1016/j.apsusc.2019.05.210>
 52. Hao R, Wang G, Jiang C, Tang H, Xu Q (2017) In situ hydrothermal synthesis of g-C₃N₄/TiO₂ heterojunction photocatalysts with high specific surface area for Rhodamine B degradation. *Appl Surf Sci* 411:400–410. <https://doi.org/10.1016/j.apsusc.2017.03.197>
 53. Mousavi M, Habibi-Yangjeh A, Abitorabi M (2016) Fabrication of novel magnetically separable nanocomposites using graphitic carbon nitride, silver phosphate and silver chloride and their applications in photocatalytic removal of different pollutants using visible-

- light irradiation. *J Colloid Interface Sci* 480:218–231. <https://doi.org/10.1016/j.jcis.2016.07.021>
54. Xu A, Tao H, Chen S, Zhu L, Zhao Y, Jiang J, Pan L, Tao J (2015) A novel approach to utilize thiol reduced graphene oxide as linker molecule for Cu₂ZnSnS₄ sensitized solar cell. *Int J Hydrog Energy* 40(46):15933–15939. <https://doi.org/10.1016/j.ijhydene.2015.09.068>
55. Suryawanshi M, Shin SW, Ghorpade U, Song D, Hong CW, Han S-S, Heo J, Kang SH, Kim JH (2017) A facile and green synthesis of colloidal Cu₂ZnSnS₄ nanocrystals and their application in highly efficient solar water splitting. *J Mater Chem A* 5(9):4695–4709. <https://doi.org/10.1039/C7TA00257B>
56. Raza A, Shen H, Haidry AA (2020) Novel Cu₂ZnSnS₄/Pt/g-C₃N₄ heterojunction photocatalyst with straddling band configuration for enhanced solar to fuel conversion. *Appl Catal B* 277:119239. <https://doi.org/10.1016/j.apcatb.2020.119239>
57. Ou M, Wan S, Zhong Q, Zhang S, Wang Y (2017) Single Pt atoms deposition on g-C₃N₄ nanosheets for photocatalytic H₂ evolution or NO oxidation under visible light. *Int J Hydrog Energy* 42(44):27043–27054. <https://doi.org/10.1016/j.ijhydene.2017.09.047>
58. Nie L, Yu J, Li X, Cheng B, Liu G, Jaroniec M (2013) Enhanced performance of NaOH-modified Pt/TiO₂ toward room temperature selective oxidation of formaldehyde. *Environ Sci Technol* 47(6):2777–2783. <https://doi.org/10.1021/es3045949>
59. Luo Y, Yan Y, Zheng S, Xue H, Pang H (2019) Graphitic carbon nitride based materials for electrochemical energy storage. *J Mater Chem A* 7(3):901–924. <https://doi.org/10.1039/C8TA08464E>
60. Akbarzadeh F, Motaghi M, Chauhan NPS, Sargazi G (2020) A novel synthesis of new antibacterial nanostructures based on Zn-MOF compound: design, characterization and a high performance application. *Heliyon* 6(1):e03231. <https://doi.org/10.1016/j.heliyon.2020.e03231>
61. Khandan FM, Afzali D, Sargazi G, Gordan M (2018) Novel uranyl-curcumin-MOF photocatalysts with highly performance photocatalytic activity toward the degradation of phenol red from aqueous solution: effective synthesis route, design and a controllable systematic study. *J Mater Sci Mater Electron* 29(21):18600–18613. <https://doi.org/10.1007/s10854-018-9978-z>
62. Li J-R, Sculley J, Zhou H-C (2012) Metal-organic frameworks for separations. *Chem Rev* 112(2):869–932. <https://doi.org/10.1021/cr200190s>
63. DeCoste JB, Peterson GW (2014) Metal-organic frameworks for air purification of toxic chemicals. *Chem Rev* 114(11):5695–5727. <https://doi.org/10.1021/cr4006473>
64. Mattioli IA, Baccarin M, Cervini P, Cavalheiro ÉTG (2019) Electrochemical investigation of a graphite-polyurethane composite electrode modified with electrodeposited gold nanoparticles in the voltammetric determination of tryptophan. *J Electroanal Chem* 835:212–219. <https://doi.org/10.1016/j.jelechem.2018.12.056>
65. Yola ML, Atar N (2019) Development of cardiac troponin-I biosensor based on boron nitride quantum dots including molecularly imprinted polymer. *Biosens Bioelectron* 126:418–424. <https://doi.org/10.1016/j.bios.2018.11.016>
66. Freitas M, Neves MMPS, Nouws HPA, Delerue-Matos C (2020) Quantum dots as nanolabels for breast cancer biomarker HER2-ECD analysis in human serum. *Talanta* 208:120430. <https://doi.org/10.1016/j.talanta.2019.120430>
67. Freitas M, Nouws HPA, Delerue-Matos C (2019) Electrochemical sensing platforms for HER2-ECD breast Cancer biomarker detection. *Electroanalysis* 31(1):121–128. <https://doi.org/10.1002/elan.201800537>
68. Lah Z, Ahmad SAA, Zaini MS, Kamarudin MA (2019) An electrochemical sandwich immunosensor for the detection of HER2 using antibody-conjugated PbS quantum dot as a label. *J Pharm Biomed Anal* 174:608–617. <https://doi.org/10.1016/j.jpba.2019.06.024>
69. Pacheco JG, Rebelo P, Freitas M, Nouws HPA, Delerue-Matos C (2018) Breast cancer biomarker (HER2-ECD) detection using a molecularly imprinted electrochemical sensor. *Sens Actuators B Chem* 273:1008–1014. <https://doi.org/10.1016/j.snb.2018.06.113>
70. Sharma S, Zapatero-Rodríguez J, Saxena R, O’Kennedy R, Srivastava S (2018) Ultrasensitive direct impedimetric immunosensor for detection of serum HER2. *Biosens Bioelectron* 106:78–85. <https://doi.org/10.1016/j.bios.2018.01.056>
71. Freitas M, Nouws HPA, Keating E, Fernandes VC, Delerue-Matos C (2020) Immunomagnetic bead-based bioassay for the voltammetric analysis of the breast cancer biomarker HER2-ECD and tumour cells using quantum dots as detection labels. *Microchim Acta* 187(3):184. <https://doi.org/10.1007/s00604-020-4156-4>
72. Ou D, Sun D, Lin X, Liang Z, Zhong Y, Chen Z (2019) A dual-aptamer-based biosensor for specific detection of breast cancer biomarker HER2 via flower-like nanozymes and DNA nanostructures. *J Mater Chem B* 7(23):3661–3669. <https://doi.org/10.1039/C9TB00472F>
73. Gu C, Guo C, Li Z, Wang M, Zhou N, He L, Zhang Z, Du M (2019) Bimetallic ZrHf-based metal-organic framework embedded with carbon dots: ultra-sensitive platform for early diagnosis of HER2 and HER2-overexpressed living cancer cells. *Biosens Bioelectron* 134:8–15. <https://doi.org/10.1016/j.bios.2019.03.043>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.