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ARTICLE

Fabrication of Photo-electrochemical biosensor for ultrasensitive screening of mono-bioactive molecules: effect of geometrical structures and crystal surfaces

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The controlled design of biosensors based on photo-electrochemical technique with high selectivity, sensitivity, and rapid response for monitoring of mono-bioactive molecules, particularly dopamine (DA) levels in neuronal cells is highly necessary for clinical diagnosis. Hierarchical carbon-, nitrogen-doped (CN) nickel oxide spear thistle (ST) flowers associated in single-head (S), and symmetric and asymmetric-double heads (D, and A, respectively) that tightly connected through micrometric dipole-like rod or trunk were fabricated by using a simple synthetic protocol. The CN-ST flower heads were decorated with dense nano-tubular like hedgehog needleshed skins in vertical alignments. These designated architectures are key features for creating biosensor surface electrodes for photo-electrochemical, ultrasensitive screening of mono-bioactive molecules. The exceptional electrode designs produced numerous catalytically active sites, large surface area, and high electron-transfer mobility. The active coating of carbon-nitrogen nanospheres significantly enhanced the photo-electrocatalytic activity of the prepared biosensor electrodes and prevented leakage of photocatalytic activity under long-term exposure to irradiation. Among all photo-electrochemical assays, the biosensors showed significant sensitivity and selectivity of DA in the presence of interfered molecules such as ascorbic acid (AA), uric acid (UA), adrenaline (A), and noradrenaline (NA). The photo-electrochemical of the CN-SST-{110} crystal surface electrode showed significant sensing performance of DA in terms of unimpeded diffusion pathways, a wide concentration-detection range, and a low detection limit, even in the coexistence of the potentially interfering molecules compared with other electrodes-modified CN-DST-{111} and CN-AST-{101} crystal surfaces. Furthermore, the CN-SST photo-biosensor electrode shows potential in the selective and sensitive determination of DA in real samples, such as human serum and secreted DA from living cells. This finding indicates that the hierarchical ST biosensor may enable analytical discrimination and monitoring of DA and can be employed for clinical diagnosis application.

1- Introduction

The ability of the human body to regulate the variables at steady or stable internal environment is known as

homeostasis.¹ The health of a human body is highly dependent upon the functioning of the organs to maintain homeostasis by regulating various negative and positive feedback mechanisms.¹ The slight inability in organs to regulate the stable internal environment may lead to serious health problems or even death. Significant progress has been created to assist in the detection of various abnormalities in the human body by control monitoring of several bio-molecules such as dopamine, uric acid (UA), ascorbic acid (AA), glucose, and hydrogen peroxide (H₂O₂).² The precise detection of these biomolecules would have the ability to provide useful information about the treatment and prevention of various genetic problems.² Therefore, keen interest has grown in developing a portable sensor for rapid, cost effective and on-site monitoring of a wide range of targets to revolutionize scientific research. Dopamine is an important catecholamine neurotransmitter in the mammalian central nervous system where it plays a key role in controlling locomotion, learning, working memory, cognition, and emotions.³ Several neurological disorders, including Parkinson's disease,

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Electronic Supplementary Information (ESI) available: [characterization analysis apparatus, schematic preparation of materials, SEM for the prepared materials, XPS spectrum with survey of N1 2P and O 1S, DPV for AA, DA and UA at ITO and CN-SST, individual and simultaneous detection of AA, DA and UA using DPV, structural stability after formation of thin film from CN-STs and after successive using in 0.5 mM DA for 20 cycles using CV measurements, and column plot for discovery of stability and reproducibility, CN-SST cytotoxicity using CCK-8 protocol, amperometric response for DA released from PC12 and the column for obtaining the current response with and without PC12, and Table S1 for real sensitive detection of DA in complicated medium (human blood serum). See DOI: 10.1039/x0xx00000x

schizophrenia, human immunodeficiency virus infection, and restless leg syndrome resulted from the abnormality in the dopamine concentrations.^{3,4} Therefore, finding a reliable analytical method is clinically important to measuring dopamine level in physiological or biological samples.

Several methods have been reported and developed to quantitatively detect dopamine using simple instruments such as Raman spectroscopy, capillary electrophoresis, fluorescence spectrometry and high-performance liquid chromatography.^{4,5} However, most such sensing devices cannot compete with the electrochemical method due to its advantages of low cost, simple operating system, high sensitivity and commercial availability to the public. Electrochemical dopamine sensors with enzymes (e.g., tyramine oxidase) play a leading role in research attempts to simplify dopamine testing and are expected to play a similar role in the move toward continuous dopamine monitoring.⁶ However, the major disadvantages of these enzymatic dopamine sensors are their lack of stability, the difficulty of recycling, and tedious fabrication processes, which are hard to overcome. Consequently, substantial attention has been focused on direct electrocatalytic oxidation of dopamine at enzyme-free electrodes with high sensitivity and stability, fast response time, simple fabrication process, and wide working range.

A major challenge in using non-enzymatic electrochemical sensor for the precise monitoring of dopamine is to find a material that can accurately sense dopamine in the coexistence of high concentration of primary interferences such as UA and AA.⁷ To overcome these challenges, two fundamental approaches have been developed: one is to pre-coat the working electrode surface with negatively charged polymer such as Nafion and charged surface. This negatively charged polymer repels the anionic species such as UA and AA while attracting the positively charged dopamine.^{8,9} However, these negatively charged polymer-based electrodes suffer from drawbacks such as very low sensitivity, poor reproducibility, and non-uniform thickness. The second approach is to modify the electrode surface by applying a variety of advanced nanostructures/microstructures with well-controlled shape that can separate the oxidation potential of dopamine from UA and AA.¹⁰ Thus far, intensive efforts have been devoted to modifying the electrode surface with various structures including gold nanowires, nanoslices and nanocorals, palladium nanoclusters, CuO nanoleaves, titanium dioxide nanosheets and reduced graphene oxide sheets modified with gold nanoparticles.^{11–15} NiCo-NPs-in-N/C, graphene fiber, and reduced graphene oxide-zinc oxide are used for simultaneous detection of AA, UA, and DA.^{16–18} However, most of these structural modifications provide high selectivity but lack sensing efficiency. Therefore, constructing electrochemical dopamine sensor is particularly challenging for designing nanostructures/microstructures with a controlled shape that can preferentially expose a great fraction of electrochemically active sites to achieve high sensitivity and selectivity.

Photo-electrochemistry possesses a different form of excitation energy (light) and detection, thus it is representing

high sensitive analytical method. Furthermore, the use of electronic detection turned the photo-electrochemical (PEC) instrument easier and low cost compared with conventional optical methods. Although photo-electrochemistry is a potentially sensitive analytical method, the limited material has photoactive property for the photo-electrochemical application.^{19,20}

Transition metal oxides have characteristic properties, such as size-tunable optical and electronic properties; therefore, it is used in the field of PEC.^{21–23} However, transition metal oxides optical properties have limitations in instability and photo corrosion.^{24,6}

Hence, to overcome these problems, carbonaceous materials (such as graphene, carbon nanotubes, and carbon dots) were used as dopant materials to enhance the visible light PEC properties and prevent the photo corrosion of the transition metal oxides.^{25–31} Improvement of ZnO NPs efficient of energy in visible light was achieved by doping ZnO NPs on functionalized SWCNTs.²⁸ In addition, graphene enhances the photocurrent enhancement of TiO₂ nanocrystals under visible light as reported, which was five times higher than that of only TiO₂ nanocrystals.²⁶ All these above-mentioned results indicate that the PEC properties of transition metal oxides can be improved effectively by appropriate doping with carbonaceous materials, and designing various attempts to prepare novel carbonaceous material-doped semiconductor nanohybrids for PEC sensing under visible light is still highly desirable and technologically important.

A few reports for PEC biosensors, such as graphene quantum dots–TiO₂ nanocomposite, were used for dopamine sensing under visible light irradiation with low detection limit and wide linear range.²⁹ Other PECs using composite materials (metal oxides with carbonaceous materials) were reported for glucose, NADH, and H₂O₂ sensing.^{24–36}

In this study, the photo-electrochemical biosensor was fabricated based on hierarchical carbon-, nitrogen-doped (CN) nickel oxide spear thistle (ST) flowers associated in single-head (S), and symmetric and asymmetric-double heads (D, and A, respectively) that tightly connected through micrometric dipole-like rod or trunk. The significant enhancement of photo-electrochemical performance for monitoring of mono-bioactive molecules, particularly dopamine (DA) levels in neuronal cells was due to the morphological CN-ST architectures. The CN-ST flower heads were decorated with dense nano-tubular like hedgehog needless skins in vertical alignments. The CN-ST structural ordination and arrangement lead to exceptional electrode designs with numerous catalytically active sites, large surface area, and high electron-transfer mobility. In addition, the CN nanosphere- doped ST acts as electron transport channels between the active centers of the electrode–electrolyte interface, leading to enhanced stabilization photo electrocatalytic activity of CN-ST biosensors under irradiation. Among all of the geometrical structures of CN-ST photo-biosensor electrodes, the CN-SST biosensor shows high photo-electrocatalytic activity, leading to create

DA-sensitive and -selective sensing assays. Significantly, the determination of DA released from the living cell (PC12) was successfully achieved with potential sensitivity and selectivity using CN-SST biosensor. This finding indicates that the hierarchical CN-SST based photo biosensor may enable monitoring of DA secreted from living cells and in human blood serum samples.

Scheme 1

2- Results and discussion

Controlling growth with directing morphology of NiO was assembled. Construction of NiO was built depending on the morphology directing agent diammonium hydrogen phosphate ((NH₄)₂HPO₄). Controlling addition of directing agent with constant flow rate (Q) forming different constructions of NiO like spear thistle (ST) flowers with (single head (S), symmetric double head (D) and asymmetric double head (A) that tightly connected through micrometric dipole-like rod or trunk as in scheme S1. The initial precursor nickel nitrate hexahydrate (Ni(NO₃)₂·6H₂O, (NH₄)₂HPO₄) and the as-obtained (ST) flowers construction can produce from successive reactions. In a typical procedure, hydrolysis of (NH₄)₂HPO₄ produce OH⁻ anions, which subsequently reacted with Ni²⁺ to form Ni-OOH ST hierarchy. The dropwise addition of PO₄³⁻ ions to the composition domains in definitely-controlled follow rate leads to control the formation of ST flower structure associated in single-head (S), and symmetric and asymmetric-double heads (D, and A, respectively), see Scheme S1.

The ST crystal growth with controlling head shapes may be due to the electrostatic attraction between positive and negative charge, where PO₄³⁻ ions possess a massive negative charge and attracted the Ni²⁺ ions. Thus, PO₄³⁻ ions are the key role in the formation of hierarchal architectures (SST, DST, and AST). The flow rate of additive (NH₄)₂HPO₄ directly affected the kinetic growth and shape of ST heads. Moreover, the ST head construction may be attributed to the stereo PO₄³⁻ shape in its solution, which reflected the crystal growth depth from inner to outer. The CN was assembled after the stable construction of ST, a thin film for polyaniline that polymerized at 0°C after dropwise addition of potassium persulfate with continuous stirring. Potassium persulfate starts the free radical monomer, with continued homogenous black precipitate was formed at ST surfaces. Carbonization of polyaniline that acts as the nitrogen and carbon source at 500 °C in Ar gas flow revealed a homogenous CN that dressed at the surface of ST samples.

2.1 Structure features of ST hierarchal biosensor

Figure 1A shows the high-angle-annular-dark-field scanning transmission electron microscopy (HAADF-STEM) of ST with the symmetric double head (D), wherein two heads spread on opposite sides and tightly connected through micrometric dipole-like rod or trunk. High-magnification HAADF-STEM image (Fig. 1B) represents ST flower heads were decorated with dense nano-tubular like hedgehog needlesh skins in vertical alignments. High magnification focused on the head

surface clearly reflects the surface contains a thick nanotube at the head surface ends with an average size of 15–20 nm as in Fig. 1C. As shown in Fig. 1D, the whole head of ST samples is uniformly covered with carbon nanospheres with an average size of 5–10 nm. High-magnification HAADF-STEM image (Fig. 1D) shows that C spheres with an average thickness of less than 10 nm have been oriented along the nanotube-like morphology.

Confirmation of the distribution and elemental contents at the surface of ST was demonstrated by EDS-STEM mapping as in Fig. 1(E–I). The surface elemental composition and distribution reflect the homogeneous distribution of C, Ni, O, and N at the CN-ST samples and dominant Ni and O atoms with high percentages, such as 46.2% and 48.05% for Ni and O, respectively, also revealed that the carbon present on the surface contents of 4.15% and small amount of doping N (1.6%). These results provide the surface containing carbon and nitrogen-ST with a homogeneous distribution that is responsible for the smooth surface of ST and the pore distribution at the surface. ED-STEM can provide the atomic arrangement, crystal plane and the active surface of CN-ST catalysts, as in Fig. 1(K–M). The finding reflects the high ordered crystalline surface with different active site plans for DST-, AST-, and SST-NiO, namely, {111}, {101}, and {110} facets, respectively. ED-STEM image of nanotubes at the surface of DST-AST and SST reflects that the crystal growth is grown along {111}, {101}, and {110} facets, respectively. Figs. 1(N–Q) shows the SAED-TEM images that revealed the diffraction spots around dominant {111}, {101}, and {110} crystal planes. Further confirmation of controlled structure was obtained from field emission- scanning electron microscope (FE-SEM) images as in Fig. S1. Figure S1A shows the homogeneous and unit geometrical structure of ST flower like, which is similar to spear thistle (SST) flowers with single head and trunk. High magnification of FE-SEM reflects that the head size was approximately 3–4 μm with a trunk size of approximately 1–2 μm, and relevant CN covers the unit surface (Fig. S1A, inset). After the flow rate was defined, ST was constructed with a double head connected by trunk in-between and the two heads spread on opposite sides like a dipole with the symmetric double head (DST) (Fig. 1SB) and asymmetric double head (AST) (Fig. S1C). Construction of ST with asymmetric double head and CN was revealed from inset Fig. S1C, where and C- nitrogen nanospheres covers the surface of NiO with the homogeneity and formality.

High resolution- Transmission electron microscope (HR-TEM) images further revealed that the hierarchical ST doped with CN was grown. The Findings obtained from TEM, EDS-STEM, and SEM profiles (Fig. 1), CN were uniformly bound on the whole surface of prepared materials through the H-bonding or electrostatic interactions preferably rearranged to form well-dispersed and homogeneous CN nanoparticles (<<10 nm).

Figure 1

Active sites for {110}, {101}, and {111} crystal planes and its surface atomic configurations, electron density difference maps, and pulse surface electrons in continuous generation modes around ST are illustrated by density functional theory

(DFT) (Fig. 2). Double layer atomic configuration of Ni^{2+} and O^{2-} around {111} crystal plane of SST provides the highly crystalline order of its surface and alignment of atomic distribution (A). The mesostructured pore windows formed around {110} SST crystal facet with center cubic $\text{Fm}\bar{3}\text{m}$ crystal symmetry were taken perpendicular to the fourfold axis and with tilt modeling to show a pronounced atomic-scale arrangement at the center and edge of the crystal lattice of the entire tube-shaped layer. Open pores at the ends of the nanotubes of SSH directly affected on the electrocatalytic nature by influencing the charge transfer and mass transport, leading to rapid strong binding with the target molecules. The contact surface was further provided with target molecules with active sites Ni^{2+} and rich- O^{2-} vacancies along with the atomic monolayer based on axial alignment, which affects the catalytic behavior of {110} plane as in Fig. 2B. The continuous electron streams of double layer on the top-level isosurface of the {111} and {101} orientations of DST and AST distributed closely to a central and focal point surface around Ni^{2+} and O^{2-} vacancy sites (Fig. 2(C&D)). The difference of crystal planes of ST because of variable geometrical shape along with atomic configuration around each crystal planes, which represents the effectiveness of catalytic activity of each surface, the power of binding with target biomolecules, and centered active atoms. The atomic configuration and charge surface was affected by the presence of CN at its surface, where a cloud of charge around {110} plane for a single layer of Ni^{2+} and rich- O^{2-} configuration (E). The surface charge characteristic of Ni^{2+} and rich- O^{2-} vacancies configuration was influenced by CN. The clouds of CN cover the surface, where the stability of dominant rich- O^{2-} vacancy sites increased. The partial polarization between N and C stabilizes and enhances the O^{2-} vacancy sites and increases the binds with positively target biomolecules, such as DA. The active doping of CN may lead to the formation of a protective layer and represents as negative charge balancers. The surface-representing localized networks of the confinement electrostatic potential and elevated the surface charge density around C, N, O, and Ni atoms and effective potential DA-to- Ni^{2+} site binding. This geometric molecule-to-surface orientation generated numerous accessible surface sites, flexible interactions, and thermodynamically stable bindings. The surface configurations of hierarchical SST oriented with nanotubes with open holes and longitudinal long-range channels can feasibly create dense Ni^{2+} atoms and more electron-rich O^{2-} vacancy sites, producing more efficient electron transport. CN facilitates the mass transport and effectiveness for binding of the target molecule at {110} exposed plane.

Figure 2

N_2 adsorption-desorption isotherm was used for detection of surface area and pore size distribution. As shown in Fig. 3A the N_2 adsorption isotherms exhibit IV feature with H1 hysteresis loop. Hysteresis between adsorption and desorption branches observed at $0.82 < p/p_0 > 1$ for CN-SST and form at $0.88 < p/p_0 > 1$ for CN-DST and CN-AST whereas form at $0.92 < p/p_0 > 1$ for SST, which demonstrated the hierarchical formation of ST architectures.³⁷⁻⁴⁰ CN has potentially effect in

the surface area and porous structure.^{41,42} Pore-size distribution was observed according to the NLDFT model (Fig. 3B), where the dominant porous structure was in the region of mesoporous. All the samples displayed very close pore size distribution, while the CN-SST exhibits the highest pore volume. The specific surface areas (S_{BET}) = 22.3, 42.3, 60.5, and $75.4 \text{ m}^2 \text{ g}^{-1}$ for SST, CN-DST, CN-AST, and CN-SST respectively (table inset in Fig.3B). A significant increase of the surface area increases in the presence of CN for SST, which prevent the agglomeration of the ST. The CN-SST has the highest surface area and provides an invasive electrolyte electrode interface and can enhance the ion diffusion kinetics. Consequently, structure formation can improve the photo-electrochemical capacitance and the charge transfer kinetics.^{22,23}

Sharp and well-resolved diffraction peaks for the ST samples are found in the WA-XRD patterns as shown in Fig. 3C, which was consistent with JCPDS no. 01-089-5881 (i.e., a face-centered cubic $\text{Fm}\bar{3}\text{m}$ symmetry with the lattice constant $a = 8.35 \text{ \AA}$). The highly ordered crystalline structure of NiO is confirmed and provides the ED-STEM photos.^{43,44} However, WA-XRD profile of carbon incorporated SST, DST and AST (Fig. 3C (red line)) were found to keep the characteristic diffraction peaks of face-centered cubic NiO (JCPDS no. 01-089-5881), along with a broad peak at $2\theta = 24.61$, indicating the existence of carbon. This finding provides the degree of crystalline structure of STs Structures and the existence of CN at its surface. Raman spectra can provide characteristic features of sample molecules that act as a fingerprint, unique to a molecule or indeed and individual molecular structure. The Raman spectra of the SST, DST, and AST samples show several bands above 400 cm^{-1} at an excitation wavelength of 532 nm as in Fig. 3A. The Raman spectra of SST, DST, and AST showed a vibrational band at approximately 532 , 750 , and 1085 cm^{-1} caused by (1P) and (2P) corresponding to the first-order transverse optical TO, 2TO, and 2LO (longitudinal optical) modes, respectively, which are characteristics for NiO.⁴⁵ However, the existence of two distinct peaks in CN-SST, CN-DST and CN-AST corresponds to D ($\sim 1350 \text{ cm}^{-1}$) and G ($\sim 1600 \text{ cm}^{-1}$) bands originating from the disordered carbon and sp^2 cluster, respectively.⁴⁶ To elucidate the surface features and components of CN-doped ST compared, ST measurements were performed. Figure S2A shows wide range XPS spectra (i.e., survey) of ST and CN doped ST components. In addition, high-resolution XPS spectra of C 1s and N 1s peaks are shown in Figs. 3E and 3F, respectively. Figure 3E shows high-resolution XPS spectra of the C 1s peaks to reveal the carbon source in ST, which represents strong peaks at 285.01 , 285.8 , 288.2 , and 289.4 eV assigned to C-C (sp^2), C-C (sp^3), C=O, and O-C=O bands, respectively, indicating the conversion of polyaniline to CN nanospheres after carbonization. The presence of pyridinic nitrogen that results from the defects caused by N-doping may enable the analyte to penetrate more rapidly and boost the shuttling of ions at the electrode-electrolyte interface.⁴⁷ The N1s spectra with four main peaks centered at 398.22 , 399.14 , 400.4 , and 402.6 eV assigned to pyridinic nitrogen (C=N), pyrrolic nitrogen (C-N), graphitic nitrogen, and N-oxide of

pyridinic nitrogen, respectively, as in Fig. 3F. The Ni 2p spectra with main peaks centered at 872.3 and 854.3 eV assigned to Ni 2p₁ and Ni 2p₃ of Ni (II) ions, respectively.⁴⁸ The peaks of Ni 2p and O 1s in the ST undergo visible shifts to lower binding energy compared with pristine ST (Fig. S2 (B&C)). This finding indicates the surface binding interactions between the CN nanospheres and ST.

Figure 3

2.2 Photo-electrocatalytic activity of CN-ST biosensor

First, the photo-electrochemical behaviors of the NiO-based (such as DST, AST, and SST), and CN-NiO-based biosensors (such as CN-DST, CN-AST, and CN-SST) deposited at ITO electrode were investigated using cyclic voltammetry in 0.1 M phosphate buffer solution (PBS) (pH 7.4) containing 1 mM [Fe(CN)₆]³⁻ within the potential range of 0.0 V to 0.6 V (vs. Ag/AgCl) at a scan rate of 100 mV/s (Fig. 4A). [Fe(CN)₆]³⁻ was used as a redox probe to investigate various factors, including electronic properties, surface structure, and surface chemistry that affect charge kinetics of the selected electrodes. As shown in Fig. 4A, the redox peak separation (ΔE_p) values of the CN-DST (95 mV), CN-AST (80 mV), and CN-SST (60 mV) photo-biosensor electrodes were significantly lower than that of the DST (120), AST (100 mV), and SST (90 mV) photo-biosensor electrodes, which can be attributed to the enhanced ion transmission performance at the electrode-electrolyte interface, thereby engaging greater fractions of catalytic active sites at the surface of the electrode for the Faradaic redox reaction.⁴⁹⁻⁵¹

The interfacial electron-transfer rate was measured using EIS to decouple the electrocatalytic activity of the ST-, and CN-ST photo-biosensor electrodes. In an EIS measurement, the semicircle diameter reflects the electron-transfer resistance (R_{et}) of the electrode, and the line reflects the diffusion of the electroactive species. In general, a large semicircle indicates a high charge transfer resistance of the electrode, implying a low electron transfer rate (higher resistivity). Typical Nyquist impedance plots of ST- and CN-ST photo-biosensor electrodes in 0.1 M PBS (pH 7.4) are shown in Fig. 4C. The SST-based photo-biosensor structures (Fig. 4C) represent a small semicircle diameter at high frequency, showing a relatively small R_{et} . The semicircle of the CN-SST photo-biosensor electrode showed a further decrease, with extremely lower R_{et} value and higher conductivity than that of SST electrodes. This finding suggests the enhanced electron-transport efficiency of the CN-SST photo-biosensor. Furthermore, the key parameters of CN-SST photo-biosensor such as high surface area, dominant {110} crystal facet play role in the low resistivity, high electron diffusion pathway, and high DA-sensitivity electrode design compared with the other CN-DST and CN-AST structures.

To investigate the effect of ST geometrical structure, and CN-doped ST electrode designs on the photo-electrochemical oxidation activity of DA molecule, we study the C.V of the NiO-based (such as DST, AST, and SST), and CN-NiO-based (such as CN-DST, CN-AST, and CN-SST) photo-biosensor electrodes in

0.1 M PBS at pH=7, 5 μ M DA at a scan rate of 100 mV s⁻¹ under UV (at λ =365 nm) light irradiation, respectively. Figure 4C shows that the photocatalytic current of SST photo-biosensor electrode with CN is higher than that of pristine NiO electrode for DA-sensing. The NiO spear thistle (ST) flowers associated in single-head (S), and symmetric and asymmetric-double heads (D, and A, respectively) that fabricated with different crystal arrangements and active surface planes (i.e., {110}, {101}, and {111} facets, respectively) play key components in the enhancement the DA photo-catalytic activity. For instance, the electrode designs with different NiO crystal facets lead to increase the DA oxidation in the following order: {110}-CN-SST > {101}-CN-AST > {111}-CN-DST, respectively, as evidenced from CVs and impedance profiles (Fig. 4). The {110}-CN-SST photo-biosensor electrode exhibiting negative shift compared with other electrodes-modified {111}-CN-DST and {101}-CN-AST crystal surfaces may be due to its high surface area, pore volume, and effectively-faceted {110} plane due to highly dense active sites and atomic configuration of Ni²⁺ and O²⁻ as presented in Scheme 1.

The UV light irradiation at λ =365 nm of the CN-ST-modified ITO electrode surfaces may lead to the formation of an electron e^-_{CB} (conduction band) and a hole h^+_{VB} (valence band) pair (Scheme 1). The mobility of generated h^+_{VB} / e^-_{CB} onto the CN-ST surfaces leads to increase the electrical conductivity of the CN-ST-modified ITO. The homogenous dispersion of CN onto the entire NiO crystal surfaces produces negative charges and then stabilizes the dense electron clouds at its surface. The dense configuration of e^-_{CB} and mobile electron clouds onto surface lead to enhance the CN-ST/ITO-to-DA binding. The facile electron movement/transfer along electrode surfaces under irradiation enhances the photoinduced oxidation of DA. This photoinduced electrode surface process boosts the sensitive, selective monitoring of low-level concentrations of DA target.²⁵⁻³⁶

The effect of solution pH on the photo-electrochemical oxidation of 5 μ M DA on CN-SST photo-biosensor electrode was investigated in the range of 6 to 9 at a scan rate of 100 mV s⁻¹ by time-dependent on-off switching response of UV light irradiation (365 nm) at an applied potential of 0.21 V (Fig. 5A). As shown in Fig. 5B, the oxidation peak current of DA increased with increasing pH from 6 to 7.4 but decreased within the range of more than 7.4. Thus, pH 7.4 was selected as the optimum solution pH for DA sensing and is approximately the physiological pH leading to easy biological control.

Controlled surface processes of CN-SST photo-biosensor electrode were investigated in the presence of 5 μ M DA using cyclic voltammetric technique under UV light irradiation (365 nm) in 0.1 M PBS (pH 7.4) at different scan rates ranging from 10–100 mV s⁻¹. As in Fig. 5C, the redox peaks of DA were increased with increasing scan rate, from 10 mV s⁻¹ to 100 mV s⁻¹. A linear relation between oxidation and reduction peak current with the square root of scan rate was observed with

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regression equations $I_a(\mu A) = -1.7 + 0.155 v^{1/2} (mVs^{-1})$ ($R^2=0.97$) and $I_c(\mu A) = 2.1 - 1.25 v^{1/2} (mVs^{-1})$ ($R^2=0.992$) (Fig. 5D). This linearity suggests that the DA electrochemical process occurred at the surface of CN-SST photo-biosensor electrode is a diffusion-controlled process.⁵²⁻⁵⁴

Figure 4, 5

2.3 Photo-electrochemical behavior of CN-ST biosensors for monitoring mono-bioactive molecules

The photo-electrochemical performance of mono-bioactive molecules such as AA, UA, A, NA, and DA on CN-SST and SST photo-biosensor electrodes were investigated using differential pulse voltammetry in 0.1 M N₂-saturated PBS (pH=7.4) under UV irradiation. As shown in Fig. S3(A–E); the strong DPV peak obtained from photo-electro-oxidation of AA, UA, A, NA, and DA on CN-SST photo-biosensor electrode provides the high sensitivity of designed photo-biosensor. The photo-oxidation peak potentials of 0.5 mM AA, 0.2 mM UA, 20 μ M, 20 μ M NA, and 5 μ M DA are at -0.01, 0.36, 0.12, 0.17, and 0.21 V in 0.1 M PBS at pH=7.4; the difference of the oxidation peak potential (peak separation) between A and NA (ΔE_{A-NA}), NA and DA (ΔE_{NA-DA}), and A and DA (ΔE_{A-DA}) were 0.05, 0.04, and 0.9 V, respectively. The monoamine neurotransmitters have similar oxidation mechanism but slightly different in the chemical structure; their separation is very difficult but CN-SST photo-biosensor electrode provides high sensitivity toward A, NA, and DA. The peak separations of AA-DA, DA-UA, AA-UA (ΔE_{AA-DA} , ΔE_{DA-UA} , and ΔE_{AA-UA}) were 0.22, 0.15, and 0.3 V, respectively. Thus, the ability for sensitive and selective detection of DA in the presence of all the coexistence bioactive-molecules such as AA and UA is remarkable. Moreover, strong DPV signal obtained at low concentrations of DA compared with AA and UA might lead to superior photo-electrochemical signaling and selective discrimination of DA in biological samples.

2.4 Photo-electrochemical assays of catecholamine neurotransmitters

Monoamine neurotransmitters such as A, NA, and DA represent the catecholamine neurotransmitters and secreted with percentage values depending on its sources. The photo electro-oxidation of each molecule on CN-SST photo-biosensor electrode under UV irradiation 365 nm in 0.1 M PBS, pH 7.4 at scan rate 100 mVs⁻¹, pulse height 60 mV and pulse width 10 ms was investigated (Fig. 6(A–C)). The oxidation photocurrent peak for A, NA, and DA increased proportionally with increasing concentration in a range from 1 μ M to 32 μ M with high sensitivity, indicating that the CN-SST photo-biosensor electrode can be used for the determination of A, NA, and DA. By plotting the concentration versus the current for A, NA, and DA, the linear regression equations are $I(\mu A) = 74.35 + 2.67 [A](\mu M)$ ($R^2=0.887$), $I(\mu A) = 68.64 + 3.02 [NA](\mu M)$ ($R^2=0.89$), and $I(\mu A) = 73.8 + 2.62 [DA](\mu M)$ ($R^2=0.88$) (Fig. 6(Aa–Cc)). The detection limit was calculated to be 0.11, 0.1, and 0.06 μ M for A, NA, and DA. Thus, the CN-SST photo biosensor is sensitive for DA, NA, and A. Overall, the homogeneous charge

distribution and strong binding along the electronically-coordinated structures of the Ni²⁺ site, O²⁻ vacancy, and CN on the surface can dominate the retention of the actively-site surface with mobile electron clouds. The high crystallinity of original SST structures without atomic surface dissolution or aggregation leads to substantially high absorptivity of DA target. All these factors are the key role for high sensitivity and selectivity for monoamine neurotransmitters (i.e DA).

Figure 6

2.5 Photo-electrochemical selectivity of DA

Evaluation of the modified electrode for individual detection of a single molecule in the tertiary mixture and simultaneous detection in one pot was successfully studied using DPV in the potential range of -0.2 V to 0.7 V in N₂-saturated electrolyte (0.1 M PBS, pH 7.4) under UV irradiation 365 nm. The reactivity of individual species was observed by varying its concentration while keeping the concentration of other two species constant on the CN-SST photo-biosensor electrode. Results (Fig. S4b) showed that the DA photo-oxidation peak centered at 0.21 V (vs. Ag/AgCl) increases linearly with the addition of DA doses from 1–128 μ M in the presence of 0.5 mM AA and 0.2 mM UA. The regression equation was calculated by drawing a calibration plot of DA concentrations versus peak photocurrents that is given as $I(\mu A) = 0.3 [DA](\mu M) + 8.87$; $R^2 = 0.95$; $N = 5$] (Fig. S4b). The detection limit was 30 nM based on regression equation. Similarly, anodic peak photocurrent increases linearly from 50 μ M to 400 μ M, from 25 μ M to 400 μ M, in the presence of 10 μ M DA and 0.2 mM UA or 10 μ M DA and 0.5 mM AA, for AA and UA concentration injections, respectively, (Fig. S4(A&C)). The detection range and sensitivities of the AA and UA were determined by plotting the calibration graph of concentrations versus peak currents (Fig. S4 (a&c); the peak currents are given as $I(\mu A) = 0.006 [AA](\mu M) + 8.26$; $R^2 = 0.989$ and $I(\mu A) = 0.014 [UA](\mu M) + 7.81$; $R^2 = 0.957$; $N = 5$] for AA and UA, respectively. The detection limits were 12 and 9 μ M. For further evidence, DA, AA, and UA were determined simultaneously using DPV under the latter conditions. The DPV anodic peak photocurrents show linear behavior with increasing injections of DA, AA, and UA concentrations in the range of 2–60, 25–800, and 100–1000 μ M, with detection limits of 0.2, 9.78, and 11.27 μ M respectively, based on $3\sigma/n$, where σ is the standard error of the intercept, and n is the slope of the linear equation (Fig. S4(D&d)). Thus, the feasibility of CN-SST photo-biosensor electrode toward selective and sensitive determination of DA, AA, and UA was indicated individually or simultaneously, detecting DA in the living cell even in the presence of the other interfering mono-bioactive molecules. The atomic configuration of Ni²⁺ and rich-O²⁻ vacancy sites with CN surface clouds around {110} crystal facet plane has the main reason for high DA sensitivity and selectivity at CN-SST photo-biosensor electrode, where the dominant surface charge is negative due to stabilized rich-O²⁻. Ascorbic acid and uric acid have low pKa (5.8 and 4.2 for UA and AA) value and act as anions, whereas DA (pKa = 8.9) acts as a cation at physiological pH. Repulsion between the surface of the proposed biosensor and AA and UA decrease the sensitivity due to negative repulsion. Otherwise,

strong binding with DA due to the electrostatic interaction between negative surface charge and DA positive charge, which enhances the sensitivity and selectivity.^{55,8}

Chronoamperometry is a highly sensitive electrochemical technique, thus it is used for screening of DA at low concentrations. Figure 7 shows the identical current time response curve upon successive injection of 0.5 μM DA in 0.1 M PBS (pH 7.4) under UV irradiation (365 nm). The high-amperometric response was observed upon addition of DA concentrations and a linear relation between the DA concentration and the reflected photocurrent was obtained. The linear regression equation was expressed as $I (\mu\text{A}) = 3.95 + 28.56 [\text{DA}] (\mu\text{M})$ ($R^2 = 0.999$) with signal-to-noise ($S/N = 3$) and $\% \text{RSD} = 2.8\%$. The detection limit was calculated to be 6 nM. The lower detection limit and fast response provide that the CN-SST photo-biosensor electrode is an ideal biosensor for sensitive monitoring and screening of DA.

Figure 7

2.6 Reproducibility of Photo-electrochemical sensing of DA

The structural stability of constructed CN-SST over ITO electrode after several 20 CV reuse/cycles was investigated by using FE-SEM, as presented in Fig. S5(A-F). The top-view FE-SEM images show evidence that the hierarchical CN-SST onto the ITO glass substrate was attained after 20 reuse/cycles (Fig. S5(A-C)). The low magnification images clearly show well-dispersed and deposition of CN-SST onto the ITO surfaces (Fig. S5(A-B)). Figures S5 (D-F) show the high-magnification FE-SEM images, which illustrate the morphological orientation of CN-SST photo-biosensor after 20 CV reuse/cycles. In general, the micrographs provide evidence of the stability of spherical broccoli-head umbrella architectures associated with abundant nano-tubular arrangements, as presented in Fig. S5(E&F). The designed structures CN-SST photo-biosensor attained the surface functionality with the designated electrodes without damage or distortion in the surface electron configuration and mobility and even used for several times under photo-irradiation.

The reproducibility of CN-SST photo-biosensor electrode was evaluated through the photo electrochemical measurements (PEC). In such experimental protocols, the photocurrent responses are examined for multiple (≥ 10) CN-SST biosensor electrode at a concentration of 10 μM DA at the optimum sensing conditions of 0.1 M PBS (pH 7.4) and 0.2 V (vs. Ag/AgCl) applied potential at 20 $^{\circ}\text{C}$. The RSD of the ten amperometric response assays was 1.6%, evidenced by the fitting plot of the response current graphs (Fig. S5G). To confirm reusability of CN-SST photo-biosensor electrode, the photocurrent responses as a function of ten different samples with a constant concentration of DA (5 μM) were measured repeatedly after every washing of CN-SST photo-biosensor electrode with distilled H_2O for three times at the optimum sensing conditions (Fig. S5H). Thus, the reused CN-SST photo-biosensor electrode showed high sensing response efficiency

(i.e., 98%), despite ten reuses/cycles with $\% \text{RSD} = 5.01$. These results provide high stability and reproducibility of CN-SST photo-biosensor electrode and can be assessed as a significant economical sensor for monitoring of DA.

2.7 Cytotoxicity of CN-SST on the PC12 cell line

The cells viability and cytotoxicity of CN-SST were investigated using the Cell Counting Kit-8 (CCK-8) assays. The cytotoxicity of CN-SST was investigated upon incubation of PC12 with various concentrations of CN-SST at 37 $^{\circ}\text{C}$ for 48 h. The metabolic activity of PC12 cells decreases with the increase of CN-SST concentration (Fig. S6). Untreated cells act as a control for all measurements. The CN-SST provides low cytotoxicity for PC12 cells. For example, 27 % cells were lost at high concentration (200 $\mu\text{g}/\text{ml}$); however, approximately 6% cells were damaged with 10 $\mu\text{g}/\text{ml}$. The result indicates that the CN-SST has no significant effect on the cells populations, leading to low cytotoxicity and cell viability. This finding confirmed that CN-SST can also be used as a real in vitro model for extracellular DA detection.

2.8 Signaling and Monitoring DA released from neuronal cells and in human serum

Monitoring DA released from neuronal cells is a key in regulating both, neural mechanism and various brain functions. To demonstrate the real applicability of the developed sensor, K^+ -induced dopamine released from PC12 cells were monitored through amperometric method under applied potential of 0.21 V and 0.1 M PBS at pH=7.4 in the presence of UV light irradiation (365 nm) on CN-SST photo-biosensor electrode (Fig. S7(A&B)). The cells were stimulated by K^+ to depolarize the cell's membrane. Cell membrane depolarization was reported by K^+ extracellular stimulation in which the cell membrane depolarized and induce an influx of Ca^{2+} and Na^+ through the opening of voltage-sensitive Na^+ channels and subsequent opening of voltage-sensitive Ca^{2+} channels.⁵⁶ Increasing the level of intracellular Ca^{2+} enhancing the release of dopamine from large dense-core-vesicles of the cells. The intracellular Ca^{2+} increased to a threshold sufficient to trigger the exocytosis when the voltage-sensitive Ca^{2+} channel opened. Releasing of dopamine to the extracellular region expressed by K^+ induces the cell membrane depolarization and dopamine containing vesicles with cell membrane fusion. The illustration of comparability of our biosensor for monitoring of dopamine by making a control sample that contains PBS (pH 7.4) only and the other sample contains 1×10^6 cells /mL of PC12 in 0.1 M PBS (pH 7.4) with 5 mM K^+ upon addition of DA. The high amperometric response of DA addition to both solutions with a difference in the current value was illustrated in Fig. S7(A & B). The columns plot in Fig. S7C show that a difference between the current of the blank and sample contains PC12, which is corresponding to the released DA from PC12 with $\% \text{RSD} = 2.6$.

The DA released from neuronal cells (i.e., PC12) was detected by using CN-SST photo-biosensor, as shown by applying

chronoamperometric method. The supernatant of the cell line (i.e., the secreted DA under K^+ stimulation) was step added at applied potential of 0.21 V in 0.1 M PBS (pH 7.4) under UV light irradiation (at $\lambda=365$ nm). Figure 8A shows significant amperometric response upon injection of 0.5 μ M DA within a few second. The same equivalent response was observed upon injection of the supernatant of 1×10^6 cell/mL, which contains DA released from PC12 under 5 mM K^+ stimulation, then confirmed by injection of 0.5 μ M DA. This finding confirmed that the proposed photo-biosensor can monitor DA released from living cells with high sensitivity and selectivity. These results indicate the ability of CN-SST photo-biosensor electrode for in-vitro monitoring of DA released from living cells.

Furthermore, monitoring of DA in a complicated medium rather than PBS by using CN-SST, while maintaining high sensitivity and selectivity, were investigated. Standard addition method was performed by spiking of 1 μ M DA in 0.1 M PBS (pH 7.4) containing human serum samples at a fixed potential of 0.21 V, as shown in Fig. 8B. Stepwise, time-additional injections of 1 μ M DA into an assay that contained 10 mL of 0.1 M PBS (pH 7.4) and 50 μ L human serum sample was carried out. The high amperometric response is observed upon stepwise addition of DA as presented in Fig. 8B. The recovery values compared with the spiking assay of DA in 0.1 M PBS (pH 7.4) containing human serum sample show high recovery percentage ranged from 97 to 118% with %RSD of 3.1, as presented in Table S1. These results clearly confirmed that CN-SST photo-biosensor can be a promising electrode for DA monitoring in real samples and can be employed for clinical application.

Figure 8

Conclusions

A simple, sensitive, and selective biosensor was successfully developed for the ultrasensitive determination of bioactive-molecules and monitoring of DA released from living cells using novel ST photo-biosensor electrodes. The building of hierarchical NiO spear thistle flower allied in single-head (S), and symmetric, asymmetric-double heads (D and A, respectively) that tightly connected through micrometric dipole-like rod or trunk were successfully fabricated. CN doped ST enhanced the intrinsic photo-electrocatalytic activity through increasing the surface area, enlarge the pore distribution, balance the surface charge (C, N, Ni^{2+} , and O^{2-} atomic configuration around {110} crystal plane with dominant O^{2-} vacancy sites and CN clouds) and improved the electrode photostability under irradiation for long-term exposure. These designated architectures are key features for generating biosensor surface electrodes for photo-electrochemical, ultrasensitive screening of mono-bioactive molecules. The exceptional electrode designs produced numerous catalytically active sites and high electron-transfer mobility. The designed photo-biosensor electrode provides significant sensitivity and selectivity for monitoring of DA among all interfered molecules

such as A, UA, A, and NA. The CN-SST photo-biosensor electrode showed highly photo-electrochemical activity for electrooxidation of DA at low detection limit up to 6 nM (S/N 3). Furthermore, the photo-biosensor electrode provides significant monitoring of DA from living cells (PC12). This finding indicates that the hierarchical ST biosensor can be employed for analytical discrimination and sensitive monitoring of DA released from living cells and in human serum for clinical diagnosis.

3 Experimental

3.1 Reagents

All chemicals were of analytical grade and were used without further purification. Nickel nitrate hexahydrate ($Ni(NO_3)_2 \cdot 6H_2O$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$), Dopamine hydrochloride (DA), aniline, uric acid (UA), potassium ferricyanide [$K_3Fe(CN)_6$], noradrenaline (NA) phosphate buffer saline (PBS), and human serum were purchased from Sigma-Aldrich Company, Ltd., USA. L (+)-ascorbic acid (AA), potassium per sulfate, HCl and L-adrenaline (A) were purchased from Wako Company, Ltd., Osaka, Japan. Indium tin oxide (ITO) glass electrodes (2 cm $\times$ 1 cm) were purchased from ALS Company, Ltd., Japan.

3.2 Synthesis of NiO based ST architectures

NiO was assembled with three different shapes like spear thistle (ST) flowers (ST with, single head (s), symmetric double head (D), and asymmetric double head (A by using a one-pot hydrothermal synthetic approach. In a detailed procedure, 1mM of ($Ni(NO_3)_2 \cdot 6H_2O$) was dissolved in a 100 mL volumetric flask containing 50 mL deionized water and stirred until dissolved at room temperature. Next, 50 ml of 1 mM ($(NH_4)_2HPO_4$ in deionized water, were added to the stirred solution of ($Ni(NO_3)_2 \cdot 6H_2O$) with different flow rate (Q) to control the shape of the particles (See Scheme S1). The prepared solution was then transferred into a 100 mL Teflon-lined stainless-steel autoclave and was sealed and maintained at 160 $^{\circ}C$ for 8 h. After required time, green precipitates were retrieved and then rinsed with ultrapure water/absolute ethanol to remove the soluble impurities. The as-prepared ST photo-biosensors dried in an oven at 80 $^{\circ}C$ and annealed at 400 $^{\circ}C$ for 4 h to obtain ST with different morphologies.

3.3 Synthesis of NiO based CN-ST photo-biosensors

To fabricate carbon nitrogen nanosphere doped NiO based ST architectures, typically, 60 mg of prepared NiO based ST and 100 mL of tris-buffer solution (pH = 8.5) were loaded into a 250 mL volumetric flask and stirred at room temperature followed by the addition of purified aniline (20 mM). Next, 20 mM of ammonium persulphate solution was added dropwise to the stirred solution to perform the polymerization reaction at 0 $^{\circ}C$. The polymerization was preceded for 12 h at room temperature, and the obtained aniline polymerized STs were washed several times with deionized water. The polyaniline coated ST were calcined at 500 $^{\circ}C$ for 4 h in an argon atmosphere to obtain CN- ST.

3.4 Fabrication of NiO based CN-ST photo-biosensor electrodes

Working electrode fabrication was assembled as follows: NiO based CN-ST architectures were dispersed in 5 ml methanol at room temperature. Thin films of CN-ST were fabricated by uniformly spreading a 15 μ l onto the surface of an ITO glass substrate (2 cm $\times$ 1 cm). To control the exposed surface area of ITO substrate and for better electrical contact a definite area (1 cm $\times$ 1 cm) of the ITO electrode substrate were masked prior to the deposition. This process was repeated five times; the resulting electrode was dried overnight at room temperature, followed by washing with deionized water to remove any unbound particles.

3.5 Cell culture and in vitro study

The pc12 cell line was obtained from PC12 (ATCC® CRL1721™) and was cultured by incubation under 5% CO₂ at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 10% horse serum. The culture medium was replaced every 3 to 4-day.

3.6 Cytotoxicity

The cytotoxicity of CN-SST photo-biosensor was investigated by Cell Counting Kit-8 (CCK-8) assay. The cells (5 $\times$ 10⁴ cells/mL) were seeded onto 96-well microplates to a total volume of 100 μ L/well and maintained at 37 °C in a 5% CO₂/95% air incubator for 24 h. Then, the CN-SST (10 μ L) with different concentrations were added (10, 20, 50, 100 and 200 ng/ml) to the cell culture, and incubated for 48 h. After that, the CCK-8 solution (10 μ L, 5mg/mL) was added to each well and incubated for another 2-4 h in the CO₂ incubator. The measurements were proceeds at an absorbance of 450 nm using a microplate reader.

3.7 Human serum analysis using CN-SST photo-biosensor

The real applicability of CN-SST photo-biosensor was investigated by using standard addition method in human serum samples. Human serum purchased from Sigma Aldrich and stored at -20 °C until used. A 50 μ L from serum sample was added to 10 ml of 0.1 M PBS (pH = 7.4) to perform the chronoamperometric method at applied potential 0.21 V with successive addition of DA concentrations under saturated N₂ at room temperature.

3.8 Photo-electrochemical measurements

Photo- electrochemical measurements (PEC) were performed with a home-built PEC system. A 500 W Xe lamp equipped with monochromator was used as irradiation source. The photocurrent was measured on Zennium/ZAHNER-Electric instrument that was controlled by Thales Z 2.0 software at room temperature. A conventional three-electrode system containing ITO glass modified (2 cm $\times$ 1 cm) as working electrodes, platinum wires as a counter electrode, and Ag/AgCl (3 M NaCl) as a reference electrode was used for electrochemical analysis. The freshly prepared electrolyte (0.1

M PBS (pH 7.4)) was de-aerated by bubbling a slow stream of purified N₂ to avoid dopamine oxidation. The N₂ flow was maintained during the entire electrochemical measurements to ensure N₂-saturated electrolyte.

References

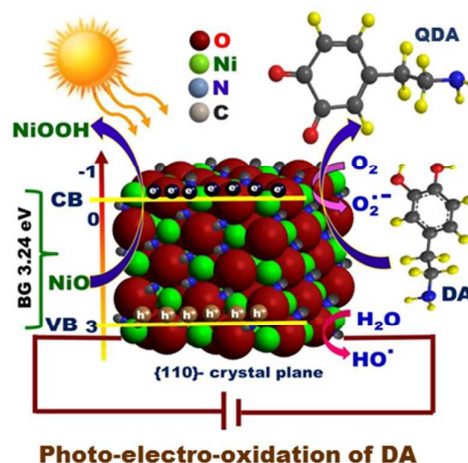
- 1 C. Riether, C. M. Schurch and A. F. Ochsenbein, *Cell death and differentiation*, 2015, **22** (2), 187-198.
- 2 C. Aicheng and C. Sanghamitra, *Chem. Soc. Rev.*, 2013, **42** (12), 5425-5438.
- 3 B.-R. Li, Y.-J. Hsieh, Y.-X. Chen, Y.-T. Chung, C.-Y. Pan, Y.-T. Chen, *J. Am. Chem. Soc.*, 2013, **135** (43), 16034-16037.
- 4 X. Zhang, et al., *Anal. Chem.*, 2015, **87** (6), 3360-3365.
- 5 E. Farjami, R. Campos, J. S. Nielsen, K. V. Gothelf, J. Kjems and E. E. Ferapontova, *Anal. Chem.*, 2013, **85**, 121-128.
- 6 J. S. Huang, Y. Liu, H. Q. Hou and T. Y. You, *Biosens. Bioelectron.*, 2008, **24**, 632-637.
- 7 T. Nakaminami, S.F. Ito, S.F. Kuwabata, H. Yoneyama, *Anal. Chem.* 1999, **71**, 1928-1934.
- 8 E. Popa, Y. Kubota, D.A. Tryk, A. Fujishima, *Anal. Chem.*, 2000, **72**, 1724-1727.
- 9 T.A.Mir, M.H. Akhtar, N.G.Gurudatt, J.-I. Kim, C.S. Choi, Y.-B. Shim, *Biosens. Bioelectron.* 2015, **68**,421-428.
- 10 J.-M. Zen, P.-J. Chen, *Anal. Chem.*, 1997, **69**, 5087-5093.
- 11 Y.X. Li. and X.Q. Lin, *Sens. & Actuat. B: Chem.*, 2006, **15**(1), 134-139.
- 12 Y. Zeng, C. Li, C. Tang, X. Zhang, G. Shen and R. Yu, *Electroanalysis*, 2006, **18**, 440-448
- 13 D. Bruns, et al., *Methods*, 2004 **8**,33(4), 312-21.
- 14 N.F. Atta, M.F. El-Kady and A. Galal, *Sens. Actuat. B: Chem.*, 2009, **141**(2), 566-574.
- 15 P. Kalimuthu and S.A. John, *Bioelectrochemistry*, 2009, **77**(1), 13-18.
- 16 X. Zhang, W. Yan, J. Zhang, Y. Li, W. Tang and Q. Xu, *RSC Advances*, 2015, **5**, 65532-65539.
- 17 W. Cai, J. Lai, T. Lai, H. Xie, J. Ye, *Sensors and Actuators B: Chemical*, 2016, **224**, 225-232.
- 18 X. Zhang, Y.-C. Zhang and L.-X. Ma, *Sensors and Actuators B: chemical*, 2016, **227**, 488-496
- 19 G.-L. Wang, J.-J. Xu and H.-Y. Chen, *Biosensors and Bioelectronics*, 2009, **24**, 2494-2498.
- 20 W.W. Zhao, Z.Y. Ma, P.P. Yu, X.Y. Dong, J.J. Xu and H.Y. Chen, *Anal. Chem.*, 2012, **84**, 917-923.
- 21 M.A. Shenashen, S. Kawada, M.M. Selim, W.M. Morsy, H. Yamaguchi, A.A. Alhamid, N. Ohashi, I. Ichinose, S.A. El-Safty, *Nanoscale*, 2017, **9**, 7947-7959.
- 22 S.A. El-Safty, M. Khairy, M.A. Shenashen, E. Elshehy, W. Warkocki, M. Sakai, *Nano Res.*, 2015, **8**(10), 3150-3163.
- 23 H.J. Chen and L.Z. Wang, *Beilstein J. Nanotech.*, 2014, **5**, 696-710.
- 24 K. Wang, J. Wu, Q. Liu, Y.C. Jin, J.J. Yan and J.R. Cai, *Anal. Chim. Acta*, 2012, **745**, 131-136.
- 25 Y. Li, Y. Hu, Y. Zhao, G. Shi, L. Deng, Y. Hou and L. Qu, *Adv. Mater.*, 2011, **23**, 776-780.
- 26 B. Sun, K. Zhang, L.J. Chen, L.T. Guo and S.Y. Ai, *Biosens. and Bioelect.*, 2013, **44**, 48-51.
- 27 X.M. Zhao, S.W. Zhou, L.P. Jiang, W.H. Hou, Q.M. Shen and J.J. Zhu, *Chem. Eur. J.*, 2012, **18**, 4974-4981.
- 28 T.T. Duong, Q.D. Nguyen, S.K. Hong, D. Kim, S.G. Yoon and T.H. Pham, *Adv. Mater.*, 2011, **23**, 5557-5562.
- 29 X. Zhang, F. Wang, H. Huang, H.T. Li, X. Han, Y. Liu and Z.H. Kang, *Nanoscale*, 2013, **5**, 2274-2278.
- 30 F. Vietmeyer, B. Seger and P.V. Kamat, *Adv. Mater.*, 2007, **19**, 2935-2940.
- 31 Y. Yan, Q. Liu, X. Du, J. Qian, H. Mao, K. Wang, *Anal. Chim. Acta*, 2015, **853**, 258-264.

ARTICLE

Journal Name

- 32 Y. Yang, K. Yan and J. Zhang, *Electrochimica Acta*, 2017, **228**, 28–35.
- 33 H. Lia, J. Lia, D. Chenb, Y. Qiu and W Wang, *Sens. Actuat. B: chem.*, 2015, **220**, 441–447.
- 34 Y. Wang, C. Gao, S. Ge, J. Yu and M. Yan, *Biosens. Bioelectro.*, 2016, **85**, 205–211.
- 35 K. S. W. Sing, D. H. Everett, R. A. W. Haul, L. Moscou, R. A. Pierotti, J. Rouqu  rol and T. Siemieni  wska, *Pure Appl. Chem.*, 1985, **57**, 603–619.
- 36 X. Bo, J. Bai, J. Ju and L. Guo, *J. Power Sources*, 2011, **196**, 8360–8365.
- 37 (a) S. El-Safty, M. Shenashen, M. Ismael, M. Khairy, *Adv. Functional Mater.*, 2012, **22**(14), 3013–3021; (b) S.A. El-Safty, Y. Kiyozumi, T. Hanaoka and F. Mizukami, *Appl. Catal., B*, 2008, **82**(3–4), 169–179; (c) M. Khairy and S.A. El-Safty, *Curr. Catal.*, 2013, **2**, 17–26.
- 38 (a) S.A. El-Safty, Y. Kiyozumi, T. Hanaoka, F. Mizukami, *Appl. Catal. A*, 2008, **337**(2), 121–129; (b) S.A. El-Safty, Y. Kiyozumi, T. Hanaoka and F. Mizukami, *Appl. Catal., B*, 2008, **82**(3–4), 169–179; (c) S.A. El-Safty, Y. Kiyozumi, T. Hanaoka and F. Mizukami, *J. Phys. Chem. C*, 2008, **112**(14), 5476–5489.
- 39 (a) S.A. El-Safty, M. Sakai, M.M. Selim, A.A. Hendi, *ACS appl. mater. interf.*, 2015, **7**(24), 13217–13231; (b) S.A. El-Safty, F. Mizukami and T. Hanaoka, *J. Phys. Chem. B*, 2005, **109**(19), 9255–9264; (c) M.A. Shenashen, S. A. El-Safty and E. A. Elshehy, *Analyst*, 2014, **139**(24), 6393–6405.
- 40 (a) S.A. El-Safty, M. Shenashen, *Sens. Actuat. B*, 2013, **183**, 58–70; (b) M. Khairy, S. A. El-Safty, M. Ismael and H. Kwarada, *Appl. Catal., B*, 2012, **123–124**, 162–173; (c) M. Khairy, S.A. El-Safty, M. Ismael and H. Kwarada, *Appl. Catal., B*, 2012, **127**, 1–10.
- 41 F. Li, C. Li, X. Liu, Y. Chen, T. Bai, L. Wang, Z. Shi, and S. Feng, *Chem. Eur. J.*, 2012, **18**, 11641 – 11646.
- 42 F. Li, C. Li, J. Liu, X. Liu, L. Zhao, T. Bai, Q. Yuan, X. Kong, Y. Han, Z. Shi and S. Feng, *Nanoscale*, 2013, **5**, 6950–6959.
- 44 (a) T. Kavitha and H. Yuvaraj, *J. Mater. chem.*, 2011, **21**(39), 15686–15691; (b) W. Warkocki, S. A. El-Safty, M. A. Shenashen, E. Elshehy, H. Yamaguchi and N. Akhtar, *J. Mater. Chem. A*, 2015, **3**, 17578–17589.
- 45 W. Liu, Y. Feng, X. Yan, J. Chen and Q. Xue, *Adv. Funct. Mater.*, 2013, **23**, 4111–4122.
- 46 S. Y. Kim, H. M. Jeong, J. H. Kwon, I. W. Ock, W. H. Suh, G. D. Stucky, J. K. Kang, *Energy Environ. Sci.*, 2015, **8**, 188–194.
- 47 S. A. El-Safty, Y. Kiyozumi, T. Hanaoka and F. Mizukami, *Appl. Catal. B: Environ.*, 2008, **82**, 169–179.
- 48 D. H. Shin, J. S. Lee, J. Jun and J. Jang, *J. Mater. Chem. A*, 2014, **2**, 3364–3371.
- 49 L. Chen, M. Feng and H. Zhan, *RSC Adv.*, 2014, **4**, 30689–30696.
- 50 (a) D. Hassen, S.A. El-Safty, K. Tsuchiya, A. Chatterjee, A. Elmarakbi, M.A. Shenashen, M. Sakai, *Sci. Rep.*, 2016, **6**, 24330; (b) M.A. Shenashen, D. Hassen, S.A. El-Safty, H. Isago, A. Elmarakbi, H. Yamaguchi, *Chem. Eng. J.*, 2017, **313**, 83–98. (c) M.A. Shenashen, D. Hassen, S.A. El-Safty, M.M. Selim, N. Akhtar, A. Chatterjee, A. Elmarakbi, *Adv. Mater. Interf.*, 2016, **3**(24).
- 51 D. Hassen, M.A. Shenashen, S.A. El-Safty, M.M. Selim, H. Isago, A. Elmarakbi, A. El-Safty, H. Yamaguchi, *J. Pow. Sources*, 2016, **330**, 292–303.
- 52 R. Nurzulaikha, H.N. Lim, I. Harrison, S.S. Lim, A. Pandikumar, N.M. Huang, S.P. Lim, G.S.H. Thien, N. Yusoff and I. Ibrahim, *Sensing and Bio-Sensing Research*, 2015, **5**, 42–49.
- 53 (a) N. Akhtar, S.A. El-Safty, M.E. Abdelsalam, H. Kwarada, *Adv. healthcare mater.*, 2015, **4**(14), 2110–2119; (b) N. Akhtar, S.A. El-Safty, M. Khairy, W.A. El-Said, *Sens. Actuat. B*, 2015, **207**, 158–166.
- 54 N. Akhtar, S.A. El-Safty, M.E. Abdelsalam, M.A. Shenashen, H. Kwarada, *Biosens. Bioelectron.*, 2016, **77**, 656–665.
- 55 M. Ghita and D.W.M. Arrigan, *Electrochimica Acta*, 2004, **49**(26), 4743–4751.
- 56 H. Shinohara, Y. Sakai and T.A. Mir, *Anal. Biochem.*, 2013, **441**, 185–189.

Figures



Scheme 1. Schematic representation of the photo-electrooxidation process of dopamine (DA) on the surface of the {110}-crystal plane. The scheme shows the atomic configuration of Ni^{2+} and O^{2-} along CN-SST, the effective charge transfer of species at the surface, active sites from rich-vacancies O^{2-} and Ni^{2+} and formation of QDA product.

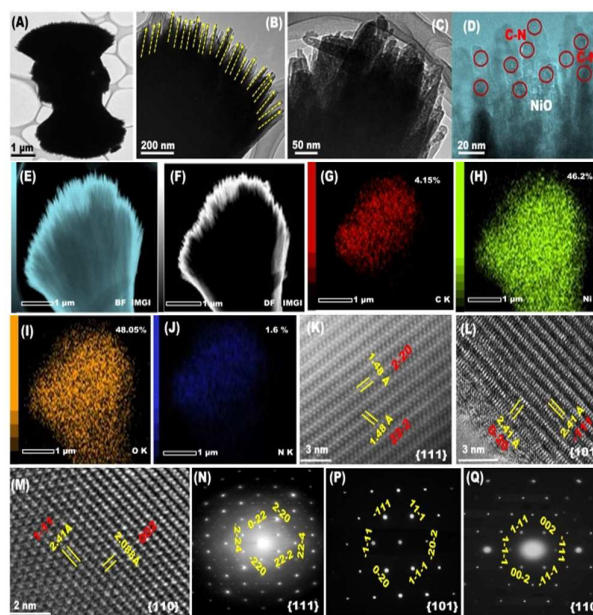


Figure 1. A) Low magnification HR-TEM micrograph illustrates the surface morphology of one of the designed ST with two heads connected by a trunk which is like spear thistle (ST)

flowers with the double head (DST). High magnification of HAADF-STEM reflects the head morphology that is like a thick serrated surface as in (B). c) HAADF-STEM photograph reflects the head serrated surface consists of a thick nanotube with an average size around 20 nm. (D) shows high magnified HAADF-STEM image focused on the nanotube surface and obtained the CN formatted at the surface of ST with average size > 2 nm and covers the surface with formality and homogeneously. The EDS-STEM imaged and elemental analysis mapping shows evidence of the homogeneous dispersion of C, N, O, Ni along the entire CN-ST flower heads (E-I). (K-M) High magnification HAADF-STEM of ST architectures which reflected the degree of the crystalline surface. High-dense {111}-ED-STEM (L) for symmetric double head, {101} for asymmetric double head, and {110} for single head. micrographs of root- and top- sides of ST samples and the corresponding atomic configuration along with {111}, {101} and {110} crystal planes for the symmetric double head, asymmetric double head, and single head, respectively.

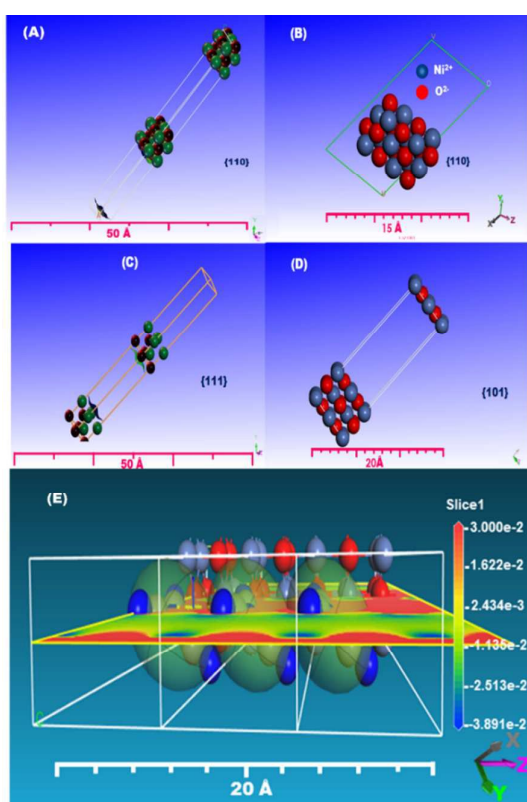


Figure 2. A) The top-view of double layer ST atomic organization along with {110} crystal plane for SST and demonstrated the Ni^{2+} and O^{2-} configuration with highly ordered crystalline surface. B) The atomic arrangement of Ni^{2+} and O^{2-} as monolayer based on axial alignment and represents the low contact angle between the target molecules and atomic active centers, which effect on the catalytic behavior of {110} plane. C and D) The top view of a double layer of atomic configuration around {111} and {101} crystal plane for DST and AST, respectively. E) Top view of multilayer of atomic arrangement around {110} crystal facet plane along with providing CN. The orientation of building atomic tower shows the possibility of the {110}- top-surface binding sites with high densely electronic clouds with the crystal edge surface and central crystal corresponding to

density functional theory (DFT) with the effectiveness of CN on the surface atomic construction and its contact with the biomolecules.

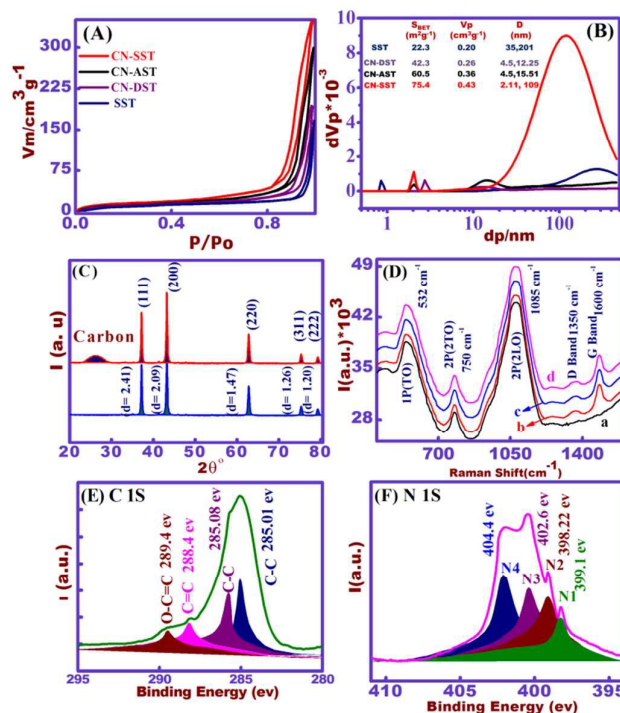


Figure 3. A) N_2 isotherms of the ST without and with CN of DST, AST, and SST, which featured type IV isotherm H1-type hysteresis loop. B) NLDFT model for pore size distribution, inset table contains the data of the surface area, pore size and pore volume and (C) WA-XRD pattern of SST (blue line) and CN-SST (red line). (D) Raman spectrum of SST (a) CN-DST (b), CN-AST (c) and CN-SST (d). XPS Survey of The CN-SST samples; (E) C 1s, (F) N 1s.

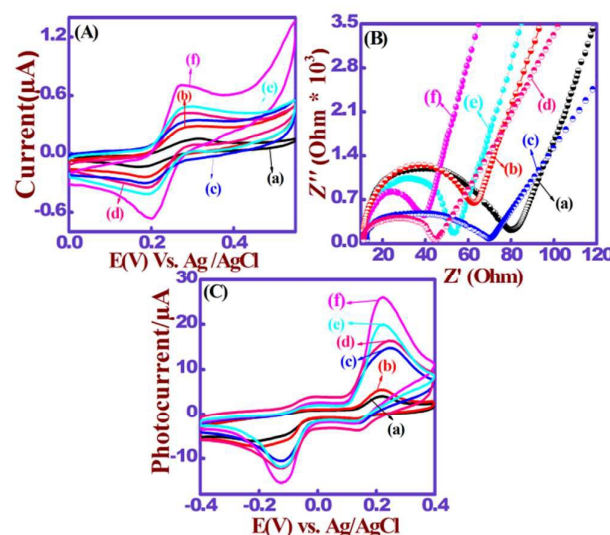


Figure 4. (A) CVs of DST & CN-DST (a, b), AST & CN-AST (c, d) and SST & CN-SST (e, f) photo biosensor electrodes in 0.1 M PBS (pH 7.4).

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=7) containing 0.1 mM of $[\text{Fe}(\text{CN})_6]^{3-}$ at 100 mVs^{-1} , (B) Electrochemical impedance of DST & CN-DST(a, b), AST & CN-AST (c, d) and SST & CN-SST (e, f) photo biosensor electrodes in 0.1 M PBS (pH = 7) and (C) CVs of DST & CN-DST(a, b), AST & CN-AST (c, d) and SST & CN-SST (e, f) photo biosensor electrodes in 0.1 M PBS pH=7.4 containing $10 \mu\text{M}$ of DA at scan rate 100 mVs^{-1} under UV irradiation (365 nm).

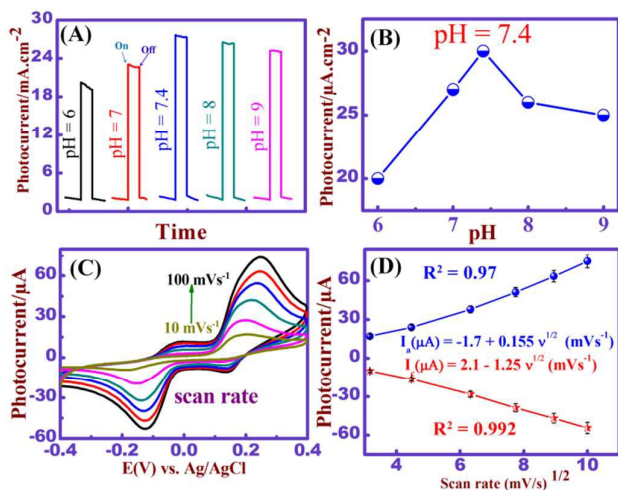


Figure 5. (A) time-dependent on-off switching response CN-SST photo biosensor electrode in PBS containing $10 \mu\text{M}$ dopamine with varying pH from 6 to 9 under UV light irradiation (365 nm) at applied potential 0.21 V, (B) the plot of pH versus the photocurrent (μA), (C) CVs of $10 \mu\text{M}$ dopamine in PBS pH = 7.4 at different scan rate from 50- 300 mVs^{-1} under UV light irradiation and (D) the linear plot of square root of scan rate versus anodic and cathodic photocurrent (μA).

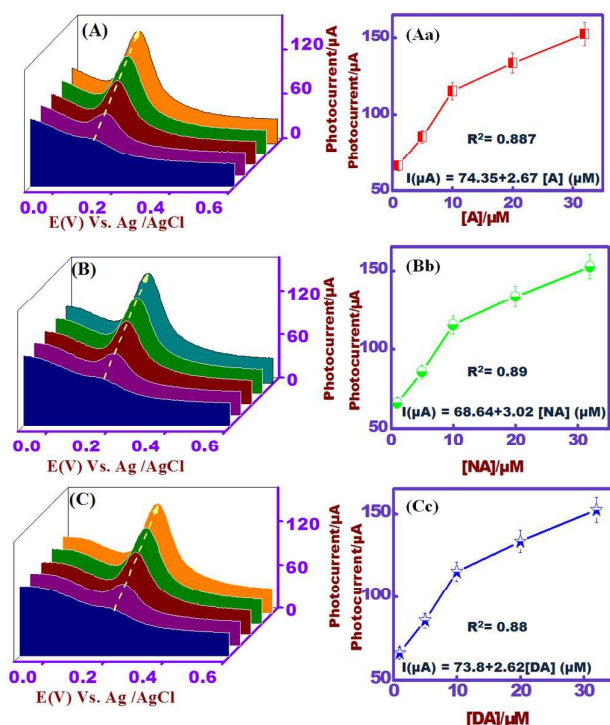


Figure 6. DPV of 1, 5, 10, 20 and $32 \mu\text{M}$ of A (A), NA (B) and DA (C) under UV light irradiation (365 nm) in 0.1 M PBS pH=7.4. (Aa), (Bb) and (Cc) the linear plot of concentration (μM) Vs the photocurrent (μA) for A, NA, and DA, on CN-SST photo biosensor electrode, respectively.

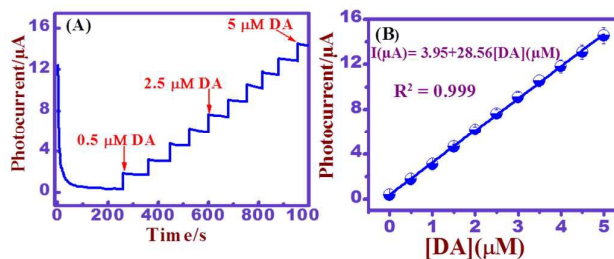


Figure 7. Amperometric response of DA with successive addition of each step $0.5 \mu\text{M}$ under UV light irradiation (365 nm) in PBS pH=7.4 and applied potential 0.21V, (B) the linear plot of concentration (μM) Vs the photocurrent (μA) on CN-SST photo biosensor electrode.

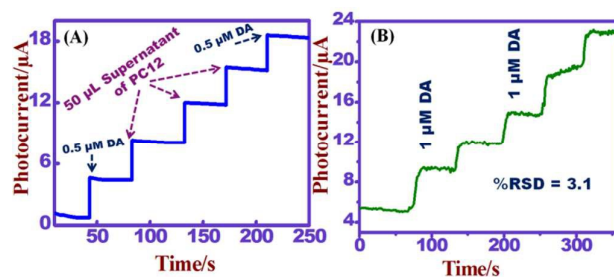


Figure 8. A) The amperometric response of $0.5 \mu\text{M}$ DA injected to N_2 -saturated 0.1M PBS (pH 7.4) at 0.21 V and continuous injection of $50 \mu\text{L}$ PC12 supernatant containing the released DA under 5 mM K^+ simulation. B) The amperometric response of spiked $1 \mu\text{M}$ DA in N_2 -saturated 0.1 M PBS (pH 7.4) containing $10 \mu\text{M}$ human serum.