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Anisotropic Gold Nanoparticle for the Highly Sensitive Colorimetric Detection of Glucose in Human Urine

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Abstract

Because of the difference in surface energies of various crystal facets that arised from a large fraction of edges, corners and vertices, the morphology of nanoparticles has its extreme potential for various applications. In this report, using poly (3,4 ethylenedioxythiophene) polystyrene sulfonate (PEDOT: PSS) functionalized anisotropic gold nanoparticles (GNPs) we have demonstrated a simple but robust method for the naked eye colorimetric detection of Glucose in human urine with high sensitivity. Glucose oxidase (GOx) was conjugated to the modified anisotropic GNPs. The controlled degree of PEDOT: PSS attached on the surface of the GNPs was crucial for the detection of Glucose. The GNPs were aggregated and the color of the solution changed from pink to blue upon addition of only 10 μg/mL of Glucose. The detection limit of the Glucose was found to be 9.8μM. Such high sensitive naked eye detection using PEDOT: PSS will be very useful for the low cost homemade sensors.

Key words: Gold nanoparticles, anisotropy, PEDOT:PSS, glucose detection, colorimetric assay.

Introduction

Optical based colorimetric detection has great importance for its high sensitivity for clinically relevant molecules[1-4]. This is mainly because of its simplicity, robustness and easy readout. Specially, the visible naked eye colorimetric detection is very effective for the fabrication of homemade and low cost sensors for rural peoples. The easy avalibility of these sensors could overcome the usage of large, sophisticated and cost effective instruments. Consequently, the requirement of pathology lab for Glucose testing will be reduced. Glucose sensing from the blood or urine of human is one of the major importance, as improper maintenance of the Glucose level in the body may cause serious health problem or even death[5-7]. Generally, The normal Glucose range in urine is 0-0.8 mmol/L. Various high sensitive electrochemical techniques are available, however, there are few reports on the colorimetric and optical based detection in blood as well as in urine[8-29].

During the last few decades, nanomaterials based optical sensors have been developed for sensing of various types of clinical molecules[30-33]. Among them gold nanomaterials were found to be very effective, because of its extensive optical properties, excellent surface recognition and their very high extinction coefficient over the organic chromophores[34-41]. Moreover, the synthetic method is also easy and simple. Though GNPs have shown its potential for various sensing applications, however, the reports on colorimetric Glucose sensor is not upto the mark[17].

Further, the anisotropic GNPs, because of differences in surface energies of various crystal facets that arising from a large fraction of edges, corners and vertices would have unique properties than the spherical GNPs[42-45]. However, the application of the anisotropic GNPs in the various relevant fields is very limited[46-50].Very few reports are available on the colorimetric sensing of Glucose in urine and blood using anisotropic GNPs[26, 51-53].

Here, we have demonstrated the use of anisotropic GNPs for optical based colorimetric sensor of Glucose from human urine. The synthesized anisotropic GNPs were surface functionalized by PEDOT:PSS, which is the most commonly used polymer for electrochemical sensors[54, 55]. The controlled surface functionalization played the major role in the high sensitive detection of Glucose, as it helped in aggregation of the GNPs by providing the optimum zeta potential value. The detection was based on the colorimetric change from pink to blue with an extensive red shift of the respective surface plasmon resonance (SPR) band. Our sensor shown the detection limit of 9.8μM estimated at a signal to noise (S/N) ratio of 3 with regression coefficient R=0.99. Such a highly sensitive assay on Glucose sensing in human urine using the biocompatible PEDOT: PSS surface functionalized anisotropic GNPs will be very beneficial for the designing of low cost Glucose sensors for third world countries.

EXPERIMENTAL SECTION

Materials: All Glasswares were washed with aqua regia (3 HCl: 1 HNO_3), followed by rinsing several times with double distilled water. Gold (III) Chloride hydrate ($HAuCl_4$, 99.99%), Sodium Citrate tribasic hydrate (> 99%), Sodium Borohydride ($NaBH_4$, 99%), L- Ascorbic Acid, Chloroform, PEDOT:PSS (1.3 wt % dispersion in water, Sigma Aldrich 483095), GOx was purchased from Sigma Aldrich. CTAB (N-Cetyl-N,N,N-trimethyl-ammonium bromide), Sodium Hydroxide Pellet purified (NaOH, 97%), were purchased from Merck. Sodium Iodide (NaI, 99%) was purchased from Fisher Scientific, Double distilled 18.3 mΩ deionized water (ElgaPurelab Ultra) was used throughout the preparation of solutions. All the experiments were performed at room temperature (25°C).

Synthesis of anisotropic GNPs

GNPs were synthesized by the established seed mediated method with minor modification by changing the ascorbic acid concentration[56]. 5nm spherical seed were synthesized by mixing 0.5mL of 10mM aqueous $HAuCl_4 \cdot 3H_2O$ solution, 1mL of a 5mM aqueous solution of sodium citrate and 1mL of a 50mM aqueous $NaBH_4$ (Ice-cold) solution in 36.5mL of deionized water with vigorous stirring till color of the solution turned red. To prepare GNPs, three labeled flasks were taken. A mixture of 108mL of 0.025M aqueous CTAB solution and 56μL of 0.1M aqueous NaI solution was divided into three containers labeled with 1, 2 and 3. 9mL of mixture was added in each container 1 and 2. The remaining mixture 90 mL was added in container 3. Finally, a mixture of 125μL of a 10mM aqueous $HAuCl_4 \cdot 3H_2O$ solution, 50μL of 50mM NaOH, and 70μL of 50mM ascorbic acid was added to each container 1 and 2. A mixture of 1.25mL of 10mM $HAuCl_4 \cdot 3H_2O$, 0.4mL of 50mM NaOH, and 0.7mL of 50mM ascorbic acid were added into container 3. 1ml of the seed solution was added to container 1 with mild shaking, followed by adding 1mL of container 1 solution into container 2. After gentle shaking, the complete solution of container 2 was added into container 3. The solution was kept overnight for complete growth.

Synthesis of CTAB stabilized spherical GNPs

CTAB stabilized spherical GNP was synthesized by using a seed-mediated growth method.[50] The container for seed synthesis held 5 mL of 0.50mM $HAuCl_4 \cdot 3H_2O$ and 5 mL of 0.20 M CTAB. The solution was reduced by addition of 600μL of ice-cold $NaBH_4$ (0.010 M). Next, the container was shaken vigorously for 2 minute and occasionally opened to vent for any evolved hydrogen gas. The seed solution was brown suspensions and was allowed to age for 2 hours.

12μL of seed solution was to a solution already containing 9.50 mL of 0.10 M CTAB, 80μL of 0.010 M $AgNO_3$, 500μL of 0.010 M $HAuCl_4 \cdot 3H_2O$ and 55μL of 0.10 M Ascorbic Acid. The mixture was stirred for 10 minutes. This resulted in a red suspension that was again left undisturbed for 24 hour to increase the yield. The extra CTAB was removed by centrifugation and resuspension of the nanoparticle into equal amount of water.

Surface functionalization of GNP by PEDOT:PSS

After synthesizing GNPs, the surface modification was done by using PEDOT:PSS[54-57]. Here, a different method was used than the reported PEDOT: PSS modification, that used for either electrochemical and spin coating application. In first step, extra CTAB was removed by carrying out centrifugation at 10,000 rpm for 10 minutes followed by discarding the supernatant and the pellete was redispersed in double distilled water. This process repeated one more time. To ensure the complete removal of CTAB, from the above solution 5mL of GNPs was equilibrated with 5mL of chloroform. The solution was gently mixed and allowed to stand for 20 minutes. In this way the CTAB was exchanged from GNP to chloroform layer. Finally the chloroform layer was discarded. In the second step for surface functionalization, 100µL of PEDOT: PSS solution (1.3 wt % dispersion in water) was added to 10 mL of double distilled water (1% PEDOT:PSS of the original solution). 1 mL of the resulting PEDOT:PSS solution was added to 2 mL of the GNP solution and allowed to stand for 10 minutes.

Conjugation of GOx on PEDOT:PSS functionalized GNP

For GOx binding to PEDOT: PSS GNPs, 100 µL of 2mg/mL GOx solution was added to 900 µL of PEDOT:PSS functionalized GNPs and mixed them gently for 5 minutes. Finally the GOx

conjugated solution was stored in the refregirator for further use. The above GOx concentration was found to be optimum as the GNP got aggregated even in the absence of Glucose, when 100 µL of 3mg/mL GOx was added to the same 900 µL of PEDOT: PSS functionalized GNPs.

Nanoparticle characterization

The size of the GNPs was determined by dynamic light scattering (DLS; Malvern Instruments Ltd., Malvern, UK). All measurements were performed at a fixed angle of 173^0.[57,58] The Zeta potential and the overall surface charge were determined by using the above instrument. The respective SPR band was measured using Shimadzu UV VIS 2450 and UV VIS NIR 3600 spectrophotometer. Finally, the particle size was confirmed by high resolution Transmission electron microscopy (TEM) using a TECNAI 200 kV TEM (FEI, Electron Optics), SEM (FEI, Electron optics) and Field Emission Scanning Electron Microscopy (FE SEM) by using FEI, Electron Optics.

Glucose Sensing

The change in the SPR absorption maxima of the GOx functionalized GNPs on adding predetermined quantities of Glucose standard was recorded and a calibration curve was plotted with the wavelength against the absorbance. For a titration experiment to observe the SPR change, different amount of Glucose (2.5 - 50 µg/mL) was spiked in a fixed 100µL of doubled distilled water in each case. Finally 100µL of the above each of the Glucose sample was added to 900µL of GOx conjugated PEDOT:PSS GNPs. Each solution was allowed to incubate for 30 minutes before measuring the SPR data. For varifying the application of this method in real smaple, we have performed the same experiment with human urine. The urine sample, which did not contain any Glucose, were collected from a healthy person. 100µL of the urine sample was spiked with 10µg/mL of Glucose and then the final solution was added to 900µL of GOx

conjugated PEDOT:PSS GNP and the absorbance was measured continously for 30 minutes. The selectivity of the method was also investigated by checking the shift in SPR absorption maxima of the GOx functionalized GNP on interacting with normal urine sample. To confirm the precision and recovery of the probe, each set of experiment was carried out in triplicate. The results were obtained within the error of 2-3% .

Result and Discussion

Figure 1a depicted the characterized SPR band of the as prepared anisotropic GNPs, PEDOT: PSS surface functionalized and also GOx conjugated PEDOT: PSS GNPs. A 4nm red shift in the SPR band was observed for PEDOT: PSS surface functionalization than the as prepared anisotropic GNPs. The DLS data **(Figure 1b**) showed an overall increase in the size of the GNPs. The increase in size supported the red shift in the SPR band. GOx conjugated PEDOT: PSS GNPs, showed further 5nm red shift in the SPR band. This was also confirmed by the DLS data. The TEM and SEM images of the as synthesized GNPs, PEDOT: PSS GNPs and GOx conjugated PEDOT: PSS GNPs showed that the anisotropic GNPs is majorly hexagonal and pentagonal in shape with the average size of 45nm (the size was determined using the 80% of the total population in TEM analysis) (**Figure 1c, 1d and Supporting Figure S1**). Few triangular shaped GNPs were also observed. However the characteristics NIR region SPR band for these GNPs was not observed, which suggesedt that the major abundance are hexagonal and pentagonal GNPs. The controlled surface modification of GNPs by PEDOT: PSS and the addition of the GOx on the modified GNPs was further confirmed by their characteristics zeta potential **(Supporting Figure S2).** The as prepared GNPs have the zeta potential of about +

68mV, which arised because of the positively charged CTAB on the GNPs surface. The zeta potential of the only PEDOT: PSS polymer was observed as -70mV. Hence, complete surface coverage by replacing all CTAB would result a complete negative zeta potential. This was observed when 2% of PEDOT: PSS (200µL of 1.3wt % PEDPT:PSS in 10 mL of water) was used. However, for the maximum efficiency of the detection limit, the complete coverage was not helpful. To get the maximum efficiency 1% of PEDOT: PSS (100µL of 1.3wt % PEDOT: PSS in 10 mL of water) was added to the GNPs system and the corresponding zeta potential was reduced from + 68mV to +15.6mV. The addition of GOx further reduced the zeta potential to +6.6mV. Here, 100µL of 2mg/mL GOx was used for optimum binding on PEDOT: PSS modified GNPs. In this case no aggregation was observed in anisotropic GNPs. However, little higher concentration of GOx (100µL of 3mg/mL) induced the aggregation on anisotropic GNPs, even in the absence of Glucose **(Supporting Figure S3)**.The TEM, SEM images **(Figure 1c & 1d)** and the observed SPR band of GOx conjugated PEDOT: PSS modified GNPs **(Supporting Figure S4)** confirm the stability of the GNP in the desired experimental condition.

Sensing ability of the anisotropic GNPs is based on the observation of the large SPR band shift either due to the changes in the local dielectric constant of the GNPs by the adsorbed biomolecules. The rationale for the colorimetric assay of Glucose is essentially based on the aggregation of anisotropic GNPs induced by Glucose through cascade reactions involving Glucose, GOx, Gluconic Acid,H_2O_2, and H^+ (**Figure 2**)[41]. Upon addition of 10µg/mL Glucose and incubated for 30 minutes**,** the SPR band of the PEDOT: PSS-GOx conjugated GNPs showed 28nm red shift. This data confirmed the interaction of Glucose with the conjugated GNPs system **(Figure 3a).** The corresponding hydrodynamic diameter measurement, indeed, showed that the size of the conjugated GNPs increases to 361nm, which is due to the GNPs aggregation. The zeta

potential also reduced to +2.6mV. The aggregation was further confirmed by the TEM, SEM images and the change in color of the GNPs from pink to blue **(Figure 3b- 3d**). To confirm that the degree of surface functionalization was responsible for maximum detection of Glucose, we functionalized the GNPs using different concentration of PEDOT: PSS solution. Our data showed that out of 0.5, 1, and 2 %, the best aggregation obtained for 1% of PEDOT: PSS solution **(Supporting Figure S5).** Further, to check that the anisotropic nature of the GNPs, indeed, is mainly responsible for such high sensitive detection of Glucose, controlled experiment using PEDOT: PSS functionalized spherical GNPs was also performed. However, no such change was observed in this case **(Supporting Figure S6),** which proved the role of anisotropic nature of the GNPs. Our speculation is based on the fact that the (111) facet of hexagonal and triangular plate GNP is of low energy and hence the extent of ligand coverage will be more on this facet[59,60]. Due to this pre-oriented functionalization, local concentration of PEDOT:PSS and its conjugation with GOx will be higher. Therefore, the degree of Glucose oxidation catalysed by GOx will be more on this facet. On the other hand because of the isotropic nature the extent of ligand binding is much less in spherical GNPs than the anisotropic GNPs[61-62]. As a results no aggregation was obserbed in spherical GNPs under the similar experimental condition.

To get quantitative analytical information on the sensitivity, the detection limit of the sensor was calculated using a titrimetric experiment, where the concentration of Glucose was varied. **Figure 4a** showed the gradual change in the SPR band from 542nm to 565nm upon addition of Glucose from 2.5-50µg/mL. A good linear correlation between absorbance change and Glucose concentration could be established in the range from 2.5 to 20 µg/mL. No further change in the SPR band was observed upon addition of more amount of Glucose to the solution. The detection

limit was found to be 9.8μM, when the change in absorbance (ΔA) was plotted against Glucose concentration (**Figure 4b**).

To evaluate the optimum time of catalytic oxidation of Glucose by the GOx-GNPs conjugate, kinetic studies were also performed. **Figure 4c & 4d** depicted the shift in SPR band with the progression of the reaction time when 10μg/mL of Glucose was added and incubated progressively till 30 minutes. Interestingly, the color change happened within 10 minutes, however, the complete shift in the SPR band occurred till saturation end point at around 30 minutes. No further change in the SPR band was observed rather the solution got precipitated when incubated beyond 30 minutes.

The application of the newly developed sensors is evaluated by carrying out the detection of Glucose in real urine sample. Fresh urine sample from a healthy person was collected and spiked with 10μg/mL of Glucose followed by the addition to GOx GNPs conjugated system. An extensive red shift in the SPR band was observed **(Figure 5)**. The color change from pink to blue was also clearly noticeable as was observed for the normal assay (**Figure 3d**). It is important to note that our present assay is able to oxidize Glucose selectively even in the complex matrix of urine sample .

To check that the added Glucose in the urine is only responsible for above sensing, similar experiment in the absence of Glucose was also performed. pH dependency of our assay was also verified, as the pH of human urine varies from person to person. No noticeable SPR shift was observed in either case **(Supporting Figure S7, S8)**. Finally, we also tested the effect of cysteine, which majorly present in urine sample. No effect was also observed in this case **(Supporting Figure S9)**[17]. All the above experiments confirmed that the developed GOx GNPs system has high selectivity for Glucose even in the presence of interfering of other agents.

The Glucose-induced GNPs aggregation was considered to be a consequence of the suppressive effect of the Glucose against the coulombic repulsion between PEDOT:PSS stabilized GNPs[63]. This was proposed from the decreased in zeta potential when the Glucose was added to the system. If all the GNPs in suspension have either large negative or the positive zeta potential the high electrostatic repulsion protected from any aggregation[64]. However, the low zeta potential for controlled PEDOT: PSS functionalization helped the GNPs to aggregate The Zeta potential of conjugated GOx PEDOT:PSS GNPs was found to be 6.6mV. On addition, of 10µg/mL Glucose it was further reduced to +2.6mV. The significant decrement in zeta potential in addition of Glucose could be explained by GOx catalyzed oxidation of Glucose to Gluconic acid via Gluconate[41]. Gluconic acid could reduce the cationic character of the anisotropic GNPs, which can help in bringing down the zeta potential of the assay. This observation is also supported by the dynamic light scattering (DLS) measurement. The size of GOx conjugate system is increased from 56nm to 361nm on the addition of Glucose. This data clearly suggested that the aggregation of anisotropic GNPs is taking place due to the oxidative degradation of the Glucose to Gluconic acid. For further verification on the mechanism that the produced gluconic acid is responsible for aggregation by reducing zeta potential, the fresh urine sample spiked only with 51µM of Gluconic acid added to the PEDOT: PSS GOx conjugated GNPs. After 10 minutes of equilibration, 14nm shift in the SPR band of the assay was observed confirming the role of Gluconic acid **(Supporting Figure S10).**

Conclusion

PEDOT:PSS surface functionalized anisotropic GNPs have been designed for colorimetric naked eye Glucose sensor.The corners and edges of anisotropic GNPs found to play a major role on the Glucose sensing. Further, an optimum surface coverage of PEDOT: PSS was also very useful for the maximum detection limit.The results will be very effective for the fabrication of homemade, low cost sensors for rural people, especially in the third world countries.

ACKNOWLEDGMENT

The authors acknowledge the home institute (IIT Mandi) and Department of Science and Technology (DST) India for their financial support (Project No: IITM/SG/CKN/003 and SR/FT/CS-152/2011) for running the experiments. AC thanks to Charu Dwivedi for helpful discussion.

Notes:

Supporting Information: Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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Figures

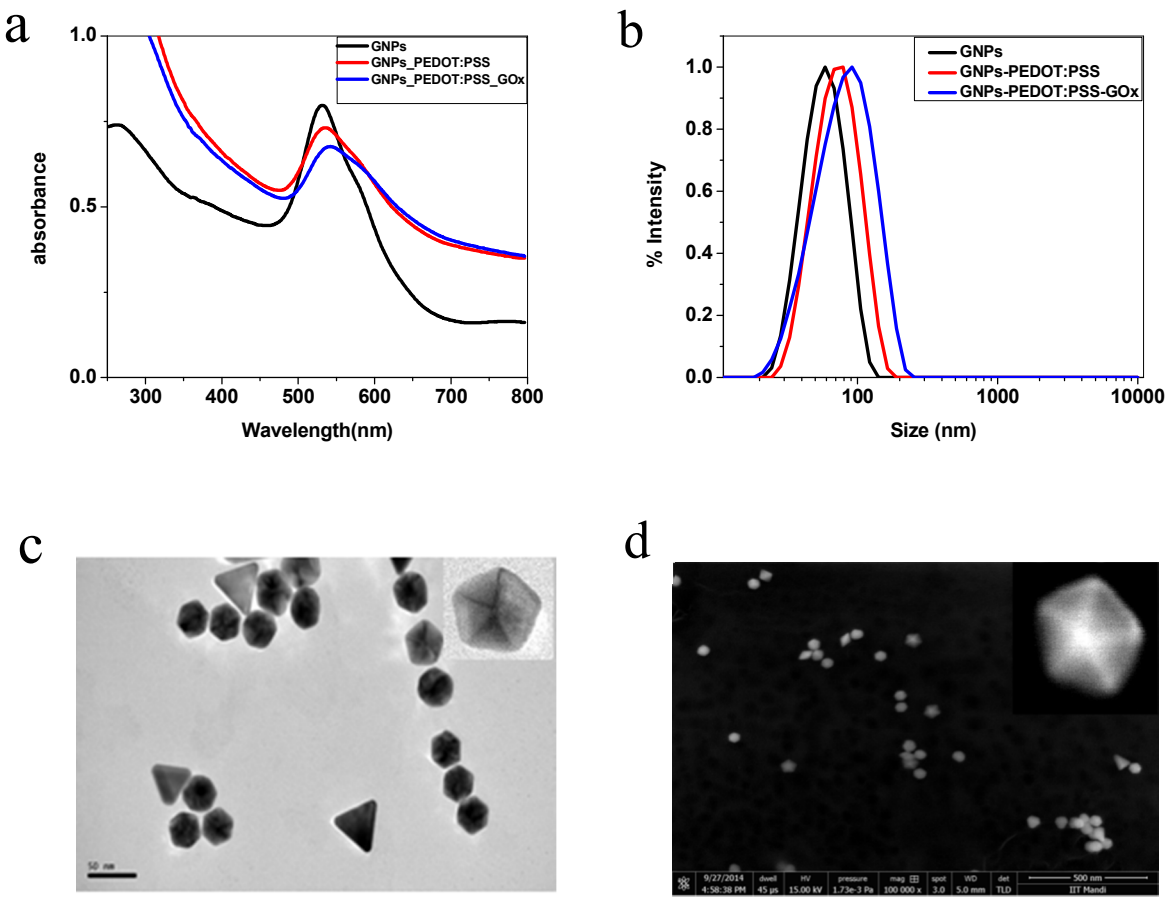

Figure 1. (a) UV-VIS absorption spectra and (b) Hydrodynamic diameter measured using DLS for the as synthesized anisotropic GNP, PEDOT: PSS modified GNP and GOx conjugated PEDOT: PSS surface functionalized GNP (c) TEM and (d) SEM images of GOx conjugated PEDOT: PSS surface functionalized anisotropic GNP; inset is showing the morphology of anisotropic GNP.

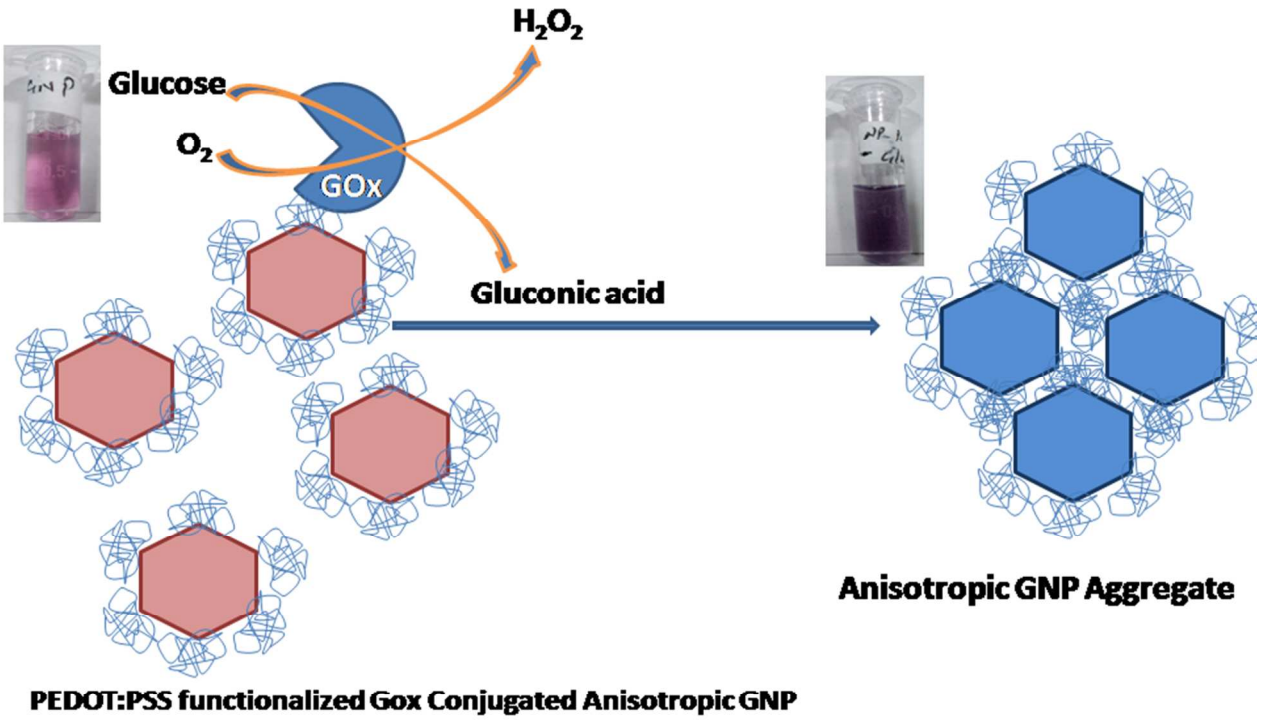

Figure 2: Schematics of the binding mechanism of GOx with PEDOT: PSS functionalized anisotropic GNP and the detection of Glucose based on the color transition from pink to blue.

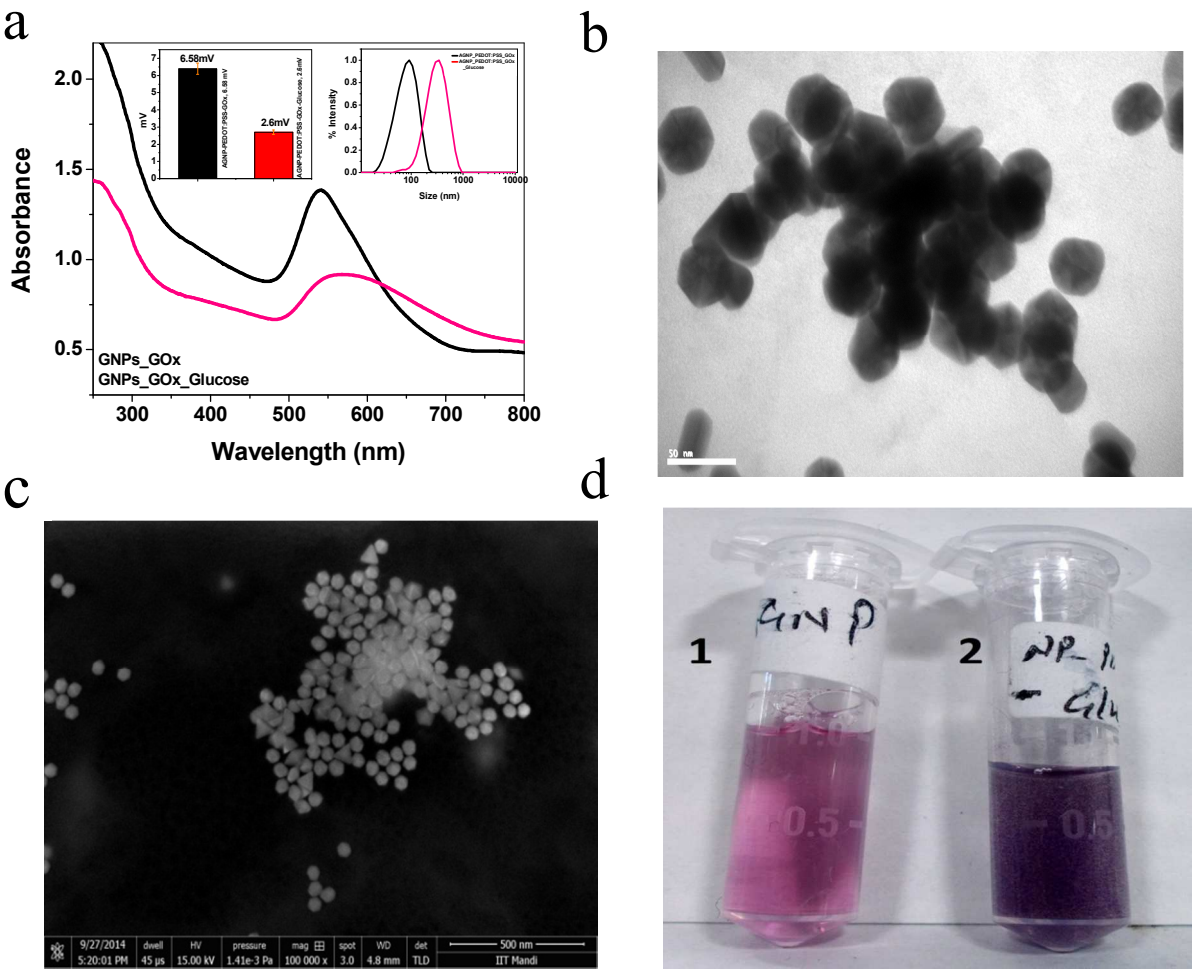

Figure 3. (a) UV-Visible absorption spectra of GOx conjugated PEDOT: PSS functionalized anisotropic GNP shows red shift on reacting with 10µg/mL of Glucose standard (the corresponding zeta potential in the left hand side inset and the right hand side inset shows hydrodynamic diameter) (b) TEM images of aggregated anisotropic GNP-PEDOT: PSS-GOx with the addition of 10µg/mL Glucose (c) SEM images of aggregated anisotropic GNP-PEDOT: PSS-GOx with the addition of 10µg/mL Glucose (d) Color changes of (1) anisotropic GNP and (2) GOx-anisotropic GNP on reacting with 10µg/mL Glucose after 10 minutes of incubation.

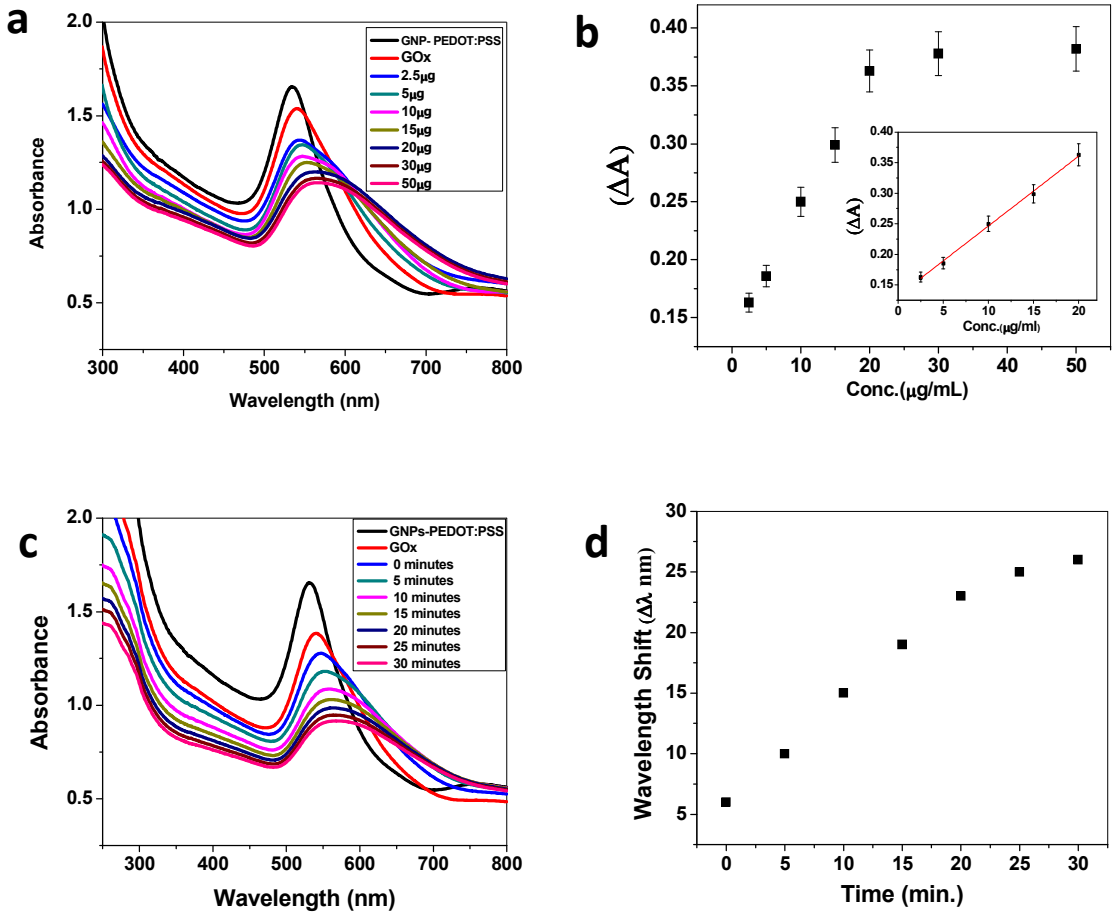

Figure 4. (a) UV-Visible absorption spectra of GOx conjugated anisotropic GNP shows red shift on reacting with different quantities of Glucose standard. (b) Plot of change in absorbance (ΔA) at 542nm against Glucose concentration for the quantitative detection of Glucose, the inset showing calibration curve of absorbance change as a function of Glucose concentration. (c) Time dependent kinetics of Glucose with GOx conjugated anisotropic GNP (d) Graphical relationship of a wavelength shift in SPR band with the progression of the reaction time.

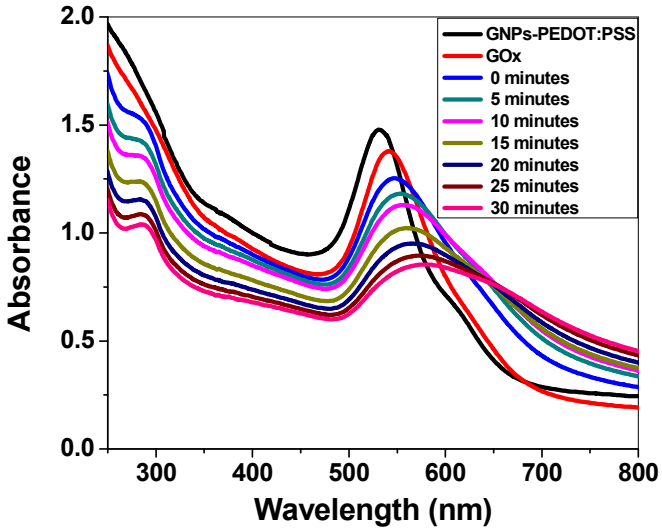

Figure 5. The SPR band shift of the GOx conjugate GNP, when a urine sample spiked with 10μg/mL of Glucose.

TOC Graphics

PEDOT:PSS modified anisotropic gold nanoparticles (GNP) for the colorimetric detection of Glucose sensing in urine.