Manuscript Details

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Abstract

Hormone mimics known as endocrine disrupting compounds (EDCs) prevalent in aquatic environment are of great environmental concern because of their endocrine disrupting and carcinogenic effects. Looking to the wide variety of natural as well as synthetic estrogen hormone mimics, a reliable in-vitro assay is required to screen the estrogenic activity. Surface plasmon resonance (SPR) is one of the most promising analytical tools to monitor high-performance biomolecular interaction in real time without labelling. Present paper demonstrates a facile SPR based affinity bioassay employing estrogen receptor-α, human (hERα) functionalized self assembled monolayer covalently bound to the gold sensor chip as recognition species. A successful interaction of suspect hormone mimic with estrogen receptor is evidenced by net rise in SPR angle in real time. The assay has been validated in terms of optimum experimental conditions and specificity with estrogen as standard showing maximum estrogenic activity. As a proof of concept, proposed affinity assay is tested for screening the estrogenic activity of progesterone, pregnenolone, tamoxifen, and bisphenol-A as representative examples of potential EDCs of different classes.

Keywords	surface plasmon resonance; label-free biosensor; estrogenic activity; estrogen mimics; endocrine disrupting chemicals; EDCs
Taxonomy	Surface Plasmon Resonance, Chemical Modification, Receptor Binding, Biosensor
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June11, 2019

To,
The Editor
Analytical Biochemistry

Subject: Submission of manuscript for publication
Title: **'Label free biosensor for screening estrogenic activity'**
Authors: Urvasini Singh, Sangeeta Loonkar, Laura Michalli Sunita Kumbhat*

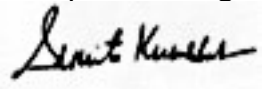
Dear Sir,

We wish to communicate above mentioned manuscript for publication in your esteemed journal, 'Analytical Biochemistry: Methods in Biological Sciences'.

We report a facile, single step SPR based affinity biosensor for screening estrogenic activity of estrogens and estrogen mimics, employing a homemade estrogen receptor conjugate covalently bonded to a surface assembled monolayer functionalized nano thin gold surface.
Work presented in this paper is original, unpublished and is not being considered elsewhere for publication.

Look forward for a positive response.

With profound regards



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Label free biosensor for screening estrogenic activity

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Highlights

- Surface plasmon resonance based affinity biosensor for screening estrogenic activity
- Covalently bonded homemade estrogen receptor conjugate over nano thin gold surface for direct affinity bioassay.
- Successful interaction of potential estrogen mimic with estrogen receptor is evidenced by net rise in SPR angle in real time
- Facile, single step affinity bioassay protocol for screening estrogenic activity of estrogens and estrogen mimics of natural and synthetic origin with reusable chip

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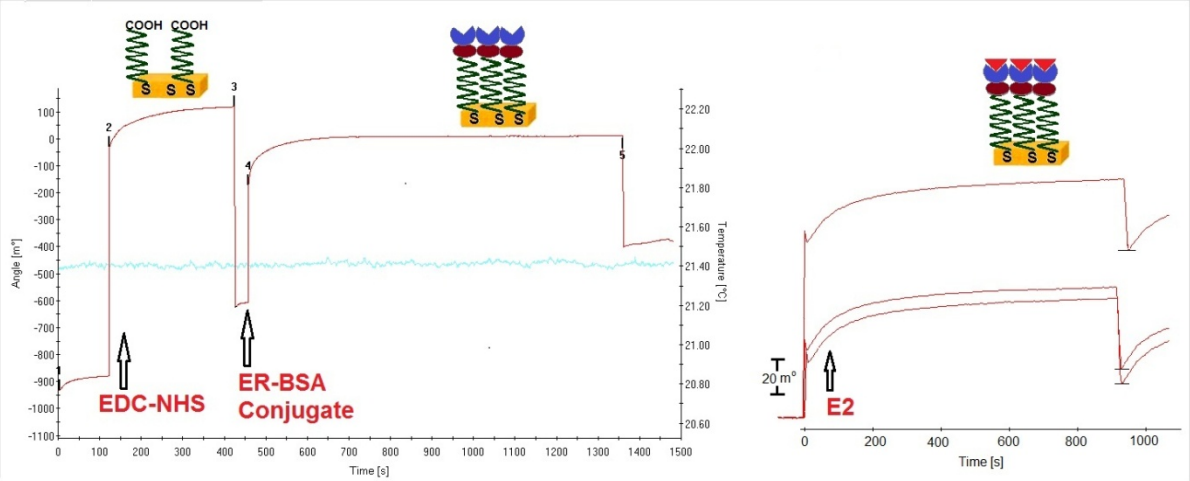
Graphical abstract



Label free biosensor for screening estrogenic activity

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Abstract

Hormone mimics known as endocrine disrupting compounds (EDCs) prevalent in aquatic environment are of great environmental concern because of their endocrine disrupting and carcinogenic effects. Looking to the wide variety of natural as well as synthetic estrogen hormone mimics, a reliable *in-vitro* assay is required to screen the estrogenic activity. Surface plasmon resonance (SPR) is one of the most promising analytical tools to monitor high-performance biomolecular interaction in real time without labelling. Present paper demonstrates a facile SPR based affinity bioassay employing estrogen receptor-α, human (hERα) functionalized self assembled monolayer covalently bound to the gold sensor chip as recognition species. A successful interaction of suspect hormone mimic with estrogen receptor is evidenced by net rise in SPR angle in real time. The assay has been validated in terms of optimum experimental conditions and specificity with estrogen as standard showing maximum estrogenic activity. As a proof of concept, proposed affinity assay is tested for screening the estrogenic activity of progesterone, pregnenolone, tamoxifen, and bisphenol-A as representative examples of potential EDCs of different classes.

Keywords: surface plasmon resonance; label-free biosensor; estrogenic activity; estrogen mimics; endocrine disrupting chemicals; EDCs

1. Introduction

Estrogens and estrogens mimics cause disruption to human endocrine systems, thus termed as endocrine-disrupting chemicals (EDCs). EDCs block or interfere with the production, metabolism and/or action of hormones in the body, thus pose a threat to aquatic life and human health [1, 2]. Occurrence of natural estrogens (estrone, estradiol, estriol and estetrol) ; natural androgens (eg. testosterone, dihydrotestosterone and androstenedione); phytoestrogen (coumestrol, genistein); pharmaceuticals (eg. 17α-ethynyl estradiol, contraceptive pill formulations); pesticides and industrial chemicals (e.g. polycyclic aromatic hydrocarbons, PAHs; polychlorinated compounds viz. PCBs, dioxins, furans; alkylphenols)

in aquatic environment are major contributors of estrogenic activity. Hormone mimics are especially prevalent in surface and waste-waters and therefore, there is a need for analytical device for screening the presence of EDCs. Among conventional analytical strategies for EDCs, fluorescence immunoassay (FIA) and radioimmunoassay (RIA) [3]; liquid chromatography and gas chromatography combined with mass spectrometry requires multistep sample preparation and elaborate instrumental setup [4]. *In-vivo* biological assays of the EDCs [5] require long time to get results and sometimes involve ethical issues for animal experiments. In present era of point-of-care testing (POCT), sensors and biosensors are most convenient *in-vitro* assay tool for field monitoring at or near point of care.

By definition, sensors are devices which can sense specific changes in physical properties or chemical properties of surrounding environment in real time with high accuracy and precision. Third/fourth generation sensors are self-contained integrated devices, capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element. Inclusion of biological element (e.g. enzyme, antibody, receptors etc) to sensor surface adds inherent specificity whereas nano films combined with advanced transducers and the processing power of modern microelectronics offer highly sensitive and specific analytical tools as nanobiosensor. Mozaz et al. [6] have reviewed different strategies used for application in biosensor technology for EDCs monitoring. The electrochemical biosensors [7] are based on depletion in voltammetric current of mediator redox probe upon binding; whereas most optical biosensors employing fluorescent label [8] and /or nanoparticles label [9]. , SPR based sensor represents a real time, highly sensitive and portable tool to confirm specific biomolecular interactions in one step

The concept of surface plasmon resonance (SPR) first introduced by Ritchie [9], has led to emergence of versatile label-free optical sensing technique [10,11]. SPR, an optical phenomenon occurs when incoming plane-polarized light hits a plasmonic metal (Au, Ag, Cu or Al) film interface under the conditions of total internal reflection. In thin (ca. 50 nm) metal layers, electrons behaves as free electron gas, the plasmon. The irradiated light on the backside may excite surface plasmon and generates a weak energy electromagnetic wave, the evanescent wave on the metal layer side. The molecular interaction at the sensor surface changes the dielectric constant and influence the surface plasmon. The evanescent field probes local changes in the refractive index (RI) of the ambient medium that are induced, for example by binding of an analyte to the surface bound macromolecular ligand. A prism coupling, known as Kretschmann configuration [12] (**Fig. 1a**), is generally used to obtain evanescent wave to excite the surface plasmon. Analogous to the prism configuration, a fiber-

optic configuration for SPR sensing has huge potential for portable and point-of care testing (POCT) devices for environmental applications [13]. In fiber optics based SPR (FO-SPR) sensing area is prepared by removing cladding from a small portion of optical fiber and is coated with a thin film of plasmonic metal, as illustrated in **Fig. 1b**. With surface plasmon resonance, molecular interactions can be recognized with a precision of the order of 10^{-6} -10^{-7} for refractive index measurement, which makes technique highly sensitive. The SPR condition depends on the angle of incidence (θ), wavelength (λ) of the light beam, the dielectric functions of metal surface and the refractive index (RI) of the dielectric medium. Angular interrogation technique is common with prism based SPR and spectral (or wavelength) interrogation is generally used in fiber optics based SPR system. **Fig. 1c** shows a plot of reflectance as a function of angle of incidence (θ) of the light beam for sensing layer with refractive indices, *ns* (refractive index of prism or optical fiber) and refractive indices after interaction with analyte (*ns* + *δns*). Increase in RI by *δns*, shifts the resonance angle by δθ and resonance wavelength shifts by δλ; corresponding record of change of RI as resonance unit (m°) with time is SPR spectrum, generally referred as 'sensogram' (**Fig.1d)** demonstrate a sensitive, specific, label free and real time detection of biomolecular interactions.

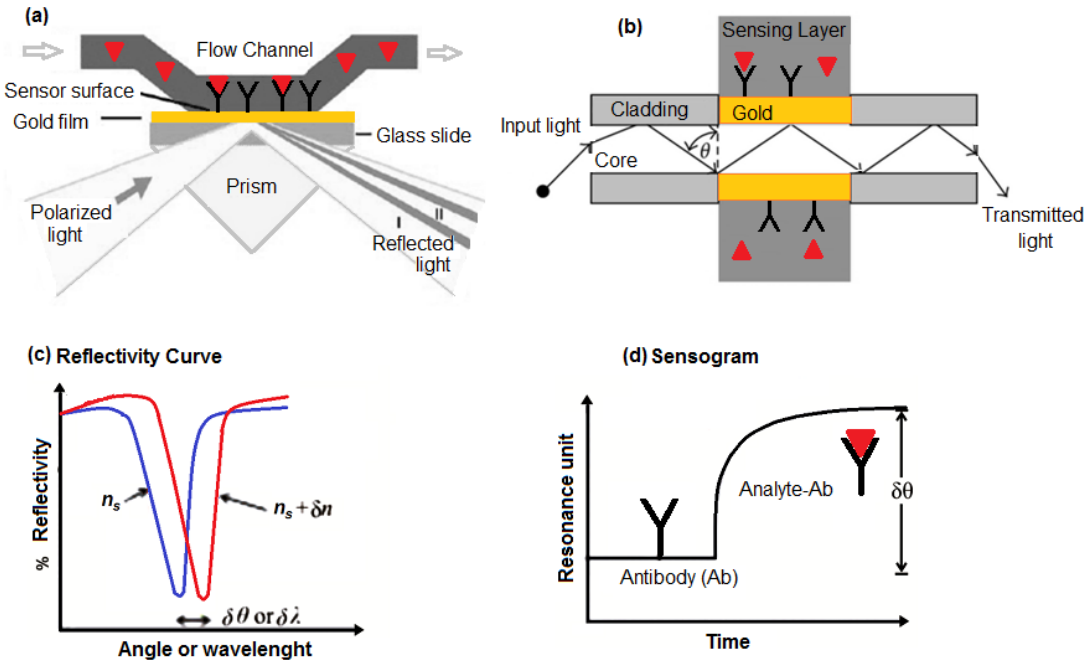

Fig. 1 Schematic illustration of surface plasmon resonance with metal-dielectric interface at a prism based Kretschmann configuration (a) and a silica optical fibre based Fiber- Optic-SPR configuration (b); SPR response as reflectivity curve (c) and sensogram .

For past more than three decades SPR has occupied top position in biosensor technology as transducer for ultrasensitive, specific, label-free and real-time monitoring [14]. Number of SPR based biomolecular interactions have been reported from our group using variety of biorecognition elements viz. enzymes [15], antibody [16, 17] and receptor [18]. Towards SPR based sensing of estrogen mimics, much of the earlier work has been focussed on individual immunoassay employing analyte based antibody [19-25]. The estrogen receptor as recognition species has been used for sensing estrogens and estrogen mimics viz. estradiol and diethylstilbestrol [26, 27]; estradiol, ethinyl-estradiol, 4-nonylphenol and tamoxifen [28]; estrone, estradiol, estriol, progesterone, testosterone, tamoxifen, diethylstilbestrol, bisphenol-A, and 4-nonylphenol [29] under indirect inhibitive assay detection format. Asano et al. [30] have screened estrogenic activity of 30 estrogens and estrogen mimics by measuring binding level of ER to ERE by pre-incubating the potential EDCs with ER. A summary of various SPR based biosensor assay protocol for EDCs found in literature are included in **table1**.

Table 1. SPR based biosensors for detection of estrogens and estrogen mimics

Sensor Chip vs Bio-recognition element	Target analyte	SPR Assay mode	Detection limit/range	References
Ab-morphine	Morphine	Inhibition immuno assay	0.1 - 10 ng/mL	Miura et al.[19] 1997
Ab- methamphetamine	Methamphetamine	Inhibition immuno assay	0.1–1000 ng/ml	Sakai et al.[20] 1999
AbE3G / gold surface (Physical adsorption)	E3G	Inhibition immune assay	10 to 150 ng/ml	Sesay et al.[21] 2001
Ab- benzo(*a*)pyrene	B*a*P	Inhibition immuno assay	0.01 -2 ng/ml	Miura et al.[22] 2003
Mixed SAM (cysteamine+2-mercaptoethanol) /BaP vs. Ab-B*a*P	B*a*P	Inhibitive immunoassay	50 pg/ml to 100 ng/ml	Gobi et al.[23] 2005
Respective Antibodies as biorecognition element on mixed SAM of C11- and C16-alkanethiols/ immobilized Sensor Chip	Atr, B*a*P, 2,4-D, 4-NP	Inhibitive immunoassay	0.05 ng /mL Atr 0.07 ng /mL BaP 0.16 ng /mL 2,4-D 0.26 ng /mL 4-NP	Dostalek et al.[24] 2007
Ab-BpA on a mixed SAM of C11- and C16 alkanethiols	BPA	Inhibitive immunoassay	0.14 ng/ml	Hegnerová et al.[25] 2010

E2-BSA conjugate/ CM5 gold sensor chip vs hER	E2, DES	Inhibitive affinity bioassay	100 µg/mL	Pearson et al.[26] 2001
E2-BSA conjugate/ CM5gold sensor chip vs hER	E2 and DES	Inhibitive affinity bioassay	200 pg./mL	Miyashita et al.[27] 2005
Engineered estrogen receptor alpha (ERα LBD)-αβ/I biotinylated peptide/ CM5 coated gold sensor chip *vs* conformation-sensitive peptides	E2, EE2, 4-NP, TEM	Inhibitive affinity bioassay	20 ng/L for E2	Spina et al.[28] 2017
E2-17PeNH /CM5gold sensor chip vs. hrERα	E1,E2,E3, T, P4, TEM, DES, BPA, 4NP, (0.1– 1 µM)	Inhibitive affinity bioassay	ER binding response in the order of E3> E2> DES> E1> TAM>>BPA >4NP>T No response for P4	Usami et al.[29] 2002
Streptavidine (SA) dextran layer/DNA fragment /biotinylated estrogen response element (ERE)/ ERα Vs E2	30 EDCs , Esteron, DHS and E2 as positive control (1µM to 10 µM)	Inhibitive affinity bioassay	Estrogenic response as Low responder eg BPA) to 1nM 10 nM to 100 nM High responder	Asano et al[30] 2004
hERα- BSA/ 11-MUA/ gold surface	E2, P4, P5, TAM, BPA,	Direct affinity bioassay	0.01 µg mL^{-1} to 100 µg mL^{-1} (E2) No response to P4 positive response to P5, TAM, BPA	Present study

SPR: Surface plasmon resonance; hERα: Estrogen Receptor-α, human; Sp1: transcriptional factor; BSA: bovine serum albumin; OG: oligoethylene glycol; 11-MUA: 11-mercaptoundecanoic acid; ERE: estrogen response element; E1: Estrone; E2: Estradiol; E3: Estriol, EE2: ethinyl-estradiol; E3G: estrone-3-glucuronide; DES: Diethylstilbestrol; BPA: bisphenol-A; Tm: Tamoxifen; BPF: bis(4-hydroxyphenyl) methane; Atr: Atrazine; BaP: benzo[a]pyrene; 2,4-D: 2,4-dichlorophenoxyacetic acid; 4NP: 4-nonylphenol; P4: Progesterone; ; P5: pregnenolone T: Testosterone; BaP: Benzo(a)pyrene; BaP-Ab: anti-BaP antibody; E2-17PeNH: aminated estradiol with a spacer molecule; Carboxymethyalted Dextran (CMD), estrogen response element (ERE), Streptavidine (SA), endocrine disrupting compounds (EDCs), ligand binding domain (LBD)

Estrogens and estrogen mimics may block the estrogen receptor and interfere with the production and action of estrogen in the body. Therefore, for environmental screening of estrogen mimics for their estrogenic activity it is desirable to look into the affinity of potential hormone mimics or EDCs towards human estrogen receptor. Herein, we report a facile SPR based direct affinity assay employing estrogen receptor-α, human as recognition species for screening the estrogenic activity of some of the potential EDCs (**fig. 2**) of different categories.

Progesterone (P4) and pregnenolone (P5) are endogenous steroidal hormones structurally similar to estrogens. Progesterone is a progestogen sex hormone and pregnenolone is a precursor/metabolic intermediate in the biosynthesis of most of the steroid hormones. Tamoxiphen (TAM) and bisphenol-A (BPA) are non-steroidal estrogen mimics. Tamoxiphen is a pharmaceutical drug, act as estrogen receptor agonist and bisphenol-A is a estrogenic chemical of industrial origin. The assay has been validated in terms of optimizing experimental conditions for direct affinity assay with estradiol as standard showing maximum estrogenic activity.

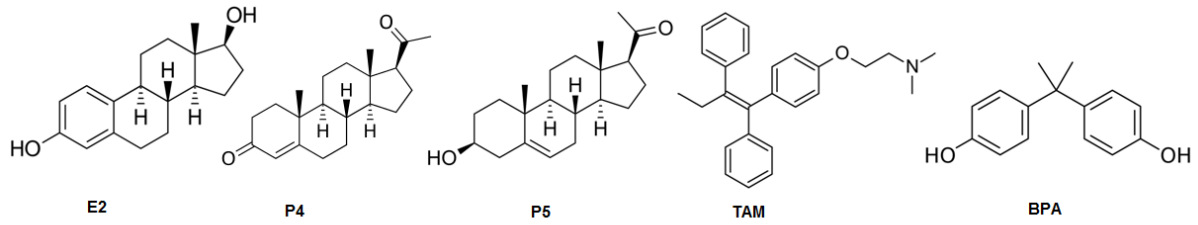

Fig. 2 Structure of Estradiol (E2); Progesterone (P4); Pregnenolone (P5); Tamoxiphen (TAM) and Bisphenol-A (BPA)

2. Experimental Section

2.1 Instrumentation

A glass prism based SPR system in Kretchmann optical configuration (Autolab Model SPRINGLE (Eco-Chemie, Utrecht, Netherlands) and circulator (Julabo, GmbH) was used for present study. Microscopic glass plates (refractive index 1.26 -1.38) with gold coating (48-50 nm) were used as sensor chips.

2.2 Chemicals, Reagents and bioreagents:

17-β-estradiol (E2), estrogen receptor-α, human (hERα) (E1528), progesterone (P4), pregnenolone (P5), bisphenol-A (BSA), tamoxifen (TAM), 11- mercaptoundecanoic acid (11-MUA), N- hydroxyl succinamide, (NHS), N-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), bovine serum albumin (BSA), ethanol amine (EA), pepsin, glycine and tris buffer were procured from Sigma Aldrich, USA. All other reagents were of AR grade from Merck. Phosphate buffer saline (PBS), pH 7.2 (0.01M disodium hydrogen phosphate and 0.01M potassium dihydrogen phosphate containing 0.8% NaCl and 0.02% KCl) prepared in deionised water, was used as carrier buffer and flowed over sensor surface throughout the SPR experiment. Pepsin solution (. 0.2 M) prepared in glycine–HCl buffer (pH 2) was used as

regeneration buffer. A stock solution of BSA (0.2g /10 ml) was prepared in 0.01M sodium acetate buffer (pH 4.5). All experiments were carried out in air conditioned laboratory maintained at 25°C and during assay sample tubes were kept in minifridge cooled 4-5 °C.

2.3 Fabrication of biosensor surface

Estrogen receptor-α human (ER) conjugate was prepared in-house with bovine serum albumin (BSA) as a carrier protein [17]. Briefly, equal quantities of BSA (0.01 gm), NHS (0.01 gm,), and EDC (0.01 gm,) were dissolved in 1 mL Sodium acetate (0.01 M, pH4.5); mixture was sonicated for 2 min in an ultrasonic bath maintained at low temperature. To the above residue, estrogen receptor (ER) as received was then added and mixture was again sonicated for 30 s and left incubated at RT for 2h. The estrogen receptor-BSA conjugate (ER-BSA) was then dialyzed in PBS buffer followed by distilled water at low temperature (3–5◦C). The product ER–BSA conjugate was lyophilized and stored at −20◦C, and designated as stock conjugate. Working dilutions of ER-BSA conjugate were made with 0.01 M sodium acetate. Clean SPR sensor chips were left immersed overnight in 1mM 11-MUA/ethanol, to allow self assembled monolayer (SAM) formation [31], which were then attached to the prism of the SPR instrument using a matching liquid (refractive index = 1.515). A buffer of low salt concentration (0.01M sodium acetate) was injected over sensor chip to stabilize the self assembled 11-mercaptoundecanoic acid monolayer. The next step was in-situ activation of SAM by introducing NHS–EDC reagent (1:1 aqueous solution of 100 mM NHS (N- hydroxy succinamide) and 400 mM EDC (N-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride) for 5 min., followed by introducing ER-BSA conjugate for 15 min. Deactivation of unbound esters was accomplished by introducing 1M EA (pH 8). All injections were of 50µL.

3. Results and Discussion

For low level quantitation of estradiol SPR based indirect inhibition assay has been reported employing different high molecular weight (HMW) interactant viz. estradiol antibodies, Estrogen receptors, estrogen response element, engineered estrogen receptor, DNA element with ERE with varied immobilization strategies (table 1). However, for screening the estrogenic activity, it is envisaged to establish the extent of affinity of potential EDCs for estrogen receptor. Therefore efforts were made to establish a SPR based direct affinity assay employing nanothin gold coated SPR sensor chip functionalized with estrogen receptor.

Homemade estrogen Receptor-BSA (ER-BSA) conjugate was immobilized onto the gold senor chip via covalent amide bonding through self assembled monolayer of a long chain thioic acid. A buffer of low salt concentration (0.01M sodium acetate) was injected over 11-MUA self assembled mono layered gold sensor chip to stabilize the SAM followed by in-situ activation of SAM by introducing NHS–EDC reagent. A net rise of resonance angle by ~120 m◦ (Fig.3A Inj 2) signifies a stable matrix of activated 11-MUA monolayer over SPR sensor surface. The strong affinity of gold for sulphur, provides a perfect matrix of orderly monolayer of long chain thioic acid (11-MUA) over the bare gold sensor chip with carboxylic acid group extending out. Following in-situ carbodiimide activation, self assembled monolayer of 11-MUA provides covalent bonding sites for amide bonding via primary amine group of ligand (protein conjugate). Step-wise immobilization of ER-BSA conjugate was performed to ascertain the optimum concentration to be used for one-step immobilization. Upon introducing 25 µg.mL− 1of conjugate onto the activated SAM, a sharp increase of resonance angle was observed. Subsequent introduction of ER-BSA conjugate shots revealed a further increase in resonance angle with a plateau at ~200 µg.mL^{-1}. Fig. 3B shows a plot of net rise in resonance angle with increasing concentration of ER-BSA conjugate in the concentration range 50-250 µg.mL-1. For ligand immobilization, an optimized concentration 100µg.mL-1 of ER-BSA conjugate introduced over the activated SAM and allowed in-situ interaction for 15 min. A sharp increase of resonance angle (ca. 200 m◦) indicated a successful binding of ER-BSA conjugate over 11-MUA functionalized sensor surface (Fig.3a Inj 4). A brief desorption of the loosely bound conjugate with PBS flow got stabilized which indicates that covalently bound estradiol conjugate is highly stable on the MUA modified sensor surface. Subsequent introduction of ER-BSA conjugate shot revealed a rise of 10 m◦ only, which shows that sensor surface is fully covered with conjugated ligand. Next is blocking step to avoid any non-specific interaction by deactivating any unbound esters, at sensor surface. The blocking step was performed by introducing one shot of ethanolamine (1M, pH 8). Next is the association phase, wherein an optimized time period of ~15 min was found suitable to get a stable SPR signal for direct biomolecular interaction of estrogens with thus prepared SPR sensor chip (ER-BSA/11-MUA/Au).

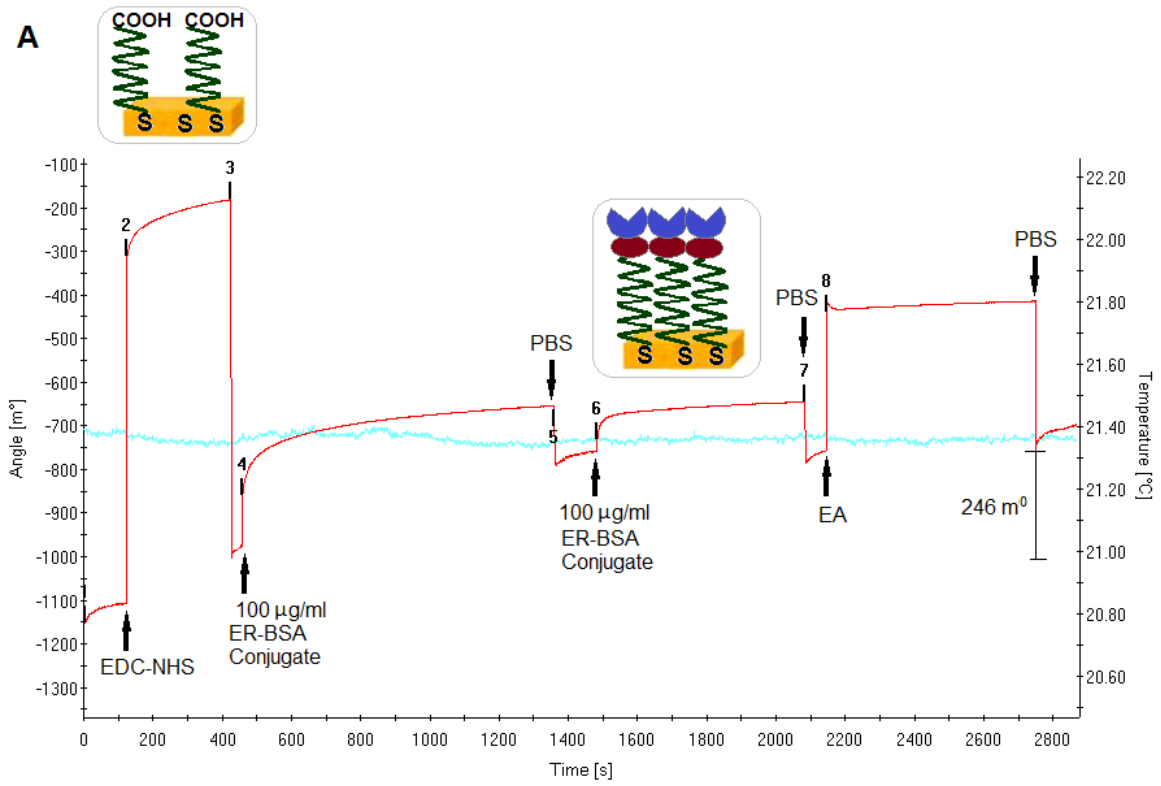

Fig. 3 SPR response for *in-situ* fabrication of affinity sensor (ER-BSA/11-MUA/Au), showing activation of 11-MUA modified gold surface followed by stepwise immobilization of E2-BSA conjugate.

To evaluate the affinity of hormone mimics towards estrogen receptor, 1µg.mL-1 each of estradiol, progesterone, pregnenolone, tamoxiphen and bisphenol-A was introduced over SPR sensor chip (ER-BSA/11-MUA/Au) to undergo association phase. Every injection was followed by a brief flow of carrier buffer, (PBS at pH 7.2) for 2 min to wash out the un-reacted species (dissociation phase). Regeneration buffer (pepsin solution) was used to remove immobilised assay components between each assay cycle.

The net rise at the end of dissociation phase with respect to the resonance angle at initiation of association phase is an affirmative sign for affinity with ERA strong affinity interaction of E2 for ER-BSA is clearly marked as rise of ~100 m° of SPR signal (fig. 4a). The rise in SPR signal for E2 interaction at proposed sensor surface may serves as a mark of highest estrogenic activity. To monitor the estrogenic activity of hormone mimics were introduced at sensor surface and allowed to interact for 15 min. Introduction of progesterone (P4) did not bring any net rise in SPR angle (fig. 4b), which shows that P4 did not bind effectively with estrogen receptor at sensor surface, and gets washed away with the flow of PBS. The binding

interaction of estrogen receptor with pregnenolone, tamoxifen and bisphenol-A is evidenced in sensogram with a net rise of SPR angle ~55 m◦, ~35 m◦ and ~30 m◦ respectively (Fig. 4c, d, e). On comparing the estrogenic activity of estrogen mimics towards estrogen receptor with respect to estradiol as standard, pregnenolone, tamoxifen and bisphenol-A are EDCs with high estrogenic activity whereas progesterone shows no estrogenic activity. Results are in concordance with reported estrogenic status of these analytes [24, 25].

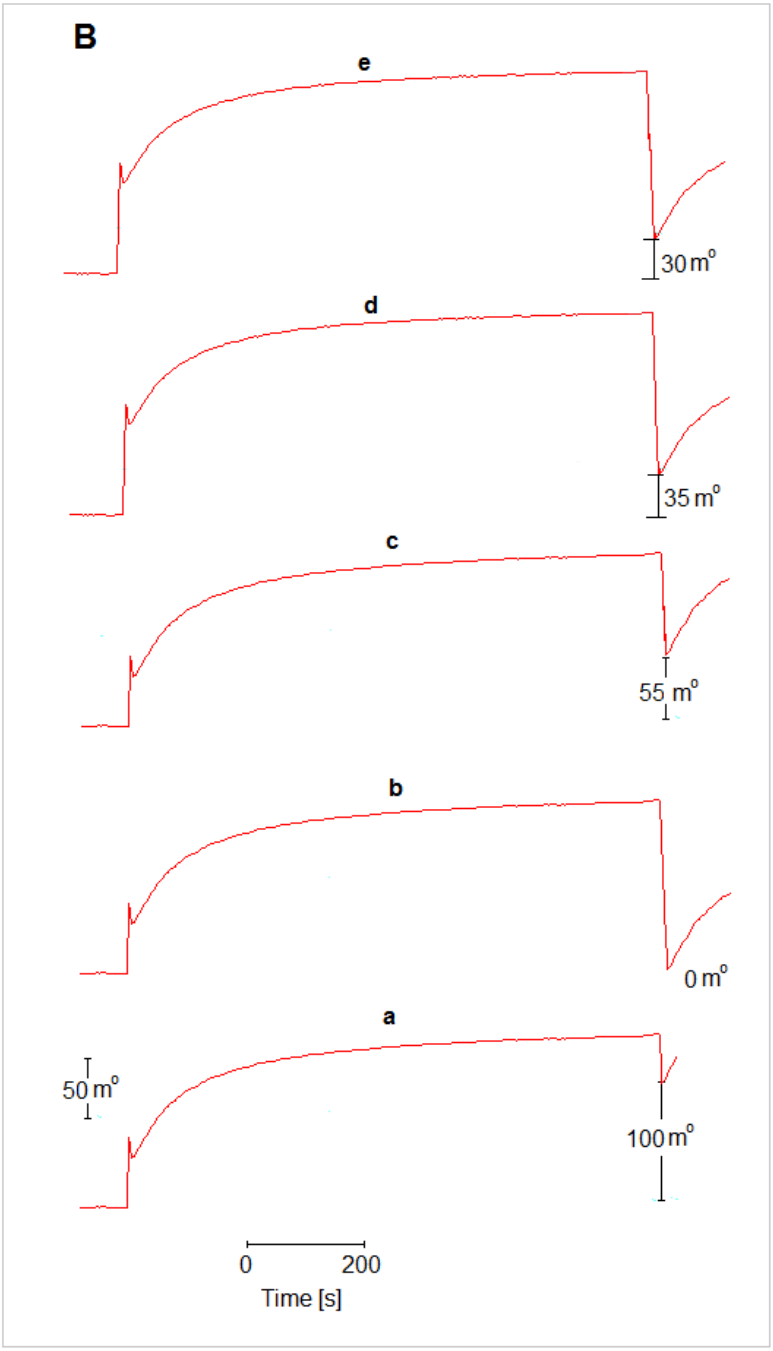

Fig. 4 Sensogram showing direct affinity interaction of 1µg.mL^{-1} each of estradiol (a, net rise 100 m◦); progesterone (a, net rise 0 m◦); pregnenolone (b, net rise 55 m◦); tamoxifen (c, net rise 35 m◦); bisphenol-A (d, net rise 30 m◦) at biosensor (ER-BSA/11-MUA/Au).

Proposed SPR based affinity sensing protocol could be used for screening the estrogenic activity of analytes in the concentration range 0.01 μg mL^{-1} to 1000 μg mL^{-1}. The regeneration capability of the platform and its adaptability to a portable SPR device makes this assay promising for screening of EDCs in field. Proposed SPR based affinity sensing protocol could be used for screening the estrogenic activity of analytes in the concentration range 0.01 μg mL^{-1} to 1000 μg mL^{-1}. The regeneration capability of the platform and its adaptability to a portable SPR device makes this assay promising for screening of EDCs in field.

4. Conclusions

The proposed SPR based affinity assay employing homemade estrogen receptor-BSA conjugate covalently bonded to a long chain thioic acid monolayer immobilized over gold sensor chip (ER-BSA/11-MUA/Au) is suitable for label free, *in-vitro* screening of estrogen activity of hormone mimics and potential endocrine disrupting chemicals. An optimum time period of 15 min was found to be satisfactory for interaction between immobilized receptor and estrogens (association phase) to get a stable SPR signal showing successful interaction and binding. Every association phase was followed by a brief flow of carrier buffer, (PBS at pH 7.2) for 2 min to wash out the un-reacted species (dissociation phase). In the presence of a chemical with estrogenic activity, the effective rise in SPR angle at the end of dissociation phase with respect to resonance angle at initiation of association phase. Further on comparing the SPR signal of hormone mimics with that of estradiol as standard, degree of estrogen activity could be ascertained in a much simple, single step, real time, label free direct affinity assay protocol. The simplicity of proposed SPR based direct affinity interaction based protocols for detection of estrogen and estrogen mimics has potential for possible transformation into futuristic fiber optics based SPR probes for on-site environmental monitoring.

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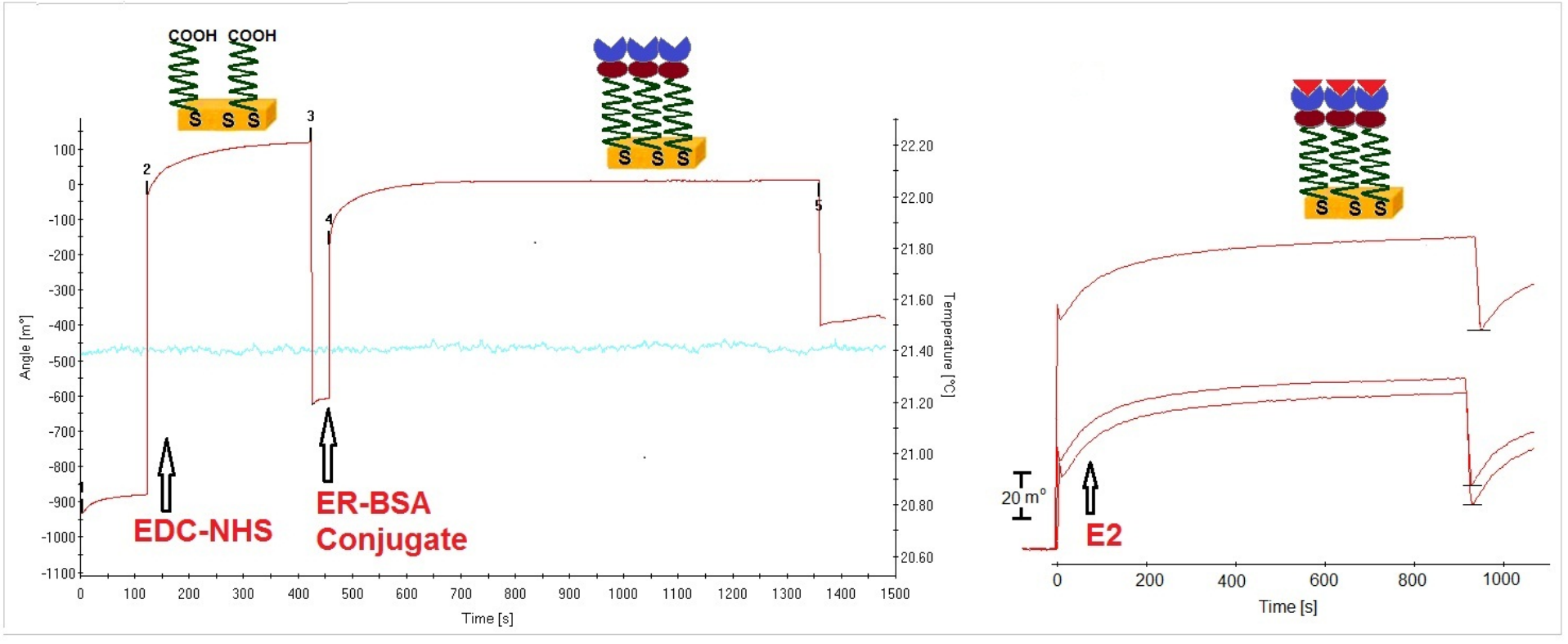
COOH COOH
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ER-BSA
Conjugate
Angle [m°]
Temperature [°C]
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