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Abstract

The grafting of 5-iodoisatin heterocycle on a cyclic olefin copolymer (COC) and a gold surface was performed using a heterogeneous phase Sonogashira reaction consisting of coupling 5-iodoisatin with an arylalkyne previously introduced onto the surfaces. This optimized strategy takes advantage of the well-established methodology to functionalize COC or gold surfaces using aryldiazonium surface chemistry. Herein, we reported the first example of an isatin decorated polymeric or metallic surface. The surfaces were analyzed with a combination of techniques such as IR (Infrared spectroscopy), XPS (X-Ray photoelectron spectroscopy) and SPR (surface plasmon resonance). Docking studies showed that isatin and two derivatives interact with AmiC, a dimeric protein produced by *Pseudomonas aeruginosa*. Bacterial adhesion on isatin-COC platform was also observed. This general strategy for robust surface functionalization represents an easy approach for patterning surfaces with compounds of biological interest, allowing access to a large panel of original biosensors.

Keywords

Isatin

Aryldiazonium salts

Biosensors

Surface functionalization

Heterogeneous Sonogashira cross-coupling

1. Introduction

Surface functionalization for the development of efficient analytical devices requires a well-controlled process in order to exactly define layer structuring [1-5]. Modification by means of organic species can be achieved by attaching a previously synthesized species directly onto the surface [6,7] or binding a scaffold to an activating material [7-9]. The orientation of the immobilized molecule is of crucial importance to facilitate the access to the partner molecule, present in solution, and to improve interface reactivity. Surface materials based on polymers such as Poly(methylmethacrylate) (PMMA) [10], cyclic olefin copolymer (COC) [11,12] or poly(dimethylsiloxane) (PDMS) [13] are particularly interesting as devices or biosensors thanks to their mechanical strength, chemical resistance and optical transparency. Nevertheless, prior to be used in biodetection, these materials need physical treatments, typically plasma irradiation [14] or UV-ozone treatment [15] for an efficient immobilization of organic molecules. Recently, we have successfully functionalized COC surfaces in absence of any plasma activation by reducing grafting of aryldiazonium salts *via* transient formation of the corresponding aryl radicals [16]. Widely used to modify metallic surfaces for their electro- or chemical grafting ability [17-20], these species also proved to be competent for the functionalization of polymeric surface [21]. From their reduced form, radicals covalently bind to the surface allowing various chemical functions (acids, nitro or bromo groups) to be introduced on the COC surface [16]. The strategy is here extended to the immobilization of an isatin derivative for designing biosensors.

Isatin and derivatives (Fig. 1a) are versatile architectures often encountered within pharmaceutical relevant compounds [22]. Among its different biological activities, isatin can counteract the effect of some natriuretic peptides both in mammals and in bacteria and contributes to decrease the virulence that these peptides induce on the *Pseudomonas aeruginosa* bacterium [23,24]. The design of a biosensor combining isatin and a physical support may allow the transduction of a measurable signal generated when isatin reacts with peptides. Although a large variety of methods exists to access isatin derivatives [22], the immobilization process is restricted because isatin is susceptible to undergo nucleophilic attack especially to the lactam moiety [25]. Moreover, once grafted isatin has to be orientated towards the liquid interface to allow interaction with molecules. Thus, the choice of suitable synthetic routes and reaction conditions , compatible with the structure of the bioactive compounds, are fundamental criteria to consider when planning surface modification programs.

Two synthetic pathways 1 and 2 based on Sonogashira and Suzuki carbon-carbon cross-coupling reactions were explored to reach our goal (Fig. 1b). These palladium-catalyzed reactions were selected since Suzuki cross-couplings tolerate an array of functional groups [26,27] and the Sonogashira reaction is usually carried out under mild conditions [28,29]. Following pathway 1, isatin was bound to an aryl derivative in homogeneous phase by a Suzuki or Sonogashira reaction. Once diazonium salt is formed, the isatin containing adduct is introduced on the surface using classical reducing grafting events. With pathway 2, the surface was pre-functionalized attaching an aryl bearing an

alkyne group via classical aryldiazonium salt chemistry. The isatin-decorated surface was then achieved by a Sonogashira reaction in heterogeneous phase.

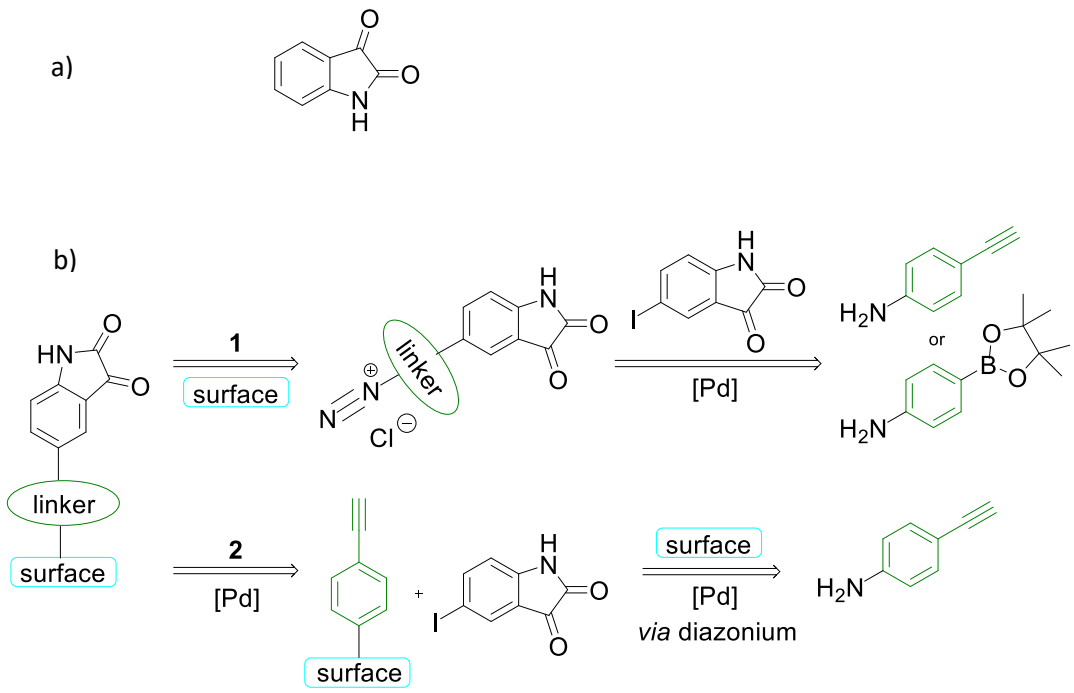


Fig 1. a) Structure of isatin and b) explored retrosynthetic strategies for surface isatin immobilization .

2. Materials & methods

2.1 Materials

Cyclic olefin copolymer (TOPAS) substrates of 2 cm^2 were purchased from microfluidic Chip Shop (Germany) and used without further treatment. Gold substrates (1 cm^2) were purchased from Schott (France) and cleaned with piranha solution and distilled water before electrografting.

All syntheses were monitored by thin-layer chromatography (TLC) on silica gel plates 60 F254 (E. Merck, Germany) and developed plates were visualized using ultraviolet light. Column chromatography was performed using silica gel 60 (0.04–0.063 mm, E. Merck).

*2.2 Synthesis of derivatives **3** and **6***

*2.2.1 5-((4-aminophenyl)ethynyl)indoline-2,3-dione **3***

In a Schlenk flask placed under argon atmosphere were added **2** (252 mg, 2.2 mmol, 2 eq.), CuI (41 mg, 0.22 mmol, 0.2 eq.) and $Pd(PPh_3)_2Cl_2$ (39 mg, 5 mol%). Anhydrous DMF (4 mL) and NEt_3 (1.5 mL) were added and the reaction mixture was

stirred at room temperature for 45 min. 5-Iodoisatin **1** (300 mg, 1.1 mmol, 1 eq.) was added and the solution stirred at room temperature for 18 h. The solution was concentrated under reduced pressure and the residue purified by column chromatography on a silica gel (dicholomethane/ethyl acetate, 8/2, v/v) to afford **3** as a dark red powder (34 mg, 0.13 mmol, 12%). mp > 400°C; IR (ν_{max}/cm^{-1}): 3448 (CO*N-H*), 3361 (NH_2), 3071(C-H), 2924 (C-H), 2208 (C≡C), 1744 (C=O), 1620-1599 (C=C). ^{1}H NMR: (300 MHz, acetone-d6) δ (ppm) 10.15 (s, 1H, NHCO), 7.69 (dd, *J* = 8.2, 1.8 Hz, 1H, H-Ar), 7.58 (d, *J* = 1.7 Hz, 1H, H-Ar), 7.25 (d, *J* = 8.7 Hz, 2H, 2 x C*H*-PhNH$_2$), 7.05 (d, *J* = 7.7 Hz, 1H, H-Ar), 6.68 (d, *J* = 8.7 Hz, 2H, 2 x C*H*-PhNH$_2$), 5.10 (br s, 2H, NH_2); TOF MS ES+: Calcd for $C_{18}H_{14}N_3O_2$ $[M+H+ACN]^+$: 304.1086, Found: 304.1074.

*2.2.2 5-(4-aminophenyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one **6***

Following the method described by Damgaard *et al*.[30] to 5-iodoisatin **1** (2.25 g, 8.2 mmol, 1 eq.) dissolved in toluene (80 mL) were added ethylene glycol (8.7 mL, 156 mmol, 19 eq.) and *p*-toluenesulfonic acid (77 mg, 0.45 mmol, 0.05 eq.). The resulting solution was refluxed at 120°C for 5 h. After cooling to room temperature, the solvent was removed by vacuum and the residue diluted with dichloromethane (30 mL) and successively washed with an aqueous saturated solution of $NaHCO_3$ (3 × 30 mL). The organic phase was dried over $MgSO_4$, filtrated and evaporated to dryness under reduced pressure. The resulting orange solid was purified by column chromatography on silica gel (cyclohexane/EtOAc 75/25 – 50/50, v/v) yielding **4** as a white solid (2.16 g, 6.80 mmol, 83%). Structural analyses are in accordance with the literature [30].

To a sealed tube were added 5-iodo sprio[1,3]dioxolaneisatine **4** (316 mg, 1.00 mmol, 1 eq.), aminophenylboronic acid pinacol ester **5** (435 mg, 2.00 mmol, 2 eq.), $NaHCO_3$ (252 mg, 3.00 mmol, 3 eq.), a 2.5/0.5 DME/H_2O mixture (7.5 mL) and the obtained solution was degassed with argon for 5 min. Pd(dppf)Cl_2 (37 mg, 5 mol%) was added, the mixture was further degassed for 2 min then stirred at 90°C for 18 h. After cooling to room temperature, the solution was filtered over a celite pad, diluted with dichloromethane (30 mL) and washed with an aqueous saturated solution of $NaHCO_3$ (3 × 30 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography on a silica gel (cyclohexane/EtOAc, 75/25 – 50/50) yielding **6** as a light orange powder (175 mg, 0.62 mmol, 62%). mp: 222-225°C; IR (ν_{max}/cm^{-1}): 3419 (CO*N-H*), 3333 (NH_2), 3065 (C-H), 2922 (C-H), 1736 (C=O), 1626 (C=C); ^{1}H NMR: (300 MHz, DMSO-d6) δ (ppm) 10.43 (s, 1H, NHCO), 7.48 (m, 2H, 2 x H-Ar), 7.29 (d, J = 8.5 Hz, 2H, 2 x C*H*-PhNH$_2$), 6.83 (d, J = 8.0 Hz, 1H, H-Ar), 6.61 (d, J = 8.5 Hz, 2H, 2 x C*H*-PhNH$_2$), 5.19 (br s, 2H, H-7), 4.38-4.26 (m, 4H, H-18, O-CH_2-CH_2-O); ^{13}C NMR: (75 MHz, DMSO-d6) δ (ppm) 174.5 (C=O), 148.1, 140.6, 135.5, 128.4, 126.9, 126.8 (2 x CH), 125.3, 122.0, 114.3 (2 x CH), 110.8, 101.8, 65.5 (2 x CH_2); TOF MS ES+: Calcd for $C_{18}H_{18}N_3O_3$ $[M+H+ACN]^+$: 324.1348, Found: 324.1333; Calcd for $C_{16}H_{15}N_2O_3$ $[M+H]^+$: 283.1083, Found: 283.1068.

*2.3 Synthesis of COC-activated surfaces **8** and **10***

To compound **2** or **6** (0.1 M) was added aqueous HCl 0.5 M and the solution was placed at 0°C. $NaNO_2$ (3 eq.) was added and the mixture was stirred over 10 min to afford derivatives **9** or **7** respectively. The COC plate was then immersed and H_3PO_2 (9 eq.) was added. The chemical reactor was incubated in a UV curing system equipped with a UV

metal halide lamp (225 mW cm^{-2}, Dymax, Germany) for 1 h. After grafting, COC plate (**8** or **10** respectively) was sonicated with acetone for 10 min.

*2.4 Synthesis of gold-activated surface **13***

According to the method described by Zhang *et al*. [31], in a round-bottomed flask, 4-ethynylaniline **2** (1.17 g, 10 mmol, 1 eq.) was dissolved in a mixture of absolute ethanol (3 mL) and a 34% aqueous solution of tetrafluoroboric acid (3.7 mL, 60 mmol, 6 eq.). *Tert*-butylnitrite (2.7 mL, 23 mmol, 2.3 eq.) was added dropwise to the solution at 0°C. The mixture was stirred at room temperature for 1 h and diethyl ether (20 mL) was added. The precipitate was filtered off and washed with cold diethyl ether to achieve derivative **12** as a beige solid (2.17 g, 10 mmol, quantitative). Structural analyses are in accordance with the literature [32].

The electrochemical grafting was performed applying a potential from 0 V to -2 V over 30 voltammetric cycles in a 10 mL acetonitrile solution of derivative **12**. After grafting, the surface was rinsed with acetonitrile and acetone.

*2.5 Synthesis of isatin-COC surface **11** and isatin-gold surface **14***

The alkyne-COC or alkyne-gold surface **10** or **13** respectively was placed in a Schlenk flask under argon atmosphere containing $Pd(PPh_3)_2Cl_2$ (5 mg, 4 mol%), CuI (10 mg, 0.05 mmol, 0.3 eq.) and anhydrous DMF (5 mL). 5-Iodoisatin **1** (50 mg, 0.18 mmol, 1 eq.) and NEt_3 (2 mL) were added and the mixture was heated at 60°C for 4 h under slight stirring to achieve surfaces **11** or **14** respectively. After grafting, the plate was sonicated with Et_2O for 10 min then rinsed with EtOH.

2.6 In silico approach

Potential ligand/protein interactions were investigated in silico by the molecular docking technique using the amidase sensor protein of *Pseudomonas aeruginosa* AmiC (Protein Data Bank 1QO0). Essential hydrogen atoms, Kollman united atom type charges and solvation parameters were added with the aid of AutoDock tools [33]. Affinity (grid) maps of 20X20X20 Å, grid points and 0.375 Å spacing were generated using the Autogrid program [33]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and electrostatic terms, respectively. Docking simulations were made using the Lamarckian genetic algorithm (LGA). Initial position, orientation and torsions of the ligand molecules were set randomly. Each docking experiment was derived from three different runs that were set to terminate after a maximum of 1,000,000 energy evaluations. The population size was set to 100.

2.7 In vitro approach

To evaluate bacterial adhesion on COC surface, the preculture of a *Pseudomonas aeruginosa* (PA14) preculture was incubated for 24h at 37 °C in LB buffer. The culture was harvested by centrifugation for 5 min at 5000×g and washed three times with water. Then, the bacteria were suspended in PBS buffer to an optical density at 580 nm (OD580)

of 0.1. Any substrates were sterilized with UV irradiation for 15 min and then immersed in bacterial suspension for 2 h at 37°C. Finally, sample plates were rinsed with sterile water and ultrasonicated to dislodge the bacteria retained. Serial decimal dilution were performed and viable counts were estimated following the surface spread plate method. Glass substrate was used as an *in vitro* experimental control.

2.8 Characterization

The original and functionalized COC and gold surfaces were fully characterized. Fourier transform IR spectroscopy was carried out using a Tensor 27 (Bruker, USA) spectrometer with a ZnSe ATR Crystal. The samples were scanned at different area to evaluate the homogeneity of the treatment, and background spectra were recorded on air. X-ray photoelectron spectra were recorded on a Vaccum Generator ESCALAB 250 (UK) spectrometer using a non-monochromatic AlKα X-ray source (150W). Pass energy was set at 160 eV for the survey and 40 eV for the high resolution spectra of N_{1s}, C_{1s}, O_{1s}, Cu_{2p} and I_{3d}. The spectra were calibrated against C_{1s} set at 285 eV. Electrografting was performed in a 3 electrode cell (platinum wire as counter and reference electrodes) using a Princeton Applied Research (US) model VersaSTAT 3 potentiostat. All sensorgrams were characterized using SPRi-Plex II from Horiba (Japan). Gold surfaces were mounted on prism using Horiba optical contact oil. Gold surfaces were incubated with 10 mM PBS solution.

3. Results and discussion

3.1 Chemistry - Synthesis of isatin derivatives

Reducing diazonium salts grafting seems to be a promising technique to access isatin modified surfaces through introduction of an aryldiazonium salt acting as anchoring point. As long as amino functional group is a suitable precursor of diazonium salt, we focused on a straightforward functionalization of isatin with an aniline group (Fig. 2). Starting from the commercially available 5-iodoisatin **1**, we firstly considered the synthesis of compound **3** by reacting isatin **1** with 4-ethynylaniline **2**. Despite the mild reaction conditions used for the Sonogashira cross-coupling process, the obtained product **3** was isolated in only 12% yield. A Suzuki reaction between **1** and 4-aminophenylboronic acid pinacol ester **5**, led only to traces of the desired cross-coupling product which confirmed the high sensitivity of isatin moiety and the need of protection of the carbonyl moiety. The Suzuki reaction of the corresponding acetal-protected isatin **4** allowed the formation of compound **6** in 62% yield and opens the way to surface modification with isatin derivatives.

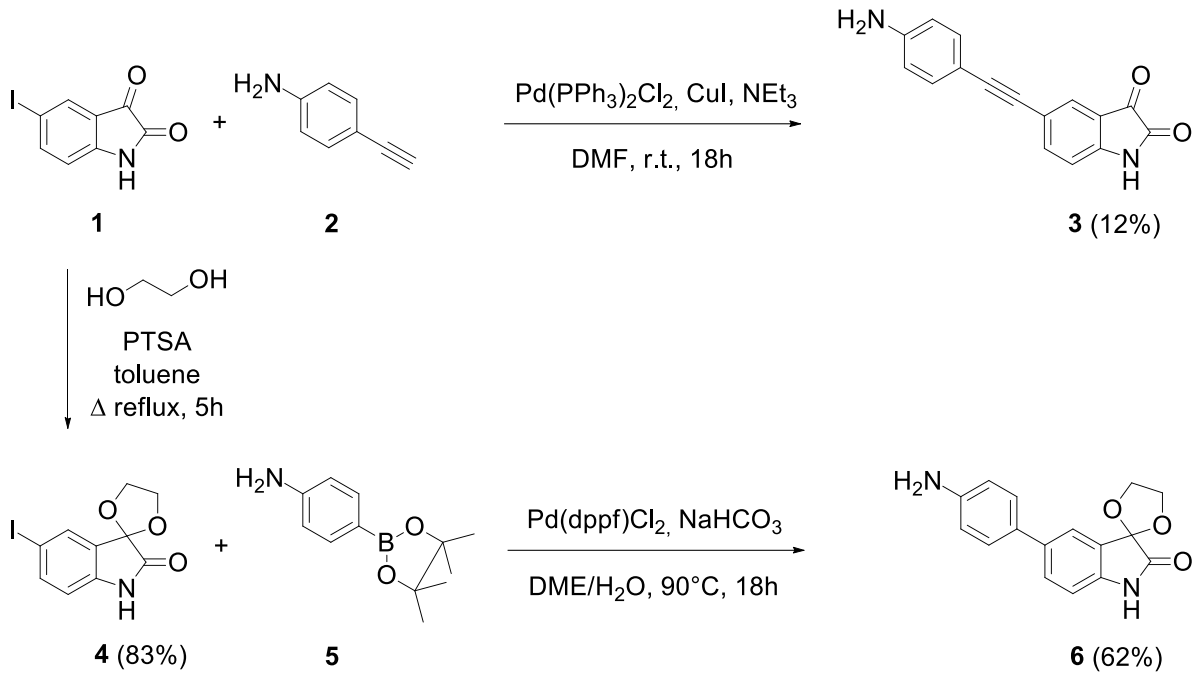
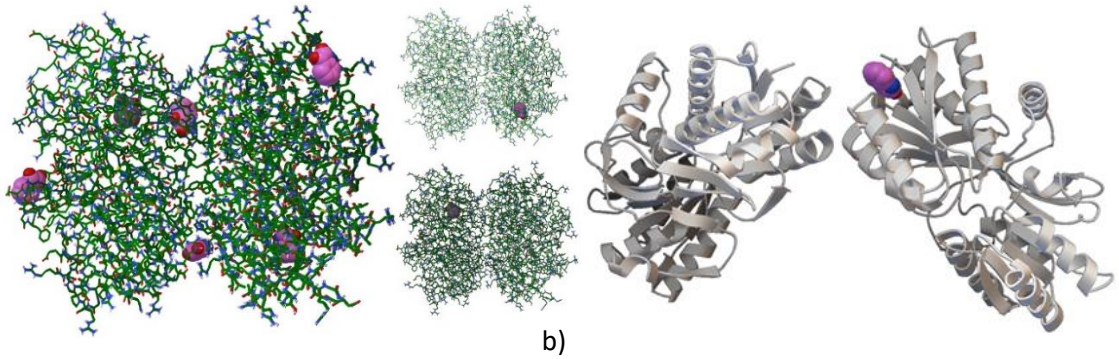


Fig. 2. Synthesis of isatin derivatives **3** and **6** bearing an aniline group.

3.2 Docking studies of isatin derivatives on Ami-C protein

AmiC is a dimeric protein expressed by *Pseudomonas aeruginosa* responsible for the detection of acetamide and natriuretic peptide hormones, thus favoring bacterium adaption to its host [34]. It was selected for this study thanks to its three-dimensional structure very close to the human receptor for natriuretic peptide C. The most used models of AmiC for docking studies are 1pEA and 1qoOp although the 1pEA model exists in the form of a single peptide chain, solely 1qoOp model being directly available in dimer form. On the 1pEA receptor, the AutoDock 4.2 software provided ten possible interaction sites distributed all over the monomer (Fig 3a). The absence of preferential affinity of isatin for one specific site suggested that two 1pEA peptide chains have to be modeled to observe an interaction between isatin and AmiC. With the entire structure of the receptor, only two sites revealed possible for location of isatin within the lobes of AmiC, as resulted from previous studies established on 1qoOp model [34]. Thus, isatin can: occupy the anchor site of the C natriuretic peptide (CNP) on the AmiC receptor, competing with the natural substrate (Fig. 3a, right top), activate AmiR favoring AmiR-AmiC binding or accommodate into the opposite end of AmiC inducing a rearrangement of the protein which hinders the access to the CNP (Fig. 3a, right bottom).



a) b)

Fig. 3. a) Docking of isatin with 1pEA model and b) 1qoOP model .

In the case of 1qoOp model, among the five favored conformations identified is the rearranged form of 1pEA. Interactions with 1qoOP exhibited K_d values in the range 300 - 400 μM unlike K_d values for 1pEA ranged from 850 to 1000 μM, refined simulations were carried out on 1qoOp model. Limiting the study to the lobes interface in a reduced box, it was possible to highlight that isatin strongly interacted with residues R77 and W306 of Ami-C through formation of hydrogen bonds (Fig. 3b).

The study, extended to compounds **3** and **6** showed all isatin and its derivatives interacted with Ami-C at the same site. However, if a hydrogen bond was established with residue R77, compound **3** displayed a second interaction with residue R77 whilst **6** with residue W99 in addition to a loss of interaction compared the carbonyl deprotected form. The binding energy (B.E) was also calculated and appeared in the same range for the three molecules, decreasing in the order isatin (B.E=-5.6 kcal·mol^{-1}) > derivative **3** (B.E=-6.7 kcal·mol^{-1}) > derivative **6** (B.E=-7.2 kcal·mol^{-1}), which indicates isatin (Kd = 73 μM) possesses weaker affinity for Ami-C than compound **6** (Kd = 5.5 μM).

3.3 Grafting on a COC surface

Following our strategy to link the isatin moiety to the surface *via* an aryldiazonium anchoring point, we compared the two pathways where the first planned binding the aryldiazonium salt to the heterocycle prior to surface grafting, whereas the second one concerned attaching the structure to a surface previously decorated by the aryl moiety.

3.3.1 Functionalization without surface pre-modification

Aryldiazonium salt **7** was prepared *in situ* from diazotization reaction of aniline derivative **6** in presence of HCl and $NaNO_2$ (Fig. 4a) without any successive purification. The grafting procedure involved aryl radical formation from **7**, adding the COC support and the reducer H_3PO_2 to the containing derivative **7** acidic solution and activating COC by UV illumination. After rinsing, the plate was analyzed by IR spectroscopy. The IR spectrum of **8** (Fig. 4b) clearly confirmed the COC functionalization with the isatin derivative, showing a distinctive strong band at 1715 cm^{-1} related to amide C=O stretching of the heterocycle and one at 1625 cm^{-1} typical for aromatic C=C bending. A significant

decrease of the signal intensity at 2950 cm^{-1} was also observed, indicating the presence of an organic layer onto COC. Two new signals were also observed at 1220 and 1080 cm^{-1} which proved that no acetal deprotection occurred, despite the acidic conditions employed for the grafting process. Further investigation would then be required to find the optimal way to release the carbonyl group at position 3 of isatin. Taking into account the multi-steps pathway involved in the general strategy, which complicates the extension to more elaborate isatin derivatives, we decided to follow a more versatile approach consisting in grafting the heterocycle *via* a heterogeneous-phase Sonogashira reaction onto pre-modified COC surfaces.

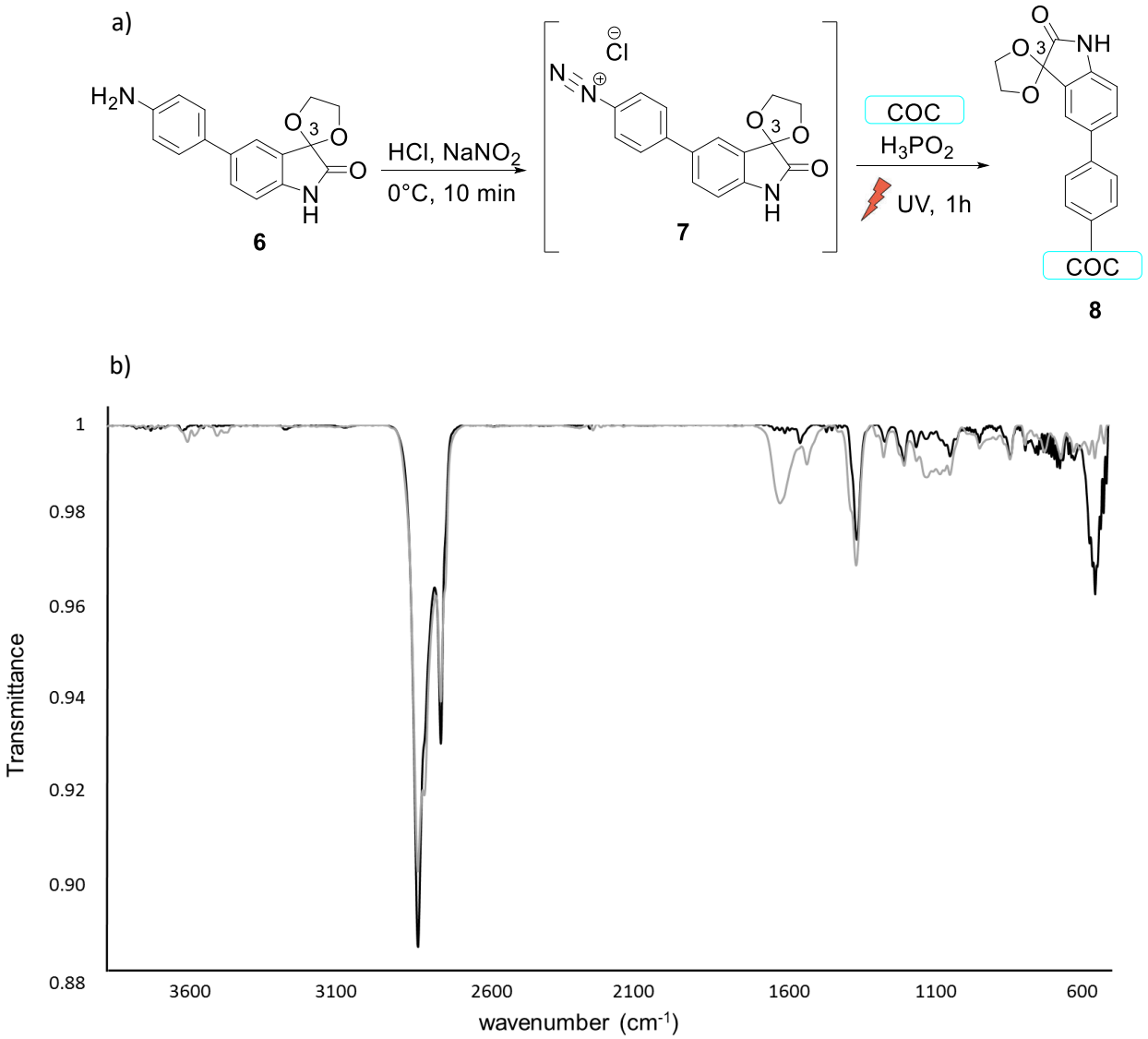
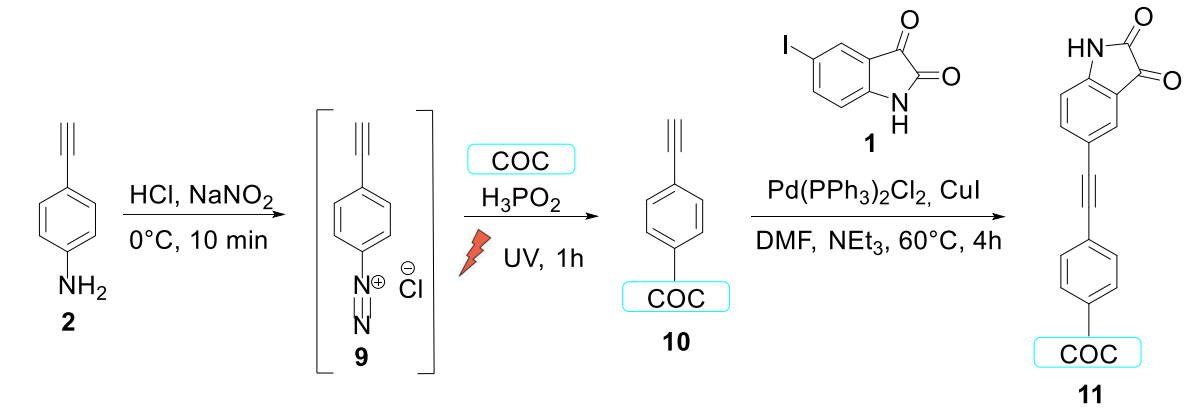


Fig. 4. a) Chemical drawing of the COC grafting process and b) the associated infrared characterizations of raw COC (black curve) and grafted COC **8** (gray curve).

3.3.2 Functionalization with surface pre-modification

Grafting of isatin using direct cross-coupling reaction of commercially available 5-iodoisatin **1** onto an activated COC surface represents the easiest and most versatile method to obtain isatin decorated surfaces (Fig. 5a). COC functionalization consisted of adding the plate to an acidic solution containing aryldiazonium **9**, activating COC by UV illumination and generating aryl radical from **9** under chemical reduction in presence of H_3PO_2. With the pre-modified surface **10** in hand, isatin was introduced by means of a heterogeneous Sonogashira cross-coupling reaction (Fig. 5a). The only two steps of this methodology would allow an extention to a wide range of heterocyclic derivatives of biological interest.

a)



b)

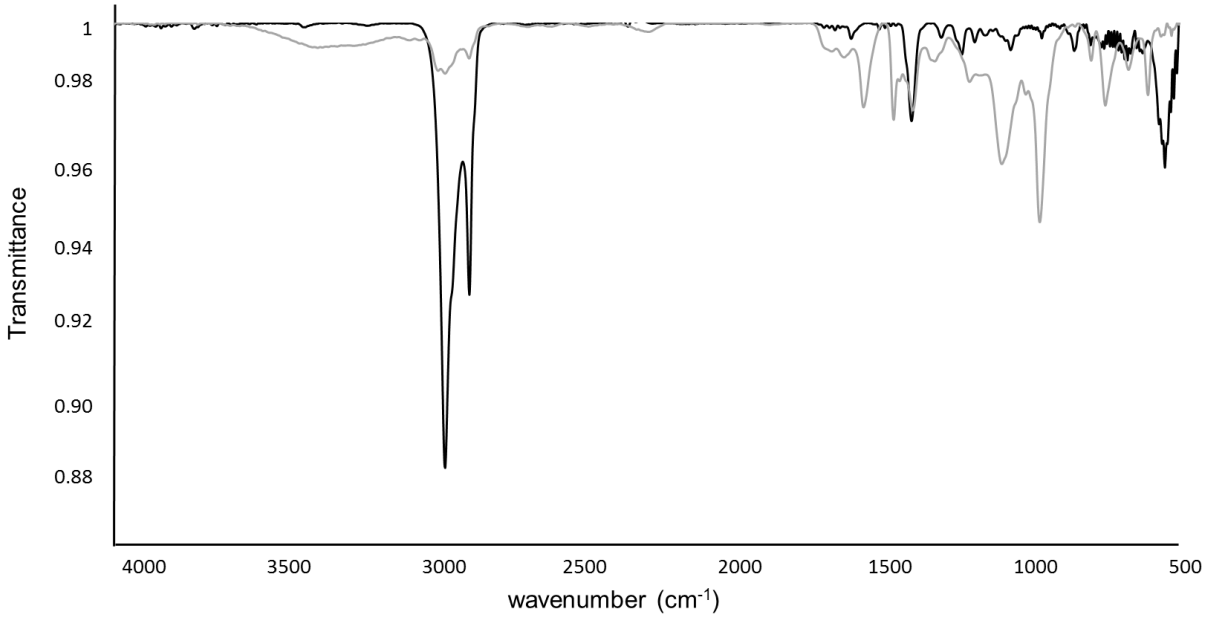


Fig. 5. a) Grafting strategy to prepare COC **11** and b) IR spectra of functionalized COC **11** by heterogeneous-phase Sonogashira coupling reaction (gray curve) compared to raw COC (black curve).

The IR spectrum (Fig. 5b) of modified surface **11** showed three bands representative of isatin, *i.e.* at 3500 cm^{-1} is the peak for amide NH stretching, bands at 1712 and 1674 cm^{-1} are typical of amide C=O stretching. Peaks at 1610 and 1454 cm^{-1} corresponded to aromatic C=C bending. Finally, the presence of the alkyne was proved by a slight signal at 2300 cm^{-1}, and the band at 1271 cm^{-1} related to amide C-N bond stretching corroborated the grafting of compound **1** onto COC **10**.

A XPS analysis of COC **11** was then performed and compared to that related to the COC substrate. The sample produced by heterogeneous-phase Sonogashira coupling reaction showed increased signal at the high energy end of the C1s spectrum, increased O1s signal, and the appearance of a N1s signal (Table 1); this is as expected for COC **11** and thus in agreement with the IR spectrum in Fig. 5b).

Oxygen was revealed as 12% of the detected atoms and nitrogen as 5% indicating two oxygen atom for each nitrogen atom as is the case for isatin (Table1). Traces of copper (0.5%) and iodine (0.8%) were also detected, derived from the copper iodide used as co-catalyst for the Sonogashira coupling

Table 1. Atomic composition obtained with a spread of 5 % from XPS analyses for raw COC and COC electrode **11**.

% atomic composition	Raw COC	COC **11**
C_{1S} at 285 eV	83	63
C_{1S} at 286 eV	5	13
C_{1S} at 291.1 eV	4	4
C_{1S} at 287.8 eV	Not found	2
N_{1s}	Close to 0	5
O_{1s}	8	12

First studies were conducted to consider the ability of isatin-COC surface **11** to act as a biosensor. Evaluation of the effect of the modified surface on bacterial adhesion was conducted on the strain PA14 of *Pseudomonas aeruginosa*, one of the most virulent clonal group in the world, by enumeration of the adherent cells. The assays, performed in quadruplicate for the isatin-COC surface, evidenced values of 44 ± 37 $x10^3$ UFC/mL for the modified surface against 19 $x10^3$ UFC/mL measured for COC surface only showing

the affinity of the isatin-surface for PA14. Although preliminary, the study is worthy to be deepened as it evidenced potential of isatin-COC in biosensing.

3.3.3 Extension of the methodology to gold surface

In order to extend this heterogeneous-phase Sonogashira cross-coupling reaction to metallic surfaces, we decided to graft isatin on a gold surface (Fig. 6a). Following analogous strategy described for COC, modified gold surface **13** was obtained by reductive electrografting of aryldiazonium **12** which was demonstrated by disappearance of the reduction peak during the second voltammetric cycle (Fig. 6b). Isatin-gold surface **14** was achieved by the metallo-catalyzed cross-coupling reaction of **13** with 5-iodoisatin **1**.

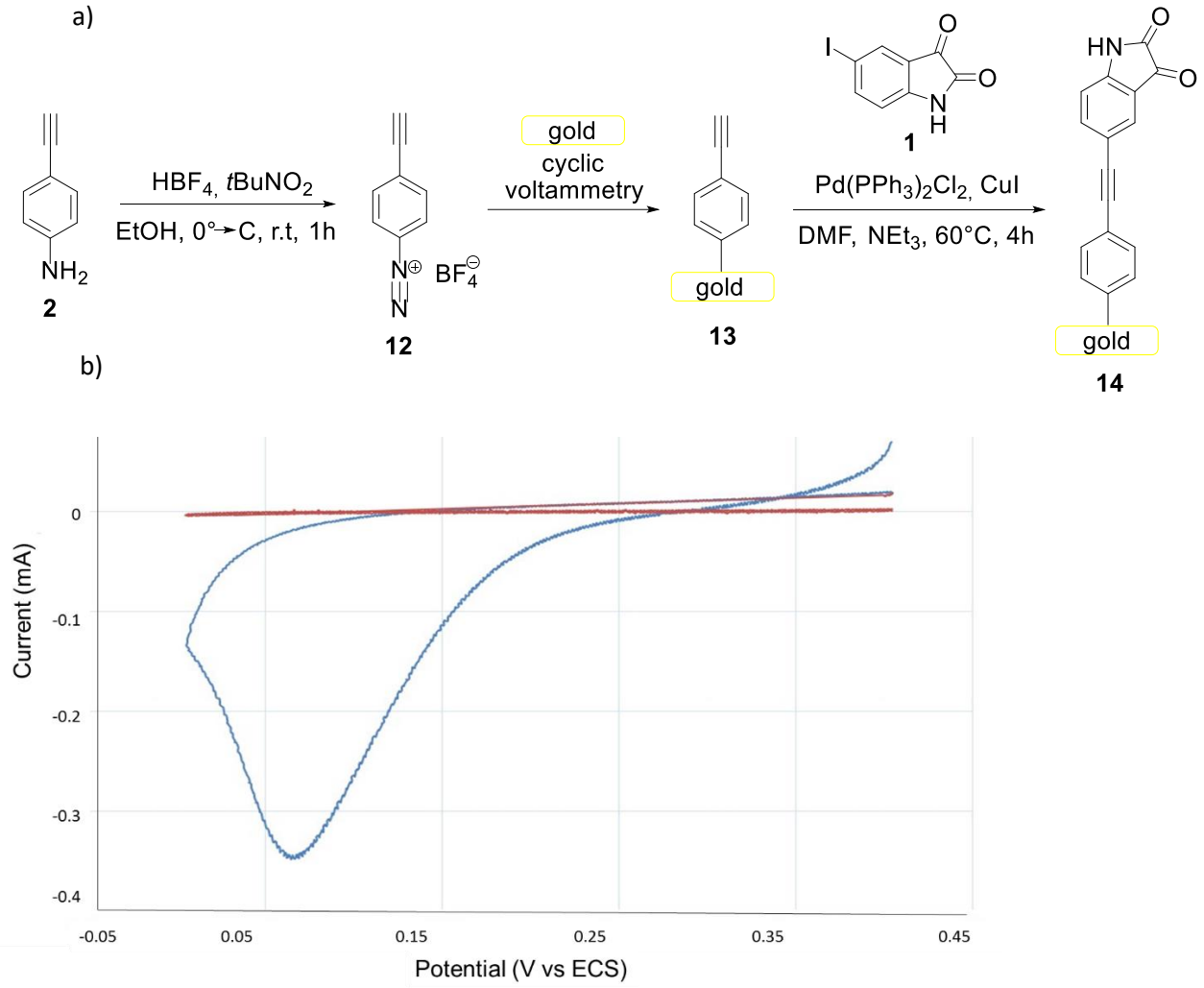
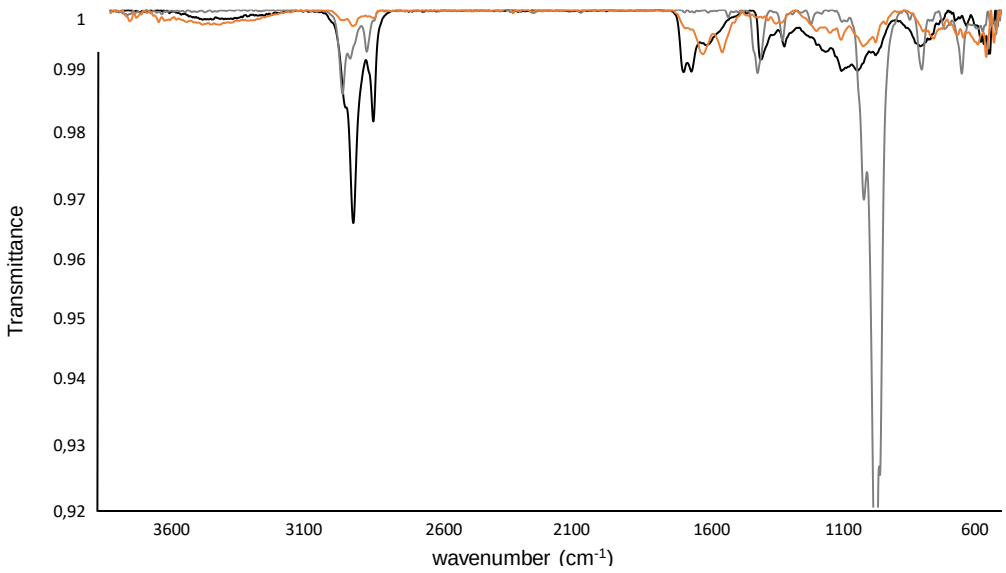


Fig. 6. a) Grafting of compound **1** on activated gold surface **13** and b) the electroreduction by cyclic voltammetry (100 $mV \cdot s^{-1}$) of diazonium **12**. Blue and red curves correspond to first and second cycle respectively.

IR spectra (Fig. 7a) were recorded for pre-activated gold surface (black line) activated surface with alkyne aryl **13** (gray line) and isatin decorated surface **14** (orange line). As the surface was covered by alkenylaryl, peak at 2950 cm^{-1} strongly decreased due to surface covering with organic layers. Reduction on peak intensity was even more important after isatin coupling. Specific signals of isatin were observed, *i.e.* a band at 3400 cm^{-1} related to amide NH stretching, and peaks at 1710 and 1675 cm^{-1} corresponding to amide C=O stretching. Aromatic C=C bond bending were also detected at 1600 and 1400 cm^{-1} whereas the presence of the pyrrole group was proven by the band associated to C-N bond stretching at 1260 cm^{-1}.The surface plasmon resonance (SPR) technique is a suitable method to evaluate biomolecular interaction and gold surface modification [35]. SPR is an optical technique, which is sensitive to any change at the liquid-solid interface. To prevent any damage of the instrument, surfaces were prepared before incubation into SPR device. Fig. 7b shows the SPR responses observed when gold surface was modified by ethynylbenzene diazonium grafting and Sonogashira coupling reaction of iodoisatin. The modification on SPR angle provided direct evidence of the successful modification of the gold surface [36]. From the angle modification, it was possible to estimate the surface coverage. Thus, SPR angle changed from 59.76° to 58.95° after diazonium grafting which corresponded to 675 ng/cm² leading us to think that the grafted layer is thin and probably multilayer. After Sonogashira coupling, the SPR angle was increased from 58.95° to 59.37° confirming the attachment of isatin on gold surface **13**. No further modification of SPR angle was observed when the modified surface was cleaned with water or phosphate buffer indicating that isatin is covalently attached to gold surface [37].

a)



b)

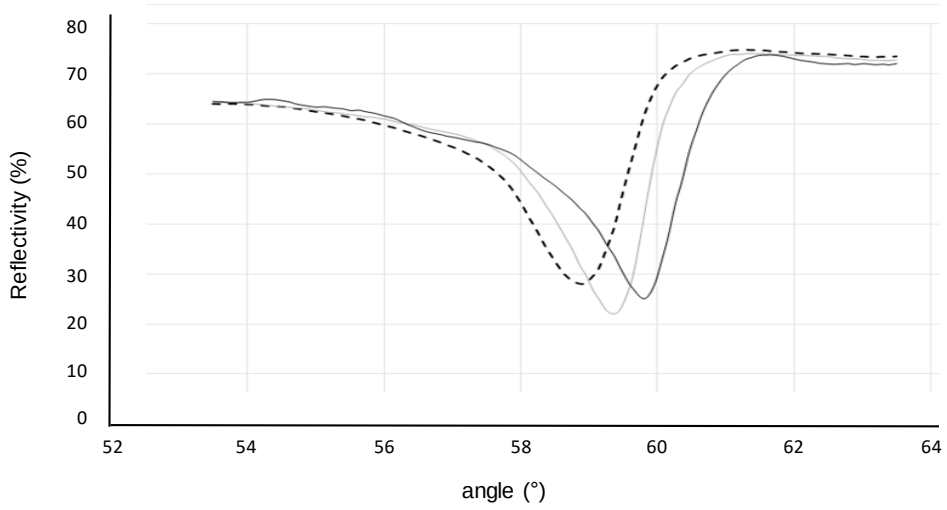


Fig. 7. a) IR spectra of gold surface (black curve), alkyne aryl modified surface **13** (gray curve) and isatin grafted gold surface **14** (orange curve) afforded by heterogeneous-phase Sonogashira cross- coupling reaction. b) Reflectivity analyses of the gold (black curve), the alkyne arylgold **13** (dashed curve) and the isatin grafted gold **14** (gray curve) electrodes.

4. Conclusion

In this work, we investigated different strategies for designing sensors with isatin moiety at their interface. The most promising strategy to introduce isatin onto surfaces involved a two-step pathway comprising (i) pre-functionalization of gold or polymeric surfaces by reductive electro- or chemical grafting of aryldiazonium salts bearing an alkyl function, followed by (ii) an original heterogeneous Sonogashira cross-coupling reaction with iodo-isatin. We assessed each modified surface by different techniques such as IR and XPS spectroscopy for instance and we tested the adhesion of PA14 bacteria on our isatin polymeric sensor. The detected affinity of the isatin-surface for PA14 is in accordance with the *in silico* study performed with the heterocycle and some of its derivatives. This point is still under investigation to clarify the way of adhesion of bacteria to isatin sensor and to identify the role of isatin in the biofilm formation and the quorum sensing of *Pseudomonas aeruginosa*. The methodology imparted high robustness to the modified surfaces as isatin covalently bound to arylalkyne, itself being covalently attached to surfaces. Thanks to the versatility of metal-catalyzed cross-coupling reactions, a wide number of biological relevant scaffolds may be easily introduced.

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Conflicts of interests

There are no conflicts of interest to declare.

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