DUAL-CONTROL MOLECULAR SWITCHES FOR BIOMEDICAL APPLICATIONS

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INTRODUCTION

Conducting polymers have attracted considerable research attention because of promising applications in biosensors, biomaterials, electronics, energy storage and conversion devices. Photo-responsive materials have great potential in biomedicine, particularly in the area of tissue regeneration and repair [1,2], as are conducting polymers, because they can provide a physical platform for the growth of several lines of living cells whose properties can be tuned by external stimulation and control [3]. The work explores the behaviour of hybrid conducting polymer/photo responsive materials, with particular emphasis for use in biomedical applications.
Terthiophene-Spiropyran polymers (**TTT-BSP, 1** and **2**) synthesized and characterised in this work are examples of such hybrid materials, in that they can be switched between two or more states (each with their own distinct characteristics) using an external stimulus (photonic or electrochemical). These materials show particular propensity to functionalize surfaces, in our case ITO (glass and PET) and QCM crystals via electrochemical deposition. Photochemical switching shows its strongest effect when the monomers of these materials are dissolved in polar solvents: the hypsochromic shift of **BSPNO$_2$acetoTTh (2)** in fig.**1(a)-(e)**. Furthermore, irradiation of the electro-grown polymer with a 254nm light source for 15 minutes also showed evidence of photoswitchable behavior. Post-synthesis electrochemical stimulation produced dramatic morphological and surface behaviour changes in the material, as evidenced by contact angle measurements and visualization with **AFM**.
This study ultimately seeks to take advantage of these induced morphology changes of the polymer and to clarify the interactions between biomolecules like fibronectin and the **TTT-BSP** based materials, with the support of elegant, modern and appealing methodologies like protein-functionalized **AFM** tips and **QCM**.

PHOTOCHROMIC PROPERTIES OF THE TTT – BSP MONOMERS

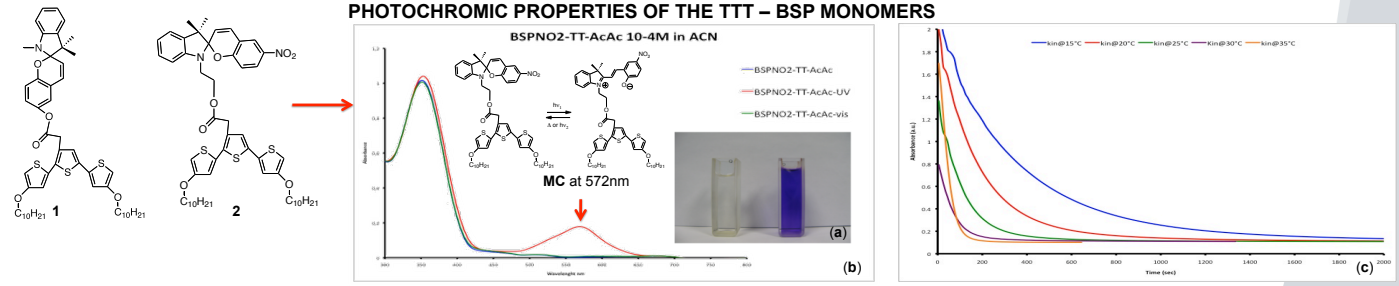

Figure 1: (a) Structure of the monomers **BSPOHacetoTTh (1)**, **BSPNO$_2$acetoTTh (2)** and their photochromic effect in Acetonitrile. **(b)** UV-*vis* of **BSPNO$_2$acetoTTh** with the wavelength of Merocyanine formation highlighted (**MC** at 572nm) in Acetonitrile; **(c)** Thermal Relaxation of **2** from 15°C to 35°C in Acetonitrile.

AFM ANALYSIS OF PROTEIN ADHESION: FIBRONECTIN VS BSPNO$_2$acetoTTh [4]

Fibronectin (fig. 2a) is a high molecular weight (440kDa) extracellular matrix glycoprotein produced by the liver, important in humans for the following reasons:
1. It binds integrins, membrane-spanning receptor proteins.
2. It binds collagen, fibrin, proteoglycans based on heparane sulfate and heparin.
3. Fundamental for effective cell adhesion.
4. Important for cell growth.
5. Essential for cell migration.
6. Role in cell differentiation.
7. Important in wound healing.
8. Important in embryonic growth.

Fig. 2(b): Nanoworld PNP-DB tips (k≈ 0.5 N/m) with gold reflective coating were functionalized with **fibronectin (FN)**. The tips were initially cleaned in plasma cleaner for 5 minutes then placed into a 1% 3-EDSPA in toluene solution for 2 hours. The tips were rinsed with toluene, then the PBS and excess fluid was drained off. The tips were then placed into a 25% GAH solution in PBS for 1 hour, then rinsed with PBS and the excess fluid drained off. The tips were then placed into a 10 mg/mL FN in PBS solution for 1 hour, then rinsed with PBS and stored in PBS in the fridge until required. Each tip was pre-calibrated before use in the experiments.
The spring constant of the cantilevers was calculated using the Sader method, which relies on the resonant frequency, quality factor and geometrical parameters of the cantilever. The sensitivity of each cantilever was measured *in situ* in PBS on a glass substrate. The protein adhesion measurements were carried out using force-distance curves of the functionalized tips onto the polymer surface in 10 mmol PBS solution. The force-distance curves were conducted over an approach range of 500 nm, at a rate of 0.5 Hz with a dwell time on the surface of 1 sec. Measurements were performed on an Asylum Research MFP-3D Atomic Force Microscope (AR Research, OR).

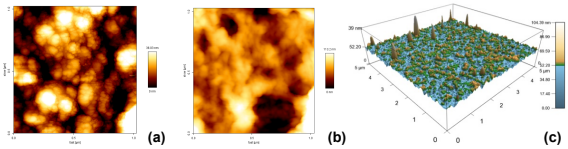

Fig. 3: Surface study of the polymer. **(a) p-BSPNO$_2$acetTTh** at reduced state; **(b) p-BSPNO$_2$acetTTh** at oxidized state; **(c)** Topography of **p-BSPNO$_2$acetTTh**.

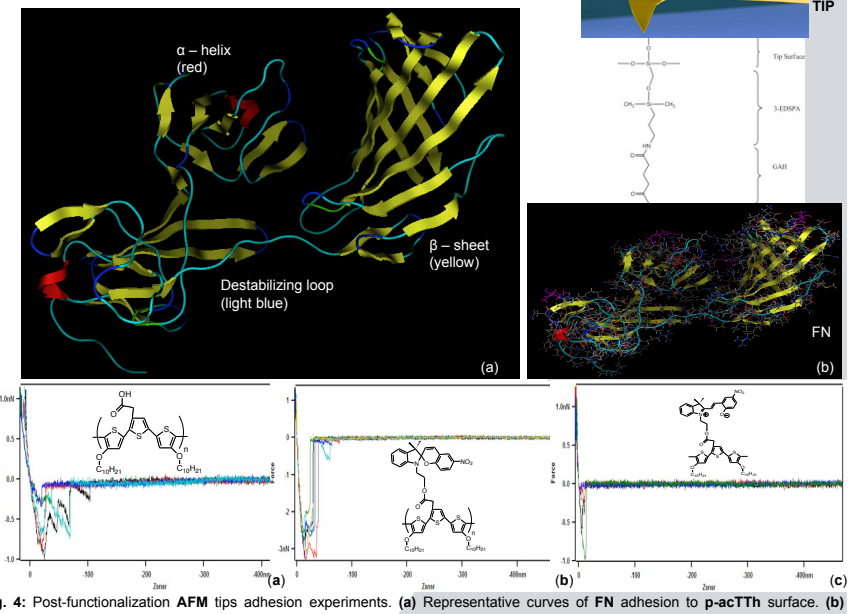

Fig. 4: Post-functionalization **AFM** tips adhesion experiments. **(a)** Representative curves of **FN** adhesion to **p-acTTh** surface. **(b)** Representative curves of **FN** adhesion to **p-BSPNO$_2$acTTh** surface. **(c)** Representative curves of **FN** adhesion to **p-MCacTTh** surface. Although **p-acTTh** exhibited ability to interact with **FN**, the adhesion of the protein was higher for **p-MCacTTh** and **p-BSPNO$_2$acTTh**.

QCM ANALYSIS OF PROTEIN ADSORPTION: FIBRONECTIN VS BSPNO$_2$acetoTTh [4]

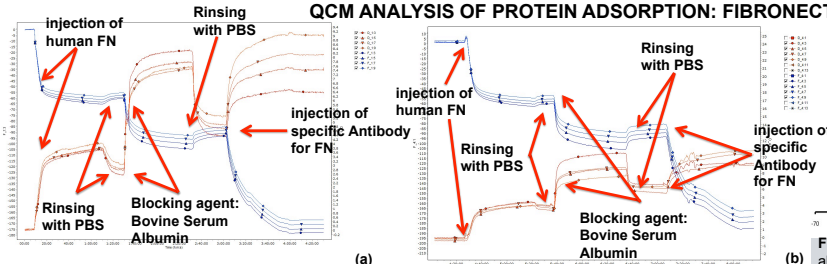

Fig.5: (a) QCM test on **p-BSPOHacTTh** and **(b) QCM** test on **p-BSPOHacTTh**. These two experiment have been performed with this procedure: **(a)** was allowed to interact with three different solutions of proteins flushed on the surface and added in this sequence: **FN** (concentration was 50µg/ml), **Bovine Serum Albumin** (concentration was 50µl/ml), **FN** specific **Antibody** (concentration was 1/150 active units), without any electrochemical stimulation for 4.20hrs. **(b)** was kept at -400mV for 8.20hrs (first 4hrs were required for standard equilibration of the system) and then allowed to interact with the three solutions of biomolecules with the same previous scheme flushed on the surface. The results showed no remarkable differences from the frequencies point of view. For what concerned the dissipation is appreciable a different progression shape when the polymer is stimulated at negative potentials. This could be due to a different morphology of the polymer at -400mV. Further study will investigate this aspect.

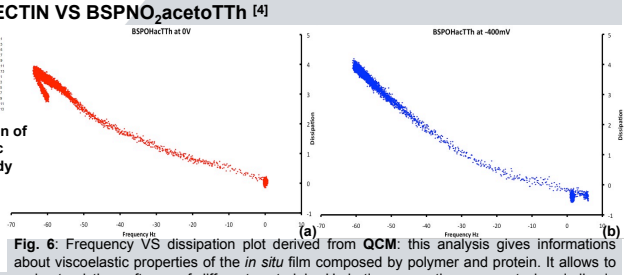

Fig. 6: Frequency VS dissipation plot derived from **QCM**: this analysis gives informations about viscoelastic properties of the *in situ* film composed by polymer and protein. It allows to understand the softness of different materials. Hydration properties appear to be similar in these conditions. **(a) p-BSPOHacTTh** at 0V; **(b) p-BSPOHacTTh** at -400mV.

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CONCLUSIONS

The target of this work was the analysis of the surface interactions between two different adaptive materials and an important biological agent like Fibronectin. The experiments thought to observe the intensity and the presence this relationship were **QCM** and **AFM**. Atomic Force Microscopy tips functionalized with **human FN** proved the presence of adhesion forces between **FN** and the hybrid conducting polymer in exam. The results were reproducible and showed higher interactions with the **BSP** isomer rather that its **MC**. **QCM** powerful technology allows to follow the adsorption kinetics of proteins to the biomaterials interface enabling the characterization of physical properties of the film, like hydration and conformation. From the preliminary data here presented we can say that we proved the presence of interactions between **BSP-TTH** hybrid conducting polymers and biological macromolecules essential for living tissues. Many aspects need to be clarified like the behaviour of the polymer at oxidised state, but more experiments have already been set up.

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