Paper-based Electrochemiluminescent Screening for Genotoxic Activity in the Environment

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Supporting Information

Patterning the paper device. Paper analytical devices (PADs) featuring hydrophilic channels with hydrophobic boundaries (wax) were made by heat pressing commercially available wax paper (Reynolds Cut-Rite) on a Whatman 1 filter paper. Briefly, the wax paper was first folded and required patterns were made by cutting with a puncher and sharp blade. The cut patterns on wax paper were marked with permanent marker pen which later enabled visualization of the hydrophilic patterns over a white background. The filter paper was first placed between wax paper template and heat pressed using a small thermal press for 60s at 350°C resulting in paper channels. Due to heat press, the wax transferred from both sides onto the paper except from the patterns. This relatively simple and inexpensive method can be done on demand due to presence of raw materials available commercially such as paper, wax paper and flat iron/ hair straighter.

Printing electrodes and assembly of paper electrochemical devices (PEDs). Paper electrochemical device consisted of two patterned filter paper which was fixed using a double sided tape. The first paper contained three analytical hydrophilic spots of 5 mm diameter separated by a distance of 1 cm from the center of the spots. The three hydrophilic spots were screen printed with a 5 mm x 40 mm rectangular carbon ink electrode. The screen printing was performed using rolling ink on a cut patterned projector paper by an applier. This was followed by 7-10 min of 60 °C baking for curing electrode. When electrode was flipped, the top hydrophilic paper analytical spots were used to make assembly of thin films containing RuPVP, DNA and RLM using layer by layer electrostatic interactions. Briefly, paper analytical spots (5 mm diameter) were incubated with 5 µL of each solutions containing positively charged RuPVP (2.0 mg/mL in 50% ethanol) for 10 min, calf thymus DNA (2.0 mg/mL in 10 mM Tris Buffer pH 7.0) for 25 min and repeated to form $(RuPVP/DNA)_2$ bilayers with D.I. water rinsing between steps. Followed by incubation of rat liver microsomes (RLM) (10 mg/mL in Tris buffer) for 30 min to form $(RuPVP/DNA)_2$/RLM. For background, PDDA was incubated instead of RLM as the final layer to form $(RuPVP/DNA)_2$/PDDA. All these steps were performed in humidifying chamber (moistened towel at the bottom of a closed plastic container) in order to avoid drying of solutions.

A second filter paper was patterned with 35 mm x 17 mm hydrophilic channel. Inside the channel, 50 mm x 2 mm printed reference (Ag/AgCl) and counter electrode (carbon ink) ran parallel to each other with the distance between electrodes to be 13 mm. After this step, holes of 4 mm diameter were punched corresponding to the analytical spots for ECL view. The second paper with holed hydrophilic channel containing reference and counter electrode was aligned and placed over the first paper electrode containing spots. The two patterned screen printed papers electrode was fixed using a cut patterned (35 mm x 20 mm) double sided tape. A 3D paper device with bottom paper for working electrode (spots containing films) and top paper containing reference and counter electrodes with channels enabled applying potential for

electrolysis and oxidation of metallopolymers for ECL. The paper channel enabled flow of buffers and test samples required for ECL measurement inside dark box.

Conversion of ECL intensity from grayscale to color. The raw ECL image from µPEDs were obtained from the Gene Snap and converted to colored scale using Adobe Photoshop. First the contrast levels were adjusted using option "Auto levels". The obtained image was then converted to color image by modifying the mode to RGB from grayscale. The gradient of the image was changed using gradient scale option to ECL scale as shown in Figure 2, 3. The image was reconstructed by cutting the layers and assembling all the cut images in one single figure (Either all time intervals together or different real samples).

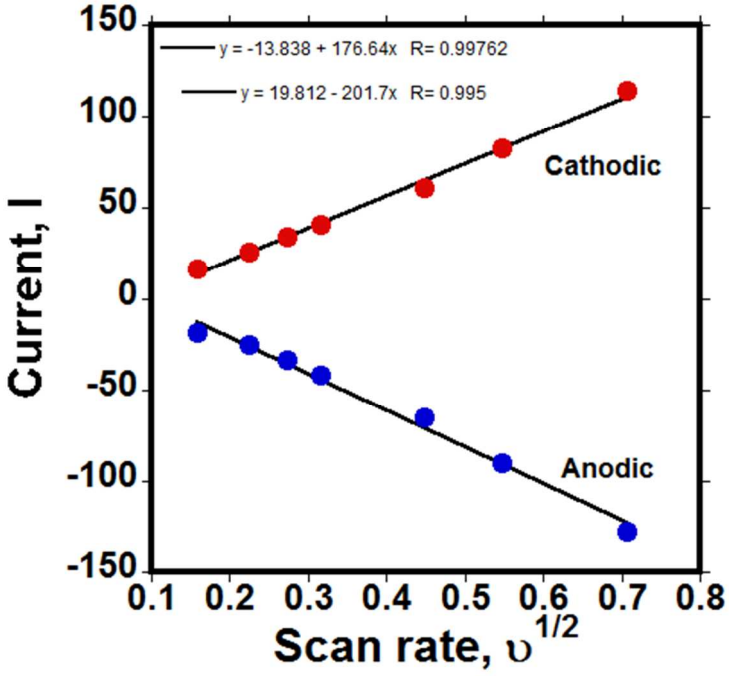

Figure S1. Plot of I_p vs square root of scan rate obtained from voltammograms in Figure 1A.

Quartz Crystal Microbalance (QCM). Adsorption of film assembly at each step was monitored using quartz crystal microbalance (QCM, USI Japan) using a 9 MHz QCM gold resonators (AT-cut, International Crystal Mfg., Oklahoma City, OK) and summarized in **Table S1**. Briefly, gold resonators were first incubated with 5 mM 3-mercaptopropanoic acid (MPA) in ethanol for a period of 18 h to form negatively charged gold surface to mimic the surface of paper. This was followed by alternate electrostatic layer by layer adsorption of RuPVP , DNA and RLM on MPA modified gold resonators with the same conditions used on µPEDs. Adsorbed mass per unit area (M/A) of dried films on the resonators was determined by Frequency change (ΔF) using Sauerbrey equation.

$$M/A\ (g/cm^2) = -\Delta F(Hz)/1.83*10^8 \quad (1)$$

And Nominal thickness (d, nm) was determined using

$$d\ (nm) = (-0.016 \pm 0.002)\ \Delta F(Hz) \quad (2)$$

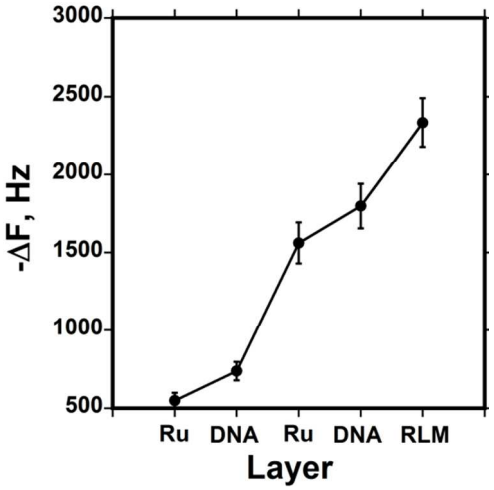

Figure S2. Quartz crystal microbalance frequency response of adsorption of layers RuPVP, DNA, and RLM used in construction of films on PEDs.

RuPVP, μg/cm^2	DNA, μg/cm^2	RLM, μg/cm^2	Total nominal thickness, (d, nm)
7.50 ± 0.7	2.31 ± 0.35	2.91 ± 0.33	37

Table S1. Summarized adsorbed total mass of each component and total nominal thickness of $(Ru/DNA)_2RLM$ films determined using equation 1 and 2.