ADVANCED MATERIALS INTERFACES

Supporting Information

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Fast Atmospheric Plasma Deposition of Bio-Inspired Catechol/Quinone-Rich Nanolayers to Immobilize NDM-1 Enzymes for Water Treatment

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Synthesis of N-(3,4-Dihydroxyphenethyl)acrylamide (DOA): A two-neck round-bottom flask was charged with 12.1 g (31.6 mmol) of $Na_2B_4O_7.10H_2O$ and 5.0 g of Na_2CO_3, and 475 mL of milli-Q water (18.2 MΩ.cm, Millipore). This basic aqueous solution was degassed in sonicator bath (Branson 2510, 100 W, 42 KHz) for 1 h, applying light vacuum followed by, bubbling with argon for another 2 h. 3 g (15.8 mmol) of Dopamine hydrochloride (Sigma-Aldrich) was added under argon atmosphere and continued stirring for 30 minutes. The flask was then cooled at 0°C before drop-wise addition of 5.1 mL (63.2 mmol) of acryloyl chloride with stirring. Another 9.0 g Na_2CO_3 was added to maintain the pH of the solution above 9 during the reaction. After stirring for 12 h at room temperature, the solution was acidified to pH 1–2 with 6N HCl and continued stirring for 1 h. The mixture was extracted five times with ethyl acetate, washed with 0.1 M HCl and dried over $MgSO_4$. The solvent was removed in vacuum to yield crude greyish paste, which was further purified by flash silica gel column chromatography eluting with dichloromethane/methanol (9:1) mixture (80% yield).

Atmospheric-pressure plasma-assisted polymerization: A solution of DOA in vinyltrimethoxysilane (VTMOS) (0.5 mg mL^{-1}) was sprayed by a 48 kHz ultrasonic atomising

nozzle (Sono-Tek Corporation). The created mist was composed of droplets of median diameter of 40 microns and the range of size was 5 to 200 microns. 0.5 mL min^{-1} of solution was injected in the nozzle by using a syringe driver. The Sonotek generator was set up to 2W to generate the mist, while at the output of the nozzle, a nitrogen flow was used in order to shape the mist, and entrain it on the substrate. Plasma polymerization in argon was then performed with a dielectric barrier discharge reactor composed of two flat alumina covered electrodes connected to high voltage and ensuring an efficient plasma surface zone of 18.72 cm^2. The samples were placed on the moving table (*i.e.* grounded electrode) ensuring a dynamic deposition mode. The table speed and the gap between the electrodes were fixed at 100 mm s^{-1} and 1 mm, respectively. The plasma discharge was ignited with a sinusoidal signal at 10 kHz chopped by a 1667 Hz rectangular signal. The power density was set up to 1.6 W cm^{-2}.

Silver nanoparticles formation protocol: Inspired by the protocol of Faure *et al.* (*Adv. Funct. Mater.* **2012**, 22, 5271-5282) plasma coated samples were immersed in the dark in an $AgNO_3$ aqueous solution (1g L^{-1}) under stirring (300 rpm) at ambient temperature during 24h and then subsequently rinsed 5 times during 5 minutes each in distilled H_2O under stirring (500 rpm) and then dried under nitrogen flux.

NDM-1 production: The poPINF plasmid containing the synthetic gene encoding *K. pneumoniae* was provided by R.Owens from the Oxford Protein Production Facility UK. The protein production and purification were performed according to the Green *et al.* protocol (2011) with an additional freezing step carried out before cell lysis with french press. The NDM-1 (28 kDa) isolation and purification were solely performed with a HisTrap FF column (GE Healthcare). Excess of imidazole was removed via three washes in Amicon ® Ultra-4

Centrifugal Filter Units 3,000 NMWL (1000 x g for 15 min). Enzymes were then resuspended in a phosphate buffered saline (PBS) solution.

Enzymes immobilization: The enzyme immobilisation was performed in PBS solution at pH 7.4 at a final concentration of 1 mg mL^{-1} during 1h at room temperature under gentle agitation (100 rpm). Samples were then washed 5 times during 5 minutes with PBS under 500 rpm stirring to remove unbound enzymes. Enzyme concentrations were doubly measured by 2D Quant Kit (GE Healthcare) and RC DC protein assay (Bio rad). The measured immobilized NDM-1 concentration on the steel discs was equal to 1.1 ± 0.2µg cm^{-2}.

Antibiotics degradation assays: Enzymatic activity was estimated by the amoxicillin degradation monitoring over time. Samples with immobilized enzymes were incubated in a degradation medium at ambient temperature (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 12.5 mM, Bovine Serum Albumin (BSA) 10 µg mL^{-1}, amoxicillin 100 µg mL^{-1} in filtered tap water (0.22 µm) with final volume of 1 mL). Each 24h, the degraded amoxicillin concentration is estimated by measuring absorbance at 210 nm with 2 Synergy microplate reader (Biotek). The medium was removed every 24 hours, wells were washed 3 times with filtered tap water and a new volume of the degradation medium was added in wells. The procedure was slightly different for the free enzymes in solution. In order to compare the activities of the free form and the immobilized enzymes, the same amounts of enzymes are introduced in the 1 mL degradation medium. The measured immobilized amount of NDM-1 on the surface was found to be about 1.1 µg cm^{-2}, *i.e.* about 3 µg of enzymes are present on the surface for the 3 cm^2 samples. Hence, the free form NDM-1 concentration was set to 3 µg mL^{-1} to maintain the same amount of enzymes in the degradation assay. In the wells used for the first measurement (t= 24h), enzymes were directly dissolved in a volume of

1 mL of the degradation medium containing 100 µg mL^{-1} of amoxicillin. For all other wells, at time t_0, enzymes were dissolved in a volume of 300 µL containing 20 µg mL^{-1} of amoxicillin in order that the enzymes are in activity. All wells were fed by this same volume every 24 hours. When wells were used to measure, 24 hours before they are amended with 100 µg mL^{-1} of antibiotic and supplemented to achieve a final volume of 1 mL.

Flow test - Resistance to shear stress: Immobilized enzymes onto plasma functionalized samples were fixed to the rotor of an annular reactor (Biofilm Reactor Annular LJ 1320 reactor, Biosurface Technologies Corporation). The samples were then subjected to laminar water flows equivalent to a 30 $km.h^{-1}$ flow rate. After 144 hours the enzymatic activity was measured and compared to the disks used for degradation assays.

Figure S1 shows that the obtained layer is covering the entire surface of the substrate.

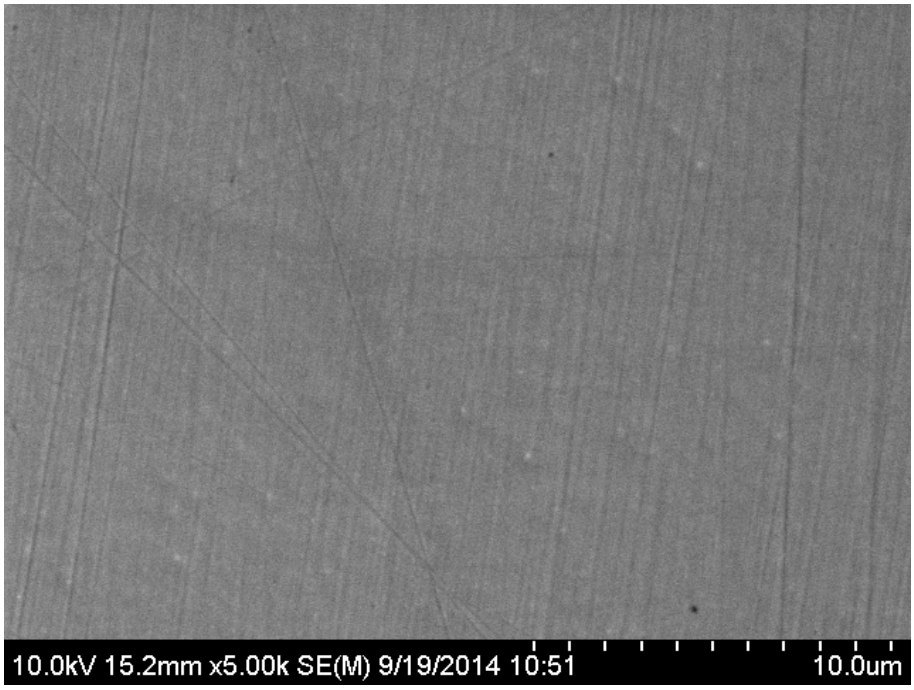

Figure S1. Scanning Electron Microscope micrograph of a DOA-VTMOS plasma deposited coating on stainless-steel substrates.

Figure S2 shows the presence of two components tentatively assigned to amide (400.1 eV) and charged ammonium groups (401.8 eV). Prior to the XPS core level deconvolution, the N1s peak position was calibrated using the C1s C-C component position at 285 eV.

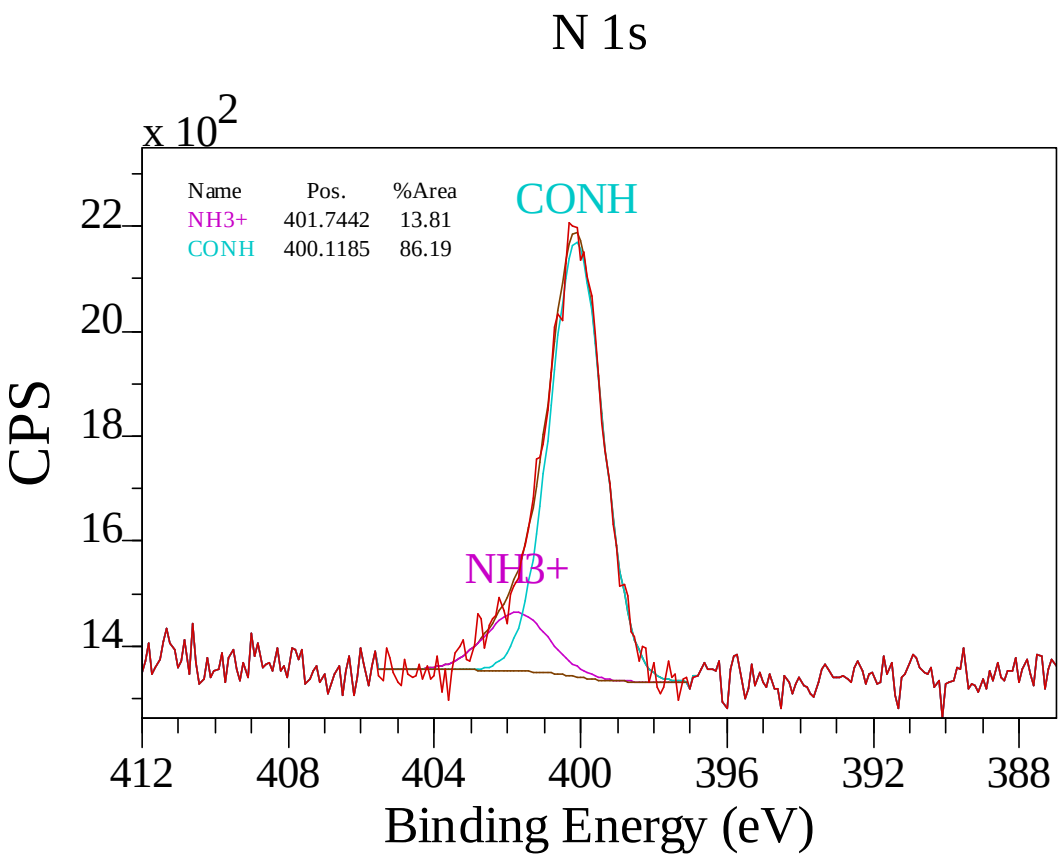

Figure S2. XPS N1s core-level deconvolution of a DOA-VTMOS layer.

Figure S3 shows the reproducibility of the system. Enzymatic degradation assays were performed on 3 independent series of 4 samples each, grafted with 3 different batches of produced NDM-1.

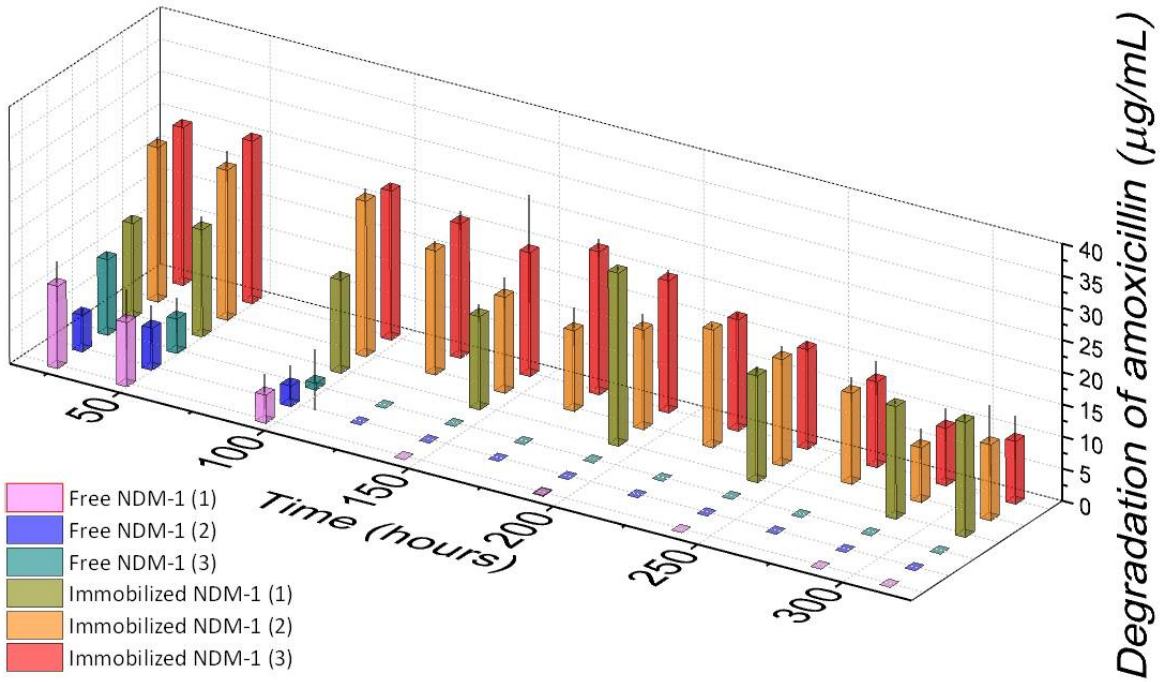

Figure S3. Amoxicillin degradation assays by free and immobilized NDM-1 on plasma coated stainless steel samples.