# Supplementary Information

**Title** Highly sensitive electrochemiluminescent detection of prostate cancer biomarker

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## Reagents

Multiwalled carbon nanotubes were purchased from Nanoamor. Nitric acid (>69 %), sulfuric acid (95 – 97 %), sodium hydroxide (≥98 %), 4-[(N-Boc)aminomethyl]aniline (≥97 %), hydrochloric acid (≥37 %), N,N-diisopropylethylamine (99.5 %), 1-hydroxybenzotriazole hydrate (HOBt, ≥97 %), sodium phosphate dibasic, sodium phosphate dibasic and all solvents (DMF, THF, EtOH, $Et_2O$, MeOH, $CH_2Cl_2$) from Sigma Aldrich. Isopentyl nitrate (97 %, stab. with 0.2 % anhyd. sodium carbonate) from Alfa Aesar. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), 4-(dimethylamino)pyridine (DMAP) and trifluoroacetic acid (TFA) from Fluorochem. N-Hydroxysuccinimide (NHS, ≥97%) and Kaiser Test kit from Fluka. Bis(2,2'-bipyridine)-[4-(4'-methyl-2,2'-bipyridin-4-yl)-aminobutyl] ruthenium(II) complex from Cyanagen. N-succinimidyl 3-maleimidoproprionate and N-Boc-2,2'-(ethylenedioxyl)diethylamine were synthesized as previously reported.[1]

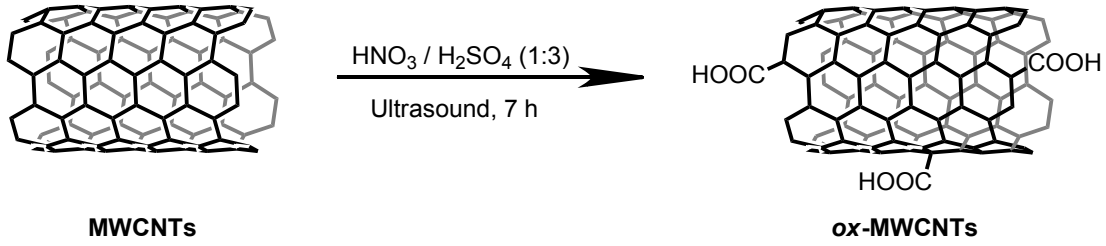
[1] G. Pastorin, W. Wu, S. Wieckowski, J.-P. Briand, K. Kostarelos, M. Prato, A. Bianco *Chem. Commun.*, **2006**, *35*, 1182.

## Instrumentation

TGA profiles were recorded on a TGA Q500 (TA instruments), under $N_2$, by equilibrating at 100 °C for 20 min, and following a ramp at 10 °C/min up to 800 °C (approximately 1 mg of each compound). TEM measurements were performed on a TEM Philips EM208, using an accelerating voltage of 100kV. Samples were prepared by drop casting from dispersion onto a TEM grid (300 mesh, nickel, carbon only). SEM measurements were performed on a SEM of type JEOL JSM-6490LV.

## Preparation of f-MWCNT@mAb

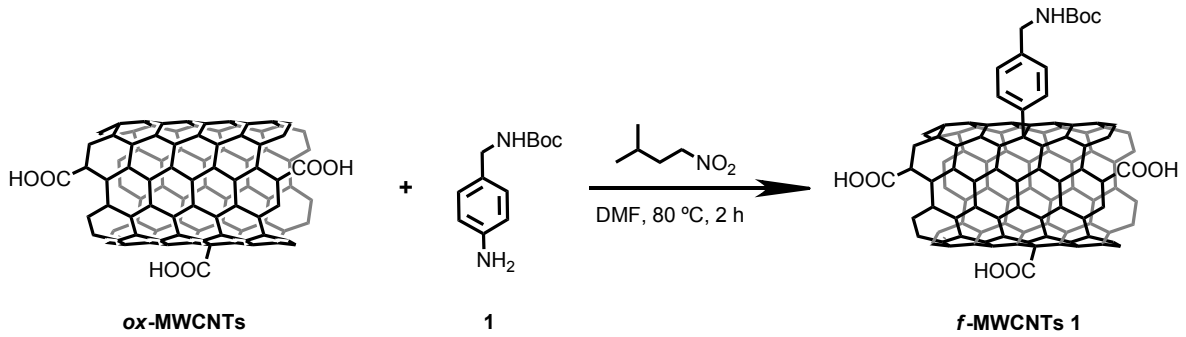
**Synthesis of ox-MWCNTs**




**MWCNTs** ***ox*-MWCNTs**

MWCNTs (200 mg) were sonicated in a mixture of nitric acid and sulfuric acid (48 mL) in a 1:3 ratio for 7 h at 10-30 ºC. After sonication, the resulting mixture was poured onto distilled water (700 mL) and filtered on a teflon membrane (Millipore, JHWP, 0.45 μm). The black solid was then washed by redispersion and filtration in distilled water, in a 0.1 M solution of NaOH, distilled water, DMF and finally in THF. The *ox*-MWCNTs were recovered from the filter as a black solid (150 mg).The weight loss at 500 ºC in TGA was 5.1 %, corresponding to a functionalization with carboxylic groups of 1142 μmol $g^{-1}$.

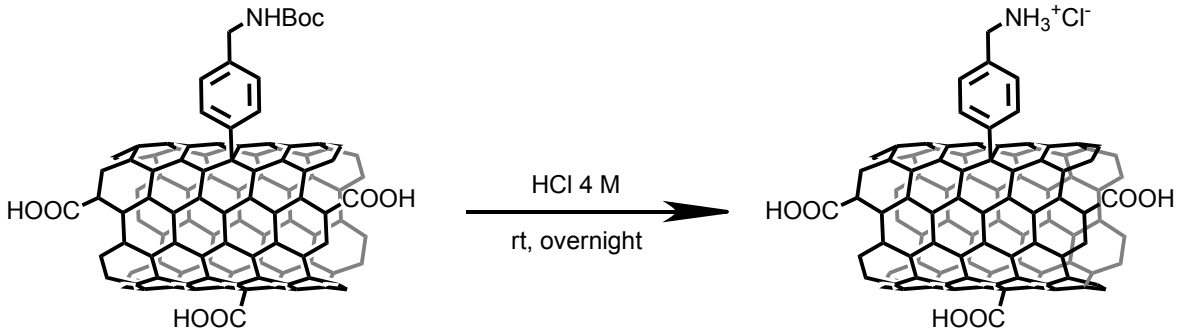
**Synthesis of *f*-MWCNTs 1**




***ox*-MWCNTs** **1** ***f*-MWCNTs 1**

The *ox*-MWCNTs (30 mg) were dispersed in DMF (30 mL) by sonication for a couple of minutes. 4-[(N-Boc)aminomethyl]aniline (660 mg, 3 mmol) was added, followed by dropwise addition of isopentyl nitrite (1.45 mL, 10.8 mmol). The resulting suspension was heated up to 80 ºC and stirred for 2 hours. Then, the mixture was filtered on a teflon membrane (Millipore, JHWP, 4.5 μm) and washed by redispersion and filtration using DMF, MeOH, distilled water, MeOH, AcOEt and finally, rinsed over the filter with $Et_2O$. The *f*-MWCNTs **1** were recovered from the filter as a black solid (25 mg). The TGA profile showed a neat weight loss of 3.6 %, corresponding to a degree of functionalization of 176 μmol of functional groups per gram of *f*-MWCNTs **1**.

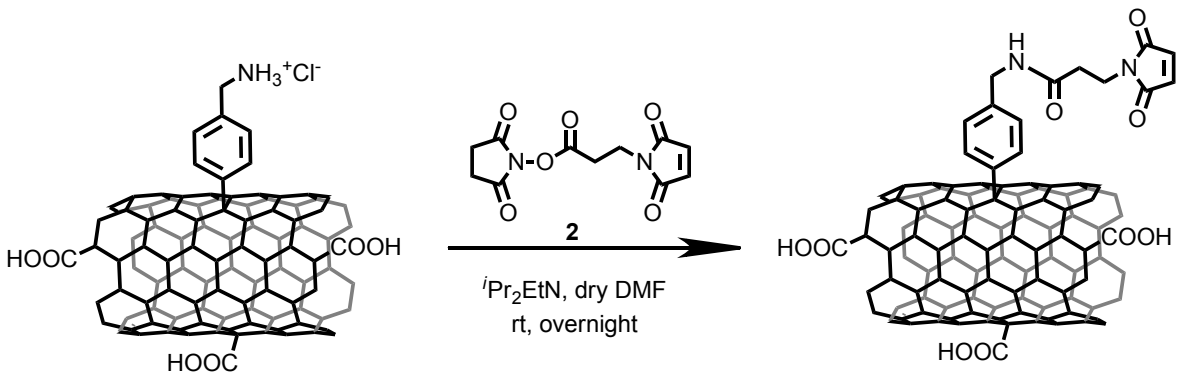
**Synthesis of *f*-MWCNTs 2**




***f*-MWCNTs 1** ***f*-MWCNTs 2**

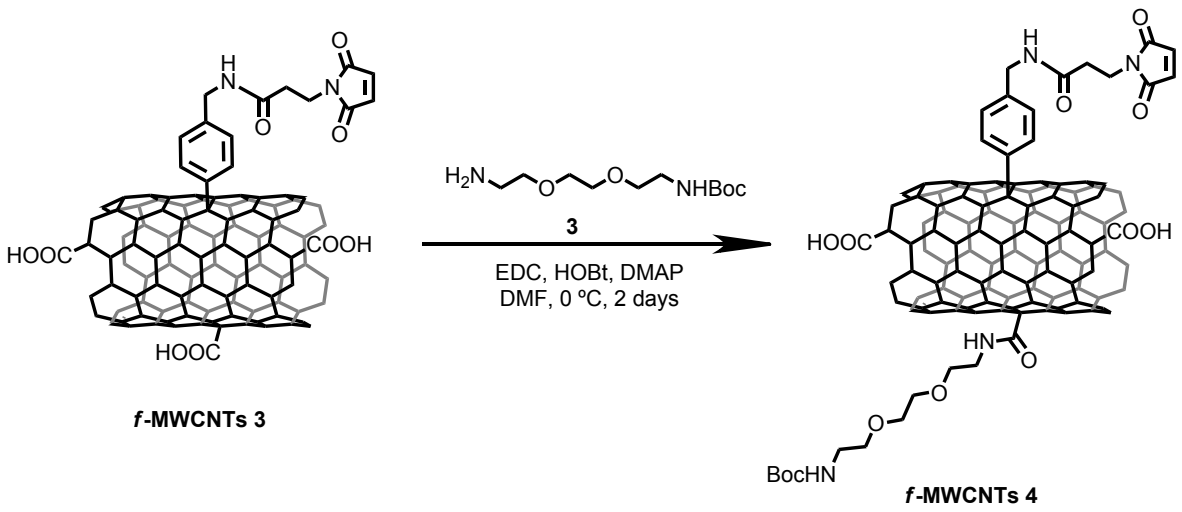
The *f*-MWCNTs **1** (20 mg) were dispersed in a 4 M HCl solution (20 mL) by sonication and mixture was stirred at rt overnight. The nanotubes were filtered on a Teflon membrane (Millipore, JHWP, 0.45 μm) and washed by redispersion followed by filtration in distilled water, EtOH, finally rinsed with $Et_2O$. The *f*-MWCNTs **2** were recovered from filter as black solid (15.1 mg). The TGA profile showed a weight loss of 8 %, which corresponds to a degree of functionalization of 202 μmol $g^{-1}$. Free amino groups presented on sample were quantified by colorimetric assay Kaiser Test (KT) giving a functionalization of 160 μmol of free amino groups per gram of sample.

**Synthesis of *f*-MWCNTs 3**



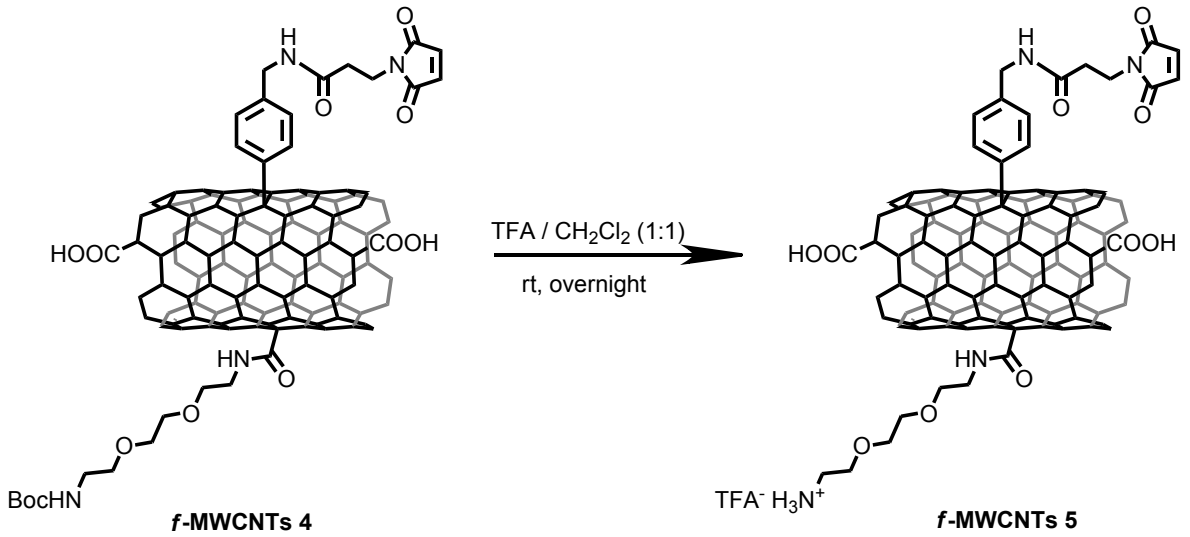

The *f*-MWCNTs **2** (13 mg) and N-succinimidyl 3-maleimidopropionate (138 mg, 520 µmol) were dissolved in dry DMF (13 mL). Then, *N,N*-iisopropylethylamine (90 µL, 520 µmol) was added and reaction was stirred at rt overnight. The mixture was filtered on a teflonmembrane (Millipore, JHWP, 0.45 µm) and rinsed with DMF. Then, the solid was washed by redispersion and filtration in distilled water, DMF, EtOH and finally rinsed with $Et_2O$, affording the *f*-MWCNTs **3** as black solid (11.4 mg). The TGA profile showed a weight loss of 9.1 %, corresponding to a degree of functionalization of 154 µmol $g^{-1}$.

**Synthesis of *f*-MWCNTs 4**



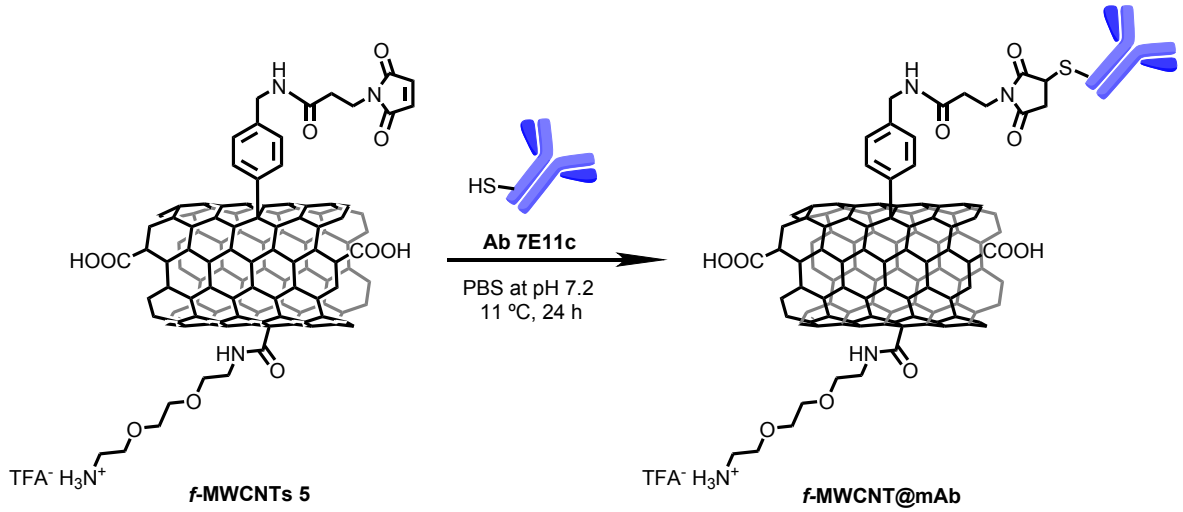

The *f*-MWCNTs **3** (9 mg) were dispersed in DMF (10 mL). *N*-Boc-2,2′-(ethylenedioxy)diethylamine (58 mg, 230 μmol), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, EDC (35.65 mg, 230 μmol), 1-Hydroxybenzotriazole hydrate (HOBt, 32 mg, 230 μmol) and 4-(Dimethylamino)pyridine, DMAP (28.06 mg, 23 μmol), were added at 0ºC. The mixture was allowed to reach rt and it was stirred for 2 days. The mixture was filtered on a teflonmembrane (Millipore, JHWP, 0.45 μm) and it was washed by redispersion and filtration with distilled water, DMF and MeOH. Finally, the CNTs on the filter were rinsed with $Et_2O$, affording of the *f*-MWCNTs **4** (8.3 mg). The TGA profile showed a neat of 6.9 %, corresponding to a degree of functionalization of 283 μmol $g^{-1}$.

**Synthesis of *f*-MWCNTs 5**




The *f*-MWCNTs **4** (7 mg) were dispersed in a mixture of TFA and $CH_2Cl_2$ (7 mL, 1:1). Then, the suspension was stirred at rt overnight. The resulting suspension was filtered on a Teflon membrane (Millipore, JHWP, 0.45 μm) and washed by redispersion and filtration with MeOH and finally rinsed with $Et_2O$. The *f*-MWCTs **5** were recovered as black solid (5.5 mg). The TGA profile showed a neat weight loss of 2.6 % from *f*-MWCTs **3**, corresponding to a degree of functionalization of 97 μmol $g^{-1}$ for *f*-MWCTs **5**. Kaiser test afforded 90 μmol $g^{-1}$ of free amino groups.

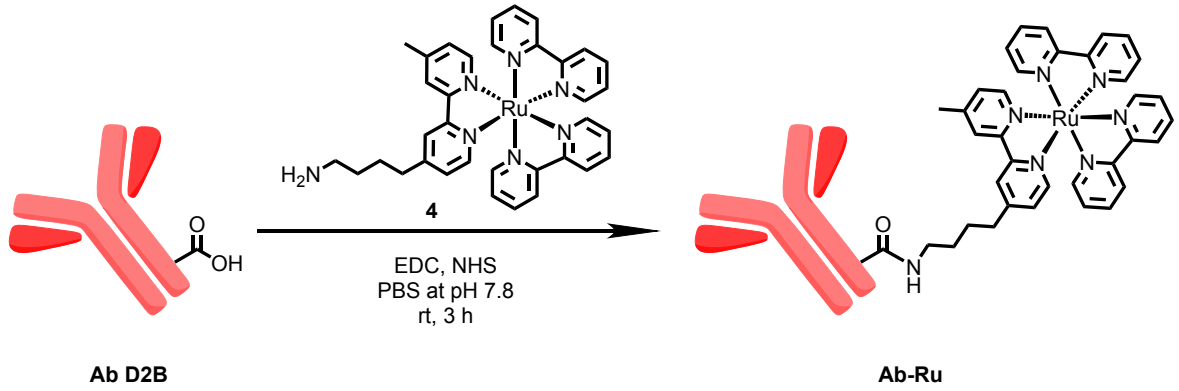
**Synthesis of *f*-MWCNT@mAb**




The *f*-MWCNTs **5** (3.23 mg) were added to a solution of Ab 7E11c in phosphate buffer saline, PBS (3 mL, 0.77 mg/mL, pH = 7.2), and was carefully sonicated for 10 min in an ice bath. The obtained suspension was gently shaken at 11 ºC for 24 hours. The mixture was centrifuged at 2000 rpm for 4 min using a centrifuge tube with membrane (Vivaspin 20, 300000Da cut-off). Centrifugation was repeated 5 times with 15 mL of fresh PBS to remove free Ab. Remaining CNTs suspension was dialyzed in PBS using a dialysis sack (300000 Da cut-off).

A small fraction of the final suspension (1 mL) was centrifuged using a centrifugal filter device (Amicon Ultra, 50,000 cut-off membrane) and then centrifuged 5 times with Milli-Q water, in order to remove PBS salts before performing TGA. After centrifugation, the sample was freeze-dried. The TGA profile showed a neat weight loss of 45.7 % for ***f*-MWCNT@mAb**, corresponding to 3 µmol of Ab 7E11c per gram of sample.

**Synthesis of Ab-Ru complex conjugate**




**Ab D2B** **Ab-Ru**

The coupling agent EDC (0.132 mg), N-Hydroxysuccinimide, NHS (0.1 mg), and bis(2,2'-bipyridine)-[4-(4'-methyl-2,2'-bipyridin-4-yl)-aminobutyl] ruthenium(II) complex (0.8 mg) were added to 1 mL of Ab D2B solution in PBS (1.5 mg $mL^{-1}$), after correcting pH to 7.8. The mixture was shaken for 3 hours at rt. The solution was diluted to 5 mL and then pH was exchanged to 7.4 by using dialysis (12,000 – 14,000 Da cut-off membrane) overnight at 4 ºC. The solution was concentrated to 0.5 mL by centrifugation with centrifugal filter device (Amicon Ultra, 50,000 Da cut-off membrane). Then, it was centrifuged with fresh PBS to remove unreacted Ru complex. The washings were performed until filtrated solution did not show Ru complex by UV-Vis spectroscopy (450nm).

## TGA analysis

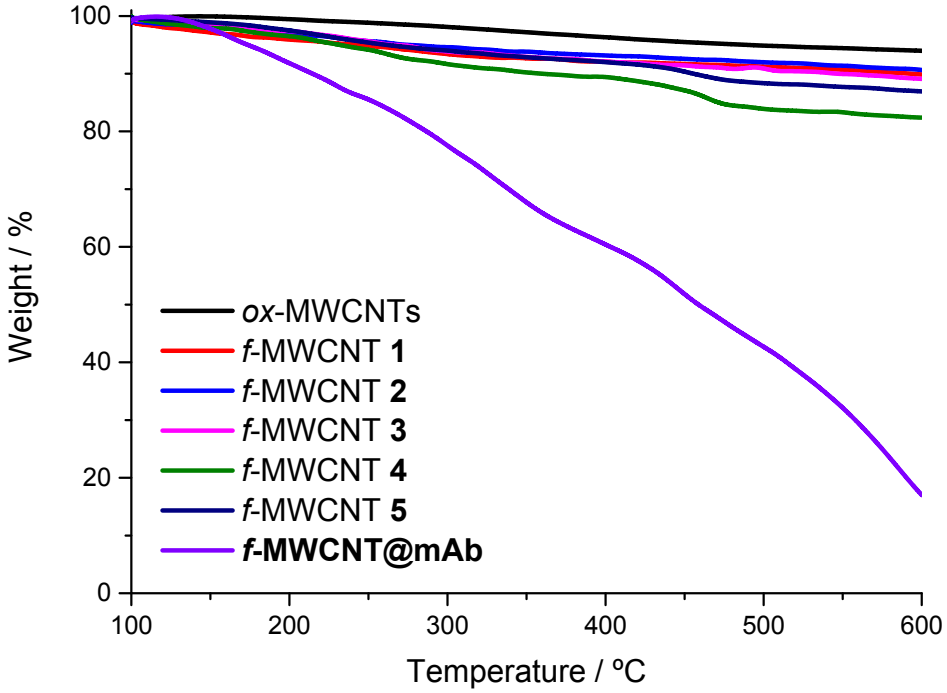



**Figure S1**. TGA profiles in $N_2$ atmosphere for *f*-MWCNTs.

**Table S1.** Average functional degree by TGA and Kaiser test.

| MWCNTs | Funct. Degree TGA (µmol FG / g MCNTs)[a)] | Funct. Degree KT (µmol $NH_2$ / g MCNTs) |
|---|---|---|
| *ox*-MWCNTs | 1142 | |
| *f*-MWCNTs 1 | 176 | |
| *f*-MWCNTs 2 | 202 | 160 |
| *f*-MWCNTs 3 | 154 | |
| *f*-MWCNTs 4 | 283 | |
| *f*-MWCNTs 5 | 97 | 90 |
| *f*-MWCNT@mAb | 3 | |

[a)] TGA-determined weight loss at 500 ° C.

In order to draw quantitative information from thermogravimetric plots, we performed the following calculation to obtain $X$ (functionalization degree):

$$X\ (\mu mol \cdot g^{-1}) = \frac{L(\%) \cdot 10^4}{M_w (g \cdot mol^{-1})}$$ Equation S1

Where *L* corresponds to the weight loss observed at 500ºC (in %), after having subtracted the analogous loss from the pristine material. In sequential functionalization steps:

- For the first step, *L* stands as described above.
- For the second step, *L* refers to the difference between first and second functionalization.

The molecular weight ($M_w$) is set for the expected desorbed moiety.

The conversion factor ($10^4$) provides data in the desired unities ($\mu mol \cdot g^{-1}$).

## TEM characterization

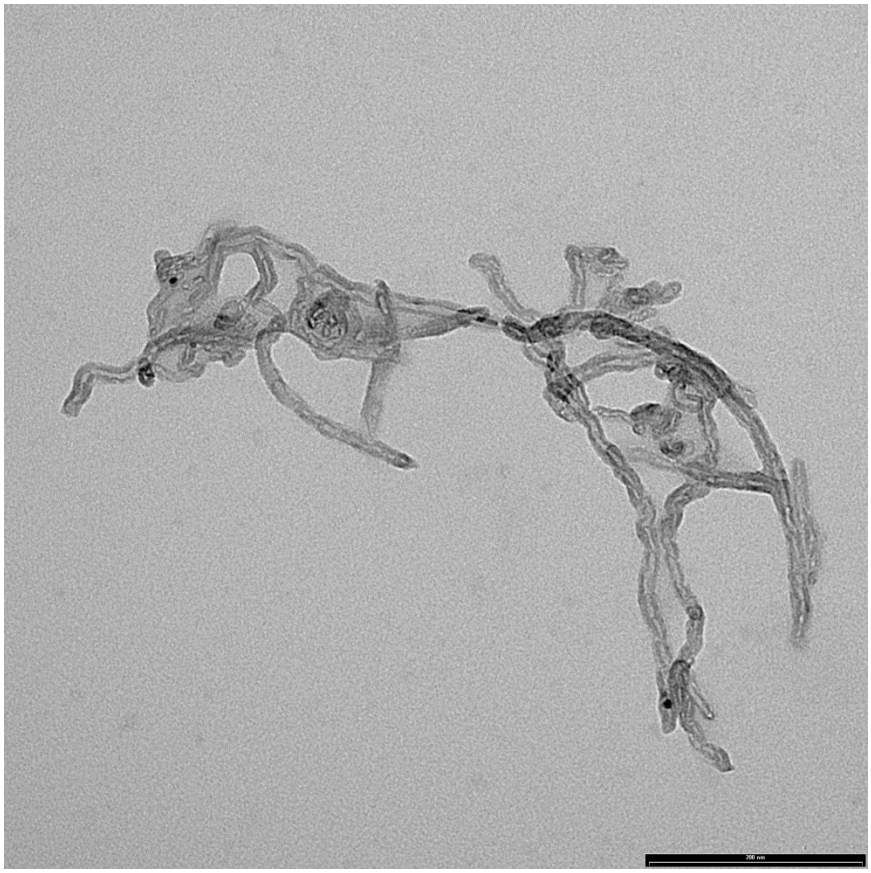
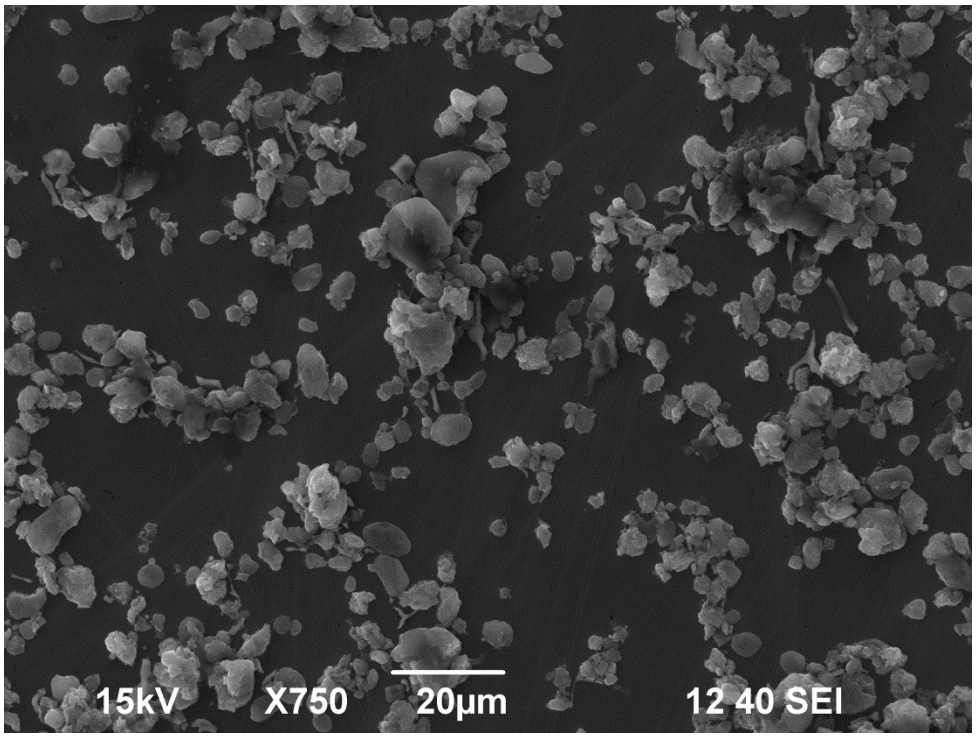
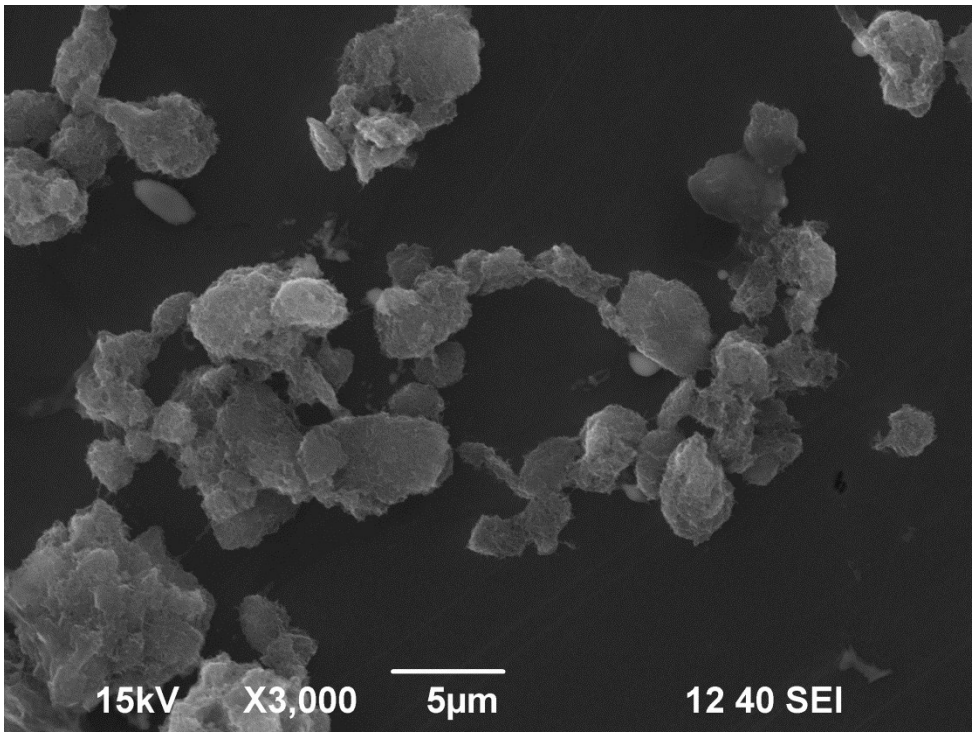


**Figure S2**. TEM image of *ox*MWCNTs.

## SEM characterization



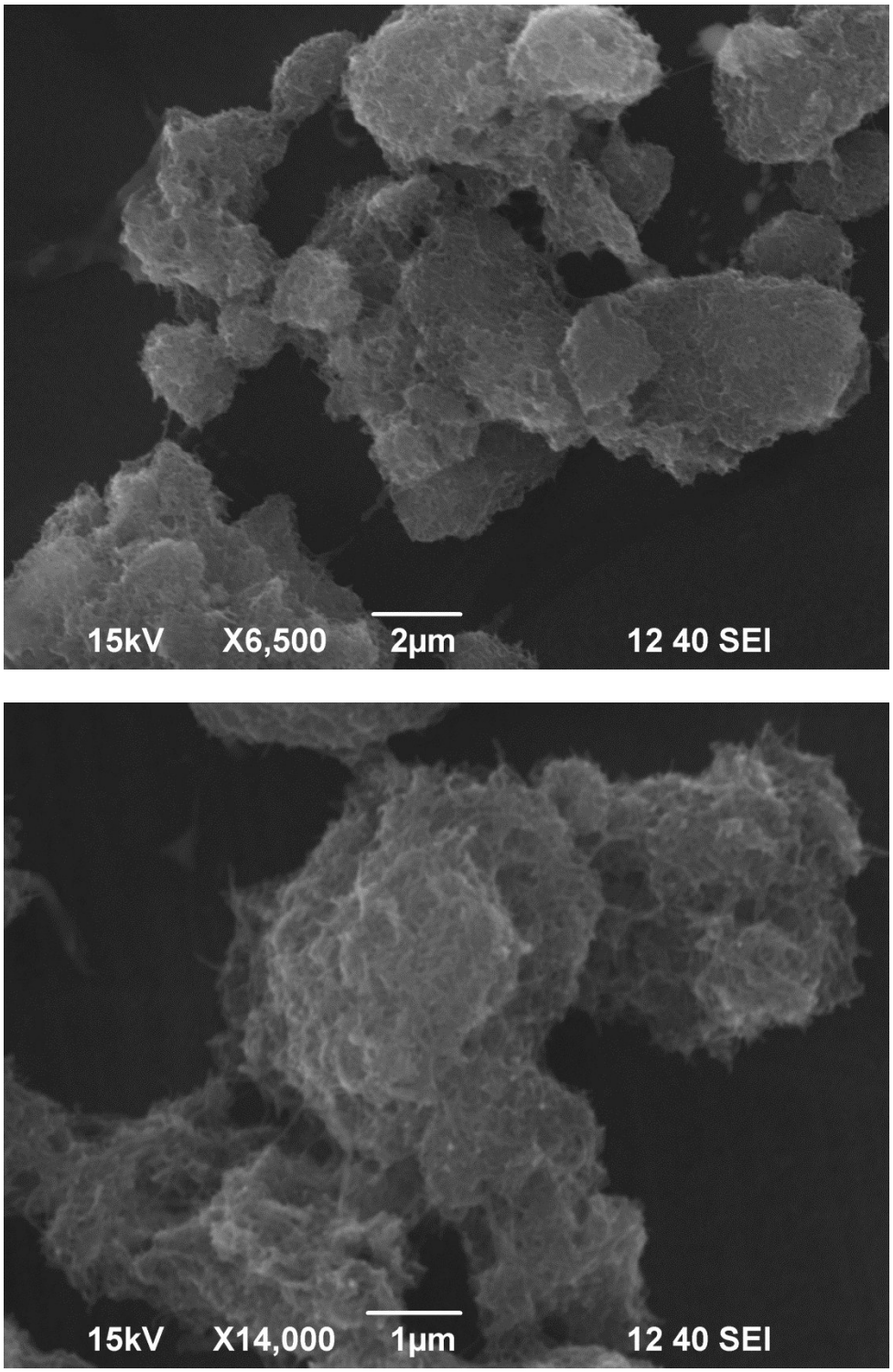



**Figure S3**. SEM images of ITO electrode modified with *f*-MWCNTs.

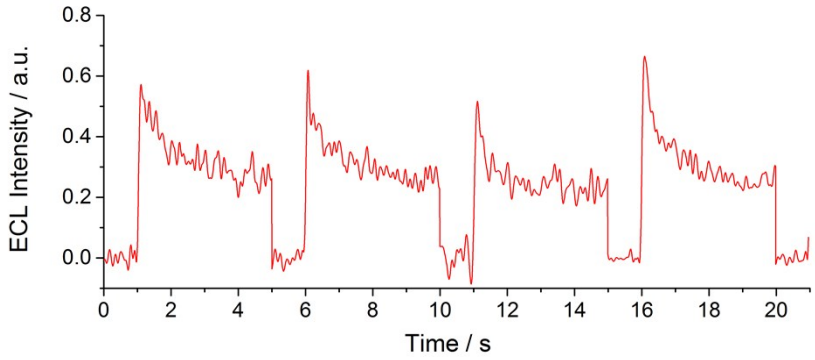



**Figure S4**. ECL intensity (red trace) vs time from 200 mM TPrA in PBS. Step potential E1 = 0 V, t1 = 1s; E2 = 1.70 V, t2 = 4s. PMT bias 750 mV. The ***f*-MWCNT@mAb** was incubated with lysate solution containing 4.508 ng $mL^{-1}$ of PSMA antigen for 2h.

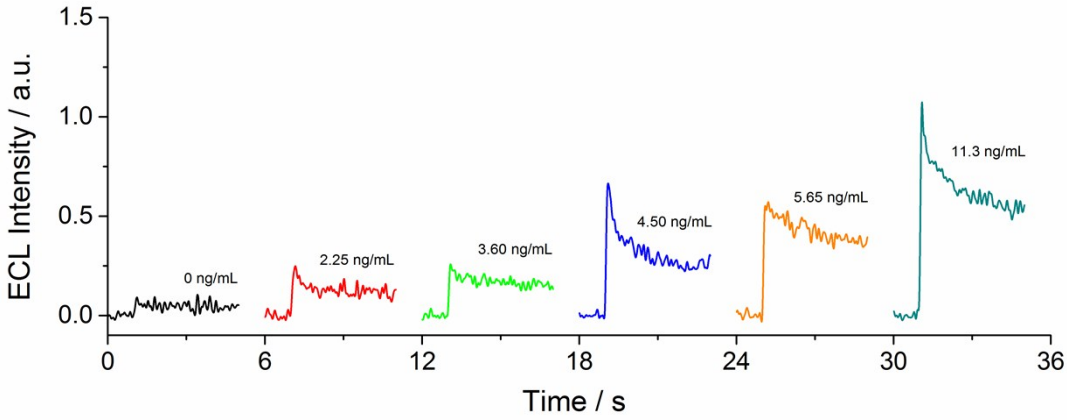



**Figure S5.** ECL intensity for different PSMA concentrations (11.3, 5.65, 4.50, 3.60, 2.25, 0 ng mL-1) in the presence of 200 mM of TPA in 0.2M PBS. ***f*-MWCNT@mAb** based immunoassay at 1.70 V. Electrochemiluminescence experimental conditions: PMT bias 750 V.