Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Fluorescent ampicillin analogues as multifunctional disguising agents against opsonization

Nalinikanth Kotagiri,[‡a,b,c] Joshua Sakon,[c,d] Haewook Han,[e,f] Vladimir P. Zharov[g] and Jin-Woo Kim*[a,b,c]

[a]*Bio/Nano Technology Laboratory, Institute for Nanoscience & Engineering, University of Arkansas, Fayetteville, AR, USA*
[b]*Department of Biological & Agricultural Engineering, University of Arkansas, Fayetteville, AR, USA*
[c]*Cell & Molecular Biology Graduate Program, University of Arkansas, Fayetteville, AR, USA*
[d]*Department of Chemistry & Biochemistry, University of Arkansas, Fayetteville, AR, USA*
[e]*Department of Electrical Engineering, Pohang University of Science & Technology (POSTECH), Pohang, Republic of Korea*
[f]*School of Interdisciplinary Bioscience & Bioengineering, POSTECH, Pohang, Republic of Korea*
[g]*Arkansas Nanomedicine Center, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

[*]*E-mail: jwkim@uark.edu*
[‡]*Current Address: Department of Radiology, Washington University School of Medicine, St. Louis, MO, USA*

Supplementary Notes

1. Quantum yield estimation

Quantum yield (Φ) gives the efficiency of the fluorescence process. It is defined as the ratio of the number of photons emitted to the number of photons absorbed:

$$\Phi = \frac{\text{Number of photons emitted}}{\text{Number of photons absorbed}}$$

We used the comparative method to calculate the Φ as reported previously.[S1] The selected standards include quinine sulfate (QS) and 9,10-diphenylanthracene (DPA) with Φ of 0.54 and 0.9, respectively. The choice of these standards was based upon their emission profiles, which match those of iAmp. Firstly, the two standards were cross-calibrated with each other to instill confidence in the method used in this study. The first standard was prepared by adding 2 mg of QS to 2 ml of sulfuric acid (0.1 M). Serial dilutions were made to determine the concentration to give a maximal optical density (OD) value of 0.1. The 0.67% solution yielded this value and thereafter five serial dilutions were prepared. Similarly for DPA, the same procedure was followed by dissolving the chemical in dimethyformamide (DMF) and preparing serial dilutions. The 0.78% solution yielded the OD of 0.1 and thereafter five serial dilutions were prepared. For each sample, the UV/Vis absorbance spectrum of a dilution was recorded and the absorbance at the excitation wavelength noted. Next, the fluorescence spectrum of the same solution was recorded using NanoDrop 3300 Fluorospectrometer (Thermo Fisher Scientific, Inc., Wilmington, DE). The integrated fluorescence intensity (*i.e.,* the area of the fluorescence spectrum) from the fully corrected fluorescence spectrum was then calculated. After repeating for all the five dilutions of each sample, a graph was plotted with integrated fluorescence intensity vs. absorbance and the slope was estimated. Using the following equation, the Φ for each sample was calculated:

$$\Phi_x = \Phi_{st}\left(\frac{Grad_x}{Grad_{st}}\right)\left(\frac{\eta_x^2}{\eta_{st}^2}\right)$$

where Φ is the fluorescence quantum yield, the subscripts x and st denote test and standard, respectively, *Grad* the gradient from the plot of integrated fluorescence intensity vs. absorbance, and η the refractive index of the solvent. The estimated Φ of QS and DPA were 0.5 and 0.9, respectively. The estimated values are in accordance with those previously reported,[S2,S3] confirming the validity of our method. Subsequently, serial dilutions of iAmp were prepared and using QS as standard, the Φ was estimated. QS was used as standard because its absorption maxima (λ_{max} = 346 nm) was closest to iAmp (λ_{max} = 340 nm).

2. Isoelectric focusing

The iAmp and ampicillin solutions were evaluated using 5% agarose gel electrophoresis. Tris/acetate/EDTA (TAE) buffer was used as the electrophoresis buffer and the gel running time was 30 min at 110 V. The gel was examined under UV light using Bio-Rad Gel Doc 2000 (Bio-Rad Laboratories, Inc., Hercules, CA).

3. ^{1}H NMR

^{1}H NMR spectrum was measured on a Bruker Avance 300MHz NMR spectrometer at an ambient temperature using a broadband observed probe with z-gradient. Samples were dissolved in D_2O and placed in a 5 mm tube.

4. FTIR

The iAmp and ampicillin solutions were dried using RapidVap Vacuum Dry Evaporation System (Labconco, Kansas City, MO) before FTIR analysis. The spectrum was recorded on a Bruker Tensor 27 Spectrometer at the resolution of 2 cm^{-1} in wavenumber.

5. HPLC-MS

HPLC-MS experiments were carried out on a Bruker Esquire LC-ESI-Ion-Trap MS. The HPLC column was a Supelcosil reverse-phase C8 packing column with a dimension of 4.6 mm × 150 mm and 5-μm particles (Sigma-Aldrich, Milwaukee, WI). The flow rate was set to 0.8 ml/min. HPLC mobile phase was a mixture of solvent A – 0.1% formic acid in water and solvent B – 0.1% formic acid in Acetonitrile. After a 5 min hold at 5% B, the gradient was programmed linearly to 100% B at 50 min. Mass spectra were collected in the default full scan mode using standard conditions for the instrument in the electrospray ionization mode.

6. Ammonia Assay

Ammonia assay was done according to the manufacturer's instructions. Briefly, to tubes with 1 ml of water marked as 'Blank', 'Test', and 'Standard' was added 100 μl of water, 100 μl of sample and 50 μl of ammonia standard, respectively. After incubation at RT for 5 min, the absorbance for each was recorded at 340 nm. 10 ml of L-glutamate dehydrogenase was added and absorbance recorded again after 5 min. The absorbance differences for blank and standard as well as blank and test were determined. Then, the absorbance difference of the blank was subtracted from those of the sample as well as standard, thereby obtaining $\Delta A_{\text{Ammonia}}$. The concentration of ammonia ($C_{Ammonia}$; g/L), based upon the extinction coefficient of NADH at 340 nm (*i.e.,* ε = 6,300 $M^{-1}cm^{-1}$), was calculated using Beer Lambert law equation:

$$C_{Ammonia} = 0.71 \times \Delta A_{Ammonia}$$

The estimated concentration values were compared to the standards and considered valid when they were within 5% of the standards. .

Supplementary References

S1. Williams, A. T. R.; Winfield, S. A.; Miller, J. N. Relative fluorescence quantum yields using a computer controlled luminescence spectrometer. *Analyst* **1983**, *108*, 1067.

S2. Melhuish, W. H. Quantum efficiencies of fluorescence of organic substances: effect of solvent and concentration of the fluorescent solute. *J. Phys. Chem*. **1961**, *65*, 229.

S3. Hamai, S.; Hirayama, F. Actinometric determination of absolute fluorescence quantum yields. *J. Phys. Chem*. **1983**, *87*, 83.

Supplementary Figures

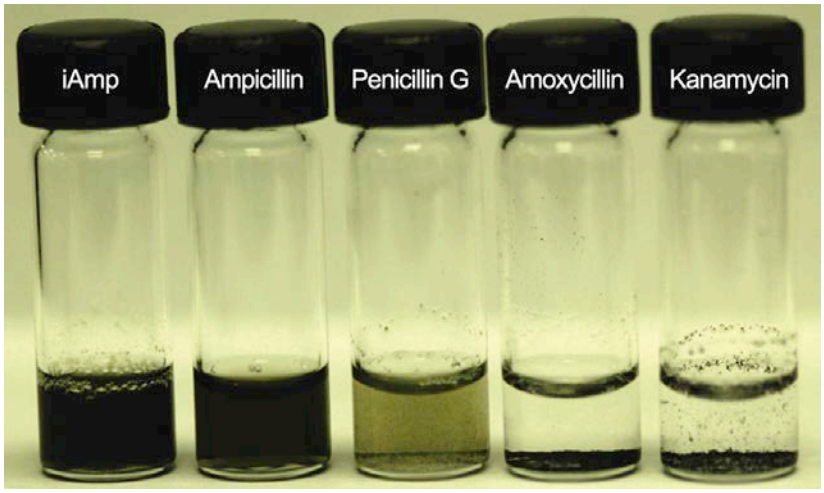

Figure S1. Dispersions of pristine SWNTs in various beta-lactam antibiotics, including amoxicillin, ampicillin, and penicillin G, and a non beta-lactam antibiotic, kanamycin.

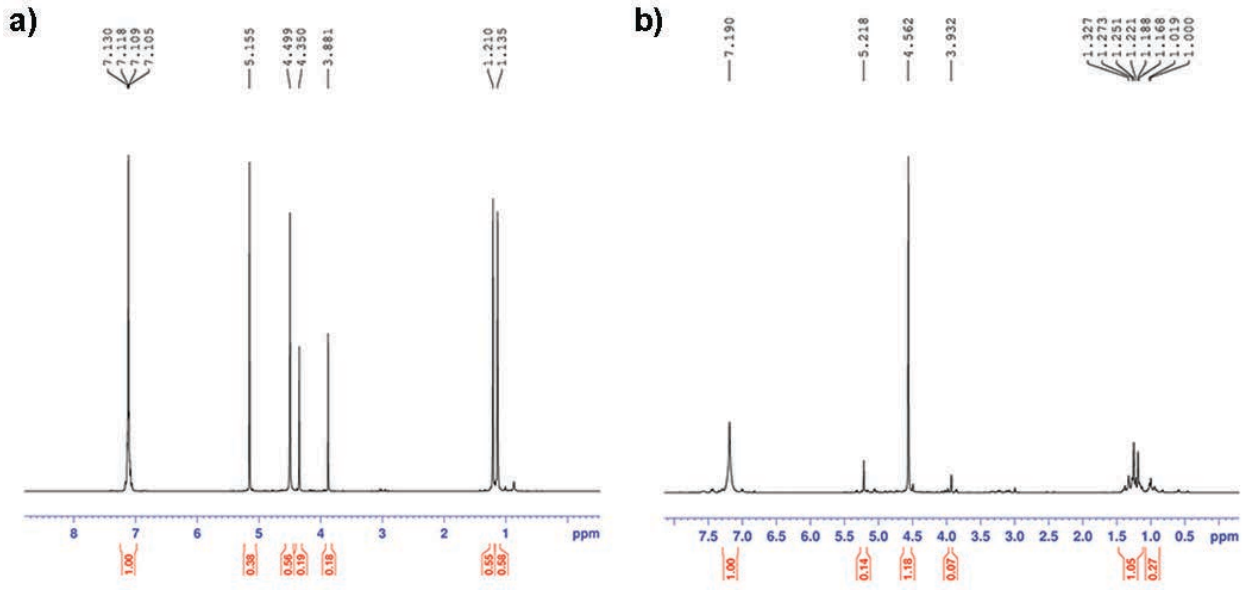

Figure S2. ^{1}H NMR spectra of ampicillin (**a**) and iAmp (**b**). Notice that the spectra are identical except for the loss of H-10 in iAmp.

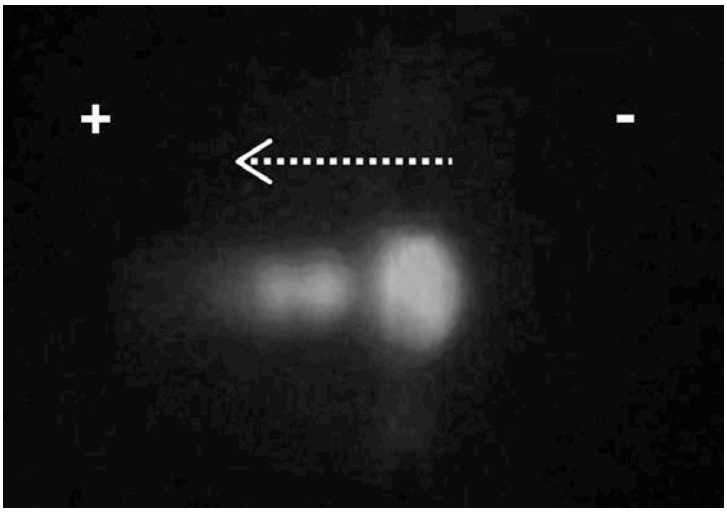

Figure S3. Isoelectric focusing showing iAmp moving towards the positive electrode.

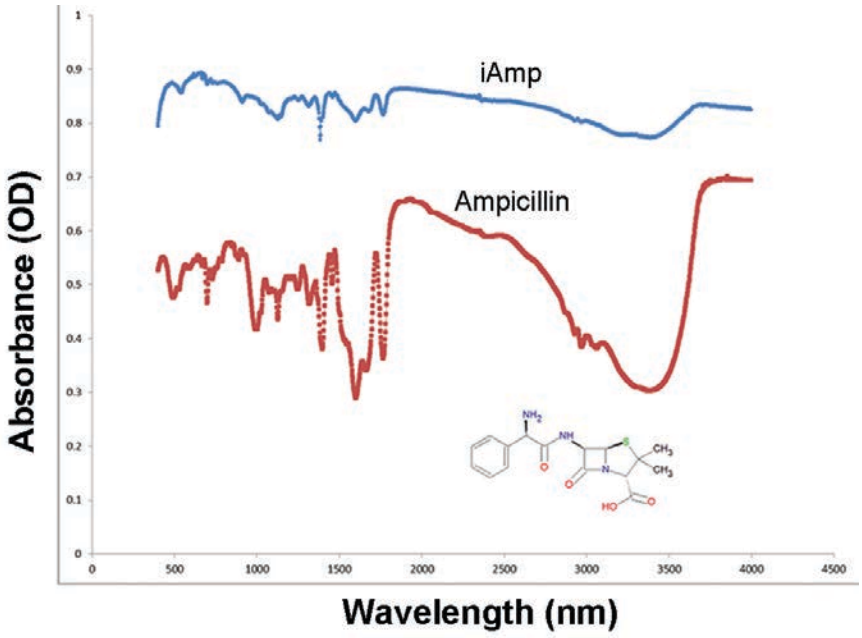

Figure S4. FTIR spectra of iAmp (**blue line**) and ampicillin (**red line**).

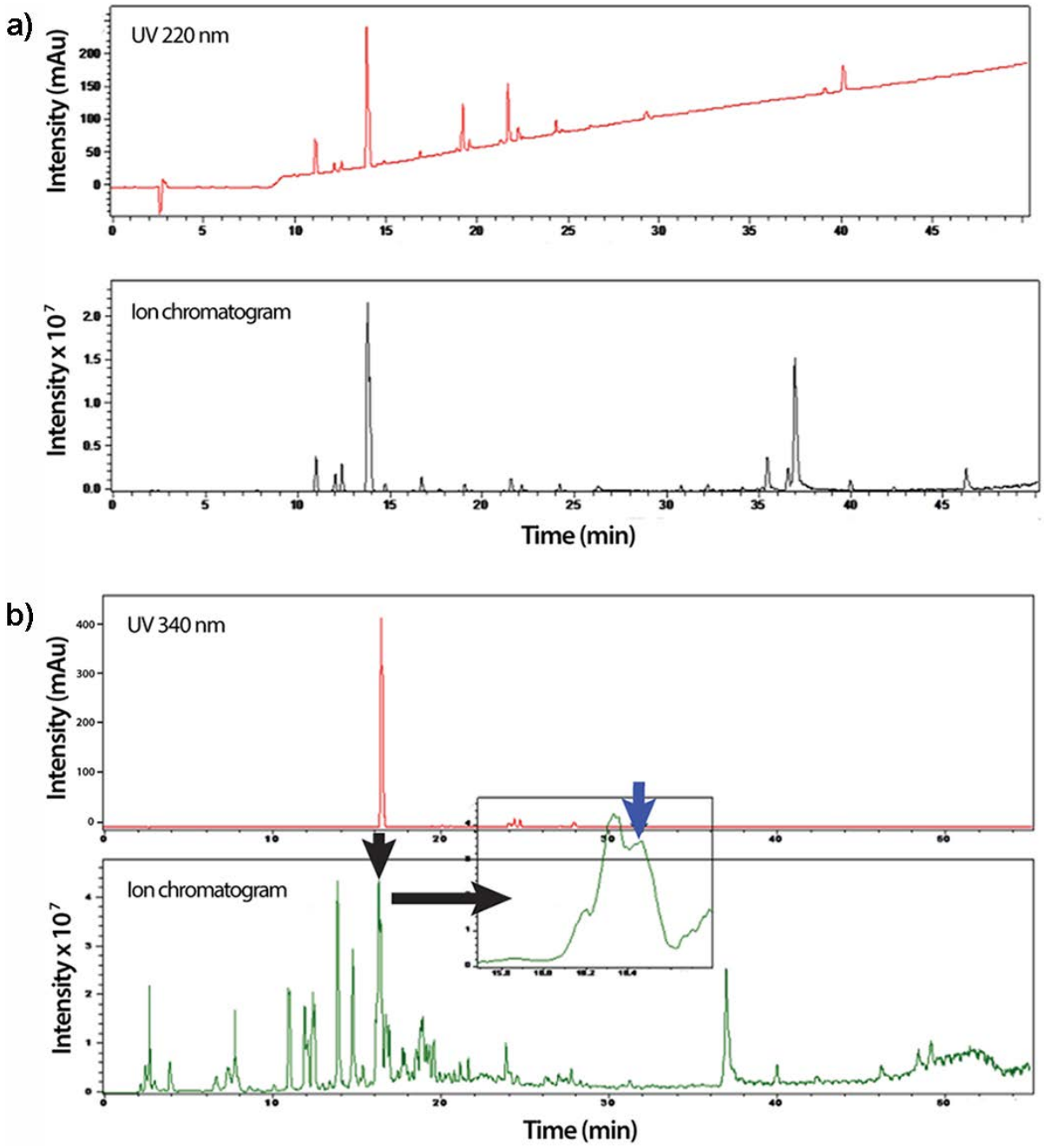

Figure S5. Photodiode array (PDA) data and ion chromatogram of ampicillin (**a**) and iAmp (**b**) using a reverse-phase C8 column.

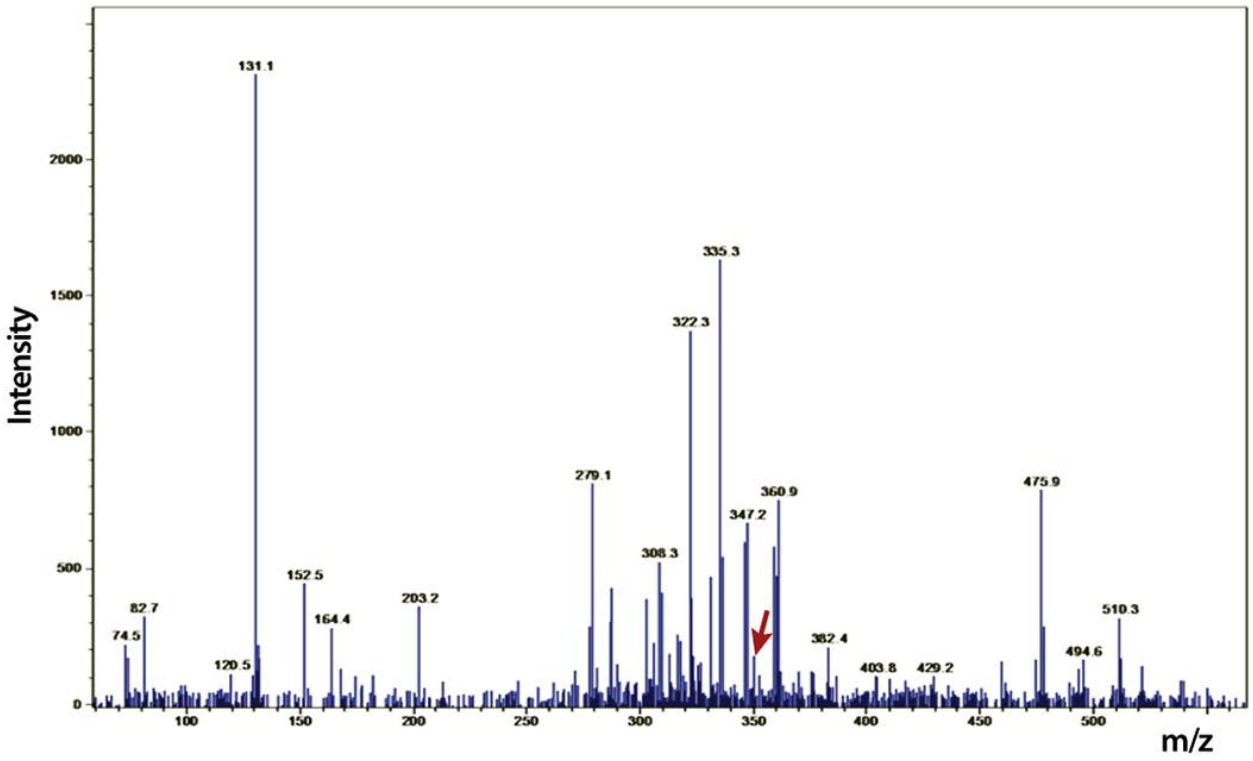

Figure S6. ESI-Ion-Trap MS of iAmp. Arrow denotes the iAmp peak.

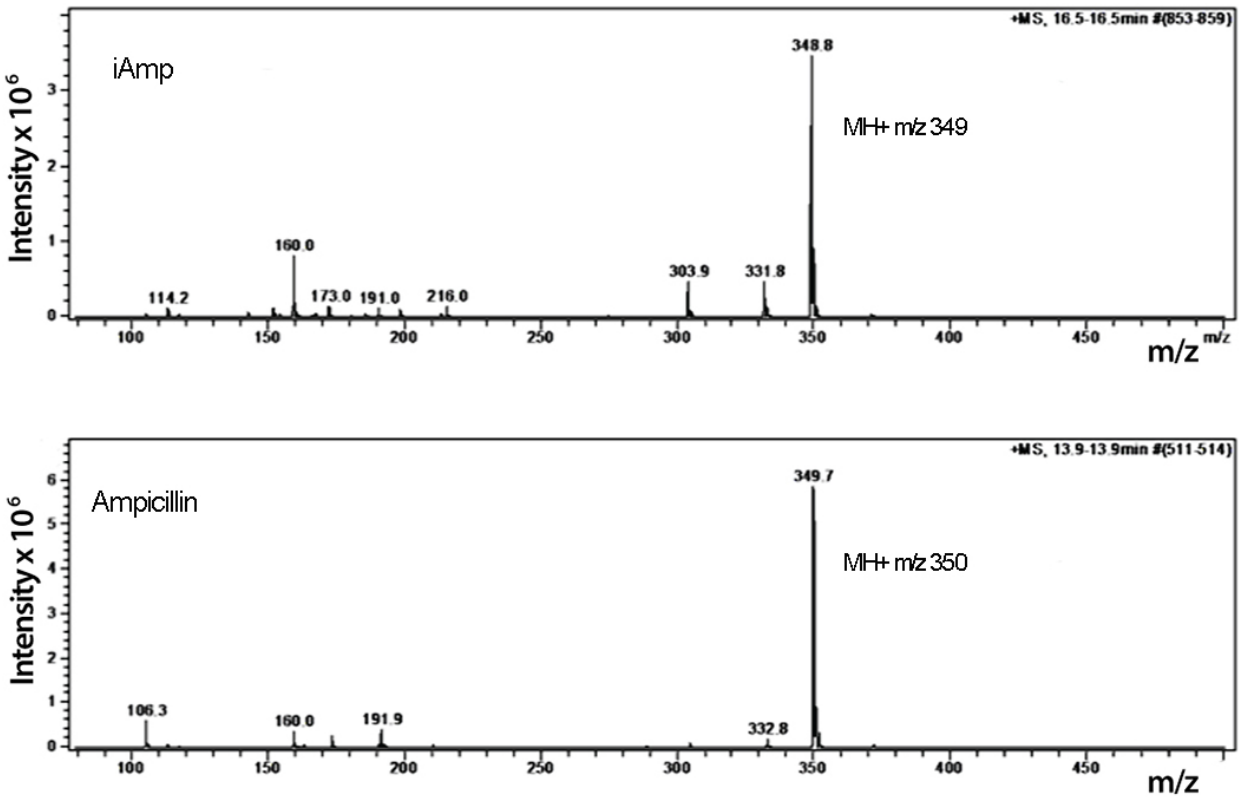

Figure S7. HPLC-MS data of iAmp (**top**) and ampicillin (**bottom**).

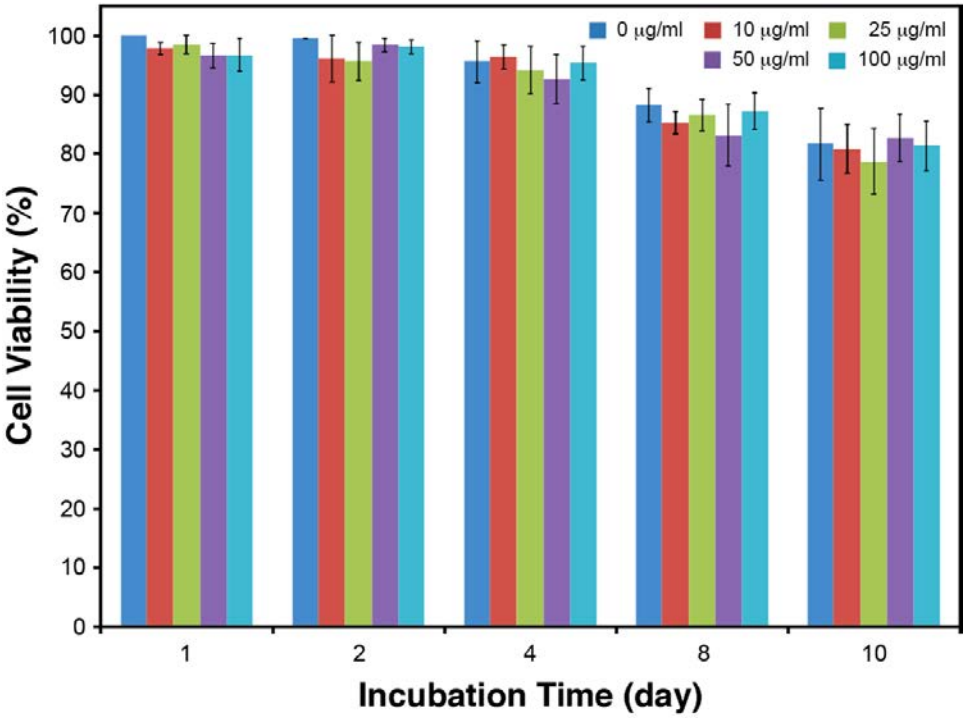

Figure S8. Cytotoxicity studies of iAmp. Note that all experiments were performed 3 times in triplets and the average has been shown as cell-viability percentage in comparison with the iAmp untreated control samples at time 0. The controls at time 0 were considered as 100% viable.

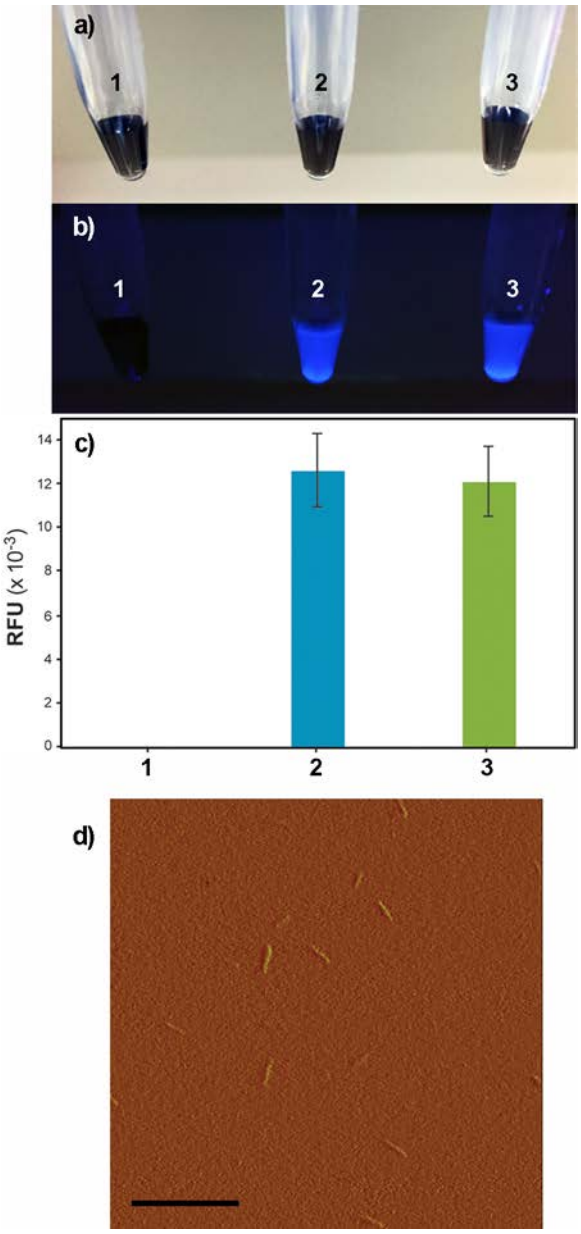

Figure S9. Bio-stability of iAmp-SWNTs. Bright light images (**a**), fluorescence images (**b**), and fluorescence intensities (**c**) of: **1** – SWNTs only in water, **2** – iAmp-SWNTs in PBS (0.1 M; pH 7.4), and **3** – iAmp-SWNTs in EMEM with 10% FBS. All samples were incubated at 37°C and 5% CO_2 for 10 days. After incubation, they were purified using the Harvard Apparatus Ultra-Fast Dialyzer with a 50-kDa MWCO cellulose acetate membrane in 0.1 M PBS buffer (pH 7.4) for 24 h at 25°C. Notice little or no change in fluorescence intensities even after 10-day incubation, implying that iAmp coating, through π-π interaction with SWNT, remains stable in such high protein and salt environment. **d**) Phase AFM image of iAmp-SWNTs of **3**. Notice a similar result as the control iAmp-SWNTs (Figure 1b), displaying uniformly dispersed SWNTs without noticeable aggregations or association of proteins. Scale bar represents 500 nm.

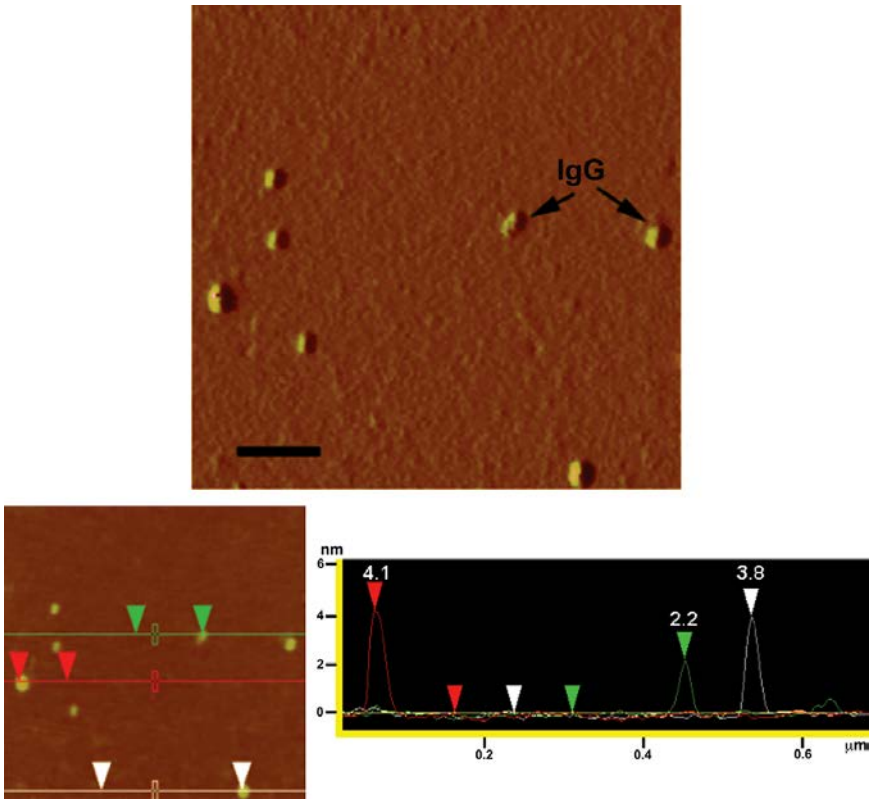

Figure S10. Phase AFM image of IgG (**top**). Scale bar represents 100 nm. AFM height image (**bottom, left**) and section analysis plot (**bottom, right**) of IgG. The average height is ~3.5 nm.

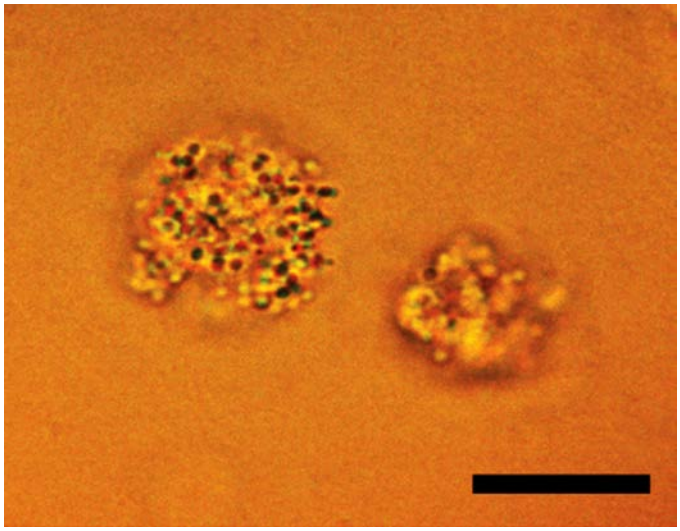

Figure S11. Light microscopy image of macrophages with uncoated dsSWNTs that were preincubated with C3b only. Scale bar represents 25 μm.

Figure S12. Epi-fluorescence microscopy image of macrophages with iAmp-SWNT-IgG that were preincubated with opsonins, *i.e.,* C3b and/or IgG, using the DAPI filter. Scale bar represents 25 μm.