

Supporting information

Fluorescence labeling of carboxylic groups

A mixed solution of 0.193 mM of PB and 0.07 mM of dibenzo-18-crown-6 (D18C6) (Aldrich) (used as a catalyst) in acetone (Fisher) was prepared in the dark. 30 ml of the solution was added into a 50 ml glass beaker which contained a sample of known mass and a Teflon coated magnetic stir bar. The reaction was run at $\sim 50^{\circ}\text{C}$ (hot plate) in the dark for 3.5 hrs, while stirring. After the reaction, a supernatant (~ 20 ml) over the labeled SWCNTs was transferred into an empty 200 ml flask. The sample was washed in the beaker with neat acetone (~ 20 ml) and supernatant transferred into the same 200 ml flask. The washing of the sample (and transferring of the supernatant) was repeated several times (4-5times). The collected supernatant was diluted up to 200ml with acetone and then diluted with acetone by a factor of 500 ($100\ \mu\text{l}$ up to 50ml). The fluorescence spectrum of the solution was recorded (excitation at 362 nm). The preceding procedure was implemented twice for as-received SWCNTs (13.7mg and 16.9mg), Air/HCl treated SWCNTs (15.1mg and 15.9mg), and nitric acid treated SWCNTs (23.1mg and 29.1mg). The depletion of the fluorescence signal was measured and compared to the fluorescence intensity of the dye after the control experiment (labeling reaction with no sample in the beaker). (Fig. S1, S2, S3, S4)

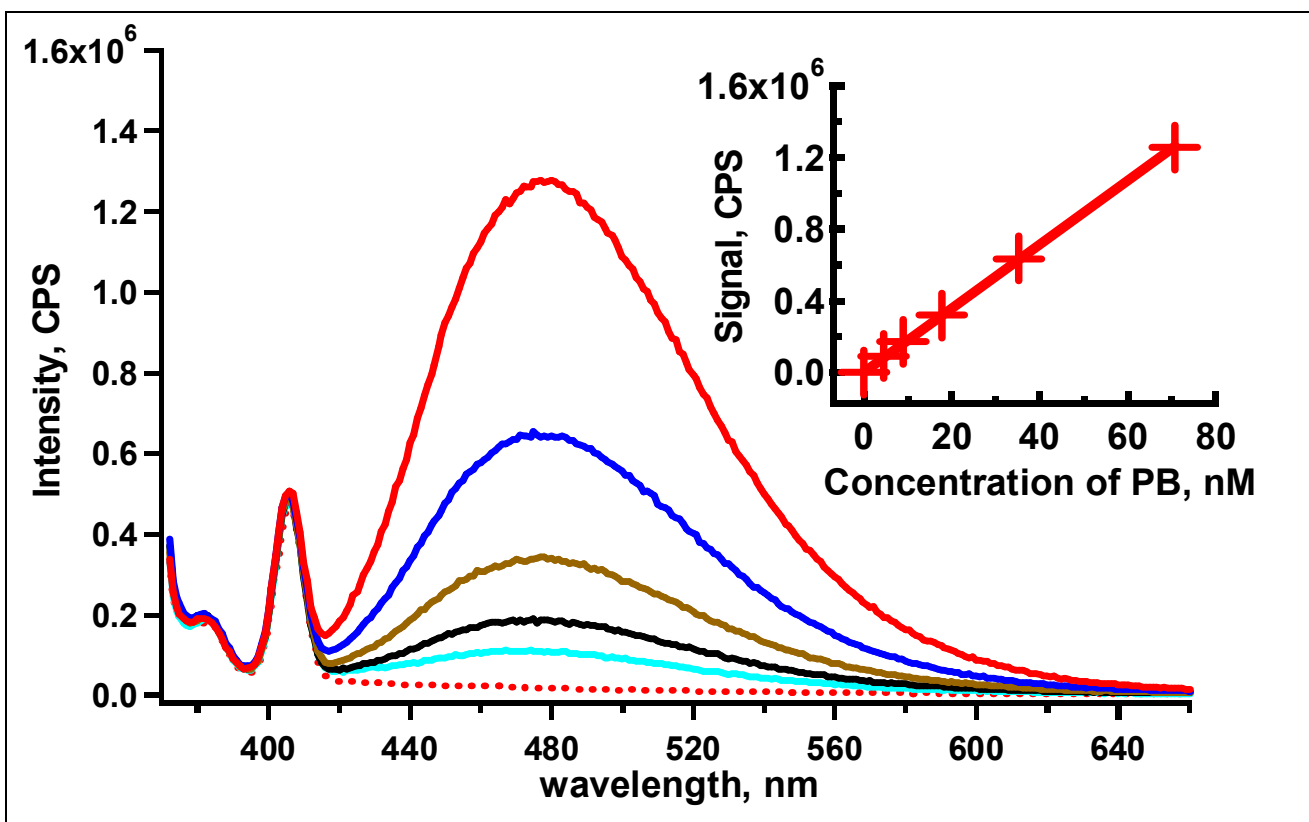


Figure S1. Emission spectra of panacyl bromide of different concentrations: 70.6nM (red solid line), 35.3nM (blue solid line), 17.7nM (brown solid line), 8.8nM (black solid line), 4.4nM (cyan solid line); neat acetone (red dotted line) excited at 362nm. (Inset: calibration plot of fluorescence intensity at 475nm vs. PB concentration)

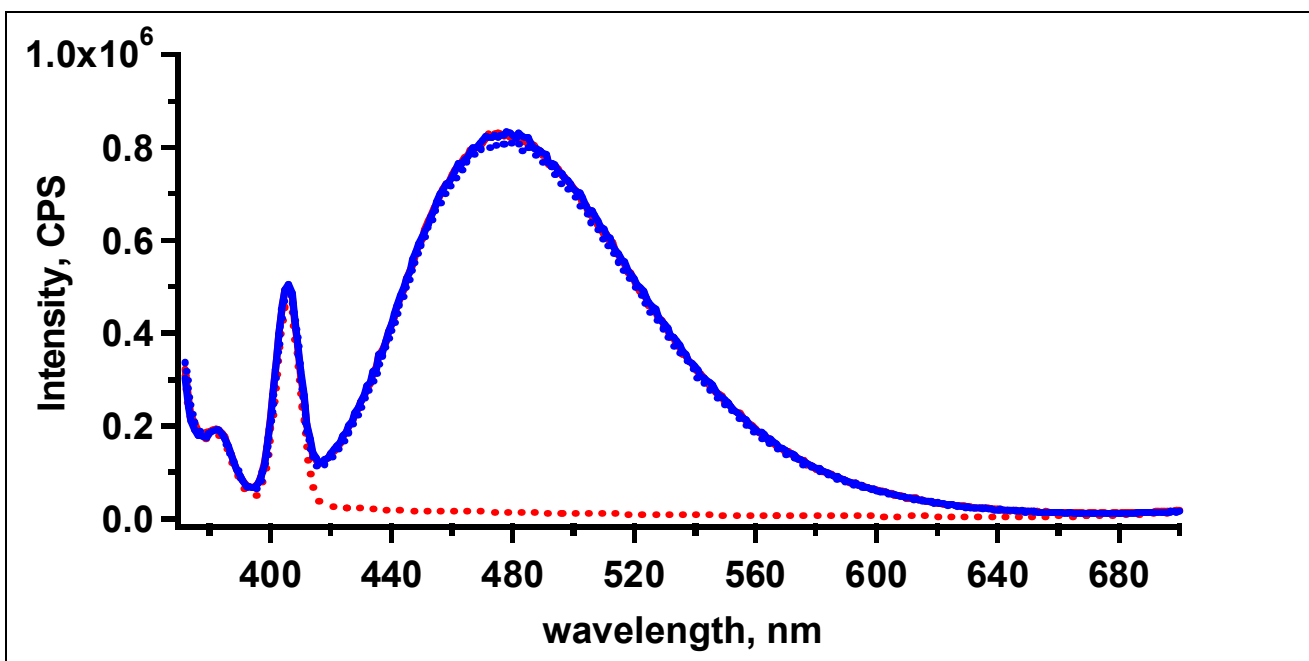


Figure S2. Emission spectra of panacyl bromide reacted with carboxyls of as-received SWCNTs: control experiment without SWCNTs (red solid line), after reaction with 16.9mg SWCNTs (blue solid line), after reaction with 13.7mg SWCNTs (blue dotted line); neat acetone (red dotted line). Excitation at 362 nm.

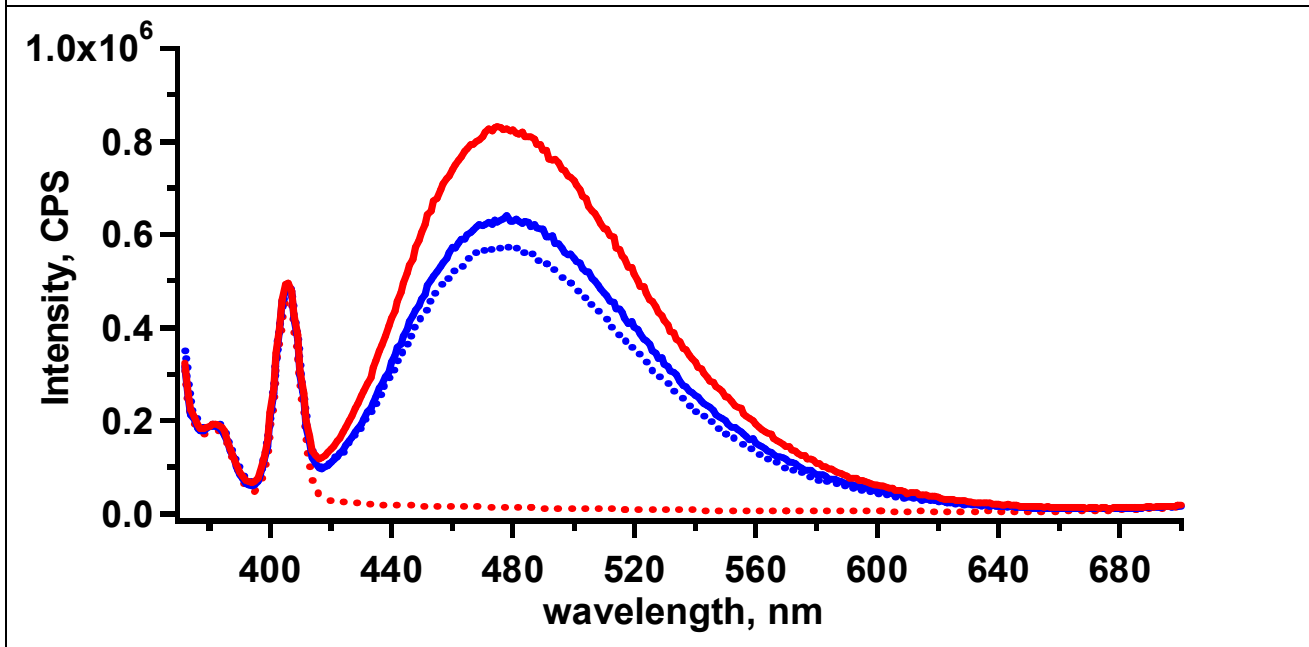


Figure S3 Emission spectra of panacyl bromide reacted with carboxyls of Air/HCl treated SWCNTs: control experiment without SWCNTs (red solid line), after reaction with 15.1mg SWCNTs (blue solid line), after reaction with 15.9mg

SWCNTs (blue dotted line); neat acetone (red dotted line). Excitation at 362 nm.

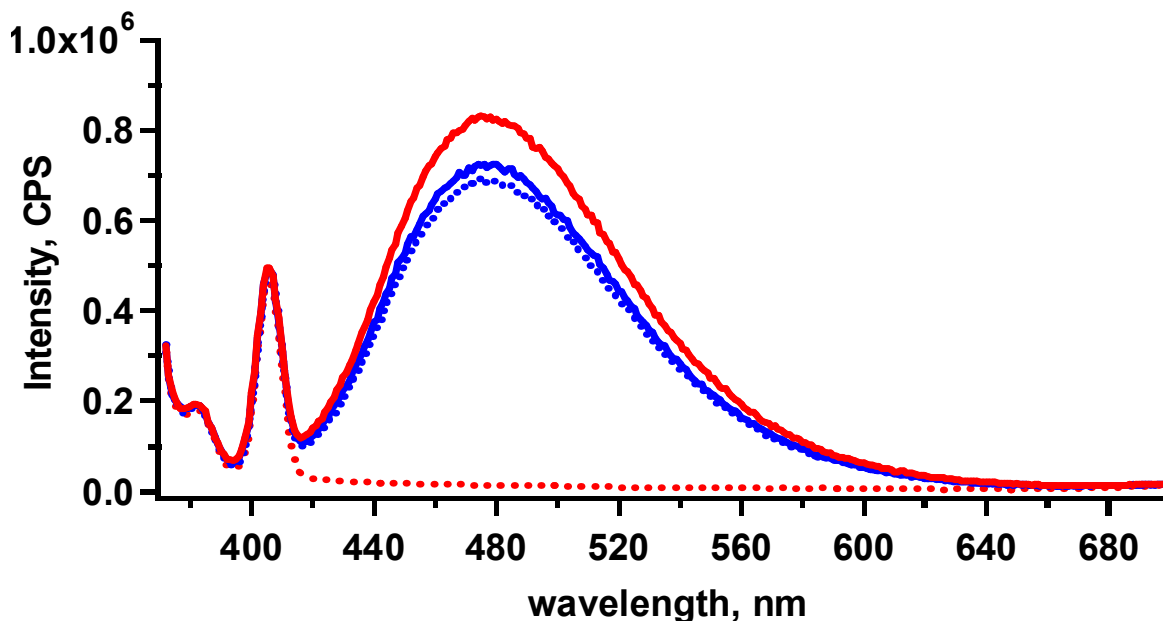


Figure S4 Emission spectra of panacyl bromide reacted with carboxyls of nitric acid treated SWCNTs: control experiment without SWCNTs (red solid line), after reaction with 29.1 mg SWCNTs (blue solid line), after reaction with 23.1 mg SWCNTs (blue dotted line); neat acetone (red dotted line). Excitation at 362 nm.

Fluorescence labeling of carbonyl groups

A solution of 0.115 mM of DH in methanol (JT Baker) was prepared in the dark. 30 ml of the solution was added into a 50 ml glass beaker which contained a sample of known mass and a Teflon coated magnetic stir bar. 1 ml of 0.1 M solution of HCl in methanol was added as a catalyst into the beaker. The reaction was run at room temperature ($\sim 25^{\circ}\text{C}$) in the dark for 62 hrs, while stirring. After the reaction, the supernatant (~ 20 ml) over the labeled SWCNTs was transferred into an empty

200ml flask. The sample was washed in the beaker with neat methanol (~20ml) and the supernatant transferred into the same 200ml flask. The washing of the sample (and transferring of the supernatant) was repeated several times (4-5times). The collected supernatant was diluted up to 200ml with methanol and then diluted with acetone by a factor of ~70 (700 μ l up to 50ml). Acetone was taken in order to react with unreacted DH, since Dansyl Hydrazine fluorescence changes upon binding. The fluorescence spectrum of the solution was recorded 3hrs. later (Excitation at 350nm). The preceding procedure was implemented twice for as-received SWCNTs (26.4mg and 23.9mg), Air/HCl treated SWCNTs (19.4mg and 15.0mg), and nitric acid treated SWCNTs (48.2mg and 56.3mg). The depletion of the fluorescence signal was measured with reference to the fluorescence intensity of the dye after the control experiment (labeling reaction with no sample in the beaker). (Figure 2, 3, 4, 5)

Fluorescence labeling of alcohol groups

Approximately 20 g of Na₂CO₃ (Fisher) were dissolved in water to get 1 L of solution with pH ~11 (SOL#1). 250 ml of 0.152 mM of 5-DTAF in SOL#1 was prepared in the dark. 30 ml of the solution was added into a 50 ml glass beaker which contained a sample of known mass and a Teflon coated magnetic stir bar. The reaction was run at room temperature (~25⁰C) in the dark for 24hrs, while stirring. After the reaction, the supernatant (~20 ml) over the labeled SWCNTs was transferred into an empty 200 ml flask. The sample was washed in the beaker with SOL#1 (~20 ml) and the supernatant transferred into the same 200 ml flask. The washing of the sample (and transferring of the supernatant) was repeated several times (4-5times). The collected supernatant was diluted up to 200ml with SOL#1 and then diluted with SOL#1 by a factor of 5000 (10 μ l up to 50ml). The fluorescence spectrum of the solution was recorded (excitation at 490 nm). The

preceding procedure was implemented twice for as-received SWCNTs (15.3mg and 15.6mg), Air/HCl treated SWCNTs (13.1mg and 13.4mg), and nitric acid treated SWCNTs (17.8mg and 21.2mg). The depletion of the fluorescence signal was measured and compared to the fluorescence intensity of the dye after the control experiment (labeling reaction with no sample in the beaker). (Figure S5, S6, S7, S8)

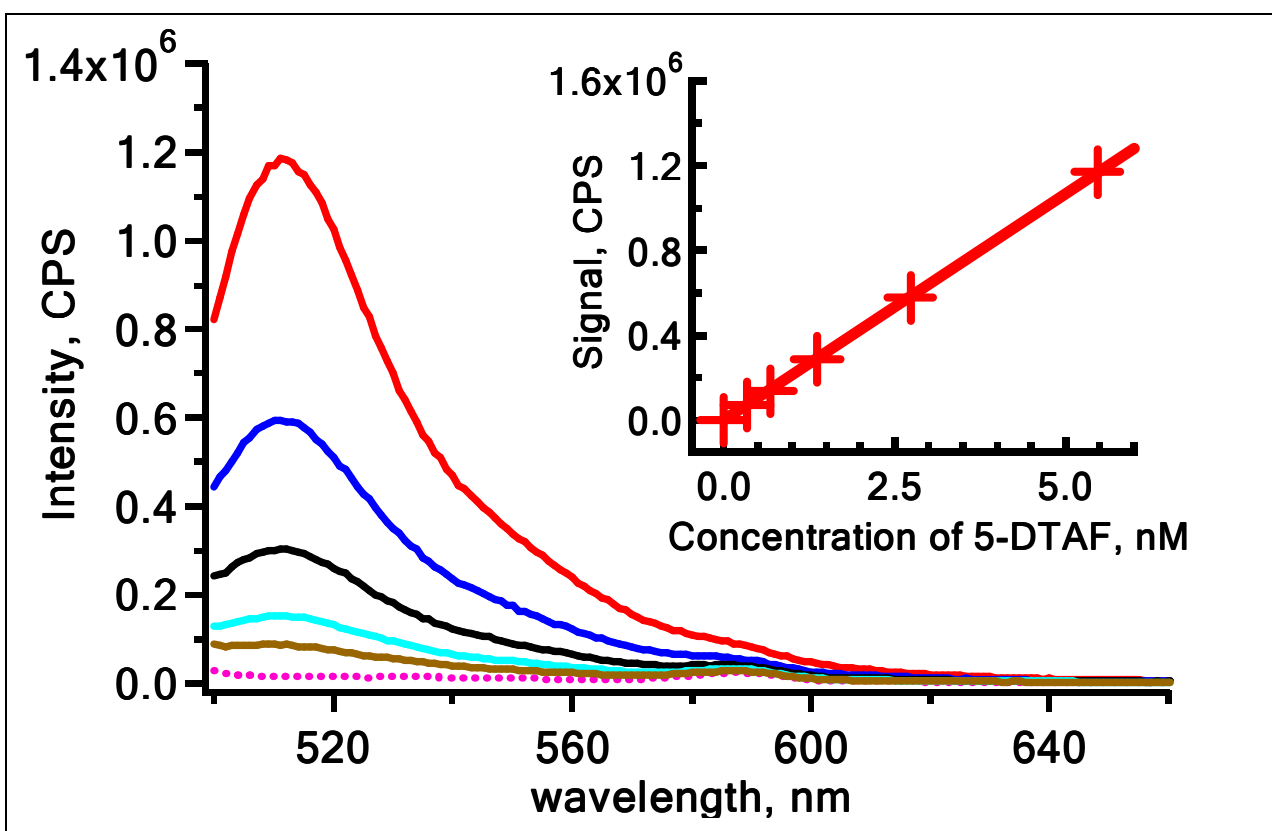


Figure S5. Emission spectra of 5-DTAF of different concentrations: 5.5nM (red solid line), 2.7nM (blue solid line), 1.4nM (black solid line), 0.7nM (cyan solid line), 0.3nM (brown solid line); Na₂CO₃+H₂O (pH~11) (red dotted line). Excitation at 490nm. (Inset: calibration plot of fluorescence intensity at 511nm vs. 5-DTAF concentration)

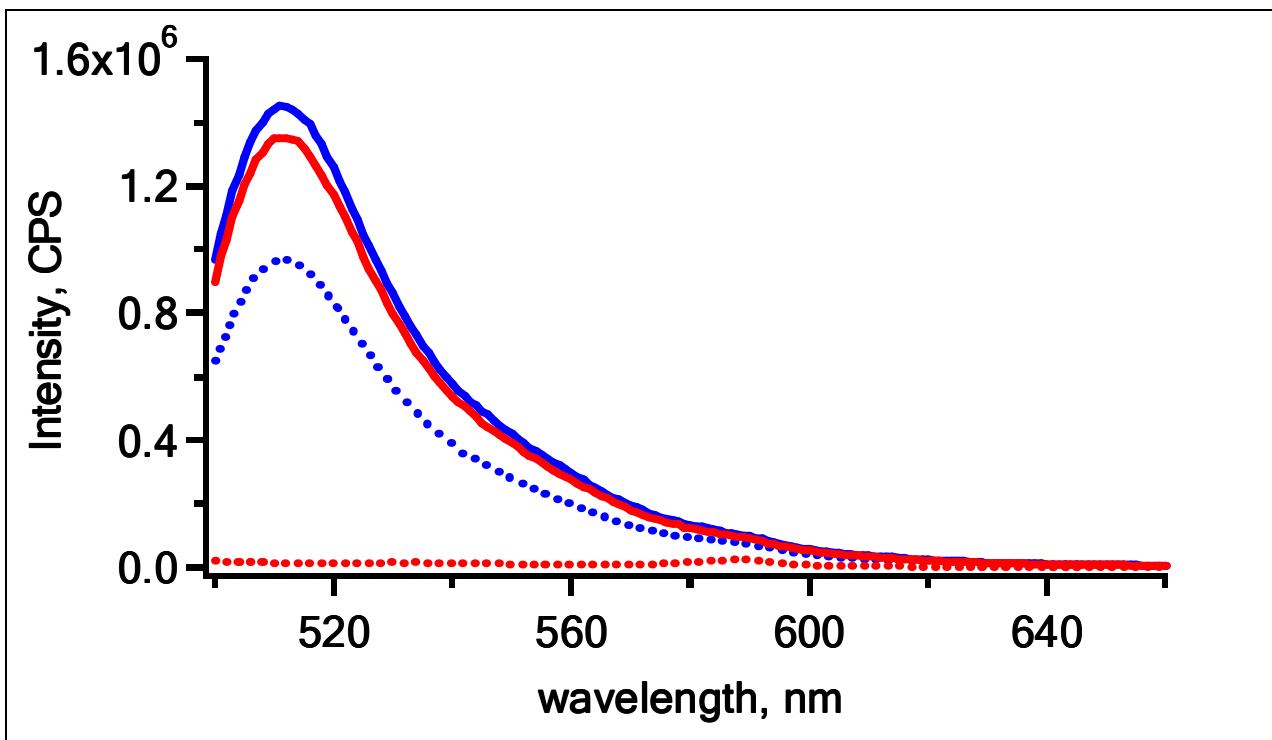


Figure S6. Emission spectra of 5-DTAF reacted with hydroxyls of as-received SWCNTs: control experiment without SWCNTs (red solid line), after reaction with 15.3mg SWCNTs (blue solid line), after reaction with 15.6mg SWCNTs (blue dotted line); Na₂CO₃+H₂O (pH~11) (red dotted line). Excitation at 490 nm.

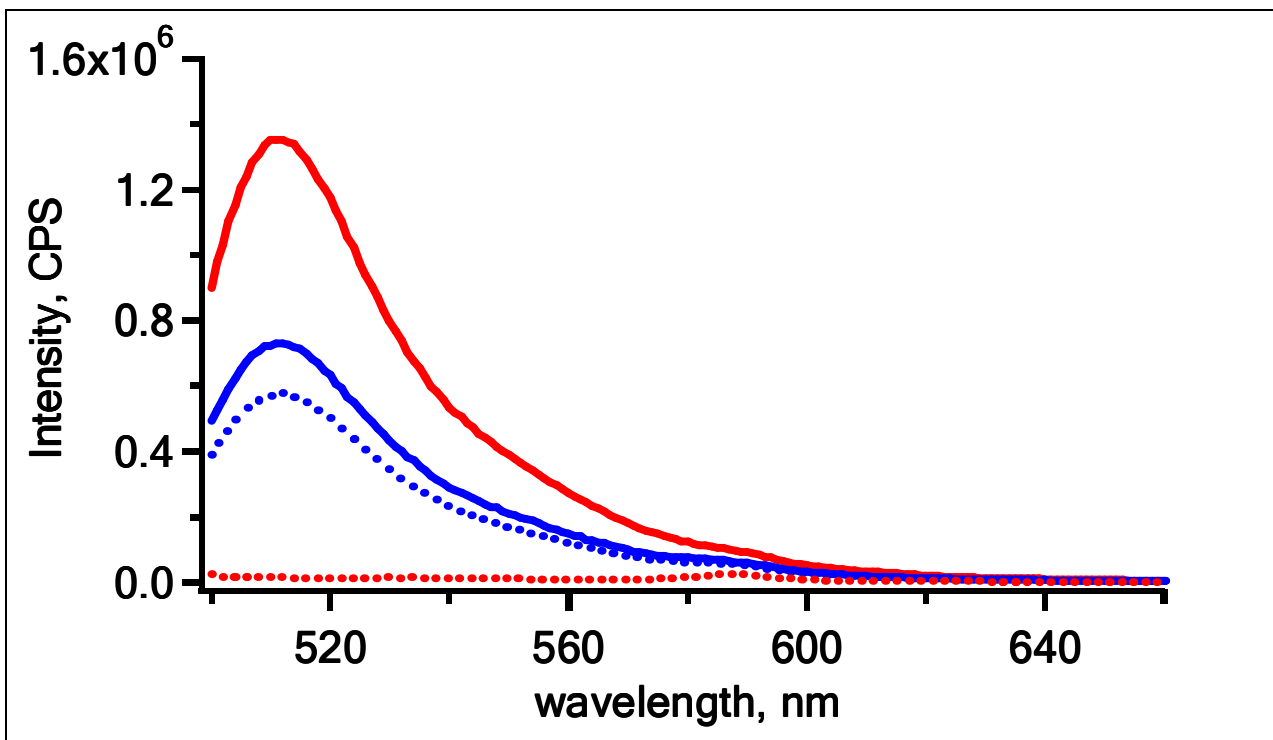


Figure S7. Emission spectra of 5-DTAF reacted with hydroxyls of Air/HCl treated SWCNTs: control experiment without SWCNTs (red solid line), after reaction with 13.1mg SWCNTs (blue solid line), after reaction with 13.4mg SWCNTs (blue dotted line); $\text{Na}_2\text{CO}_3 + \text{H}_2\text{O}$ (pH~11) (red dotted line). Excitation at 490 nm.

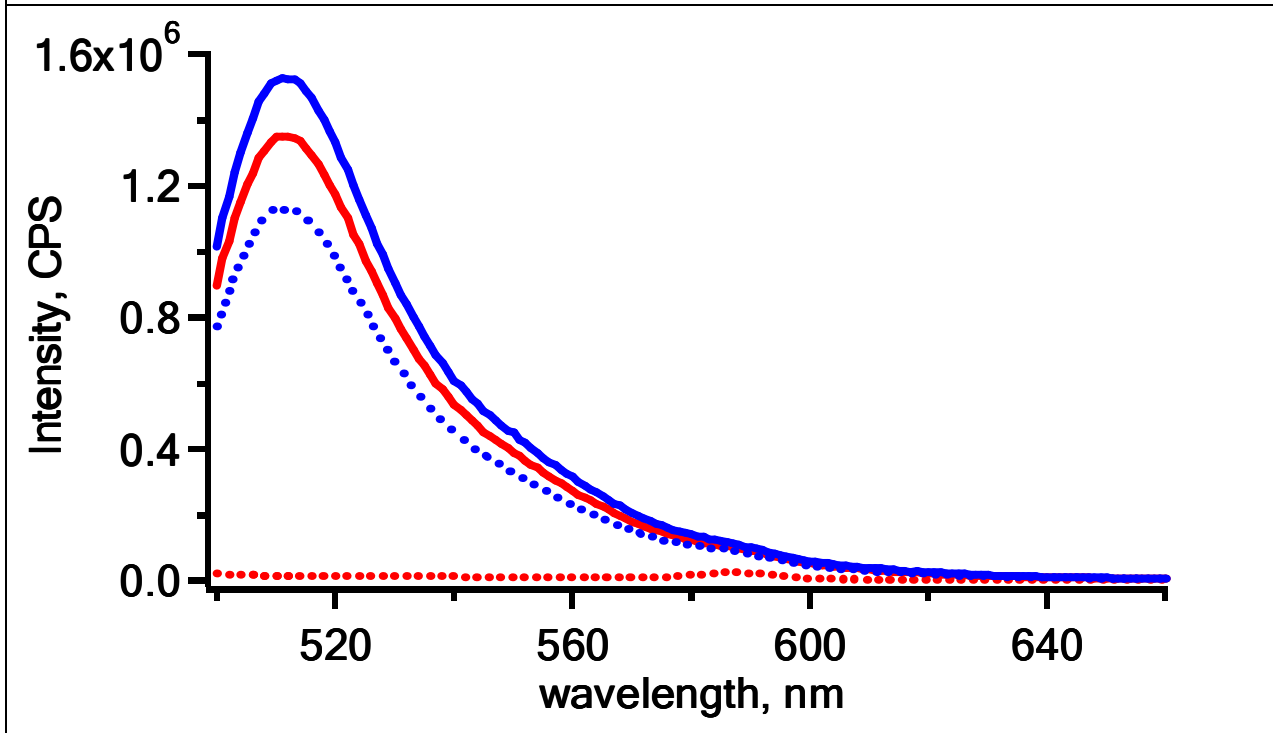


Figure S8. Emission spectra of 5-DTAF reacted with hydroxyls of nitric acid treated SWCNTs: control experiment without SWCNTs (red solid line), after reaction with 17.8mg SWCNTs (blue solid line), after reaction with 21.2mg SWCNTs (blue dotted line); Na₂CO₃+H₂O (pH~11) (red dotted line). Excitation at 490 nm.

It is worth noting that the labeling of –OH groups is not as straightforward as the labeling of other functional groups (e.g., carbonyls and carboxyls) on SWCNTs. Dansyl Chloride (DC) (5-dimethylaminonaphthalene-1-sulfonyl chloride, Molecular Probes), the dye, originally chosen to label –OH groups in this study, showed extremely high reactivity with water (both, from the ambient and the solvent). Thus, DC was abandoned in favor of 5-DTAF. To the best of our knowledge, 5-DTAF is the only dye which selectively reacts with –OH groups of alcohols without concomitant reaction with –OH groups of water (provided pH~9-11). However, 5-DTAF exhibited strong physisorption toward SWCNTs, significantly affecting the FLOSS results for –OH detection. Thus the results in Table 3 should be considered upper limits rather than absolute values. We hypothesize that the fluorescein-like skeleton of 5-DTAF is responsible for the strong π – π interaction of the dye molecules with SWCNTs. This speculation is consistent with our observations (results not shown) of the strong non-specific adsorption of dye F-121 (fluorescein-5-thiosemicarbazide, Molecular Probes) on SWCNTs, which (F-121) also possess a fluorescein-like structure. F-121 was

originally used to label carbonyls and was finally abandoned in favor of Dansyl Hydrazine. Even though in some applications (sensors, light-harvesting systems, composites, etc.) strong non-specific adsorption of the moieties to SWCNTs might be appreciated, the fluorescein-like species, in our opinion, should be avoided when covalent attachment, unobscured by physisorption, is needed (e.g. FLOSS).

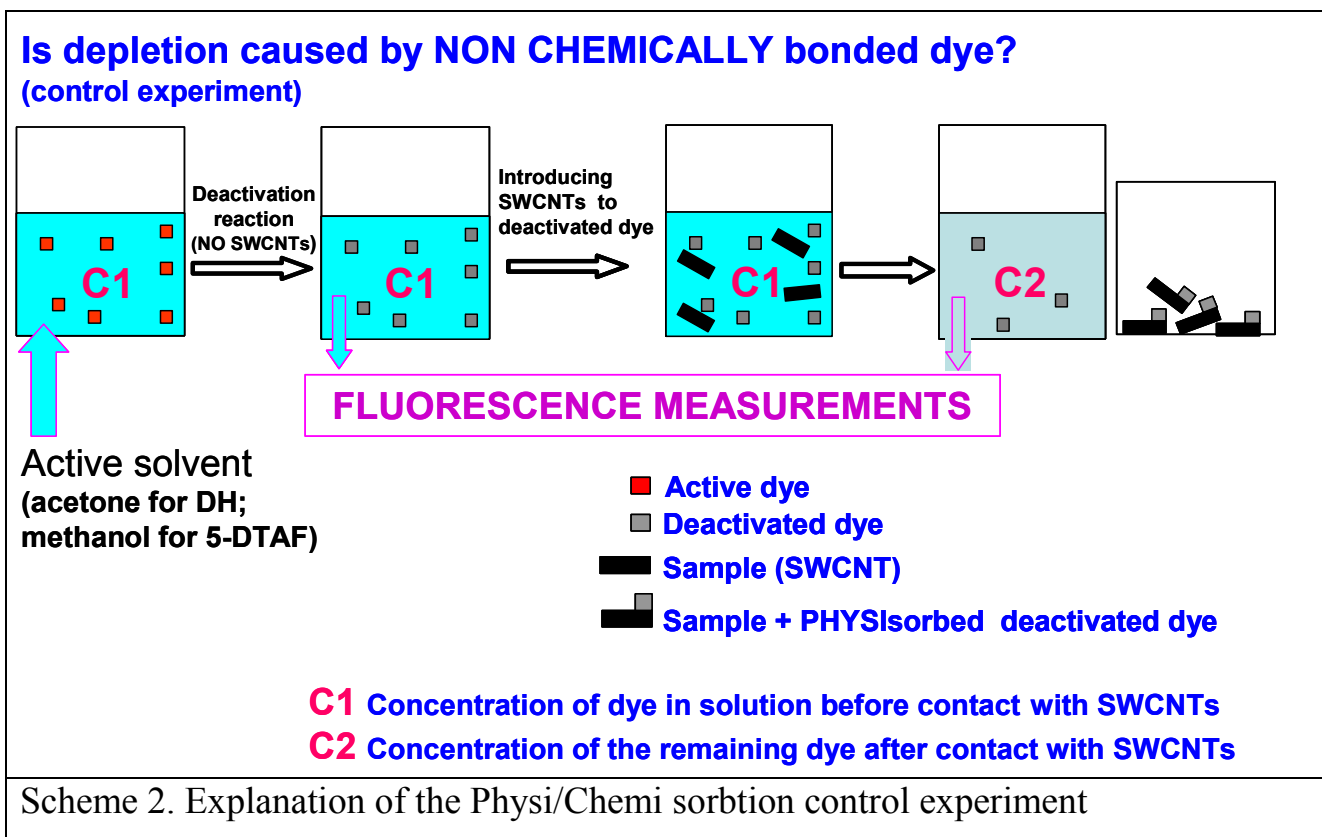
Surprisingly, for two samples, FLOSS labeling of –OH groups with 5-DTAF resulted in negative values of depletion (-5.8×10^{12} groups/cm² for as-received and -1.3×10^{14} groups/cm² for HNO₃ treated SWCNTs, Table 2, Figures S6, S8). Enhancement of the intensity of the fluorescence in these cases might be explained by the presence of some fraction of the labeled SWCNTs in the analyzed supernatant. SWCNTs due to their high absorption cross section may possibly increase quantum yields of the fluorophore molecules been attached to the nanotubes. The explanation is consistent with the visual observation of the dark (but transparent) supernatants after the 5-DTAF labeling reactions.

The phenomenon of negative depletion was also observed for 5-DTAF labeled Multi Walled Carbon Nanotubes (MWCNTs) (paper in preparation) and is under the further investigation.

Control experiments

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In order to check if depletion after the covalent attachment of the labels is not obscured by physisorption of the dyes to samples, a series of control experiments was run. Fresh (non-labeled) samples were subject to contact with deactivated (i.e. pre-reacted) dyes for the period of the FLOSS reaction time (or longer).(Scheme 2) Control experiments did not show any significant depletion for DH and PB dyes, but did show depletion for 5-DTAF (Figure S2, S9, S10, S11, S12, S13, S14)



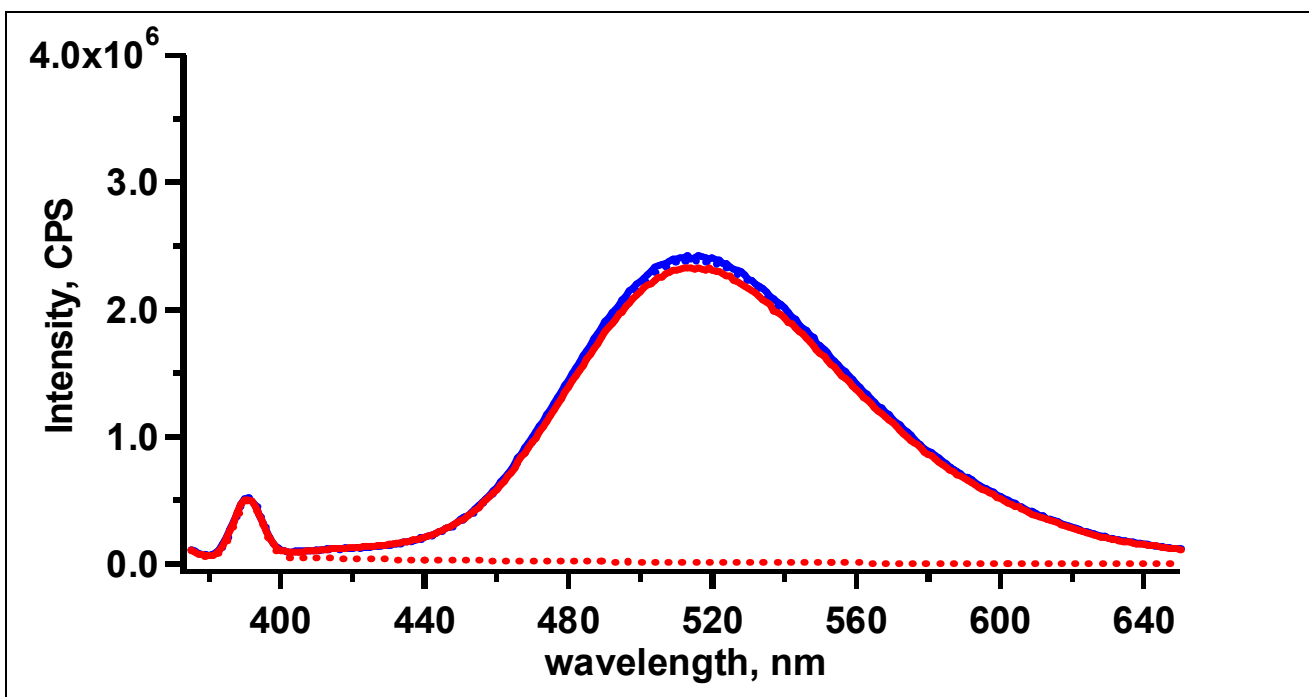


Figure S9. Emission spectra of deactivated dansyl hydrazine after contact (~144 hr.) with as-received SWCNTs: control experiment without SWCNTs (red solid line), after contact with 22.3mg SWCNTs (blue solid line), after contact with 23.9mg SWCNTs (blue dotted line); acetone (red dotted line). Excitation at 350 nm.

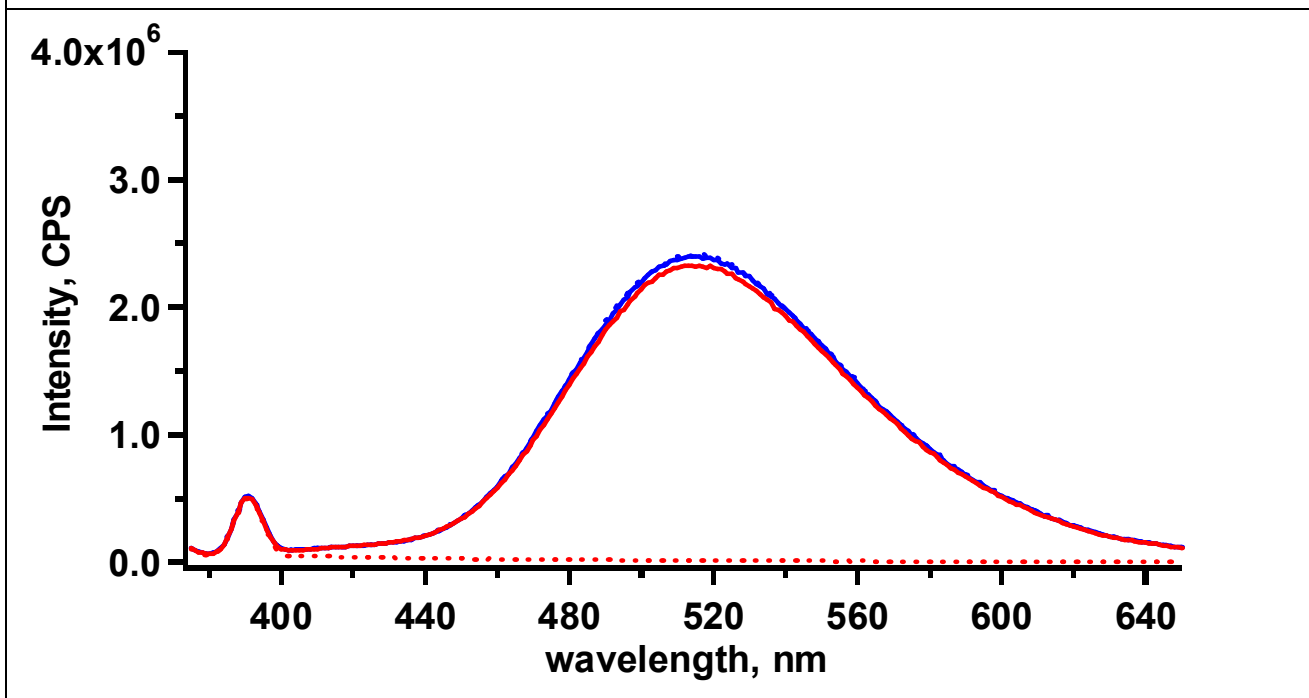


Figure S10. Emission spectra of deactivated dansyl hydrazine after contact (~144 hr.)

with Air/HCl treated SWCNTs: control experiment without SWCNTs (red solid line), after contact with 16.1mg SWCNTs (blue solid line), after contact with 16.5mg SWCNTs (blue dotted line); acetone (red dotted line). Excitation at 350 nm.

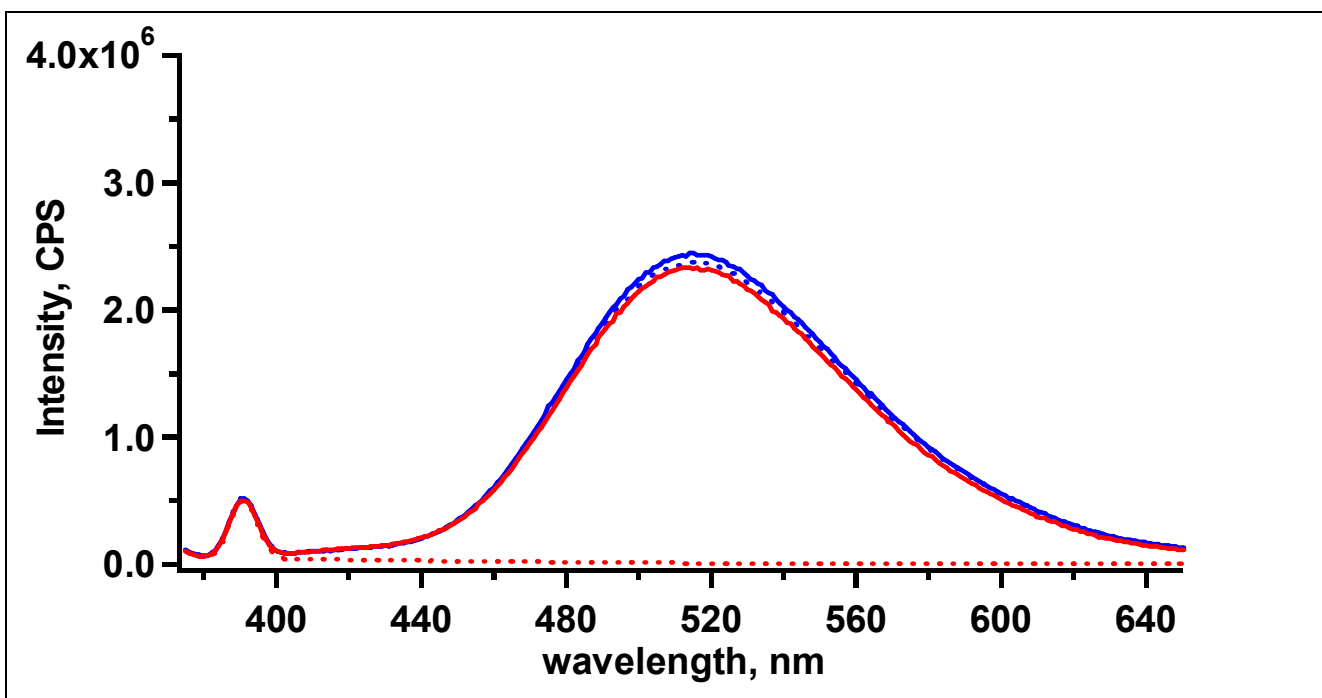


Figure S11. Emission spectra of deactivated dansyl hydrazine after contact (~144 hr.) with nitric acid treated SWCNTs: control experiment without SWCNTs (red solid line), after contact with 45.5mg SWCNTs (blue solid line), after contact with 54.4mg SWCNTs (blue dotted line); acetone (red dotted line). Excitation at 350 nm.

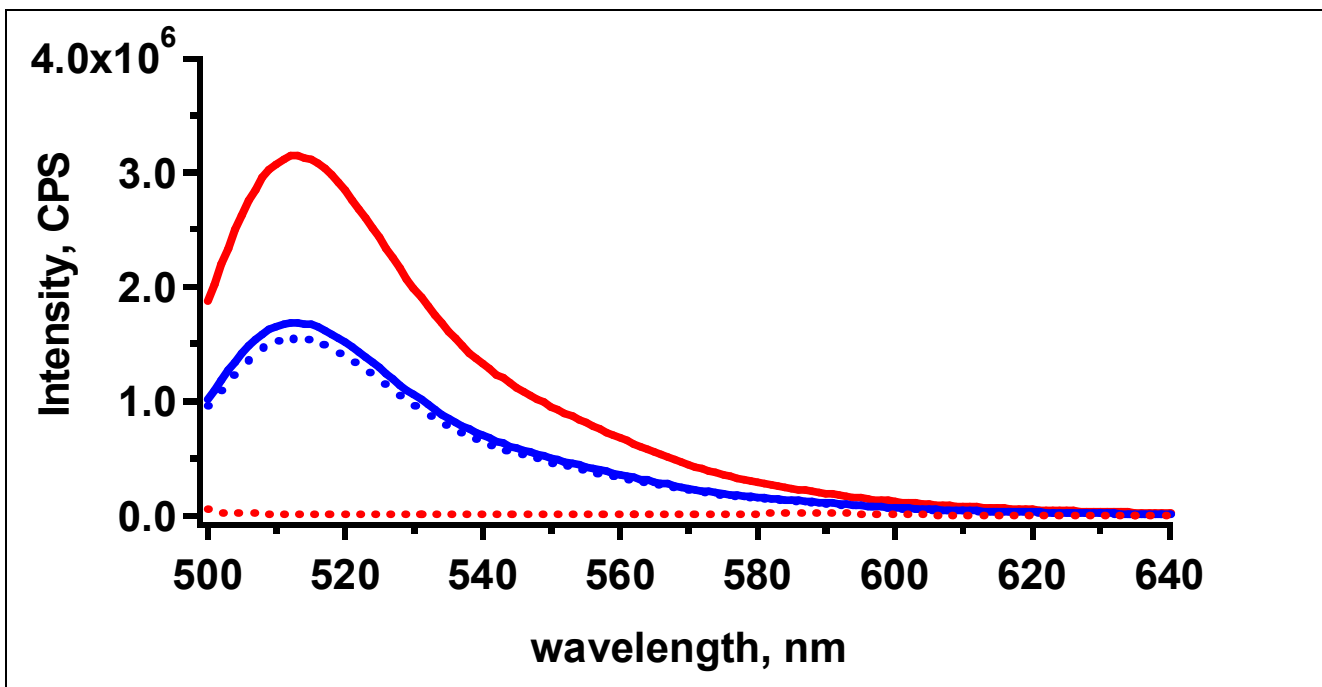


Figure S12. Emission spectra of deactivated 5-DTAF after contact (~24 hr.) with as-received SWCNTs: control experiment without SWCNTs (red solid line), after contact with 14.8mg SWCNTs (blue solid line), after contact with 15.7mg SWCNTs (blue dotted line); Na₂CO₃+H₂O+MeOH (red dotted line). Excitation at 490 nm.

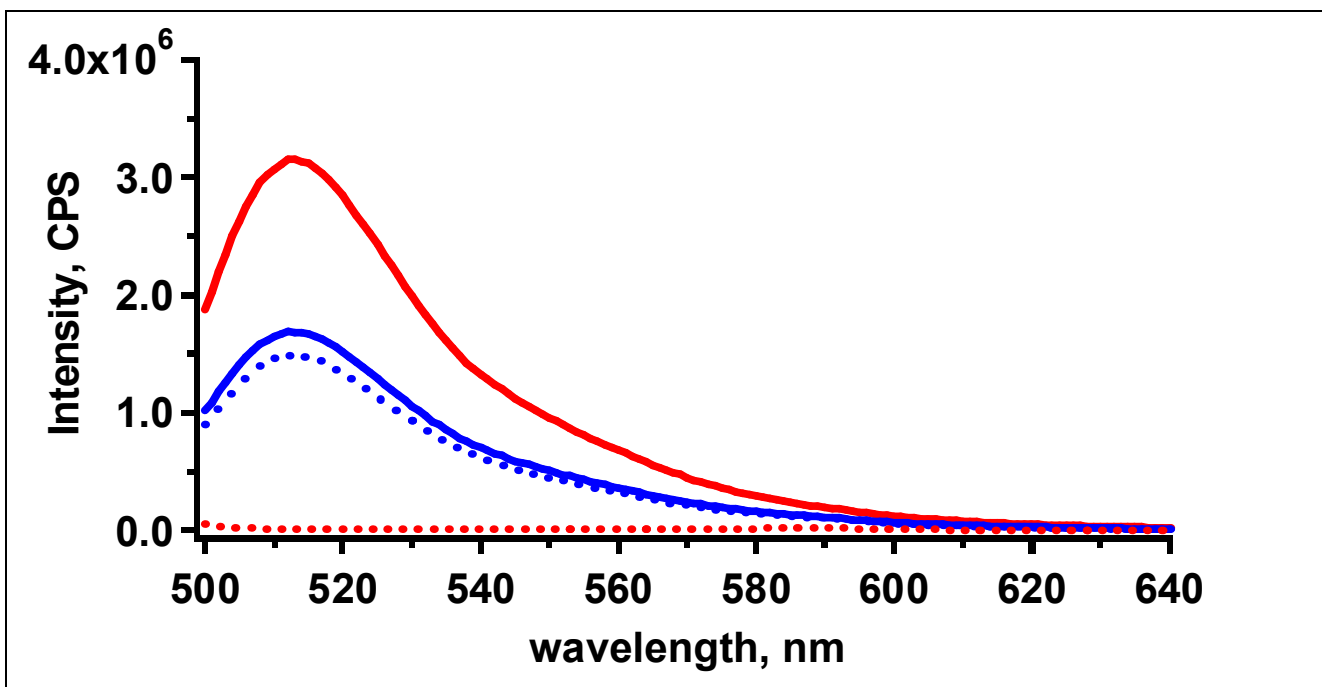


Figure S13. Emission spectra of deactivated 5-DTAF after contact (~24 hr.) with as

Air/HCl treated SWCNTs: control experiment without SWCNTs (red solid line), after contact with 8.6mg SWCNTs (blue solid line), after contact with 11.5mg SWCNTs (blue dotted line); Na₂CO₃+H₂O+MeOH (red dotted line). Excitation at 490 nm.

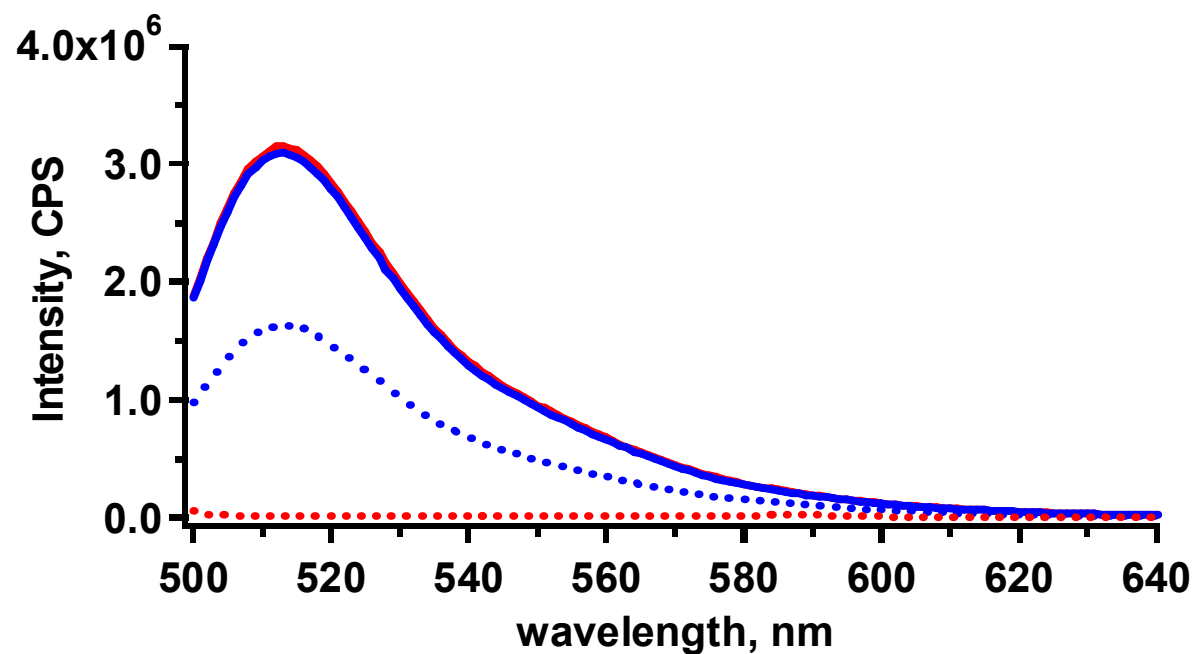


Figure S14. Emission spectra of deactivated 5-DTAF after contact (~24 hr.) with nitric acid treated SWCNTs: control experiment without SWCNTs (red solid line), after contact with 18.4mg SWCNTs (blue solid line), after contact with 18.0mg SWCNTs (blue dotted line); Na₂CO₃+H₂O+MeOH (red dotted line). Excitation at 490 nm.

The supernatant inevitably contains some SWCNTs in suspension (~0.0003 mg/ml at most), that could modify the observed fluorescence of the dye solution. For example, the fluorophores covalently attached to the SWCNT could add to fluorescence of free dye in solution, leading to an underestimation of the depletion. In addition, some dye may physisorb to the SWCNT, with a resultant reduction in fluorescence if adsorption is accompanied by quenching, leading to an overestimation of depletion. We estimate, based on the concentration of SWCNTs left in suspension and the density of functional groups, that the fluorescence from

covalently bound dyes on SWCNT remaining in suspension could at most contribute to 0.01% of the observed fluorescence of the supernatant.

We performed control experiments where solutions with and without SWCNTs were prepared with identical dye concentrations to assess the effect residual SWCNTs on fluorescence. We observe a 20-60 % decrease, depending on the type of SWCNT, in fluorescence from physisorbed dye in the case of 5-DTAF (label for hydroxyl groups), and less than 7% in the case of panacyl bromide (label for carboxylic groups) and dansyl hydrazine (label for carbonyls). Thus, for the labeling reactions involving panacyl bromide and dansyl hydrazine, FLOSS results apparently are not significantly affected by the presence of small quantities of SWCNTs in the supernatant. The measurements with 5-DTAF, however, are more significantly affected with an apparent overestimate of depletion that could result in overestimates of the surface hydroxyl groups by a factor of 2 in some cases. This compounds the problems associated with 5-DTAF physisorption.

Time control experiments

To check if the duration of the labeling reaction was chosen correctly, labeled samples were subject to contact with a fresh portion (i.e. non-reacted) of the solution of each of the dyes. Second run did not show significant additional depletion, which means that the reaction time is long enough the functional groups to be labeled. (Figure S15, S16)

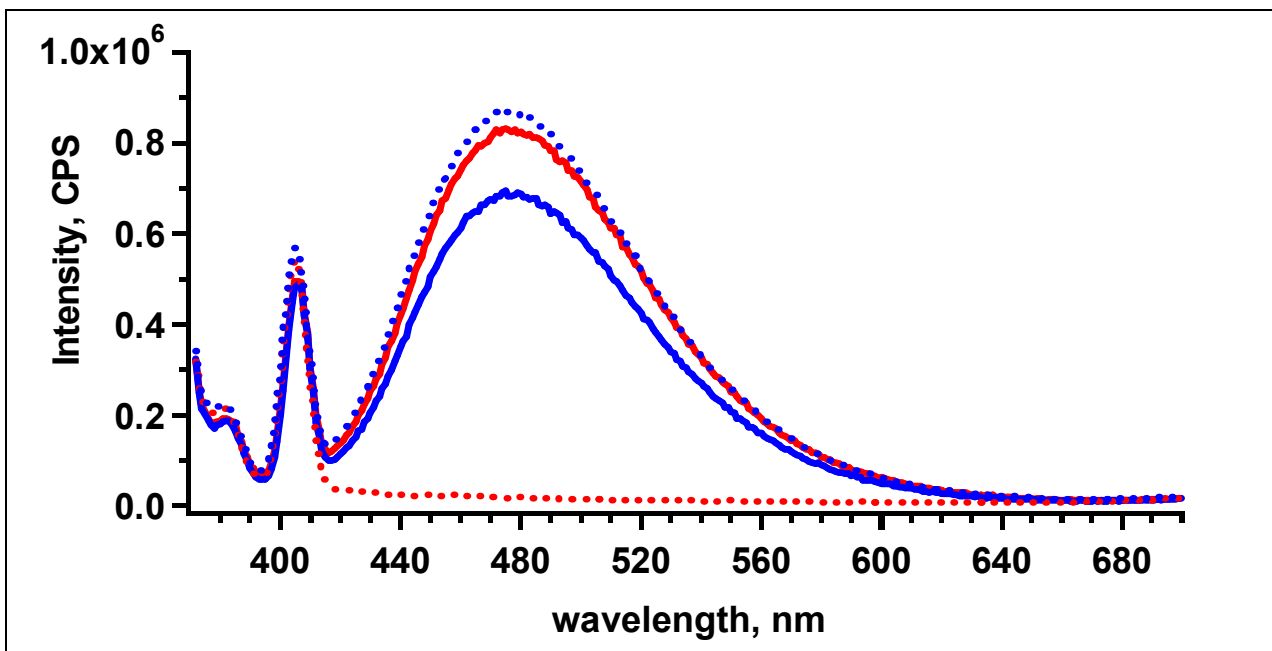


Figure S15. Emission spectra of Panacyl Bromide after first run of the reaction with 29.1mg of non-labeled nitric acid treated SWCNTs for 3.5hrs. (blue solid line) and second run with 29.1mg of labeled nitric acid treated SWCNTs for 3.5hrs. (blue dotted line); control experiment without SWCNTs (red solid line), acetone (red dotted line). Excitation at 362nm.

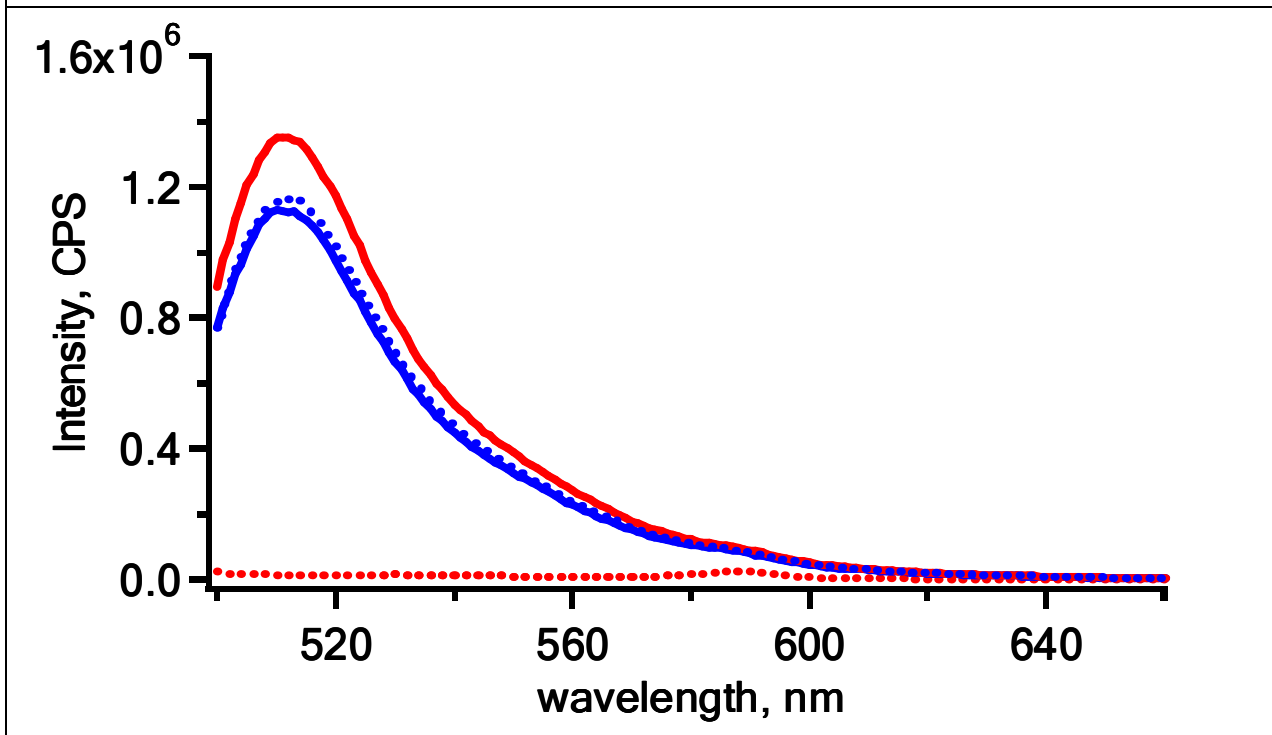


Figure S16. Emission spectra of 5-DTAF after first run of the reaction with 21.2mg

of non-labeled nitric acid treated SWCNTs for 24hrs. (blue solid line) and second run with 21.2mg of labeled nitric acid treated SWCNTs for 7days (blue dotted line); control experiment without SWCNTs (red solid line), $\text{Na}_2\text{CO}_3+\text{H}_2\text{O}$ (pH~11) (red dotted line). Excitation at 490nm.

Thermogravimetric analysis (TGA)

TGA was run for each of the samples (i.e. as-received, Air/HCl treated, nitric acid treated SWCNTs), heat rate $10^\circ\text{C}/\text{min.}$; 20ml/min of air flow. TGA curves represent the dynamics of weight loss of the sample with increasing temperature.[1] The presence of the multiple peaks or dips on TGA and DTG curves of a complex sample, comprised of different components, usually indicate distinct temperature ranges of weight loss (e.g. due to combustion) for the different components. Thus, each region with constant, non zero slope on TGA curve is associated with the transformation a certain component of the system.[1] In the case of SWCNTs, the low temperature region ($25\text{-}300^\circ\text{C}$) weight loss is related to the desorption of physisorbed water. In the same range of the temperatures, metal catalyst impurities are oxidized, leading to weight gain, and, sometimes, to the appearance of a small peak on TGA curve (peak at 150°C in Fig.S19). The region of biggest weight loss ($500\text{-}700^\circ\text{C}$) is associated with burning of carbonaceous impurities and, partially, carbon nanotubes, while the high temperature region ($700\text{-}900^\circ\text{C}$) is mostly related to the loss of the carbon nanotubes.

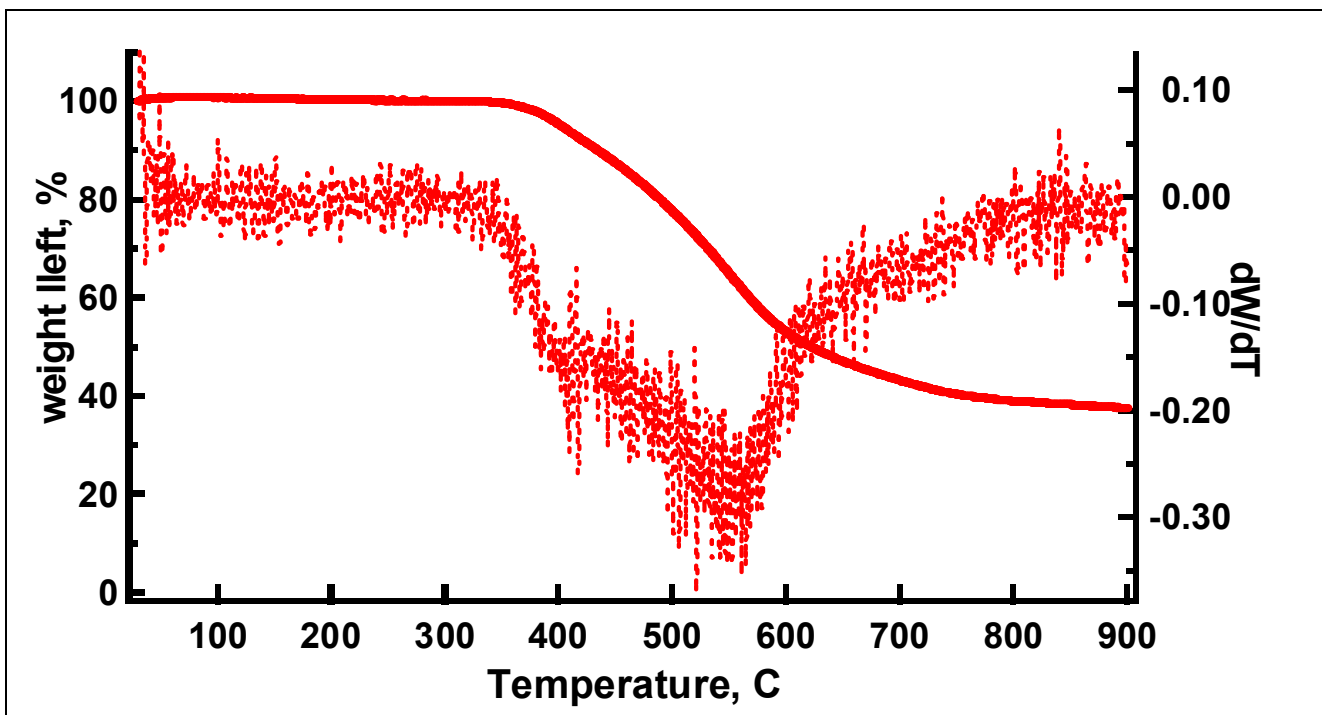


Figure S17. Thermogravimetric (TGA, red solid line) and differential rate (DTG, red dotted line) curves for as-received SWCNTs

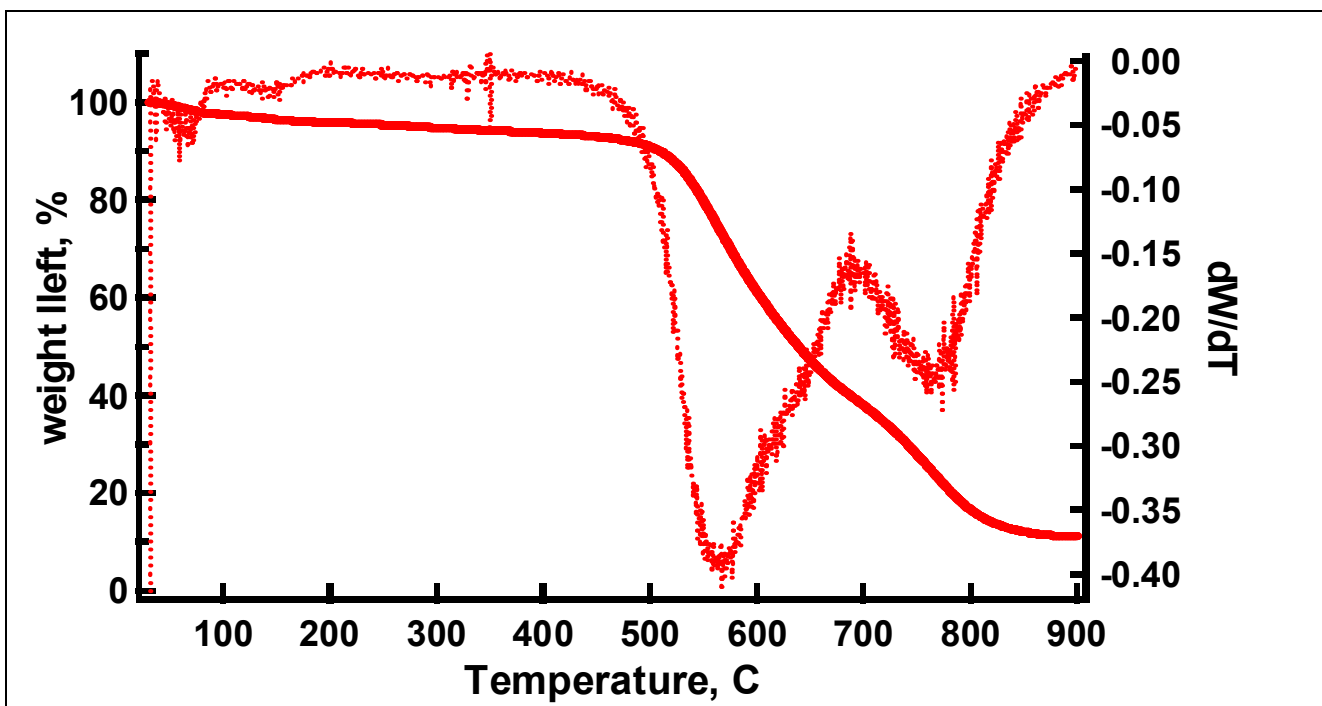


Figure S18. Thermogravimetric (TGA, red solid line) and differential rate (DTG, red

dotted line) curves for Air/HCl treated SWCNTs

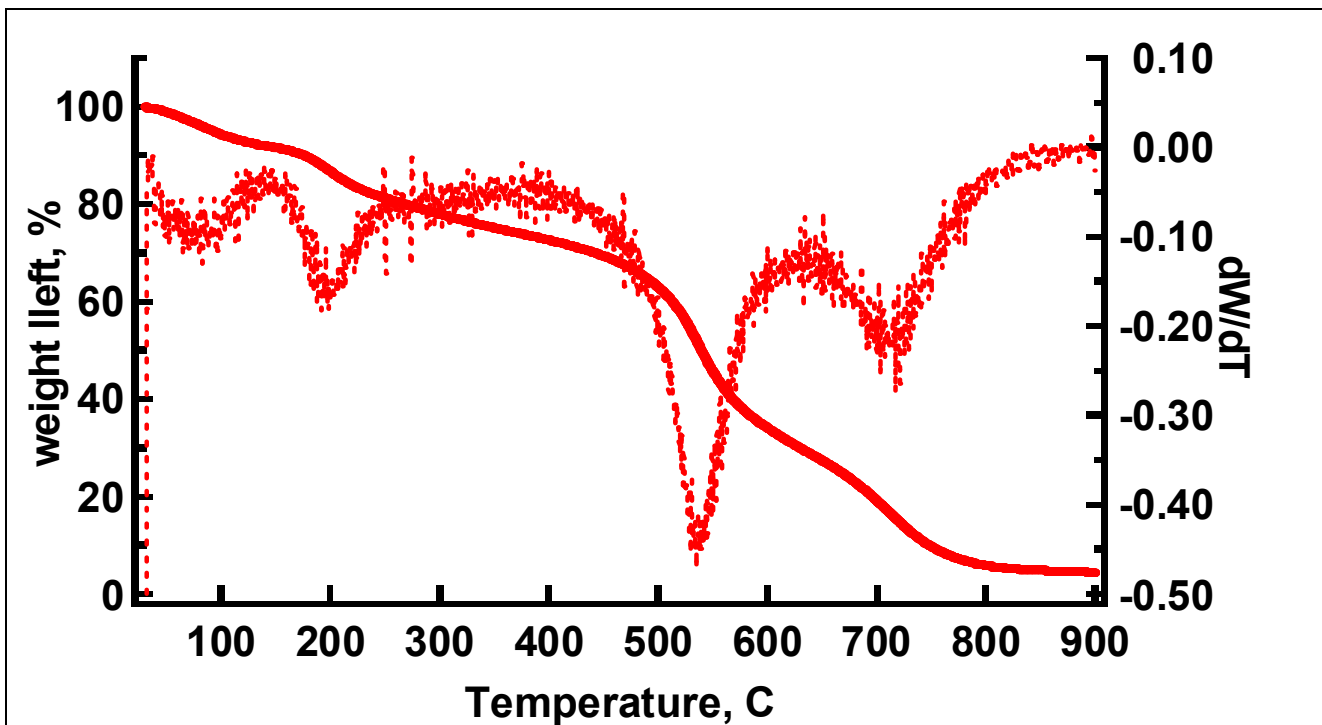


Figure S19. Thermogravimetric (TGA, red solid line) and differential rate (DTG, red dotted line) curves for nitric acid treated SWCNTs

References

1. Itkis, M.E., et al., *Comparison of analytical techniques for purity evaluation of single-walled carbon nanotubes*. Journal Of The American Chemical Society, 2005. **127**(10): p. 3439-3448.