Environmental Science and Technology: June 16th 2010.

Supporting Information for:

Identifying Components in Dissolved Humic Acid that Bind Organofluorine Contaminants using $^{1}H\{^{19}F\}$ Reverse Heteronuclear Saturation Transfer Difference NMR Spectroscopy

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This supporting information contains 6 pages and 2 Figures.

S1: Integrations of RHSTD Spectra

The constituents of a humic acid extract are derived from complex biomolecules and their degradation products. The ^{1}H NMR spectra of those components, even in isolated forms are similarly complex, with signals typically spread across the entire chemical shift range. This means that simple deconvolutions are not easily performed because the signals in any chemical shift region arise from multiple components. In cases where a single constituent dominates the signal in any one region a semi-quantitative analysis can be performed by focusing on that one peak, or spectral region. This is the case for lignin and lignin-derived material in peat humic acid, which gives rise to most of the aromatic signal in the ^{1}H spectra. Protein is not as easily separated out from a total humic acid spectra, however the peak at 0.8 ppm is dominated by methyl groups from proteins in this particular sample (*1-2*). In the perfluoronaphthol spectra, complete isolation of this peak from the neighboring aliphatic peak is not possible because of overlap. Lignin also contributes some signals to the spectra in this region. Nevertheless, integration of the RHSTD spectra in terms of the total signal, compared to the contributions from aromatic and aliphatic regions is informative.

Integrations of the total RHSTD spectra at different organofluorine loading ratios show that both organofluorine compounds exhibit a strong initial rise in total RHSTD signal as the loading concentration increases shown in figure S1. For perfluorooctanoic acid, this initial rise is followed by a plateau. In the RHSTD Spectra itself (Fig. 2), the qualitative line-shape does not change for perfluorooctanoic acid after this inflection

point. For perfluoronapahthol, the initial rise is followed by a more gradual rise that continues at increasing concentrations.

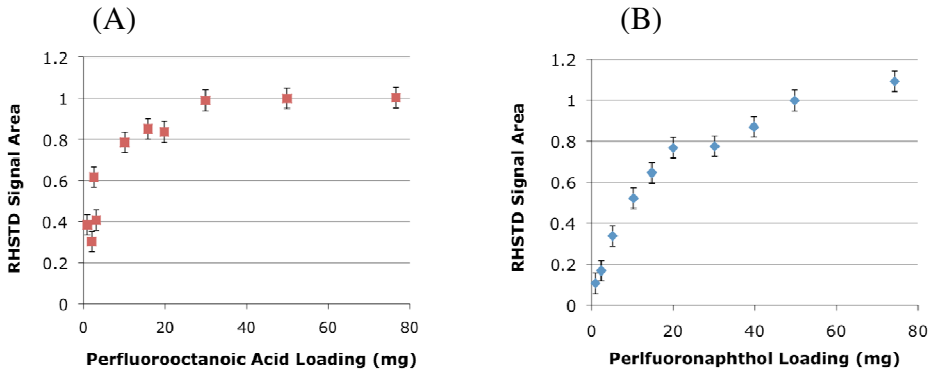


Figure S1. Total RHSTD signal of humic acid as a function of different loading concentrations of (A) perfluorooctanoic acid, and (B) perfluoronaphthol. Integrations are scaled to the 50 mg loading of each compound, respectively.

Integration of the aromatic region, as shown in figure S2A, shows that the signal from aromatic alone, which is predominantly from lignin-derived components, does not continue to increase after the inflection point signifying a saturation of available interaction sites. The aliphatic region continues to increase in intensity as the concentration increases. For the initial concentrations up to the inflection point, the signal in the aromatic region increase at a faster rate suggesting that interactions with aromatic material are preferred over those of aliphatic material. It is important to note that because the integration of the aliphatic material (protein, aliphatics, and lignin) is not as domain specific as the integration of the aromatic material (mostly lignin), some of that rise is due to interactions not at aliphatic sites, such as lignin, meaning that the slope shown is a maximum, and the true extent of interaction at aliphatic and protein-derived components is less than that suggested by the integrations.

Also showing the preference for aromatic material over aliphatic material by perfluoronaphthol is figure S2B, which relates the changes in signal at different regions of the RHSTD spectra relative to a quantitative reference ^{1}H spectrum of the same sample. The relative signal is calculated using

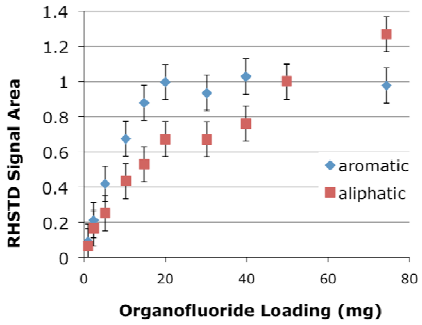
$$\text{Relative signal} = (S - S_o)/S_o \quad (1)$$

where S is the % contribution of the region of interest to the total integral of the RHSTD spectrum and S_o is the same value for a quantitative reference ^{1}H spectrum. Overall, the

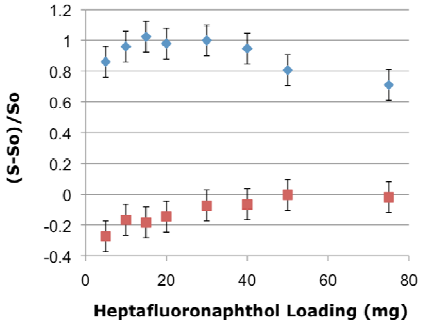
aromatic region gives a positive result, signifying that this region contributes more to the RHSTD spectrum than it does in the reference spectrum, whereas the aliphatic region generally gives a negative result, signifying that this region gives less signal in the RHSTD spectrum than the same region in the reference spectrum. The aliphatic region gradually increases as the perfluoronapthhol concentration increases signifying that interactions at these domains gradually become more important, where as the aromatic region increases slightly at the beginning, then holds steady, after which it starts to decline, signifying that interactions at another region are becoming slightly more important.

(1) Kelleher, B.P.; Simpson, A.J. Humic substances in soils: are they really chemically distinct? *Environ. Sci. Technol.* **2006,** *40* (15)*,* 4605-4611.

(2) Simpson, A.J.; Simpson, M.J.; Smith, E.; Kelleher, B.P. Microbially derived inputs to soil organic matter: are current estimates too low? *Environ. Sci. Technol.* **2007,** *41* (23)*,* 8070-8076.



(A)



(B)

Figure S2. Semi-quantitative deconvolutions of the perfluoronaphthol RHSTD spectra as a function of loading (mg). (A) Absolute signal at aromatic and aliphatic domains. (B) Normalized signal area of aromatic and aliphatic signal regions relative to the same signals in a quantitative reference spectrum. Aromatic is blue diamonds, aliphatic is red squares.