Supplementary Information for:

Accessibility of humic-associated Fe to a microbial siderophore: implications for bioavailability

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Contents: 12 total pages including 4 tables and 1 figures

1. Description of dissolved organic matter samples and isolation.
2. Detailed method development for metal removal experiments.

1. Detailed Description of Dissolved Organic Matter Samples and Isolation.

Aquatic organic matter (OM) isolated by our group from three blackwater rivers by reverse osmosis (RO) and sorption to XAD-8 and -4 (for two rivers) resins was used in DFOB-mediated metal removal experiments (see Table S-1 for a list of isolates by source environment). OM isolates were collected in the Spring of 1998 from a stream in McDonalds Branch (MDB) basin, a small freshwater fen in the New Jersey Pine Barrens (USA).[1] Water was collected from two hydrologically distinct sites: an upstream site draining a hardwood swamp that was a potential ground-water recharge zone (Site 2; S2) and a downstream site draining an Atlantic White Cedar swamp that was a potential ground-water discharge zone (Site 10; S10). OM also was isolated from Nelson's Creek (NLC), a first order stream in the Ottawa National Forest (Michigan, USA), in the Summer of 2002.[2] The upland region of the NLC drainage basin contains northern

hardwood forests, while the wetland region sampled contains both deciduous and evergreen plants. Finally, OM was isolated in June 2012 from the Suwannee River (SR) in southeastern Georgia (USA), a blackwater stream draining the Okefenokee Swamp and the source of many standards and reference samples made available by the International Humic Substances Society (IHSS).[3] All of the OM samples were freeze-dried upon collection/isolation and stored in a desiccator prior to use in experiments.

RO has been used to concentrate a broad range of aquatic NOM from surface waters as it has a high recovery, thus results in little DOM fractionation.[4] At each location, RO isolates were created on-site using a RealSoft PROS/IS system build by E.M. Perdue (previously described in detail[4,5]). Briefly, raw surface water was pumped through a series of three high volume cartridge filters of decreasing pore size (10, 1, and 0.4 micron) and a column of Chelex 100® cation exchange resin (Na^+ form) that removed a substantial portion of coagulation-inducing metals such as Ca^{2+}, Fe^{3+}, and Al^{3+} to prevent membrane fouling. After passing through the RO system, the liquid retentate was stored on ice then refrigeration, in amber glass bottles, lyophilized and stored in a desiccator.

To isolate and fractionate raw filtered surface water via sorption to macroporous XAD resins, surface water was collected from NLC, MDB, and SR and filtered through the same filters used for RO isolations. The filtrate was transported in high-density polyethylene carboys on ice to G. Aiken's U.S. Geological Survey laboratory (Boulder, Colorado, USA). Details of the XAD isolation procedures can be found elsewhere.[6] Briefly, water samples were acidified to pH 2.0 with HCl and passed through a two-column setup containing Amberlite XAD resins—an XAD-8 resin to remove the

48 “hydrophobic acid” fraction followed by an XAD-4 resin to remove the “transphilic acid”
49 fraction. The organic material sorbed to the resins was back-eluted from each of the
50 columns using 0.1 M NaOH. Liquid isolates were freeze dried and stored in desiccators.

51 The number- and weight- average molecular weights (M_n and M_w, respectively)
52 and polydispersities (ρ) of chromophoric OM components (Table S-1) were calculated
53 from UV/Vis fractograms (λ=254 nm) from the AsFlFFF system using previously
54 published methods.[7] Absorbance at λ=254 nm has been widely used as a surrogate for
55 [DOC] in HS-rich waters[8] and tends to be highly sensitive and reproducible for small
56 samples (such as come off the AsFlFFF) at low [DOC]; direct measurement of [DOC]
57 was found to be impractical given the small sample volumes and low [DOC]. Moreover,
58 previous MW measurements by HPSEC also relied on UV absorbance at 254 nm (see
59 below). While HS-rich samples tend to have mostly chromophoric OM, it is possible that
60 the XAD-4 samples, in particular, may have some non-chromophoric components. In
61 Table S-1, M_n, M_w, and ρ of isolates previously measured by high-pressure size exclusion
62 chromatography (HPSEC, with UV/Vis detection at λ=254 nm; see previous studies for
63 detailed methodology[9]) are provided to compare the overall trends between source
64 environments and isolation techniques. As these two analytical methods are chemically
65 and physically quite different, these techniques often do not agree in absolute MW values
66 (although they often do in trends between samples) and readers are directed to previously
67 published literature for detailed descriptions of such differences.[7,10-12] However, the
68 general trends in M_n, M_w, and ρ measured by HPSEC and AsFlFFF are comparable for
69 the different source environments and isolation techniques. Specifically, RO and XAD-8

isolates have larger values of M_n, M_w, and ρ of chromophoric components than XAD-4 isolates, as expected.

2. Detailed Method Development for Metal Removal Experiments.

Injection volume (100 μL) and mobile phase composition, pH, and ionic strength (15 mM ammonium carbonate, pH 7) were previously optimized to enhance sample recovery, trueness of size distribution, and detection for isolated OM.[13] However, optimization of the asymmetrical flow field-flow fractionation (AsFlFFF) system to investigate OM-associated metal removal by desferrioxamine B (DFOB) had not been previously undertaken. In this section, we discuss the methodology developed to (1) optimize the time of equilibration between OM samples and DFOB before injection into the AsFlFFF system, (2) determine retention volumes and detection for DFOB and Fe-DFOB in the absence of OM, and (3) mitigate the potential impact of Fe-DFOB molecules on OM fractograms. Calculated ICP-MS and UV/Vis signal recoveries calculated before (Table S-2) and after (Table S-3) DFOB addition are presented here.

The retention volumes and detection ability of the AsFlFFF system for DFOB and Fe-DFOB were determined by introducing DFOB and Fe-DFOB (at 1:1 and 1:2 molar ratios) into the AsFlFFF system at 50 and 100 μM concentrations of DFOB and UV/Vis signals (λ=254nm) was monitored for sample recovery and the specific retention volume. As expected, DFOB and Fe-DFOB eluted between ~550 and 700 g mol^{-1} (corresponding to 0.5 to 1.0 mL elution volume), as the molecular weight of DFOB as the mesylate salt is 656 g mol^{-1} (Figure S-1). The conversion between retention volume and molecular weight was made by calibrating the AsFlFFF with polystyrene sulfonate (PSS) standards following previous research,[7,13] and it is noteworthy that the PSS standard calibration

resulted in appropriate MW results for DFOB. The DFOB UV/Vis signal was quite low when compared to the UV-Vis signal of the Suwannee River RO isolate (Figure S-1), but DFOB and Fe elute together, suggesting their association, which is anticipated given the high affinity of DFOB for Fe.

The final stage of method development for the DFOB-mediated metal removal experiments was to resolve and mitigate potential contributions of Fe-DFOB and DFOB with OM UV/Vis fractograms. Fe-DFOB and DFOB were introduced into the AsFlFFF system and the relative heights and elution volumes of UV/Vis fractograms were compared to those of OM (no DFOB added). From Figure S-1, it is established that the UV/Vis signal for Fe-DFOB and DFOB spikes between 0.5 and 1.0 mL, but the signal intensity is much less than 3% of that of the average OM UV/Vis signal intensity in that region (see OM UV/Vis signal response in Figure S-1 for comparison); this difference was deemed not substantial enough to contribute to the OM UV/Vis signal. Additionally, the positioning of the UV/Vis peaks in Figure S-1were used to assess whether the Fe-DFOB complex had been completely removed from the OM mixture by looking for Fe peaks in the 0.5-1 mL elution volume region.

Detailed ICP-MS and AsFlFFF equipment specifications and operational parameters that are referenced and discussed in the main text are presented in Table S-4.

Tables and Figures

Table S-1. Compilation of raw filtered surface water (RFSW) and reverse osmosis (RO), XAD-8, and XAD-4 isolated organic matter physiochemical properties from the Suwannee River (SR), Nelson's Creek (NLC), and Sites 2 (S2) and 10 (S10) from McDonald's Branch (MDB).

Source	Date	Isolation	Cond.	pH	HPSEC (λ=254nm)			AsFlFFF (λ=254nm)		
					M_n	M_w	ρ	M_n	M_w	ρ
SR	May 2012	RFSW	196	3.71	NM	NM	NM	1574	2901	1.84
		RO			NM	NM	NM	1637	3091	1.86
		XAD-8			NM	NM	NM	1591	2831	1.78
		XAD-4			NM	NM	NM	1094	1878	1.72
NLC[2]	June 2002[14]	RFSW	25.8	5.93	886	2034	2.43	NM	NM	NM
		RO			1215	2356	1.94	2227	4033	1.81
		XAD-8			1208	2317	1.92	2358	4174	1.77
		XAD-4			981	1518	1.55	1606	2611	1.63
MDB S2[3]	Spring 1997[1]	RFSW	65	4.0	1102	1954	1.77	NM	NM	NM
		RO			1432	2618	1.83	2335	3932	1.68
		XAD-8			1387	2383	1.72	2240	3650	1.63
MDB S10[3]	Spring 1998[1]	RFSW	69	4.2	1048	1837	1.75	NM	NM	NM
		RO			1263	2196	1.74	1989	3262	1.64
		XAD-8			1150	2028	1.76	1948	3159	1.62

M_w is the weight-average molecular weight (g mol^{-1}), M_n is the number-average molecular weight (g/mol), and ρ is the polydispersity, M_w/M_n for chromophoric NOM molecules (detected by UV/Vis, λ=254nm). Molecular weight data analyzed by HPSEC for NLC[2] and MDB[15] have been published, thus are reported here for reference purposes. Conductivity (Cond.) is reported as μS/cm. Molecular weights labeled NM were not measured.

Table S-2. Injected sample DOC and AsFlFFF recovery (%) of UV/Vis absorbance (UV) and ICP-MS signals of raw filtered surface water (RFSW) and reverse osmosis (RO), XAD-8, and XAD-4 NOM isolates from Nelson's Creek (NLC), and sites 2 (S2) and 10 (S10) at McDonald's Branch (MDB), and the Suwannee River (SR).

Sample	% Recovery				
	UV	ICP-MS			
	λ = 254nm	Fe	Al	Cu	Zn
SR RFSW	80.4	94.2	21.5	90.4	90.6
SR RO	79.8	100	58.2	100	100
SR XAD-8	81.8	100	51.1	100	100
SR XAD-4	70.7	89.9	38.9	46.7	100
NLC RO	86.6	85.1	50.8	94.2	100
NLC XAD-8	84.4	81.6	39.1	100	100
NLC XAD-4	75.3	85.5	30.0	99.2	91.8
MDB S2 RO	83.7	98.3	39.7	100	100
MDB S2 XAD-8	84.7	89.2	57.8	100	100
MDB S10 RO	78.2	88.3	57.0	100	100
MDB S10 XAD-8	80.8	96.9	47.5	100	83.4

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Table S-3. Changes in AsFlFFF recovery of UV/Vis (UV) and ICP-MS signals of reverse osmosis (RO), XAD-8, and XAD-4 organic matter isolates from Nelson's Creek (NLC), Sites 2 (S2) and 10 (S10) of McDonald's Branch (MDB), and the Suwannee River (SR) after the addition of 20 μM DFOB.

Sample	UV (%)	ICP-MS (%)			
	λ= 254nm	Fe	Al	Cu	Zn
SR RO	+2.6	-90.3	-13.2	-41	-16.6
SR XAD-8	-10.7	-83.8	-24.4	-51.2	-23.6
SR XAD-4	+8.5	-80.5	-61.1	-7.5	-18.6
NLC RO	-13.3	-74	-39.1	-37.2	0
NLC XAD-8	-12.5	-71	-10.5	-23.5	-17
NLC XAD-4	-9.4	-80.6	+7.7	-39.6	-22.8
MDB S2 RO	-9.2	-84.3	-4.1	-11.8	0
MDB S2 XAD-8	-10.9	-77.7	-34.1	-17.1	-23.6
MDB S10 RO	-8.7	-72.4	-37.8	-30.9	-7.7
MDB S10 XAD-8	-11	-82.2	+24.2	-30.7	-1.3

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Table S-4. ICP-MS and asymmetrical Flow FFF (AsFlFFF) operational parameters and equipment specifications.

ICP-MS	
ICP-MS	Agilent Technologies 7000x
RF power (W)	1550
Carrier gas (L/min)	1.06 (Ar)
Collision cell gas (mL/min)	4 (He)
Torch	Quartz
Nebulizer	MicroMist (borosilicate glass, ID 0.5 mm)
Spray Chamber	Quartz
Sample and skimmer cones	Ni
Dwell Time (s)	0.05
Tuning solution	1 g/L Li, Mg, Y, Ce, Tl, Co in 2 wt% HNO3
AsFlFFF	
AsFlFFF System	Eclipse 3+ AsFlFFF System (Wyatt Technology)
AsFlFFF Membrane	300 Da PES (Postnova Analytics)
Autosampler	Agilent Technologies 1200 Series
Sample Pump	Agilent Technologies 1200 Series with a micro-vacuum degasser
UV/Vis detector	Diode array, Agilent Technologies 1200 Series
Fluorescence detector	Agilent Technologies 1200 Series FLD
Mobile phase	15mM ammonium carbonate, pH 7
Tip to tip channel length (cm)	19.5
Spacer (μm)	750
Focus flow rate (mL/min)	1.5 (same as cross flow rate)
Focus time (min)	2
Channel flow rate (mL/min)	1
Cross flow rate (mL/min)	1.5

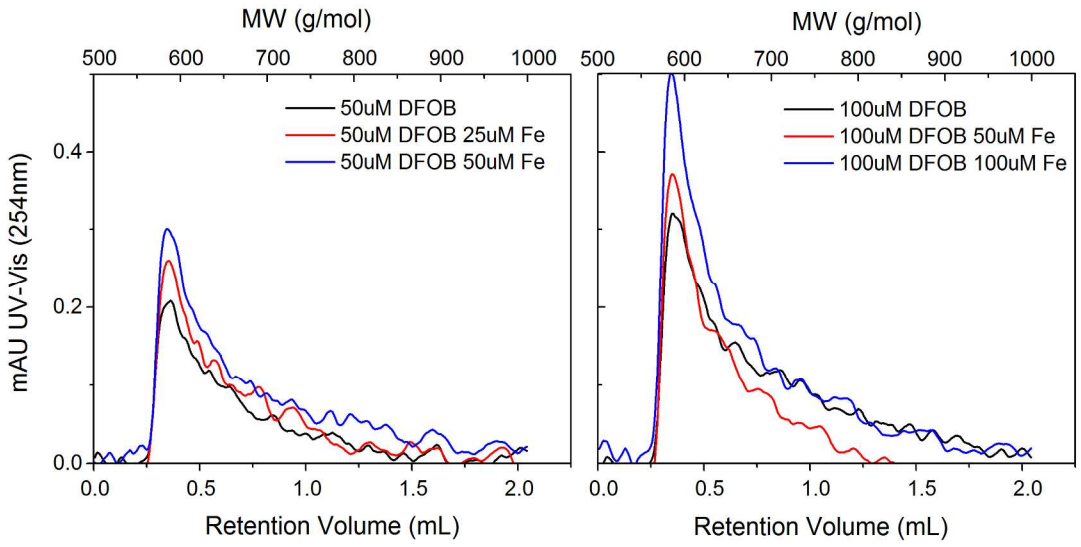

Figure S-1. Fractograms of DFOB and Fe-DFOB as measured by UV/Vis (λ=254nm) showing a single peak eluting between ~0.25 and 1 mL, which was equivalent to an approximate molecular weight (MW) between 550 and 700 g mol^{-1}. The maximum UV/Vis signal response was less than 1% of that observed for OM samples in this region of retention volumes.

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