

## Electronic Supplementary Information

### Pattern-based sensing of sulfated glucosaminoglycans with a dynamic mixture of iron complexes

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#### Content

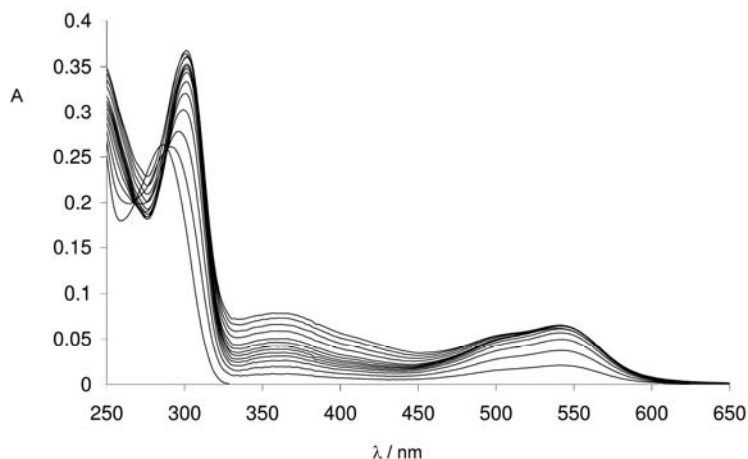
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## 1. General

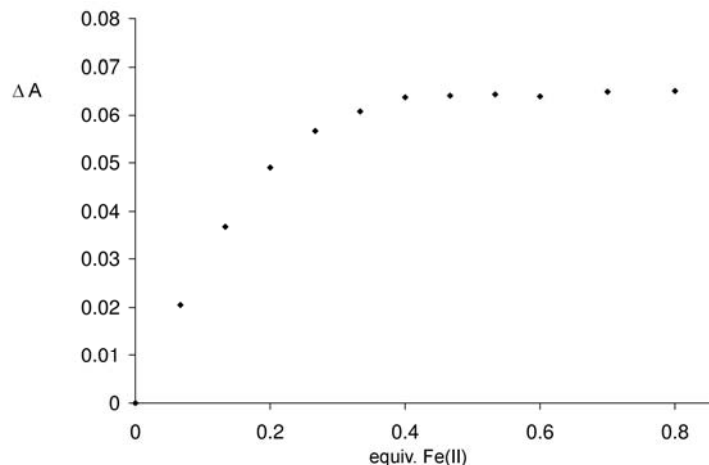
Dipicolylamine (Sigma), Evans Blue (Fluka),  $[\text{FeCl}_2(\text{H}_2\text{O})_4]$  (Sigma), MOPS buffer (Fluka), low molecular weight heparin (Fluka), unfractionated heparin (Applichem), dextran sulfate (Sigma), heparan sulfate (Sigma), chondroitin sulfate A (Sigma) and dermatan sulfate (Sigma) were used as received. The ligand *N*-(6-aminohexyl)-4'-methyl-2,2'-bipyridine-4-carboxamide hydrochlorid (**1**) was synthesized starting from 4,4'-dimethyl-bipyridin (Fluka) as described in the literature.<sup>10</sup> Stock solutions of the ligands and the metal salt were prepared with a concentration of 10 mM in bidistilled water. Stock solutions of the sulfated glycosaminoglycans were prepared with a concentration of 1 mg/ml. MOPS buffer (100 mM, pH 7.0) was prepared with bidistilled water and used for all experiments. All UV/Vis spectra were recorded on a Lambda 35 spectrometer (Perkin Elmer) using disposable cuvettes (Brand). Quartz cuvettes (Hellma) were used for spectrophotometric titrations.

## 2. Spectrophotometric titration with $[\text{FeCl}_2(\text{H}_2\text{O})_4]$ and ligand **1**

A stock solution (1.0 mM) of  $[\text{FeCl}_2(\text{H}_2\text{O})_4]$  was prepared by dissolving the appropriate amounts of  $[\text{FeCl}_2(\text{H}_2\text{O})_4]$  in water. The titration was performed by adding aliquots of this stock solution to a buffered aqueous solution of ligand **1** ( $[\mathbf{1}] = 30 \mu\text{M}$ ,  $[\text{MOPS}] = 10 \text{ mM}$ , pH 7.0). Each aliquot contained 0.0667 equivalents of  $[\text{FeCl}_2(\text{H}_2\text{O})_4]$  with respect to ligand **1**. After each addition, the solution was equilibrated for 30 min at room temperature before measuring the absorption spectra in the range of 250 to 800 nm.



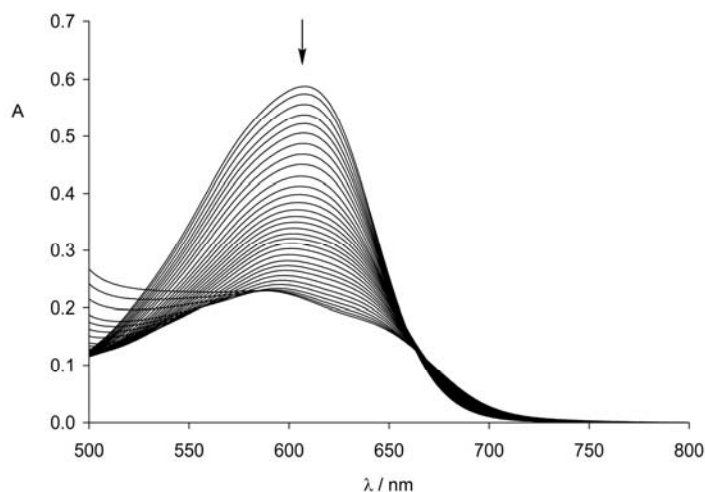
**Fig. S1** Absorption spectra of a buffered aqueous solution (MOPS, pH 7.0) containing ligand **1** (30  $\mu\text{M}$ ) upon addition of increasing amounts of  $[\text{FeCl}_2(\text{H}_2\text{O})_4]$  (0 – 30  $\mu\text{M}$ ).



**Fig. S2** Absorption change at 538 nm for the titration shown in Figure S1. The data suggest that a complex with the stoichiometry Fe:ligand **1** = 1:3 is formed. This result is in line with the known tendency of Fe(II) to form  $ML_3$  complexes with bipy ligands.<sup>1</sup>

### 3. Spectrophotometric titration with $[Fe(dpa)_2]^{2+}$ and Evans Blue

A stock solution (1.0 mM) of  $[Fe(dpa)_2]^{2+}$  was prepared by dissolving the appropriate amounts of  $[FeCl_2(H_2O)_4]$  and dpa in water. The solution was equilibrated for 24 hours at room temperature before utilization. The titration was performed by adding aliquots of this stock solution to a buffered aqueous solution of Evans Blue ( $[EB] = 7.5 \mu M$ ,  $[MOPS] = 10 \text{ mM}$ , pH 7.0). Each aliquot contained one equivalent of  $[Fe(dpa)_2]^{2+}$  with respect to Evans Blue. After each addition, the solution was equilibrated for 20 min at room temperature before measuring the absorption spectra in the range of 250 to 800 nm.

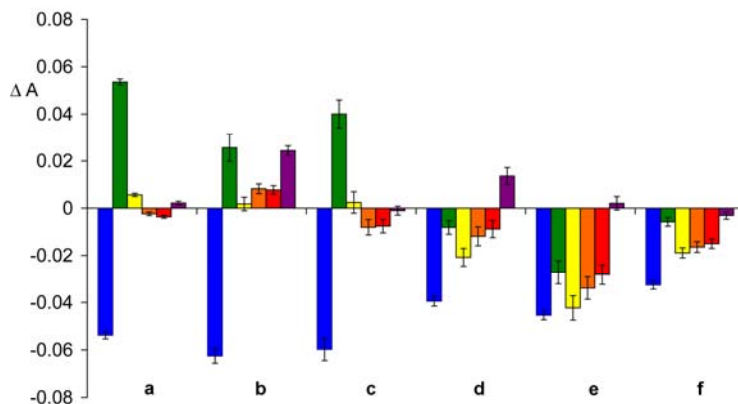


**Fig. S3** Absorption spectra of a buffered aqueous solution (MOPS, pH 7.0) containing Evans Blue (7.5  $\mu M$ ) upon addition of increasing amounts of complex  $[Fe(dpa)_2]^{2+}$  (0 – 600  $\mu M$ ).

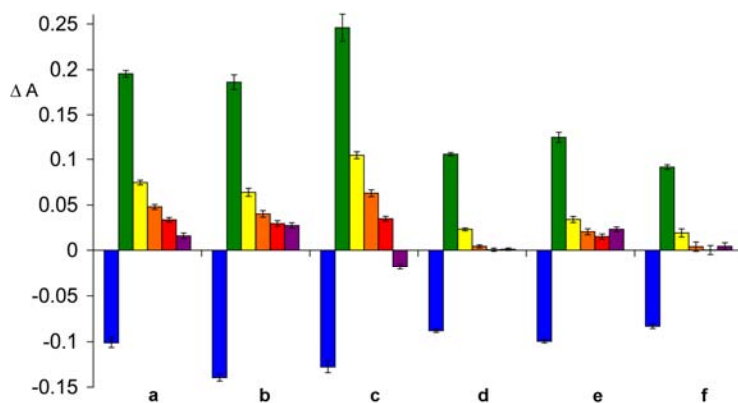
[1] H. Irving and D. H. Mellor, *J. Chem. Soc.*, 1962, 5222–5237.

#### 4. Sensing of sulfated glycosaminoglycans

The sensor was prepared by mixing appropriate amounts of stock solutions containing the ligands, the metal salt and the buffer. After equilibration for 15 h at room temperature, a UV/Vis spectrum was recorded. Subsequently, the analytes were added and a second UV/Vis spectrum was recorded after equilibration for 90 min. Each measurement was repeated six times. The final concentrations were: [1] = 90  $\mu\text{M}$ , [dpa] = 300  $\mu\text{M}$ , [Evans Blue] = 3.75  $\mu\text{M}$ , [ $\text{Fe}^{2+}$ ] = 30  $\mu\text{M}$ , and [MOPS] = 10 mM. The concentrations of the polysulfated sugars were either 10 or 50  $\mu\text{g/ml}$ .



**Fig. S4** Response of the sensing ensemble composed of  $[\text{FeCl}_2(\text{H}_2\text{O})_4]$ , dpa, **1**, and Evans Blue (for conc. see text) after addition of different glycosaminoglycans (final conc. = 10  $\mu\text{g/ml}$ ). Blue: 431 nm, green: 545 nm, yellow: 585 nm, orange: 612 nm, red: 632 nm, and purple: 669 nm. a = LMWH, b = UFH, c = dextrane sulfate, d = chondroitin sulfate A, e = chondroitin sulfate B/dermatan sulfate, f = heparan sulfate. The values represent averages of six independent measurements.



**Fig. S5** Response of the sensing ensemble composed of  $[\text{FeCl}_2(\text{H}_2\text{O})_4]$ , dpa, **1**, and Evans Blue (for conc. see text) after addition of different glycosaminoglycans (final conc. = 50  $\mu\text{g/ml}$ ). Blue: 431 nm, green: 545 nm, yellow: 585 nm, orange: 612 nm, red: 632 nm, and purple: 669 nm. a = LMWH, b = UFH, c = dextrane sulfate, d = chondroitin sulfate A, e = chondroitin sulfate B/dermatan sulfate, f = heparan sulfate. The values represent averages of six independent measurements.