Supporting Information

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GENERAL

All commercial chemicals were used as received without further purification. DGL G3 was provided by the COLCOM company (batch #1204-03). GAGs were purchased from Sigma-Aldrich: Heparin sodium salt from porcine intestinal mucosa (207 USP/mg, ref: H3399-50KU), Chondroitin sulfate A sodium salt from bovine trachea (ref: C9819-5G), Hyaluronic acid sodium salt from streptococcus equi (ref: 94137-10MG), Chondroitin sulfate B sodium salt from porcine intestinal mucosa (ref: C3788-25MG), Dextran sulfate sodium salt from Leuconostoc spp. (ref: D6924-1G). All solutions were prepared in 10 mM HEPES buffer, pH 7.8. The buffer was prepared using MilliQ deionized water (18 MΩ). HPLC purifications were performed using an Armen SPOT Prep system, on a Reveleris flash cartridge (reversed phase C18, 40 µM particles, 40 g) operated at 9 $mL.min^{-1}$. For the purification procedure, a gradient from 90:10 H_2O (0.1 % TFA)/MeCN (0.1 % TFA) to 10:90 H_2O (0.1 % TFA)/MeCN (0.1 % TFA) over 30 minutes was operated, with UV/Vis monitoring at 215 and 437 nm. LC/MS analyses were performed on an UPLC Acquity H-Class equipment (Waters), with a Kinetex column (Phenomenex, reversed phase C18, 100 x 2.1 mm, 2.6 µm particles). Chromatograms were recorded by a TUV Photodiode detector at 210 nm, and mass spectra were recorded by a Synapt G2-S mass spectrometer (Waters, ESI, positive ions introduction). For the analysis procedure, the following gradient was operated at 0,5 $mL.min^{-1}$:

Time (min)	% A (H_2O + 0.1% Formic acid)	%B (MeCN + 0.1% Formic acid)
0	90	10
12	20	80
13	0	100

Fluorescence measurements were performed on a Berthold Tristar LB 941 microplate reader (λ_{ex} = 485 nm and λ_{em} = 535 nm), at 20°C. All fluorescence experiments were performed at least in triplicate.

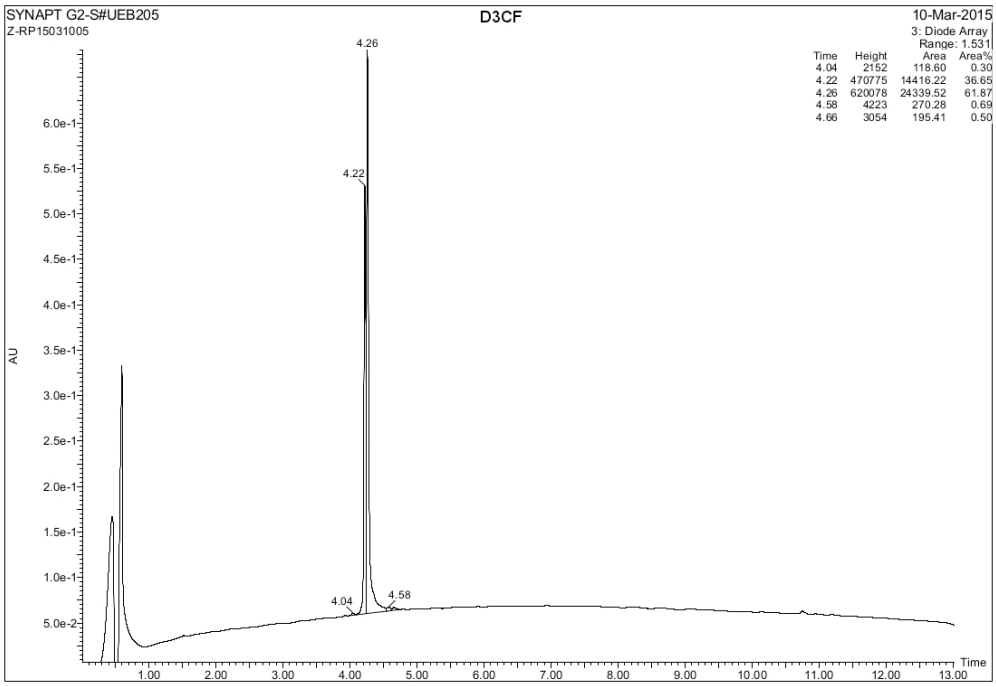
SYNTHESIS

Assembly of the linear protected peptide Fmoc-Asp(OtBu)-Asp(OtBu)-Asp(OtBu)-ChloroTrityl™ resin was carried out manually with a traditional Fmoc/tBu strategy in a glass reactor with a glass frit. 50 mg of Chlorotrytil resin (1.3 mmol/g loading) were made to swell in 5 mL of dichloromethane (DCM). Fmoc-Asp(OtBu)-OH (15

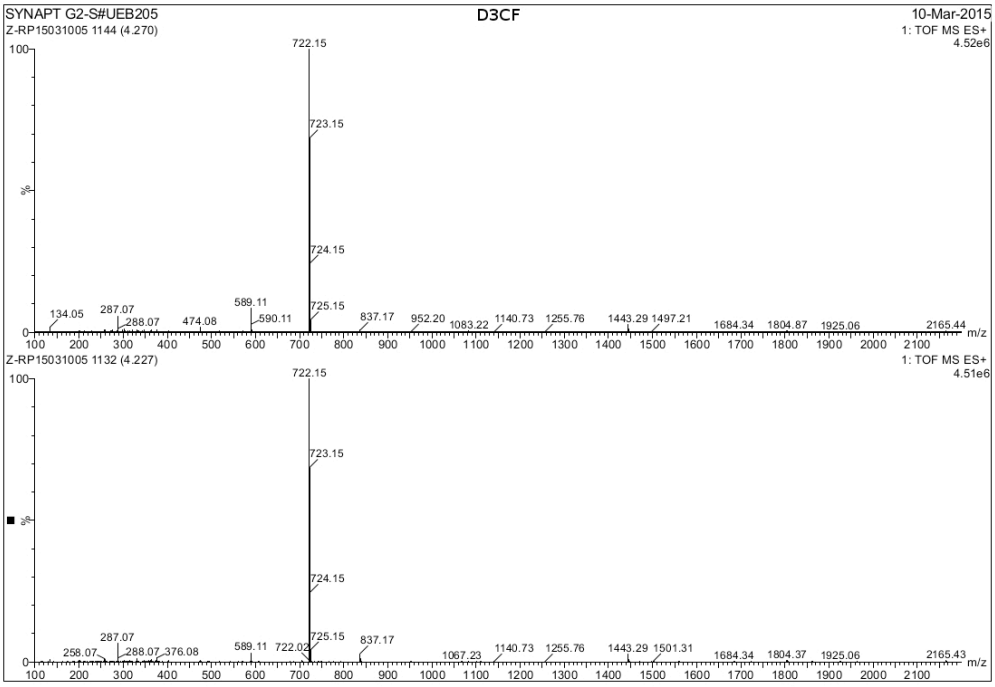
mg, 0.036 mmol) was added, followed by DIEA (0.02 mL, 0.12 mmol). The suspension obtained was stirred for 2h at room temperature. The supernatant was removed by suction and then the resin was capped with DCM/MeOH/DIEA (17:2:1, 5 mL, overnight), then DCM/MeOH/DIEA (17:2:1, 5 mL, 2h). Fmoc deprotection reactions were performed with a mixture DMF/piperidine/formic acid 75:20:5 (1 x 2 mL x 1 min; 3 x 2 mL x 3 min; 1 x 2 mL x 15 min). The resin was washed with DMF (5x3 mL, 1 min each). A suspension of Fmoc-Asp(OtBu)-OH (25 mg, 0.06 mmol), PyBop (31 mg, 0.06 mmol) and DIEA (0.02 mL, 0.12 mmol) in 2 mL of DMF was stirred for 2 h. Each coupling reaction was performed in duplicate in order to avoid the risk of deletion. The final introduction of the 5(6)-carboxyfluorescein (CF) label was performed as follow, reducing lighting exposure as much as possible:[1] all the previously prepared quantity of H_2N-Asp(OtBu)-Asp(OtBu)-Asp-(OtBu)-ChloroTrityl™ was made to swell in 5 mL of DMF. CF (113 mg, 0.3 mmol), DIC (0.05 mL, 0.3 mmol) and HOBt (46 mg, 0.3 mmol) were added and the suspension was stirred for 1 h. The fully deprotected and labeled peptide was recovered directly upon acid cleavage with TFA/TIPS/H_2O (95:2.5:2.5, 4 mL, 1 h), followed by TFA/TIPS/H_2O (95:2.5:2.5, 2x3 mL, 1 min each). The combined filtrates were concentrated under reduced pressure. Crude D3CF was obtained by precipitation with Et_2O.

Analyses and purification of the synthesized D3CF were performed by LC/MS, as previously mentioned (see GENERAL section). Retention time = 4.22 and 4.26 min (two main peaks were observed since 5(6)-carboxyfluorescein is a mixture of two regio-isomers). Yield = 86%.

HPLC trace:



MS spectrum:



m/z:722.15 → $[M + H^+]$

ANALYTICAL METHODS

Fluorescence titration of G3 with D3CF

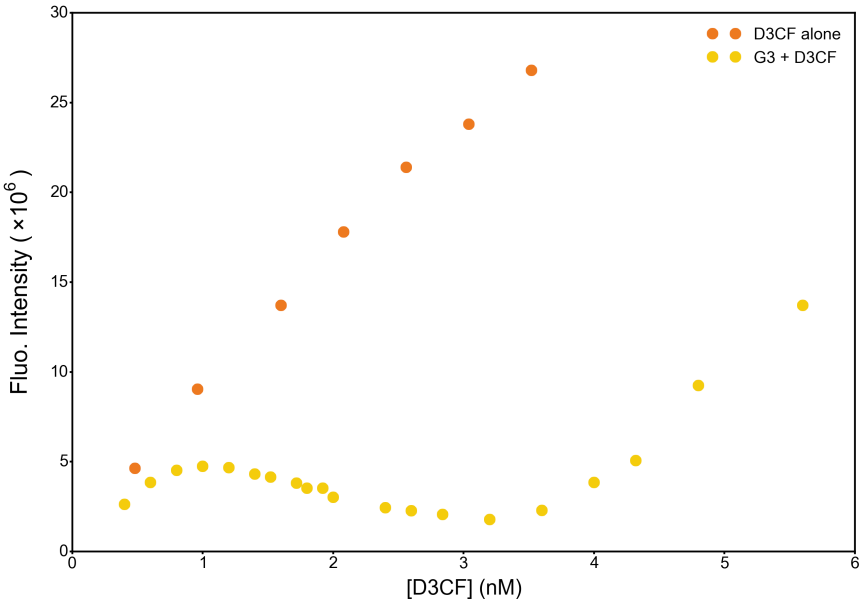


Figure S1. Fluorescence titration of the G3 with D3CF. Conditions: 10 mM HEPES buffer, pH = 7.8, [G3]=163 nM ([lysine] = 20 mM), λ_{ex} = 485 nm, λ_{em} = 535 nm.

Fluorescence titrations of G3-D3CF complexes with GAGs

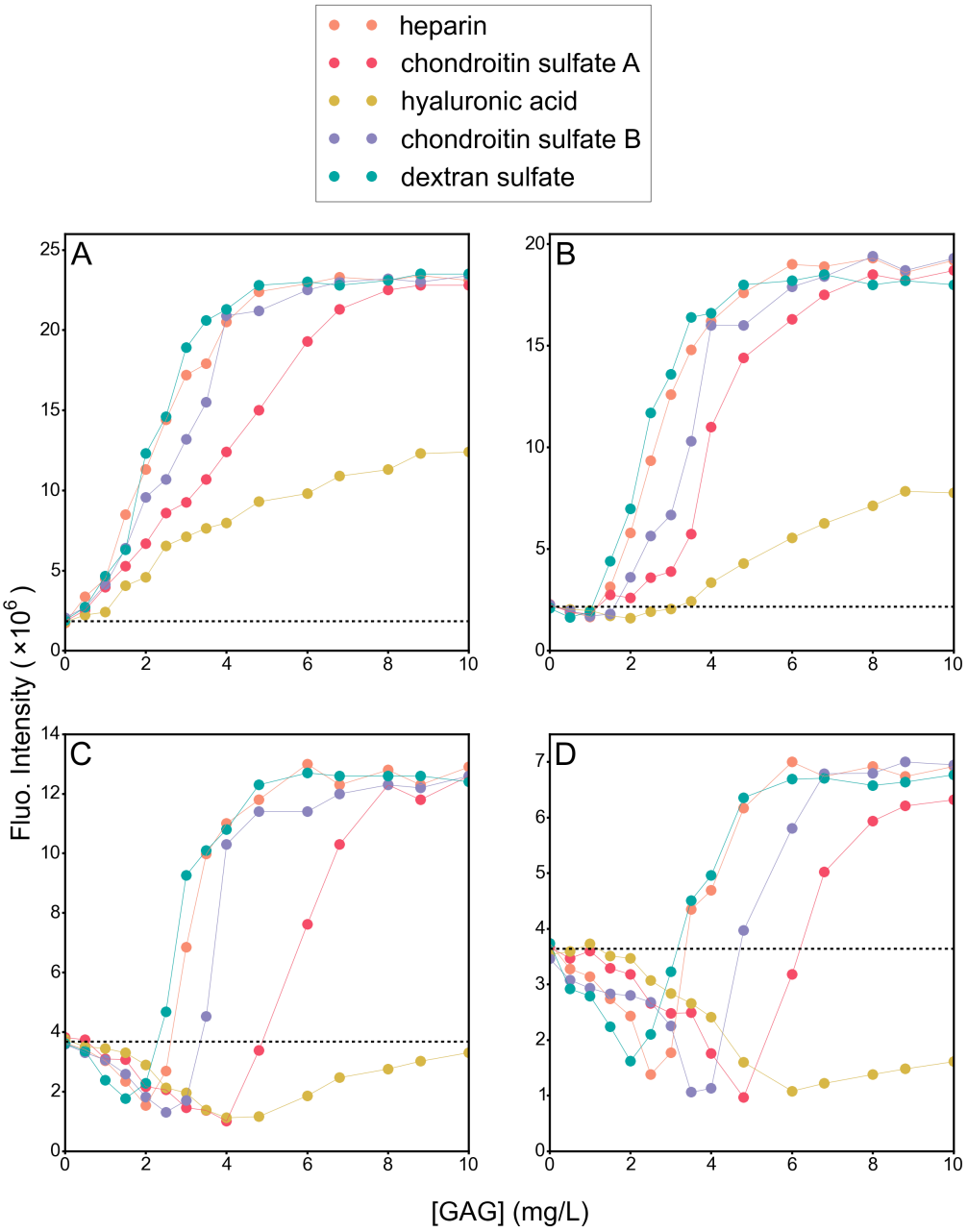


Figure S2. Fluorescence titrations of the G3-D3CF complexes with GAGs. Loadings: 100% (A), 75% (B), 50% (C), 25% (D). Conditions: 10 mM HEPES buffer, pH = 7.8, [G3]=163 nM ([lysine] = 20 mM), λ_{ex} = 485 nm, λ_{em} = 535 nm.

Typical fluorescent sensing experiment

In a typical sensing experiment (for 100% loading), microplate wells were filled with 55 µL of buffer, 10 µL of a G3 solution in buffer (final concentration in the well: 163 nM), and 20 µL of D3CF solution in buffer (final concentration in the well: 3.2 µM). The mixture was allowed to equilibrate for 15 min. Finally, 35 µL of a 15 $mg.L^{-1}$ GAG solution were added (final concentration in the well: 3.5 $mg.L^{-1}$). The fluorescence intensity was then followed for 45 min, with measurements every 2 min. The fluorescence intensities finally used in the treatments were a mean of the last 3 points of a measurement.

COMPUTATIONAL METHODS

The following procedure does not provide accurate 3D structures of DGL G3-D3CF complexes. However, it does provide a good representation of their topology, and of the distribution of their charges.

The appropriate number of D3CF molecules was positioned around a "naked" DGL G3 molecule (for the generation of the topology of a DGL G3 molecule, see ref. 2). Then, Spartan'10 at the MMFF level of theory in vacuum was used to minimize the structure.[3] The Discovery Studio software, with the force field MMFF94, was used to assign the partial charges of the atoms. Finally, VMD[4] (version 1.9.1) and the APBS[5] plugin (version 1.3, default settings) allowed us to generate electrostatic potential maps.

REFERENCES

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