Electronic Supplementary Material (ESI) for Chemical Communications

AFM investigation of *Pseudomonas aeruginosa* lectin LecA (PA-IL) filaments induced by multivalent glycoclusters

Delphine Sicard, Samy Cecioni, Maksym Iazykov, Yann Chevolot, Susan E. Matthews, Anne Imberty, Jean-Pierre Praly, Eliane Souteyrand, Sébastien Vidal, and Magali Phaner-Goutorbe**

Supporting Information

1-Preparation of the LecA/glycocluster samples

10 µL of a solution of lectin LecA (PA-Il) was added (25 pM final concentration) to 20 µL of $CaCl_2$ in ultrapure water, (final concentration of 0.3 µM) in a 1.5 mL eppendorf. A 10 µL of a solution of glycoclusters (tetra-galactosylated 1,3-alternate calix[4]arene **1** or tetra-mannosylated 1,3-alternate calix[4]arene **2**) was then added (final concentration of 25 pM).
The solution was incubated without agitation, eppendorf in vertical position, during 1 h, at room temperature.
20 µL of the final solution was deposited on a freshly cleaved mica surface. The sample was then dried overnight in a desiccator with silica gel.

2-Structure of the tetra-mannosylated 1,3-alternate calix[4]arene-based glycocluster 2

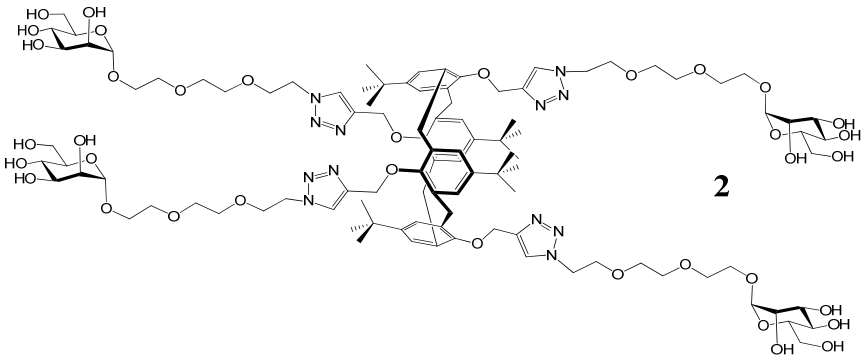

Figure S1: Structure of the mannosylated tetravalent 1,3-alternate calix[4]arene-based glycocluster **2**.

3- AFM image of the LecA/glycocluster 2 sample

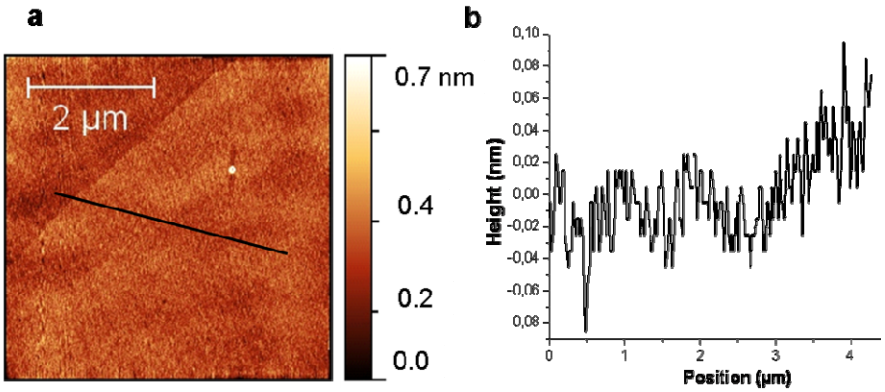

Figure S2: (a) AM-AFM image after the deposition of LecA/tetra-mannosylated 1,3-alternate calix[4]arene-based glycocluster **2** on the mica substrate. Image size is of 5 x 5 µm^2. RMS = 0.080 nm. **(b)** Profile of the surface related to the black line in **(a)**.

For these negative control experiments, four different samples have been studied. For each experiment, new final solutions and new samples have been prepared. AFM observations have been realised with different tips. The image presented in **Figure S2** is a typical image observed on these samples. No filaments were observed on the surface of the mica substrate.

4-AFM experiments

AFM experiments were performed in air with two different atomic force microscopes, a Di-Cp-II (Brucker) AFM microscope and a stand alone SMENA (NT-MDT) AFM microscope. Images are taken in the Amplitude Modulation (AM) AFM mode with working amplitude/free amplitude ratio around 55-80%. Mikromash NSC 21 tips are used, typically with a spring constant of around k = 0.5 N/m. Images were performed at a scanning frequency ranging between 0.7 and 1 Hz. Images contain 256 x 256 pixels or 512 x 512 pixels. Data analysis and image treatments are realised with Gwyddion Software.

5-Molecular modeling

Modeling of the linear aggregates formed by the interaction between LecA and the 1,3-alternate calix[4]arene-based glycocluster was performed by expanding the modeling approach previously described (Cecioni and *al.*, 2009). All details for building the glycocluster, editing atomic charges and optimizing geometry using TRIPOS force field (Clark and *al.*, 1989) with addition of carbohydrate parameters (Imberty and al., 1999) have therefore been reported.

Tetrameric LecA was prepared from the X-ray structure (PDB code: 1OKO) after removal of water molecules and carbohydrate ligands. Hydrogen atoms were added and Pullman charges computed, except for the calcium ions that were treated with a charge of 2. The positions of hydrogen atoms were optimized with Tripos force-field.

In order to generate linear aggregates, one galactose residue of the glycocluster was fitted into one binding site of LecA by overlaying its atoms with the “ghost” of galactose molecule from the PDB structure (dummy atoms). A systematic conformational search was performed around 12 rotatable bonds with distance constraint between atom O4 of the second galactose

and the calcium ion in neighbouring binding site. The ligands with appropriate conformations where optimized with inclusion of constraint for perfect fit with the "ghost" galactose. After removal of these dummy atoms, several steps of energy minimization were performed. The last cycle included full optimization of the whole ligand, with no constraints, in order to check the stability of the proposed interaction. The resulting conformation was copied to the two other arms so that their galactose moieties can be directly fitted in neighbouring binding sites of another LecA tetramer. The structure was then propagated by translation to generate the linear filament.

The occurrence of "branches" was modeled by breaking the symmetry of one of the glycocluster arms. A manual conformational search was performed on all torsional angles of the linker of one arm. The conformations that exposed the galactose away from the linear filament were selected and this galactose was fitted in the binding site of an additional LecA tetramer. The selected final model corresponds to a conformation of the linker that resulted in no steric conflict between the "branched" LecA tetramer and the linear filament.

References

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