ON-CELL SATURATION TRANSFER DIFFERENCE NMR FOR THE IDENTIFICATION OF FIMH LIGANDS AND INHIBITORS

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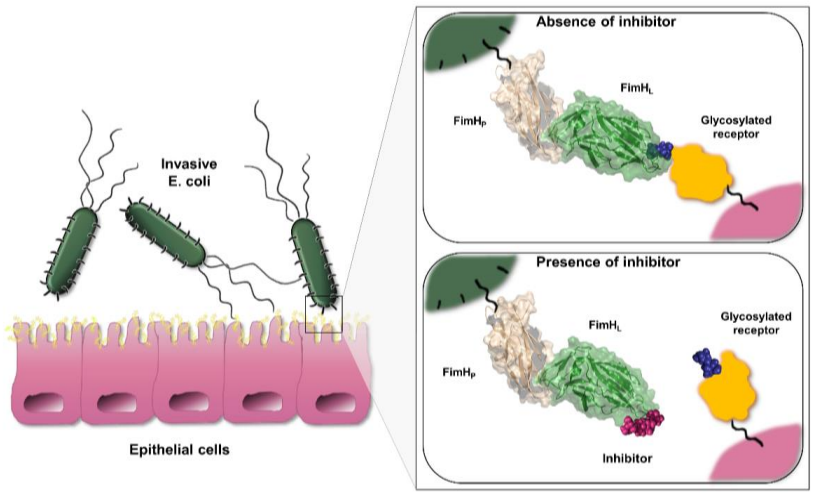


Figure 1. FimH-mediated adhesion of UPEC cells to host mammalian cells.

Introduction

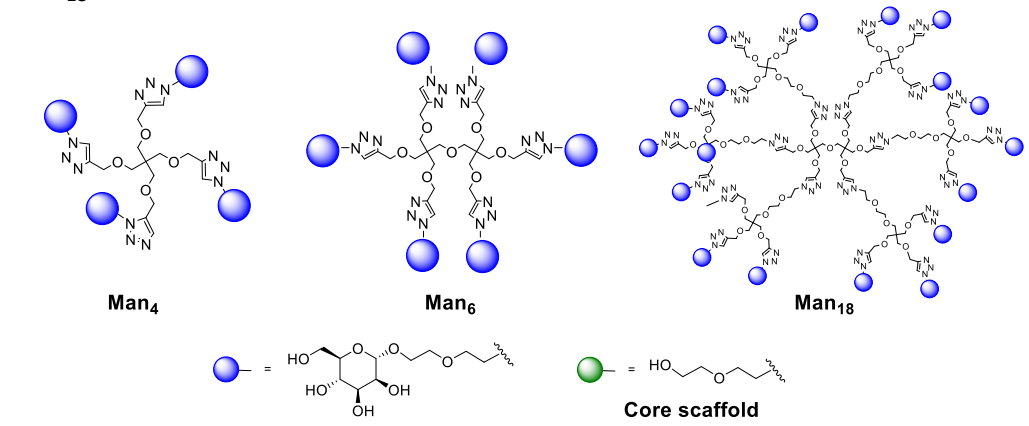
Bacterial adhesion is the first step in pathogen infection and bacterial adhesins are prime candidates as targets for antibacterial therapeutics such as specific-ligand-like inhibitors and vaccines.

FimH is a mannose-specific binding protein located at the apical end of uropathogenic *Escherichia coli* (UPEC) type 1 fimbriae that confer bacterial binding to mannosylated glycoproteins on host surface. It is considered a virulence factor and an attractive therapeutic target for urinary tract infection (UTI) and Crohn's Disease (CD).

Aim of this work was the development of an NMR-based assay allowing a very rapid screening of FimH ligands and the structural characterization of their binding mode to living bacterial cells expressing the protein on their surface.

Results

Monodispersed glycodendrimer based on pentaerythritol core and bearing different units of D-mannose, a FimH natural ligand, were synthesised through a convergent modular strategy exploiting the so-called "click chemistry" and achieving a small library of potential FimH multivalent ligands (Man_4, Man_6, Man_{18}).



STD-NMR experiments acquired on samples containing bacterial living cells (*E. coli* CFT073, a uropathogenic strain expressing a high level of FimH) were set up to test the ability of synthesized compounds to bind FimH on cell surface. A *E. coli* CFT073 ΔFimH strain was employed as negative control (Fig. 1). This approach represents a new rapid and sensitive method allowing the efficient screening of potential FimH ligands.

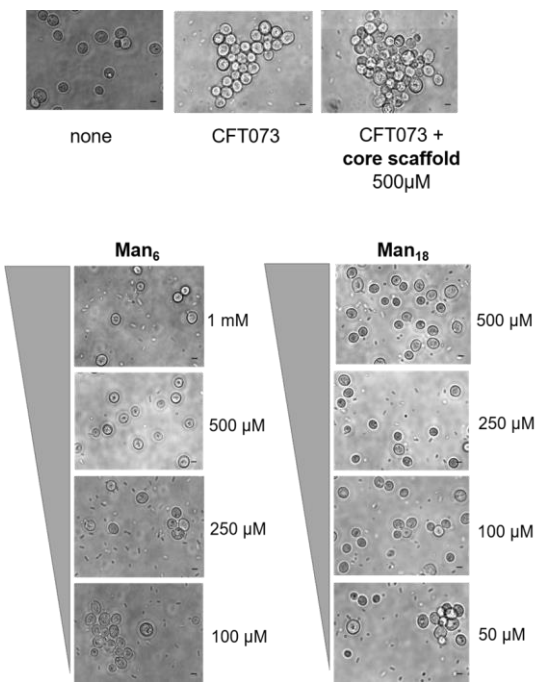
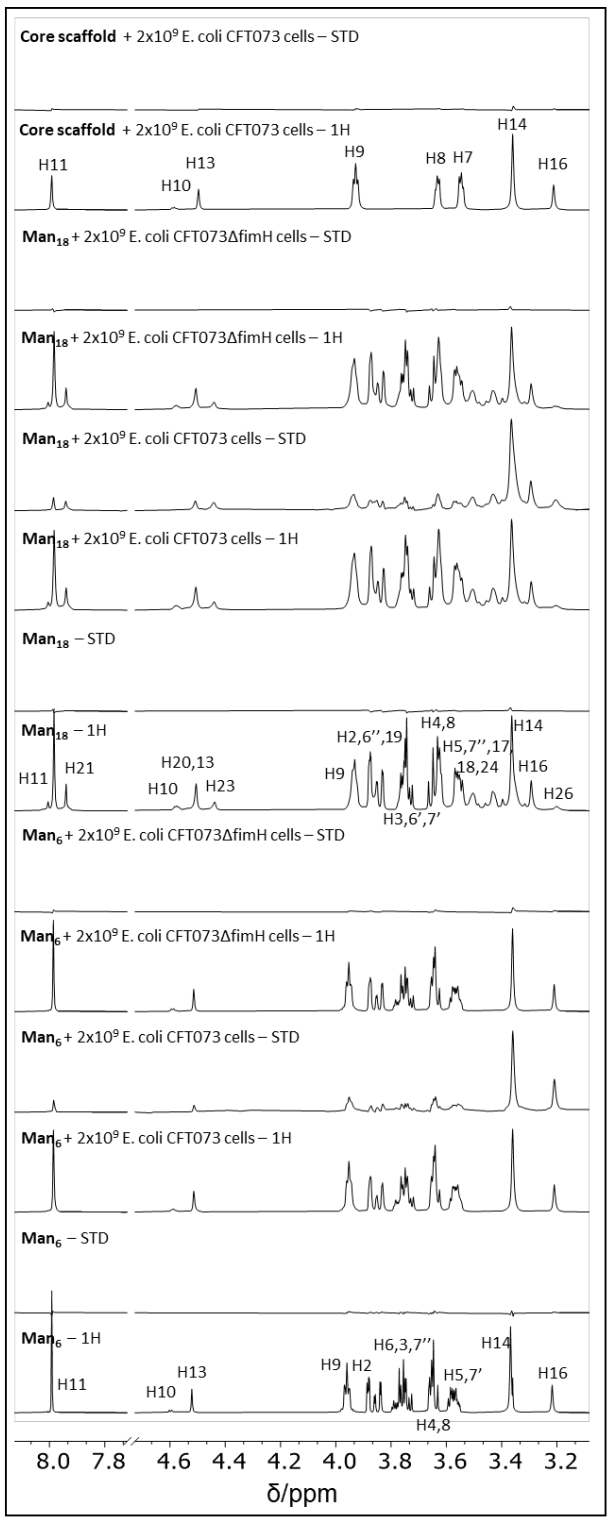


Figure 2. Yeast agglutination inhibition assay.

Compound inhibition of D-mannose sensitive adhesion was tested by yeast agglutination inhibition assay (Fig. 2). As expected, we found a significant correlation between the number of D-mannose units and compound affinity potency. In particular, Man_{18} showed a MIC of 63 μM (Tab. 1).

Table 1. Minimal inhibitory concentration (MIC) for the yeast agglutination assay.

Ligand	MIC (μM)
Scaffold	>500
Man_4	500
Man_6	250
Man_{18}	63



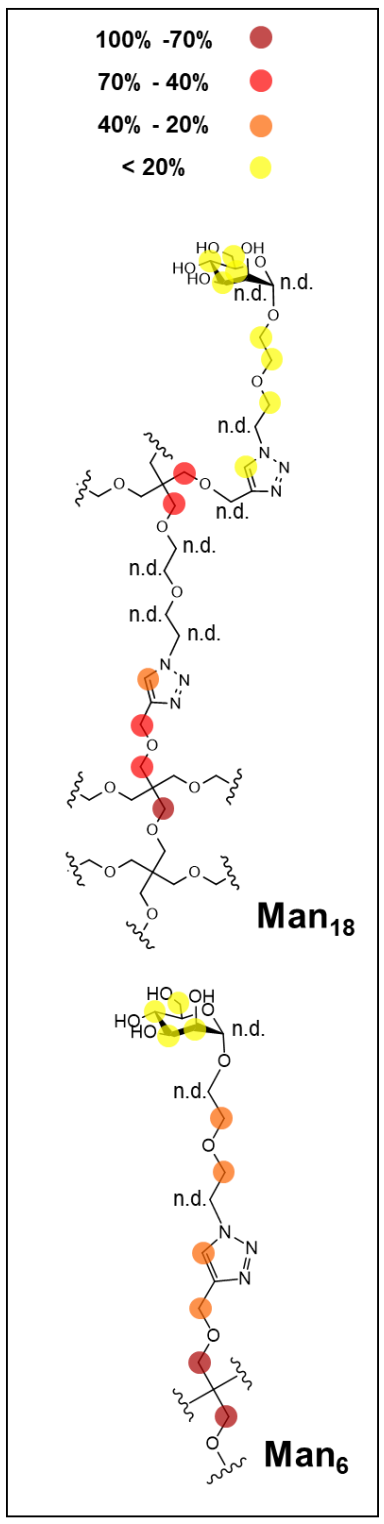


Figure 1. STD-NMR experiments on E. coli cells and ligand binding epitope mapping. All spectra were acquired at 600 MHz and 25 °C. ^{1}H-NMR spectra were acquired with 16 scans; STD NMR spectra were acquired with 256 scans, selective irradiation frequency 0.0 ppm, saturation time 3.0 s, off-resonance irradiation frequency 30 ppm, and reported with a 10x signal enhancement.

Conclusion

We demonstrate the feasibility of on-cell STD NMR experiments to characterize molecular recognition events involving the bacterial adhesin FimH. This method allows to verify and identify the structural determinants of the binding of new potential receptor ligands in a very fast and reliable way and, most importantly, under physiologically relevant conditions. Moreover, the dendrimer Man_{18} emerges as hit compound for the rational design of new multivalent FimH inhibitors.

Reference

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