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Miniaturized Affinity Chromatography (WAC) coupled to Mass Spectrometry in drug discovery to decipher glycoclusters from dynamic combinatorial libraries

Chromatographie d'affinité miniaturisée couplée à la spectrométrie de masse appliquée à la recherche de médicaments : criblage de glycoclusters à partir de bibliothèques combinatoires dynamiques

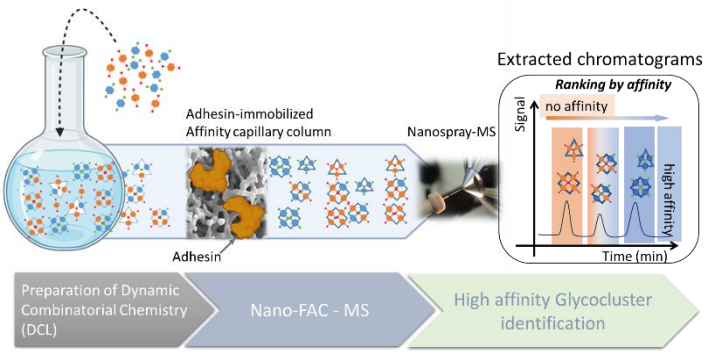
Context: Bacterial infections are the most eminent public health challenge of the 21st century. For example, *Pseudomonas aeruginosa* (PA) is responsible for 20% of clinical acquired infections and is part of the ESKAPE family of pathogens under surveillance by the WHO[1]. The primary step leading to infection is bacterial adhesion to the surface of host cells, due to protein-oligosaccharides interactions[2]. Bacterial flagellins and lectins, also called adhesins, are among the most important bacterial proteins involved in these early events of adhesion. As we approach the limits of the antibiotic era, acquisition of a deeper knowledge in bacteria-host cells/(bio)materials surface adhesion is crucial for the identification of new ligand-binding events and its assessment as novel targets for alternative anti-infective strategies. The design of anti-adhesive glycoconjugates blocking this adhesion provides innovative routes to anti-infectious therapeutic therapies [4]. While an adhesin-carbohydrate interaction is typically in the mM to µM range, multivalent glycoconjugates (e.g. glycoclusters, glycodendrimers, glycopolymers) with high affinity (up to nM) have already found biomedical applications[3].

The general strategy to identify multivalent glycoclusters of high affinity is to synthesize a family of candidates and evaluate their affinity one by one. Dynamic Combinatorial Chemistry (DCC) has already proven to be a valuable alternative approach to synthesize multivalent glycoconjugates through the formation of a ***dynamic combinatorial library*** (DCL) of all possible glycoclusters (in equilibrium) obtained from ***reversible covalent bonds*** (disulfide bonds). In the presence of the adhesin, equilibria in solution are shifted due to the interactions of high affinity glycoclusters with the adhesin, leading to an amplification of the corresponding ligand in the mixture.

However, the complexity of such DCL requires powerful analytical methods to decipher the information from the DCL mixture and to identify the best ligands for a specific adhesin. The current method (HPLC-MS)[4] is based on the comparison of the composition of the DCL obtained in the presence/absence of the adhesin[5–7] but this approach nevertheless suffers from many limitations: high protein (adhesin) and ligand consumption (mg scale) , tedious and time-consuming workflow, no information on the affinity range…

Thesis project*: To make the most of dynamic combinatorial chemistry, we propose to develop a* ***powerful analytical workflow*** *for the amplification/selection and* ***identification*** *of the best hits while reducing reagent consumption. Miniaturized affinity chromatography in-line coupled with mass spectrometry (mAC-MS) will be at the heart of the analytical strategy.*

The DCL mixture will be deciphered on an affinity column on which an adhesin is immobilized. The specific interaction of a glycocluster for the adhesin induces retention times proportional to the affinity (Ka). Glycoclusters with no and/or low affinity for the protein are not retained and eliminated (simplification of the mixture). The glycoclusters are then eluted in ascending order of affinity and detected and identified by mass spectrometry coupled on-line to the column.



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We recently reported a **proof-of-concept** of the application of **mAC-MS** for the identification of glycoclusters (using Concanavalin A as an adhesin model) in a DCL.[8] These preliminary results were obtained on a simple DCL of glycoclusters without any amplification step. One of our aims is to extend the role of **mAC-MS** to the amplification stage. Indeed, the affinity column can also be used for the "on column" amplification of the DCL mixture.

This work will be carried out in collaboration with other team specialized in the design of multivalent glycoconjugates by DCC as ligands of lectins (ICSN/ Paris Saclay).

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