

## DENDRIMERS AS POTENT INHIBITORS OF *STREPTOCOCCUS PNEUMONIAE* AUTOLYSIS AND CELL DIVISION

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*Streptococcus pneumoniae* (pneumococcus) is an important pathogen that causes the death of millions of people every year [1]. Autolysis and cell division are important processes for the virulence of these microorganisms that are carried out by hydrolytic enzymes belonging to the choline-binding protein family (CBPs) [2]. These proteins contain a choline binding module (CBM) that anchors the enzyme to choline residues present in the cell wall [3, 4]. The activity of these enzymes can be inhibited by the competition of high concentrations of free choline or choline-analogues [5, 6].

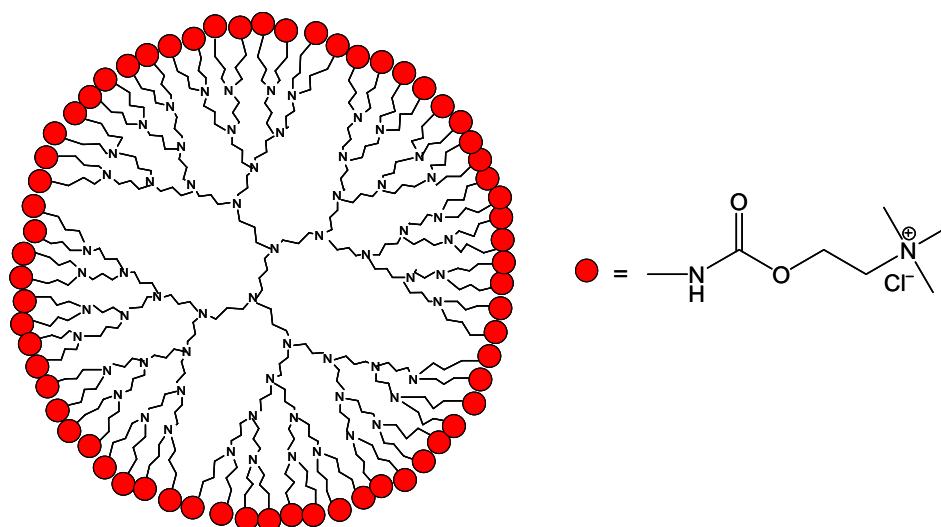
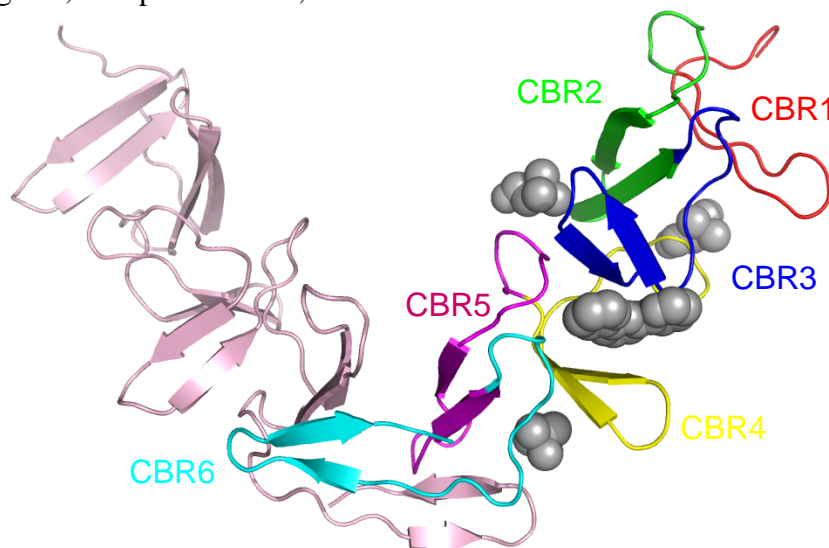
As CBMs can contain from 4 up to 14 choline binding sites, we devised a strategy to use multivalency to create a high affinity ligand for pneumococcus hydrolytic enzymes. For this, dendrimers were chosen as a scaffold, due to their high monodispersity and easiness of derivatization [7]. Therefore, choline dendrimers (ChDs) were synthesized by derivatizing polypropylene dendrimers with choline residues (Fig. 1).

The binding of ChDs to the major representative CBM, the protein C-LytA (Fig. 2), was monitored using surface plasmon resonance (SPR), fluorescence anisotropy and circular dichroism. These compounds presented 3 to 4 orders of magnitude higher affinity than free choline. Additionally, micromolar concentrations of ChDs were able to inhibit *in vitro* the activity of several of the most important pneumococcus cell wall hydrolases of the CBP family, and to prevent autolysis and cell division in *S. pneumoniae* cultures (Fig. 3).

These results present a clear example of how multivalency can considerably increase binding affinity and open the way for the development of new drugs against pneumococcus.

### References:

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**Figures:****Figure 1** – Generation 5 choline dendrimer structure.**Figure 2** - C-LytA structure based on PDB record 1H8G. C-LytA is formed by six choline binding repeats (CBRs). Each CBR forms a  $\beta$ -hairpin and two consecutive hairpins form a choline binding site, except for CBR6, which forms the dimerization interface.**Figure 3** – Micromolar concentrations of generation 5 choline dendrimers inhibit pneumococcus cell division, promoting the formation of long cell chains. Milimolar concentrations of choline are needed to achieve the same effects.