

CHOLINE DENDRIMERS AS VERSATILE TOOLS FOR THE DEVELOPMENT OF NEW ANTIBIOTICS AND TISSUE IMAGING

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Dendrimers are non-linear, branched polymers that display multiple copies of a single ligand on its surface. They are regular, monodisperse macromolecules of well-defined structure that can be easily functionalized and employed for the efficient molecular recognition of proteins. The multiple applications of these molecules range from *in vivo* drug-delivery to imaging agents [1,2].

Streptococcus pneumoniae (pneumococcus) is a major human pathogen [3] that contains multiple copies of the aminoalcohol choline (Fig 1A). These multivalent architectures serve as attachment sites for a variety of surface-exposed choline-binding proteins (CBPs) that are involved in essential processes for virulence such as cell-wall separation, the release of bacterial toxins and adhesion to the host [4]. All CBPs contain a characteristic choline-binding module (CBM) that directs the adsorption of the protein to the bacterium surface (Fig 1B). In order to mimic the structure of the pneumococcal cell wall we synthesized choline-functionalized poly(propyleneimine) (PPI) dendrimers [5] (Fig 2A) that increased their affinity to the CBMs about 10 000-fold compared to free choline. Concomitantly, the dendrimers also turned out to be very efficient inhibitors of the enzymatic activity of CBPs *in vitro* (by competing with the cell walls for binding to the enzyme), and arrested separation of daughter cells after cell division when assayed in liquid cultures of *S. pneumoniae* (Fig 2B). Therefore choline dendrimers constitute a promising starting point for the development of new antibiotics against pneumococcal diseases.

Taking advantage of the strength and specificity of the interaction choline dendrimers and CBPs, we have constructed a fusion protein containing the choline-binding module C-LytA and the collagen-binding protein CNA35 from *Staphylococcus aureus* [6]. The protein bound to fluorescein-labeled choline dendrimers with micromolar affinity. This non-covalent protein-dendrimer complex was used to specifically label collagen tissue samples that were subsequently visualized by fluorescence microscopy (Fig 3).

Our results therefore show that choline dendrimers can be used for specific, non-covalent, high affinity binding of fusion proteins containing choline-binding modules. These molecules may constitute attractive tools for a variety of biotechnological and biomedical applications.

References:

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