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Symmetric cationic γCD as extracellular inhibitor for bacteria

The worldwide spread of antibiotic-resistant microorganisms can be viewed as an ecological consequence of the systematic use of antimicrobial agents and presents an enormous challenge to society with opportunity for innovative solutions.

Various alternative strategies are under development, such as plasmid containing engineered DNA to destroy genes of pathogens [1], antimicrobial cationic and cyclic peptides all of which involve interaction with the bacterial cell membrane leading to cell death [2], enhancing the host's response to infectious agents through vaccination and immunomodulation [3], blockade of bacterial intercellular communication by using agents attacking quorum sensing molecules [4], bacteriophages therapy, where pathogens may be targeted through manipulation of phage DNA [5], non-antibiotics, compounds having potential to modify cell wall permeability with broad spectrum antimicrobial activity, antitumor antibiotics, compounds like azinomycins that show potent activity against multidrug resistant bacteria [6], phototherapy by using differential phototoxicity of photosensitizers in bacterial and human cells [7].

Cyclodextrins improve the solubility, stability and bioavailability of many antibiotics. CD-based formulations enable the controlled release of a variety of antibiotics such as vancomycin, tetracycline and doxycycline.

The advantages to use CDs for antibiotic delivery are clearly demonstrated by the formulations already in the market such as Pansporin-T (Takeda, Japan), Meiact (Meiji Seika, Japan), Mito Extra (Novartis, Europe) and Clorocil (Oftalder, Poland).

Recent studies have shown additional properties of the CDs. Indeed, these versatile molecules can be considered more than simple excipients, since they could work as antibiotics themselves. In a recent paper appeared in Nature Chemistry [8] Hagan Bayley, Ben Davis and co-workers showed how the adaptation and application of the latest technologies to new biological problems can result in novel contributions to the drug discovery process. The main idea of this work is based on the discovery systems able to weakening the outermost sugar protective coating around Gram-negative bacteria. With the modification of the outer membrane, the bacteria can be recognized and suppressed by the mammalian immune system.

So far, researchers have not been able to develop drugs capable of disrupting the capsular

polysaccharide layer. The pathogenic strain of *E. Coli* selected by the team from the university of Oxford produces the capsular polysaccharides for its protective coating by connecting sugar repeat units together inside the cell and then transporting the resulting polymers to the cell surface through a pore. This channel is formed by the trans-membrane protein Wza complex, an eight-fold symmetric bottomless vase-shape assembly that spans the outer cell membrane (Fig. 1).

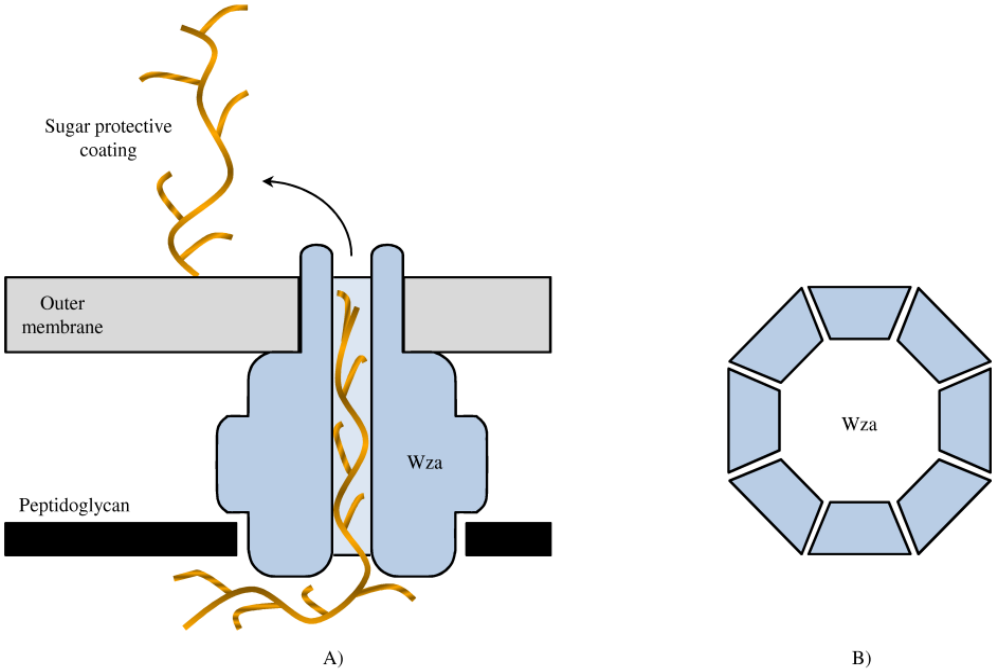

Fig. 1: A) Schematic representation of the transport of capsular saccharides through the Wza pore, B) Cross-section view of the Wza pore showing the octameric superstructure (drawn after Kong et al. [8]

The researchers have screened by single channel electrical recording in planar lipid bilayers (a recognized model for discovering channel blockers [9-10]) a series of glycomimetic molecules as potential channel inhibitor. In order to have a more effective screening test, the team had to engineer an open form of Wza.

After expression of the wild type Wza in a cell-free system, purification of the octamer by SDS-Page, careful modeling of some mutants pores and combination of the obtained data with the X-ray structure of the wild type Wza, they could produce an open form of the protein and start the screening.

Among the tested molecules the most effective Wza inhibitor (dissociation constant ~13 μM) proved to be the octakis(6-deoxy-6-amino)-γ-cyclodextrin (6-Am_8-γCD) (synthesis and structure in Fig 2). Inhibition is thought to have been achieved by binding of 6-Am_8-γCD to a previously unknown binding site of the Wza alpha helix barrel, a site that is directly accessible from the external medium.

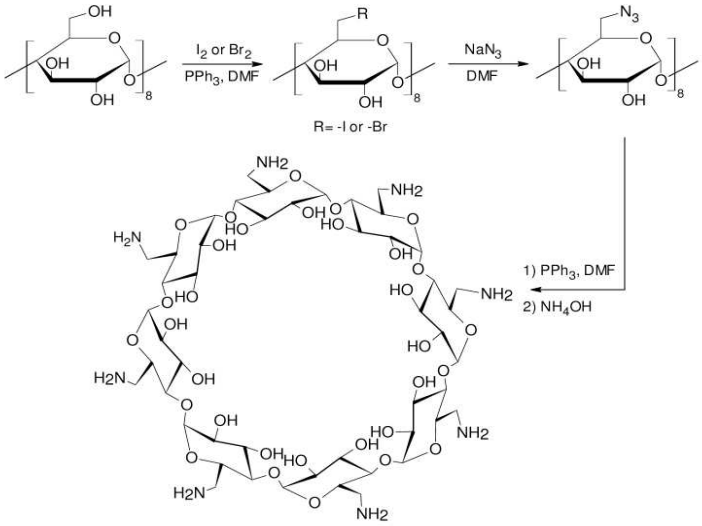

Fig. 2: Synthetic scheme and structure of octakis(6-deoxy-6-amino)-γ-cyclodextrin from the external medium.

The binding would occur by exploiting charge-charge and hydrogen bonding interactions and would be facilitated by the matching rotational symmetry and equivalent outer diameter (~17 Å) of the inhibitor with the internal diameter of the Wza pore (Fig 3). In order to gain detailed information about the binding site of the 6-Am$_8$-γCD, the team studied its interactions with several mutants of the Wza pores. By supporting the results with computational docking simulations, they could identify the amino acids involved in the blocker binding. Furthermore, the researchers demonstrated that 6-Am$_8$-γCD blocks Wza under simulated physiological conditions and that the inhibition causes defects by thinning the capsular polysaccharide layer in concentration dependent manner, allowing the bacteria to be recognized and killed by the complement of the human immune system. Specificity for the microbial target was demonstrated by the weak toxicity of the 6-Am$_8$-γCD towards a human cell line.

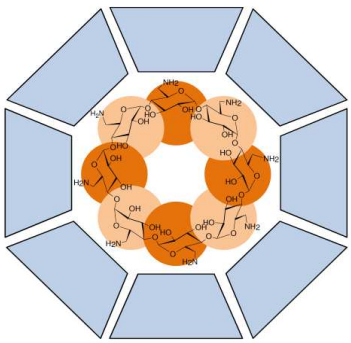

Fig. 3: Schematic representation of the interaction of Am$_8$-γCD with Wza pore (cross- section view, drawn after Kong et al. [8])

These studies represent the first examples of inhibitors against an important new class of antimicrobial drug target. Future plans of the Oxford team are evaluating the efficacy and the safety of their glycomimetics in animals and, if positive results will be achieved, moving the potential drugs into human trials.

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Milo Malanga

CycloLab R&D Laboratory Ltd., Budapest, HUNGARY

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Vesicles, Nanoparticles, Networks, Micelles, Carriers, Release

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Cholesterol, Cryotop, Slow-Freezing, Vitrification

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Semen, Frozen-Thawed Sperm, Freezing, Goat, Caprine

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Beta-Cyclodextrin, Dyeing, Sleather

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Imazalil, Thiabendazole And O-Phenylphenol, PAN Membranes, Ceramic Membranes

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Pullulan, Encapsulation, Aroma Compounds, Controlled Release, Nanofibers, Active Packaging, Cyclodextrin-Aroma Complex

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PAH Oxidation, Modified Fenton-Reaction, Activated Persulfate, Column

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Beta-Cyclodextrin, Adsorbent, Biodegradable, Adsorption, Heavy Metal Removal

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Beta-Cyclodextrin, Benzyl Azide, Benzyl Thiocyanate, Magnetic Nanoparticles, Nucleophilic Substitution

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Beta-Cyclodextrin; Aqueous-Solution; Semiconductor Photocatalysis; Exchange Bias; Bisphenol-A; Removal; Microspheres; Shell; Phosphopeptides; Nanocrystals

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Herbicide Norflurazon; Contaminated Soil; Enhanced Solubilization; Phenylurea Herbicides; Organic Amendments; Sandy Soil; Biodegradation; Bioavailability; Phenanthrene

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Magnetic; Transition-Metal-Free; N-Bromosuccinimide; Catalyst;

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Muscle Creatine-Kinase; Guanidine-Hydrochloride; Binding; Surfactants; Pathways; Urea

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Perfluorohexanoic Acid Pfhxa; Imprinted Polymer Adsorbents; Perfluorooctane Sulfonate; Reverse-Osmosis; Waste-Water

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Poly(Butylene Adipate); Polymorphic Crystals; Poly(Tetramethylene Adipate); Enzymatic Degradation; Melt Crystallization; Morphology; Poly(L-Lactide); Transformation; Metastability

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Pyrrole, Paal-Knorr, Beta-Cyclodextrin, Water

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Sorption Isotherm, Terpolymer, Beta-Cyclodextrin, Chitosan, Glutaraldehyde, Arsenate Dianion, 4-Nitrophenolate

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4-Nitrophenol, Films, Oxide, Isomers

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Beer, Mannose, Maltosaccharides, Hpaec, Pad

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Beta-Cyclodextrin, 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone, Charge Transfer, Fluoroquinolones, Honey, Inclusion Complexes

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Beta-Glucan, Immune-System, Polysaccharides, Innate, Interleukin-12, Macrophages, Ganoderma, Dectin-1, Dietary, Tlr2

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Capillary Electrophoresis, Chiral Separation, Enantioseparation, 2,8-Dimethyl-6H,12H-5,11-methanodibenzo[b,f][1,5]diazocine

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Capillary-Electrophoresis, Chiral Selectors, Enantiomers, Enantioseparation, Clenbuterol, Atenolol, Recognition, Separation, Blockers, Tlc

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Capillary Electrophoresis, Chiral Separation, Fluoxetine, Guanidine, Chiral Separation, Beta-Cyclodextrin, Amlodipine, Ce

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Chiral Selector, Separation, Clenbuterol, Resolution

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Chiral Separation, Methoxytolterodine, Phosphated Cd, Sulfated Cd, Tolterodine, Chiral Separation, Sulfated Cyclodextrins, Tolterodine, Selectors, Samples, Ce, Derivatives, Resolution, Drugs

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Protonated Molecule, Forensic Science, Drug Identification

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Graphene, Electrocatalysis, Electrochemistry, Supramolecular Assembly

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Non-Enzyme, D-Glucose, Thionine, Concanavalin A, Electeochemical Biosensor

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Photonic Crystal, Molecular Imprinting, Molecularly Imprinted Photonic Polymer, Beta-Cyclodextrin, Phenylalanine, D-Phe, L-Tyrosine, L-Tryptophan

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Reporter Ligands, Naphthyridine-Dansyl Linked Ligand, Naphthyridine-Dbd, Pyridine-Dbd

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Silicon Nanowire Biosensors, Label-Free, Molecular Printboards, Electrical Detection, Host-Guest, Beta-Cyclodextrin, Protein-Ligand, Whole-Blood, Real-Time, Sensor

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Sol-Gel Process, Bisphenol-A, Beta-Cyclodextrin, Polymers, Tio2, Recognition, Layers, Units, NMR

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