MULTIVALENT GLYCOCALIXARENES

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We herein briefly introduce the potentials of glycocalixarene as multivalent ligands. Their ability to inhibit specific proteins or to stimulate the immuno response, in fact, discloses the important role they might play in bionanotechnology and in nanomedicine

Glicocalixareni multivalenti
Nella presente rassegna vengono brevemente presentate le potenzialità dei glicocalixareni come leganti multivalenti. La loro capacità di inibire specifiche proteine o stimolare la risposta immunitaria mette in luce l'importante ruolo che questi leganti possono avere nel campo delle bionanotecnologie e della nanomedicina.

Calixarenes[1] are one of the most important classes of macrocycles developed in supramolecular chemistry[2], the chemistry of noncovalent interactions. Their name is due to the vase-like shape of these macrocycles which resembles that a Greek calix crater[3]. Especially in the case of the smallest macrocycle, the calix[4]arene, the functionalisation of the lower rim (phenolic O-atoms) with units larger than ethyl groups fixes the macrocycle in four different structures named by Gutsche as cone, partial cone, 1,3-alternate and 1,2-alternate, that are characterised by a different orientation of phenolic units into the space[3] (Fig. 1).

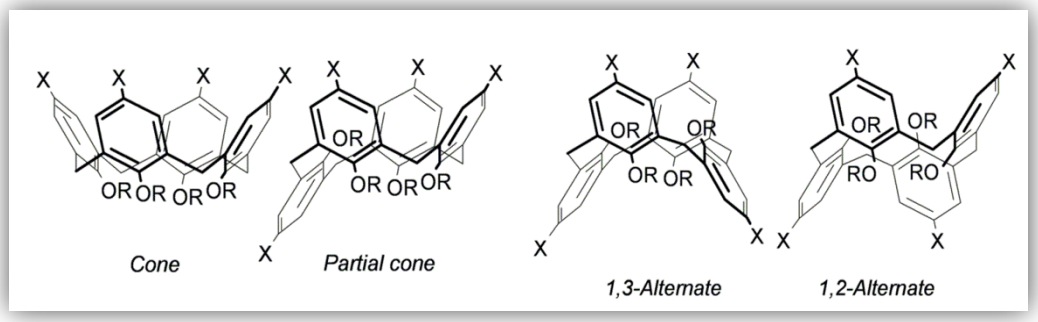

Fig. 1
Different structures of calix[4]arenes (when R>Et)

The success of calixarenes in supramolecular chemistry is due, beside to their easy preparation even in kilo-scale, to their ability to selectively interact with cations[4], anions and neutral molecules[1]. More recently they have been also used as scaffolds for the construction of multivalent ligands[5] taking advantage of the different valency they can reach by tuning the size of the macrocycle (Fig. 2) or of the different stereochemical orientation of the ligating units they can achieve by changing conformation.

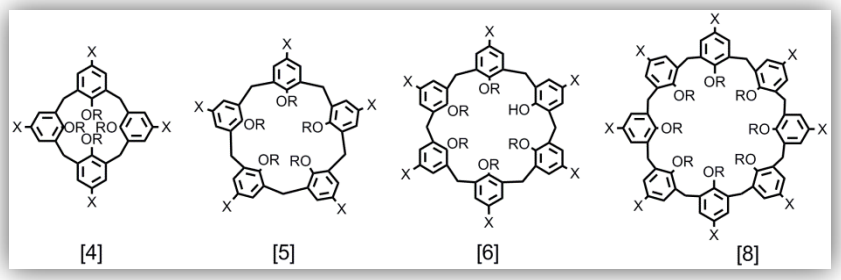

Fig. 2
Different size and valency of the calix[n]arenes (n≥4)

Multivalency[6], the ability of an entity/molecule to bind another entity/molecule via simultaneous and multiple interactions is an important tool also widely used by Nature to make binding more efficient and selective. In particular, cells exploit multivalency to communicate and interact with other cells or entities through carbohydrate-protein interactions. These phenomena are of great importance in many physiological and pathological processes such as cell-cell communication, virus, toxin and bacterial invasion and tumour progression[7,8]. The particular type of multivalency based on carbohydrate-protein interactions is also called cluster glycoside effect[9] and involves, from one side, the glycocalyx, a complex array of oligosaccharides (glycoproteins and glycolipids) present on the cell surface and, from the other side, specific proteins named lectins present on the interacting species[10]. The design and synthesis of multivalent glycosylated structures, glycoclusters, thus appears to be a promising strategy to provide novel high-affinity ligands able to interfere and inhibit all the pathological processes where carbohydrate-protein interactions are involved[7,8]. Calixarenes have therefore also been used as scaffolds for the preparation of multivalent glycoconjugates by linking carbohydrates, through a proper spacer, at the upper or lower rim and thus originating glycocalixarenes. By inserting on the glycocalixarene sugar moieties specific for the target lectin it is therefore possible to obtain potent multivalent inhibitors of these proteins. Different successful studies have been reported in the literature dealing with glycocalixarenes and the inhibition of a series of pathological processes of the cell. A first example refers to a tetra-sialylated calix[4]arene that protects cells from cytopathic effects when incubated with the influenza A virus and showing an anti-adhesive activity with an inhibition potency up *ca.* 300 times that of a monomeric model[11]. Another study reports on the synthesis of a series of galactosyl- and lactosylthioureido calix[*n*]arenes for the inhibition of medically relevant VAA plant toxin and human galectins. The results obtained show that glycocalix[6]- and -[8]arenes are able to inhibit the binding of galectin-4 to human pancreatic carcinoma cells with high potency. Moreover the 1,3-alternate calix[4]arene results the most efficient inhibitors of the binding of galectin-1 but does not recognize galectin-3, while its isomeric cone derivative (Fig. 3), simply differing in the stereochemical orientation of the lactose units in the space, strongly inhibits the adhesion of galectin-3 but not of galectin-1[12].

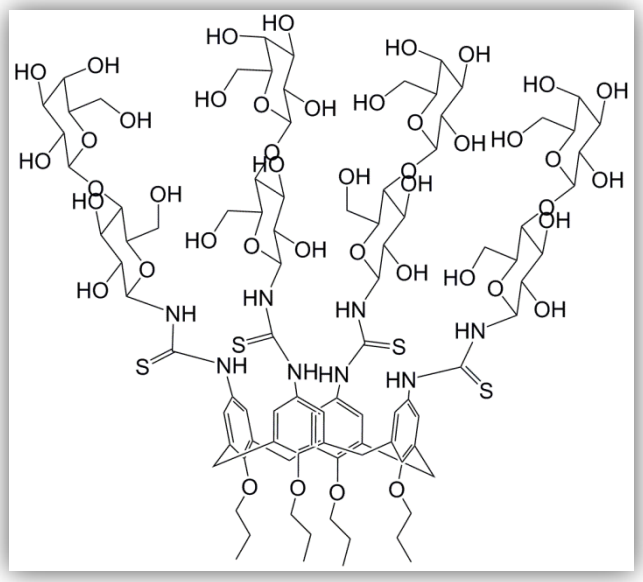

Fig. 3
Lactosylthioureido calix[4]arenes that inhibit galectin-3

Also, quite interestingly, galactose units clicked at the lower rim of calix[4]arenes strongly interact with *Pseudomonas Aeruginosa* Lectin A (PA-IL) with a ca. 200 time increase in the binding compared to its monomeric counterpart and thus suggesting the use of these compounds as possible disaggregation agents of biofilms formed by this opportunistic human pathogen[13]. Glycocalixarenes have been also anticipated as potential site-specific drug-delivery systems. Their use for the noncovalent functionalization of liposome was explored and the *1,3-alternate* glucosylated calix[4]arene resulted an useful bolaamphiphile to stabilize and rigidify liposome. Furthermore the presence of the glucosylcalixarene derivative in the liposome structure reduces the leakage of calcein from the liposome internal aqueous compartment and greatly enhances the entrapment of a lipophilic drug in the lipid bilayer.
Moreover, these glucosylated liposomes also show a specific multivalent interaction with Concanavalina A (a plant lectin used as model)[14], thus demonstrating that the noncovalent functionalisation of liposome with

multivalent glycosylated ligands might give the possibility to prepare efficient and stable drug delivery systems, potentially able to target specific lectins/cells (Fig. 4).

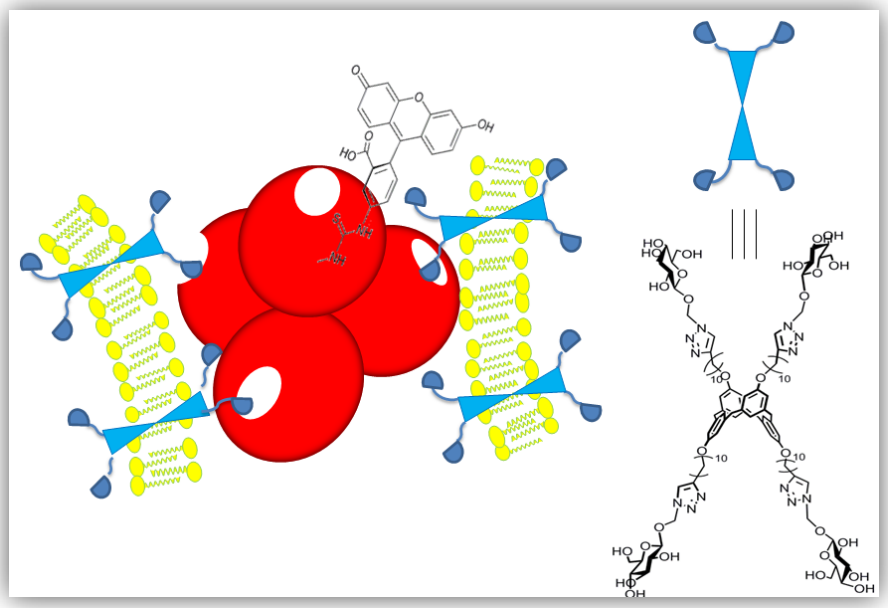

Fig. 4
Interaction of the glucosyl units of glicocalixarene bolaamphiphiles included in a DOPC bilayer with ConA lectin

Finally, glycocalixarenes for the activation of the immune response were also developed. For example a cone calix[4]arene bearing four tumor associated antigen S-Tn at the upper rim and the immunoadjuvant PC_3S at the lower rim was studied. This derivative induces a more effective immune response than the monovalent reference compound when tested both at the same and at 4-fold higher concentration[15].
In conclusions, glycocalixarenes strongly interact with biomacromolecules and especially with carbohydrate binding proteins, exploiting multivalency. The increasing number of publications and patents on this class of compounds testifies the growing interest for their application in nanomedicine for antiadhesion therapy, activation of the immuno response, pathogen inhibition and targeted drug delivery.

Acknowledgements
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