"Supra"molecular Recognition of Galectin 1

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In this issue of *Chemistry & Biology*, Belitsky et al. [1] elegantly describe the use of supramolecular complexes of sugars to multivalently target Galectin-1 (Gal-1), a lectin involved in the organization of cell surface glycoproteins, signaling, cell death, and cancer metastasis.

The images of proteins and nucleic acids in biochemistry textbooks are often adapted from crystal structures or Irving Geiss illustrations. These images, however, represent only single frames in a movie. In reality, biomolecules are dynamic and exhibit motions that are either stochastic or carefully orchestrated, as in cases of chemical catalysis. On a larger scale, this dynamic nature is also illustrated by the constant reorganization of proteins and lipids on cell surfaces.

Carbohydrates displayed on cell surfaces are essential for signaling and adhesion, but they are also gateways for viral and bacterial infections. The hallmark of these interactions is multivalency, in which multiple weak interactions are formed simultaneously to increase affinity and specificity. Taking cues from nature, many laboratories have used multivalency as a strategy to find inhibitors of a variety of interactions, including those between a virus and a host cell by multivalently displaying sugars on a polymer's backbone [2]. In some cases, crystal structures of carbohydrates bound to their receptors can guide the design of multivalent compounds, illustrated by the elegant work of the Bundle group to neutralize Shiga-like toxins by STARFISH ligands [3].

The most common multivalent assemblies used to target cell surface interactions display ligands on flexible polymeric scaffolds. The flexible nature of the scaffolds is advantageous since it allows some conformational flexibility in how multivalent ligands are displayed. These flexible polymers, however, do not exhibit flexibility on a scale that would allow them to adapt toward the dynamic distribution of glycoproteins on cell surfaces. Because of the weak monovalent affinity of carbohydrates and lectins (K_ds $\geq$ 1 mM) and the use of multivalency to increase binding affinity, the impacts of cell surface dynamics on molecular recognition are likely to be significant.

In this issue of *Chemistry & Biology*, Belitsky et al. [1] elegantly describe the use of adaptable supramolecular pseudopolyrotaxanes that multivalently display mobile ligands, analogous to "beads on a string." The ligands have rotational freedom about the polymer and limited translational freedom. This allows the ligands to optimally orient themselves with regard to the target and to each other (Figure 1). Specifically, they used pseudopolyrotaxanes displaying lactosides to multivalently target galectin-1 (Gal-1), which normally binds glycoproteins expressed on cell surfaces. Information from studies of a series of sugar-displaying pseudopolyrotaxanes gives insight into the optimal spacing between ligands and ligand adaptability.

Galectins play important roles in cell adhesion, signaling, angiogenesis, and apoptosis. Thus, galectins have long been an intriguing target for the development of cancer therapeutics. Specifically, intra- and extracellular Gal-1 contribute to tumor progression through cell adhesion and migration and to tumor escape from the immune system. Gal-1 is overexpressed in cancer cells and binds to lactosides expressed on the surfaces of T cells causing their aggregation and eventual apoptosis. This leads to the weakened immune response that is associated with tumor progression and metastasis. Interestingly, inhibition of Gal-1 gene expression suppresses tumor proliferation [4] and is associated with improved immunoregulation and a corresponding decrease in tumor mass [5]. These results suggest that inhibition of Gal-1 may be a viable therapeutic avenue for treatment of cancer.

Like other cell surfaces, the organization of proteins expressed on T cell surfaces is dynamic. Despite this, Gal-1 effectively binds lactosides displayed on the cell surface and nucleate T cell agglutination. Thus, the coauthors hypothesized that multivalently displayed lactosides that mimic dynamic reorganization at T cell surfaces might bind in a similar fashion and be effective inhibitors of Gal-1. An additional challenge to multivalently targeting Gal-1 is the protein itself. Like other members of its protein superfamily, Gal-1 is a rigid dimer [6] with two lactoside binding sites that are spaced far apart and point in opposite directions. Belitsky and coworkers showed that pseudopolyrotaxanes can dynamically display lactosides, accommodate distal binding sites, and inhibit T cell agglutination (Figure 1) [1].

The pseudopolyrotaxane system used to multivalenlty target Gal-1 was based on the pioneering work of Harada [7] and Wenz [8] on cyclodextrin (CD)-based pseudopolyrotaxanes. Belitsky et al. [1] used a polyviologen backbone encircled by cyclodextrin (CD) functionalized with lactosides (LCD). Polyviologen copolymers are repeating units of decamethylene and positively charged bipyridinium units. CDs are naturally occurring cyclic oligosaccharides that encircle hydrophobic compounds in aqueous solutions.

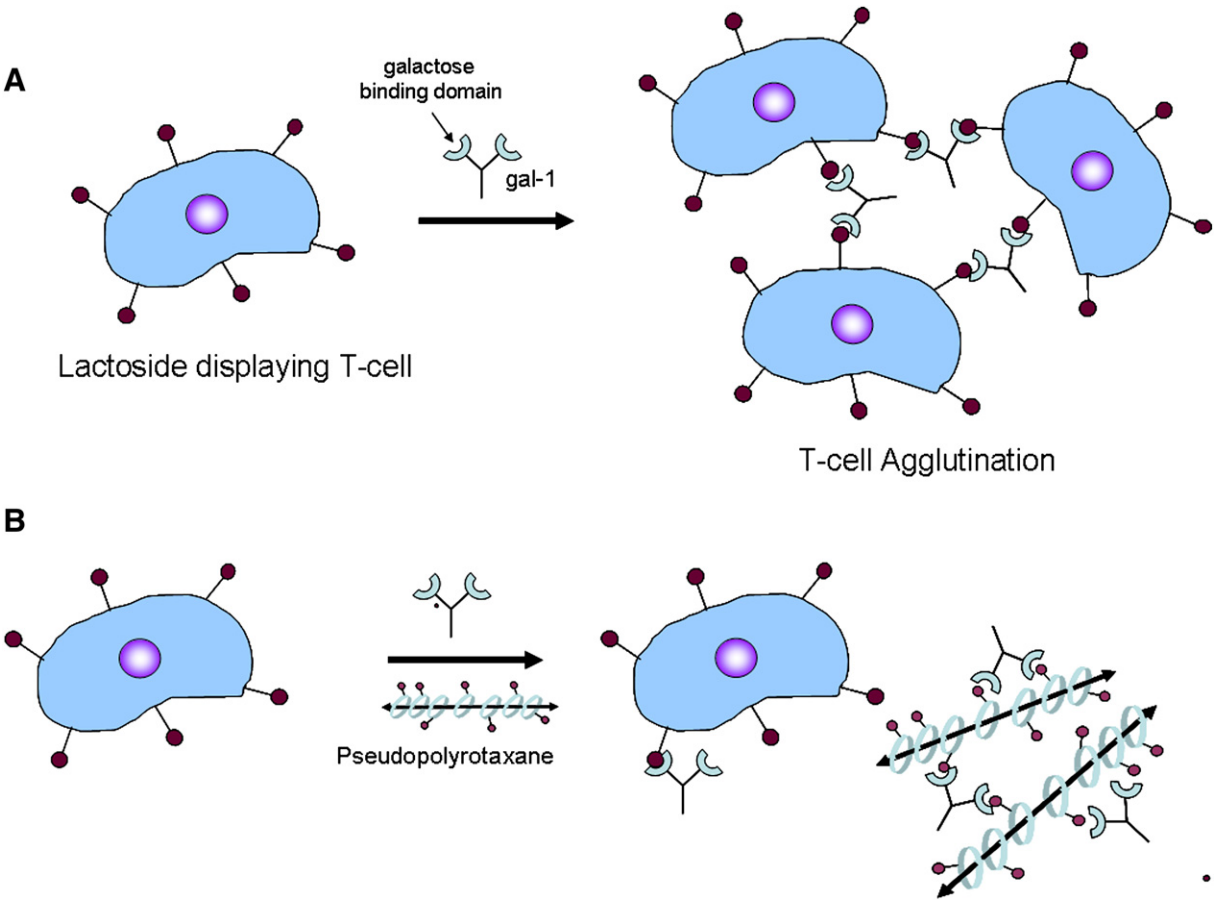

Figure 1. Supramolecular Recognition of Gal-1 by Lactoside-Displaying Pseudopolyrotaxanes
Schematics of (A), T cell agglutination caused by the lactoside binding protein Galactin-1 (Gal-1) and (B), inhibition of T cell agglutination by adaptable pseudopolyrotaxanes that display lactoside-functionalized cyclodextrins (LCDs). LCDs can rotate 360° about the polymer and have limited translational freedom.

The LCDs have both translational and rotational freedom around the polymer backbone. They can rotate 360°, however, their translational movement is limited by the presence of the bipyridium units, which serve as "speed bumps."

In order to determine the optimal length of the polymer backbone (related to the distance between binding sites) and the loading of LCDs (related to multivalent binding), the authors synthesized and studied three polymers. By altering the amount of polymer present in a constant concentration of LCDs, the amount of loading or threading was controlled. Nearly fully threaded polymers were synthesized by adding equimolar amounts of LCDs to repeating polymer units. Analogously, half-threaded and quarter-threaded polymers were created by having twice the amount of polymer repeating units as LCDs and four times the amount of polymer, respectively. Fully threaded pseudopolyrotaxane with one equivalent of free LCD were created from solutions containing a 2:1 ratio of LCD:polymer repeating units.

The polymers were then tested for their ability to inhibit T cell agglutination and compared to the monovalent lactoside-functionalized CD. At 100 μM concentration of lactoside, the longest polymer with quarter threading showed the largest multivalent enhancement over monovalent lactose (30-fold) and free LCD (20-fold) and was 2-fold better than the fully threaded polymer. This is consistent with a supramolecular statistical effect, in which multivalent binding is dependent on the number of connected ligands. Though the lactosides displayed on the pseudopolyrotaxanes are not covalently connected, they are mechanically connected by encapsulating the same polymer backbone.

Though there are still some questions to be answered such as the mechanism of binding, these studies are a firm foundation for future investigations that use adaptable ligands to multivalently bind other biomolecules.

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