

Interactions of the human anti-HIV-1 Antibody 2G12 with Oligomannosides and their Multivalent Glyconanoparticles in solution as studied by NMR

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Human antibody 2G12 neutralizes a broad range of human immunodeficiency virus type 1 (HIV-1) isolates by binding an unusually dense cluster of carbohydrate moieties on the “silent” face of the gp120 envelope glycoprotein.[1] Crystallographic studies revealed that 2G12 is highly specific for terminal Man α 1 \rightarrow 2Man [1,2], but that its carbohydrate specificity is less restrictive than originally believed [1], enhancing binding to a cluster of oligomannose moieties and relaxing the constraint of an exact match of the oligosaccharides with respect to the multivalent binding site of the antibody [2].

Although 2G12 binds to clusters of oligomannoses with nM affinity, the binding kinetics of the individual oligosaccharides is amenable to NMR-ligand-based techniques [3]. With the aim of gaining a better understanding of the influence of multivalency on the molecular recognition of oligomannosides by the antibody 2G12 we are carrying out NMR studies in solution, using synthetic oligosaccharides and their corresponding multivalent functionalized gold nanoclusters (glyconanoparticles, GNPs) prepared in our group, following a two-step approach:

(1) The study of the binding of the oligomannosides (di-, tri-, tetra-, penta- and heptamannosides) to 2G12 by using Saturation Transfer Difference NMR (STD NMR) and exchange-transferred NOE experiments; STD NMR allows the determination of their binding epitopes and their relative affinities [3]; using tr-NOESY experiments it is possible to determine their conformations in the bound state, and compare them with those in the free state [3], key structural information for the atomic characterization of these flexible ligands.

(2) The study of the affinity enhancement afforded by the multivalent presentation of the oligomannoside ligands on the surface of the GNPs by the use of competitive STD NMR experiments through the addition of the nanoclusters to the protein-oligosaccharide complex. For these systems, the relative affinity of the GNP can be determined monitoring the disappearance of the STD signals of the monovalent ligand, the latter acting as an indicator molecule, provided that conditions are used to assure the selectivity of the protein saturation.

[1] D. A. Calarese, *et al.*, *Science* 300, 2065 (2003).

[2] D. A. Calarese, *et al.*, *Proc. Natl. Acad. Sci. USA* 102, 13372 (2005).

[3] B. Meyer and T. Peters, *Angew. Chem. Int. Ed.* 42, 864 (2003).