

Carbohydrate based Affinity Ligands for Product-Protective Glycoprotein Purification



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Affinity capture is a critical step in downstream processing of almost all biopharmaceuticals. Typically, these steps are based on highly specific and/or selective, multivalent affinity interactions between target molecule and affinity ligand providing high yield and strong purification factors. On the flip side these multivalent affinity interactions require denaturing elution conditions that can impact product quality. Recently, investigators have found that elution from protein A affinity resins introduces higher order oligomers and aggregates into monoclonal antibody manufacturing lots. Thus, it seems that denaturing elution conditions on high capacity resins should rather be avoided. Some Boronate and transition metal complexes of carbohydrates are useful for affinity capture and allow competitive elution under non-denaturing conditions. Here we investigate the potential of modified carbohydrates to serve as affinity ligands for glycoproteins in a cell culture matrix.

The Affinity Capture Problem

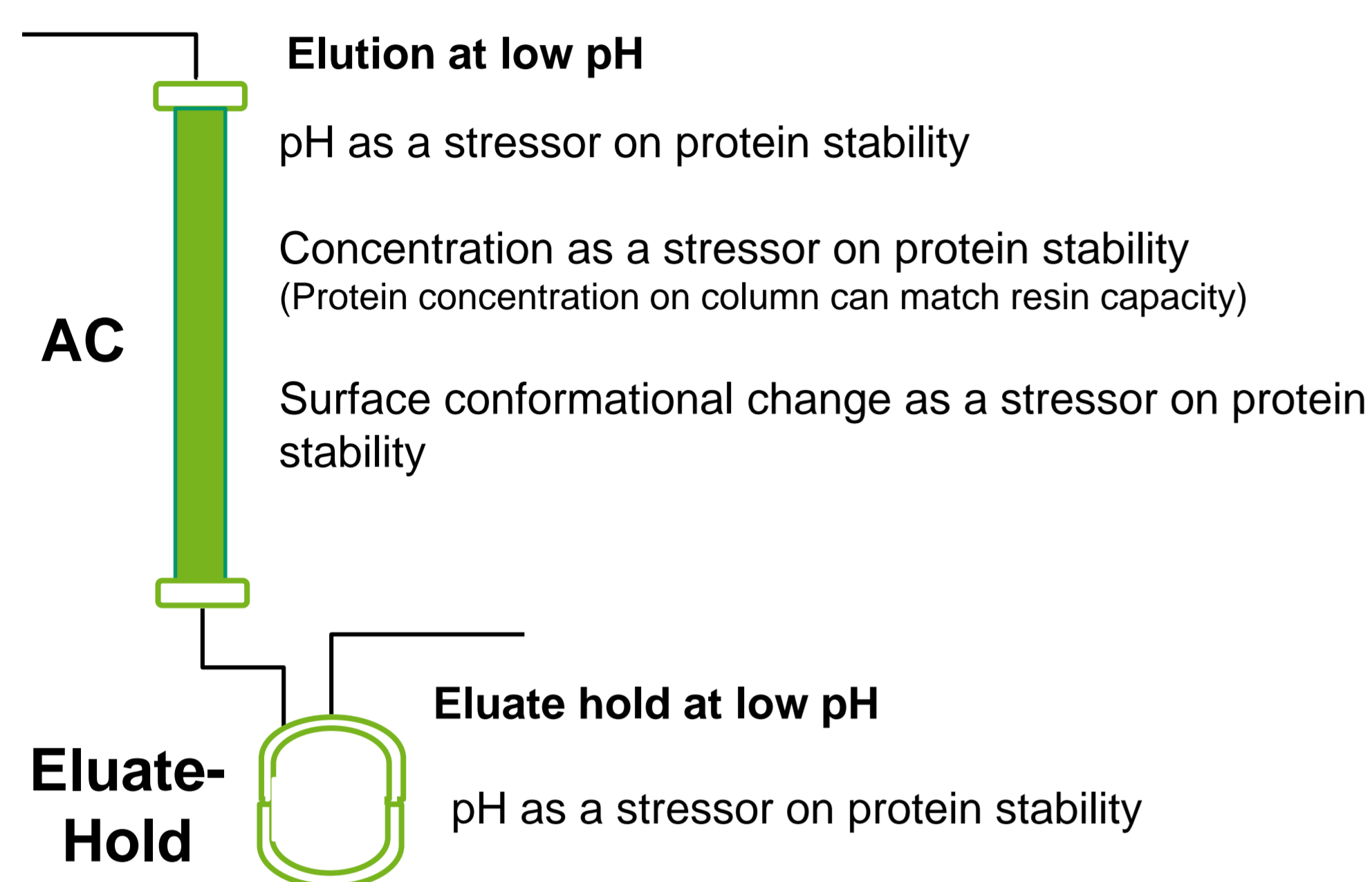


Fig. 1: Affinity capture, low pH hold and their impact on protein stability

Protein A induced Aggregate Formation?

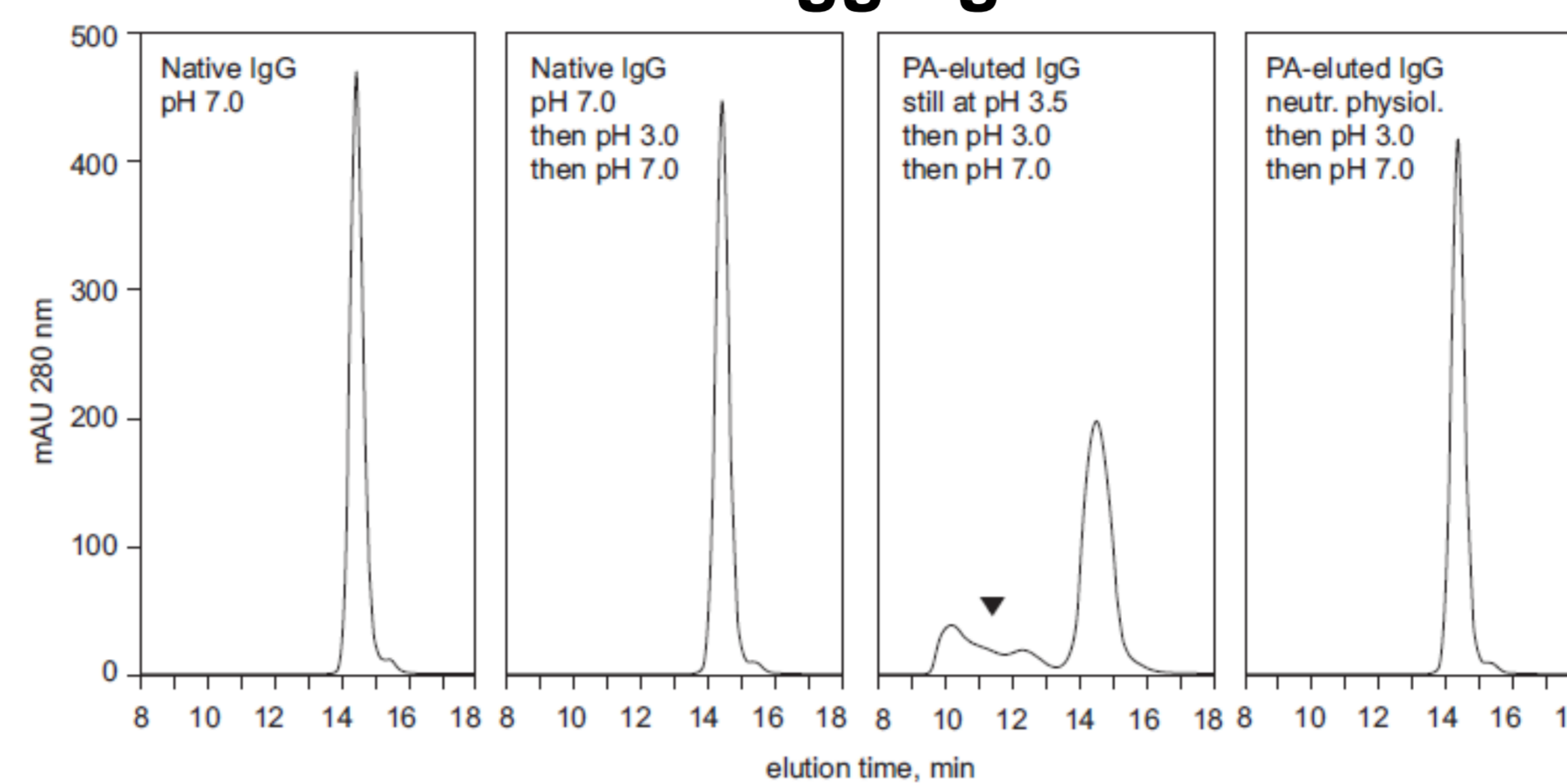


Fig. 2a: SEC Profiles illustrating increased vulnerability of protein A-purified IgG by exposure to pH 3.0 (Original figure from Gagnon et al., 2015 [1]).

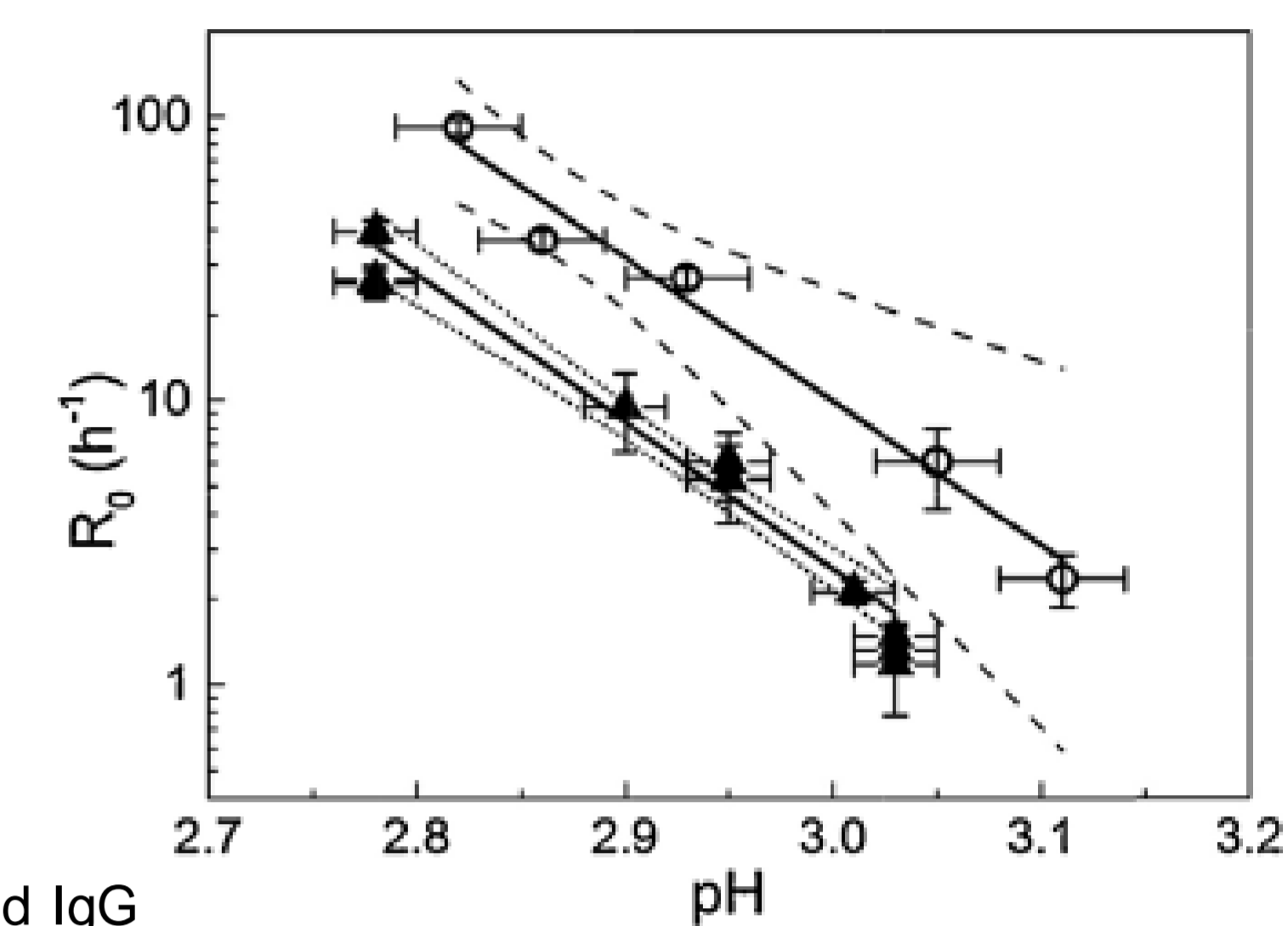


Fig. 2b: Semi-log plot of monomer decay rate, R_0 , against pH for solution-only experiments (filled triangles) and experiments including a prior protein A chromatography step (open circles). (Original figure from Mazzer et al., 2015 [2]).

Search for Alternative Capture Ligands

Prerequisites:

Reasonable selectivity
Mild non-denaturing elution

Possible Solution:

→ multivalent ligand binding
→ competitive elution

Basic Approach: Branched Oligosaccharide as a bivalent ligand scaffold

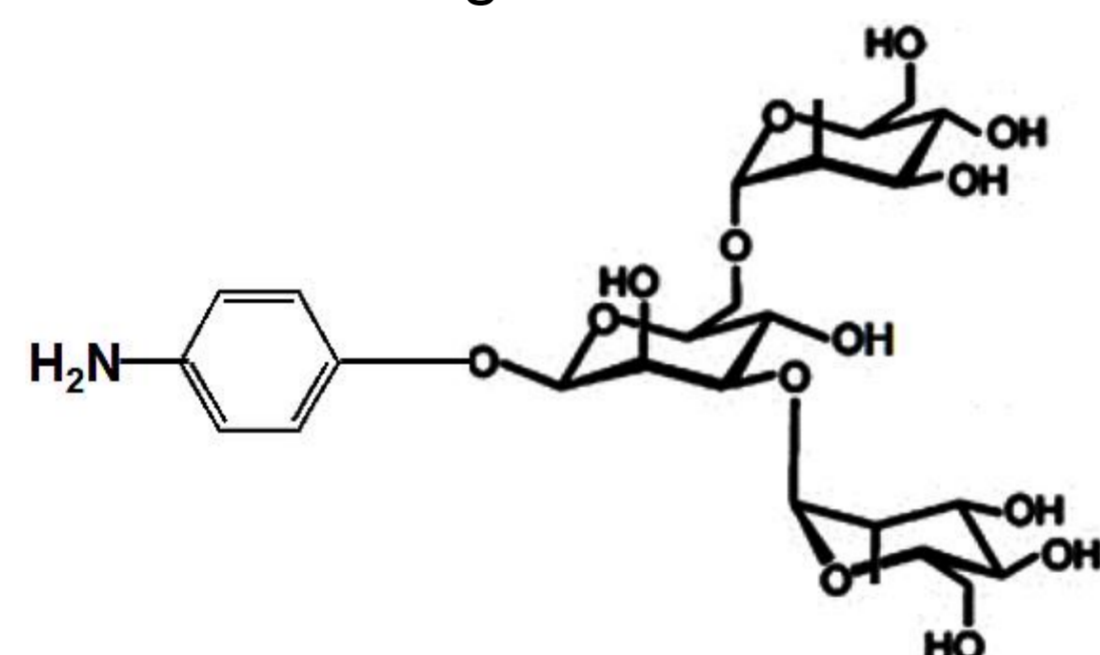


Fig. 3: 4-Aminophenyl-3,6-Trimannose as a branched oligosaccharide ligand scaffold

Option 1: Boronate Affinity

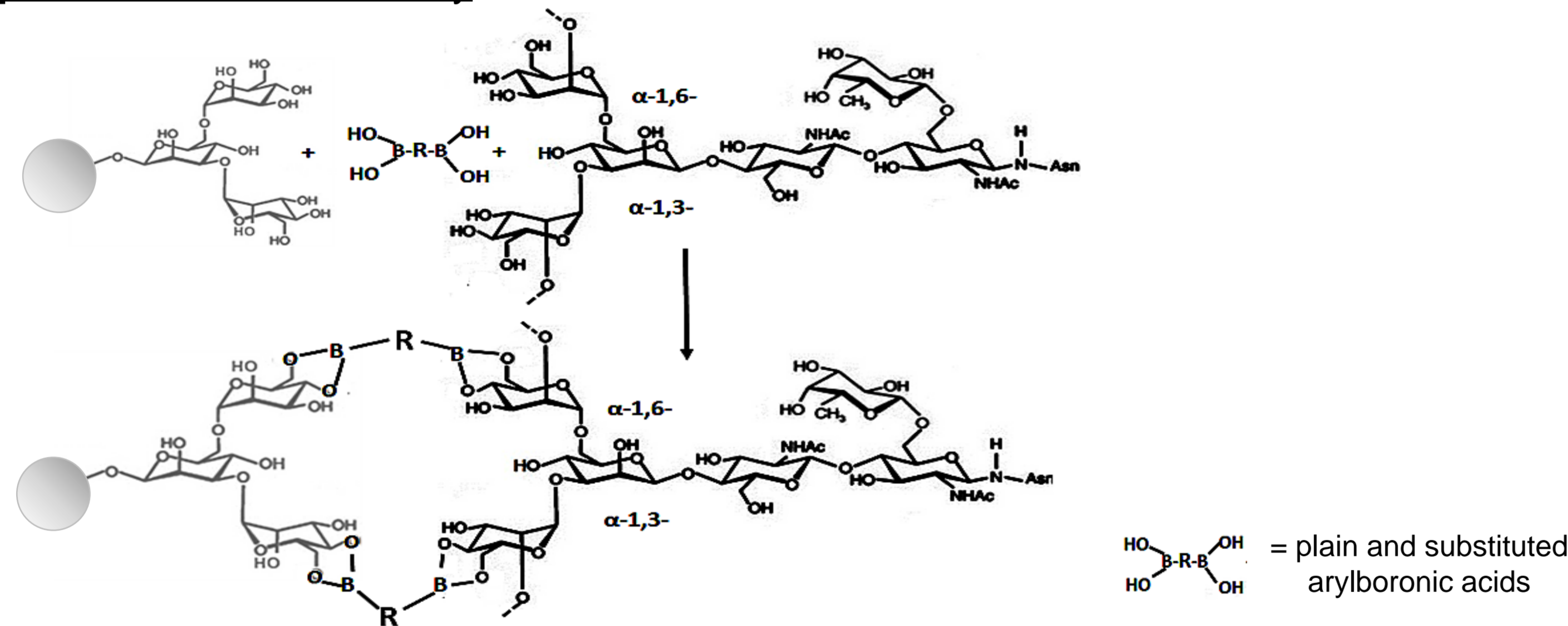


Fig. 4: Working Hypothesis for bivalent binding between boronate charged Trimannose-scaffold and protein-bound biantennary N-glycan. Example: Sugar-Diol-Boronic Acid Complexation [3,4]

Option 2: Carbohydrate Metal coordination complexes

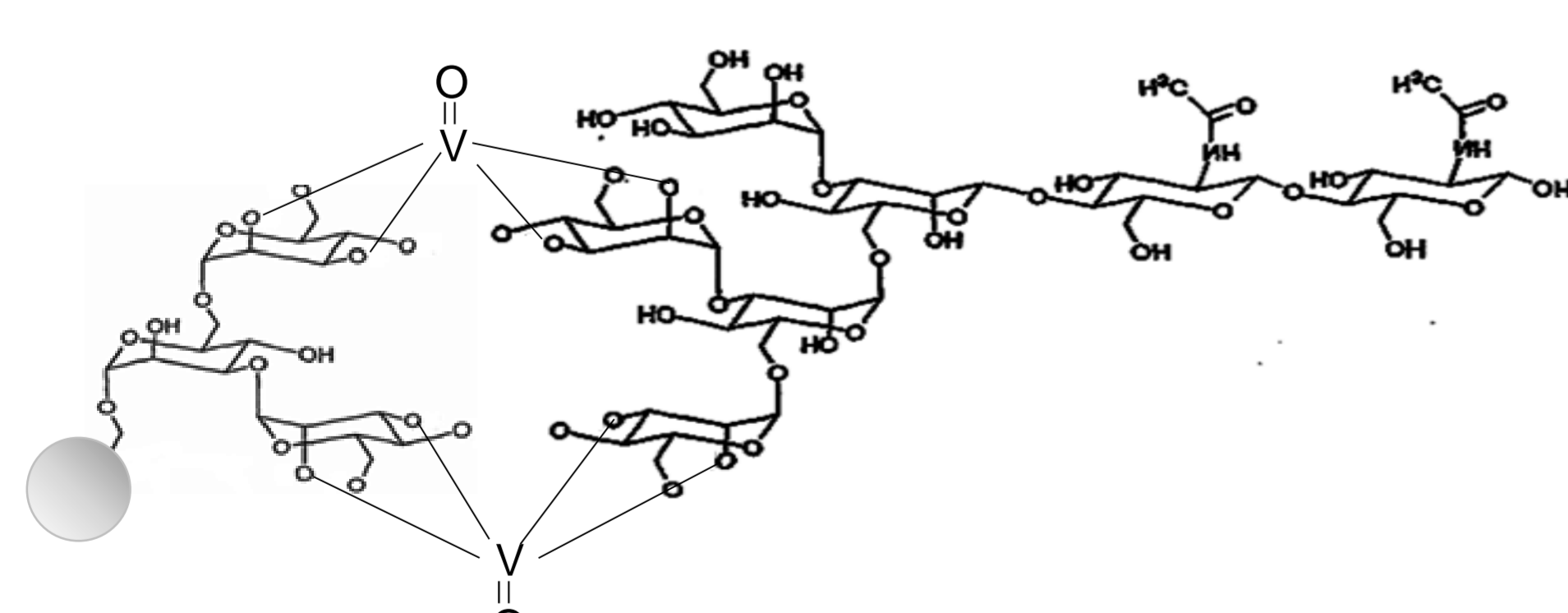
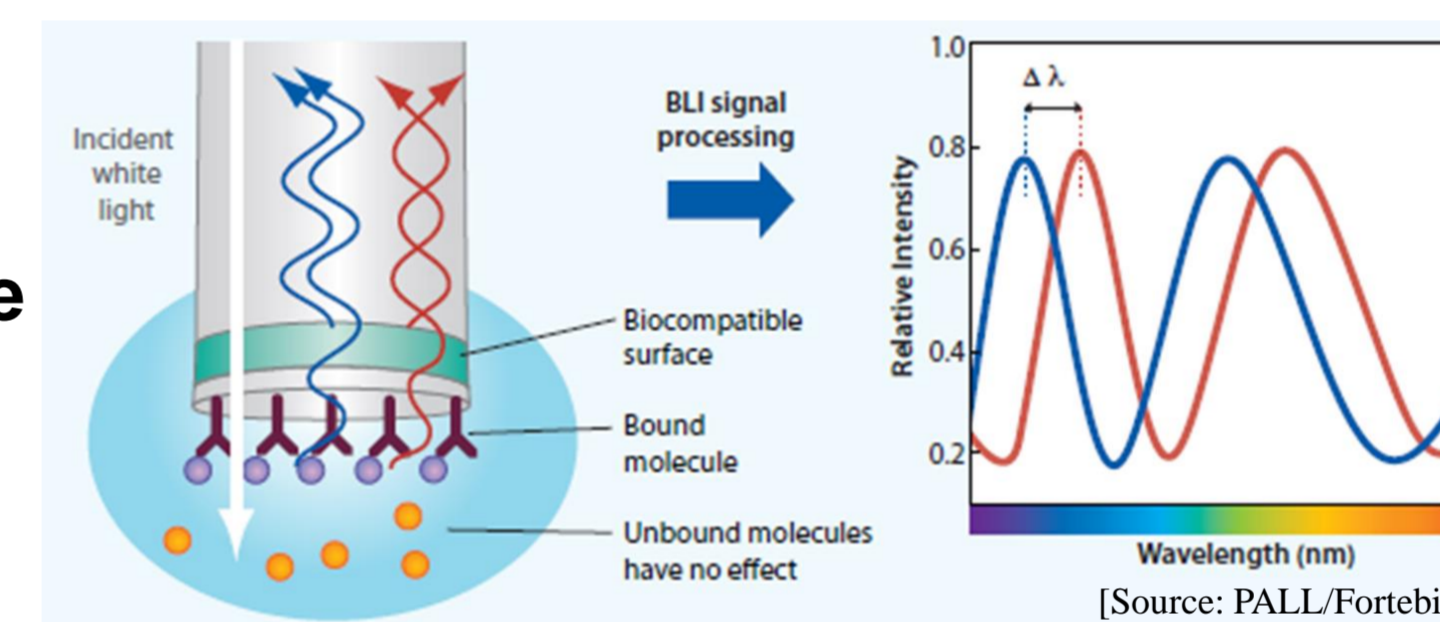


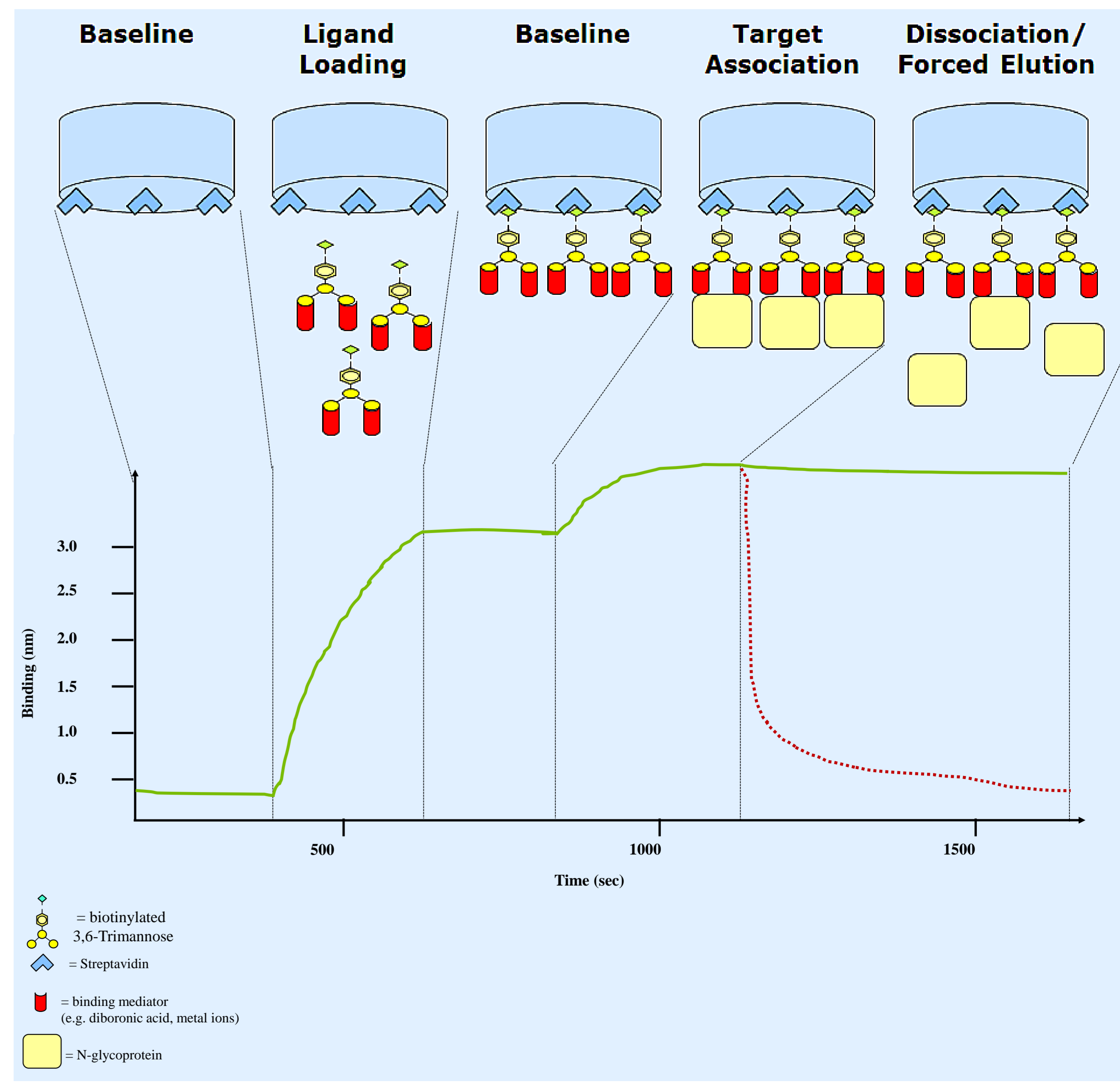
Fig. 5: Working Hypothesis for bivalent binding between metal charged Trimannose-scaffold and protein-bound high-mannose N-glycan (Man5). Example: Sugar-Oxovanadium(IV) coordination [5]

Initial Approach: Analysis of binding and elution kinetics by Biolayer Interferometry

Measuring Principle



Operating Principle:



Perspectives

Modified carbohydrates hold a great potential to serve as affinity ligands for glycoprotein capture from cell culture supernatants. Knowledge of detailed binding and elution kinetics are of particular importance for identifying ideally suited ligands for this purpose. These kinetics will be investigated by biolayer interferometry as well as by orthogonal methods. In addition, the selectivity of ligand binding will be investigated. Of particular interest is the investigation of the binding selectivity of the oligosaccharide ligands towards high-mannose structures as present on Man5-glycosylated product variants and certain adventitious agents e.g. enveloped viruses.

Literature

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