Electronic Supplemental Information

Probing multivalency for the inhibition of an enzyme:
Glycogen phosphorylase as a case study

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Determination of $K_i$ values

The activity of glycogen phosphorylase was assayed into the direction of glycogen synthesis. It means that the enzyme catalyzes the addition of glucose residues to a glycogen primer from an α-D-glucose-1-phosphate (G1P) substrate. Glycogen serves as a primer in this reaction and binds to the glycogen storage site of the enzyme. G1P is bound to the catalytic site and by the liberation of phosphate, the glucose residues are attached to glycogen. Therefore G1P concentration is varying between 2 and 20 mM in the assay (as given in the methods), since the affinity of the enzyme for G1P is about 4-6 mM and the glucose analogue inhibitors are competing to this site. Glycogen has an affinity toward the enzyme around 0.1-0.3 % (depending on the molecular mass of glycogen) thus 1 % glycogen used in the assay saturates the storage site of the enzyme.

The poor solubility of the investigated inhibitors limits the concentration used in the kinetic experiments, since DMSO at higher concentrations (more than 5 %) strongly reduces the catalytic activity of glycogen phosphorylase. Only inhibitors 9 and 14 reached more than 50 % inhibition in the highest concentration applied (2.5 mM) therefore the $K_i$ could be determined. The details are given in the methods, shortly 4 different inhibitor concentrations were used in the presence of varying concentrations of G1P. Thus the competition between the substrate and inhibitor can be calculated using the Dixon plot. This procedure could not be applied to inhibitors 5, 7 and 15 due to their poor solubility.
Atom numbering of molecule