Understanding the donor selectivity of the *Salmonella enterica* Virulence Factor SseK1 by STD NMR Spectroscopy

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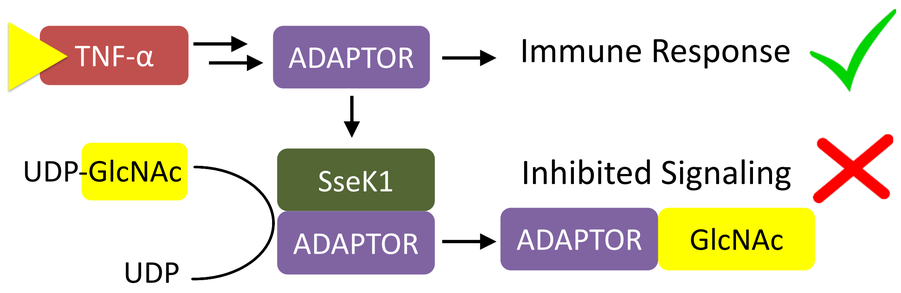
Introduction

SseK1 is a virulence factor expressed by the human pathogen, *Salmonella enterica*. SseK1 suppresses host innate immune response by interfering with the TNF-α pathway [1]. This suppression is achieved via a unique glycosyltransferase activity, which targets host proteins by tagging them with a single N-Acetylglucosamine (GlcNAc) sugar residue [2]. This reaction involves GlcNAc transfer from the donor substrate, UDP-GlcNAc, to the guanidine functional group of arginine residues within the adaptor proteins, rather than the canonical O- or N-glycosylation at serine, threonine or asparagine residues [2].



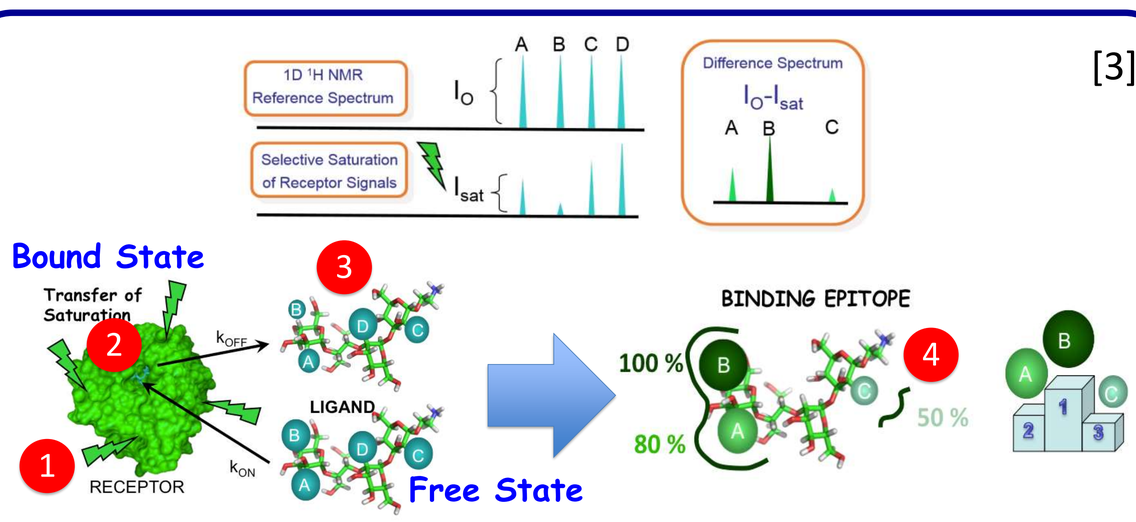
Aims

To explore the donor selectivity of SseK1 by measuring the binding of the accepted donor, UDP-GlcNAc, three related sugar nucleotides and UDP nucleotide.

To explore the binding of the ligands this study sought to satisfy the following aims:

- Explore if binding is the discriminating process: do all ligands bind?
- Produce a binding epitope map of the ligands to uncover binding mode
- Use STD NMR to identify differences in binding kinetics between the ligands

Saturation Transfer Difference NMR

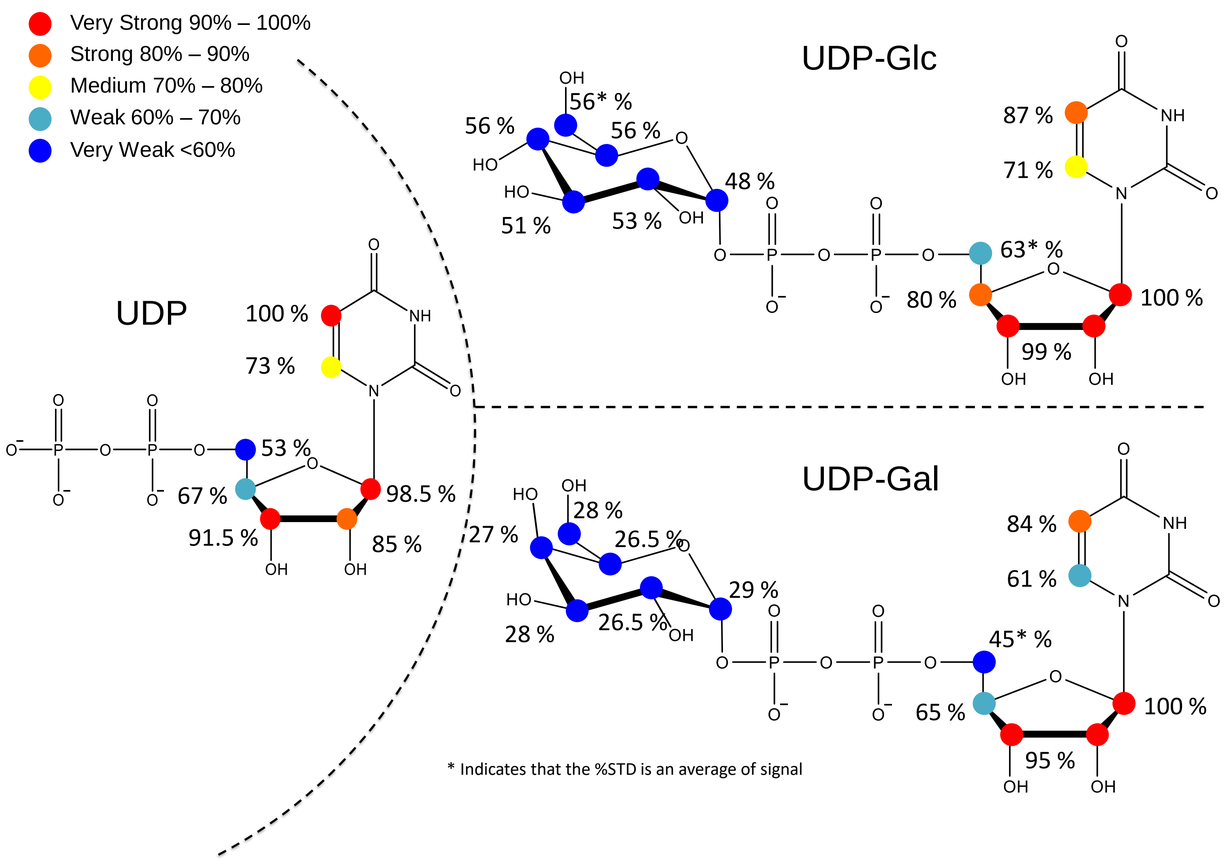


[3]

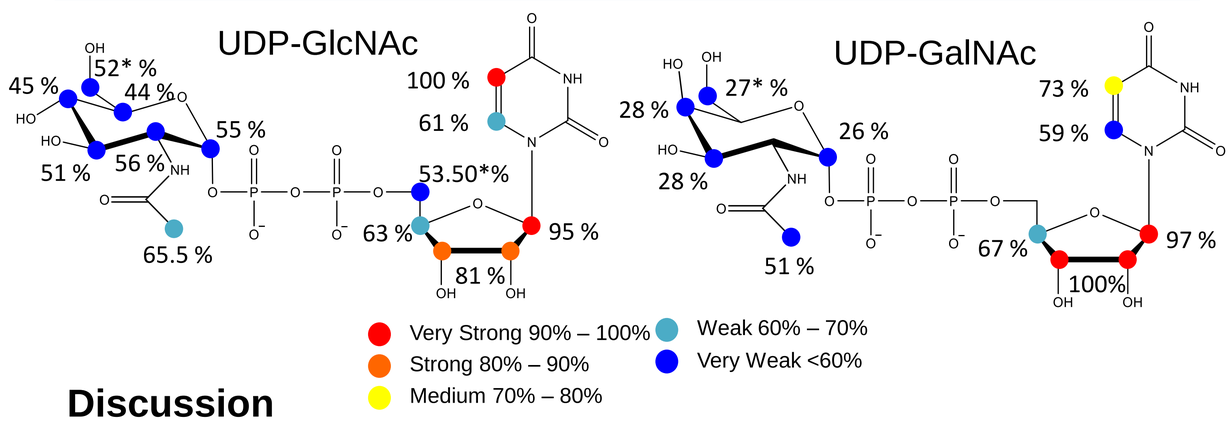
1 Selective Protein Saturation
2 Ligand Saturation Transfer **(BOUND STATE)**
3 Saturation Accumulation **(FREE STATE)**
4 Difference Intensities (**Binding Epitope**)

Intensities in the difference spectrum reflect **proximity to the protein surface** in the bound state (hot spots or **"binding epitope mapping**")

Binding Epitope Mapping: UDP, UDP-Glc/Gal



Binding Epitope Mapping: UDP-GlcNAc/GalNAc



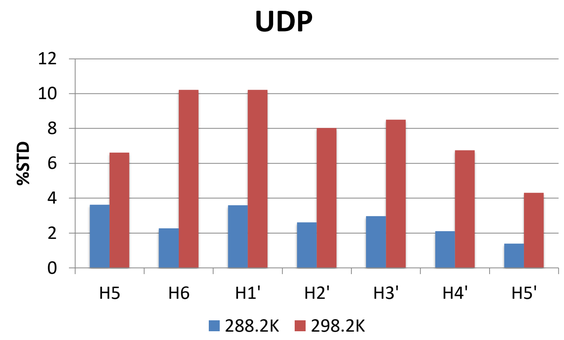
Discussion

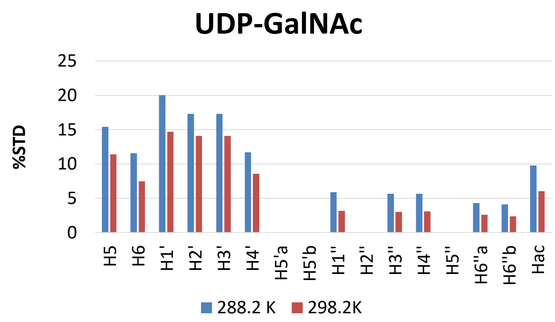
- UDP, UDP-Glc, UDP-Gal, UDP-GlcNAc, and UDP-GalNAc, all bind to SseK1
- SseK1 shows similar overall recognition of all the ligands. However, some important differences are observed regarding proximity to protein of the sugar rings
- UDP-Glc and UDP-GlcNAc have significantly higher %STD at the sugar ring indicating that a "glucose" configuration of the sugar seems to be a needed for efficient binding
- In the same line, N-Acetyl side chain shows a higher %STD in UDP-GlcNAc compared to UDP-GalNAc

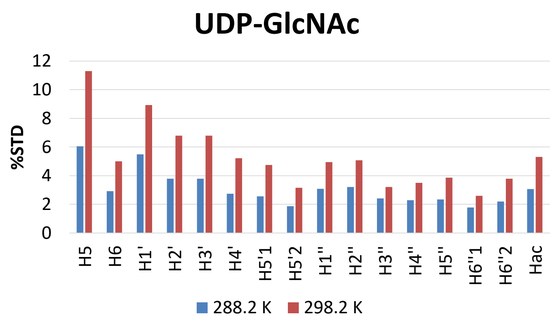
Binding Kinetics from VT STD NMR

Variable temperature (VT) STD-NMR experiments were performed to reveal differences in binding kinetics between the ligands. STD NMR experiments (2s saturation time) were carried out at 288 K and 298 K at 1mM ligand, 25 μM SseK1 protein and 250 μM $MgCl_2$.

- Increased STD with elevated temperature will occur for "strong" binders (we speed up chemical exchange)
- Weaker ligands will reduce STD intensity with increases in temperature







The ligands showing increase in %STD with temperature were UDP and UDP-GlcNAc. All the other sugar nucleotides had their %STD decreased (UDP-Glc andUDP-Gal not shown). This results is in excellent agreement with the kinetic data for this system, obtained by ITC [2], showing VT STD NMR is viable for probing kinetics of protein-ligand interactions.

Conclusions and Future Work

- Although only GlcNAc is transferred to the acceptor, other sugar nucleotides do bind to SseK1. Discrimination for transfer must therefore occur at the catalytic step
- Their binding epitopes indicate similar modes of recognition
- Glucose configuration is key for close recognition and kinetics/affinity
- 3D molecular models will be generated by Docking calculations

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References
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