Hit finding and assay enablement for MGAT1, a novel glycosyltransferase involved in cancer cell immune evasion

Kasia Handing, Associate Director

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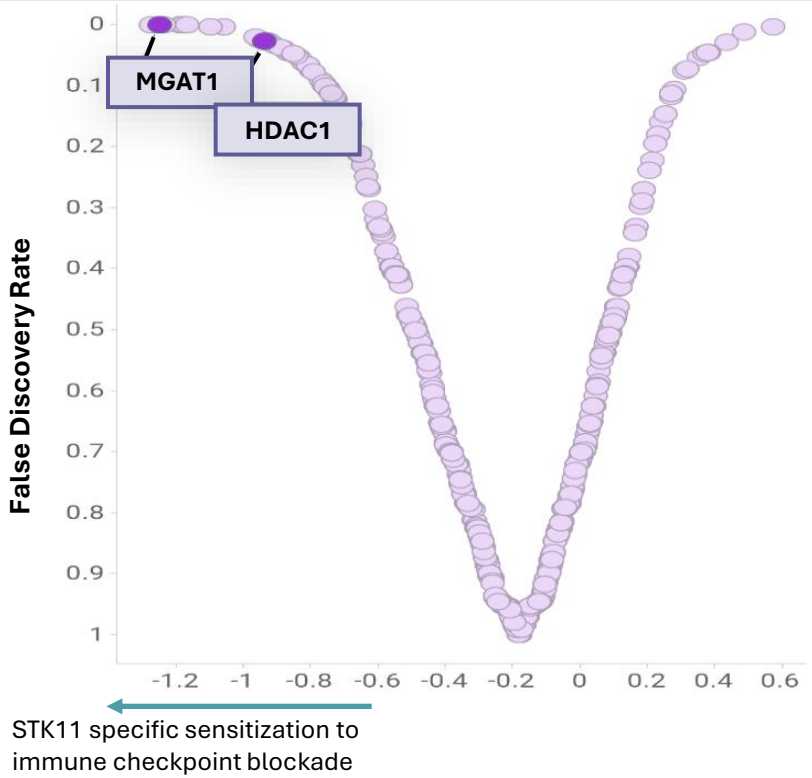
TANGO therapeutics™

MGAT1 knockout re-sensitizes STK11-mutant mouse tumors to immune checkpoint blockade

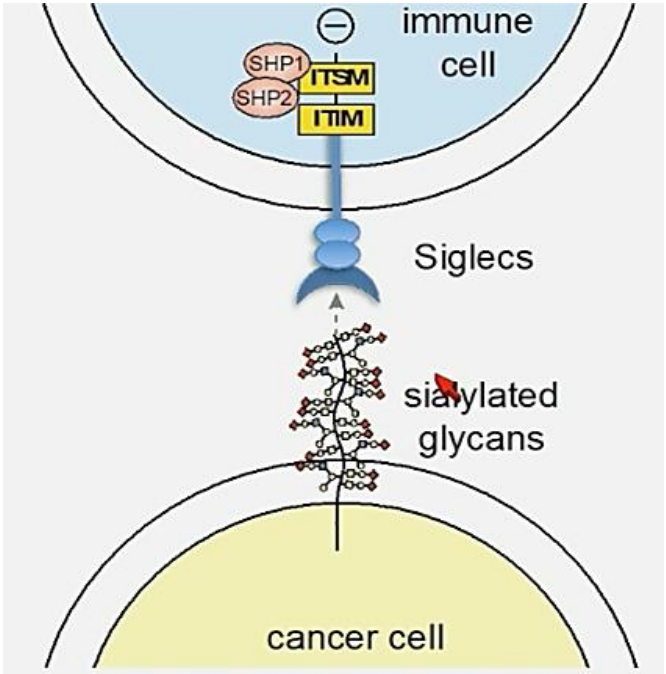
In vivo CRISPR screen



MGAT1, an essential N-glycosyltransferase, is a top hit from in vivo STK11 null MC38 screen

MGAT1 knockout sensitizes cancer cells to T-cell mediated killing in vitro

AACR 2024 Presentation



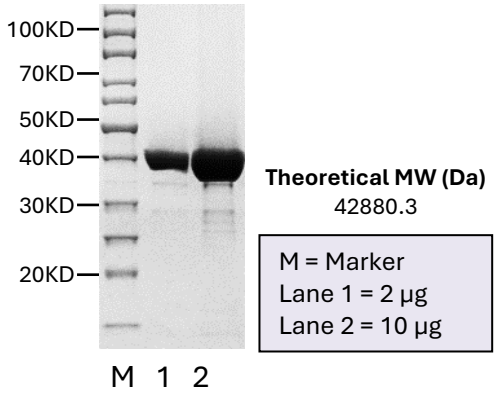
Dr. Carolyn R Bertozzi

Enzymatic removal of terminal sugars sensitizes cancer cells to PD-L1 therapy

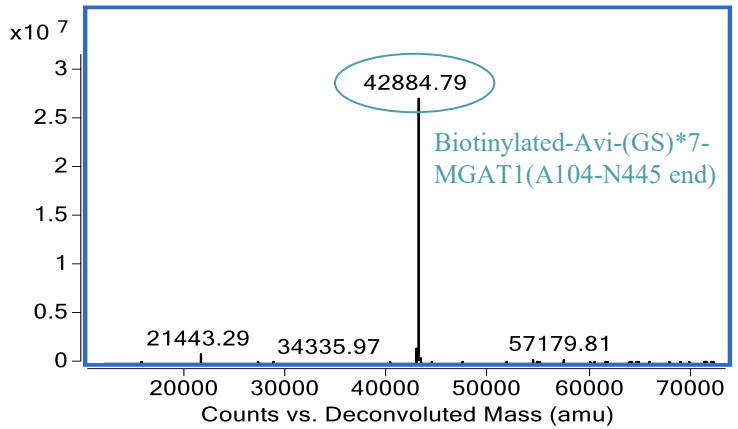
Provides additional support for targeting MGAT pathway

High-quality protein produced to support drug discovery

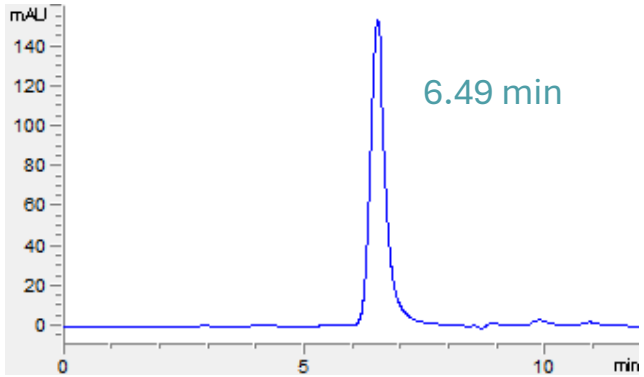
SDS-Page

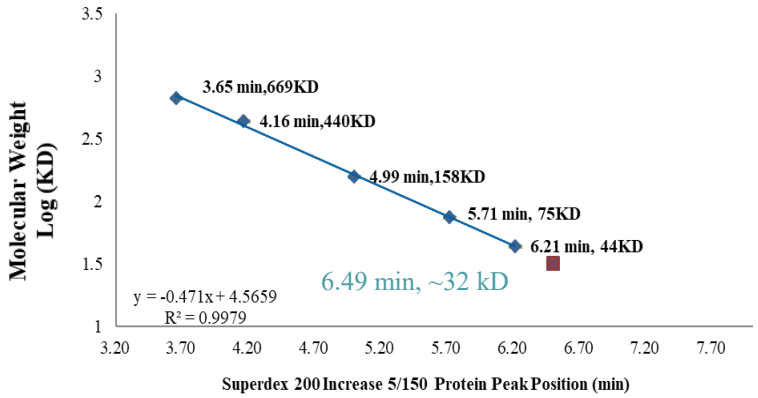


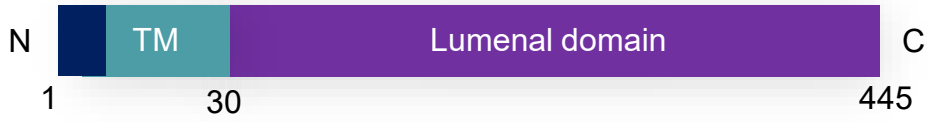
LC-MS



Analytical SEC







N-terminally truncated, biotinylated, AVI-tagged MGAT1 used in SPR assay.

Protein is >95% pure (SDS-Page), without post-translational modifications (LC-MS), and monomeric (Analytical SEC).

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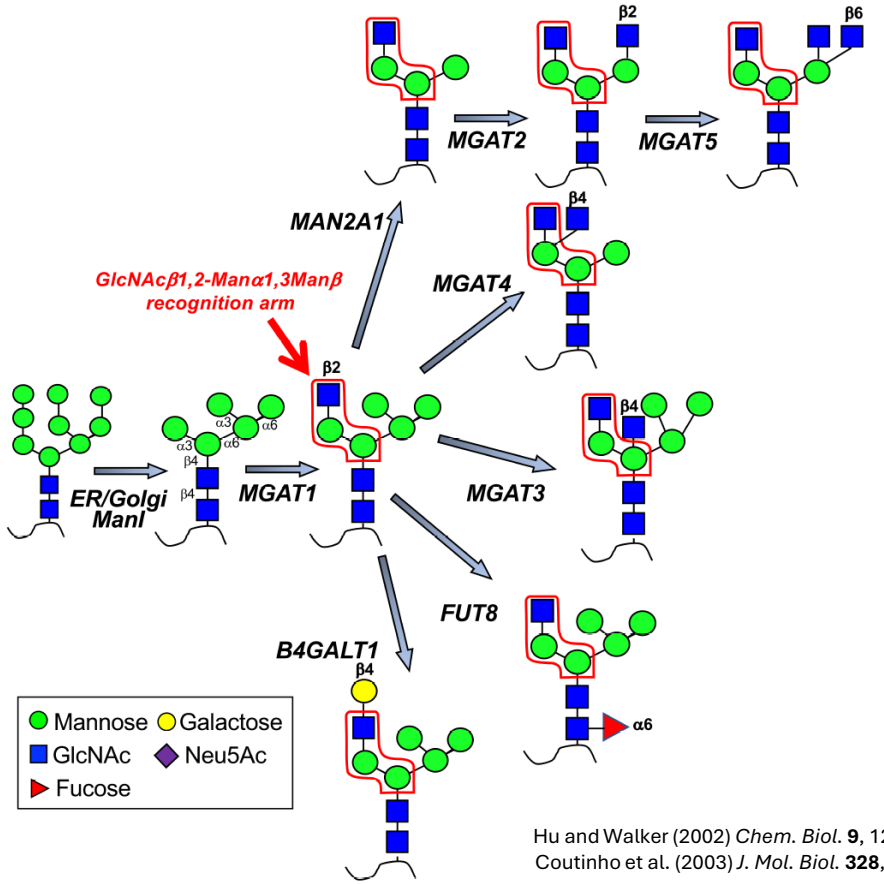
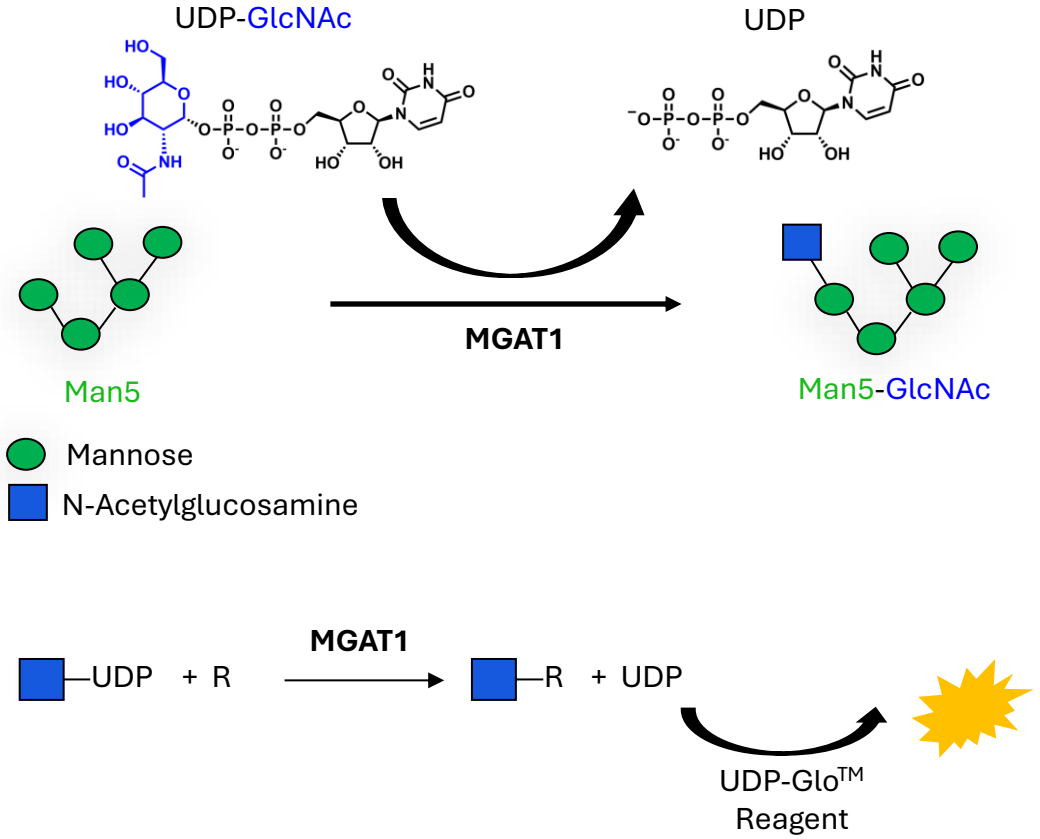
MGAT1 transfers N-acetylglucosamine to its MAN5 substrate producing UDP as a product

MGAT1 catalyzes first and rate-limiting step of N-glycan branching pathway



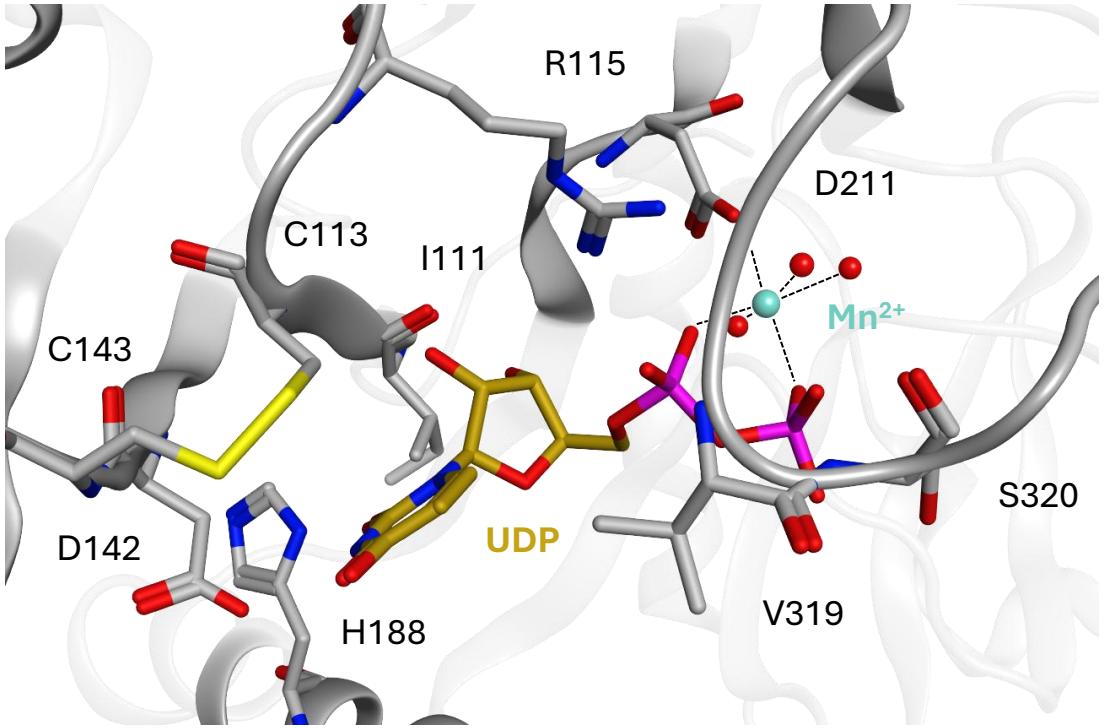
Hu and Walker (2002) *Chem. Biol.* **9**, 1287-1296
Coutinho et al. (2003) *J. Mol. Biol.* **328**, 307-317

UDP production can be measured in UDP-Glo biochemical assay



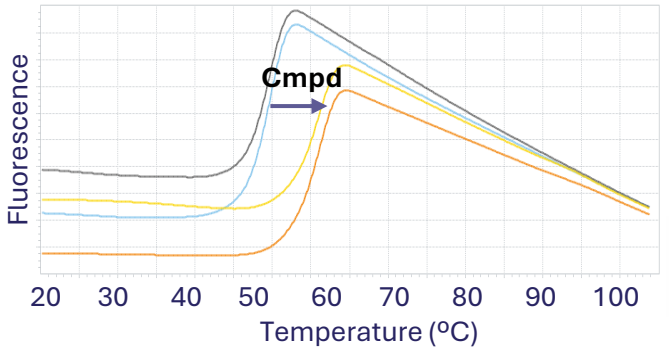
Expanded biophysical toolbox developed for characterization of compounds engaging MGAT1

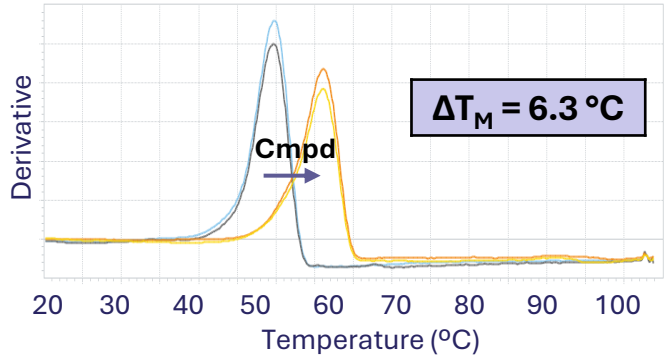
Detail molecular interactions
X-ray crystallography



First X-ray structure of human MGAT1 lumenal domain in complex with UDP at 1.6 Å resolution.

Thermal stability assessment
DSF





Sample	T_M Mean (°C)
5% DMSO	49.5
100 µM binder	55.8

DSF and nanoDSF assays developed to test changes in protein thermal stability upon compound binding

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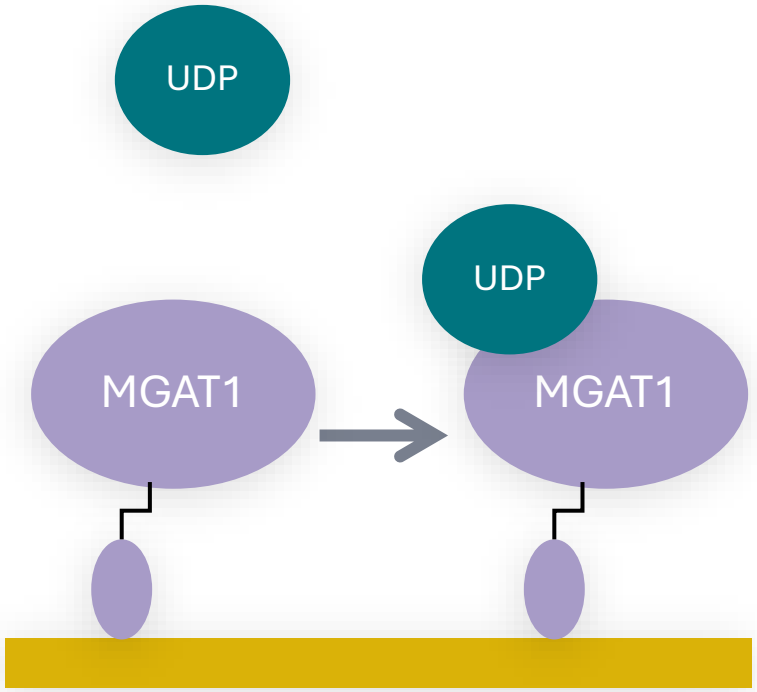
UDP used as a positive control for SPR assay development

Principle



Running conditions

Sensor chip: SA

Instrument: Biacore S200

Temperature: 20°C

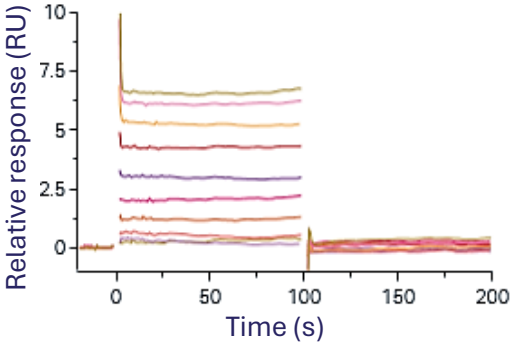
Flow parameters:

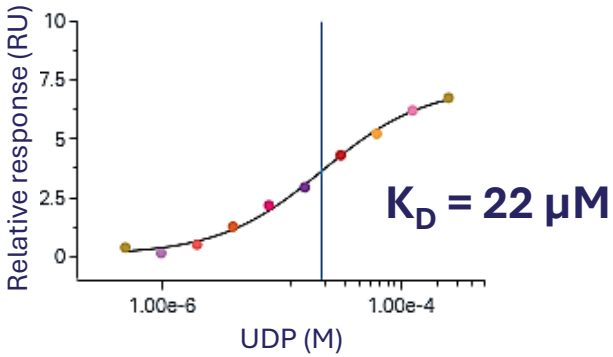
- **Flow rate** = 30 µl/min
- **Association time** = 90 s
- **Dissociation time** = 360 s

Running buffer: 25 mM Bicine (pH 7.6), 150 mM NaCl, 2 mM MnCl2 , 2 mM DTT, 0.05% P20, 3% DMSO

Protein construct: BP18135 Avi-(GS)*7-MGAT1(A104-N445 end)

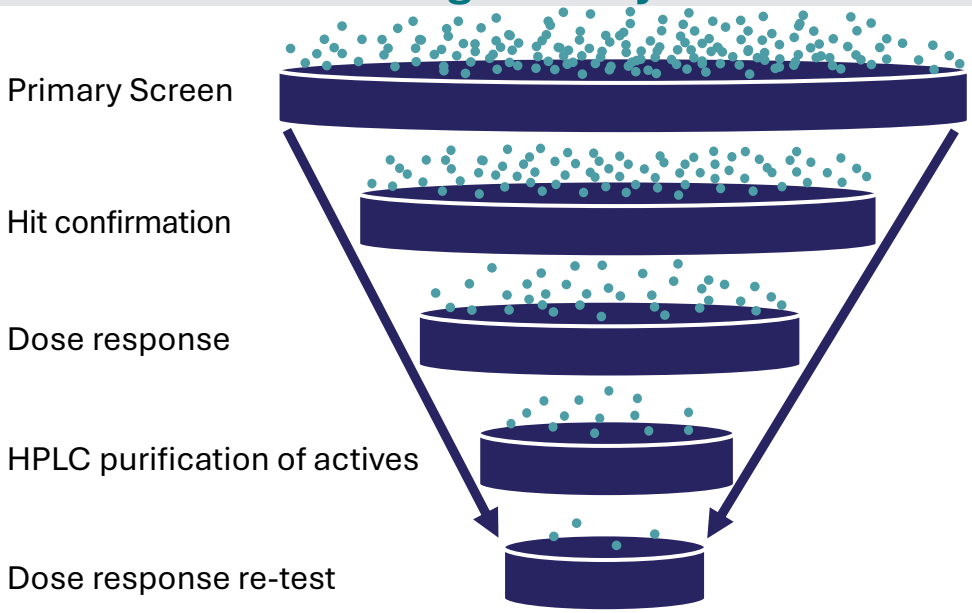
Positive control - UDP





Novel small-molecule compounds targeting MGAT1 can be identified in activity and binding screens

Biochemical HTS
500k Tango Library



2 hits with weak potency

UDP-Glo
SPR
DSF
X-ray structure

1 validated hit

HTS-1

IC_{50} = 197 µM
SPR K_D = 34 µM (*)
ΔT_M = 0.4 °C

Biophysical DEL Screen
3-billion compound library

DEL affinity selection
PCR amplification
High Throughput DNA sequencing
Feature analysis and hit proposal
Off-DNA compound synthesis

41 compounds ordered off-DNA

UDP-Glo
SPR
DSF
X-ray structure

1 validated hit

DEL-1

IC_{50} = 35 µM
SPR K_D = 1.1 µM
ΔT_M = 0.8 °C

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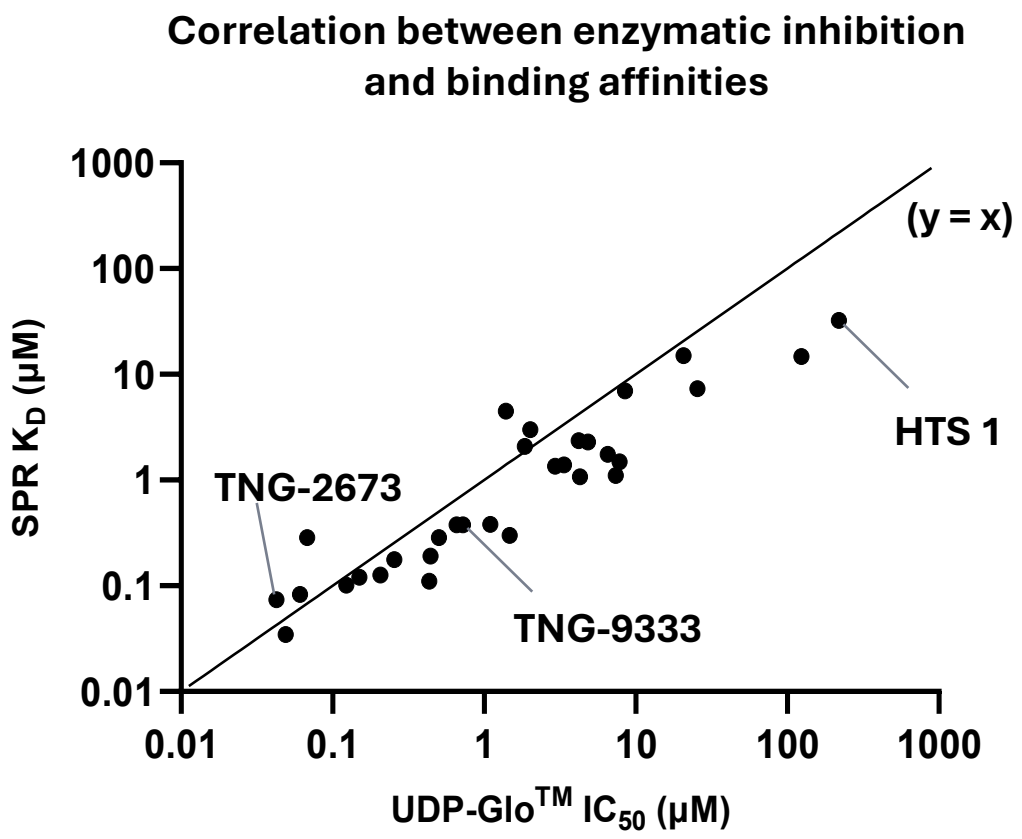
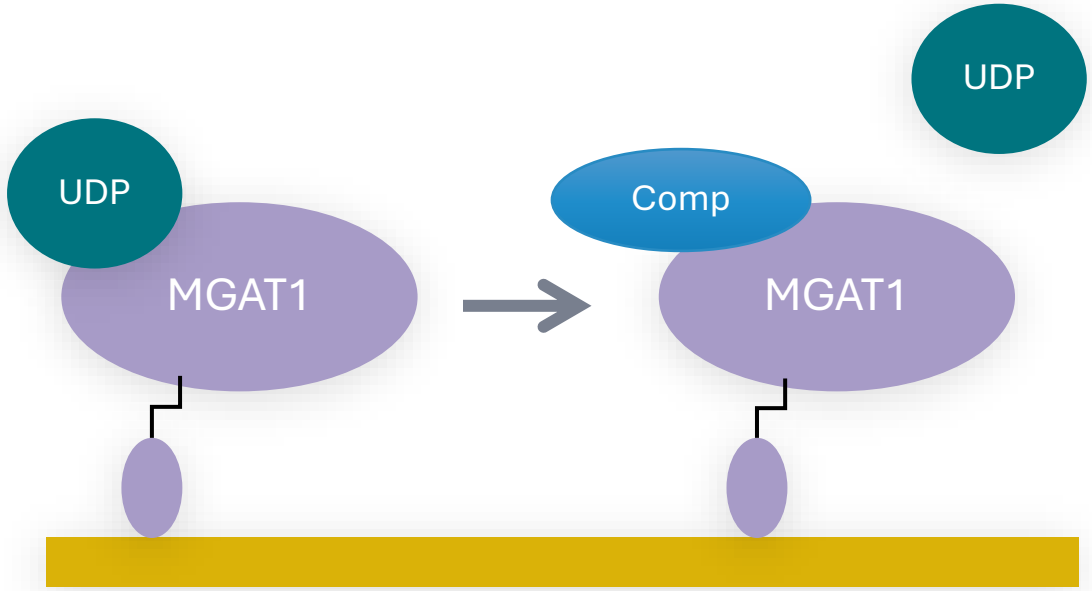
SAR development of MGAT1 HTS compound series results in nanomolar inhibitors

	HTS 1	TNG-9333	TNG-2673
MGAT1 UDP-Glo™ IC_{50} (μM)	197	0.814	0.043
MGAT1 SPR Steady-state K_D (μM) Kinetic K_D (μM)	32.2 -	0.38 0.38	0.07 0.03
MW, LogD, TPSA	335, 2.6, 82	419, 2.2, 110	478, 2.6, 108
Kinetic solubility (μM)	11.8	178	96.4
Human hepatocyte clearance (mL/min/1x10^6 cells)	54.4	3.2	<1.35



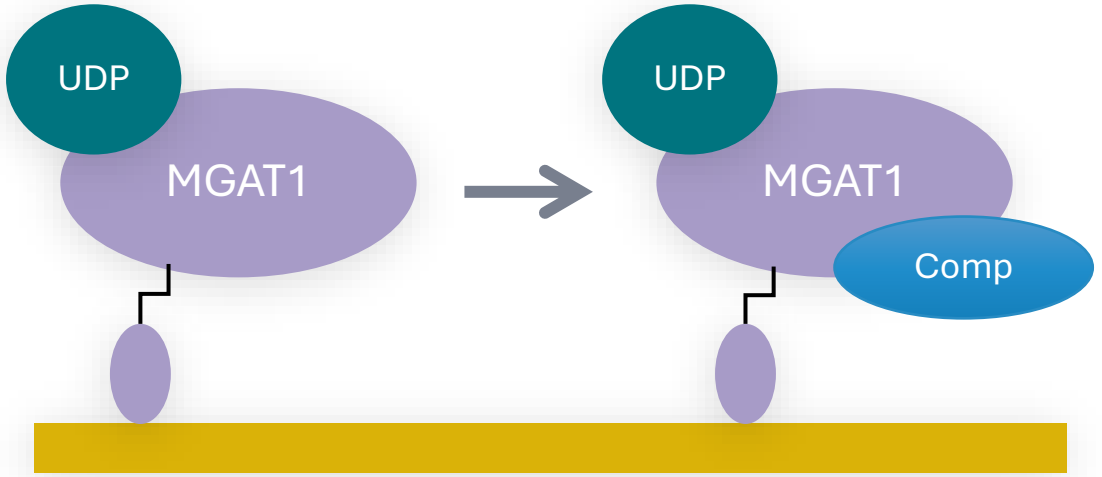
Are identified compounds orthostatic binders?

What do we expected if hits compete with UDP for binding?



When competing with UDP, K_D should be weaker as compared to binary assay since they bind to the **same** site

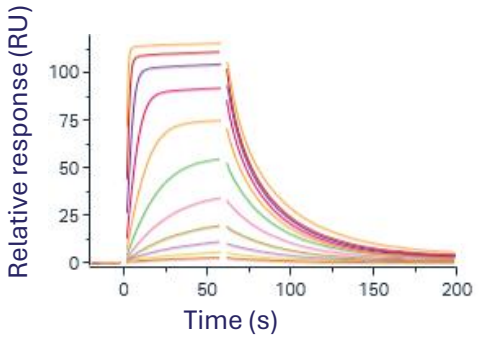
What do we expected if hits do not compete with UDP for binding?

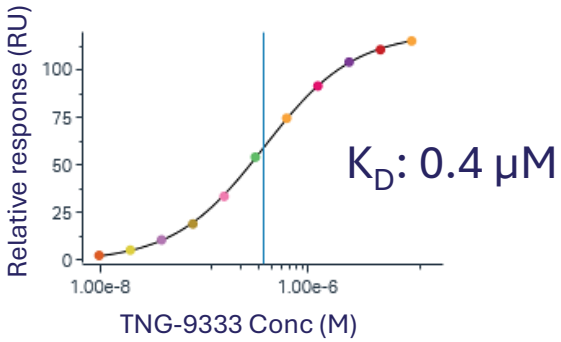


When binding to different site than UDP, K_D should not change as compared to binary assay since they bind to **different** site

HTS compound TNG-9333 binds in allosteric pocket

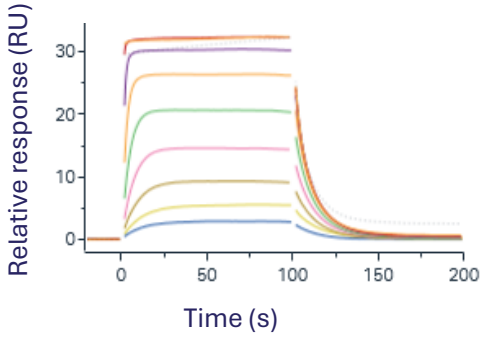
TNG-9333 - Direct binding

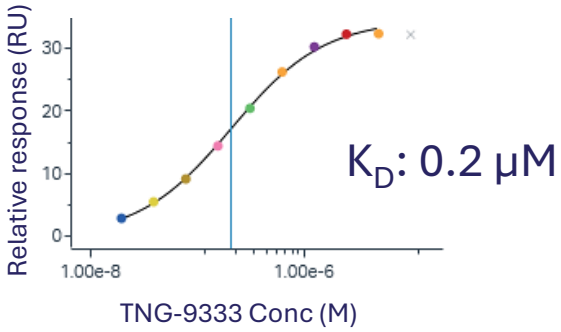




K_D: 0.4 µM

TNG-9333 – Competition with UDP (5000 µM)





K_D: 0.2 µM

Runs were performed separately with new surface preparation for every experiment. Protein immobilization levels differ.

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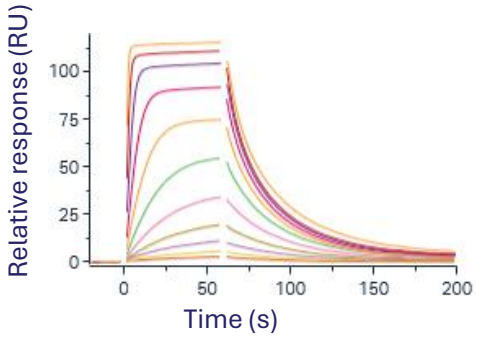
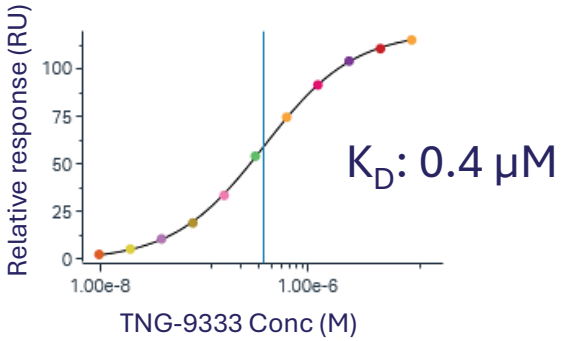
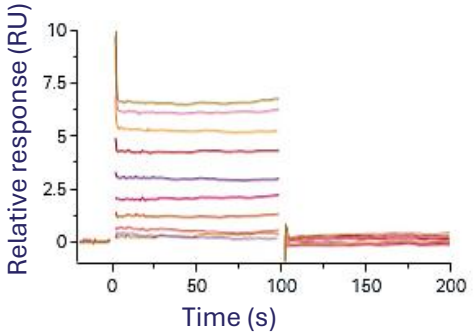
HTS compound TNG-9333 binds in allosteric pocket

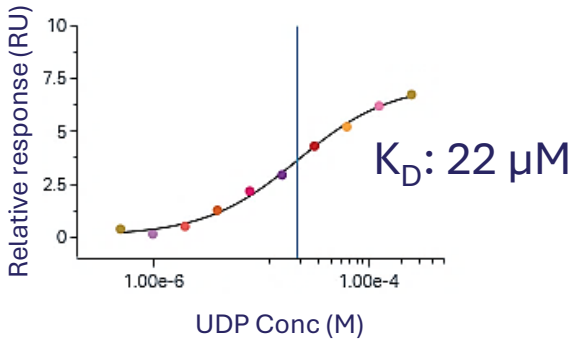
TNG-9333 - Direct binding



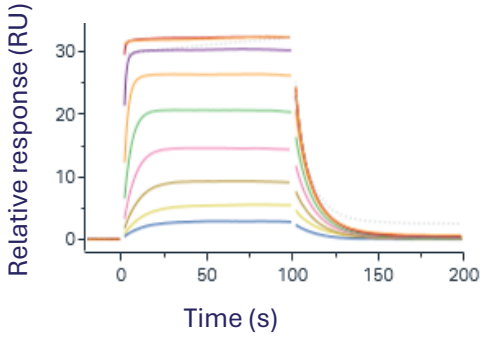


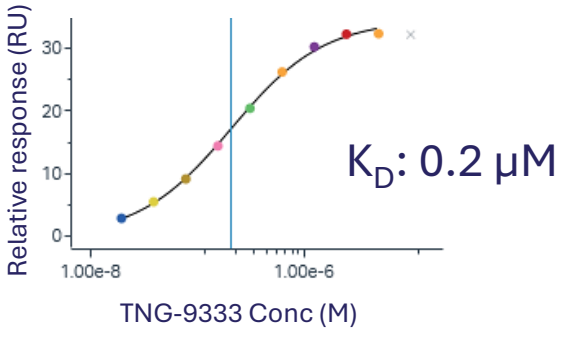
UDP - Direct binding



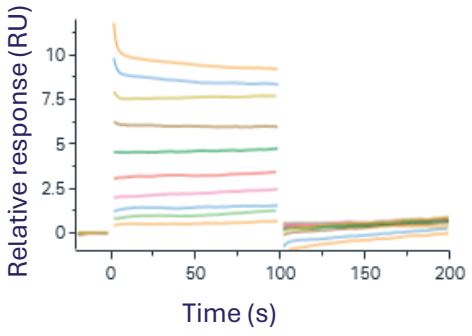


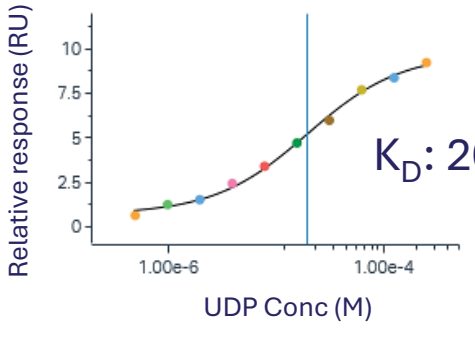
TNG-9333 – Competition with UDP (5000 µM)





UDP – Competition with TNG-9333 (20 µM)





Runs were performed separately with new surface preparation for every experiment. Protein immobilization levels differ.

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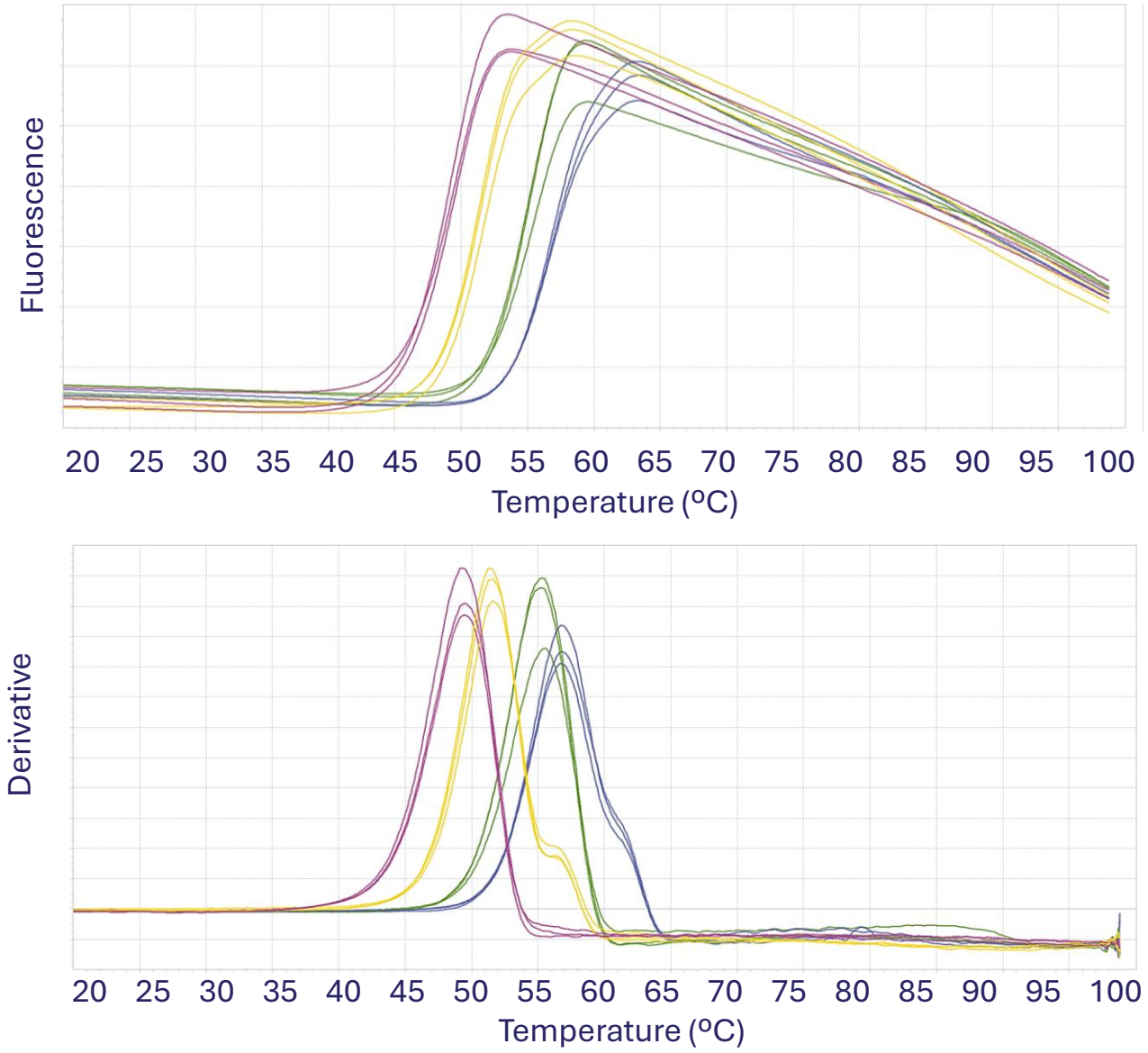
Additive thermal stabilization effect observed by HTS compound and UDP binding in DSF assay

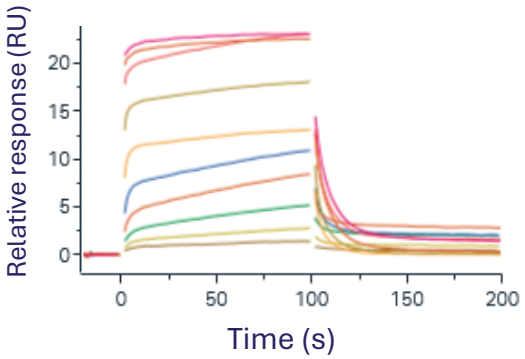


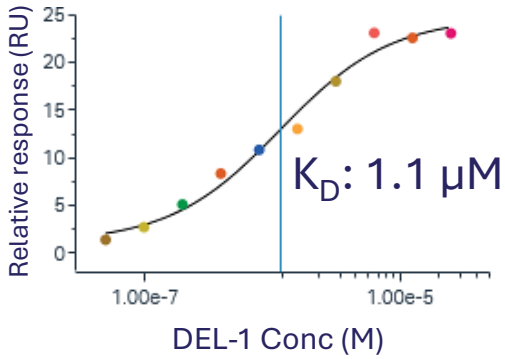
- 5% DMSO
- 5000 µM UDP
- 100 µM HTS TNG-9333
- 100 µM HTS TNG-9333 + 5000 µM UDP

Sample Name	T_M Mean (°C)	ΔT_M (°C)
5000 µM UDP	51	2
100 µM TNG-9333	55	6
100 µM TNG-9333 + 5000 µM UDP	57	8 **(additive effect)**

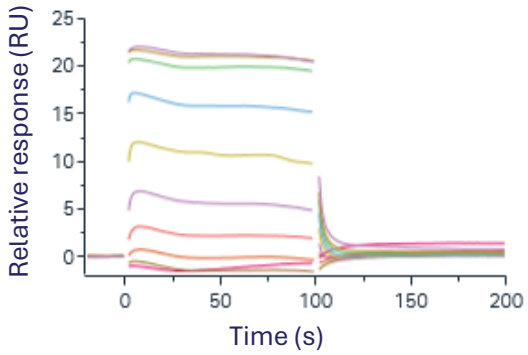
DEL-1 compound binds in allosteric pocket and compete for binding with HTS compound but not UDP

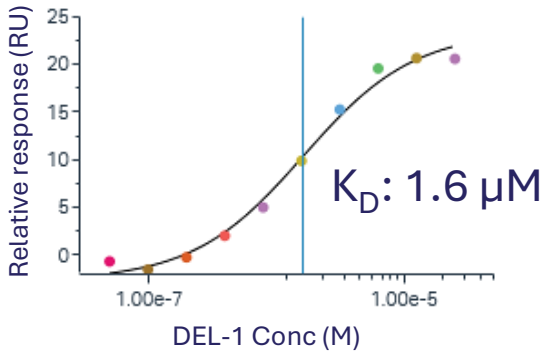
Direct binding





Competition with UDP (5000 µM)





DSF orthogonal validation

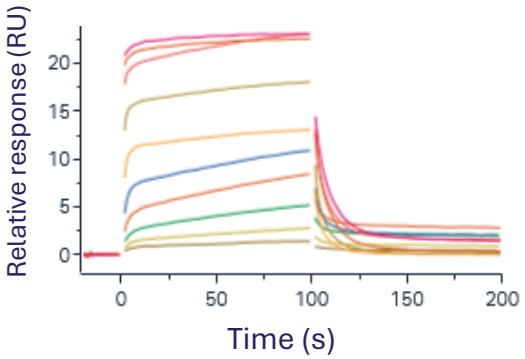
	T_M Mean (°C)	ΔT_M (°C)
5000 µM UDP	51.4	2.2
100 µM DEL-1 compound	50.8	0.8
100 µM DEL-1 compound + 5000 µM UDP	52.4	3.1 **(additive effect)**

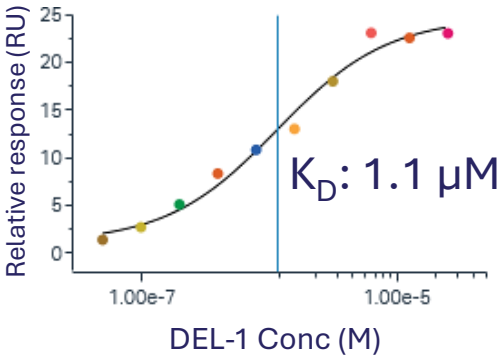
Runs were performed separately with new surface preparation for every experiment. Protein immobilization levels differ.

TANGO therapeutics

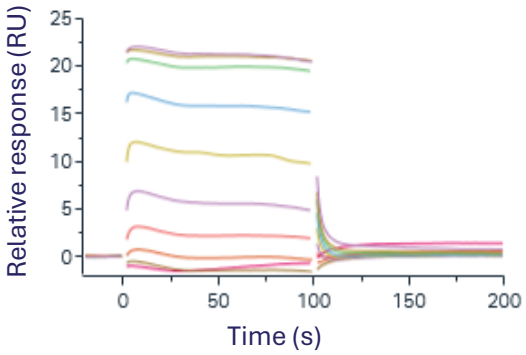
DEL-1 compound binds in allosteric pocket and compete for binding with HTS compound but not UDP

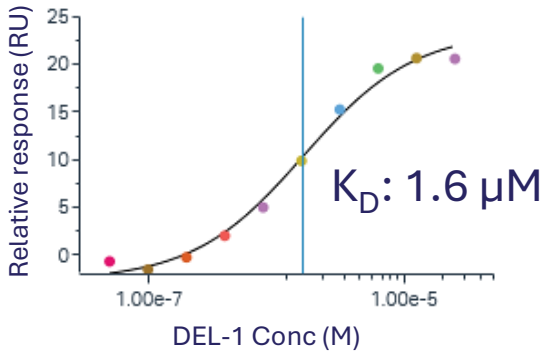
Direct binding



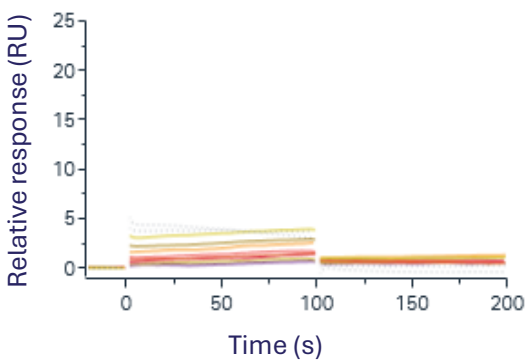


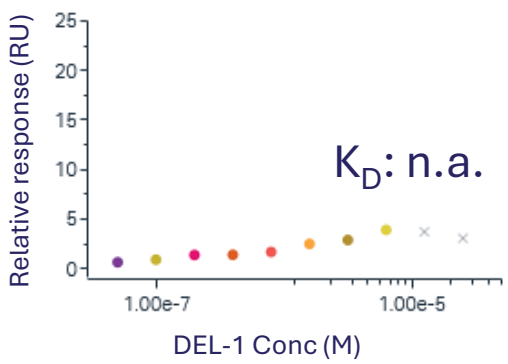
Competition with UDP (5000 µM)





Competition with TNG-9333 (20 µM)





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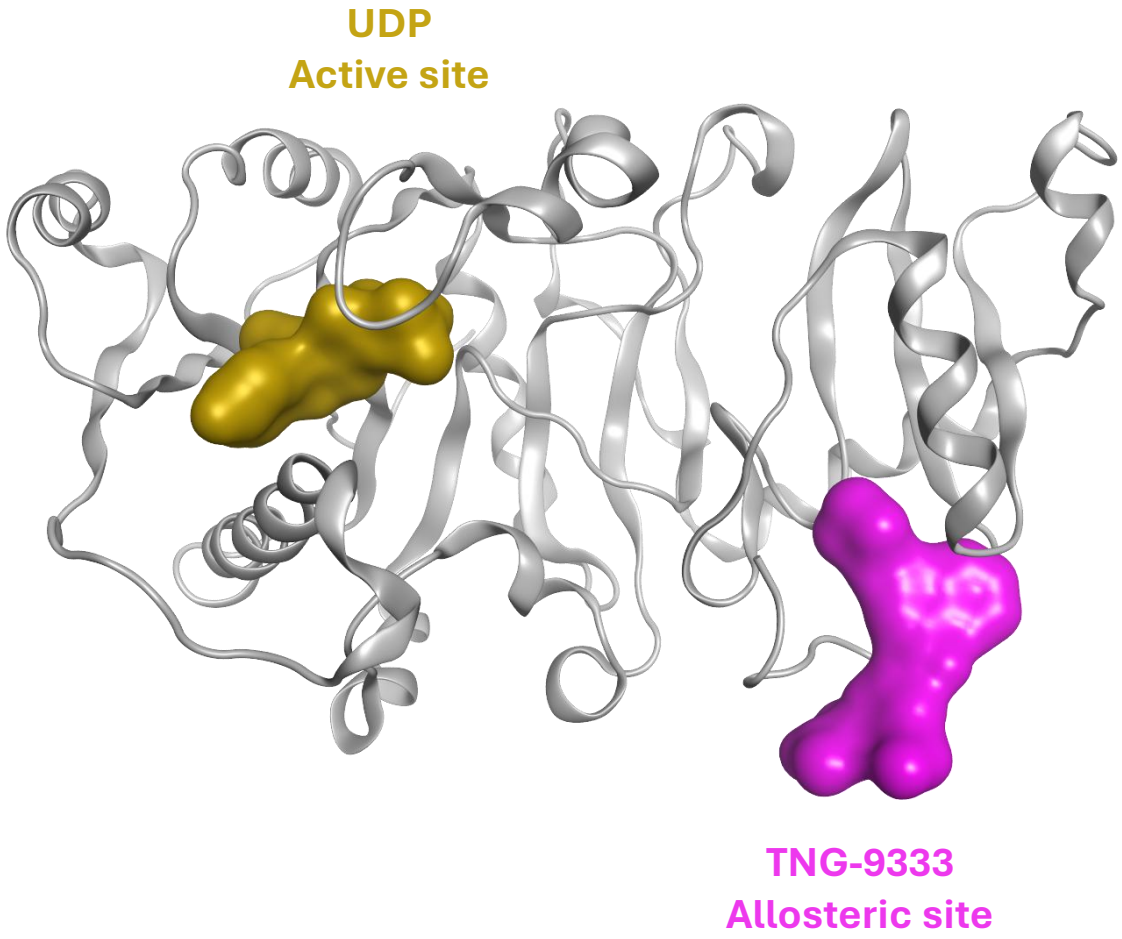
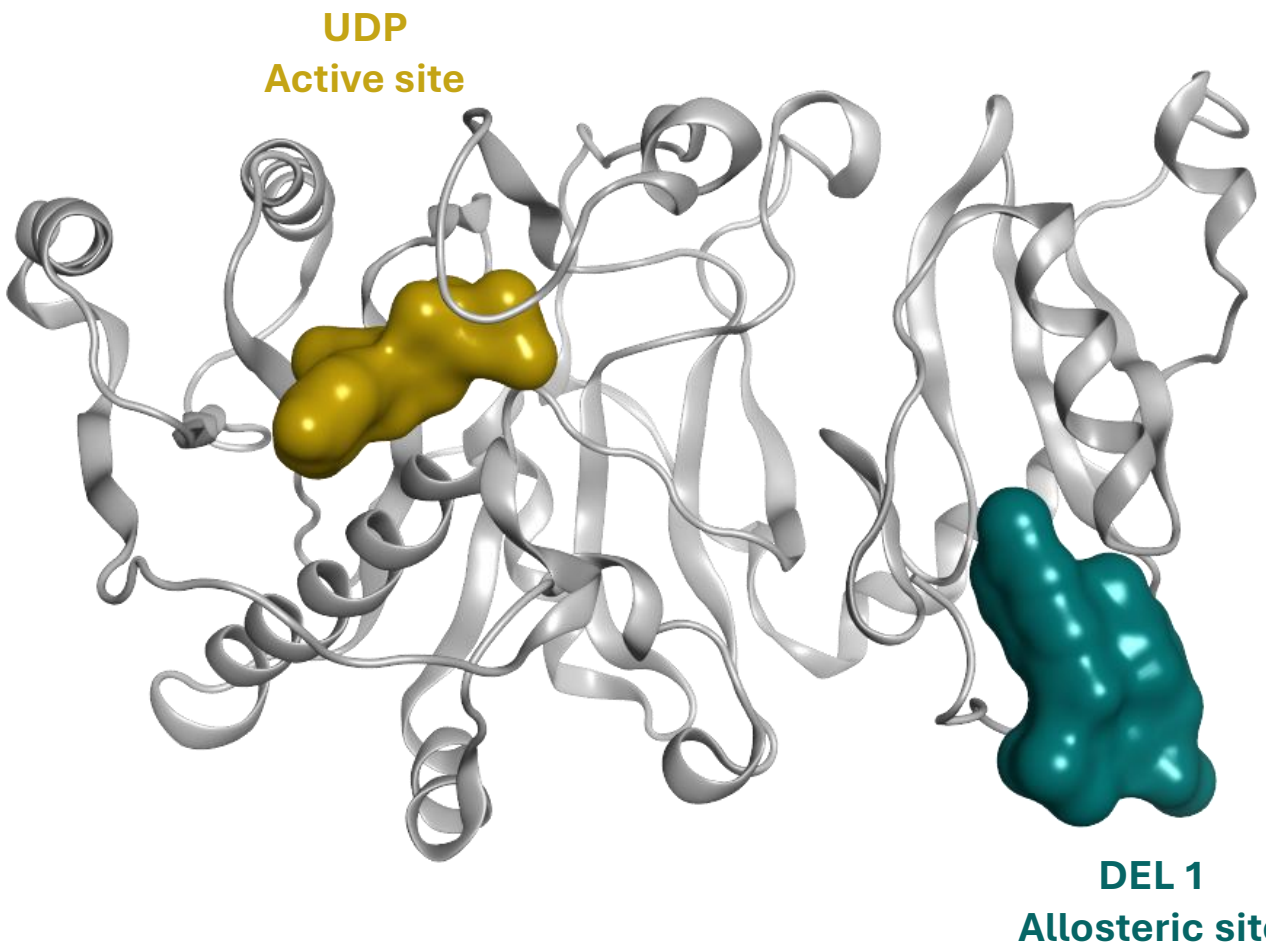
Structural biology confirms allosteric binding of HTS and DEL compounds



2.0 Å resolution



1.3 Å resolution

TANGO therapeutics

Summary

- MGAT1 is a novel, druggable, and tractable immune evasion target in STK11-mutant cancers
- Robust protein production, biophysical/biochemical assays established
- First human MGAT1 co-crystal structure solved
- Novel chemical matter identified via orthogonal approaches yielding nanomolar inhibitors
  - UDP-Glo™ HTS for enzymatic inhibition
  - DEL screen for direct binders
- SPR binding and competition studies were critical in defining mechanism of action
  - HTS- and DEL-derived compounds converged on a shared allosteric pocket
- Manuscript in preparation
- MGAT1 program is available for partnering

Acknowledgements

Tango Therapeutics

CRO Partners

HitGen Inc.

Biortus Biosciences

Wuxi AppTec

We gratefully acknowledge the contributions from all Tango associates, members of the MGAT1 Project Team, the DEL Working Group members, as well as the contributions from our CRO partners


TANGO
therapeutics™