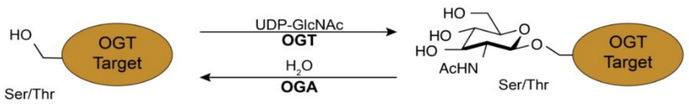
Using a photoactivatable OGT library to locate adaptor protein binding sites

Huy Vo Huynh[1], Hahns Huebsch[1], Madi Rognerud[1], Dr. Cassandra Joiner[2]
[1]CURI Program, St. Olaf College, Northfield, MN 55057
[2]Chemistry Department, St. Olaf College, Northfield, MN 55057

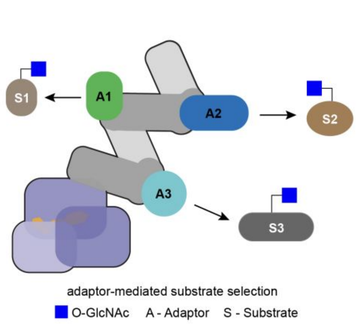
Abstract

O-linked beta-N-acetylglucosamine (O-GlcNAc) transferase (OGT) is the only human enzyme that transfers the O-GlcNAc sugar from UDP-GlcNAc to nuclear and cytoplasmic proteins.[1] OGT glycosylates over 1,000 substrates involved in nearly all cellular processes, and misregulated protein O-GlcNAc levels have been implicated in cancer and neurodegenerative disease.[2,3] How OGT selects its protein substrates is unknown, but it is proposed that OGT interacts with adaptor proteins that facilitate substrate selection through its tetratricopeptide (TPR) domain.[4] We aim to locate adaptor binding sites along the TPR domain using a photoactivatable OGT mutant library. Here, we showed that the unnatural amino acid Bpa (benzoyl-L-phenylalanine)[5] can be incorporated into 26 different locations along the TPR domain and these OGT mutants can be purified from *E. coli*. Upon UV irradiation of these mutants, we have covalently captured the dimerization of OGT and identified a binding site along the solvent-exposed surface for the TPR domain for the known adaptor, CARM1.

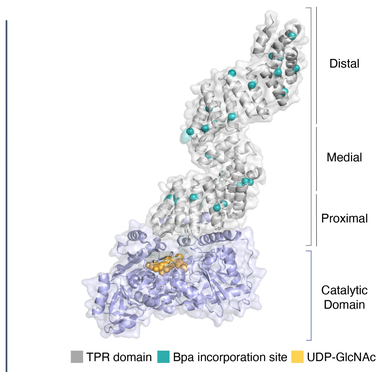
Background and Significance



OGT (O-GlcNAc transferase) is an essential enzyme in humans that transfers O-GlcNAc from the nucleotide-sugar UDP-GlcNAc to nuclear and cytoplasmic proteins, while its cognate enzyme OGA (O-GlcNAcase) removes O-GlcNAc from its targets.



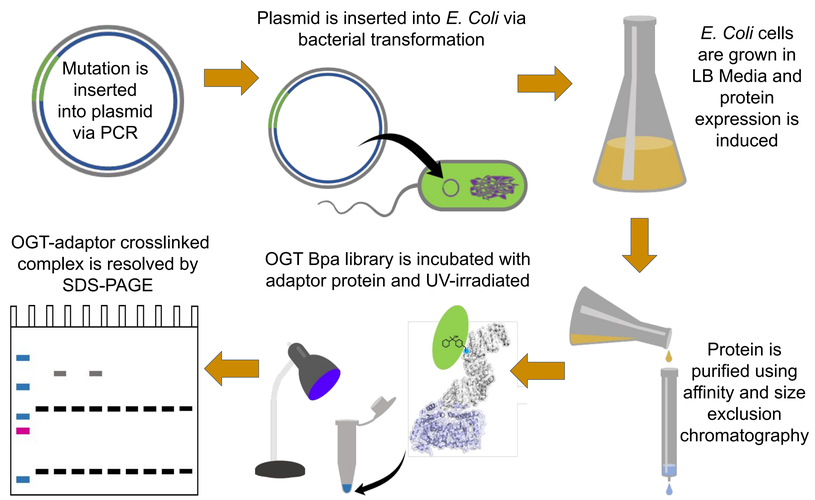
Adaptor proteins are proposed to interact with OGT's TPR domain (gray) to regulate substrate selection. The location where these adaptors bind along the TPR domain is unknown.



Bpa has been incorporated into 26 locations on OGT's TPR domain. These locations are divided into three groups: proximal, medial, and distal in relationship to the catalytic domain.

Where do adaptor proteins bind along the TPR domain?

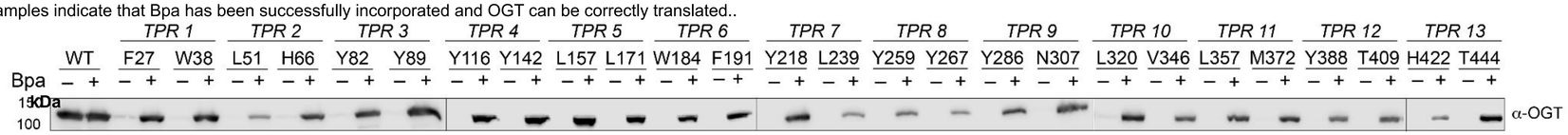
Methods



Results

Site-specific Bpa incorporation along OGT's TPR domain

Figure 1. Photoactivatable OGT library. Bpa was incorporated into 26 sites along the TPR domain of OGT and visualized via western blotting using an anti-OGT antibody. Full-length protein in +Bpa samples indicate that Bpa has been successfully incorporated and OGT can be correctly translated..



Covalent chemical capture of OGT dimerization and optimization

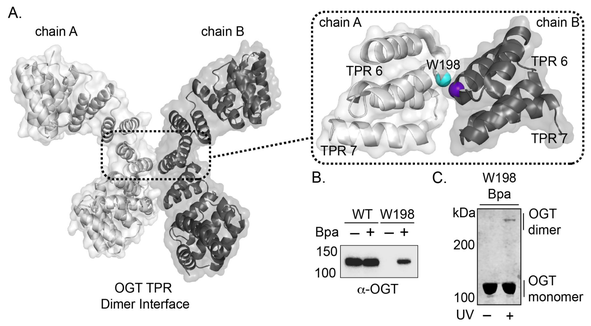
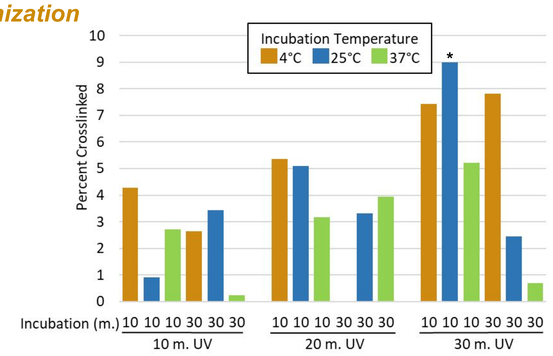


Figure 2 (left). Capture of OGT dimerization.

A. Crystal structure of OGT dimerization (PDB 1W3B). Interaction mediated by position W198 in TPR 6.
B. Incorporation of Bpa at site W198 visualized via western blot using anti-OGT antibody.
C. Covalent chemical capture of OGT dimer using W198Bpa upon UV irradiation. OGT dimer visualized by SDS-PAGE.

Figure 3 (right). OGT dimer Crosslinking optimization.

OGT dimerization procedure was optimized by varying protein incubation temperature, incubation time, and UV irradiation time. Condition with highest percent of crosslink was selected for future crosslinking experiments (*).



Mapping of CARM1 adaptor binding along TPR domain using photocrosslinking

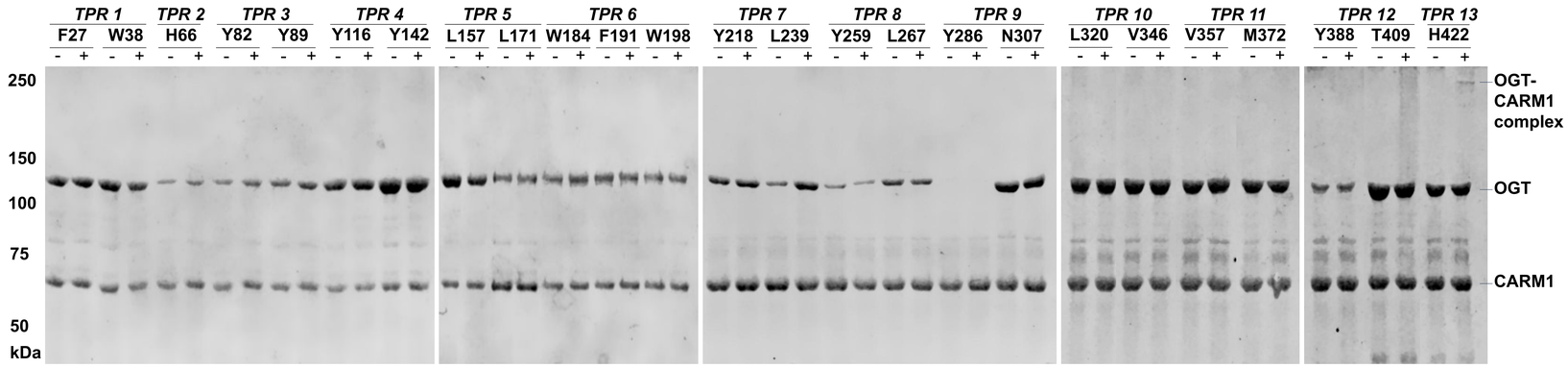


Figure 4. CARM1 crosslinking with OGT Bpa library. CARM1 was incubated with each Bpa-containing OGT construct and UV-irradiated. Crosslinked OGT-CARM1 complexes were then visualized via SDS-PAGE. Variation in OGT band appearance represents different levels of protein concentration.

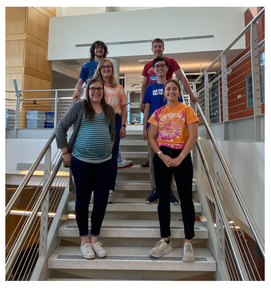
Conclusions

- Bpa can be successfully incorporated at all 26 positions along the TPR domain of OGT.
- OGT's proposed dimer was covalently captured using the OGT W198Bpa construct.
- Optimized conditions were determined using the OGT dimer and can be used to interrogate OGT-adaptor interactions.
- OGT H422Bpa construct covalently captured an OGT-CARM1 adduct upon UV irradiation, suggesting that CARM1 interacts in the proximal region of the TPR domain, but not the medial and distal regions of the domain.

Future Directions

- Insert epitope tags into adaptor expression plasmids, such as CARM1, to simultaneously visualize both proteins (OGT and adaptor) via western blot.
- Use affinity and size-exclusion chromatography to purify Bpa-containing OGT constructs that are low or show inconsistency in expression.
- Run crosslinking experiments with other known adaptors, such as mSin3a, to map their binding sites along the TPR domain.
- Use a global crosslinking approach and mass spectrometry proteomic studies to identify new protein interactors of OGT.

Acknowledgements



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