Mapping CARM1 interaction on O-GlcNAc transferase tetratricopeptide (TPR) domain using photocrosslinking

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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. OGT can O-GlcNAcylate over one thousand substrates that participate in almost every cellular process, and misregulation of O-GlcNAc levels can lead to a variety of diseases. Despite its large scope of substrates, how OGT chooses its many targets is poorly understood. It has recently been proposed that adaptor proteins drive selection by interacting with the tetratricopeptide (TPR) domain to direct OGT to subsets of substrates. Recently, CARM1 (Coactivator Associated Arginine Methyltransferase 1) was shown to interact with OGT's TPR domain, but the exact binding site of CARM1 along OGT's TPR domain is unknown. To answer that question, we used an OGT protein library where we incorporated a photoactivatable unnatural amino acid at 26 locations along the TPR domain to covalently map CARM1's interaction along the TPRs. Using this library, we found two main binding locations of CARM1 on OGT TPR 2 (H66) as well as TPRs 11-13 (V346, M372, T409). These results provide insights into how CARM1 interacts with OGT and will help guide future functional studies on CARM1-directed substrate selection.

Background and Significance

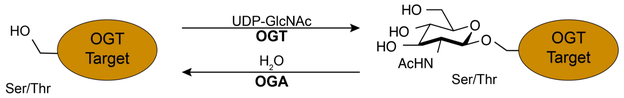

Figure 1. Regulation of protein O-GlcNAc levels is moderated by OGT and O-GlcNAcase (OGA). OGT transfers O-GlcNAc to serine and threonine residues of nuclear and cytoplasmic proteins, while OGA removes it. Maintaining a proper balance of protein O-GlcNAc levels is critical for cell homeostasis.[1]

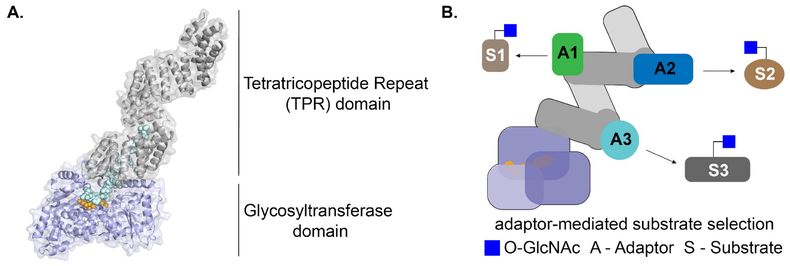

Figure 2. A) Full length crystal structure of human OGT with a peptide substrate (teal) and UDP-GlcNAc (gold) bound in the active site.[2-3] (PDB 4N3C, 1W3B) **B)** Adaptor-mediated substrate selection mechanism in which adaptor proteins are proposed to recruit specific substrates to OGT through TPR interactions.[4-5]

How and where does CARM1 bind along OGT's TPR domain?

Creation of Photoactivable OGT Library

Expression method for photoactivatable OGT library

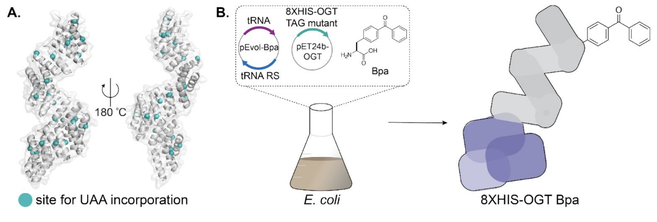

Figure 3. A) The photoactivatable unnatural amino acid, p-benzoyl-L-phenylalanine (Bpa), is site-specifically incorporated into OGT TPR domain (PDB 1W3B). Teal spheres represent Bpa incorporation sites. **B)** 8XHIS-OGT Bpa-containing mutant constructs are expressed recombinantly in *E.coli* using amber nonsense suppression. Transformation of both pEvol-Bpa and 8XHIS-OGT TAG mutant plasmids in *E. coli* allows for the site-specific incorporation of Bpa in the OGT protein.[6-7]

Bpa incorporation along OGT TPR domain

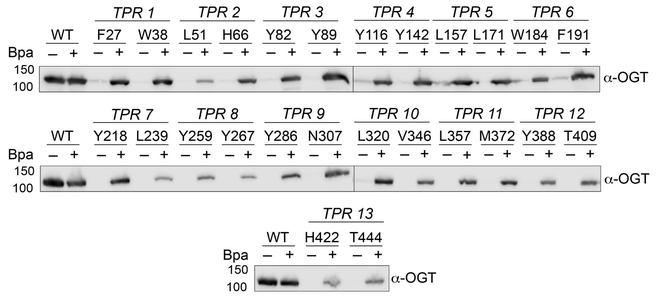

Figure 4. Bpa was incorporated into 26 sites along the TPR domain of OGT and visualized via western blotting using an anti-OGT antibody. Full-length protein in +Bpa samples indicate that Bpa has been successfully incorporated and OGT can be correctly translated.

Purification Example of Bpa-incorporated OGT Mutants

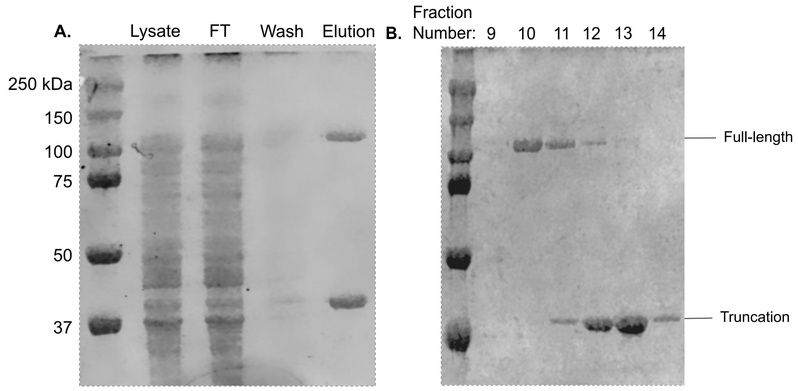

Figure 5. A) Nickel affinity chromatography of 8xHis-OGT-M372Bpa; **B)** Size-exclusion chromatography of 8xHis-OGT-M372Bpa..

CARM1-OGT Photocrosslinking

Purification of CARM1

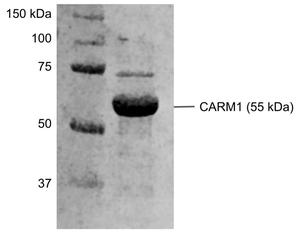

Figure 6. SDS-PAGE gel analysis of purified CARM1.

Method to covalently capture OGT-adaptors interactions

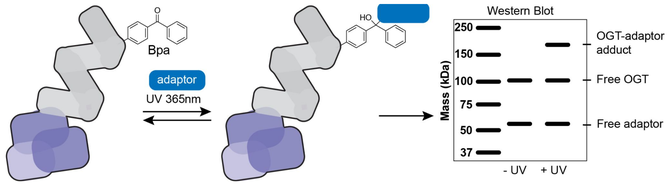

Figure 7. Library of OGT Bpa mutants are incubated with an adaptor and irradiated with 365 nm UV light. Samples were visualized by duplex western blotting using 680 nm Near-IR conjugated antibodies for the adaptor protein and 800 nm near-IR conjugated antibodies for OGT. If the adaptor interacts where Bpa is incorporated, a crosslinked adduct will form with the MW of both proteins.

CARM1 interacts with OGT at TPRs 2, 10, 11, 12

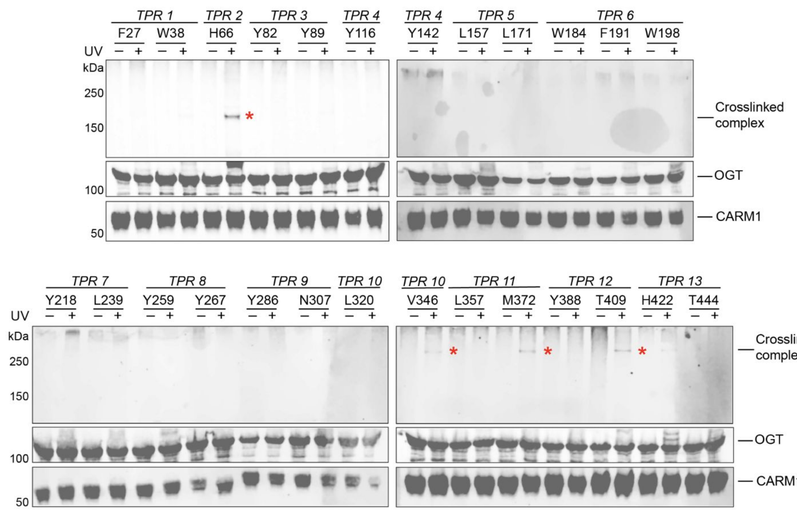

Figure 8. *In vitro* crosslinking of CARM1 and OGT reveals 4 potential binding sites at **Top Left:** TPR 2 captured by 8xHis-OGT H66Bpa, **Bottom Right:** TPRs 10, 11, and 12 captured by 8xHis-OGT V346Bpa, M372Bpa, and T409Bpa. The molecular weight for TPR10, 11, and 12 is >250 kDa suggesting the presence of two OGTs covalently bound to CARM1.

Proposed Model of CARM1-OGT Interaction

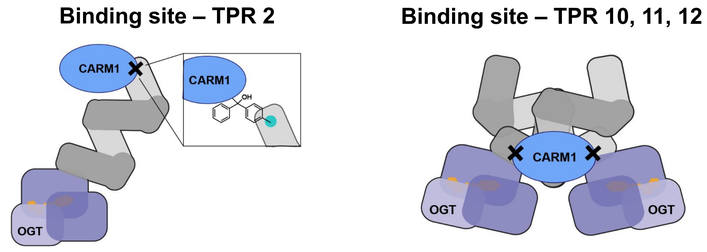

Figure 9. Proposed model of CARM1 mono-crosslinking **(left)** and dimer-crosslinking **(right)** along OGTs TPR domain. **Left:** The MW for TPR2-OGT crosslink is ~165kDa, which suggests 1 OGT covalently bound to 1 CARM1. **Right:** The MW for TPR10/11/12-OGT crosslink is ~275kDa, which suggests 2 OGT covalently bound to 1 CARM1.

Future Directions

- Continue replicating crosslinking reactions of CARM1 along OGT's TPR domain to confirm crosslinking results.
- Perform OGT dimer null mutation to confirm the dimer crosslinking between CARM1 and OGT's TPRs 10, 11, 12.
- Perform crosslinking CARM1 truncated version with OGT's TPRs 10, 11, 12..

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References

1. Joiner, C., et al. Curr. Opin. Struct. Biol. 2019, 56, 97. 2. Jinek, M., et al. Nat. Struct. Mol. Biol. 2004, 11, 1001. 3. Lazarus, M., et al. Science 2003, 342, 1235. 4. Yang, X., et al. Nat. Rev. Mol. Cell Biol. 2017, 18, 452. 5. Zhao, L., et al. Molecules 2018, 23, 1967. 6. Wang, L., et al. Science 2001, 292, 498. 7. Young, S., et al. J. Mol. Biol. 2010, 395, 361.