Isolation, Characterization, and Imaging Analysis of Human Mediator Complexes Using HaloTag Technology.

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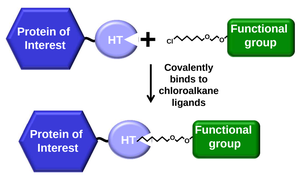
Abstract and introduction

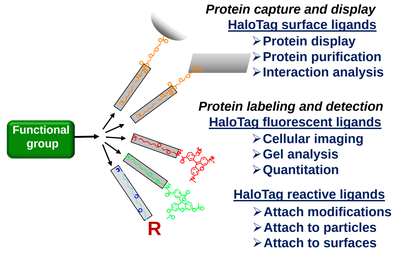
The understanding of protein complex assembly and mapping of protein interactions has rapidly grown in recent years due to significant advances in mass spectrometry. As experiments turn toward characterization of the human proteome and the complexity it presents, a need remains to efficiently capture complexes intact, particularly weak or transient interactors as well as large multiprotein complexes. Here we present a new technology based upon the use of a protein fusion tag, termed HaloTag, which allows for highly specific and covalent immobilization of proteins complexes, as well as the ability to do correlative cellular localization studies using fluorescent ligands. In studies presented here, this technology was used to further understanding of the multi-protein Mediator complex assemblies and their cellular function.

HaloTag technology

HaloTag fusion protein

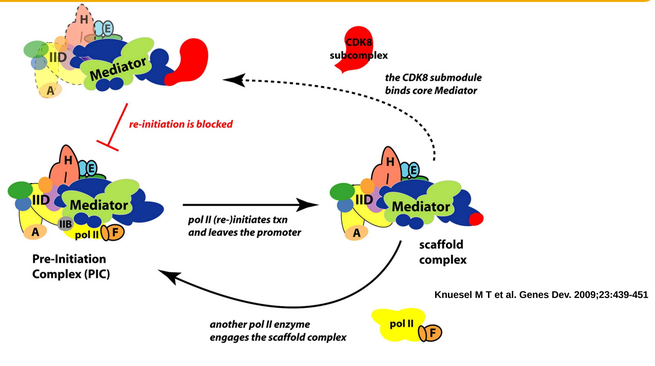


HaloTag ligands and applications



❖HaloTag (HT) is a genetically encoded 33kDa protein fusion tag.
❖Engineered to covalently bind various ligands, imparting multi-functionality.

Mediator Transcriptional Co-activator and Design

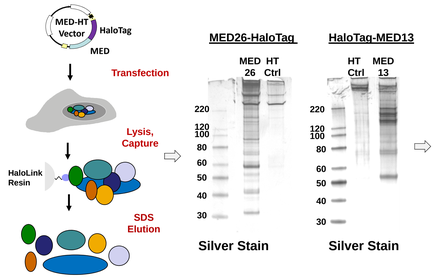


Knuesel M T et al. Genes Dev. 2009;23:439-451

❖Mediator exists in two forms: Core Mediator and Mediator+Kinase Module
❖Place HaloTag on Core component MED26, and Kinase Module component, MED13

Mediator isolation using MED26-Halo and Halo-MED13

Expression and pulldown
Halo-Mediator fusions in HEK293

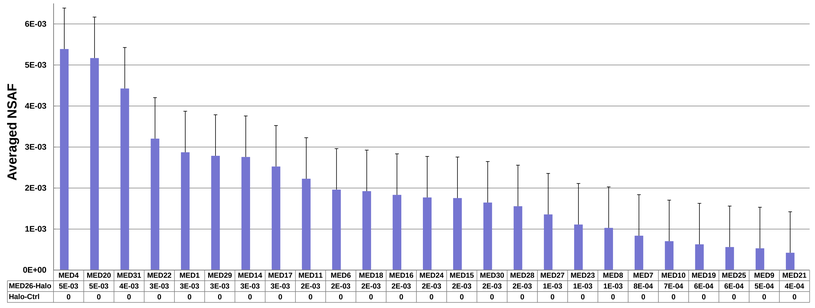


Mass Spectrometry Analysis

- Run pulldown samples on gel
- Excise into 10 segments per sample.
- Trypsin digestion
- Nano LC-MS/MS (LTQ Orbitrap Velos)
- Database search (Mascot)
- Database processing (Scaffold)
- Data Analysis (Spectral Counting and Normalized Spectral Abundance Factors (NSAFs))

❖Significant enrichment over control of proteins from both HaloTag constructs.
❖Successful expression and capture of MED13 (239kDa) as a HaloTag fusion.

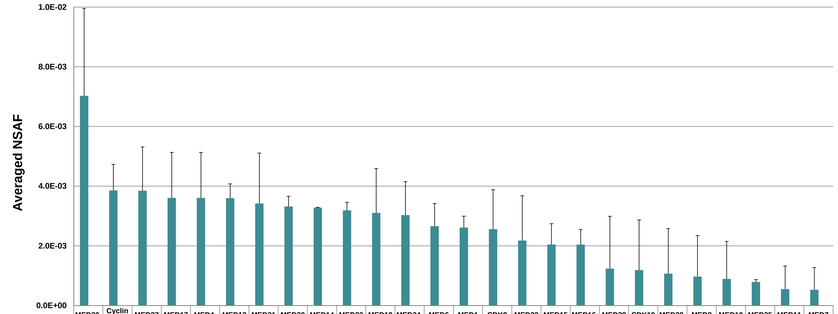
NSAF plot of Mediator subunits from MED26 Isolations



	MED4	MED20	MED31	MED22	MED1	MED29	MED14	MED17	MED11	MED6	MED18	MED16	MED24	MED15	MED30	MED28	MED27	MED23	MED8	MED7	MED10	MED19	MED25	MED9	MED21
MED26-Halo	5E-03	5E-03	4E-03	3E-03	3E-03	3E-03	3E-03	3E-03	2E-03	2E-03	2E-03	2E-03	2E-03	2E-03	2E-03	2E-03	1E-03	1E-03	1E-03	8E-04	7E-04	6E-04	6E-04	5E-04	4E-04
Halo-Ctrl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

❖Excellent enrichment and capture of core Mediator subunits in biological triplicates.
❖No Mediator subunits identified in HaloTag alone control.

NSAF plot of Mediator subunits from MED13 Isolations

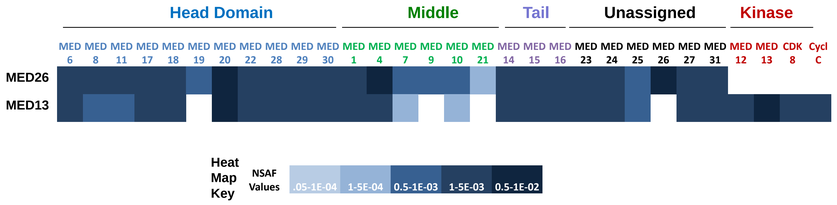


	MED20	Cyclin C	MED27	MED17	MED4	MED12	MED31	MED30	MED14	MED23	MED18	MED24	MED6	MED1	CDK8	MED22	MED15	MED16	MED29	CDK19	MED28	MED8	MED10	MED25	MED11	MED7
Halo-MED13	7E-03	4E-03	4E-03	4E-03	4E-03	4E-03	3E-03	3E-03	3E-03	3E-03	3E-03	3E-03	3E-03	3E-03	3E-03	2E-03	2E-03	2E-03	1E-03	1E-03	1E-03	1E-03	9E-04	8E-04	5E-04	5E-04
Halo-Ctrl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

❖Excellent enrichment and capture of core Mediator subunit is biological duplicates.
❖Additional capture of CDK8 Module subunits (CDK8, Cyclin C, and MED12)

Highly specific and distinct Mediator complex capture

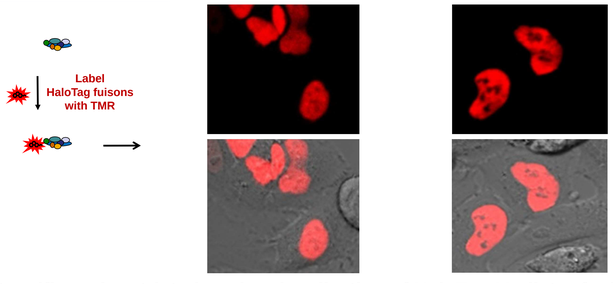
NSAF Heat Map of Mediator Subunit Coverage based upon Mediator Domains



❖Kinase module members excluded from core Mediator complexes containing MED26.
❖MED26 excluded from Mediator-Kinase module complexes.

Correlative cellular localization studies of Mediator

Cellular imaging of HaloTag Mediator fusions



❖Specific and restricted nuclear localization of HaloTag Mediator fusion

Summary

❖HaloTag technology allows for broad study of protein function both *in vitro* and *in vivo*, including complex isolation and cellular imaging.

❖Rapid and covalent isolation of complexes promotes maintenance of complex integrity, particularly macromolecular complexes.

❖Efficient isolation of core Mediator and Mediator bound to it is kinase module using MED26-HaloTag and Halo-MED13, respectively.

❖Mass spectrometry analysis showed distinct complexes associated with different Mediator subunits.

❖Cellular imaging revealed discrete nuclear localization of MED26 and MED13.

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