EFFICIENT ISOLATION AND IDENTIFICATION OF INTRACELLULAR PROTEIN COMPLEXES FROM MAMMALIAN CELLS USING HALOTAG TECHNOLOGY AND MASS SPECTROMETRY.

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Cellular development and growth is largely regulated through a complex network of protein interactions and modifications and to fully understand these processes, de-convolution of protein interactions is necessary. Many of the methods currently used allow for analysis of binary protein interactions but are less efficient for analysis of protein complexes. Furthermore most of the methods do not allow for easy visualization and analysis of protein localization in live cells, which is an important aspect of understanding of protein function and interactions. Here we present the use of the HaloTag fusion protein for efficient isolation and cellular imaging of several multi protein complexes including RNA polymerase, mediator complex and ribosome. We employed MudPIT and quantitative mass spectrometry to demonstrate successful capture and detection of subunits of these complexes using HaloTag pull-down method. Expression studies were done to analyze effects of expression levels of HaloTag fusion proteins on protein complex capture and revealed very efficient capture could be achieved even at levels near endogenous. In addition, to known RNAP II interacting partners we also identified specific interactions between RNAS polymerase and three previously uncharacterized protein interactions. Correlative in vivo imaging studies show proper localization of fusion proteins and their respective complexes. The multiple capabilities of this technology enable study of multi-protein complexes, discovery of novel interactions, characterization of protein function and cellular localization.