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Mapping transient, short linear motif-mediated protein-protein interactions using photo-crosslinking top-down mass spectrometry

Protein–protein interactions (PPIs) govern countless biological processes and range from stable, permanent complexes to transient, short-lived ones. Among the latter, short linear motifs (SLiMs) play a critical role by mediating low-affinity, transient interactions. These are essential for cellular functions like directing protein localization through targeting signals. However, due to their small, transient interfaces, SLiM-mediated interactions are challenging to study using traditional structural biology techniques or current machine learning tools. Despite their importance, methods to specifically discover and characterize SLiM-mediated interactions remain limited. Herein, we developed a new photo-crosslinking strategy to map SLiM-mediated interactions in the HSP90-HOP model system, where binding is known to be mediated by the C-terminal MEEVD motif of HSP90 and the TPR2A domain in HOP. Using chemical biology techniques and solid-phase peptide synthesis, we substituted specific residues within the MEEVD sequence with diazirine-containing unnatural amino acids. Diazirine generates highly reactive carbene species upon UV-irradiation, which covalently insert into nearby bonds within the TPR2A domain, thereby stabilizing the transient interaction for downstream analysis. We initially verified that diazirine containing peptides retained the ability to bind to TPR2A by native mass spectrometry. After UV-irradiation crosslinking was verified by LC-MS before crosslinked complexes were subsequently examined by top-down mass spectrometry (TD-MS). TD-MS allowed us to locate crosslinking site of MEEVD with the TRP2A domain with single amino acid spatial resolution. Interestingly, different diazirine-containing peptide displayed different crosslinking efficiencies and patterns.

The results allowed us to build a picture of binding orientation in the peptide-protein complex and our findings aligned with the binding interface observed in the crystal structure (PDB: 1ELR). These findings demonstrate a new powerful strategy for capturing and mapping SLiM-mediated interactions. In the future, this approach could be extended to map SLiMs across broader PPI networks, advancing our understanding of transient signaling interactions at the proteome level.

User consent

yes

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