# Mapping Synphilin-1 interaction networks using chemical cross-linking and mass spectrometry

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Abstract

Synphilin-1 is one of the major components of Lewy bodies and is implicated in the pathogenesis of Parkinson disease (PD). Over-expression of Synphilin-1 readily forms cytoplasmic protein aggregates, which are transported to aggresome upon proteasome inhibition. Capturing aggregates of abnormal proteins in the aggresome removes their toxicity, therefore clarifying mechanisms of their formation is essential for understanding the origin of many protein misfolding disorders, including PD. Previously, we identified and quantified Synphilin-1 interacting proteins using tandem affinity purification and LC-MS/MS. Here, we cross-linked Synphilin-1 protein complexes and used LC-MS/MS to further characterize the Synphilin-1 interaction network.

Synphilin-1 was constructed with a His-tag and overexpressed in HEK293T cells. The cell extracts were loaded on cobalt beads. The cross-linking reaction was performed by incubation with isotopically labeled d0/d12-BS3 cross-linkers at a ratio of 1:1. Cross-linked Synphilin-1 protein complexes were purified and digested by trypsin and analyzed by LC-MS/MS on a Q Exactive mass spectrometer (Thermo). The proteins were identified using Proteome Discoverer (Nonlinear) integrated with MASCOT (Matrix Science). Isotopically labeled cross-linked peak pairs were extracted using Progenesis LCMS (Nonlinear Dynamics) to generate inclusion lists for performing targeted MS/MS. The cross-linked peptides were assigned using xQuest (ETH Zurich).

A total of 161 protein groups were found in the cross-linked Synphilin-1 protein complex. Twenty isotopically tagged peak pairs were targeted for fragmentation yielding assignment of 6 cross-linked modified peptides. As an example, the transcription factor GATA-6 (at position K485) was observed to be cross-linked with elongation factor 1-alpha 1 (at position K443). In summary, the synphilin-1 interacting network could be mapped by cross-linking on affinity beads followed by mass spectrometry. Our goal is to gain a better understanding of the formation of aggresomes and protein misfolding disorders.