Protein Interface Catalysed Capture (PICC) to Resolve the Structure of the Tau-PSD95 Protein Complex

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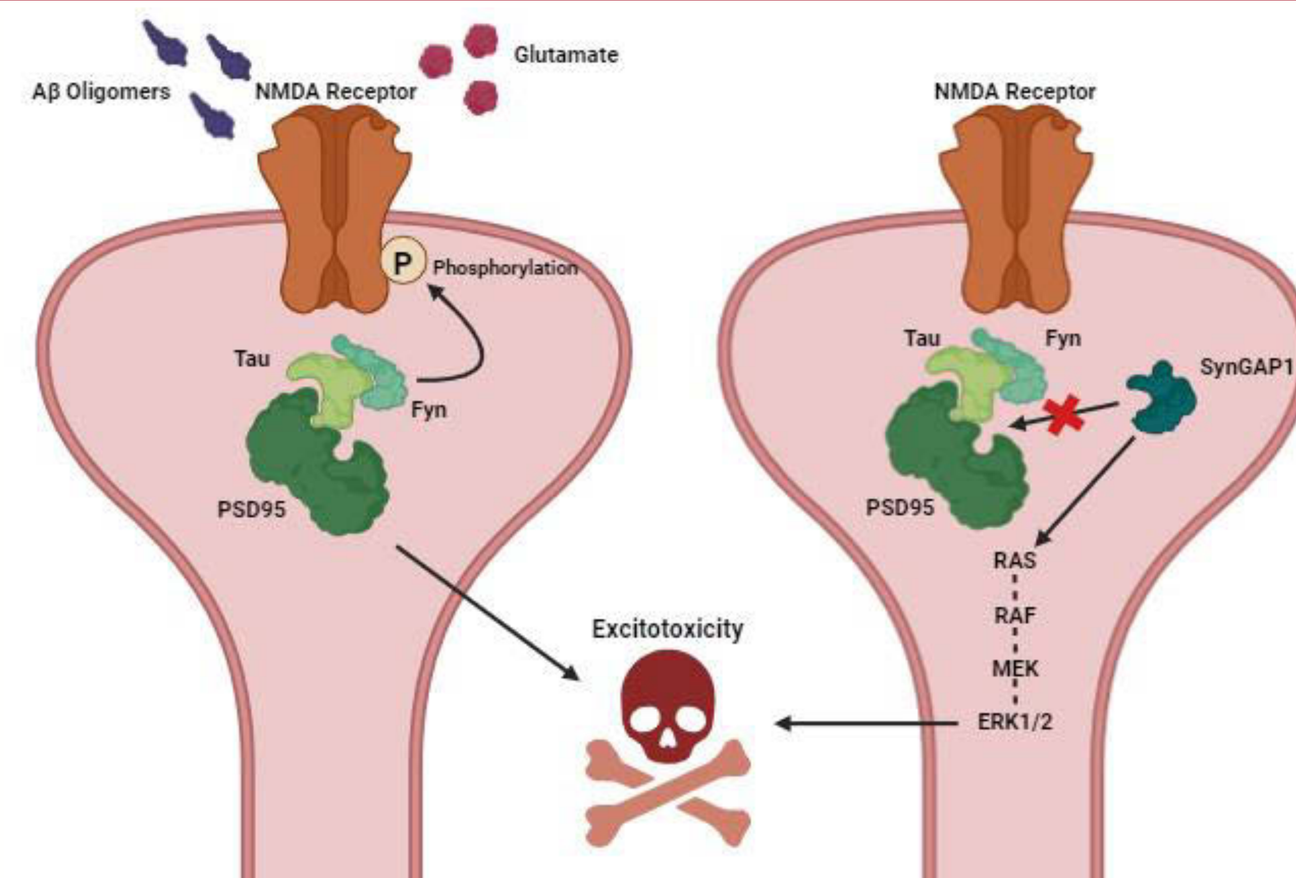
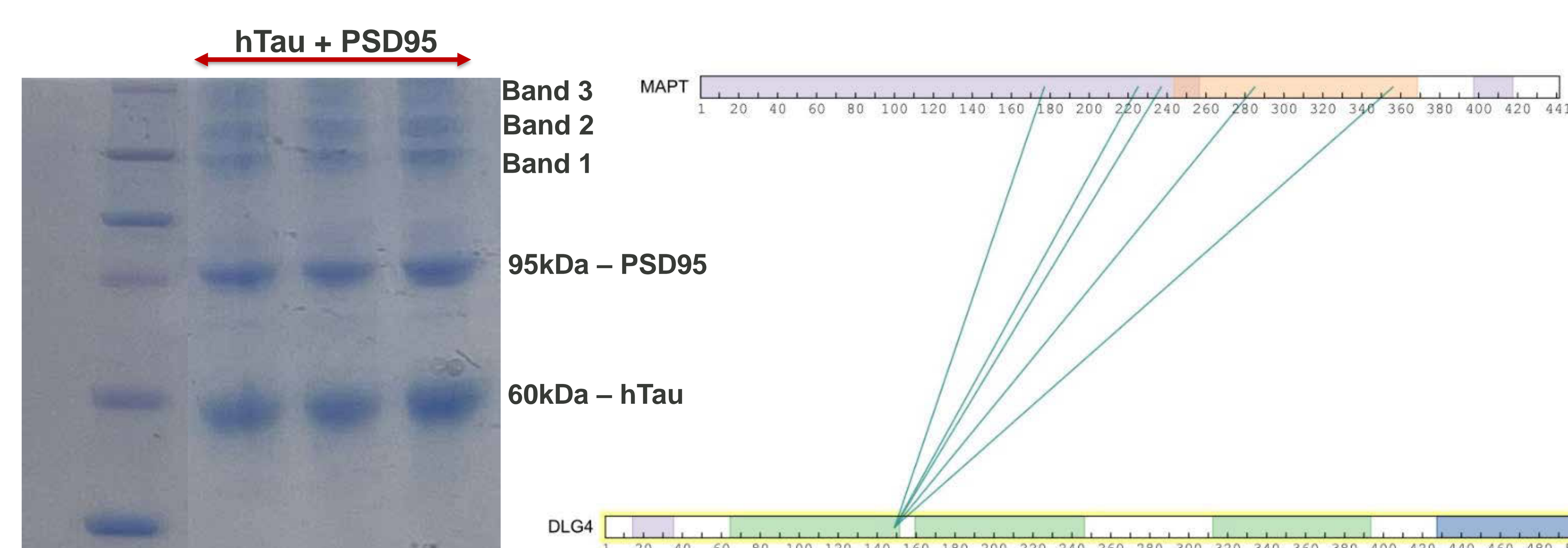
TAU IN NEURODEGENERATIVE DISEASES

The microtubule associated protein tau (MAPT) has been implicated in multiple neurodegenerative conditions, such as Alzheimer's Disease (AD), Parkinson's Disease and a range of Frontotemporal Dementia's. The tau protein is intrinsically disordered in nature which severely limits our understanding of tau biology and hinders drug development efforts. Post-synaptic density protein (PSD95) is a key interaction partner of tau and is linked to disease progression through the mediation of post-synaptic excitotoxicity[1]. Misfolded tau aggregates have been shown to directly correlate to neuronal deficits in AD mouse models and that reducing the levels of post-synaptic tau mitigates excitotoxic effects[2]. Therefore, the resolution of tau's structure in complex is crucial for the development of effective therapies for AD and other neurodegenerative diseases.



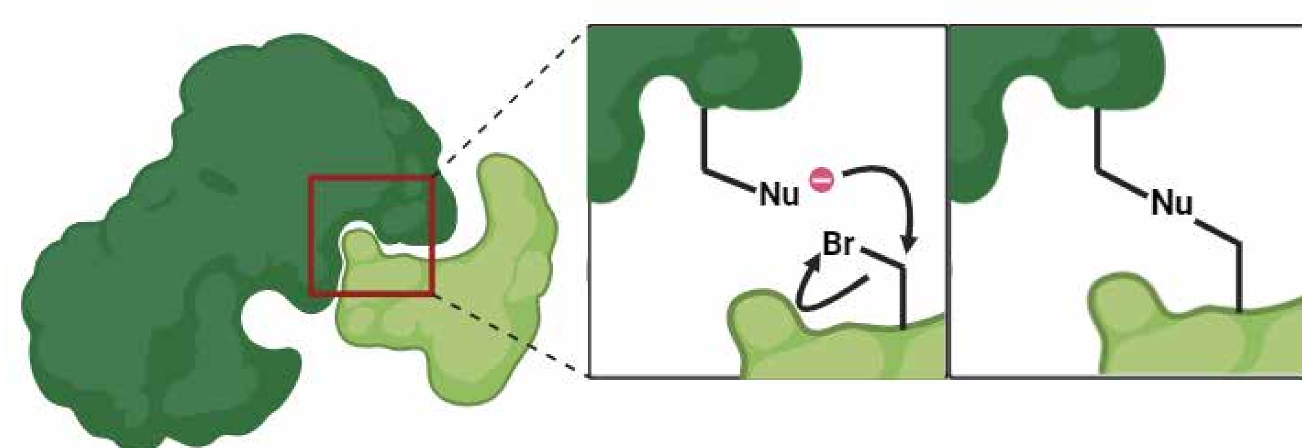
TAU – PSD95 BINDING REGIONS



We performed an unbiased, low-resolution screen of hTau-PSD95 interaction sites, by applying crosslinking mass spectrometry (XL-MS) to the protein-protein complex. We utilized disuccinimidyl sulfoxide (DSSO), a MS cleavable crosslinker containing amine-reactive N-hydroxysuccinimide (NHS) ester groups. NHS esters react efficiently with primary amine groups and form stable amide bonds. Digestion and MS of bands 1-2 identified peptide sequences from the proline rich regions P1, P2, and the microtubule repeat region R2 of tau, which interact with PSD95's PDZ1 domain.

PROTEIN INTERFACE CATALYSED CAPTURE

Recent advances in protein chemistry allows for the installation of unnatural amino acid side chains into any position of a protein's backbone in a constitutionally 'scarless' manner[3]. The introduction of electrophilic haloalkyl side chains as native amino acid mimetics enables protein interface catalysed capture **(PICC)** of protein-protein complexes. In this process, the haloalkyl acts as a context-dependent 'protein alkylator' with cross linking reactivity for interacting nucleophiles at the protein-protein interface. This grants specificity for interacting residues as cross-linking reactions are restricted to genuine protein-protein interfaces.

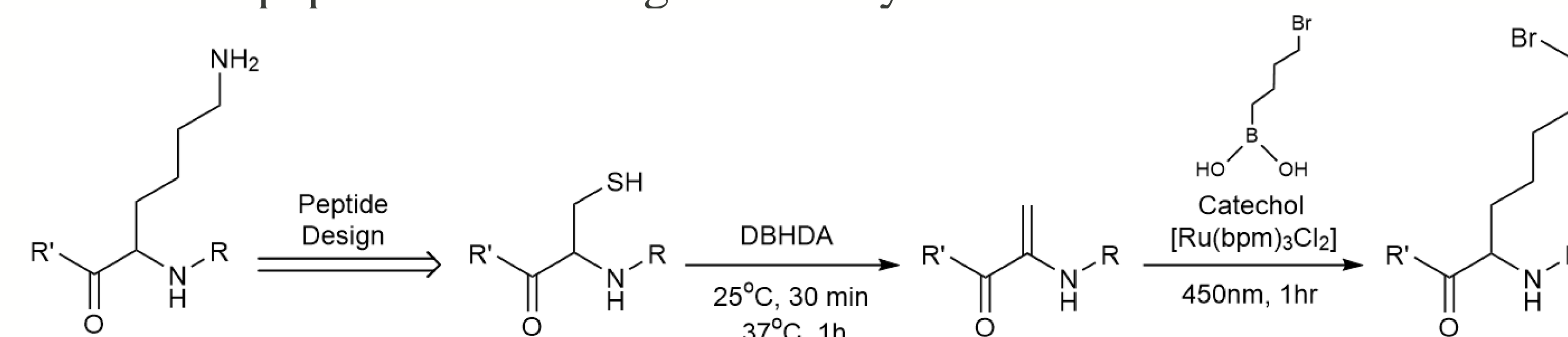


IN SILICO MODELING

We use a chymotrypsin digest followed by high resolution mass spectrometry analysis of the PICC complex. Initial experiments have identified a folded tau interface comprised of three anti-parallel regions of tau that interact with PSD95's PDZ1 domain. Experiments are ongoing to identify specific amino acid residues that are crucial to the tau-PSD95 interactome.

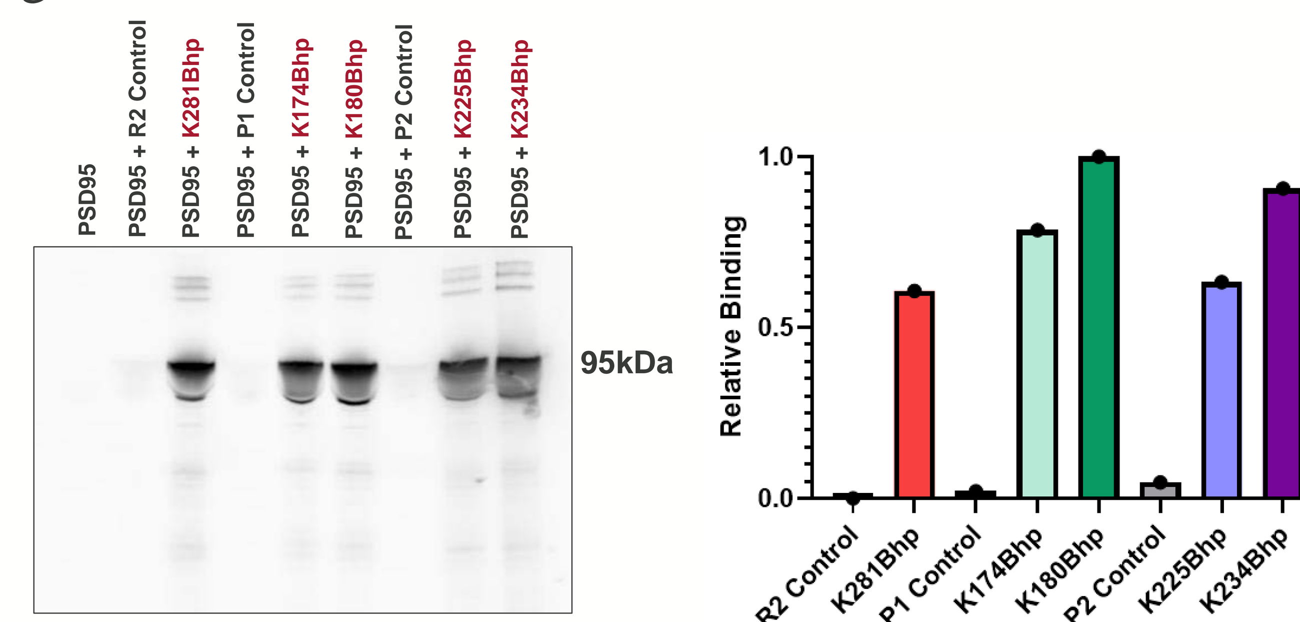
TAU PEPTIDE MODIFICATION

We designed biotinylated human tau peptides to represent the P1, P2 and R2 regions and installed PICC haloalkyl sidechains. We focused on five lysine residues **(K174, K180, K225, K234, K281)** and introduced single cysteine mutations in these positions. Reduction to Dha followed by light-driven radical chemistry using 4-bromobutyl boronic acid afforded five tau mutant peptides containing reactive lysine mimetics.



TAU - PSD95 BINDING RESIDUES

PICC tau peptides were incubated with full length PSD95 protein at low micromolar concentrations (2hr, 37ºC) and run on an SDS-gel under denaturing conditions, followed by western blot and Coomassie staining. Samples are treated with streptavidin to reveal biotinylated tau peptide signals crosslinked to PSD95.



CONCLUSION

We demonstrate that PICC is a powerful tool that can be used to trap and study protein-protein interactions. Furthermore, we present the **first folded structure of tau protein fragments in complex** with an interacting protein. Future work seeks to resolve the structure of full-length tau and to develop specific PPI inhibitors for the treatment of Alzheimer's disease.

REFERENCES

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2. Ittner, et al. *Cell*, **2010**, Dendritic functions of tau mediates Aβ toxicity in Alzheimer's disease mouse models, 142 (3), 387-397.
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