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Using native, top-down mass spectrometry to characterize the interaction of amyloidogenic proteins with assembly modulator CLR01

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Alzheimer's disease (AD) is a complex neurodegenerative disorder that manifests itself through neuronal death and loss of synaptic transmission. Its complex pathophysiology includes a double proteinopathy characterized by aggregation of the amyloid -protein (A) and neurofibrillary tangles (NFT) of the microtubule-associated tau protein. Our group has effectively implemented high resolution mass spectrometry to study the interaction of amyloidogenic proteins with lysine specific molecular tweezer (MT) compound CLR01. CLR01 preferentially binds to Lys residues on unstructured proteins and modifies their assembly into non-toxic states. We have employed top down MS methods to gain structural insight into tau and tau:CLR01 complexes. In addition, we have discovered that performing top down ECD MS of noncovalent tau: CLR01 complex can reveal the site of inhibitor binding. Noncovalent interactions are generally stable enough for transition into the gas phase for structural and stoichiometric analysis. Furthermore, electron capture dissociation (ECD) based fragmentation preserves the labile post-translational modifications (PTMs) and only dissociates the covalent bonds of the noncovalent complexes, which is especially well suited to assigning the sites of ligand binding. Our top-down MS based methods have been successfully used to characterize the effects of CLR01 binding to 4R tau protein (45.8 kDa) and tau fragment (11 kDa). ESI-MS spectra were obtained for the unmodified and phosphorylated 4R repeat domain of tau fragment and tau/CLR01 complex in a 1:1 stoichiometric ratio. The intact protein-inhibitor complex was further subjected to ECD-MS to obtain sequence information and pinpoint the sites of inhibitor binding. ECD-MS data point to CLR01 binding sites in the microtubule binding region, implicated in aggregation. Since phosphorylation plays an important role in tau aggregation, we have also tested phosphorylated tau to map the sites of phosphorylation. ECD-MS confirmed phosphorylation at Ser-235. Our ion mobility experiments on the tau fragment revealed a shift towards a more compact structure in the presence of CLR01.

Biography

Michael Nshanian has completed his PhD in Biochemistry and Molecular Biology at the University of California, Los Angeles, under the guidance of Professor Joseph Loo. He is currently a Postdoctoral research fellow at Stanford University School of Medicine. He has spent several years in the Pharmaceutical industry in the San Francisco bay area, where he helped develop and characterize drug candidates using various analytical techniques. While working in the industry, he has also completed an MS in Chemistry under the guidance of Professor Joseph Pesek. He has published in JACS, Analytical Chemistry, International Journal of Mass Spectrometry and Electrophoresis. His most recent research on using native mass spectrometry and ion mobility spectrometry to study protein-inhibitor complexes will be published in the upcoming issue of the Journal of the American Society for Mass Spectrometry.

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