

# Probing Peptide-metal Ion Interactions on a Phosphorylated $\alpha$ -Synuclein Fragment

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**ABSTRACT:** To understand the causal relationship between phosphorylation and aggregation of  $\alpha$ -synuclein, a protein involved in Parkinson's Disease, it is important to know how the phosphorylation of the protein alters its metal binding properties and tendency towards aggregation. Synthetic peptides corresponding to a C-terminal segment of  $\alpha$ -synuclein containing a phosphorylated tyrosine residue (pY125) have been studied extensively by using terbium ( $\text{Tb}^{3+}$ ) luminescence spectroscopy, ESI-mass spectrometry, circular dichroism and  $^{31}\text{P}$  NMR. The 1:1 dissociation constant for  $\text{Tb}^{3+}$ : pY125 peptide was determined by fluorescence and ESI-MS titrations to be  $K_D = 0.3(5) \mu\text{M}$  at pH 7.0. Two additional metal binding events were observed at higher  $\text{Tb}^{3+}$  concentration. Interaction of this peptide with other di- and trivalent metal ions was monitored by competitive displacement of  $\text{Tb}^{3+}$ . The pY125 peptide has a high binding affinity for  $\text{Fe}^{3+}$ , which was confirmed by ESI-MS studies.  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  are metal ions that uniquely form cross-linked species of the pY125 peptide.