Investigating the Synergistic Effect of Acetylation and Phosphorylation on Alpha-Synuclein Function Using Bioorthogonal Chemistry

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Abstract

Alpha-synuclein (αS) is a 14 kDa neuronal protein found at synaptic termini. Its physiological roles are synaptic vesicle trafficking and managing neurotransmitters. Aggregates of αS are commonly found in Parkinson's Disease (PD), Multiple System Atrophy (MSA), and Dementia with Lewy Bodies (DLB). Recent results show that αS fibrils formed under different disease environments behave differently, but the mechanism of this is not fully understood.

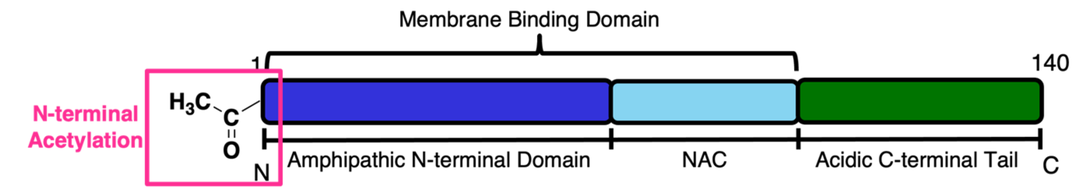
It has been suggested that various post-translational modifications (PTMs) contribute to these differences. Prior works have investigated how a single modification of αS affects its function, structure, and aggregation kinetics. It is important to know how combinations of PTMs on αS affect its native function, structure, and aggregation kinetics. Our ability to understand how combinatorial PTMs regulate αS functions in health and disease has been hindered by the absence of tools for generating site-specific PTMs.

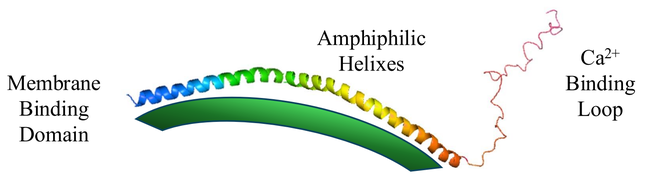
Herein, we present a novel approach to the study of αS. By utilizing genetic code expansion, enzymatic modification, click chemistry and other biochemical approaches, we have synthesized triple PTM αS and labeled the C-terminus with a fluorophore. This innovative method has allowed us to further characterize PTM effects, providing new insights into the behavior of αS.

Background

αS Background:

αS is a 140 amino acid, intrinsically disordered, and physiologically monomeric protein.

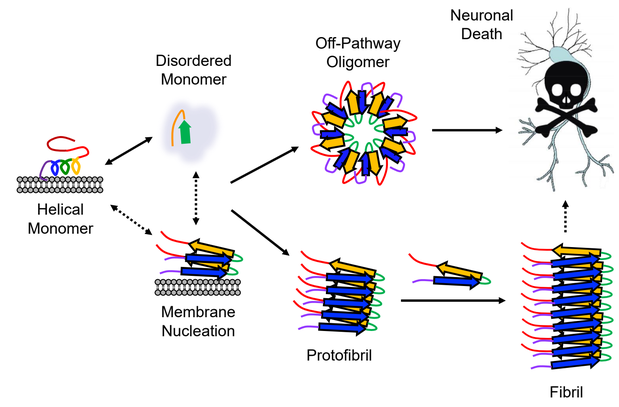


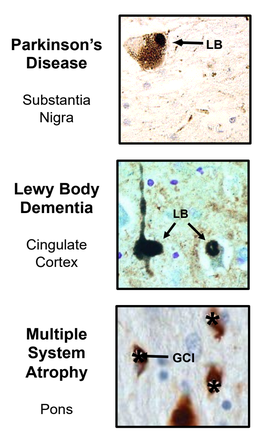


When bound to membranes, αS adopts an α-helical secondary structure. It is involved in neurotransmitter release, SNARE complex assembly, and vesicle trafficking.

αS Misfolding Diseases:

αS can oligomerize and form aggregates that are associated with Parkinson's Disease.

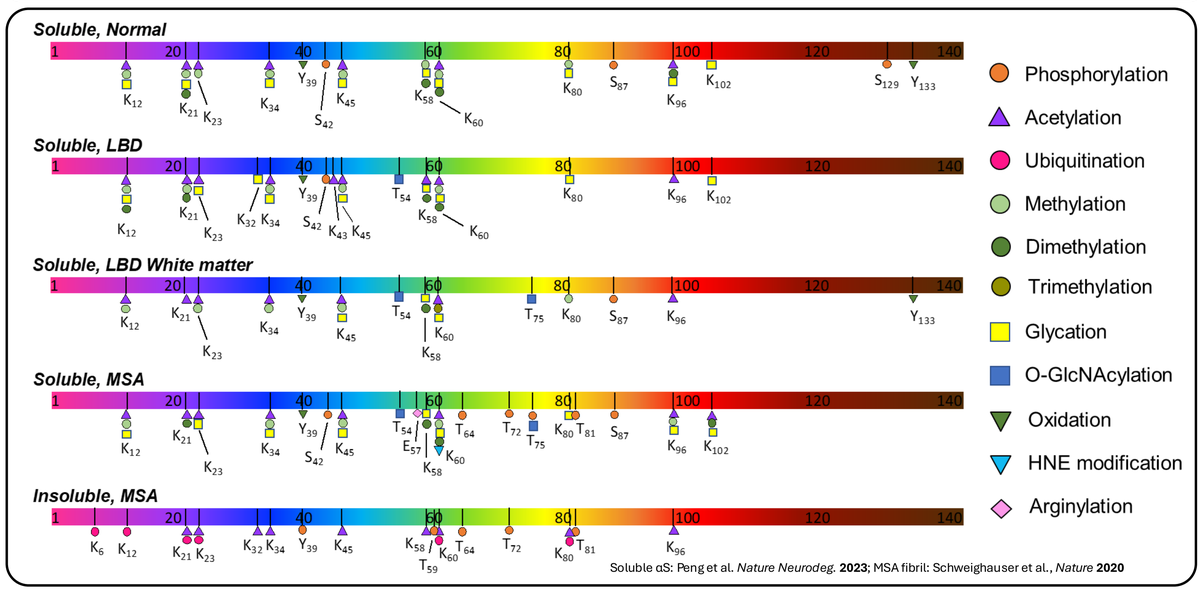




In different pathological contexts, αS can have different activities and conformations. This may be due to various combinations of PTMs.

Lindquist et. al., *Annu. Rev. Cell. Dev. Biol.* **2010;** Spillantini et al., *Nature* **1997**

αS PTMs in LB Disease/MSA Patients



Soluble αS: Peng et al. *Nature Neurodeg.* **2023;** MSA fibril: Schweighauser et al., *Nature* **2020**

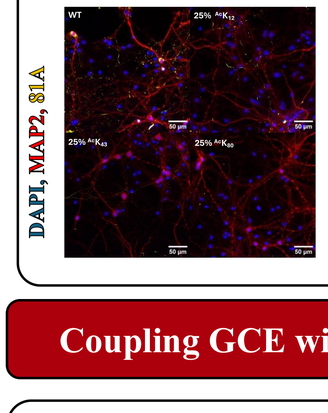
Acetylation has Differential, Site-Specific Effects on αS Aggregation

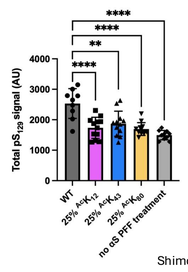
Sites of Interest:

Acetylation of αS has been demonstrated to affect both its aggregation kinetics and seeding.

GCE can be used to authentically and site-specifically scan through different acetylation sites and determine their effects.

Aggregation Seeding in Primary Neurons:

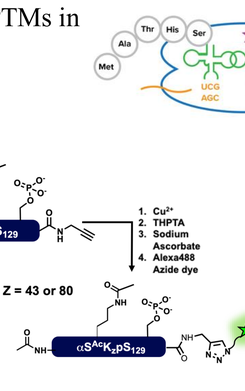




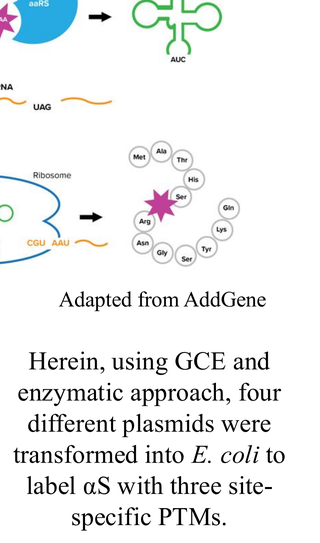
Shimogawa et al; ***2025***

Methods:

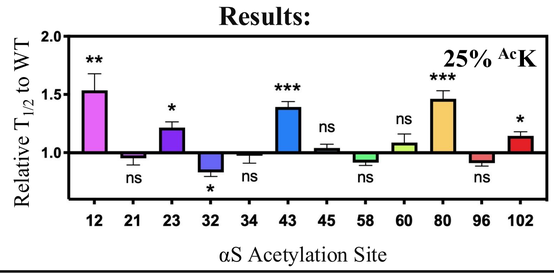
As αS begins to form aggregates, ThT binds to the beta-sheets and the fluorescent signal increases.



Thioflavin T (ThT)



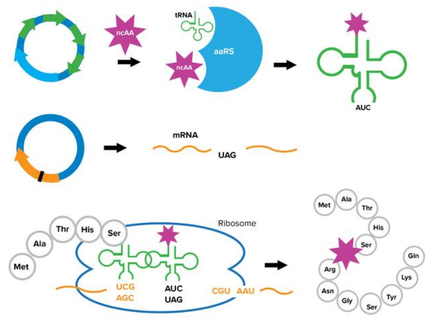
Results:



Coupling GCE with Enzymatic Methods Allows for Triple Labeling of αS

PTM studies often use protein fragments, non-specific overall modification, PTM mimics, or native chemical ligation (NCL).

Genetic code expansion (GCE) integrates modified amino acids into proteins.
Therefore, GCE can be utilized to study the role of PTMs in the function and pathology of αS.

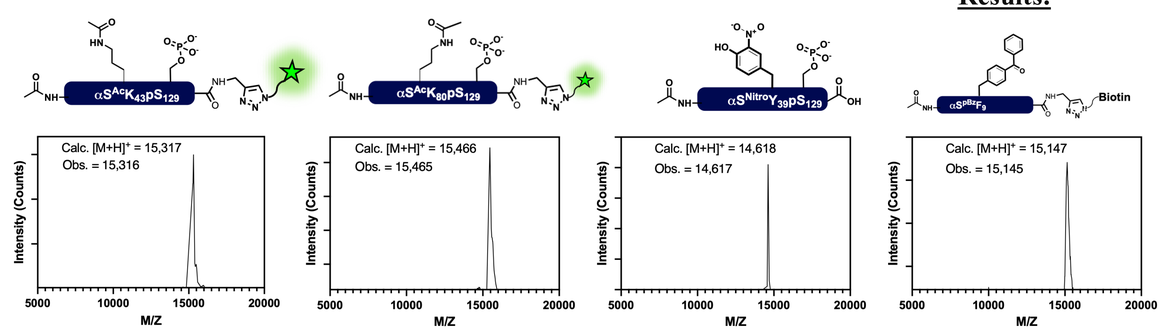


Adapted from AddGene

Herein, using GCE and enzymatic approach, four different plasmids were transformed into *E. coli* to label αS with three site-specific PTMs.

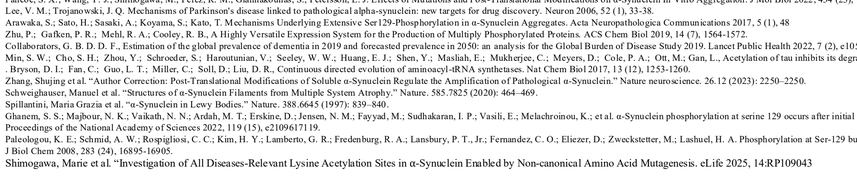
Multiply Modified αS Generated by Combing GCE with Enzymatic Approach

Results:



^{Ac}K80 has an Aggregation Rescuing Effect on αS

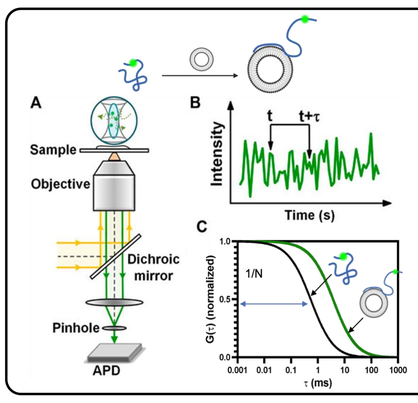
***In Vitro* Aggregation Kinetics**



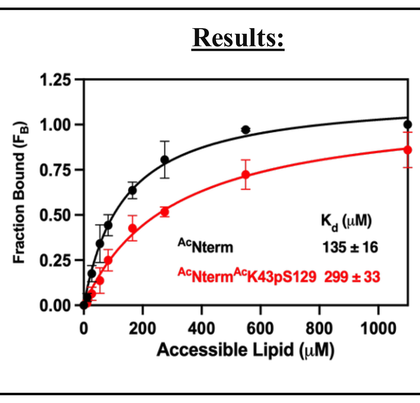
Results:

Incorporated acetylation at N-terminal, K80, and phosphorylation of S129 successfully. Using ThT as a fluorescence readout, preliminary seeded aggregation in vitro show that acetylation at K80 rescues aggregation.

^{Ac}Nterm^{Ac}K43pS129 αS Binds Less to Membranes



Results:



- Fluorescence Correlation Spectroscopy was used to determine the effect of multiple αS PTMs on vesicle (60:25:15 POPC:POPE:POPS) binding.
- ^{Ac}Nterm^{Ac}K43pS129αS binds with less affinity to the membranes compared to ^{Ac}NtermαS.

Acknowledgements

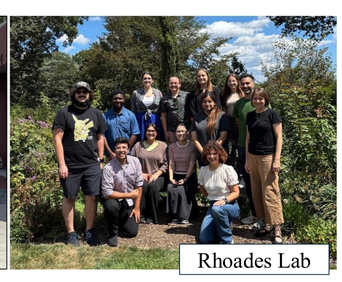
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Part of the data included in this poster was collected by:



Dr. Marie Shimogawa





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