Aggregation and Fibrillar Structure of α-synuclein

Literature Seminar

2020/12/17

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Contents

- Introduction
  - α-synuclein
- Aggregation
  - Fibril
  - Familial PD
- Oxidation of α-synuclein
- Summary

Parkinson's Disease

- Symptom
  - tremor, rigidity, stiffness, postural instability, constipation
- Number of patients
  - 6.9 million (world, 2015)
  - 160,000 (Japan, 2017)
- Cause
  - Aggregation and accumulation of misfolded α-synuclein

  →Formation of Lewy body

  →Death of dopamine producing neurons in substantia nigra

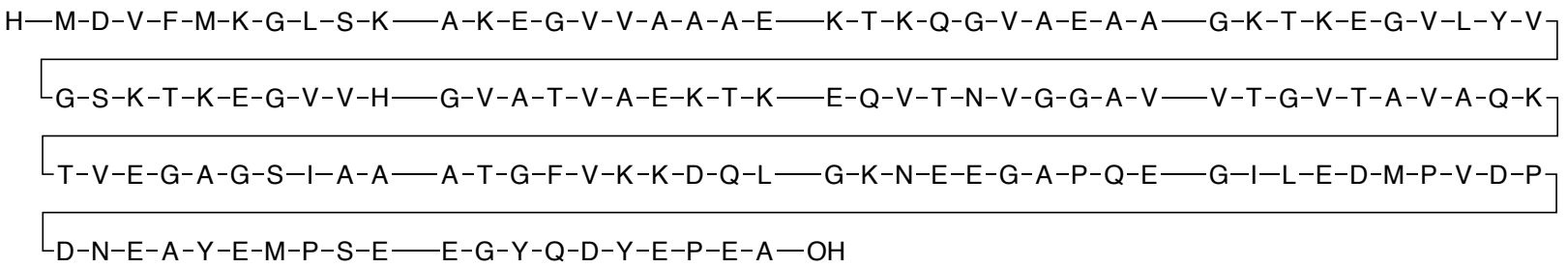
  →Decrease of dopamine

Other synucleinopathies

- Dementia with Lewy bodies
  - Symptom: memory problems, Parkinsonism, hallucinations
  - Cause: Lewy body
- Multiple System Atrophy (MSA)
  - Symptom: Shy-Drager syndrome, striatonigral degeneration, olivopontocerebellar atrophy (OPCA)
  - Cause: glial cytoplasmic inclusions (GCI) in oligodendroglia

α-synuclein

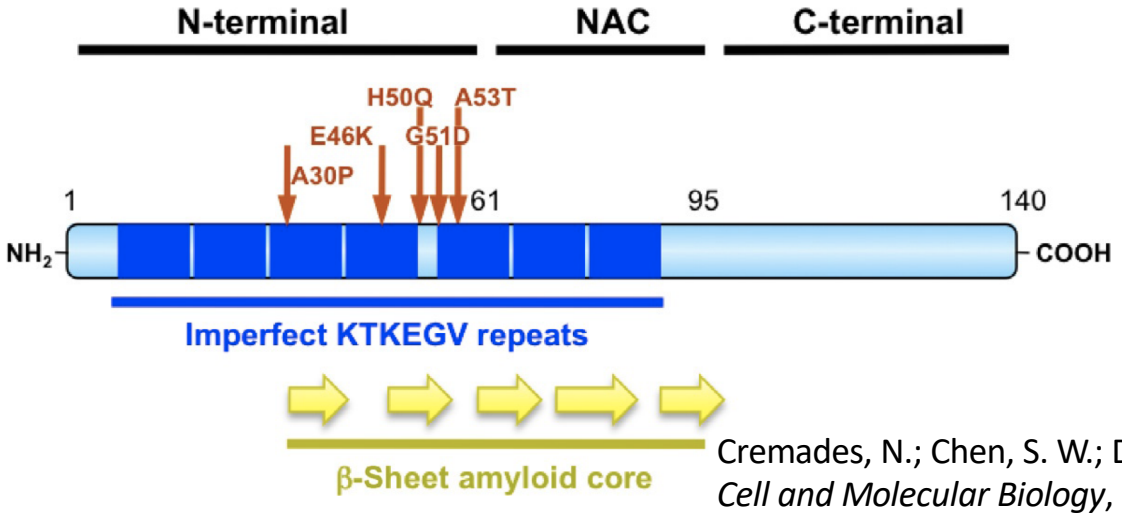
- 140-residue protein in the brain
- In neurons, located at the presynaptic termini
- In the cytosol, monomeric and no persistent structure at physiological conditions



Cremades, N.; Chen, S. W.; Dobson, C. M., *International Review of Cell and Molecular Biology*, **2017**, *329*, 79-143.

α-synuclein

- Three regions
  - N-terminal region (residues 1–60)
  - NAC (nonamyloid-β component) region (residues 61–95)-- hydrophobic→fibril formation
  - C-terminal region (residues 96–140)--highly acidic



Cremades, N.; Chen, S. W.; Dobson, C. M., *International Review of Cell and Molecular Biology*, **2017**, *329*, 79-143.

α-synuclein

- N-terminal is generally acetylated as a post-translational modification
- In solution, α-synuclein adopts an α-helical conformation in the presence of membranes with acidic phospholipid headgroups or high curvature
- Loss of all three synucleins does not produce parkinsonism or any typical form of neurodegeneration.

Cremades, N.; Chen, S. W.; Dobson, C. M., *International Review of Cell and Molecular Biology*, **2017**, *329*, 79-143.

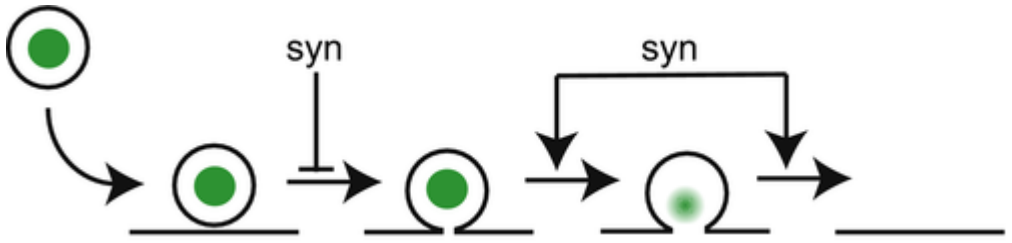
D. Sulzer and R. H. Edwards, *J. Neurochem.,* **2019**, *150*, 475-486

Possible role of α-synuclein

- Regulating synaptic trafficking, homeostasis, neurotransmitter release and so on
- Exocytosis
  - acts at membrane fusion

    ↑synuclein (α-and β-) inhibits regulated exocytosis
  - synuclein normally serves to promote dilation of the fusion pore

    ↑Loss of synuclein delays the release of peptide cargo from dense core vesicles, and increases the likelihood that the fusion pore will close



D. Sulzer and R. H. Edwards, *J. Neurochem.,* **2019**, *150*, 475-486

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Monomer

- Monomeric α-synuclein is dynamic and populates an ensemble of conformational states
- Each conformation has a life span that is dependent on intramolecular interactions
- Intramolecular interactions are stabilized by hydrogen bonds, electrostatic and hydrophobic interactions (depend on surrounding conditions)
- All the conformations α-synuclein adopt are in equilibrium

Alam, P., et al, *J. Neurochem.*, **2019**, *150*, 522-534

Tetramer of α-synuclein

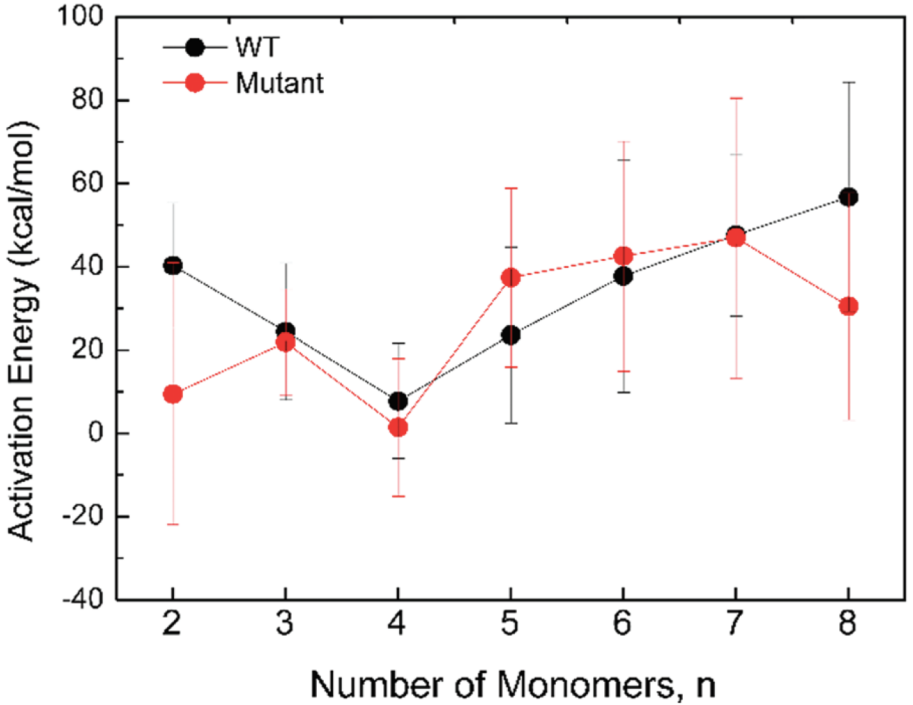
- α-synuclein could exist in large part (up to 70%) as a folded tetramer with α-helical structure
- This tetrameric structure resists aggregation

→ Destabilization of the tetramer precedes α-synuclein misfolding in vivo

Cremades, N.; Chen, S. W.; Dobson, C. M., *International Review of Cell and Molecular Biology*, **2017**, *329*, 79-143.

Stability of tetramer

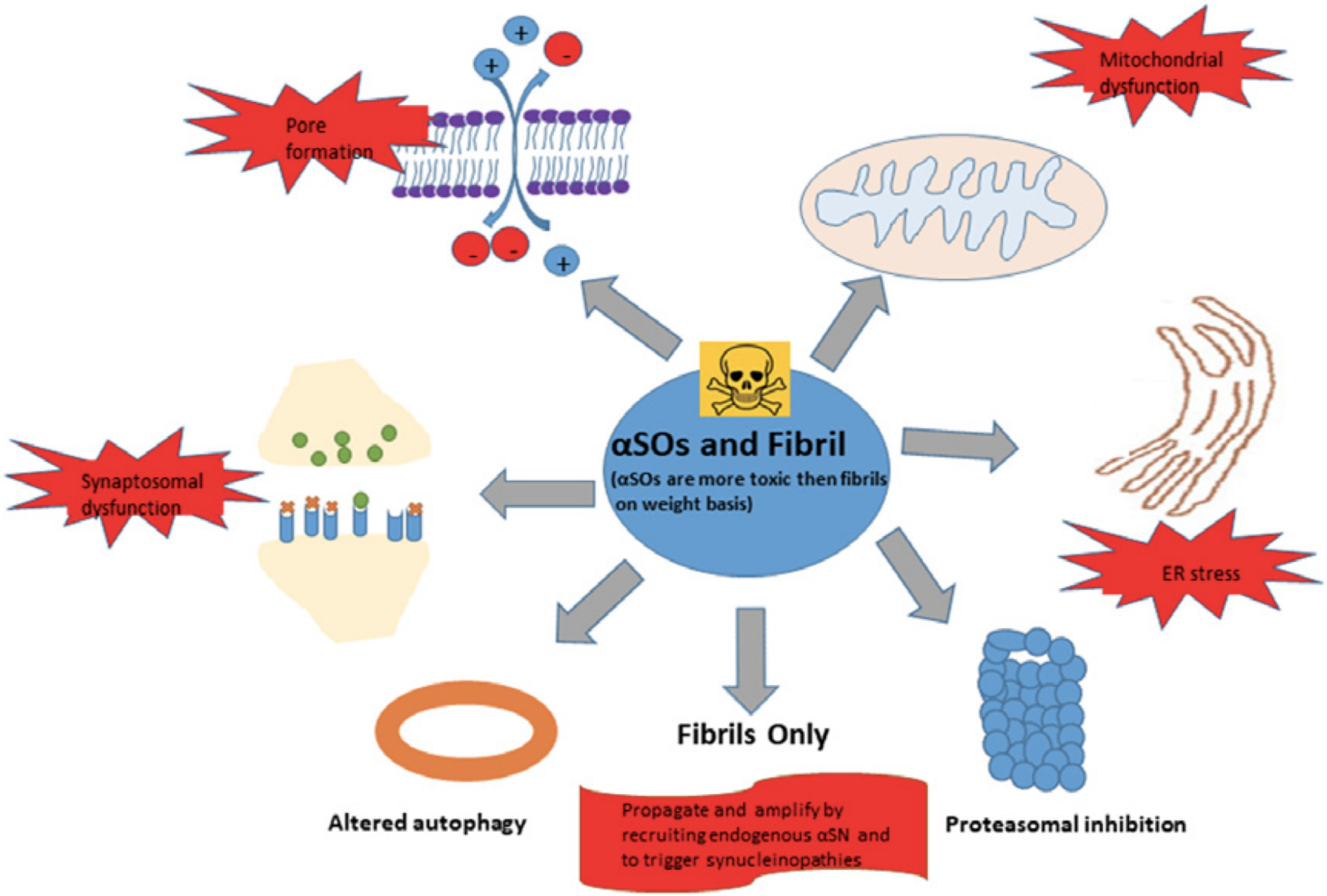
- Helical tetramer has the most balanced structure with the lowest activation energy
- Familial mutations destabilize α-helical tetramers and induce neurotoxicity and inclusions.



Calculation by molecular dynamics computer simulations

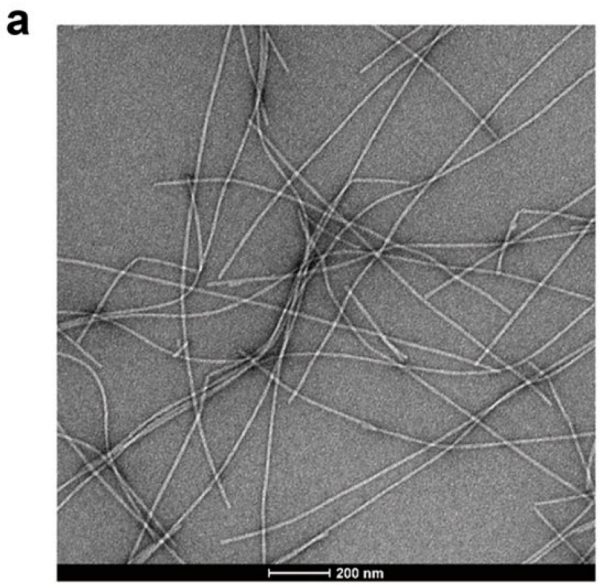
Xu, L., Bhattacharya, S. and Thompson, D., *Phys. Chem. Chem. Phys.*, **2019**, 21, 12036-12043

Toxicity of oligomer and fibril



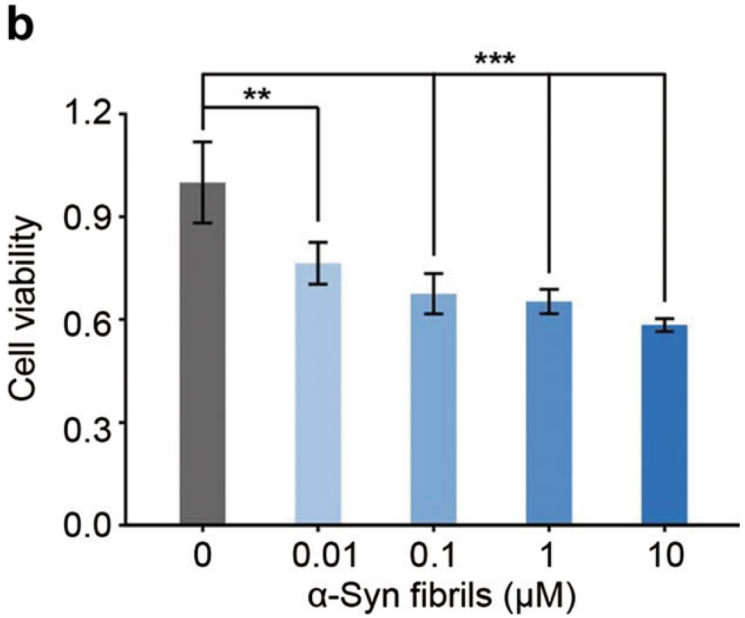
Alam, P., et al, *J. Neurochem.*, **2019**, *150*, 522-534

Pathological fibrils of α-synclein



Negative-staining TEM image of the recombinant α-syn fibrils

Incubated in a buffer containing 50mM Tris, pH 7.5, 150mM KCl and pre-formed fibril seeds, 37 ℃, 3 days



Cytotoxicity of the fibrils to HEK 293T cells

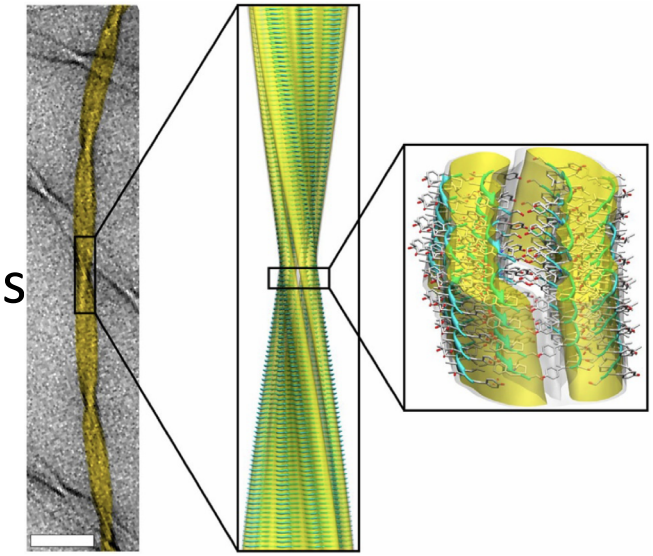
Assessed by the MTT assay.

Cells were treated with indicated concentration of α-syn fibrils for 24 h

Y. Li et al., *Cell Research*, **2018**, *28*, 897–903

Structural characteristics of fibril

- Fibril polymorphs vary in the number and disposition of the protofilaments
- Amyloid cross-β structure
- Core structure generally include residues 30-110
- Monomers adopt an antiparallel in-register β-sandwich fold
- C- and N-terminal could be involved in interactions between protofilaments

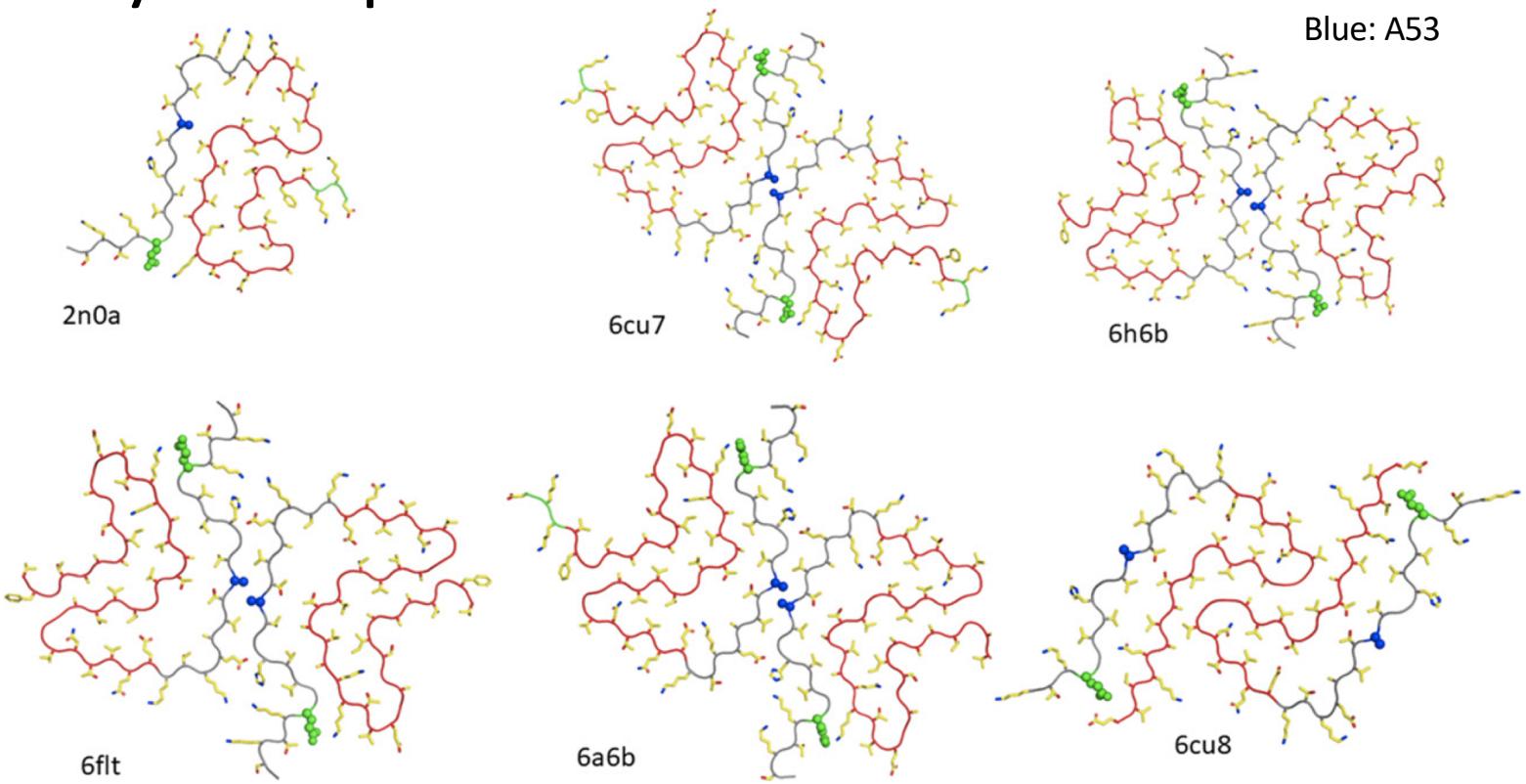


Obtained by the combination of cryo-EM imaging with solid-state NMR analysis

Cremades, N.; Chen, S. W.; Dobson, C. M., *International Review of Cell and Molecular Biology*, **2017**, *329*, 79-143.

Structure of different fibrillar polymorphs

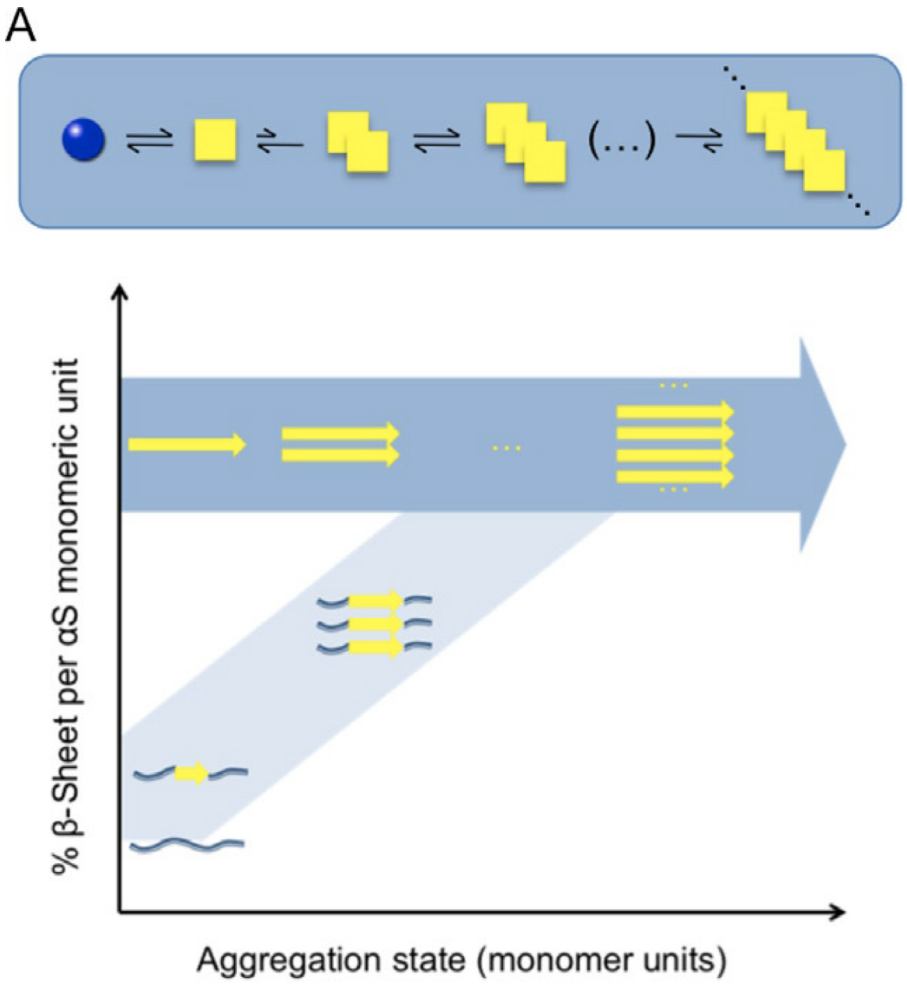
Red: NAC region(60-95)
Green: E46
Blue: A53



- The structures of fibrillar α-synuclein obtained by solid-state NMR [pdb ID# 2n0a] or Cryo-Electron Microscopy [PDB id# 6cu7, 6h6b, 6flt, 6a6b, and 6cu8] Alam, P., et al, *J. Neurochem.*, **2019**, *150*, 522-534

Models for acquisition of amyloid structure

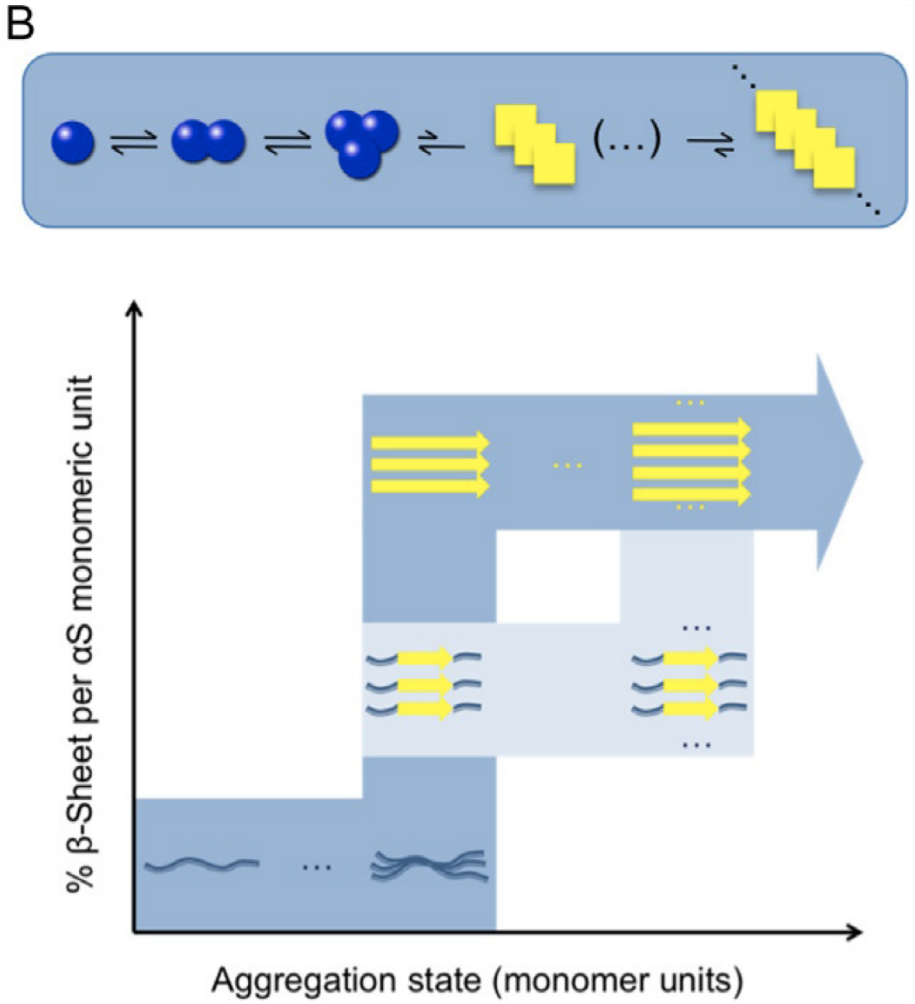
- Nucleation–polymerization model
- The structural conversion from random coil to β-sheet structure take place at the monomeric level



Cremades, N.; Chen, S. W.; Dobson, C. M., *International Review of Cell and Molecular Biology*, **2017**, *329*, 79-143.

Models for acquisition of amyloid structure

- Nucleation–conversion–polymerization model
- The structural conversion occurs at the oligomeric level.

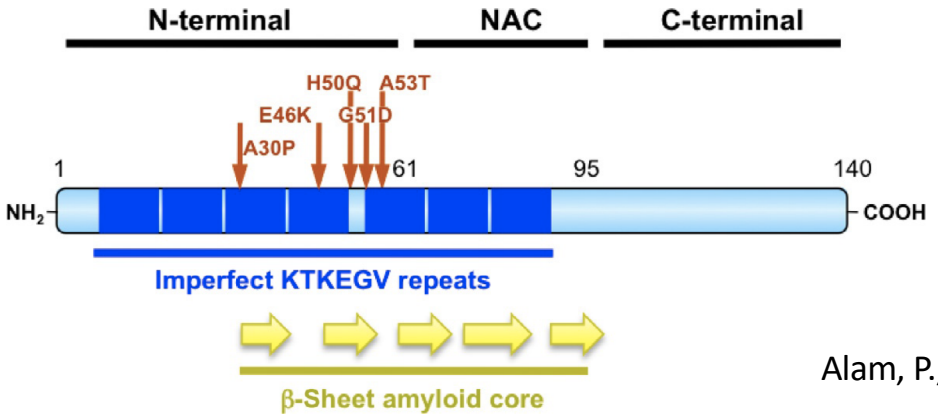


Cremades, N.; Chen, S. W.; Dobson, C. M., *International Review of Cell and Molecular Biology*, **2017**, *329*, 79-143.

Familial early onset PD

- Point mutations (A30P, E46K, H50Q, G51D, A53T)
  - Increase the number of possible conformations

    →Increase the life span of assembly-competent conformers→Aggregation
- Duplication and triplication of SNCA gene
  - Increase the concentration of assembly-competent conformers→Aggregation



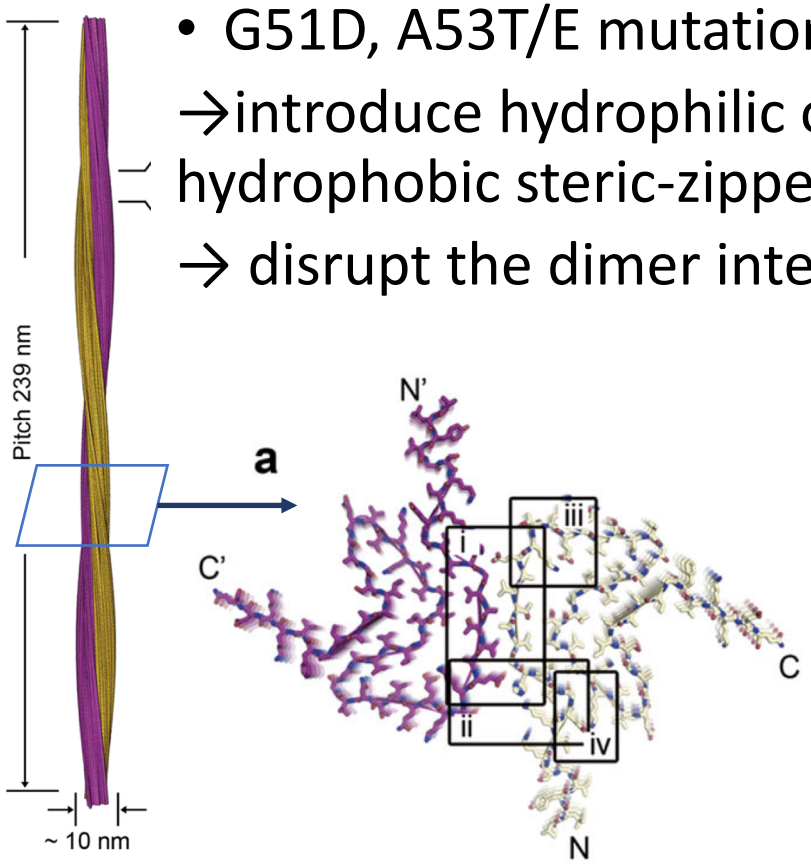
Alam, P., et al, *J. Neurochem.*, **2019**, *150*, 522-534

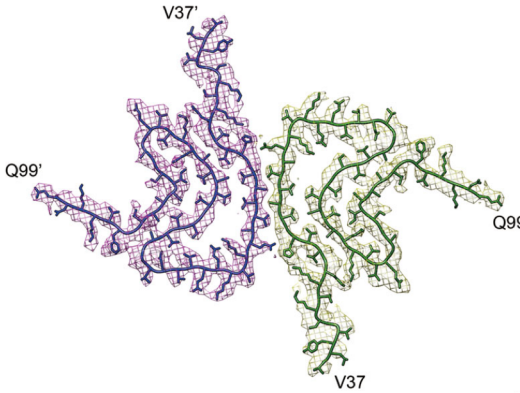
Structure of Familial PD mutation sites

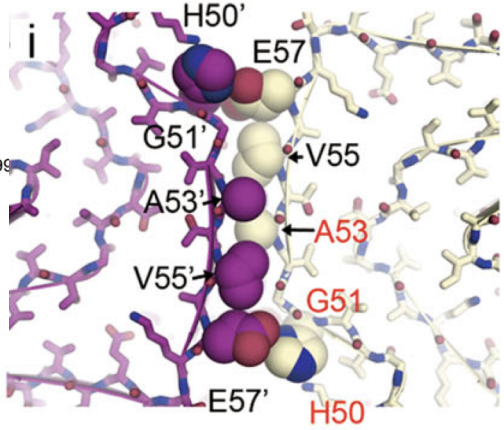
- Four mutations (H50Q, G51D, A53T/E) change the dimer interface
  - G51D, A53T/E mutations

  →introduce hydrophilic or charged residues into the hydrophobic steric-zipper interface

  → disrupt the dimer interface





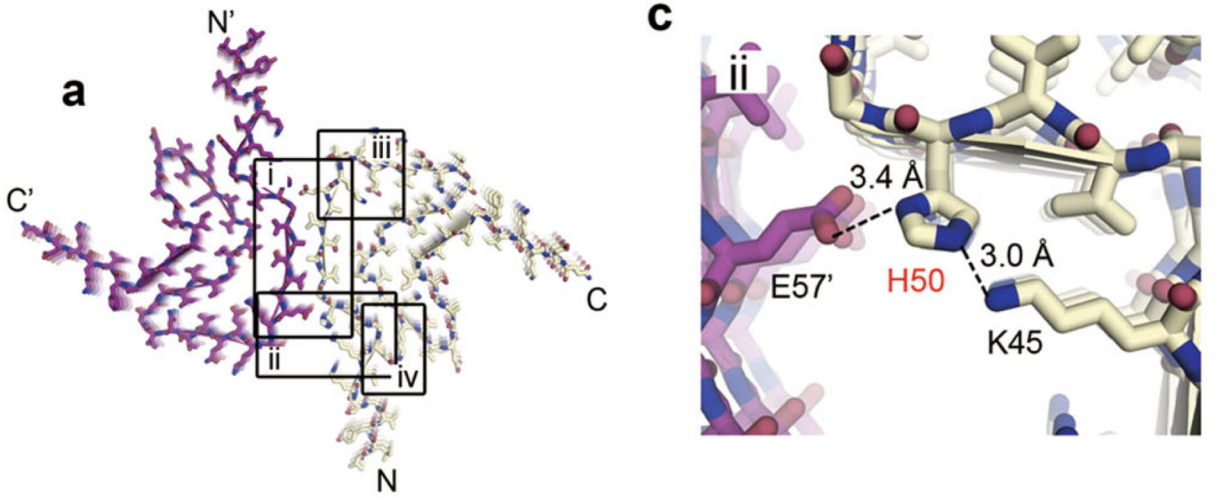


Y. Li et al., *Cell Research*, **2018**, *28*, 897–903

Structure of Familial PD mutation sites

- Four mutations (H50Q, G51D, A53T/E) change the dimer interface
  - H50Q mutation

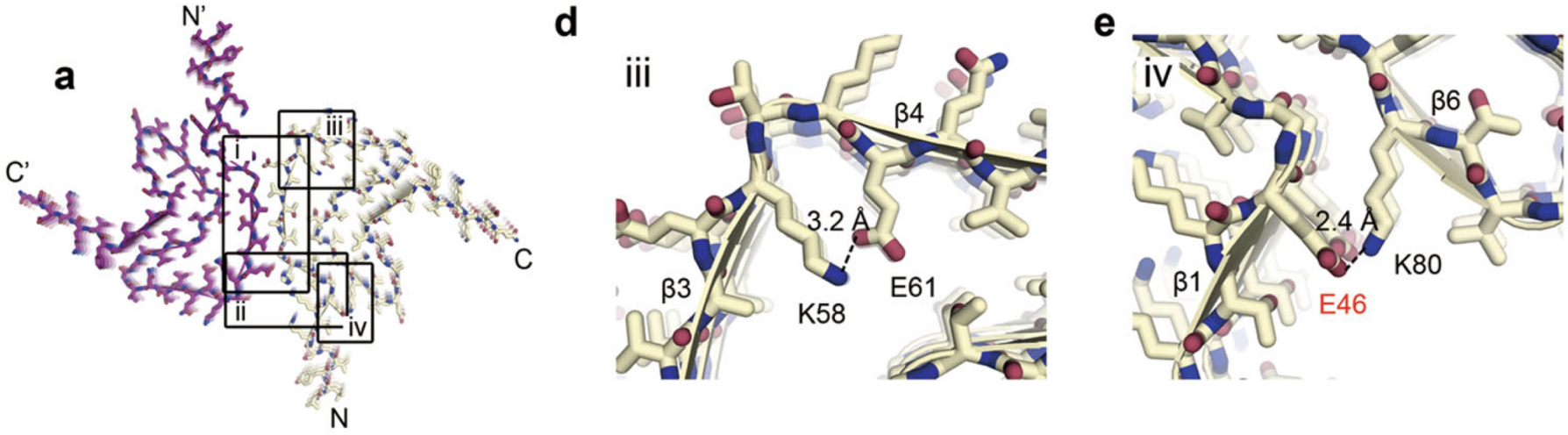
  →break electrostatic interactions among H50, K45, E57’



Y. Li et al., *Cell Research*, **2018**, *28*, 897–903

Structure of Familial PD mutation sites

- K58-E61 and E46-K80 form intramolecular salt bridges, important for the folding of the Greek-key topology
- E46K mutation break the salt bridge between E46 and K80

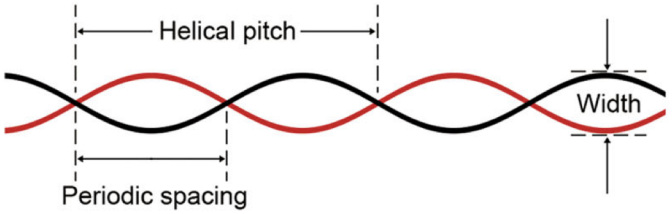


Y. Li et al., *Cell Research*, **2018**, *28*, 897–903

Structures of mutant fibrils

- Mutations (except for A30P) lead to polymorphic fibril structures with distinct features
- Fibrils formed by A30P mutant showed no difference from that of WT

(A30 is not involved in the formation of fibril core)



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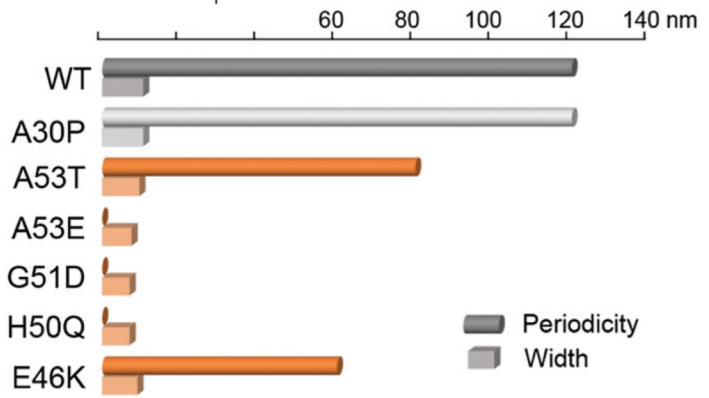
Variant fibrils	Fibril width (nm)	Periodicity (nm)	Chirality
WT	10.5 ± 0.8	120 ± 14	Left-handed
A30P	10.5 ± 0.7	120 ± 19	Left-handed
A53T	9.5 ± 0.3	80 ± 13	Left-handed
A53E	7.5 ± 0.4	ND	ND
G51D	7.0 ± 0.4	ND	ND
H50Q	7.0 ± 0.5	ND	ND
E46K	9.0 ± 0.5	60 ± 8	Right-handed



Measured by AFM

Y. Li et al., *Cell Research*, **2018**, *28*, 897–903

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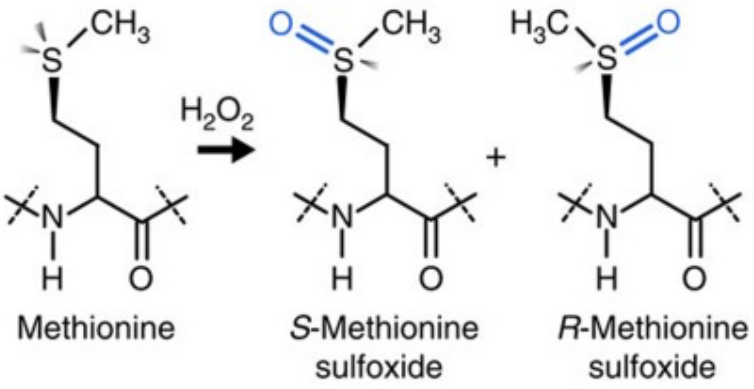
Oxidation of methionine

- Methionine side-chains are oxidation-prone and react with physiological oxidants
- H_2O_2 oxidizes all four methionines
- Lewy body contains oxidative modifications, such as nitrated tyrosines and oxidized methionines
- Methionine oxidation triggers the formation of intermediate oligomer species

Ac-MDVFMKGLS . . . EDMPVDPDNEAYEMPSEEGYQDYEPEA
1 5 116 127 140

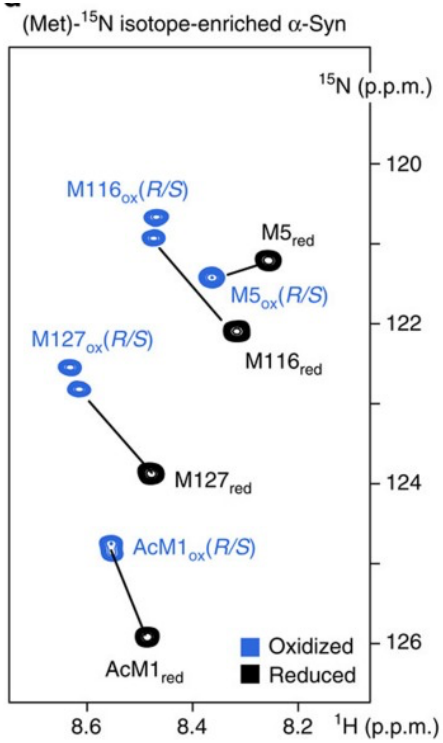
Binolfi, A. et al. Nat. Commun. **2016** 7:10251 doi: 10.1038/ncomms10251



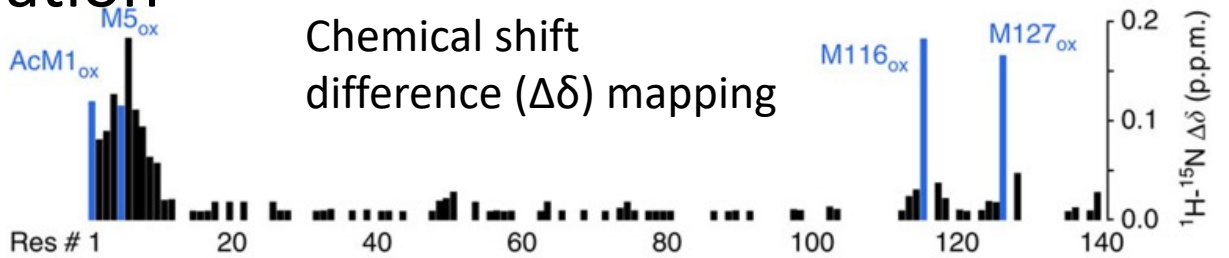
Oxidation of methionine

- Structural alterations by methionine oxidation is greater on N-terminus than C-terminus
- methionine oxidation diminished the increase in residual α-Syn helicity that occurs in response to physiological N-terminal acetylation

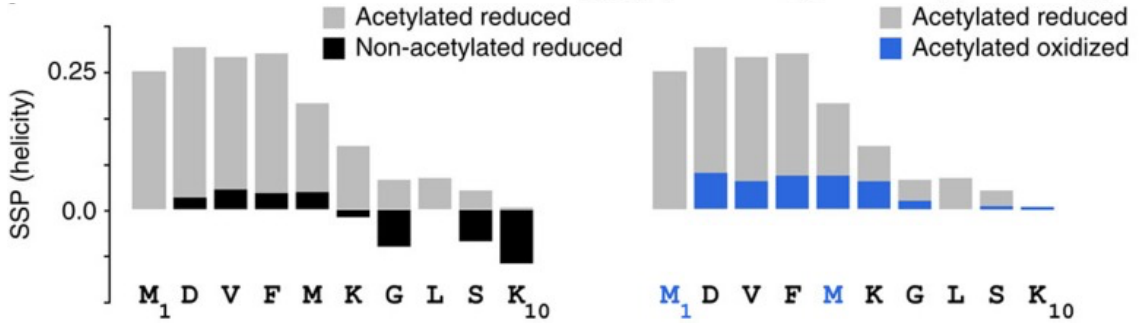
2D NMR spectra



Chemical shift difference (Δδ) mapping



secondary structure propensity (SSP) score

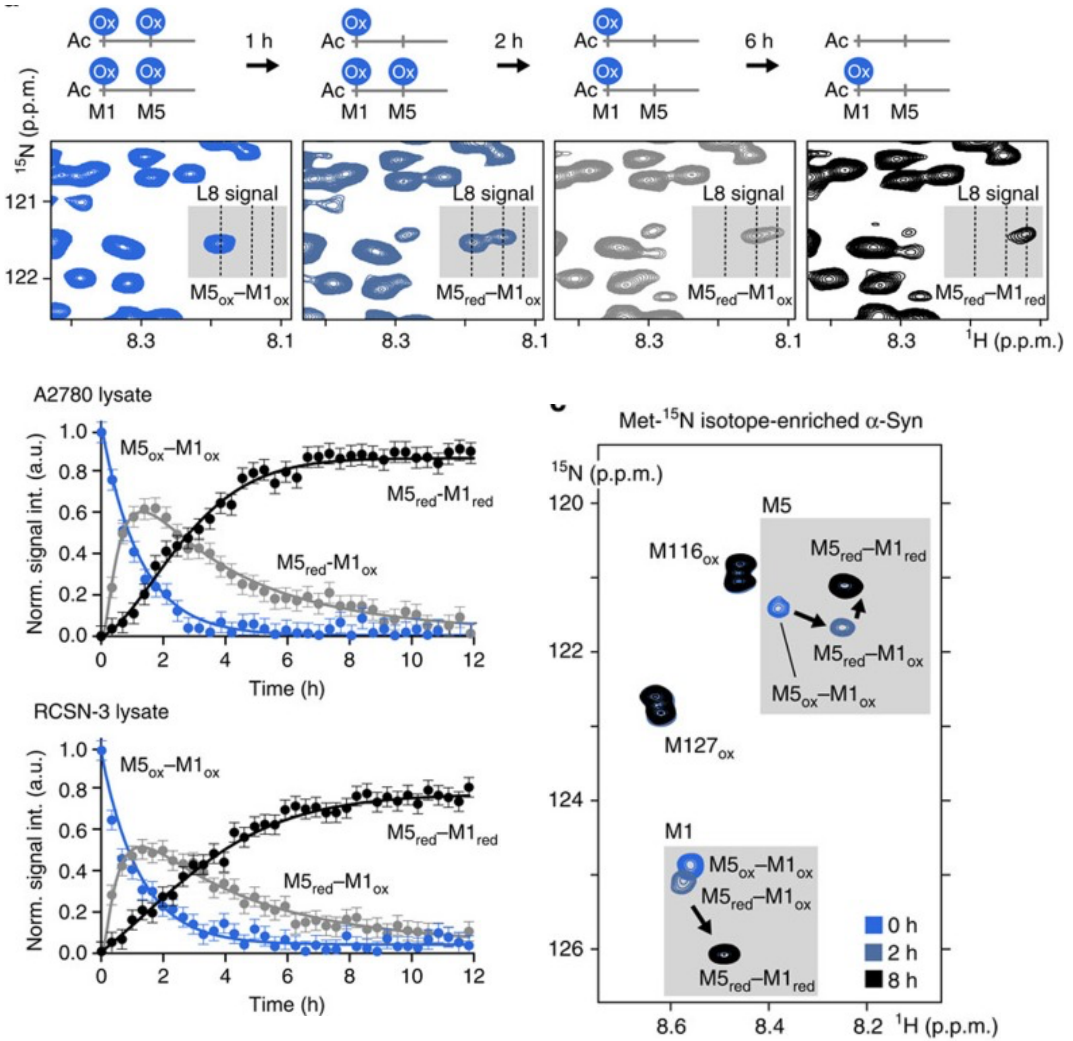


Binolfi, A. et al. Nat. Commun. **2016** 7:10251 doi: 10.1038/ncomms10251

Stepwise repair of N-terminal methionine sulfoxides

- Sulfoxide reduction occurred at Met5 before Met1
- Met1 was not reduced as long as oxidized Met5 was present
- R/S diastereoisomers were repaired equally well
- Met116 and Met127 sulfoxides stably persisted

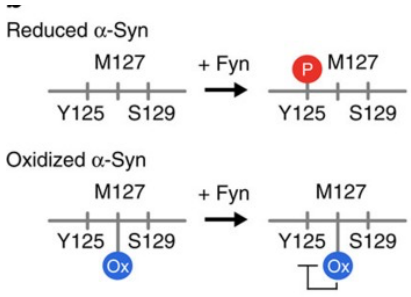
time-resolved NMR (A2780 lysate)

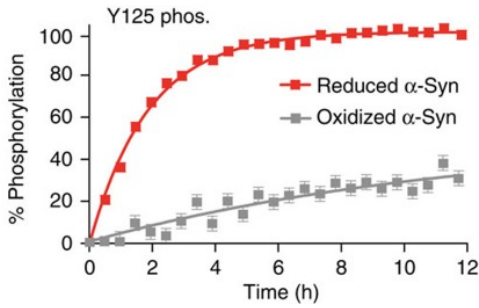


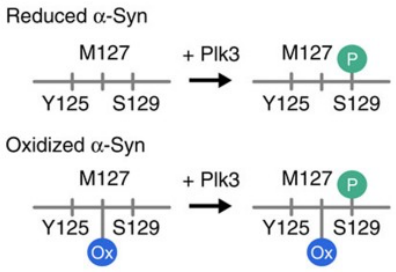
Binolfi, A. et al. Nat. Commun. **2016** 7:10251 doi: 10.1038/ncomms10251

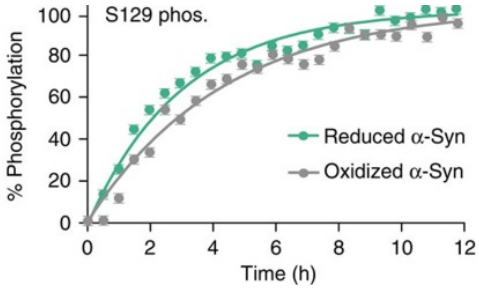
C-terminal methionine sulfoxides impair phosphorylation

- Tyr125 phosphorylation of oxidized α-synuclein was impaired
- Oxidized C-terminal methionines did not compromise phosphorylation of Ser129 by Plk3
- An age- and disease-dependent decline of Tyr125 phosphorylation is reported









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Contents

Summary

- Aggregation of α-synuclein is explained by two models
- Familial PD mutations break local interactions and change fibril structure
- C-terminal α-synclein sulfoxides are stably preserved