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Iron-mediated interaction of alpha synuclein with lipid raft model membranes

Loredana Casalis

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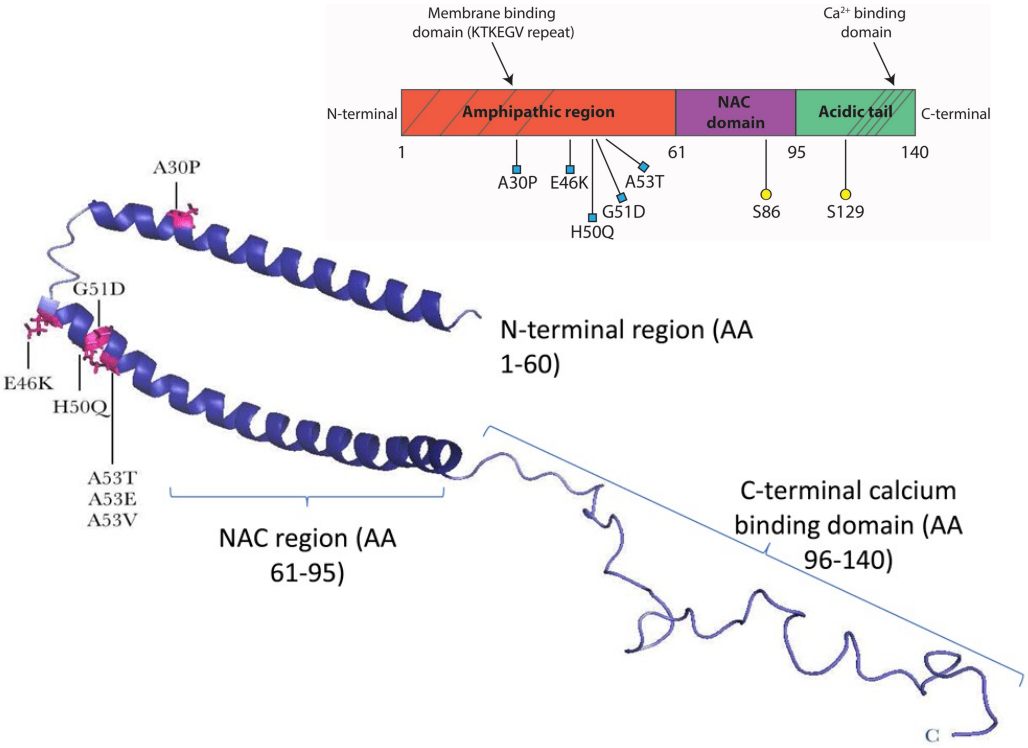
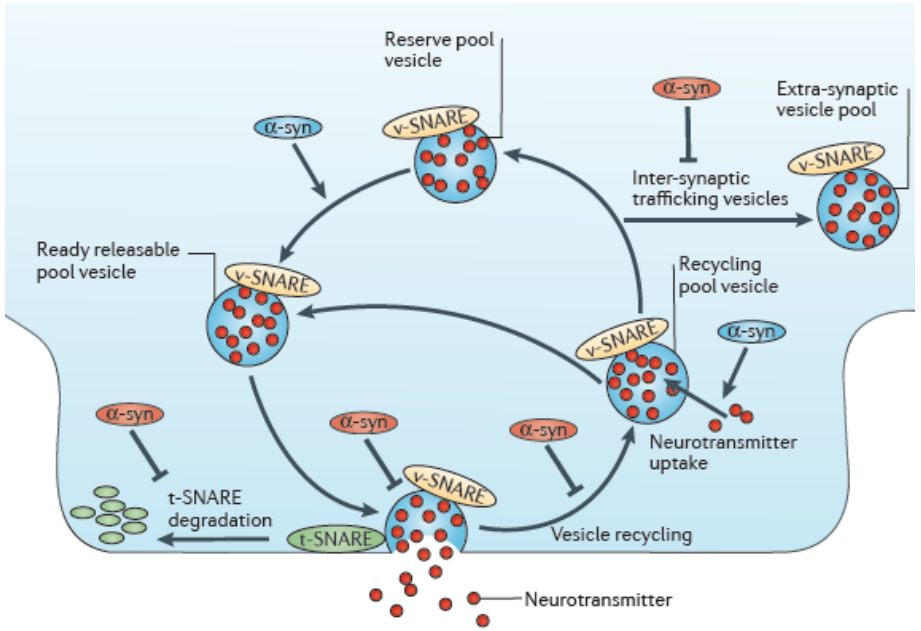
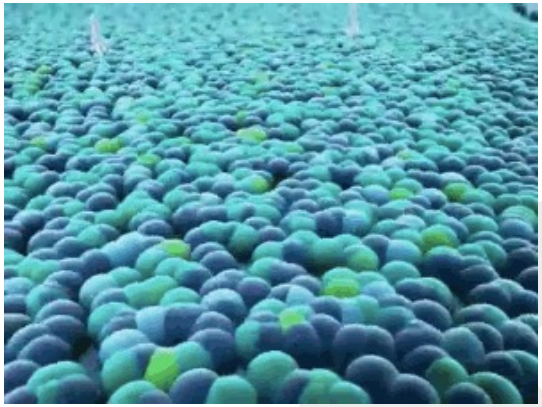
Alpha Synuclein





Lashuel et al. Nat. Rev. Neurosci. (2012). 14, 38–48.

- 140 aa neuronal cytosolic intrinsically disordered protein
- Regulates presynaptic vesicle homeostasis
- Is supposed to bind membrane vesicles at specific lipid microdomains, the lipid rafts, crucial for its synaptic localization and physiological activity



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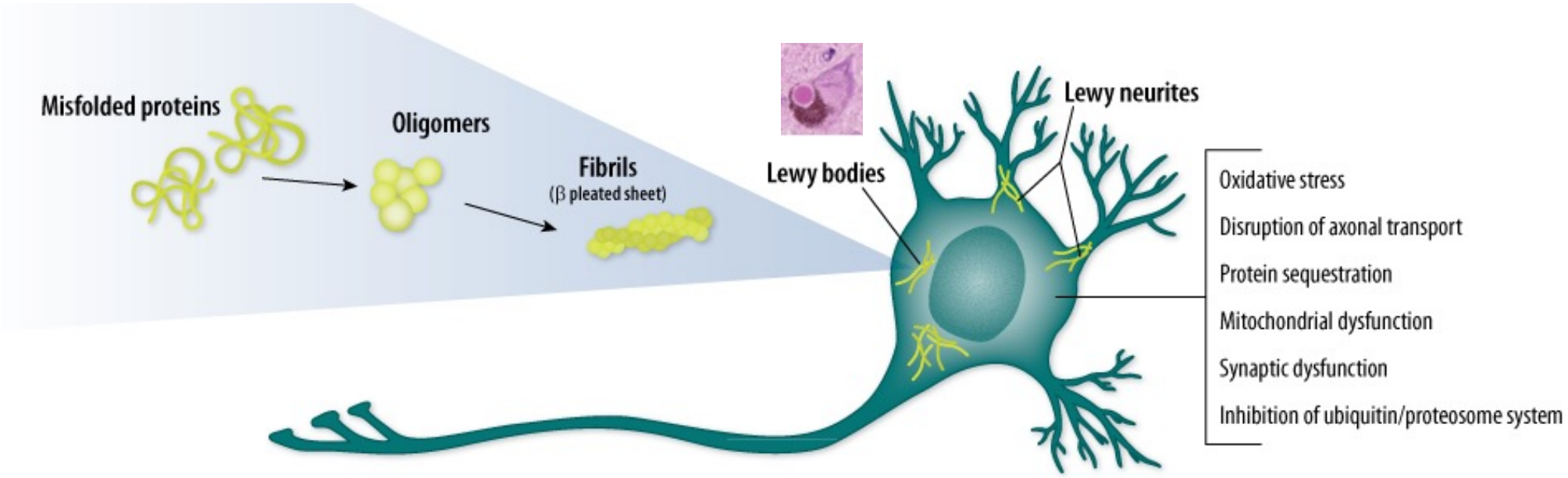
Alpha Synuclein aggregation in Parkinson's disease

➢ Misfolding and aberrant aggregation of Alpha synuclein

The main hallmark of PD is the presence of Lewy bodies, which are cytosolic abnormal depositions of alpha synuclein (αS) aggregates (oligomers, fibrils), iron and other ubiquitinated proteins



Recent findings point to **prefibrillar oligomers**, intermediate products of the αS aggregation pathway, as the **main toxic species**.
Oligomers have been proposed **to induce destabilization and permeabilization of cell membranes.**

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Alpha Synuclein aggregation in Parkinson's disease

➢ Genetic and environmental factors promoting **αS** aggregation

- Overexpression of αS
- Missense mutations (es: A53T)
- Interaction with lipid membrane
- Local changes of pH
- Metal ions
- Phosphorylation

Collaboration with Prof. G. Legname Group SISSA, Trieste

Pathological accumulation of iron in PD

J Neural Transm (Vienna). 2017 Aug;124(8):973-981. doi: 10.1007/s00702-017-1695-x. Epub 2017 Feb 6.

Alpha-synuclein and iron: two keys unlocking Parkinson's disease.

Lingor P[1,2], Carboni E[3,4], Koch JC[3].

Front Neurol. 2017 Jan 16;8:1. doi: 10.3389/fneur.2017.00001. eCollection 2017.

Iron Deposition Leads to Neuronal α-Synuclein Pathology by Inducing Autophagy Dysfunction.

Wan W[1], Jin L[1], Wang Z[2], Wang L[3], Fei G[1], Ye F[1], Pan X[1], Wang C[1], Zhong C[1].

Biochim Biophys Acta. 2016 Apr;1862(4):518-525. doi: 10.1016/j.bbadis.2016.01.002. Epub 2016 Jan 6.

Differential interaction between iron and mutant alpha-synuclein causes distinctive Parkinsonian phenotypes in Drosophila.

Zhu ZJ[1], Wu KC[1], Yung WH[1], Qian ZM[2], Ke Y[3].

AIM: Investigate the ability of αS to interact with raft-like model membranes and the role of iron (II) in these interactions

(A). *In-vitro* aggregation analysis of wild-type **αS** and A53T **αS** **mutant** species in presence of **iron (II)**

(B). Interaction of **monomers** and **iron(II)-oligomers** of αS with **lipid raft model membrane**

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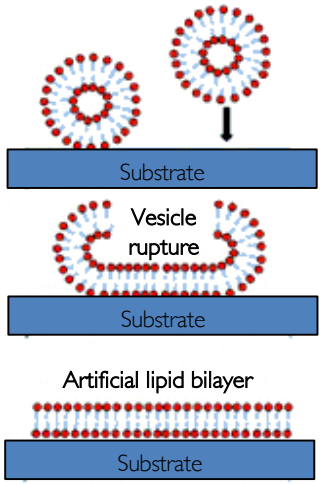
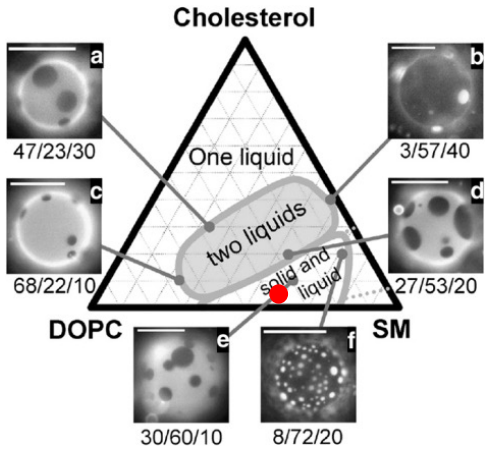
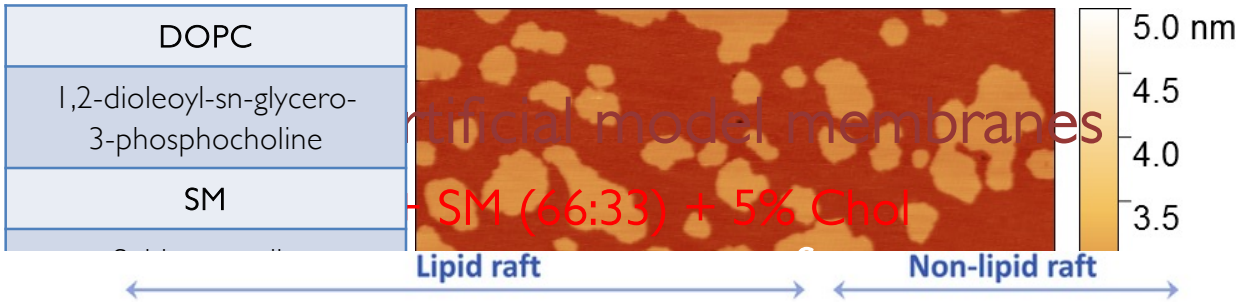
Supported lipid bilayers

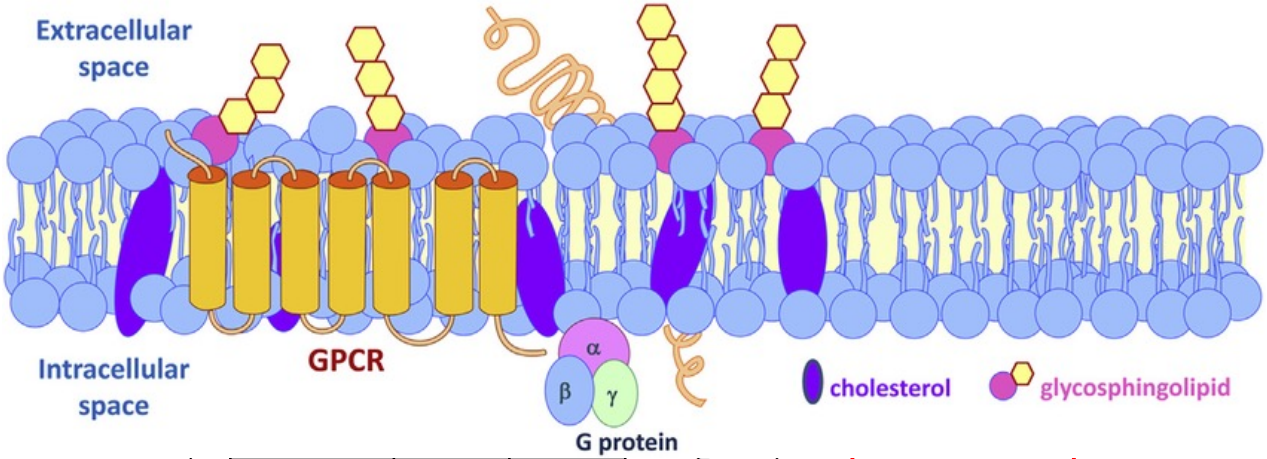
Vesicle fusion method

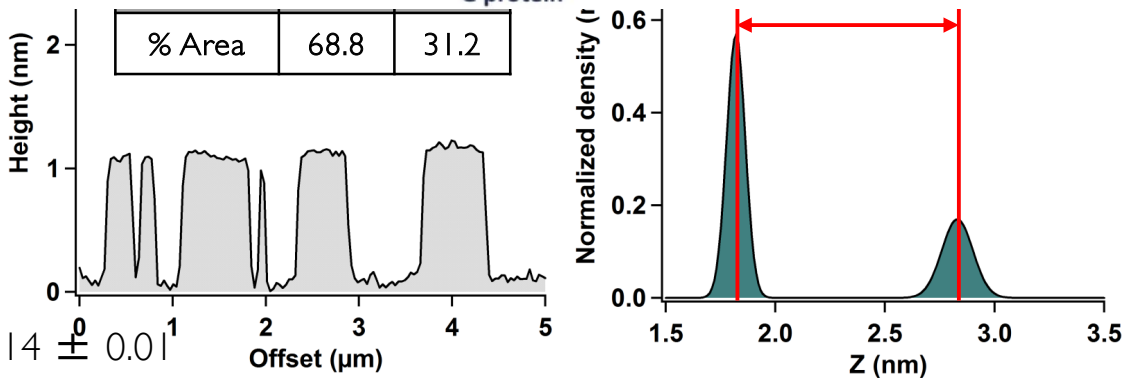




Adapted from Veatch and Keller. *Phys. Rev. Lett. (2005). 94, 148101*





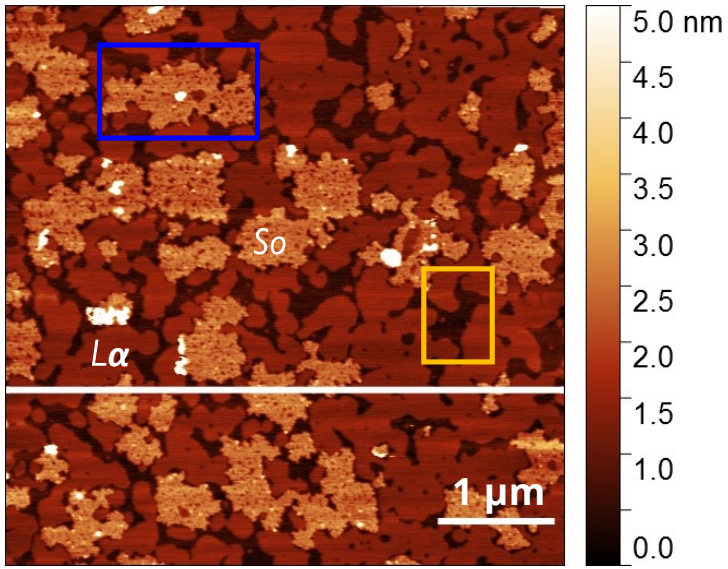


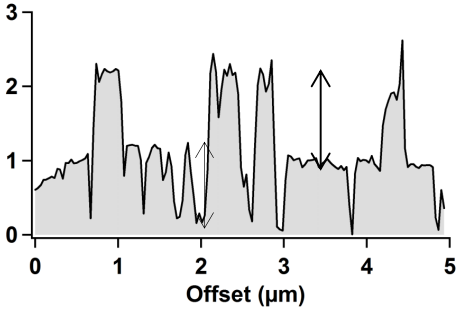
surface roughness: Lα=0.16 ± 0.01 nm, So=0.14 ± 0.01

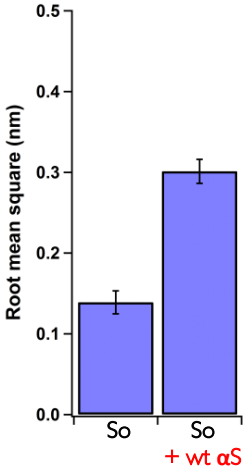
Monomeric α-syn: membrane binding interaction

➢DOPC + SM (66:33) + 5% Chol

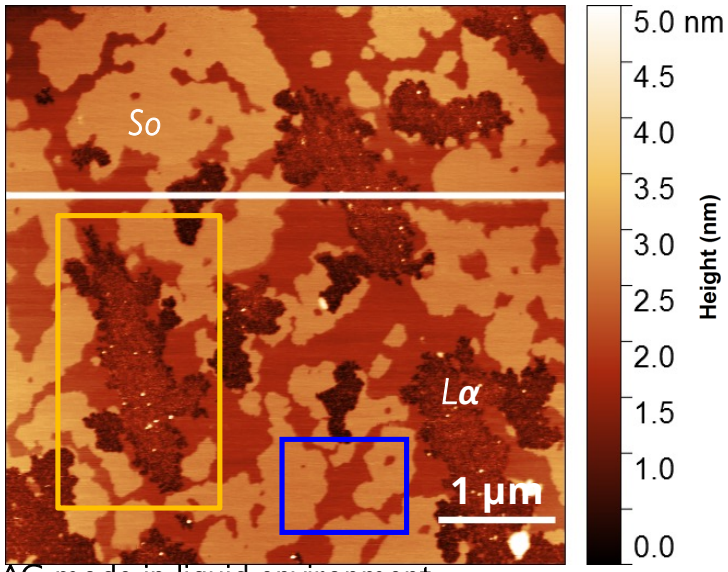
wild-type αS monomer

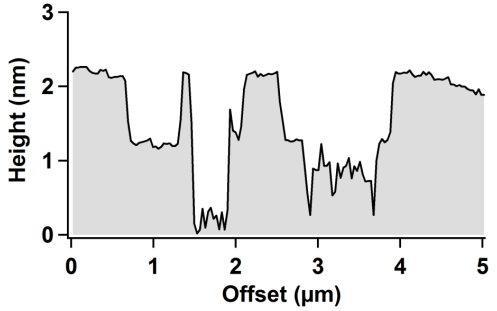


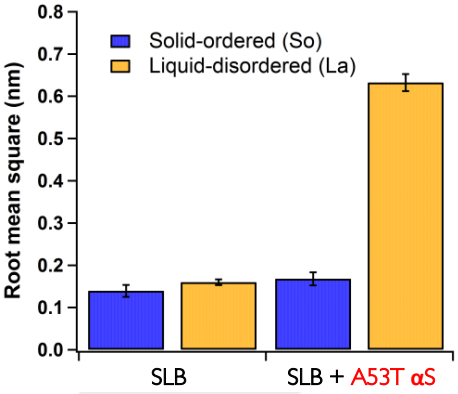




A53T αS monomer







preferential interaction of the A53T mutant with the DOPC-enriched phase

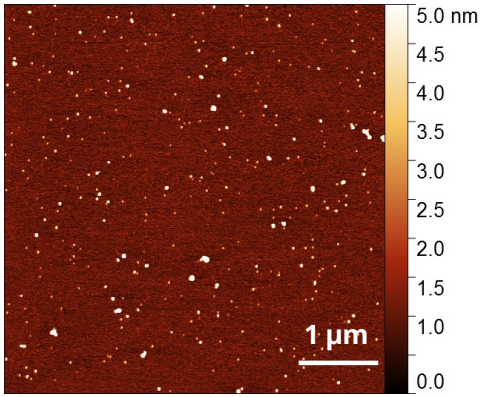
AFM imaging in dynamic AC-mode in liquid environment

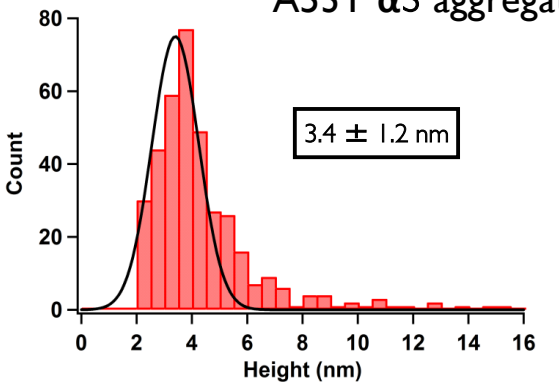
In-vitro α-syn aggregation mediated by Fe(II)

35 μM monomeric αS + 2 mM $FeCl_2$ (1 h at 37 ° C, under

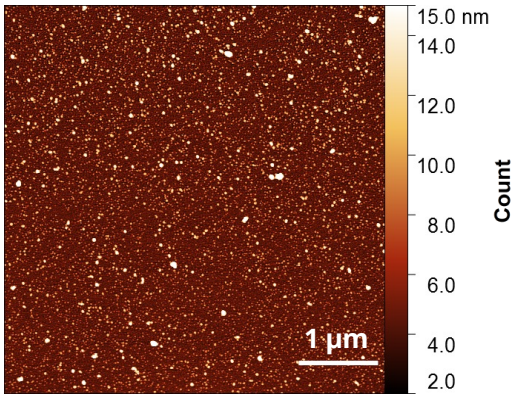
➢AFM morphological analysis

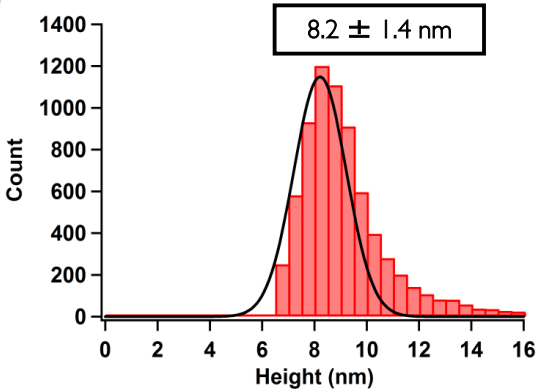
wild-type **α**S + Fe(II)





A53T **α**S + Fe(II)

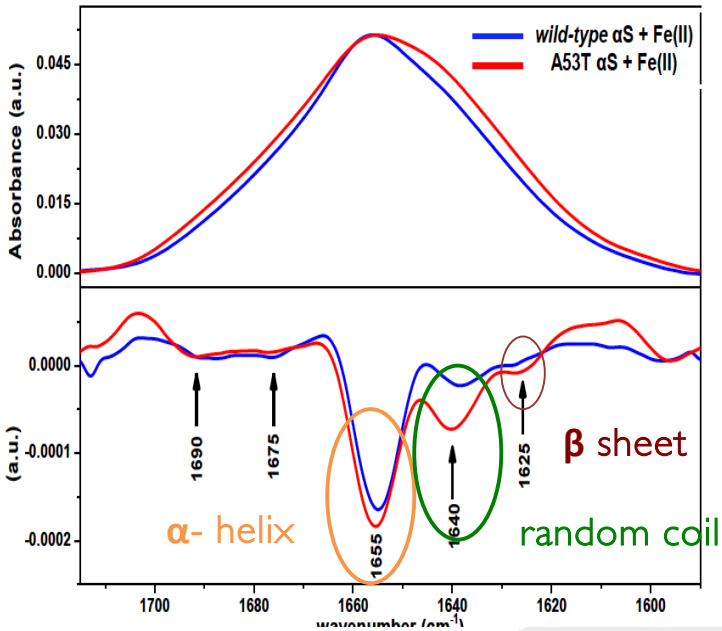




AFM imaging in dynamic AC-mode in air.

➢FTIR-ATR Analysis

A53T **α**S aggregates show higher structural disorder then WT **α**S



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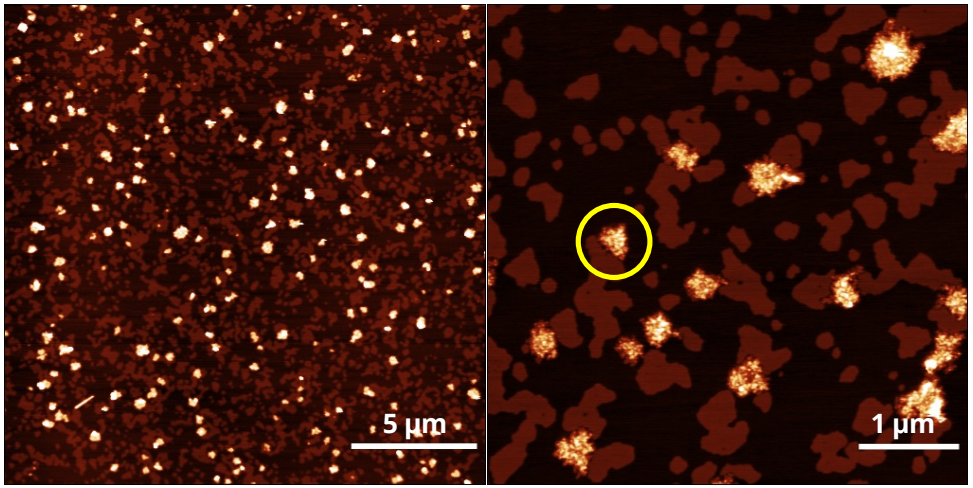
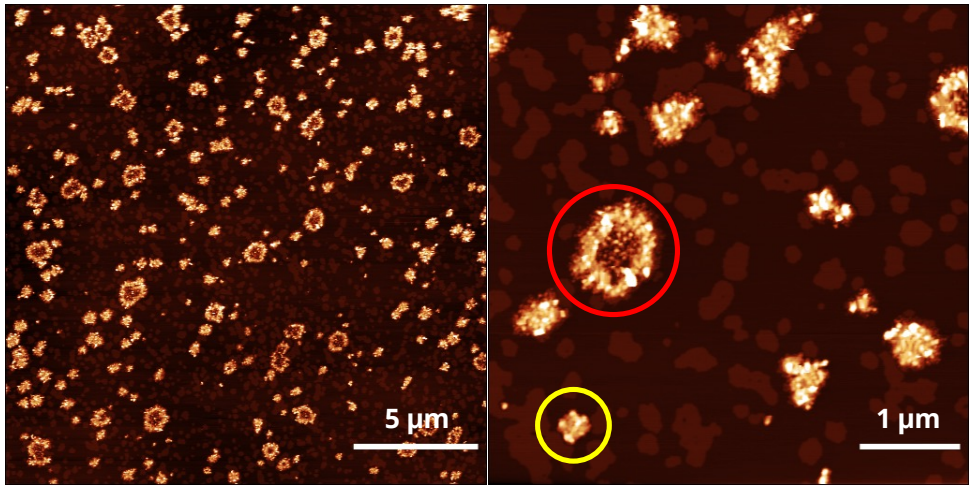
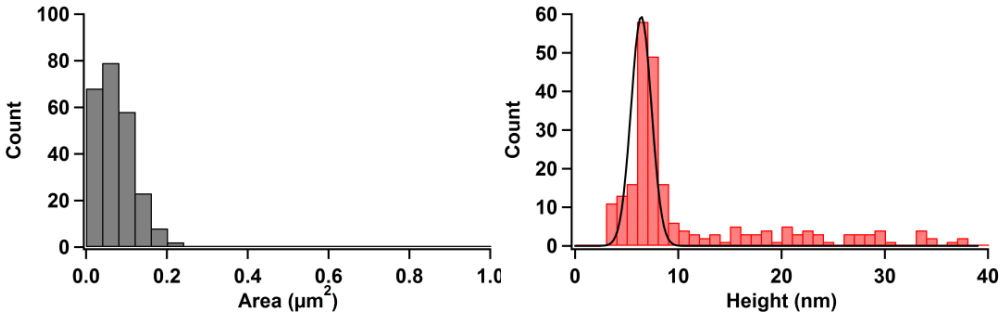
Fe(II) α-syn oligomers: membrane binding interaction

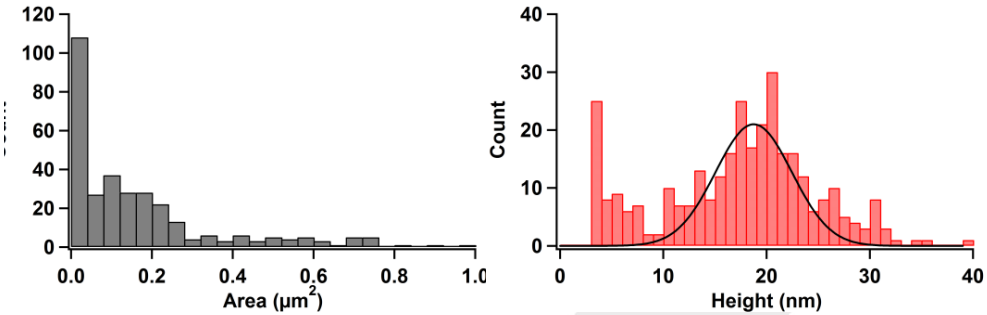
wild-type **αS**
Fe(II)-oligomers

A53T **αS**
Fe(II)-oligomers







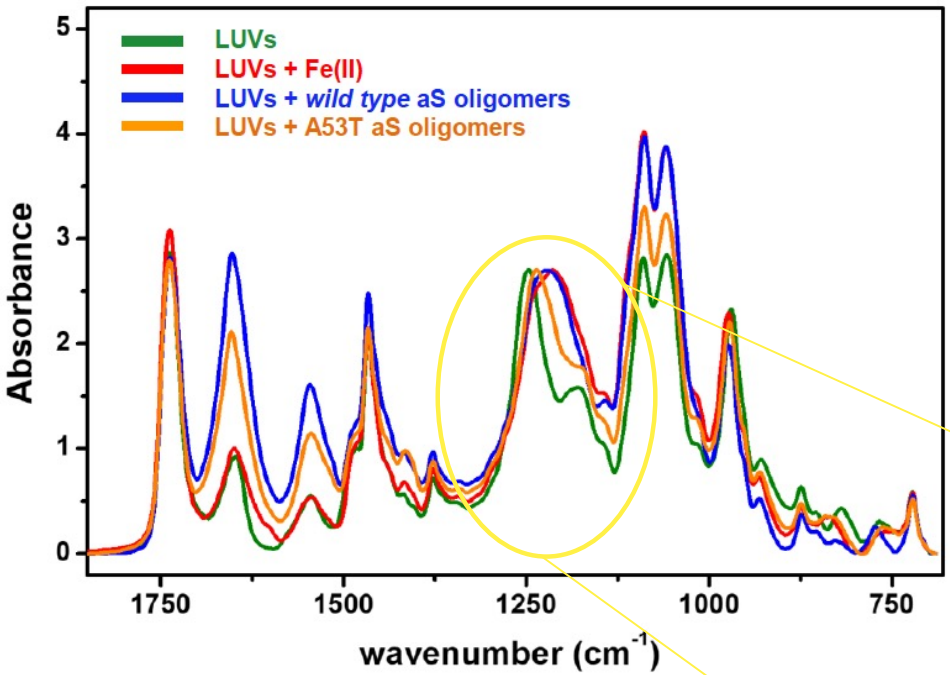


6.4 ± 1.4 nm from the raft domains

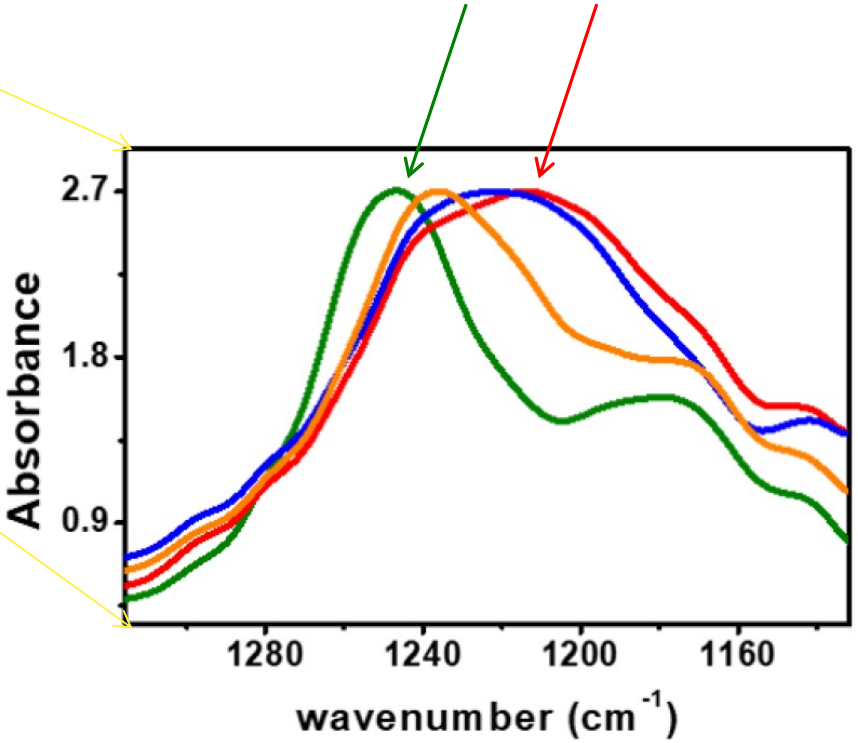
Aggregates of bigger size

AFM imaging in dynamic AC-mode in liquid environment

Fe(II) α-syn oligomers: membrane binding interaction



The main spectral difference was observed in the region 1330–1150 cm^{-1}, dominated by the absorption peak of the $(-PO_2)^-$ asymmetric stretching mode



When **Fe (II)** is added to the **LUV**, a pronounced shift is observed, to indicate the formation of complexes between iron ions and the phosphates headgroups of the lipids.

Fe(II) - WT αS oligomers on LUV behave as Fe(II) LUV, indicating **low binding affinity of Fe(II) with WT αS** .

Fe(II)-A53T αS oligomers on LUV behave as LUV alone (although shifted) suggesting **a stronger interaction of Fe(II) with A53T αS**

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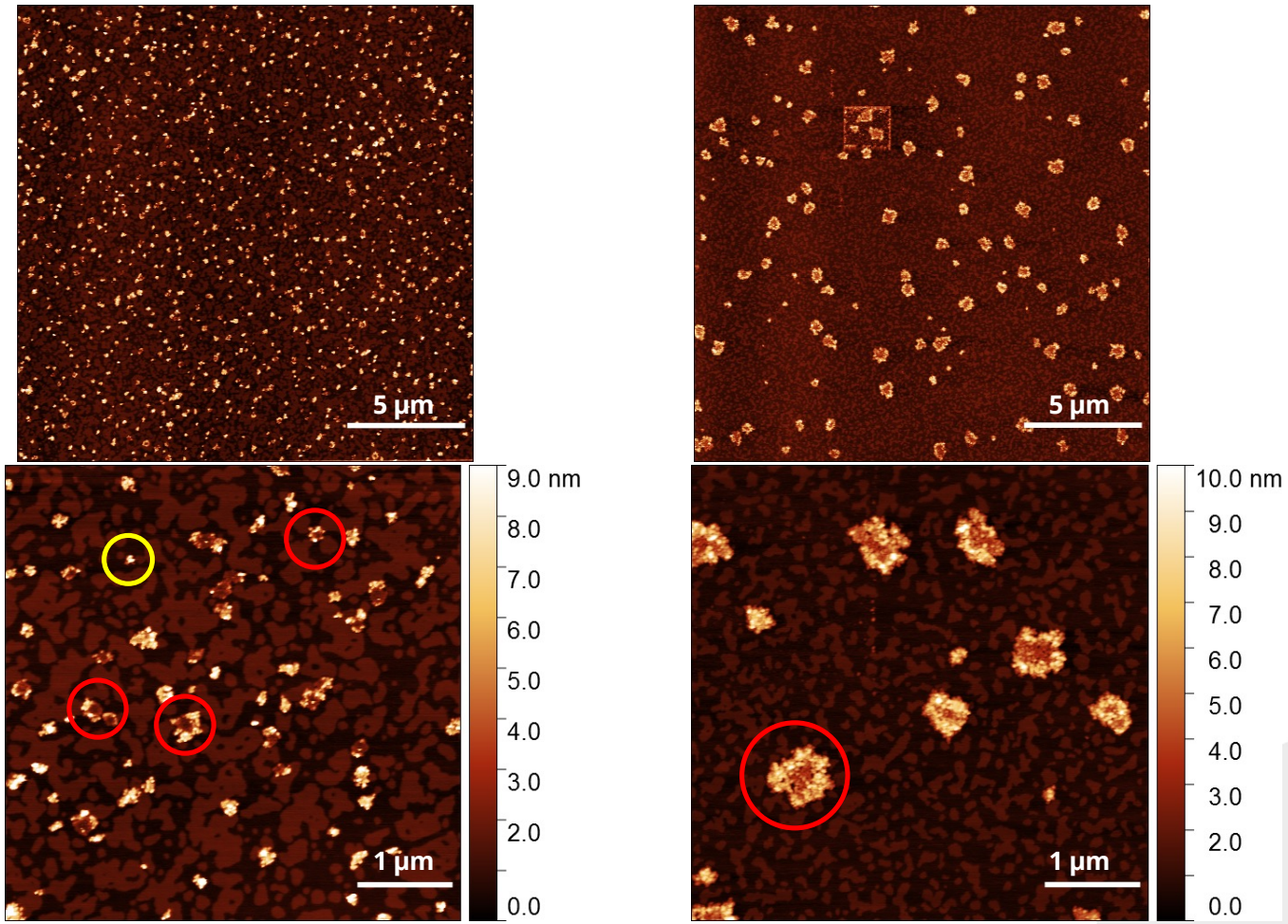
Wild-type and A53T α-syn co-incubation

wild-type **αS** + A53T **αS** (1:1)

- Co-incubation
- Co-aggregation



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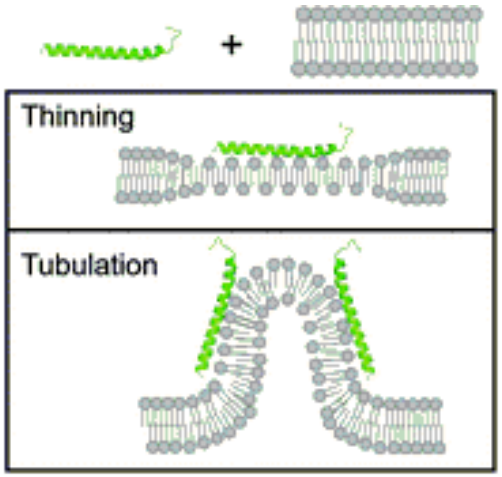
Conclusions

➢ Monomeric alpha synuclein induces membrane thinning with different raft membrane-binding capability. (A53T αS affects correct assembly of synaptic vesicles?)



➢ Strong correlation between iron accumulation and αS aggregation. Iron leads to early-stage alpha synuclein oligomers formation. Higher structural disorder might confer to A53T αS a more aggressive behavior

➢ Mutated A53T displays a stronger propensity to iron-induced aggregation than wild-type αS

➢ Oligomers of both proteins accumulate on raft domains of SLBs (impairment of molecular pathways involving lipid rafts?)