Lipid Induced Aggregation of Alpha-Synuclein

Daniela Leon-Alvarez, Jemil Ahmed, Sunil Kumar
Department of Biochemistry, University of Denver

Introduction

- Alpha synuclein (αS) is an intrinsically disordered protein that is expressed in dopaminergic neurons in the brain.
- The aggregation of αS is associated with neurodegenerative disorders like Lewy Body Dementia and Parkinson's Disease, the second most common neurodegenerative disorder.
- In the cell, αS interacts with lipids to carry out its function, but in the disease state the interaction with lipids is compromised.
- In invitro conditions, lipids can induce αS aggregation. Our lab is interested in developing small molecule ligands against lipid induced aggregation.

Physiological Role of αS

αS chaperones SNARE assembly. The protein binds to a biological lipid vesicles and
SNARE, to help maintain neurotransmitter release.

Pathophysiological Role of αS

Due to genetic mutations or post translational modifications, αS goes through structural changes that adapt into B-sheet rich fibrils that are toxic to the cell. This causes aggregation on the membrane and no longer regulates the release of dopamine.

States of αS

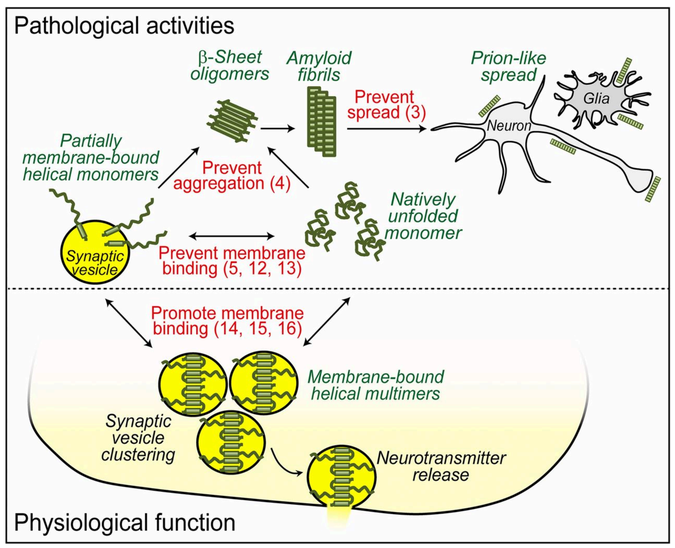


Figure 1. Physiological and Pathological States of αS

Methods & Results

- We induced αS aggregation in the presence of lipids or detergent to create a high-throughput screening assay. Aggregation was followed over time with Thioflavin T dye (ThT), a molecule which fluorescence increases upon intercalating fibrils. In the presence of lipid or detergent, αS aggregated significantly fast as compared to uninduced αS in 20mM phosphate with or without 150mM NaCl. The aggregation reached plateau in less than an hour.

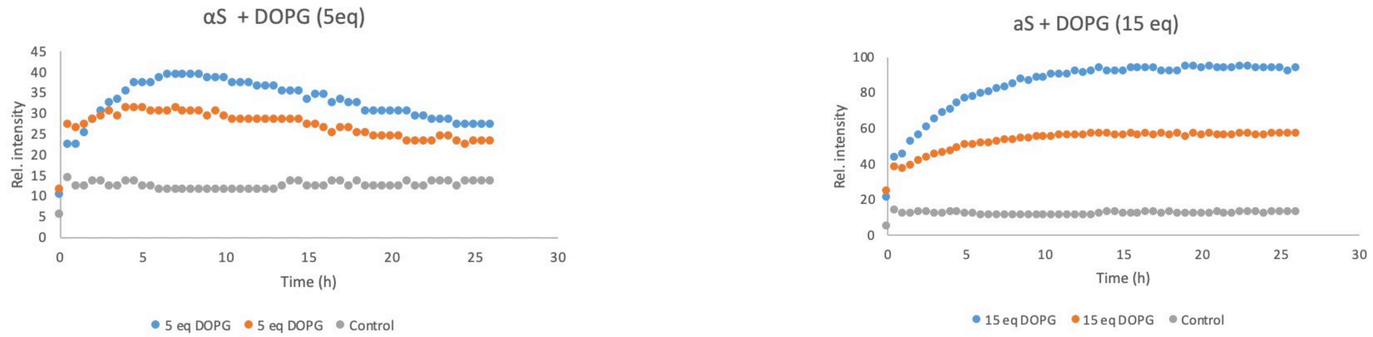


Figure 2. ThT based aggregation assay was carried out in duplicates at 50uM αS. Aggregation was induced using 5 eq. and 15 eq. DOPG, an anionic lipid. At both DOPG concentrations, aggregation starts in less than 2 hrs.

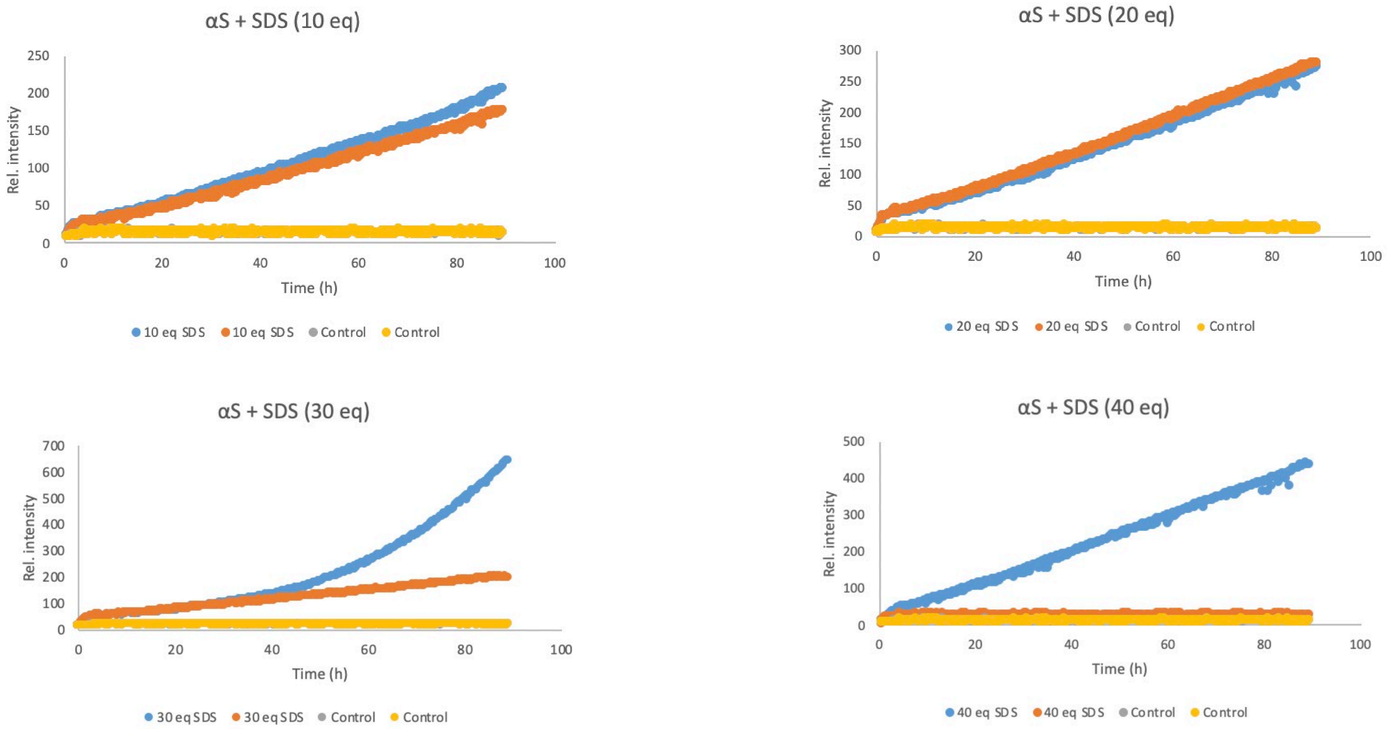


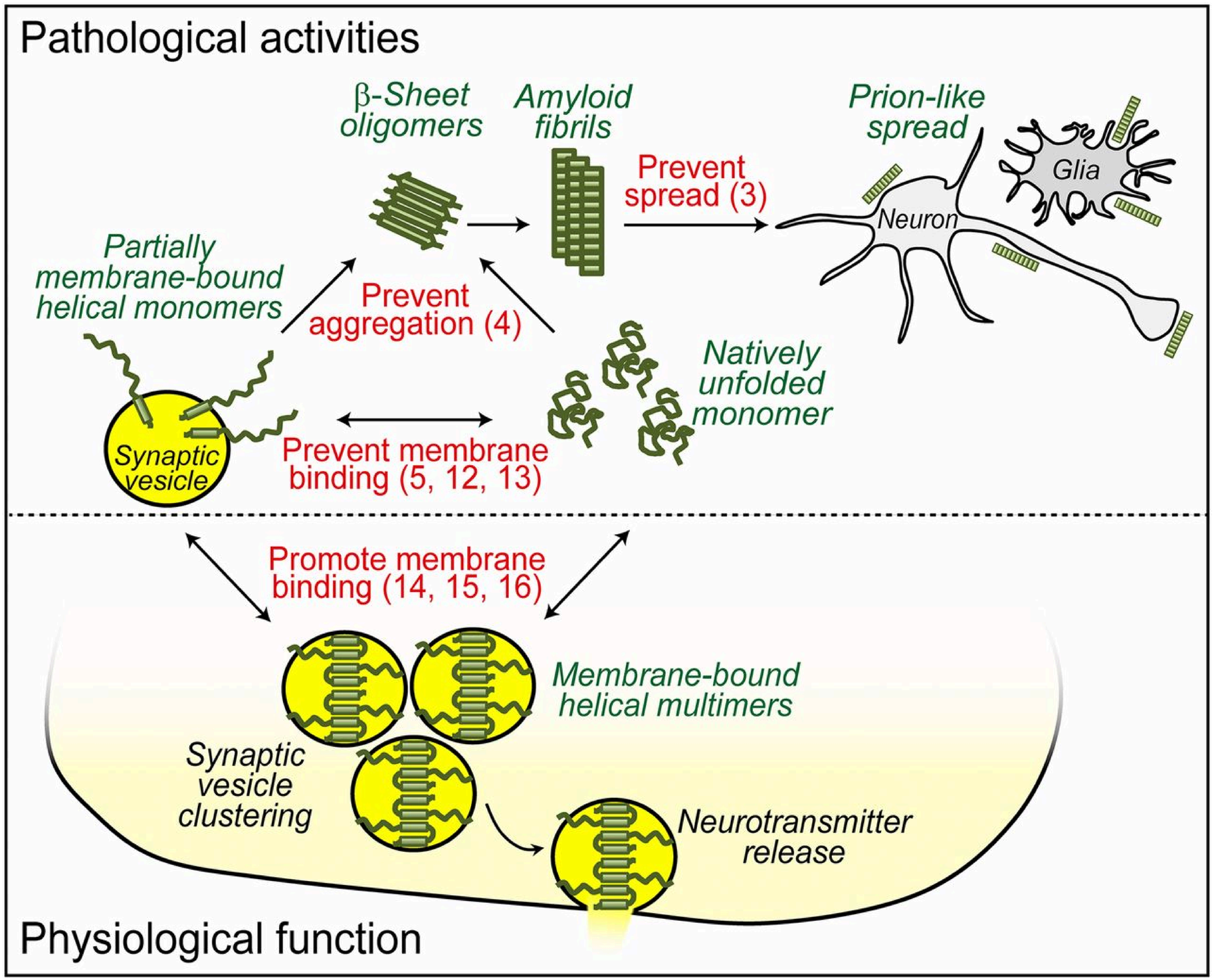
Figure 3. ThT based aggregation assay was carried out in duplicates at 20uM αS. Aggregation was observed using 10, 20, 30, and 40 eq. of SDS detergent. At all the concentrations, aggregation started in less than an hour.

Conclusion & Future Directions

- The tested lipids and detergent induced αS aggregation.
- Optimize lipid induced αS aggregation.
- Screen small molecule ligands against lipid induced aggregation.
- Perform leakage assays of dye containing lipid vesicles in the presence of αS.

[1]: Pineda et al. *PNAS*, 2017

Acknowledgements: Research made possible thanks to Colorado Wyoming Alliance for Minority Participation (CO-WY AMP).


Pathological activities
β-Sheet oligomers
Amyloid fibrils
Prion-like spread
Prevent spread (3)
Glia
Neuron
Partially membrane-bound helical monomers
Prevent aggregation (4)
Natively unfolded monomer
Synaptic vesicle
Prevent membrane binding (5, 12, 13)
Promote membrane binding (14, 15, 16)
Membrane-bound helical multimers
Synaptic vesicle clustering
Neurotransmitter release
Physiological function

Lipid Induced Aggregation of Alpha-Synuclein

Daniela Leon-Alvarez, Jemil Ahmed, Sunil Kumar
Department of Biochemistry, University of Denver

Introduction

- Alpha synuclein (αS) is an intrinsically disordered protein that is expressed in dopaminergic neurons in the brain.
- The aggregation of αS is associated with neurodegenerative disorders like Lewy Body Dementia and Parkinson's Disease, the second most common neurodegenerative disorder.
- In the cell, αS interacts with lipids to carry out its function, but in the disease state the interaction with lipids is compromised.
- In invitro conditions, lipids can induce αS aggregation. Our lab is interested in developing small molecule ligands against lipid induced aggregation.

Physiological Role of αS

αS chaperones SNARE assembly. The protein binds to a biological lipid vesicles and
SNARE, to help maintain neurotransmitter release.

Pathophysiological Role of αS

Due to genetic mutations or post translational modifications, αS goes through structural changes that adapt into B-sheet rich fibrils that are toxic to the cell. This causes aggregation on the membrane and no longer regulates the release of dopamine.

States of αS

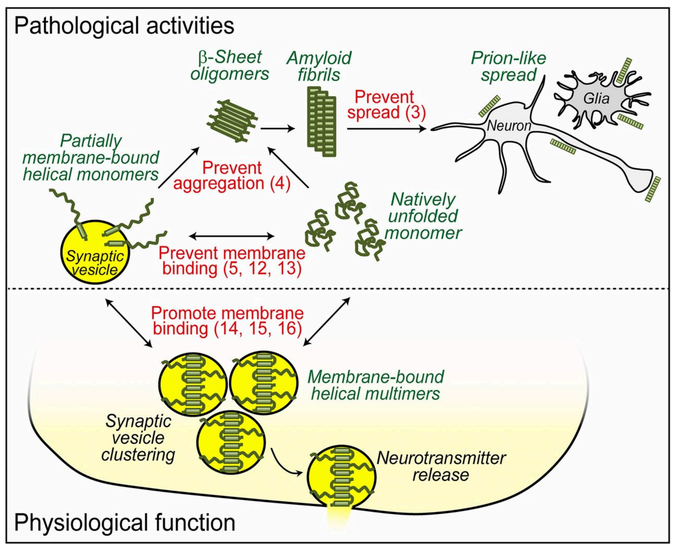


Figure 1. Physiological and Pathological States of αS

Methods & Results

- We induced αS aggregation in the presence of lipids or detergent to create a high-throughput screening assay. Aggregation was followed over time with Thioflavin T dye (ThT), a molecule which fluorescence increases upon intercalating fibrils. In the presence of lipid or detergent, αS aggregated significantly fast as compared to uninduced αS in 20mM phosphate with or without 150mM NaCl. The aggregation reached plateau in less than an hour.

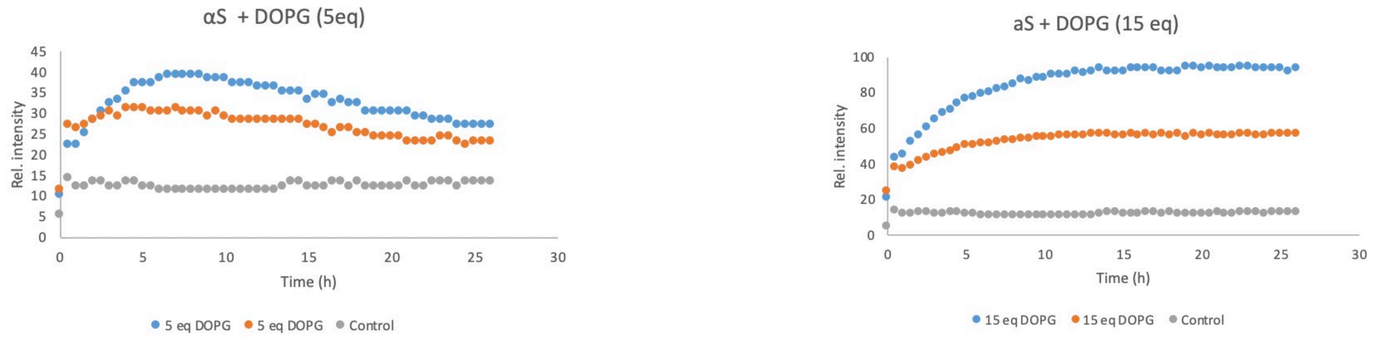


Figure 2. ThT based aggregation assay was carried out in duplicates at 50uM αS. Aggregation was induced using 5 eq. and 15 eq. DOPG, an anionic lipid. At both DOPG concentrations, aggregation starts in less than 2 hrs.

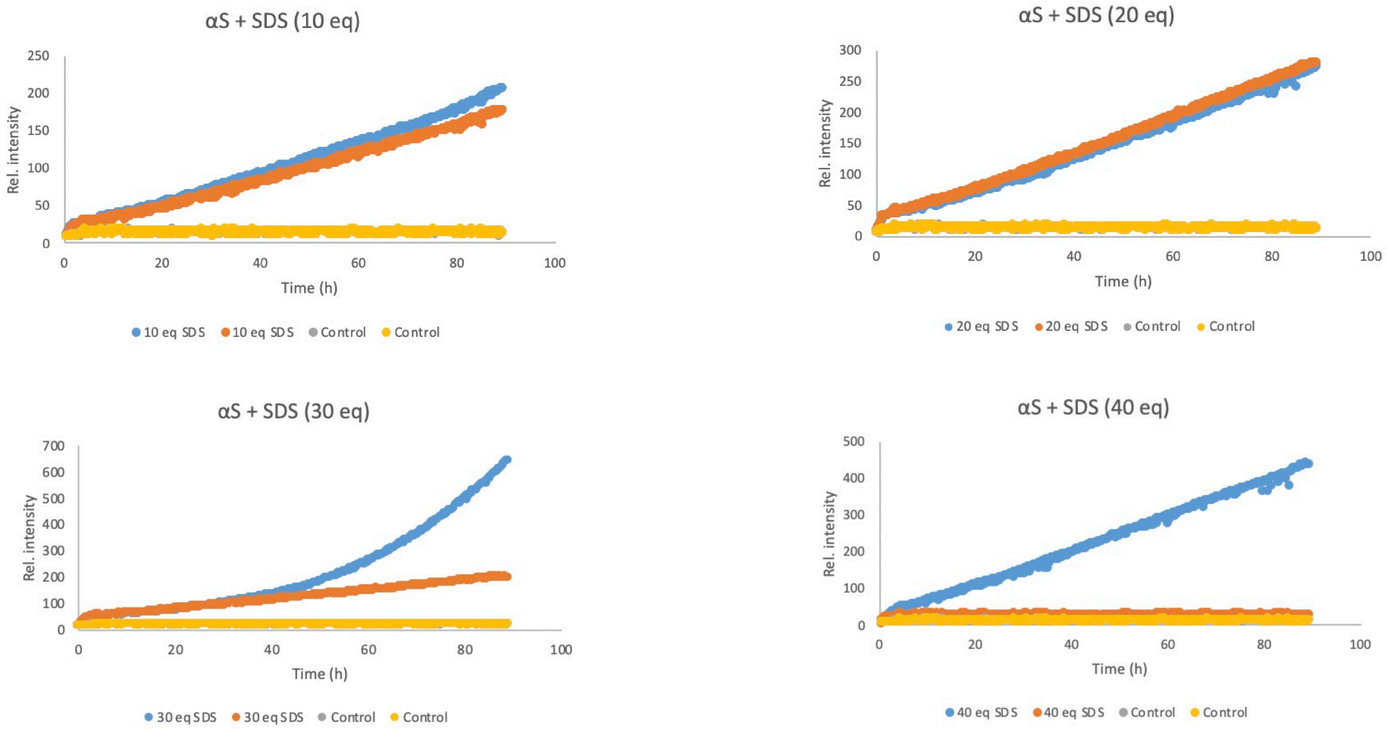


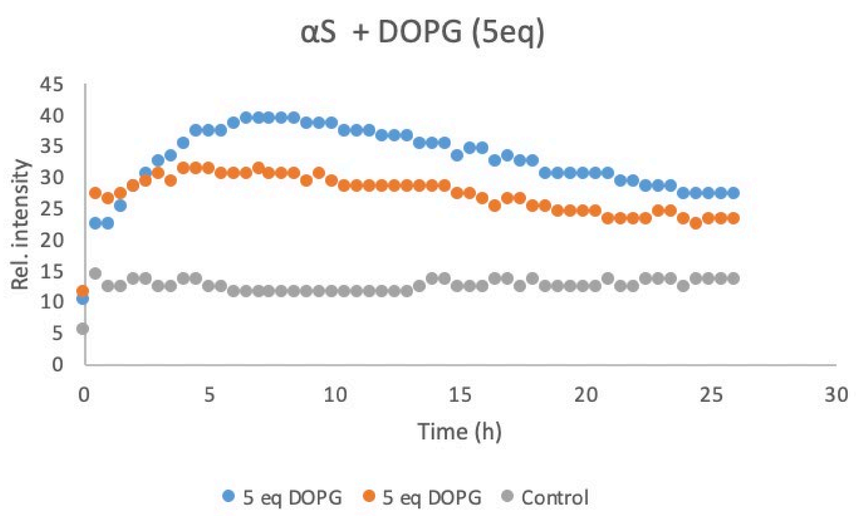
Figure 3. ThT based aggregation assay was carried out in duplicates at 20uM αS. Aggregation was observed using 10, 20, 30, and 40 eq. of SDS detergent. At all the concentrations, aggregation started in less than an hour.

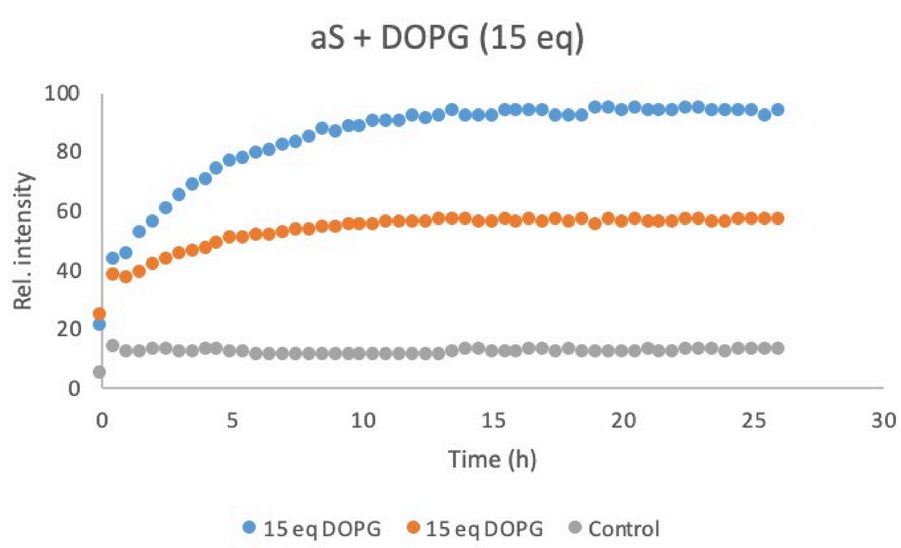
Conclusion & Future Directions

- The tested lipids and detergent induced αS aggregation.
- Optimize lipid induced αS aggregation.
- Screen small molecule ligands against lipid induced aggregation.
- Perform leakage assays of dye containing lipid vesicles in the presence of αS.

[1]: Pineda et al. *PNAS*, 2017

Acknowledgements: Research made possible thanks to Colorado Wyoming Alliance for Minority Participation (CO-WY AMP).


αS + DOPG (5eq)
Rel. intensity
0
5
10
15
20
25
30
35
40
45
0
5
10
15
20
25
30
Time (h)
5 eq DOPG
5 eq DOPG
Control


aS + DOPG (15 eq)
Rel. intensity
0
20
40
60
80
100
0
5
10
15
20
25
30
Time (h)
15 eq DOPG
15 eq DOPG
Control

Lipid Induced Aggregation of Alpha-Synuclein

Daniela Leon-Alvarez, Jemil Ahmed, Sunil Kumar
Department of Biochemistry, University of Denver

Introduction

- Alpha synuclein (αS) is an intrinsically disordered protein that is expressed in dopaminergic neurons in the brain.
- The aggregation of αS is associated with neurodegenerative disorders like Lewy Body Dementia and Parkinson's Disease, the second most common neurodegenerative disorder.
- In the cell, αS interacts with lipids to carry out its function, but in the disease state the interaction with lipids is compromised.
- In invitro conditions, lipids can induce αS aggregation. Our lab is interested in developing small molecule ligands against lipid induced aggregation.

Physiological Role of αS

αS chaperones SNARE assembly. The protein binds to a biological lipid vesicles and
SNARE, to help maintain neurotransmitter release.

Pathophysiological Role of αS

Due to genetic mutations or post translational modifications, αS goes through structural changes that adapt into B-sheet rich fibrils that are toxic to the cell. This causes aggregation on the membrane and no longer regulates the release of dopamine.

States of αS

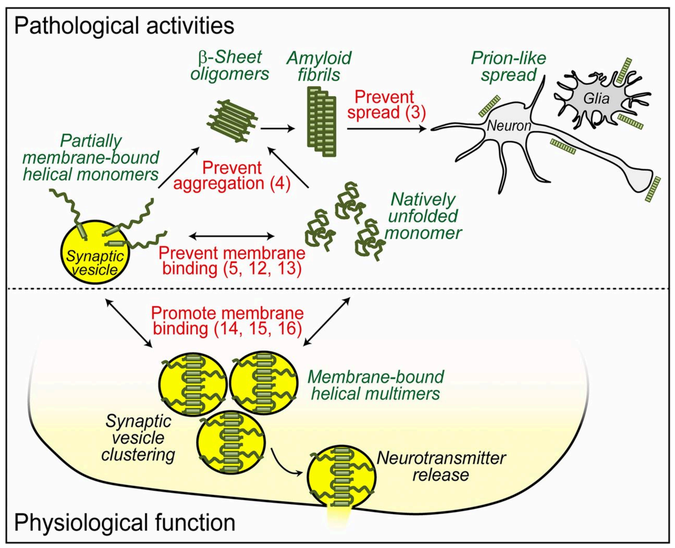


Figure 1. Physiological and Pathological States of αS

Methods & Results

- We induced αS aggregation in the presence of lipids or detergent to create a high-throughput screening assay. Aggregation was followed over time with Thioflavin T dye (ThT), a molecule which fluorescence increases upon intercalating fibrils. In the presence of lipid or detergent, αS aggregated significantly fast as compared to uninduced αS in 20mM phosphate with or without 150mM NaCl. The aggregation reached plateau in less than an hour.

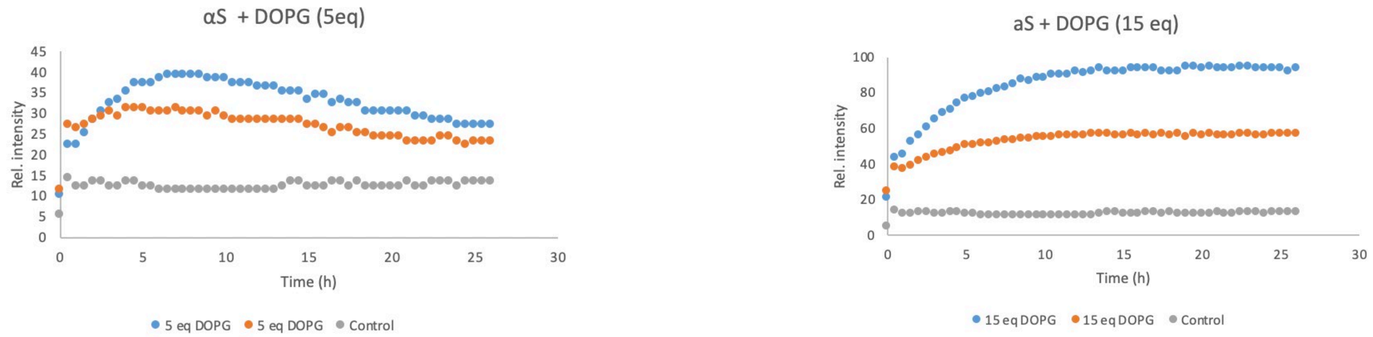


Figure 2. ThT based aggregation assay was carried out in duplicates at 50uM αS. Aggregation was induced using 5 eq. and 15 eq. DOPG, an anionic lipid. At both DOPG concentrations, aggregation starts in less than 2 hrs.

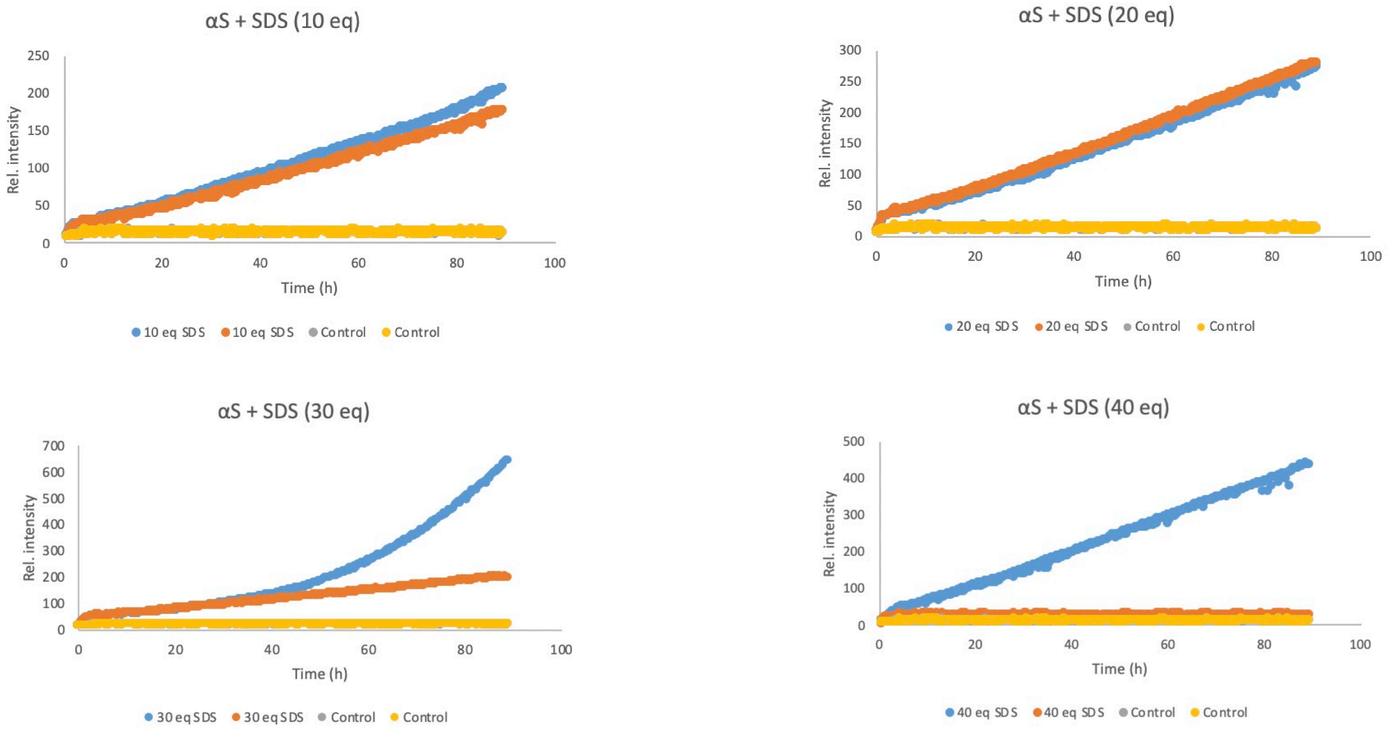


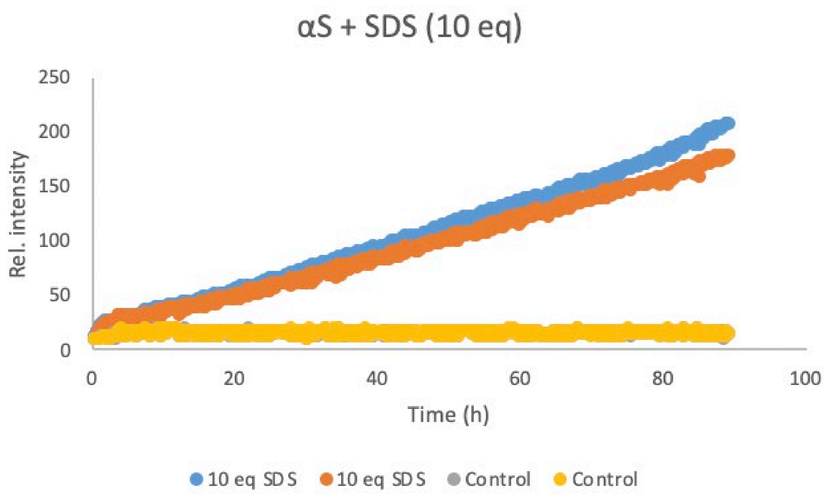
Figure 3. ThT based aggregation assay was carried out in duplicates at 20uM αS. Aggregation was observed using 10, 20, 30, and 40 eq. of SDS detergent. At all the concentrations, aggregation started in less than an hour.

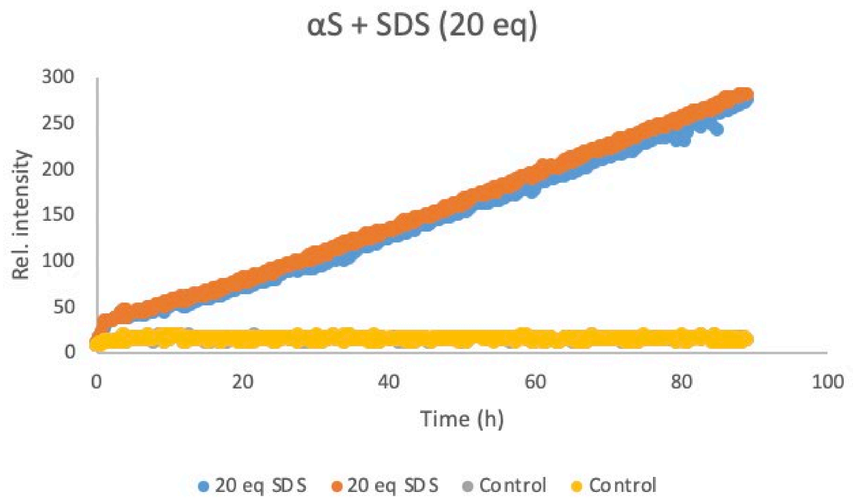
Conclusion & Future Directions

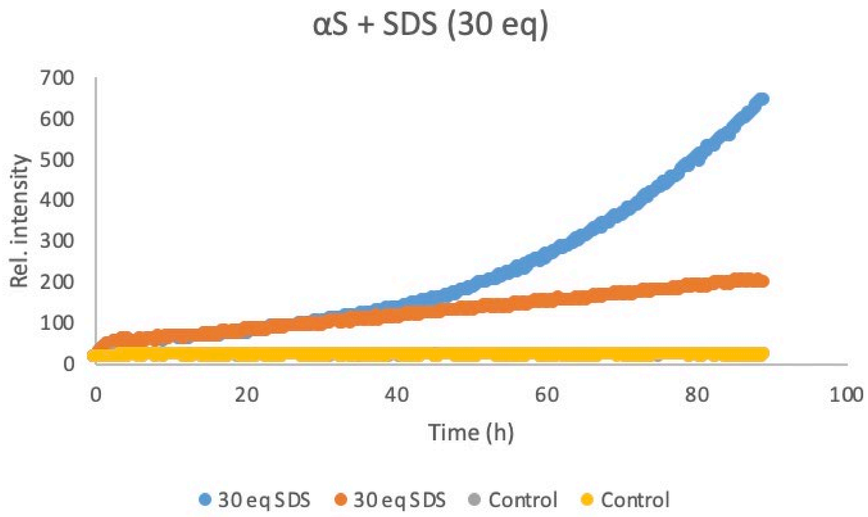
- The tested lipids and detergent induced αS aggregation.
- Optimize lipid induced αS aggregation.
- Screen small molecule ligands against lipid induced aggregation.
- Perform leakage assays of dye containing lipid vesicles in the presence of αS.

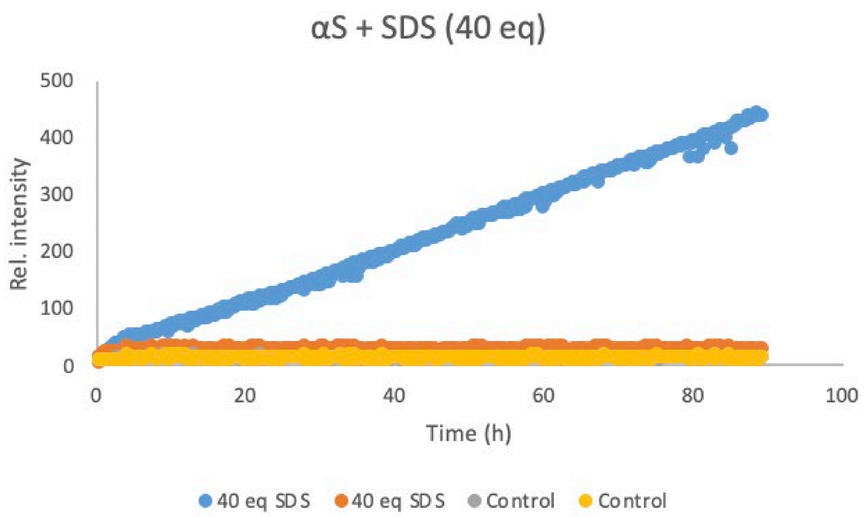
[1]: Pineda et al. *PNAS*, 2017

Acknowledgements: Research made possible thanks to Colorado Wyoming Alliance for Minority Participation (CO-WY AMP).


αS + SDS (10 eq)
Rel. intensity
250
200
150
100
50
0
0
20
40
60
80
100
Time (h)
10 eq SDS
10 eq SDS
Control
Control


αS + SDS (20 eq)
Rel. intensity
300
250
200
150
100
50
0
0
20
40
60
80
100
Time (h)
20 eq SDS
20 eq SDS
Control
Control


αS + SDS (30 eq)
Rel. intensity
700
600
500
400
300
200
100
0
0
20
40
60
80
100
Time (h)
30 eq SDS
30 eq SDS
Control
Control


αS + SDS (40 eq)
Rel. intensity
500
400
300
200
100
0
0
20
40
60
80
100
Time (h)
40 eq SDS
40 eq SDS
Control
Control

Lipid Induced Aggregation of Alpha-Synuclein

Daniela Leon-Alvarez, Jemil Ahmed, Sunil Kumar
Department of Biochemistry, University of Denver

Introduction

- Alpha synuclein (αS) is an intrinsically disordered protein that is expressed in dopaminergic neurons in the brain.
- The aggregation of αS is associated with neurodegenerative disorders like Lewy Body Dementia and Parkinson's Disease, the second most common neurodegenerative disorder.
- In the cell, αS interacts with lipids to carry out its function, but in the disease state the interaction with lipids is compromised.
- In invitro conditions, lipids can induce αS aggregation. Our lab is interested in developing small molecule ligands against lipid induced aggregation.

Physiological Role of αS

αS chaperones SNARE assembly. The protein binds to a biological lipid vesicles and
SNARE, to help maintain neurotransmitter release.

Pathophysiological Role of αS

Due to genetic mutations or post translational modifications, αS goes through structural changes that adapt into B-sheet rich fibrils that are toxic to the cell. This causes aggregation on the membrane and no longer regulates the release of dopamine.

States of αS

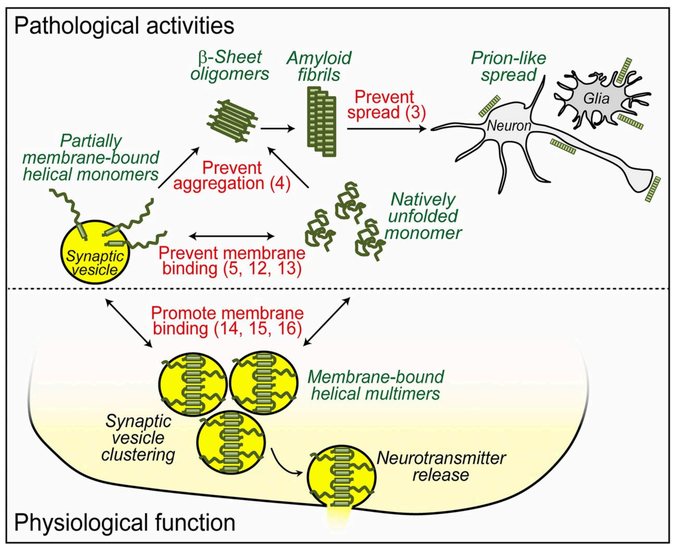


Figure 1. Physiological and Pathological States of αS

Methods & Results

- We induced αS aggregation in the presence of lipids or detergent to create a high-throughput screening assay. Aggregation was followed over time with Thioflavin T dye (ThT), a molecule which fluorescence increases upon intercalating fibrils. In the presence of lipid or detergent, αS aggregated significantly fast as compared to uninduced αS in 20mM phosphate with or without 150mM NaCl. The aggregation reached plateau in less than an hour.

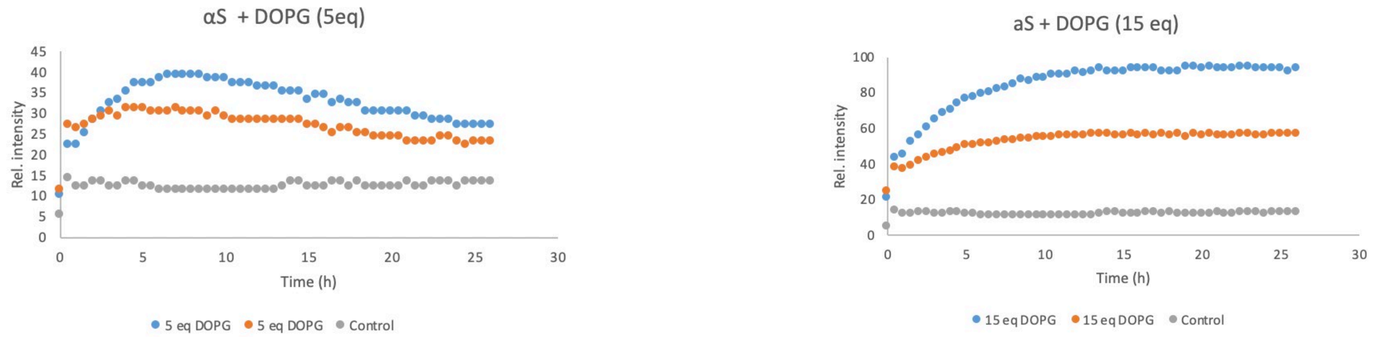


Figure 2. ThT based aggregation assay was carried out in duplicates at 50uM αS. Aggregation was induced using 5 eq. and 15 eq. DOPG, an anionic lipid. At both DOPG concentrations, aggregation starts in less than 2 hrs.

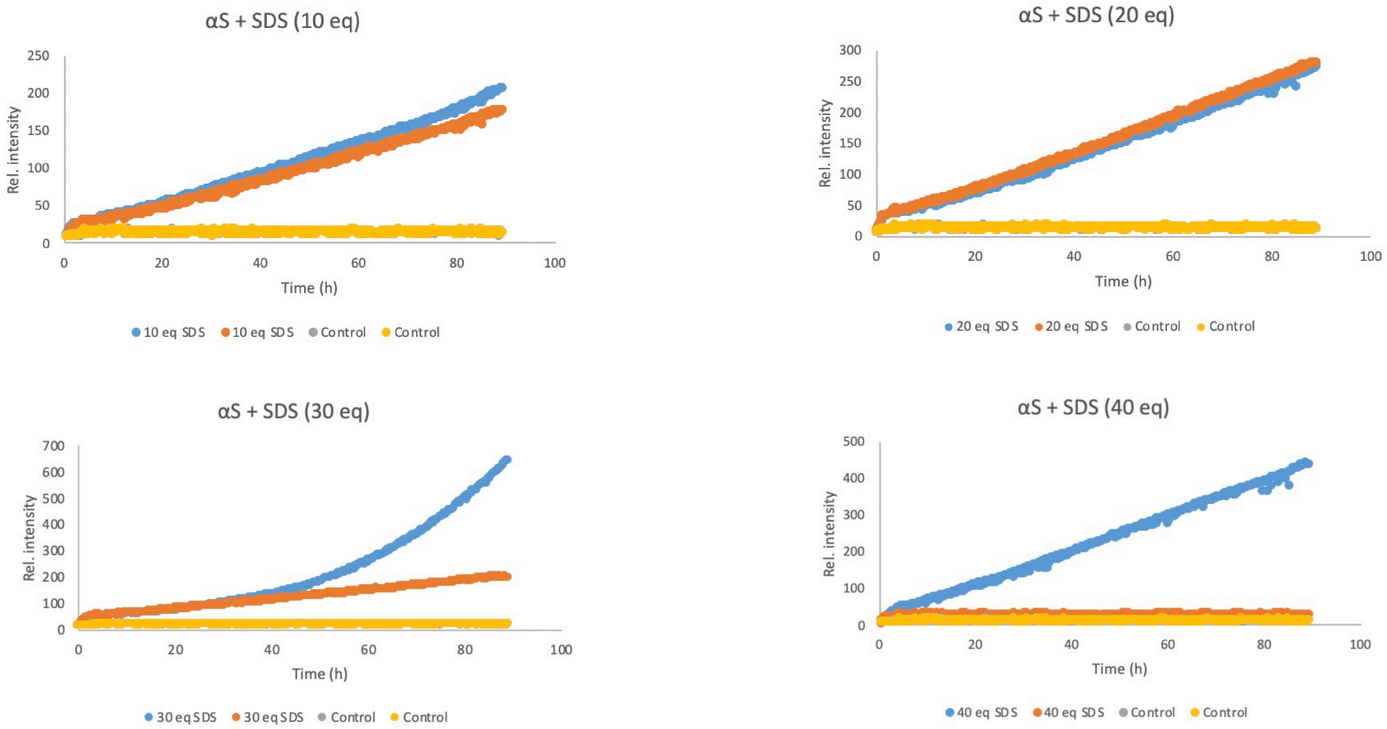


Figure 3. ThT based aggregation assay was carried out in duplicates at 20uM αS. Aggregation was observed using 10, 20, 30, and 40 eq. of SDS detergent. At all the concentrations, aggregation started in less than an hour.

Conclusion & Future Directions

- The tested lipids and detergent induced αS aggregation.
- Optimize lipid induced αS aggregation.
- Screen small molecule ligands against lipid induced aggregation.
- Perform leakage assays of dye containing lipid vesicles in the presence of αS.

[1]: Pineda et al. *PNAS*, 2017

Acknowledgements: Research made possible thanks to Colorado Wyoming Alliance for Minority Participation (CO-WY AMP).