Fluorescent labeled alpha-synuclein conformers for intracelular studies

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Introduction

Alpha-synuclein (aS) is the major component of fibrillar aggregates within neurons in various neurodegenerative diseases, known as synucleinopathies.

Cellular uptake of aS implies different unsolved mechanisms, thus is important to generate fluorescent aS species as an approach to study these processes and the interactions of aS *in cellula* . In this study, fluorescent aS species were generated by labeling monomer aS with Alexa^{488} fluorophore (Sigma-Aldrich Mix-n-Stain^{TM} CF^{TM} 488^{TM}).

The use of fluorescent labeled aS will allow further studies on the mechanism that modulates the cellular uptake for the different aggregated species.

Acknowledgements

ANII Agencia Nacional de Investigación e Innovación

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PAYS Programa en Alimentos y Salud Humana

Universidad de la República Uruguay

Results

1. Alpha-synuclein monomer labeling

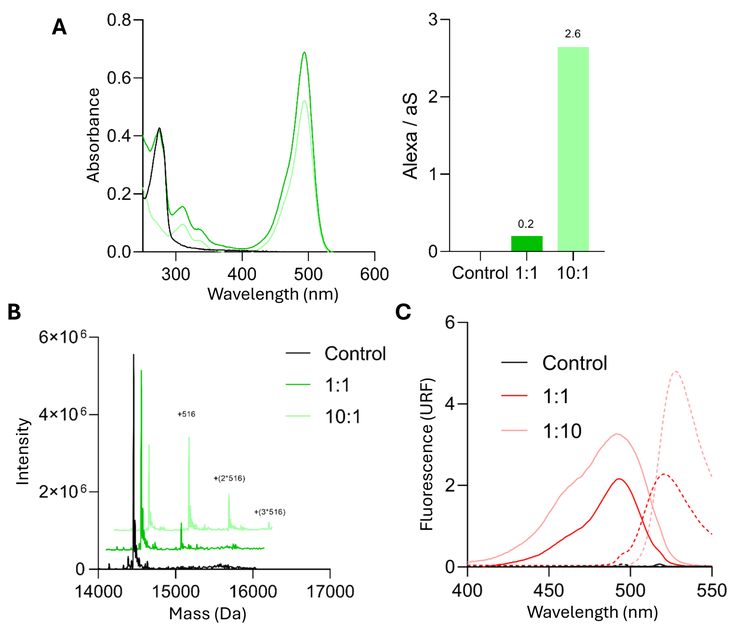

Figure 1. aS monomer labeling with Alexa^{488}.
Ratios of Alexa:aS of 1:1 and 10:1 were tested for optimization of the labeling conditions. **A.** Absorbance spectra and quantification using equation: [aS] = (A_{280} – 0.11A_{494})/5960 and [Alexa^{488}] = A_{494}/71000. **B.** Intact protein mass spectrometry of samples in A. **C.** Fluorescence spectra (λ_{ex} = 482 nm, λ_{em} = 525 nm) of the samples in A.

2. Fluorescent Alpha-synuclein fibril formation

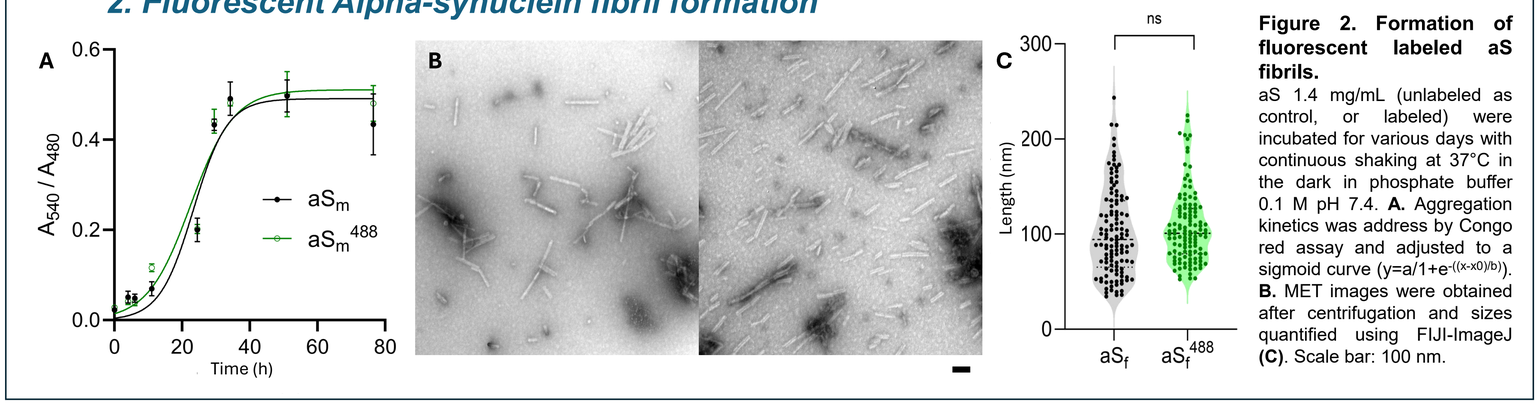

Figure 2. Formation of fluorescent labeled aS fibrils.
aS 1.4 mg/mL (unlabeled as control, or labeled) were incubated for various days with continuous shaking at 37°C in the dark in phosphate buffer 0.1 M pH 7.4. **A.** Aggregation kinetics was address by Congo red assay and adjusted to a sigmoid curve (y=a/1+e$^{-((x-x0)/b)}$). **B.** MET images were obtained after centrifugation and sizes quantified using FIJI-ImageJ **(C)**. Scale bar: 100 nm.

3. Alpha-synuclein fluorescent labeled aggregated species

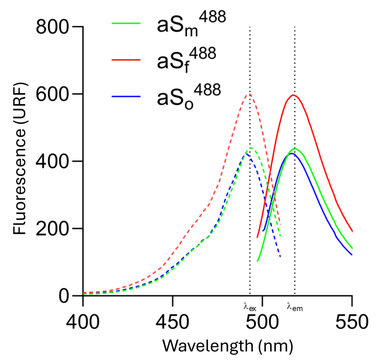

Figure 3. Fluorescent aS species labeled with Alexa^{488}.
Fluorescence properties of the different aS labeled species: aS monomer (aS_m), fibril (aS_f) and oligomer (aS_o). Fluorescence spectra were recorder to 1.4 mg/mL of protein diluted in PBS, in a spectrofluorimeter (λ_{ex} = 482 nm, λ_{em} = 525 nm).

4. Alpha-synuclein interaction with BV-2 cell line

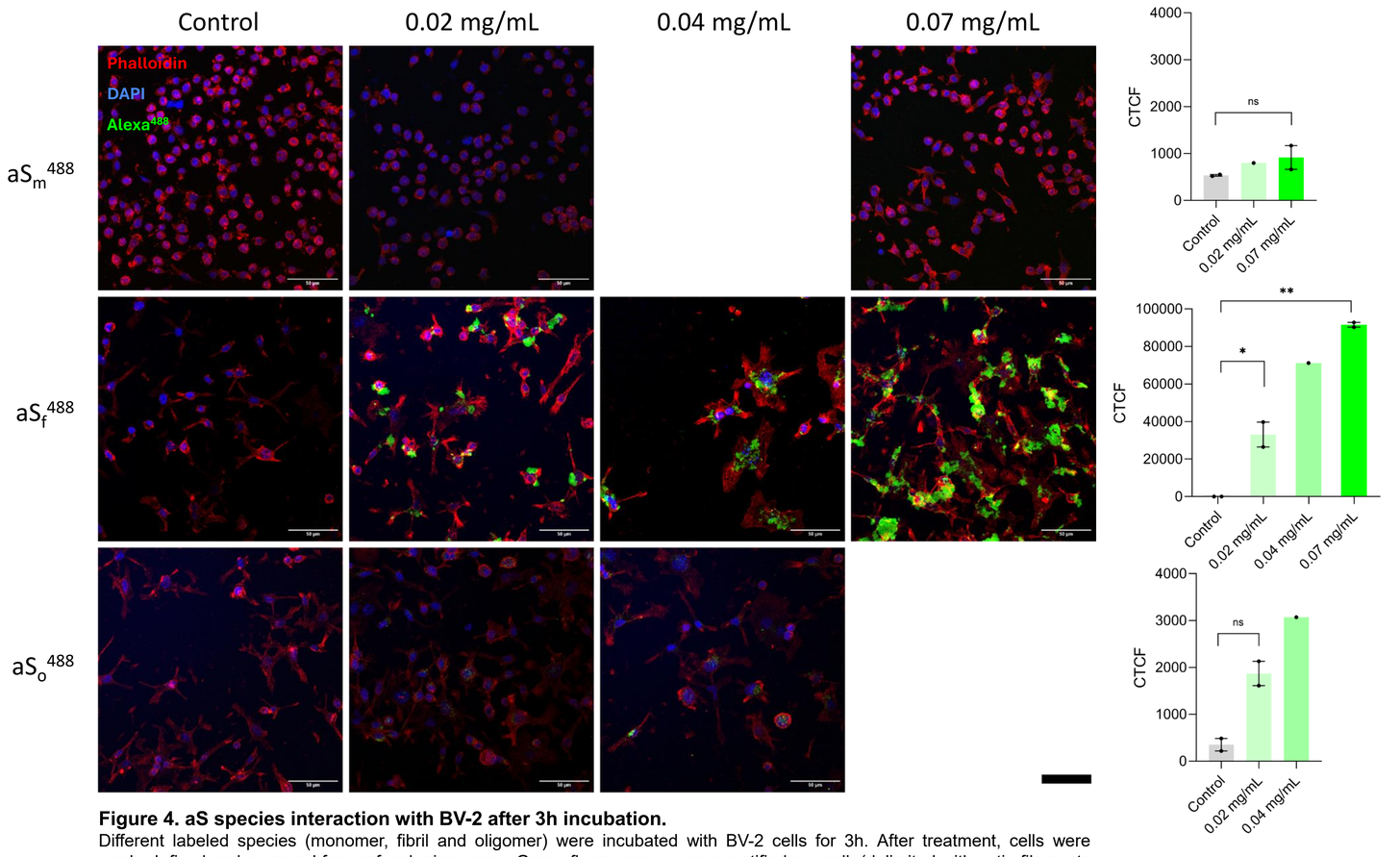

Figure 4. aS species interaction with BV-2 after 3h incubation.
Different labeled species (monomer, fibril and oligomer) were incubated with BV-2 cells for 3h. After treatment, cells were washed, fixed and prepared for confocal microscopy. Green fluorescence was quantified per cell, (delimited with actin filaments in red) as CTCF = integrated density – (cell área * average background intensity). Scale bar: 50 µm. 10 cells per frame were measured. n = 3.

5. Alpha-synuclein intracellular localization

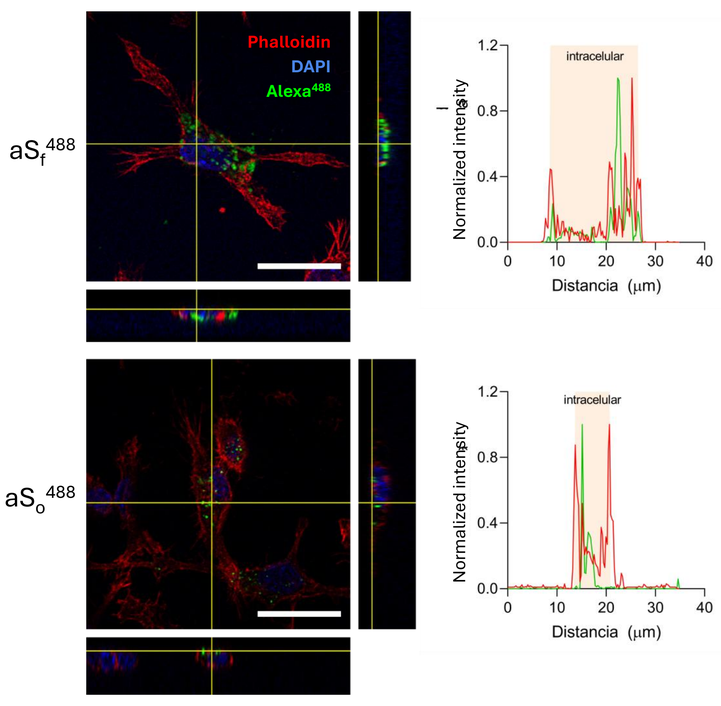

Figure 5. aS fibrils and oligomers uptake in BV-2 cells.
Labeled aS fibrils and oligomers at 0.02 mg/mL were incubated with BV-2 cells for 3h. After incubation, cells were washed, fixed and prepared for confocal microscopy. Z-stacks images were obtained (left) and intensity plots are represented for the red and green channels (right).

6. Alpha-synuclein and BV-2 cells interaction address by flow-cytometry

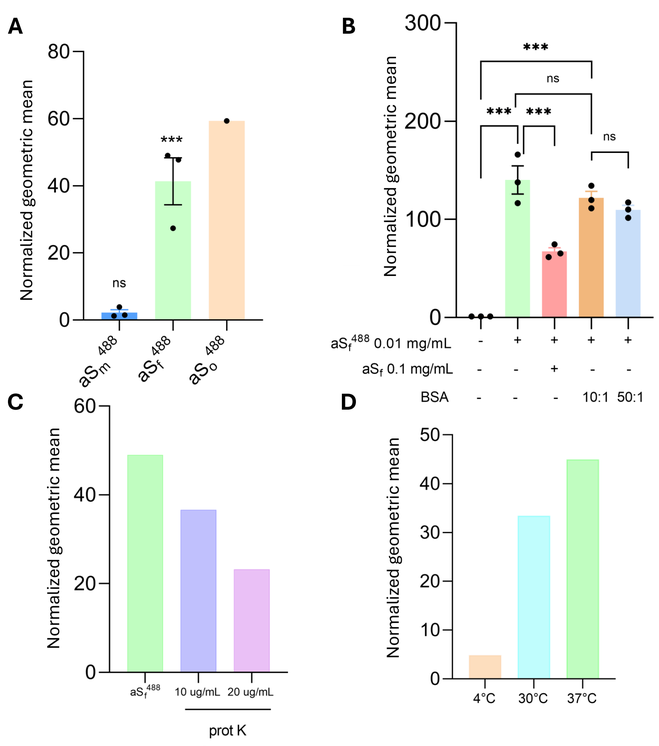

Figure 6. Labeled aS species interact with BV-2 cells.
Labeled aS at 0.01 mg/mL were incubated with BV-2 cells for 3h. After incubation, cells were washed, and prepared for flow citometry. **A.** aS monomer, fibril and oligomer were tested for interaction with BV-2 cells. n = 3 **B.** Ligand binding assay by co-incubating labeled and non labeled aS fibrils. n = 3 **C.** Pre-treatment of BV-2 cells with protease K to digest protein membrane receptors. n = 1 **D.** Temperature dependace of aS fibrils uptake. n = 1.

Conclusions

Alpha synuclein was labeled with Alexa^{488} fluorophore with an 1:1 stechiometry and obtained an average yield of 0.2 molecules of fluorophore per aS monomer.

Fluorescent aS monomer, fibrils and oligomers were generated and their fluorescent properties were similar between them.

An interaction between aS fibrils and oligomers with BV-2 cells was observed, whereas no monomeric species were detected by confocal microscopy or flow cytometry.

Uptake of aS fibrils could be explained by endocytosis iniciated by interaction with a membrane receptor.

This approach is usefull for studying the uptake and spreading of aS, a protein relevant in the progression of neurodegenerative diseases.