https://doi.org/10.17952/37EPS.2024.P1244

POM-peptide hybrid against Alzheimer's Disease

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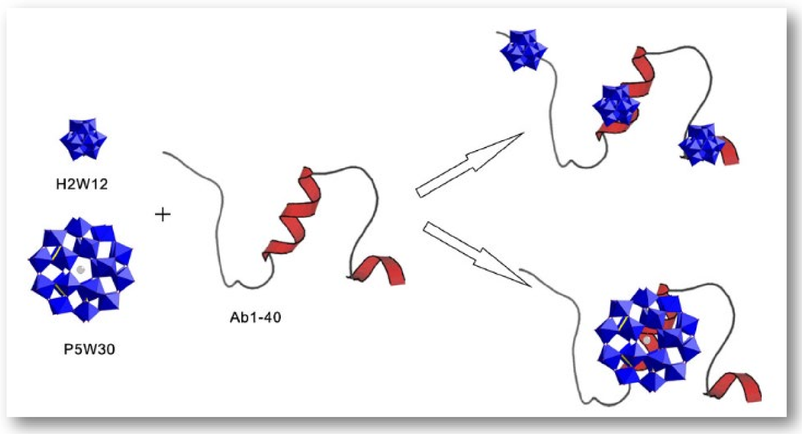
Alzheimer's disease (AD) is the sixth leading cause of disability due to its high incidence and represents a huge health and social problem. AD, which culminates in cognitive decline, involves various pathological events, including accumulation in the brain **of beta-amyloid protein (Aβ) aggregates** responsible for triggering oxidative stress, synaptic degeneration, and neuronal death. Furthermore, increasing evidence posits that AD pathogenesis involves strong interactions with immunological mechanisms in the brain and microglia activation. Despite the current knowledge, the few drugs available can only mitigate symptoms without counteracting the multiple causes of AD. Within this scenario, a novel drug discovery paradigm, involving **multifunctional agents** able to interact with AD-relevant targets and compromised networks simultaneously will enable an effective therapeutic intervention.

POMs

Polyoxometalates (POMs) are nanosized metal oxides that exhibit promising biomedical applications properties. Due to their substantial polyanionic surface area, certain POMs are capable of interacting with the positively charged region of the Aβ peptide, thereby **preventing its aggregation** (1).

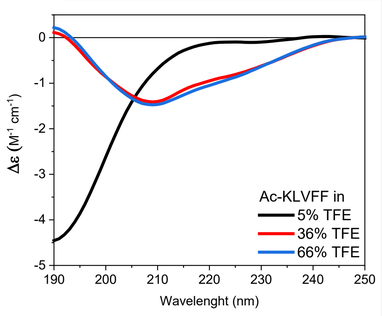
The purpose was to **develop hybrid POM-peptide** nanodrugs able to inhibit the formation of Aβ aggregates from the earliest stages.



To this end, the KLVFF peptide, which has been shown to counteract the aggregation of the Aβ peptide, and its acetylated or fluorophore-labelled analogs were synthesized. The characterization of POM-peptide complexes was conducted through the use of circular dichroism (CD), dynamic light scattering (DLS), and transmission electron microscopy (TEM). Subsequently, these complexes were evaluated in a Drosophila model to assess their capacity to permeate the blood-brain barrier.

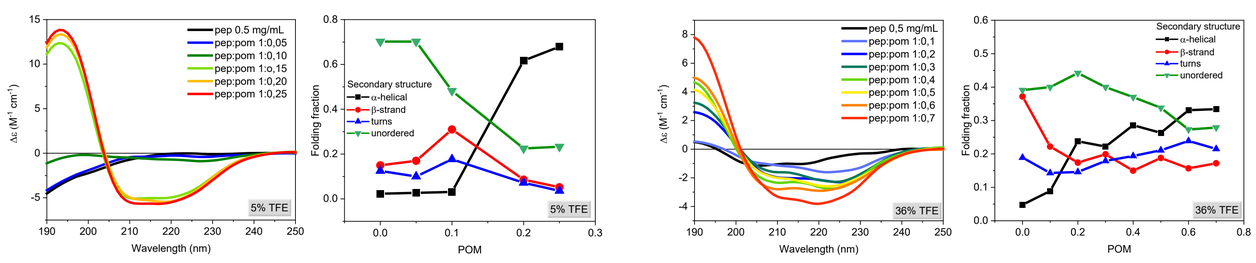
CIRCULAR DICHROISM

CD studies were carried out in the presence of trifluoroethanol (TFE) as a cosolvent due to the poor solubility of peptides in aqueous solutions. TFE facilitates intramolecular hydrogen bonding, thereby promoting folded conformations. As an illustration, the CD spectra of the Ac-KLVFF peptide at varying TFE concentrations are presented.
At a low TFE percentage (5%), the CD signature was indicative of an unordered structure. An increase in the TFE content (36%) resulted in a red shift of the negative band at approximately 210 nm, accompanied by the appearance of a positive band at 190 nm.



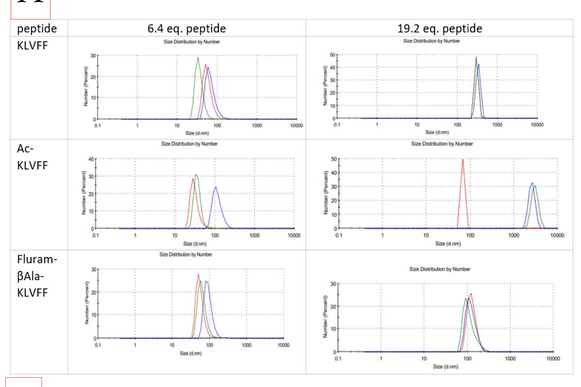
Far-UV CD spectra of the Ac-KLVFF peptide at different percentages of TFE at 25°C , cell 0.01 cm optical path.

Consequently, the conformational effects of POM on the secondary structure of peptides were investigated at two distinct TFE percentages (5 and 36%, respectively). The CD spectra of the Ac-KLVFF peptide in the presence of varying quantities of POM at different TFE percentages indicate that the **polyoxymetalate induces an ordered structure**.



A and C) Far-UV CD spectra of Ac-KLVFF peptide at different percentages of TFE in presence of increasing amount of POM at 25°C, cell 0.1 cm pathlengtht. B and D) Secondary structure estimation.

DYNAMIC LIGHT SCATTERING



*Hydrodynamic radius values of POM-peptide hybrids obtained under different conditions: (**A**) an excess of peptide and (**B**) an excess of POM.*

Hydrodynamic diameter and Zeta potential measurements were used to investigate the interaction of the KLVFF peptide and its derivatives with POM and identify the optimal conditions for forming POM-peptide hybrid systems compatible with administration in Drosophila. Experiments were carried out under two distinct conditions:

A) in the presence of an excess of peptide, keeping the number of moles of POM constant (50 µm);

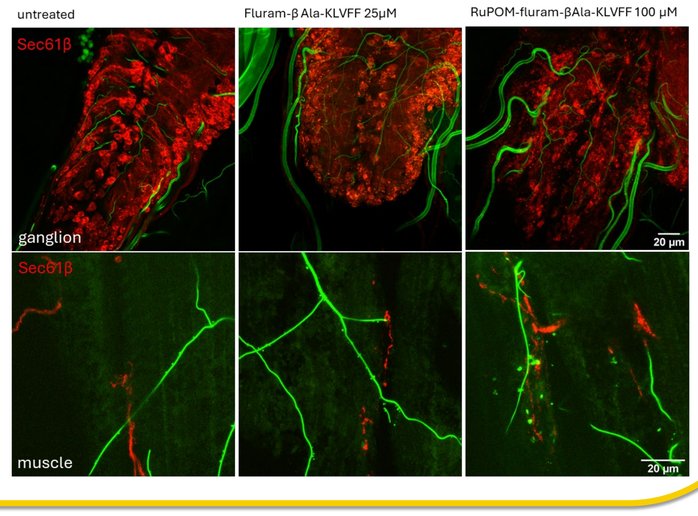
B) in the presence of an excess of POM, keeping the number of moles of peptide constant (60 µm).

The data obtained indicate that Fluram-βAla-KLVFF peptide-POM hybrid systems are **compatible** with the administration in Drosophila.

EX VIVO EXPERIMENTS IN DROSOPHILA

In order to evaluate the capacity of the peptide and POM-peptide systems to cross the blood-brain barrier (BBB) in Drosophila, *ex vivo* experiments were conducted. Third-instar larvae expressing an endoplasmic reticulum membrane marker (td-Tomato-Sec61β, red) in neurons were dissected and incubated for one hour with HL3 (untreated), Fluram-βAla-KLVFF (25 µM), and POM-fluram-βAla-KLVFF (100 µM).

The confocal microscopy images of the brain ganglia and muscle tissues indicate that **neither the peptide nor the POM-peptide hybrid is able to cross the BBB**, as no green signal is visible in the neuronal cells.

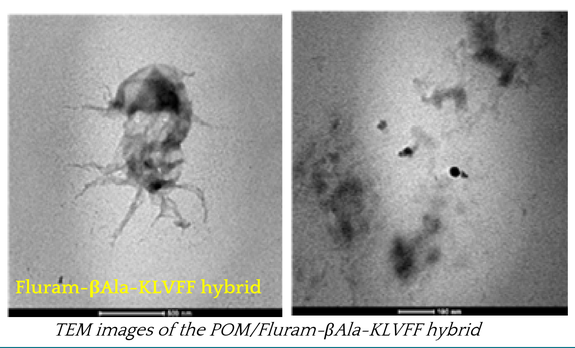


Representative images of dorsal ganglion and muscle tissue of untreated Drosophila tomato/elav III stage larvae treated with Fluram-βAla-KLVFF and POM/Fluram-βAla-KLVFF hybrid. Scale bar 20 µm.

Samples from the DLS analysis were lyophilised and subsequently subjected to TEM analysis. The sample comprising the hybrid POM KLVFF exhibited the presence of **extensive aggregates**, with dimensions spanning the micrometre range, and spherical nanoparticles, measuring approximately 100 nm. Hybrids comprising Ac-KLVFF and Fluram-βAla-KLVFF peptides also display large aggregates, albeit of a smaller size (approximately 500 nm) and in lower quantities.

The latter are predominantly characterised by **spherical particles** with a size of **100-200 nm**, exhibiting notable similarity.

TEM



TEM images of the POM/Fluram-βAla-KLVFF hybrid

1. *H. Chaudhary, I. A. Iashchishyn, N. V. Romanova, M. A. Rambaran, G. Musteikyte, V. Smirnovas, M. Holmboe, C. A. Ohlin*, Ž. M. Svedružić*, and L. A. Morozova-Roche ACS Appl. Mater. Interfaces 2021, 13, 26721−26734*

This work was supported by PRIN 2022 National Project (Grant N. 2022CAS9ZT, Development of HYbrid and Multifunctional Bio-conjugates against Alzheimer's Disease - HYMBAD).