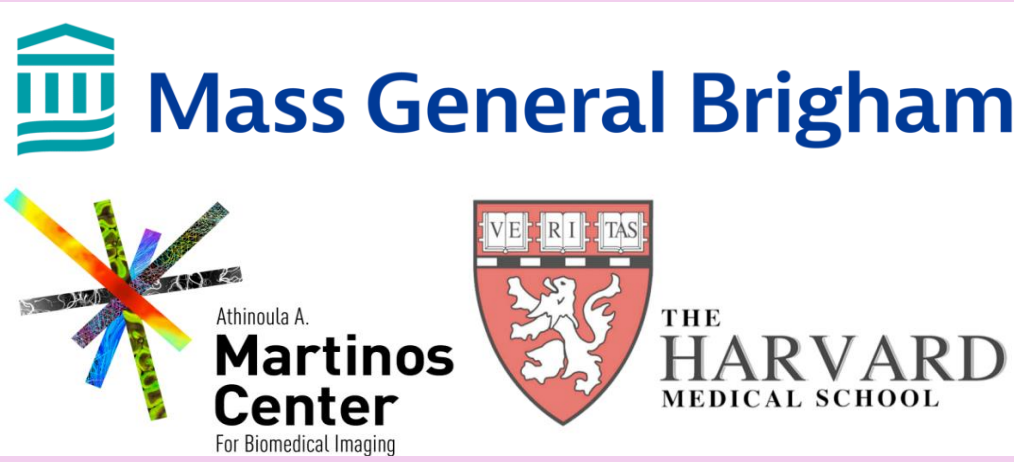


Molecular Light Degradar: Molecularly Generated Light for Photo-oxidation/degradation of Misfolded Proteins

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Abstract Misfolded proteins are tightly associated with various neurodegenerative diseases, and removing these misfolded proteins is one of the actively pursued approaches for seeking therapeutics for these diseases.

In this study, we demonstrated that molecularly produced light (molecular light) from ADLumin-5, a self-photosensitizing chemiluminescence compound, could induce photo-oxidation and photodegradation of misfolded proteins, including beta-amyloid, tau, alpha-synucleins, and TDP-43 proteins in vitro. We validated the oxidation and degradation via LC-MS, MADLI-MS, and western blotting. Using beta-amyloid as a showcase, we demonstrated that, upon photo-oxidation and photodegradation, the toxicities of this misfolded protein were significantly reduced.

To investigate the therapeutic effects of ADLumin-5 in vivo, we used the 5xFAD mouse model for longitudinal treatment for 4 months. In vivo molecular imaging results indicated that ADLumin-5 could reduce the accumulation of A β proteins.

Our study presents a novel approach to seek therapeutics for neurodegenerative disease via molecular light-induced degradation of misfolded proteins. In addition, because ADLumin-5 is dual-functional — enabling both photodegradation and in vivo imaging of misfolded protein changes — it can be considered a theranostic agent for neurodegenerative diseases, representing a novel approach to drug discovery for neurodegenerative diseases.

Research Question Can molecular light generated from chemiluminescent probes be used for unimolecular photo-therapy via self-photosensitized oxidation and degradation of misfolded proteins in neurodegenerative diseases?

Background Photodynamic therapy has been extensively explored for various diseases, but its application in neurodegenerative diseases remains limited due to the limited light penetration into the brain. However, if light is generated from molecules, it could be delivered to the targets in a manner similar to drug molecules, offering new possibilities for treatment.

Here, we dubbed the molecularly produced light “molecular light”. Although the total photon flux generated by “molecular light” is much smaller, it can be delivered with remarkable precision, positioning it within zero to nanometer proximity of the target for effective absorption and energy transfer. Since “molecular light” possesses dual functions, e.g. both as a molecule and as a light source, it is logical to leverage its molecular properties for precise targeting and its light-emitting properties for phototherapy (Fig.1)

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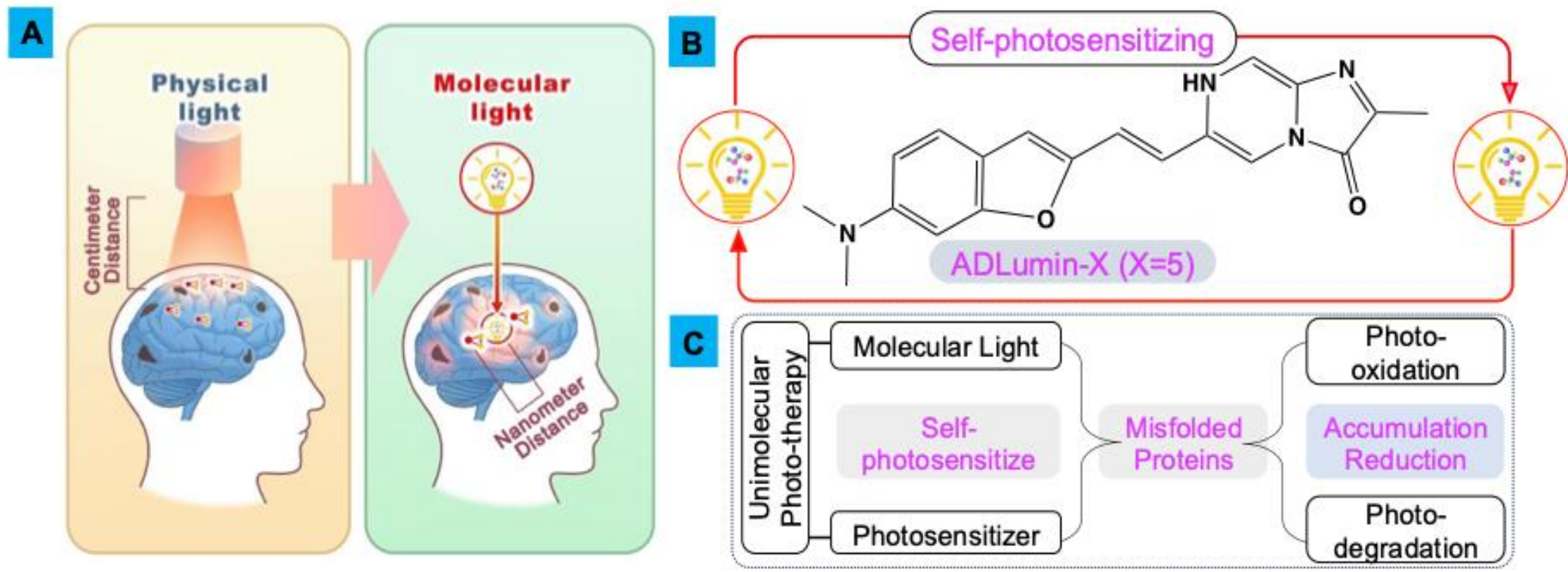


Fig.1 A) Illustration of phototherapy with physically produced light and molecularly generated light (“molecular light”). Molecular light can be delivered as molecules to avoid tissue penetration limitations of light; B) Typical chemical structure of ADLumin-X for self-photosensitizing; C) Schematic illustration of unimolecular phototherapy with molecular light, in which self-photosensitizing agents induce photo-oxidation and degradation of misfolded proteins, thereby reducing their accumulation and associated pathology in vivo.

Results

1. ADLumin-5: Molecular light degrader for A β s

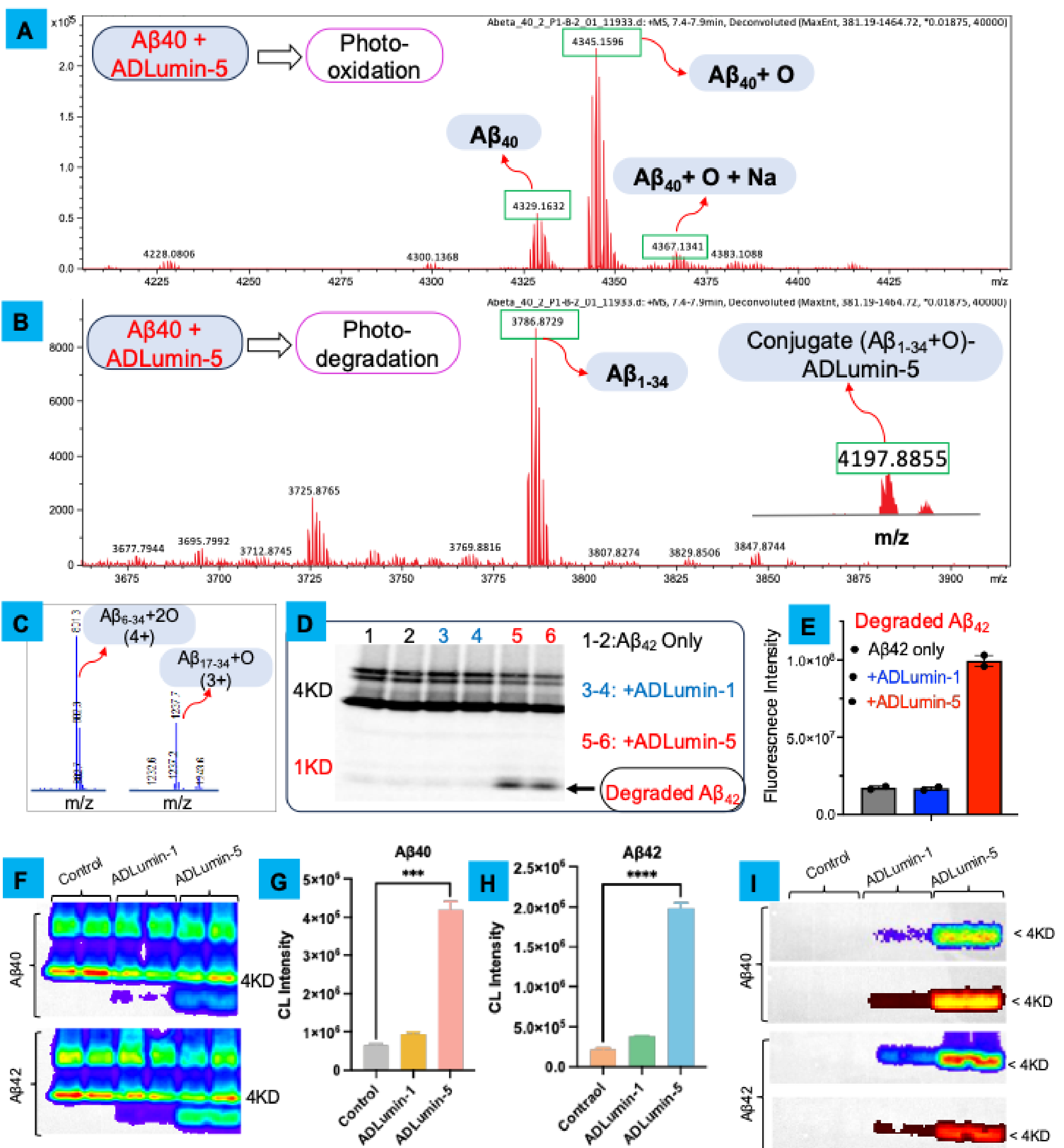


Fig. 2 Photo-oxidation and degradation of A β s with ADLumin-5. **A-B)** High resolution LC-MS analysis of A β ₄₀ incubated with ADLumin-5; **C)** LC-MS analysis of A β ₄₀ incubated with ADLumin-5 after trypsinization; **D-E)** SDS-PAGE gel and quantification of the degraded bands of FAM-A β ₄₂ incubated with ADLumin-Xs; **F-H)** Western blot assay (after antibody incubation) and quantification of A β s with different ADLumin-Xs; **I)** Western blot (before antibody incubation) of A β s with different ADLumin-Xs, image were obtained in both chemiluminescence and fluorescence. Error bar: $\pm$ SD, n = 3. ***p < 0.001, ****p < 0.0001

2. ADLumin-5: Unimolecular phototherapy to reduce A β burden in vivo with 5xFAD model

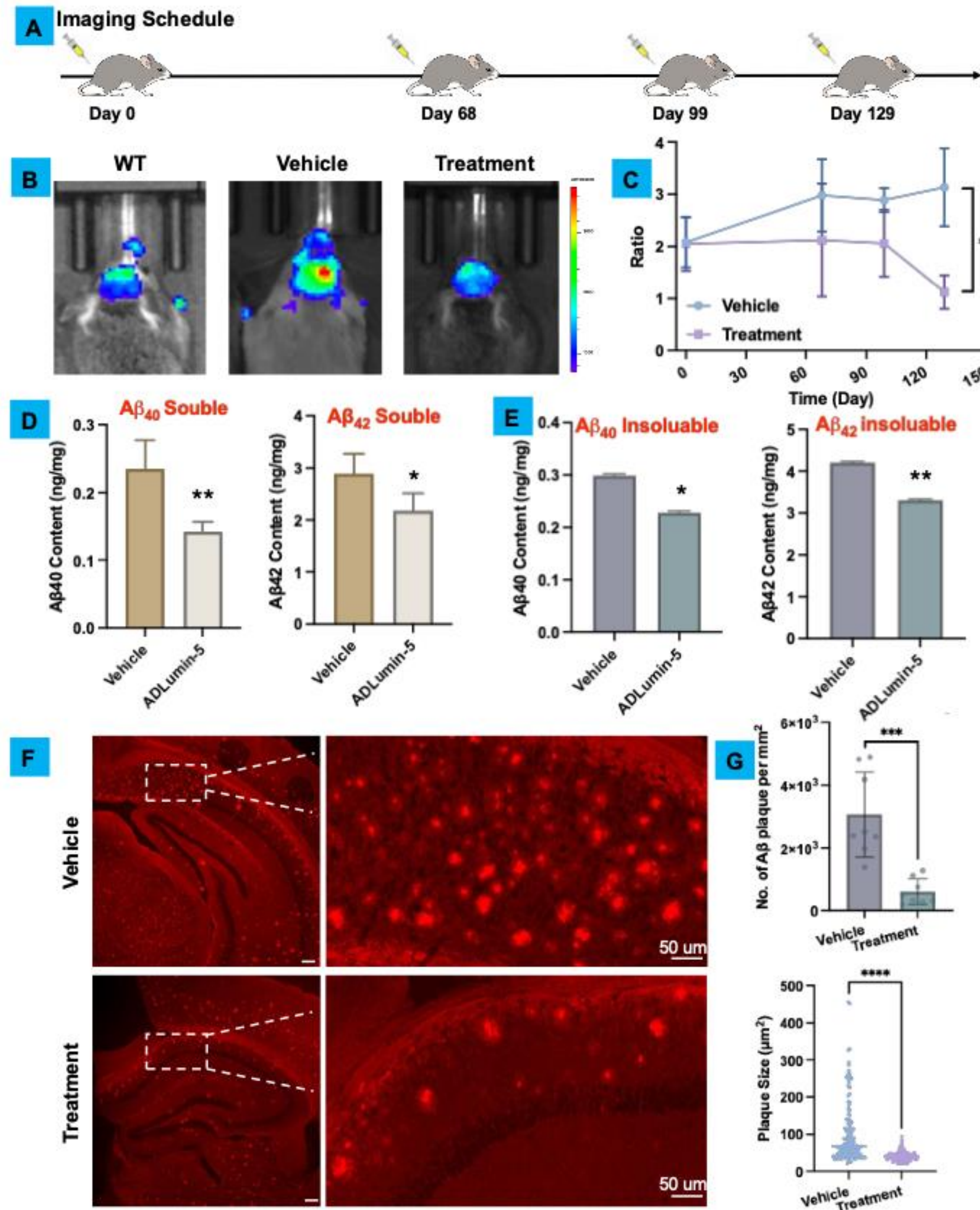


Fig. 3 In vivo therapy, imaging and ex vivo evaluation. **A)** Treatment and imaging monitoring schedule; **B)** Representative in vivo images showing A β -associated signals in different groups; **C)** Quantification of the ratio (AD/WT) brain imaging signals over time (n=3-5). **D)** ELISA analysis of brain soluble A β s after 4-Mon. treatment; **E)** ELISA analysis of insoluble A β s after 4-Mon. treatment. **F)** Ex vivo fluorescence imaging of A β burdens in brain sections with vehicle or ADLumin-5 treatment. **G)** Quantitative analysis of plaque density and size in brain sections with vehicle or ADLumin-5 treatment. Error bar: $\pm$ SD, n = 3. *p < 0.05, **p < 0.01.

Summary and Conclusions

- Molecular light from ADLumin-5 is self-photosensitizing.
- ADLumin-5 alone could induce photo-oxidation and degradation of misfolded proteins, including A β , Tau, α -syn, and TDP-43.
- Altering A β 's structure, reducing A β seeding efficiency, accelerating A β degradation and attenuating neurotoxicity.
- Reducing A β burdens in vivo (5xFAD).
- Molecular light is adequate for in vivo photo-therapy

Unimolecular Photo-therapy with Molecular Light is Feasible in Vivo

Reference

- [1] Kuang S, et al. Angew Chem 2023, 62(45): e202312519.
- [2] Zhang J, et al. PNAS. 2023, 20(50): e2310131120.
- [3] Umeda H, et al. Angew Chem 2024, 3(31): e202405605.
- [4] Ran C et al. Angew Chem 2024, 63(6): e202314468.

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