Microscopy-guided spatial protein purification identifies novel amyloid-β aggregate-associated proteins Lon protease and DDX3X helicase

Chien-Chang Huang, Weng-Man Chong, Hsiao-Jen Chang, Chun-Kai Huang, Yi-De Chen, Chia-Wen Chung, Jung-Chi Liao*

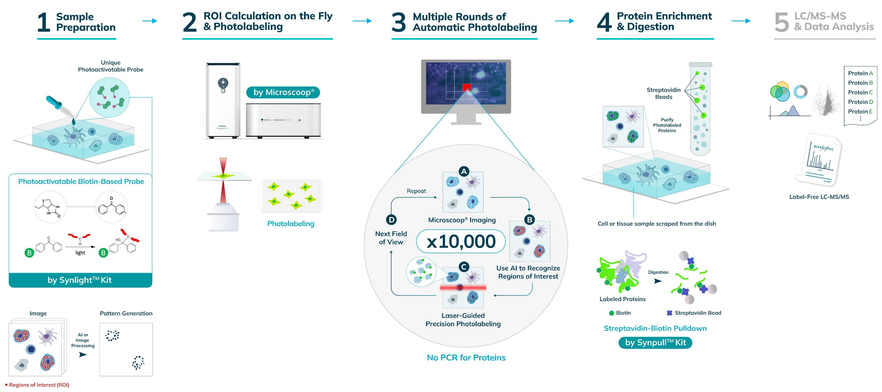
Syncell Inc., Taipei, Taiwan

For more information, please visit www.syncell.com , *Corresponding author (Email: jcliao@syncell.com)

Abstract

Aggregation of amyloid-β peptides (Aβ) is a prominent feature of Alzheimer's disease (AD). However, our understanding of the proteome of Aβ aggregates and their interactions with associated proteins remain incomplete. Existing spatial proteomics methods often rely on antibody panels/arrays, limiting de novo proteomic discovery with high sensitivity and subcellular precision. To address this gap, here we employ Microscoop®, a novel microscopy-based proteomics platform, for ultra-content microscope-guided photo-biotinylation and subsequent pulldown of subcellular Aβ-associated proteins. This platform enables subcellular spatial protein purification from thousands of fields of view for subsequent LC-MS/MS-based proteome identification. Using Aβ1-42 overexpression inhuman neuroblastoma SH-SY5Y differentiated cells as an AD model, we perform photo-biotinylation on millions of Aβ1-42 aggregates with locations of aggregates calculated on the fly fully automatically with Microscoop®. The proteomic results show that we not only find known Aβ-associated proteins, but also identify proteins not previously reported in the literature. Two of the newly identified proteins, Lon protease and DDX3X helicase, are colocalized with Aβ1-42shown in antibody staining. Colocalization with the animal amyloid plaques is further positively validated using brain sections of the 5XFAD mouse, a familial Alzheimer's disease mouse model. Our study unveils that at least Lon protease and DDX3X, two proteins that are rarely regarded as Aβ-associated proteins, are localized with Aβ, suggesting further hypothesis testing needed for their roles in Aβ.

Microscoop® : A novel platform enabling microscopy-guided automated photo-biotinylation for spatial proteomic discovery



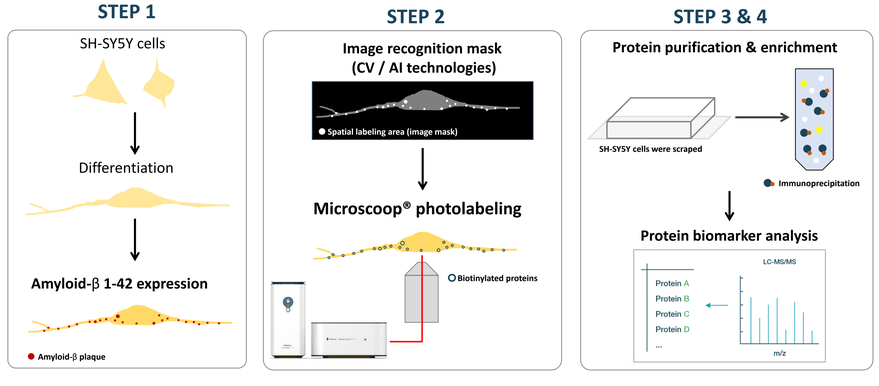
STEP 1. Regions of interest (ROIs) in cell or tissue samples were marked by immuno-fluorescence staining. Fluorescence images were acquired and segmented using conventional image processing or AI-based pattern recognition.

STEP 2. Samples were incubated with photoactivatable biotin probes. Photolabeling at subcellular ROIs were performed with two-photon illumination of the Microscoop® system.

STEP 3. Biotinylated proteins were enriched by affinity pulldown and analyzed by LC-MS/MS.

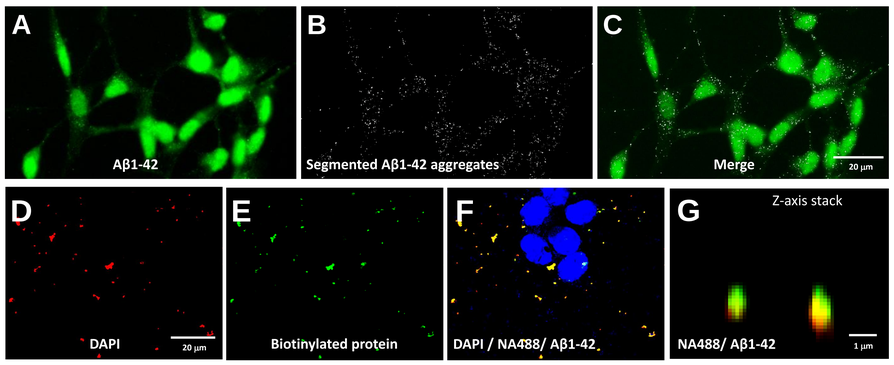
STEP 4. Hypothesis-free protein biomarkers were identified with proteomic analysis.

Proteomic discovery assay for in vitro amyloid-β plagues

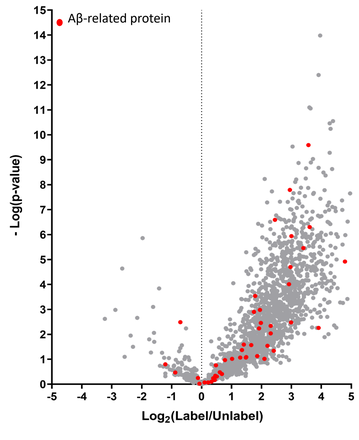


Locations of Aβ1-42 aggregates were segmented in real time and targeted photo-induced biotinylation using Microscoop®

SH-SY5Y cells were differentiated for 5 Days. Aβ1-42 were expressed by lentivirus infection for 72 hrs. The Aβ1-42 aggregated spots were indicated by immunostaining. A modified otsu thresholding method was first applied to images after image pre-processing. A following white spot extraction method by logic operation was implemented to segment Aβ1-42 aggregates with high precision (Fig. A-C). The cells were then incubated with a photo-activatable probe, and the spatial photolabeling process was performed at the segmented locations for thousands of fields of view fully automated with Microscoop® to biotinylate proteins in the regions of Aβ1-42 aggregates. Confocal imaging validated the precision of photolabeling (Fig. D-F). Fig. G indicates precise labeling viewed from the z direction. High-speed photolabeling for thousands of fields of view was necessary to assure collection of a large number of proteins of interest enough for mass spectrometry sensitivity.



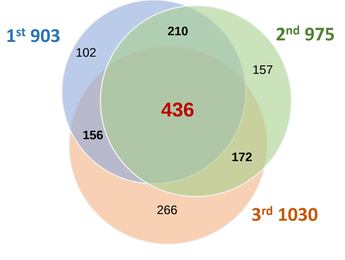
Spatial protein composition of Aβ1-42 aggregated plaques were identified by Microscoop®



LC-MS/MS results of well-known Aβ-related proteins

- 3-hydroxyacyl-CoA dehydrogenase type-2
- Clathrin heavy chain 1
- CDK5
- Histone deacetylase 1
- Histone H2B type 2-E
- Histone H4
- Rab11A
- TMED10
- Ubiquitin-60S ribosomal protein L40
- VDAC1
- hnRNP A2/B1
- Poly [ADP-ribose] polymerase 1
- Tubulin alpha-1B chain
- Tubulin beta-4A chain
- Delta(24)-sterol reductase
- Gelsolin
- Reticulon-1 / 3 / 4
- Lysozyme C
- Drebrin
- OSTC
- Tyrosine-protein kinase Yes
- Na+/K+ ATPase subunit alpha-3
- USP9X

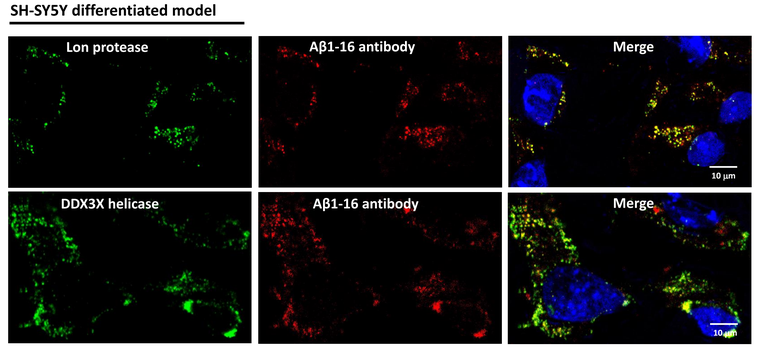
Protein overlap in biological replicates of Aβ 1-42 aggregates

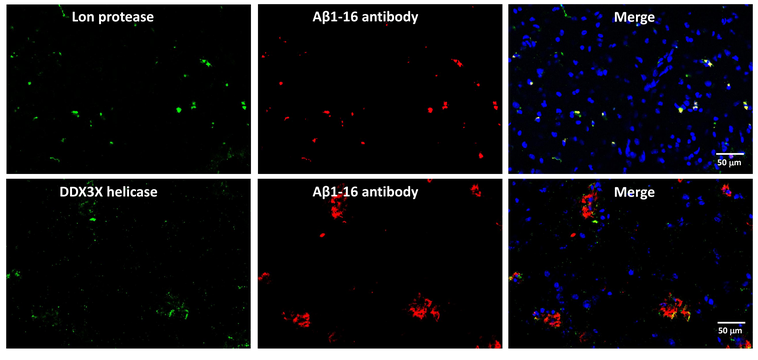


	Protein ID	%
ALL	1499	100.0%
2-overlap	974	65.0%
3-overlap	436	29.1%

The proteome of the photo-labeled (PL) sample by two-photon illumination was compared with that of the unlabeled sample (UL). In total, 1499 proteins were identified in the photo-labeled enriched group. 40 proteins were found to map to the existing Aβ-related protein database in UniProt. Among them, these proteins are well-studied and highly related to Aβ aggregates. Further validation of high-ranked candidates, we chosen 6 proteins by immunofluorescence staining will be performed to identify possible novel Aβ-associated proteins. After immunostaining validation, **Lon protease** and **DDX3X helicase** were localized with Aβ in the SH-SY5Y differentiated cell. We also validated these two protein candidates in the brains of 6-month-old 5xFAD mouse, a model of amyloid pathology that overexpresses two key human proteins associated with familial Alzheimer's disease (FAD), specifically amyloid precursor mutants and presenilin1 mutants. The figure demonstrates the localization of Lon protease and DDX3X helicase in relationship to various stages of amyloid plaque formation in 6-month-old 5xFAD mouse brain.

Novel candidates validated by immunofluorescence staining





References:

Microscopy-guided subcellular proteomic discovery by high-speed ultra-content photo-biotinylation. Chen et al. bioRxiv 2023.12.27.573388.

Conclusion

SYNCELL Microscoop® is a new technology platform for hypothesis-free spatial protein biomarker discovery. It has been used to identify novel Aβ-related proteins in this study. Microscoop® was used to precisely biotinylated proteins in Aβ1-42 aggregates of the SH-SY5Y differentiated cell model in high content. We have further performed LC-MS/MS and identified the proteome of these Aβ1-42 aggregates. This proteome not only contained known Aβ-associated proteins, but also repeatedly revealed proteins that are not considered as Aβ-related proteins so far. Further validation is in progress to check whether novel Aβ-associated biomarkers can be identified by SYNCELL Microscoop®.