Identifying novel mitochondria-lipid droplet interface proteins using microscopy-guided subcellular spatial protein purification

Yen-Ming Lin, Weng-Man Chong, Chun-Kai Huang, Hsiao-Jen Chang, Chantal Hoi Yin Cheung, Jung-Chi Liao*
Syncell Inc., Taipei, Taiwan, *Corresponding author (Email: jcliao@syncell.com)

SYNCELL

syncell.com

Abstract

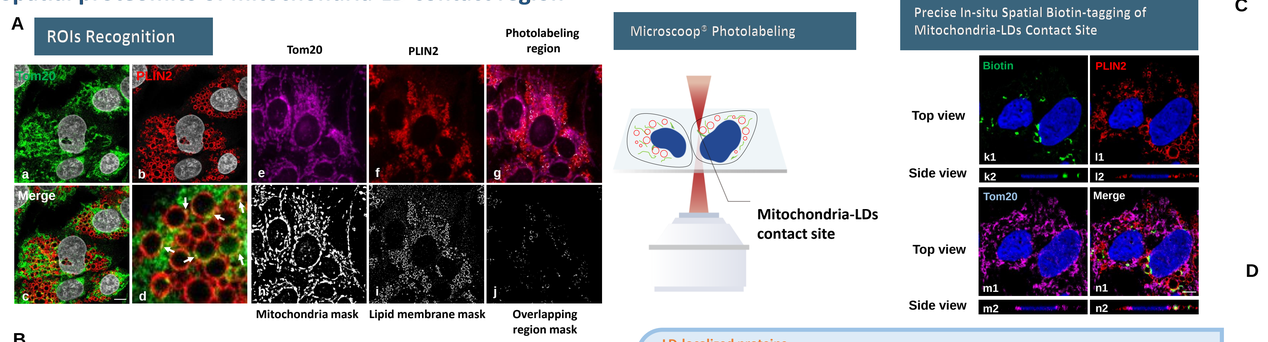
Background: Lipid droplets (LDs) serve as energy storage depots and interact with various organelles, including mitochondria. Peri-droplet mitochondria (PDM) play a critical role in lipid homeostasis and Non-alcoholic fatty liver disease (NAFLD) progression. However, the dynamic and complex nature of mitochondria-LD interactions has posed challenges in identifying new protein constituents and understanding their functions.

Method: We addressed these challenges using Microscoop®, a new microscopy-guided spatial protein purification platform, to isolate proteins from millions of mitochondria-LD interaction sites. With AI or traditional image processing, Microscoop® recognizes the specified regions of interest (ROIs) within cellular or tissue samples and induces precise photo-biotinylation at 300-nm resolution via its femtosecond pulsed two-photon illumination. The real-time and automatic "ROI recognition to photo-biotinylation" cycles render biotin-tagging of sufficient proteins for subsequent pulldown and protein identification via LC-MS/MS.

Result: Through this innovative approach and subsequent mass spectrometry analysis, we mapped the proteome of the mitochondria-LD interface in oleic acid (OA)-treated HepG2 cells. In addition to recovering well-characterized LD-associated proteins, we also identified previously known proteins involved in mitochondria-LD interactions. From the top 30 ranked common proteins across three independent experiments, five novel candidates with no prior LD association were selected for further validation. Intriguingly, immunofluorescence staining revealed their localization around lipid droplets and at mitochondria-LD contact sites, more obviously in OA-treated HepG2 cells as compared to vehicle-treated cells. Notably, when FHL3, a protein among the five candidates was suppressed in OA-treated HepG2 cells, leading to reduce mitochondria-LD contacts and result in elongated mitochondrial. This observation suggests a decrease in fatty acid β-oxidation activity.

Conclusion: Facilitated by the Microscoop®'s spatial protein purification, our study identifies previously unrecognized protein constituents at the mitochondria-LD interface, paving the way to further functional examination associated with lipid regulation associated with NAFLD pathogenesis.

Spatial proteomics of mitochondria-LD contact region



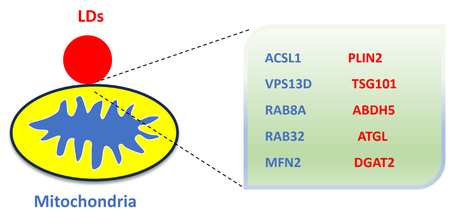
LD-localized proteins

ELMOD2	RAB5C	RBP1	TMT1A	AIFM2	SPAST	HSD17B7	PLIN2	CASP14	OPA1
HSDL1	TRAF6	RAB10	LSS	RAB18	BAX	ACSL3	RAB8A	DHRS3	IRAK1
RAB3GAP2	RAB7A	UBXN4	DGAT2	TMEM263	PISD	HSD17B11	YWHAZ	LDAH	ARL1
NSDHL	SIGMAR1	RHOA	TUBB	STARD7	RAB12	MAIP1	CDC42	RDH10	SPART
STARD13	ABHD5	PRR11	SNAP29	JUP	METTL9	ARF5	TUBA1C	TAGLN2	RAB1A
DSP	UQCRQ	CYB5R3	AUP1	RAB27A	NDUFB6	LPCAT1	APOO	PNPLA2	DHRS1
RAB43	PKMYT1	YWHAQ	RDH11	WIPI2	CIDEB	RAB3B	TMT1B	NDE1	
TXN	RAB33B	ANXA2	COPS3	RHOG	PITPNM1	CFL1	SCCPDH	VAT1	

Mitochondria proteins

GK2	IRF3	SUGCT	NDUFB1	MT-CO3	TIMM17A	RNASEH1	NDUFA7	ARF5	POLDIP2
ARMC10	HTRA2	HOGA1	COQ9	PARP9	DHX32	NDUFA4	AFMID	ARMC1	C2orf49
PLEKHN1	DIABLO	ACSM2B	MGST3	TIMM17B	ABCG2	PTGR3	NDUFA11	MRPS12	RNF5
PTCD2	MPC1	CCDC127	FIS1	SNAP29	NDUFB8	BCKDHB	RPIA	EIF4E2	NME3
BOK	POLG2	PLPBP	FBXL4	NDUFS5	NME6	MRM1	HINT2	ALKBH1	ADPRS
TOMM20	COA1	TRAF6	RAB24	MT-CO2	DOP1B	METTL17	RPUSD3	MRPL22	MTARC1
COX5A	RSAD1	OXNAD1	ARG2	FASTKD3	MOCS1	ATP5PF	RBFA	TMEM70	PNPLA8
CLPP	NDUFAF3	PLGRKT	TMEM126B	FABP1	NBR1	SFXN5	METTL15	CYB5B	SELENOO

C **Proteins involved in mitochondria-LDs association**



D **List of top 14 putative proteins**

GDAP2	HPF1	PIP	FHL3	SAAL1	MBNL3	S100A16
KLHL18	NAA10	TUBB2B	TATDN1	PPP6R3	DCTN3	DCAF16

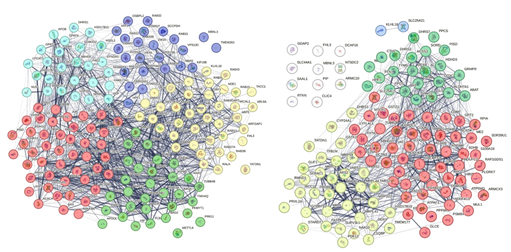
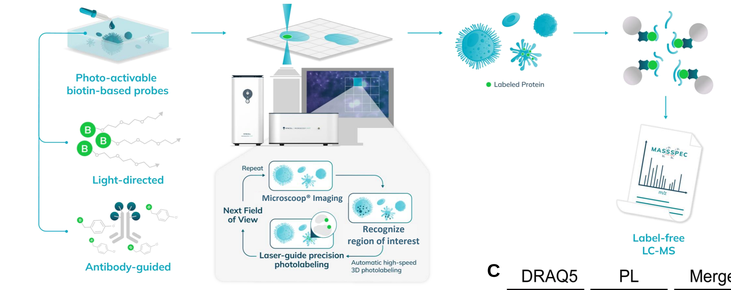


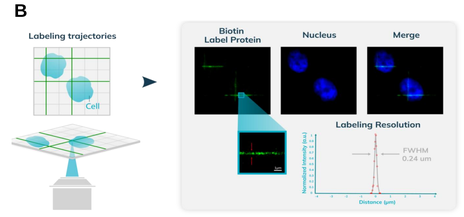
Fig. 2 | (A) Left panel. Tom20 (a, green) and PLIN2 (b, red) were immuno-stained to mark mitochondria and LD membrane respectively. The overlapped regions (c and d) between mitochondria and LD signal indicates potential mitochondria-LD contact sites. Images of mitochondria and LD (e and f) are recognized by traditional image processing and computer vision algorithm (h and i) to identify ROIs (g and j, mitochondria-LD overlapping region). The confocal images demonstrate the precise and accurate photo-biotinylated proteins (k1, stained with NeutrAvidin-488) in mitochondria-LD overlapping region at lateral (xy)- and axial (z) directions (k1-n1 and k2-n2, right panel). Bar: 5μm. **(B)** A distribution of overall protein abundances by the ratio of photolabeled (PL) sample to those in a control (CTL) sample annotated as PL/CTL ratio, showing that known LD-localized (blue) and mitochondria proteins (red) are enriched in the PL group (left panel). List of a few well-known LD-localized proteins and mitochondria proteins identified by Microscoop®. (middle panel), and the Gene Ontology analysis of biological process category of these proteins (right panel). **(C)** The list of the well-characterized proteins mediating mitochondria-LD association identified by Microscoop®. **(D)** Fourteen proteins (table list) that are neither mitochondria nor LD proteins selected from Top 30 common proteins, are combined with 157 enriched LD-associated or 111 mitochondria proteins then subjected to STRING to reveal protein-protein interaction network, suggesting that these proteins are more co-related with LD-associated protein (left panel) than with mitochondria (right panel).

Microscoop® : a hypothesis-free subcellular protein discovery platform

A



B



D

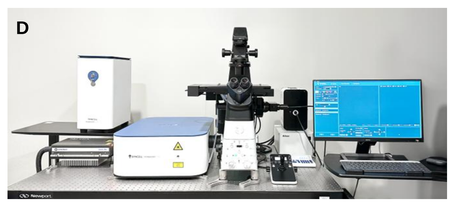
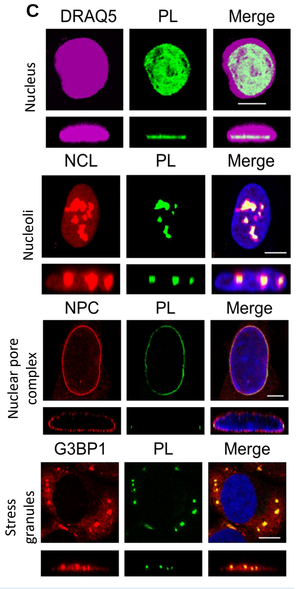


Fig. 1 | **(A)** Schematic workflow of Syncell Microscoop®. A total-sync ultra-content microscopic platform that integrates image acquisition, photochemistry, microscopy, optics, and FPGA-based mechatronics enable high-content in situ photolabeling followed by mass spectrometry analysis. **(B)** PFA fixed U-2 OS cells were photo-biotinlylated with a thin "cross" pattern, and photolabeling resolution was measured by super-resolution structured illumination microscopy. **(C)** Top- (xy) and side- (z) views of photolabeled subcellular compartments. **(D)** Microscoop® system.

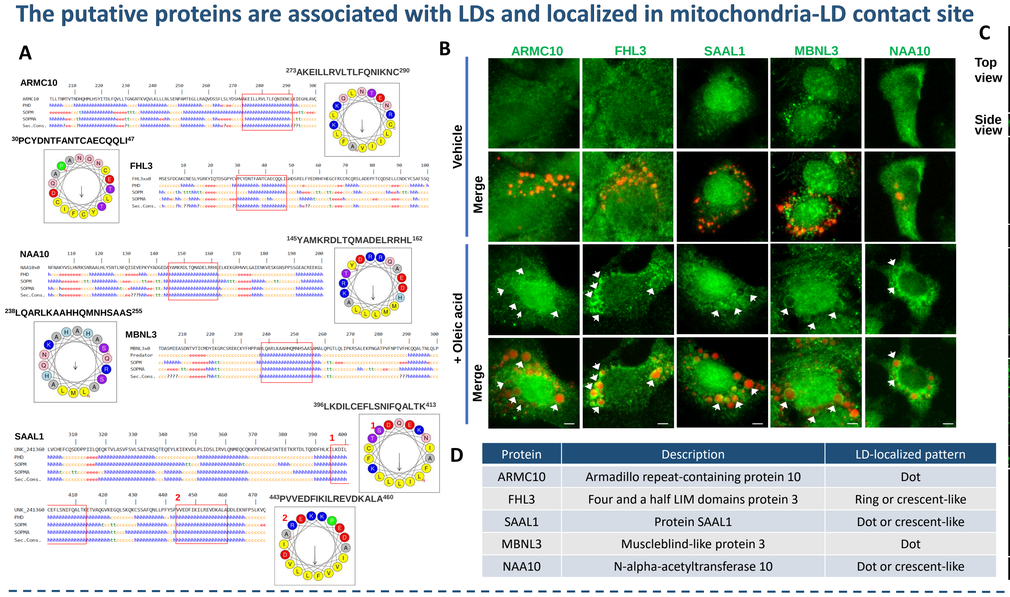
C



References

1. Cohen, S., Valm, A.M., and Lippincott-Schwartz, J. (2018). Interacting organelles. Current opinion in cell biology 53, 84-91. 10.1016/j.ceb.2018.06.003.
2. Yang, M., Luo, S., Yang, J., Chen, W., He, L., Liu, D., Zhao, L., and Wang, X. (2022). Lipid droplet – mitochondria coupling: A novel lipid metabolism regulatory hub in diabetic nephropathy. Frontiers in endocrinology 13, 1017387. 10.3389/fendo.2022.1017387.
3. Enkler, L., and Spang, A. (2024). Functional interplay of lipid droplets and mitochondria. FEBS letters 598, 1235-1251. 10.1002/1873-3468.14809.\
4. Chen, Y.-D., Chang, C.-W., Cheung, C.H.Y., Chang, H.-J., Sie, Y.-D., Chung, C.-W., Huang, C.-K., Huang, C.-C., Chong, W.M., Liu, Y.-P., et al. (2023). Microscopy-guided subcellular proteomic discovery by high-speed ultra-content photo-biotinylation. bioRxiv, 2023.2012.2027.573388. 10.1101/2023.12.27.573388.

The putative proteins are associated with LDs and localized in mitochondria-LD contact site



D

Protein	Description	LD-localized pattern
ARMC10	Armadillo repeat-containing protein 10	Dot
FHL3	Four and a half LIM domains protein 3	Ring or crescent-like
SAAL1	Protein SAAL1	Dot or crescent-like
MBNL3	Muscleblind-like protein 3	Dot
NAA10	N-alpha-acetyltransferase 10	Dot or crescent-like

Fig. 3 | (A) Secondary structure and amphipathic helix prediction of the 5 putative LD-associated proteins selected from Top 30 common proteins using algorithms from the NPS@ website and HeliQuest program respectively, indicating the potential amphipathic α-helix regions of 5 proteins. **(B)** HepG2 cells were treated with oleic acid (OA) or vehicle for 16h, followed by LDs staining with BODIPY 558/568 (red) and the 5 candidate proteins with corresponding antibodies. Epi-fluorescence images revealed their localization around lipid droplets (arrows), more obviously in OA-treated HepG2 cells as compared to vehicle-treated cells. **(C)** Confocal images verified the co-localization of all 5 candidate proteins with lipid droplet membrane (PLIN2 as a marker), as well as their presence at mitochondria-LD contact sites, suggesting a potential implication of these novel proteins in mitochondria-LD regulation. **(D)** Table list of candidate proteins and their LD-localized pattern. Bar: 5μm

Mitochondria morphology alteration and reduction of mitochondria-LD interaction upon FHL3 depletion

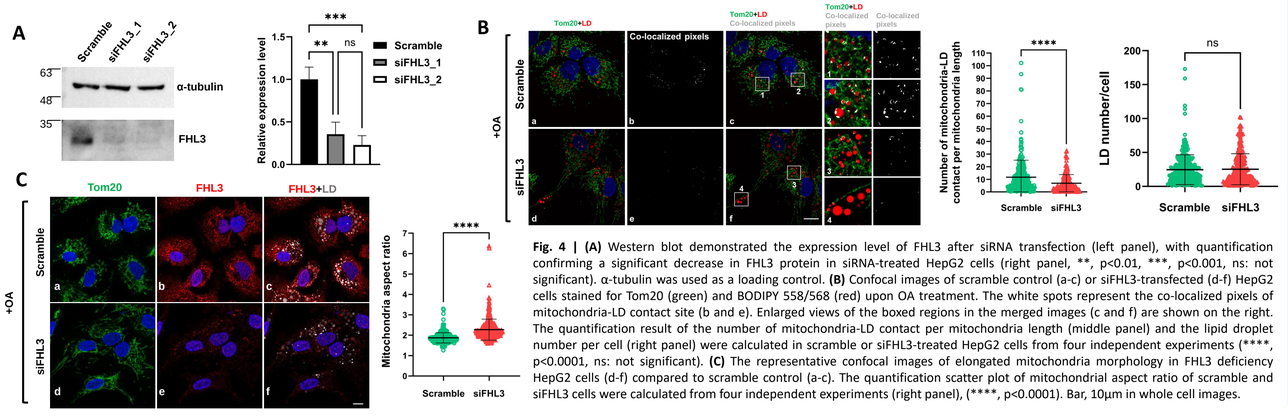


Fig. 4 | (A) Western blot demonstrated the expression level of FHL3 after siRNA transfection (left panel), with quantification confirming a significant decrease in FHL3 protein in siRNA-treated HepG2 cells (right panel, **, p<0.01, ***, p<0.001, ns: not significant). α-tubulin was used as a loading control. **(B)** Confocal images of scramble control (a-c) or siFHL3-transfected (d-f) HepG2 cells stained for Tom20 (green) and BODIPY 558/568 (red) upon OA treatment. The white spots represent the co-localized pixels of mitochondria-LD contact site (b and e). Enlarged views of the boxed regions in the merged images (c and f) are shown on the right. The quantification result of the number of mitochondria-LD contact per mitochondria length (middle panel) and the lipid droplet number per cell (right panel) were calculated in scramble or siFHL3-treated HepG2 cells from four independent experiments (****, p<0.0001, ns: not significant). **(C)** The representative confocal images of elongated mitochondria morphology in FHL3 deficiency HepG2 cells (d-f) compared to scramble control (a-c). The quantification scatter plot of mitochondrial aspect ratio of scramble and siFHL3 cells were calculated from four independent experiments (right panel), (****, p<0.0001). Bar, 10μm in whole cell images.

Summary

1. SYNCELL Microscoop® is a novel platform to enable hypothesis-free subcellular spatial proteomics.
2. The platform integrates microscopy, deep learning, two-photon illumination, and mechatronics to facilitate high-content image-guided photo-labeling.
3. Spatially specified proteins from hundreds of thousands of individual cells can be rapidly and precisely labeled by Microscoop® to achieve sensitivity of mass spectrometry.
4. In mapping the proteome of mitochondria-LD interaction region, 5 high-ranked proteins identified with Microscoop® are localized with LDs and within the mitochondria-LD contact site, suggesting their potential role mitochondria-LD communication or lipid metabolism.