Novel stress granule proteins identified by Microscoop, a microscope-guided hypothesis-free subcellular protein discovery platform

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Abstract

Stress granules (SGs) are dynamic, non-membrane-bound assemblies of protein and RNA formed in response to cellular stress. Their pathological implications in cancer and neurodegenerative disease render it crucial to study SG composition for therapeutic applications. Previous SG proteomic studies have been achieved by biochemical fractionation or proximity labeling prior to mass spectrometric (LC-MS/MS) analysis; however, the procedures are usually labor intensive and technically challenging due to the small-sized and membrane-less nature of SGs. Moreover, the essence that SG markers mostly have multiple sub-cellular localizations, further complicates the studies. In this work, we took advantage of Microscoop, an automatic microscope system integrated with a machine learning-based algorithm, to explore the SG proteome. Microscoop enables site-specific protein identification by inducing photo-labeling precisely at the selected region-of-interest. SGs were first induced in U2-OS cells by arsenite and stained for G3BP1, a common SG marker. Immunofluorescence images of G3BP1 were applied to generate a computer vision (CV) based algorithm to differentiate G3BP1 in stress granules from that in the cytoplasm. A sequential process of fluorescence imaging, CV-driven pattern generation, and photochemical labeling was automatically implemented to achieve SG protein-specific biotinylation until sufficient cells was labeled for subsequent protein enrichment and LC-MS/MS analysis. In total, 2,614 proteins were found in three biological replicates. 77% of the triplicate-overlapped proteins were identified as SG proteins, showing a high specificity of the enrichment. STRING analysis of the proteins with a stringent threshold further disclosed 8 highly confident proteins as the core interactors in the SG interaction network. More importantly, these 8 proteins have no prior annotation as SGs. Immunofluorescence staining confirmed that 6 of them, i.e. EIF3CL, DDX17, PPIA, RPSA, YWHAE, and RPLP0 showed SG pattern and co-localized with G3BP1 in stressed cells but not in normal cells, confirming the discovery capability of our method. Collectively, our study unravels novel SG proteins, which possibly serve as core SG components, and demonstrates the capability of the Microscoop technology to achieve subcellular in situ protein discovery.

Microscoop™: hypothesis-free subcellular protein discovery platform

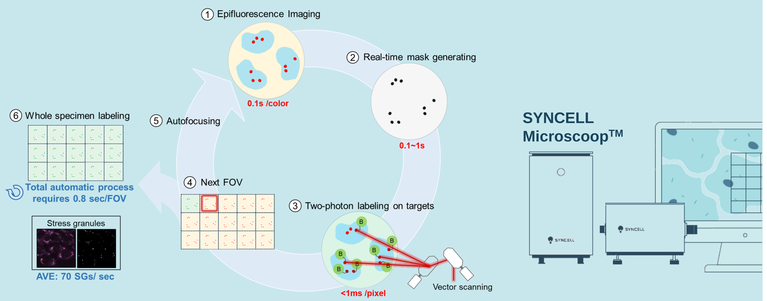

Fig. 1 | Schematic overview of the Microscoop™ workflow. A total-sync ultra-content microscopic opto-biotinylation platform only requires 0.8 Sec/FOV including image acquiring, mask generation, photolabeling to robustly photolabels an average of 70 SGs per second.

Speed and accuracy are the keys for protein labeling

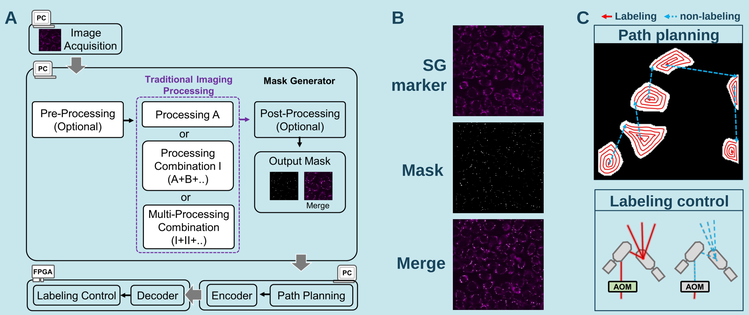

Fig. 2 | A, Image processing is applied to recognize the regions of interest of acquired images. B, Stress granules are processed by filtering and segmentation by image processing. C, The path planning algorithm generates the labeling path and the non-labeling path for an input mask, which can also apply for the labeling control on the galvanometer system and the AOM.

Nanometer-scaled photolabeling of stress granules

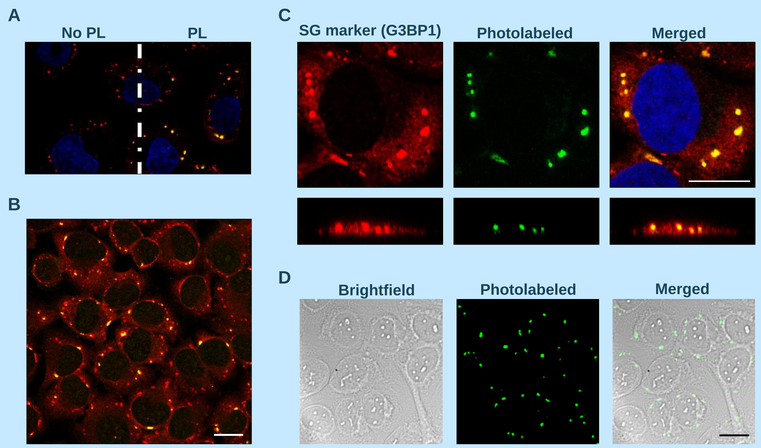

Fig. 3 | A, Confocal micrographs of photolabeled (PL) and non-photolabeled (No PL) at user defined stress granules. B, A field of view of photolabeled stress granules with Microscoop™. C-D, Confocal micrographs depicting precise and accurate photolabeled stress granules at top- and side- view. Red: G3BP1, Green: NeutrAvidin-488, Blue: DAPI. Scale bar: 10 µm.

Microscope-guided spatial proteomic workflow

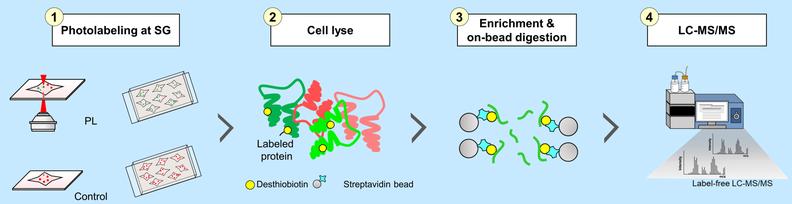

Fig. 4 | Overview of proteomic profiling of photolabeled region of interest. Cells are seeded on a glass chamber and photolabed by Microscoop™. Then, the photolabeled (PL) cells are lysed, enriched by streptavidin beads and digested by trypsin prior to LC-MS/MS measurement.

Proteomic mapping of stress granule with Microscoop™

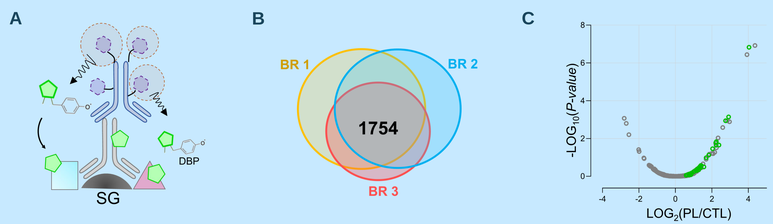

Fig. 5 | A, Schematic diagram of photolabeling mechanism. B, Venn diagram of three biological replicates of stress granule proteomics. C, Volcano plot of relative protein levels in photolabeled samples to control samples (PL/CTL ratio) in $\log_2$ scale. Over-represented (enriched) proteins are shown in green.

Biological process and protein interaction network of stress granule proteome

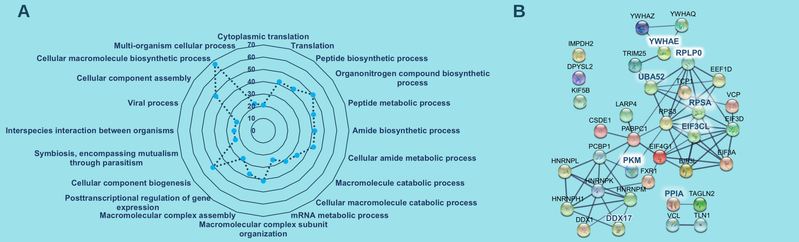

Fig. 6 | A, The enriched 124 proteins were subjected to Gene ontology to reveal SG related biological process. B, The highest abundant proteins were used as seed to visualize physical interaction between the proteins. Each node represents a protein, and each edge represents a degree of physical and functional interaction. Highlighted proteins: no prior annotation as SG.

Discovery of novel stress granule proteins

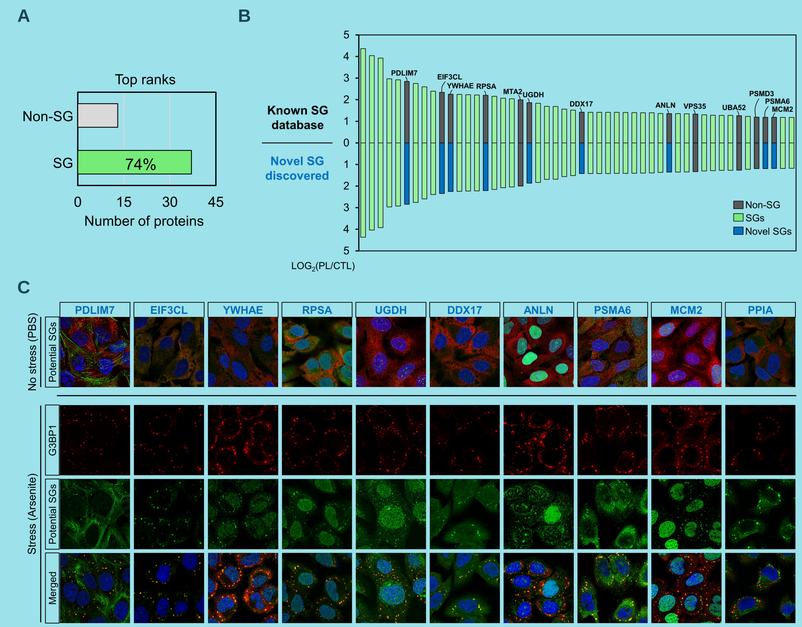

Fig.7 | A, 74% of true positive SGs are found in the top 50 proteins ranked by PL/CTL ratio. B, Proteins have no prior annotation as stress granule (gray) from the top 50 ranked proteins. Novel SG proteins (blue) discovered by Microscoop™. C, Confocal micrographs depicting SG formation of potential stress granule proteins in U-2OS cells with or without arsenite stress. Ten potential SG proteins (green) are highly co-localized with G3BP1 SG markers. lens: 63x oil. PL: photolabed (biotinlyated) proteins stained with NeutrAvidin-488, Red: G3BP1, Blue: DAPI.

Summary

- SYNCELL Microscoop™ is a novel platform to enable hypothesis-free subcellular spatial proteomic discovery
- The platform integrates microscopy, deep learning, two-photon illumination, and mechatronics to facilitate high-content image-guided photo-labeling
- Spatially specific proteins from hundreds of thousands of individual cells could be labeled precisely enough for mass spectrometry sensitivity
- Novel stress granule proteins were identified with Microscoop™ with high specificity