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Chapter 15

Chlorophyll-Based Optogenetics to Control Membraneless Organelles

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Abstract

Membraneless organelles (MLOs) formed via protein phase separation have garnered significant attention recently due to their relevance to cellular physiology and pathology. However, there is a lack of tools available to study their behavior and control their bioactivity in complex biological systems. This chapter describes a new optogenetic tool based on water-soluble chlorophyll protein (WSCP), a red light-induced singlet oxygen-generating protein, to control synthetic MLOs. Upon exposure to red light, WSCP generates singlet oxygen, which triggers the crosslinking of the proteins in the MLOs, resulting in their liquid-to-solid phase transition. The effective delivery of chlorophylls enables the successful reconstitution of WSCP in living cells, thus offering a potential approach to biological regulation at the subcellular level.

Key words Optogenetics, Photo-responsive protein, Chlorophyll, Membraneless organelle, Protein phase transition

1 Introduction

In recent years, membraneless organelles (MLOs) have gained significant attention due to their diverse physiological functions and implications in disease pathologies. MLOs are typically formed by liquid-liquid phase separation (LLPS) of biomacromolecules and can be found in various subcellular compartments, such as P bodies, postsynaptic density (PSD), and stress granules (SGs) [1–3]. Aberrant phase separation, particularly of intrinsically disordered proteins like Tau, FUS, and TDP-43, can result in pathological aggregation, which is associated with neurodegenerative diseases [4–7]. During pathological aggregation, the phase-separated condensates transform from dynamic liquid-like droplets

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to static solid-like particles, similar to material aging [8]. This phase transition can reduce molecular exchange and limit molecular dynamics, leading to loss of normal function. Although significant progress has been made in studying the solid state of protein condensates and their pathological relevance, there is a lack of tools to control the phase transition of MLOs in complex living systems.

Optogenetic tools based on photo-responsive proteins, such as cryptochrome 2 (Cry2) and Dronpa, have been developed to control protein behaviors in living cells [9, 10]. However, most optogenetic tools for phase separation rely on blue light illumination, which penetrates poorly into deep tissues. Here, we describe a red light-dependent optogenetic tool based on a water soluble chlorophyll protein (WSCP) for controlling MLOs. The WSCP derived from the *Brassicaceae* family is a photosensitizer that can generate singlet oxygen upon red light illumination [11, 12]. WSCPs exist as tetramers, harboring 2 to 4 chlorophylls, and are soluble in aqueous solutions, unlike many membrane-bound chlorophyll-binding proteins involved in photosynthesis [11]. WSCP tetramers are also super stable and remain intact under harsh conditions such as extreme pH or high temperature, making them an excellent candidate to be a robust optogenetic tool.

The synthetic MLO system is created via the LLPS of a recombinant protein RGG-Mfp-3-RGG (RMR). The RGG domain, an intrinsically disordered region (IDR), serves as the scaffolding domain to drive phase separation, while Mfp-3, a tyrosine-rich protein from *Mytilus edulis*, is amenable to singlet oxygen-mediated crosslinking [13, 14]. The recombinant RMR protein can undergo liquid–liquid phase separation in a concentration/temperature-dependent manner in vitro, and the resulting protein condensates can be decorated with WSCP via hydrophobic interactions. Red light-induced generation of singlet oxygen by WSCP facilitates crosslinking within RMR condensates, leading to the liquid-to-solid phase transition of protein condensates. This red light-triggered phase transition system can be introduced into living cells, providing a new strategy to study biological aging and pathology at the subcellular level (Fig. 1).

2 Materials

Milli-Q ultrapure Type 1 water is used for all buffer solutions, except when working with DNA, where we used nuclease-free water. All chemicals are purchased from Sigma Aldrich unless otherwise noted.

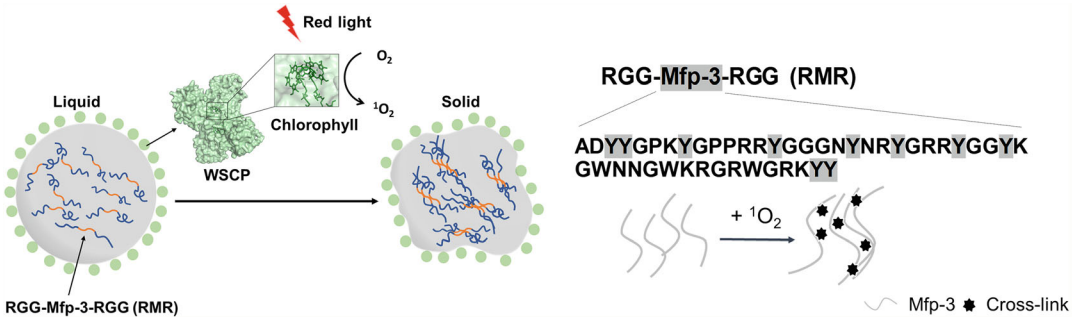

Fig. 1 *Schematic representation of a synthetic protein condensate's liquid-to-solid phase transition induced by red light.* RGG-Mfp-3-RGG (RMR) acts as the scaffold protein and undergoes LLPS to form a synthetic protein condensate. The protein condensate is decorated with WSCP via hydrophobic interactions. WSCP acts as a photosensitizer that generates singlet oxygen upon red light exposure. The resulting singlet oxygen triggers the crosslinking of RMR, resulting in the liquid-to-solid phase transition of the protein condensate. (Adapted from Ref. [14] with permission from Nature Publishing Group)

2.1 Plasmid Construction

1. pQE80l::*His6-RGG-mfp-3-RGG*: The plasmid, previously described by Li and coworkers [14], was used to express the RGG-Mfp-3-RGG (RMR) protein in *Escherichia coli*. The *mfp-3* gene (gBlocks) and the *RGG* gene (amplified from the construct pET::*RGG*, Addgene, Plasmid #124929) were inserted into pQE80l::*AAA* [15] using the restriction sites, NheI/SalI, SacI/SpeI, and XhoI/KpnI. The corresponding amino acid sequence is shown in the Appendix.
2. pCAGGS::*RGG-mfp-3-RGG-mCherry*: The plasmid, previously described by Li and coworkers [14], was used for the RMR expression in mammalian cells. The gene of *RGG-mfp-3-RGG* was amplified from pQE80l::*His6-RGG-mfp-3-RGG* and then inserted into pCAGGS:: *hPhyBY276H-mCherry-HRasCT* (Addgene, Plasmid #100540).
3. pET22b::*WSCP* and pEGFP-N1::*WSCP-egfp*: The plasmids, previously described by Li and coworkers [14], were used to express WSCP in *E. coli* and mammalian cells, respectively. The WSCP domain was derived from *Lepidium virginicum*, with the N-terminal signaling and C-terminal extension sequences removed [16, 17]. The corresponding amino acid sequence is shown in the Appendix. The *WSCP* gene was synthesized by GENEWIZ and cloned into pET22b and pEGFP-N1.
4. *E. coli* DH5α and BL21(DE3) strains (New England Biolabs) were used for plasmid amplification and protein expression, respectively.

2.2 Protein Expression and Purification

1. Luria Broth (LB): Mix 5 g/L yeast extract, 5 g/L NaCl, 10 g/L tryptone, and 15 g/L bacteriological agar (for solid media only) in water. Autoclave the mixture for sterilization.

2. Isopropyl β-D-1-thiogalactopyranoside (IPTG) Solution: Dissolve IPTG in water to prepare a 1 M stock solution. Filter the solution through a 0.22 μm filter for sterilization.
3. Liquid nitrogen.
4. RMR Lysis Buffer: 500 mM NaCl, 20 mM Tris-HCl, 0.1 mM PMSF, pH 7.5. Filter it into a sterile bottle with a 0.22 μm pore bottle cap filter.
5. RMR Wash Buffer: 500 mM NaCl, 20 mM Tris-HCl, 25 mM imidazole, 8 M urea, pH 7.5. Filter it into a sterile bottle with a 0.22 μm pore bottle cap filter.
6. RMR Elution Buffer: 500 mM NaCl, 20 mM Tris-HCl, 500 mM imidazole, 8 M urea, pH 7.5. Filter it into a sterile bottle with a 0.22 μm pore bottle cap filter.
7. RMR Storage Buffer: 500 mM NaCl, 20 mM Tris-HCl, pH 7.5. Filter it into a sterile bottle with a 0.22 μm pore bottle cap filter.
8. WSCP Lysis Buffer: 300 mM NaCl, 20 mM Tris-HCl, 0.1 mM PMSF, pH 7.5. Filter it into a sterile bottle with a 0.22 μm pore bottle cap filter.
9. WSCP Elution Buffer: 300 mM NaCl, 20 mM Tris-HCl, 500 mM imidazole, pH 7.5. Filter it into a sterile bottle with a 0.22 μm pore bottle cap filter.
10. Phosphate Buffered Saline (PBS): 100 mM Na_2HPO_4, 18 mM KH_2PO_4, 1.37 M NaCl, and 27 mM KCl, pH 7.4.
11. Fresh Spinach Leaves (from local market).
12. Acetone (Sigma-Aldrich, 179973).
13. HisTrap HF 5 mL column (Cytiva).
14. Fast Protein Liquid Chromatography (FPLC) system (ÄKTA pure, GE Healthcare).
15. Branson Sonifier 450 (Marshall Scientific).
16. Size-exclusion Chromatography (SEC) column (Cytiva, Superose 6 Increase 10/300 prepacked column & Superdex 200 pg 26/600 prepacked column).
17. Amicon® Ultra-15 Centrifugal Filter Unit.
18. Dialysis tubing cellulose membrane (Sigma-Aldrich, D9652).
19. Whatman Grade 2 Qualitative Filter Papers (Cytiva).

2.3 Mammalian Cell Culture and Transfection

1. HEK293 cell [American Type Culture Collection (ATCC)].
2. Hela cell (ATCC).
3. Decco's Modified Eagle Medium (DMEM) (Gibco™, catalog number:12100046): with additives: high glucose, glutamine, and phenol red; without additives: HEPES, sodium pyruvate, and phenol red.

4. Fetal Bovine Serum (FBS) (Gibco™, catalog number: 26140079).
5. Penicillin-streptomycin solution (Sangon Biotech): 10,000 units/mL penicillin and 10 mg/mL streptomycin.
6. Trypsin-EDTA solution (Sangon Biotech): 2.5 mg/mL trypsin and 0.53 mM ethylenediaminetetraacetic acid (EDTA).
7. Hydrophobic PTFE Membrane Filters (Sartorius, Type 11807).
8. Glass bottom confocal dish (SPL Life Science, 200350).
9. Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, D8418).
10. Lipofectamine 3000 reagent (ThermoFisher).
11. Confocal microscope system (Nikon® C2+) with a cell incubator (Chamlide TC).
12. Photometer: Power meter (PM100D-THORLABS) with a standard photodiode power sensor (S120VC-THORLABS).

2.4 Imaging Processing and Data Analysis

1. NLS-Elements viewer (Nikon®).
2. ImageJ.

3 Methods

3.1 RMR Protein Expression

1. Transform pQE80l::*His6-RGG-mfp-3-RGG* into competent *E. coli* BL21(DE3) cells using the INOUE method [18]. Plate the transformed cells on LB agar supplemented with ampicillin (100 mg/L). Incubate the plate at 37 °C overnight for colony formation.
2. Inoculate 10 mL of LB supplemented with ampicillin (200 mg/L) with a single colony. Incubate the culture overnight at 37 °C and 220 RPM.
3. Inoculate 1 L of LB supplemented with ampicillin (100 mg/L) in a baffled conical flask using the overnight culture. Grow the culture in a shaking incubator at 37 °C and 220 RPM until the OD600 reaches 0.8–1.0.
4. Induce the culture with IPTG at a final concentration of 400 μM. Continue to culture overnight at 16 °C and 220 RPM in a shaking incubator.
5. Harvest the culture by centrifugation at 4200 *g* and 4 °C for 20 min. Discard the supernatant and flash-freeze the pellet with liquid nitrogen. Store the frozen pellet at −80 °C before purification.

3.2 RMR Protein Purification

1. Resuspend the pelleted *E. coli* cells from Subheading 3.1 in the RMR Lysis Buffer. Sonicate the sample using a sonifier with the following settings: 20% duty cycle, 30% output power, 5-min sonication, and 5-min cooling. Repeat the sonication process four times. Keep the sample in an ice bath during sonication.
2. Centrifuge the homogenized solution at 4 °C and 18,000 *g* for 1 h. Discard the supernatant and wash the cell pellets 2–3 times with the RMR Lysis Buffer supplemented with 1 M urea.
3. Resuspend the resulting pellets in the RMR Lysis Buffer with 8 M urea. Incubate the solution at 30 °C for 1 h. Centrifuge the solution again at 4 °C and 18,000 *g* for 1 h to separate the supernatant from the insoluble fraction.
4. Load the resulting supernatant onto a Ni-NTA gravity flow column with a 5-mL column volume (CV). Wash the column with five CVs of the RMR Wash Buffer. Elute the protein with two CVs of the RMR Elution Buffer. Assess the purity of the RMR protein using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).
5. Dialyze the purified RMR protein against the RMR Storage Buffer at 4 °C to prevent phase separation. After dialysis, aliquot the protein and flash-freeze each aliquot with liquid nitrogen. Store the aliquots of the protein at −80 °C for later use.

3.3 WSCP Expression

1. Transform the pET22b::*WSCP* plasmid into *E. coli* BL21 (DE3) cells. Plate the transformed cells on LB agar supplemented with ampicillin (100 mg/L). Incubate the plates at 37 °C overnight for colony formation.
2. Pick a single colony and inoculate 10 mL of LB with ampicillin (200 mg/L). Grow the culture overnight at 37 °C and 220 RPM.
3. Inoculate 1 L of LB supplemented with ampicillin (100 mg/L) in a baffled conical flask using the overnight culture. Grow the culture in a shaking incubator at 37 °C and 220 RPM until the OD600 reaches 0.8–1.0.
4. Induce the culture with IPTG at a final concentration of 400 μM. Continue to incubate the culture at 37 °C and 220 RPM for 4 h.
5. Harvest the bacterial cells by centrifugation at 5000 *g* and 4 °C for 30 min. Weigh the wet pellet. Discard the supernatant. Flash-freeze the pellet with liquid nitrogen. Store the frozen pellet at −80 °C before further purification.

3.4 Reconstitution of WSCP and Purification of Reconstituted WSCP

1. Combine the *E. coli* pellet from Subheading 3.3, fresh/frozen spinach leaves, and WSCP lysis buffer at a mass ratio of 1:8:8. Blend the mixture using a kitchen blender.
2. Sonicate the blended material using a sonifier in an ice water bath in the dark. Use the following settings: 20% duty cycle, 70% output power, 2-min sonication, and 3-min cooling. Repeat the sonication process 10 times.
3. Centrifuge the homogenized solution at 35,000 *g* at 4 °C for 1 h. Discard the pellet and carefully decant the supernatant. Sterile-filter the supernatant using a 0.45 μm syringe filter. Keep the filtered supernatant on ice under dark conditions.
4. Load a HisTrap HP 5 mL column onto an FPLC system. Set the flow rate at 5 mL/min. Turn off the flow restrictor in the loop. Wash the column with 50 mL of the WSCP Elution Buffer. Equilibrate the column with the WSCP Lysis Buffer (~50 mL).
5. Inject the filtered supernatant into the column at a flow rate of 4 mL/min. Keep the column away from light during this process.
6. Run the preprogrammed FPLC program (*see* **Note 1**). Collect the eluent in the dark and immediately store it at 4 °C before subsequent analysis.
7. Analyze the eluent using a Superose 6 Increase 10/300 prepacked SEC column (Fig. 2).
8. Further, purify the protein using a Superdex 200 pg 26/600 prepacked SEC column. Assess the protein purity using SDS-PAGE.

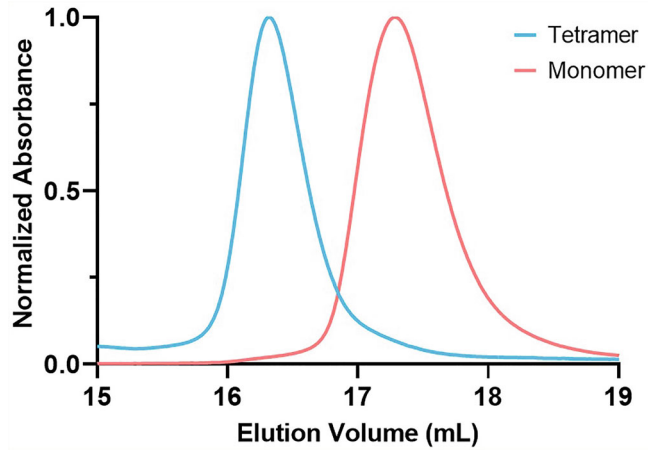

Fig. 2 *Size-exclusion chromatography (SEC) analysis comparing reconstituted WSCP in its tetrameric form and apoWSCP in its monomeric form.* (Reproduced from Ref. [14] with permission from Nature Publishing Group)

9. Remove salts and imidazole from the protein solution via dialysis. For each round of dialysis, dialyze the protein solution (10 mL) against 1 L of MilliQ water at 4 °C for 2 h. Perform four rounds of dialysis. The final concentration of imidazole should be below 1 nM.
10. Concentrate the protein to ~250 μM using a Centrifugal Filter. Perform buffer exchange with PBS using the same centrifugal filter unit.
11. Filter-sterilize the resulting protein solution using a 0.22-μm pore syringe filter. Store the protein solution in the dark at 4 °C before use. The protein solution can be stored for up to 2 weeks.

3.5 Phase Transition of RMR In Vitro

1. Thaw the frozen RMR protein (~25 μM) obtained from Subheading 3.2 with a 52 °C water bath. Initiate the liquid-to-liquid phase separation of RMR by cooling it at room temperature (23 °C) (Fig. 3) (*see* **Note 2**).
2. Add WSCP (obtained from Subheading 3.4) to the phase-separated RMR solution (Fig. 4).
3. Trigger the liquid-to-solid phase transition of RMR condensates by illuminating red LED light (630 nm) (Figs. 5 and 6) (*see* **Notes 2** and **3**).

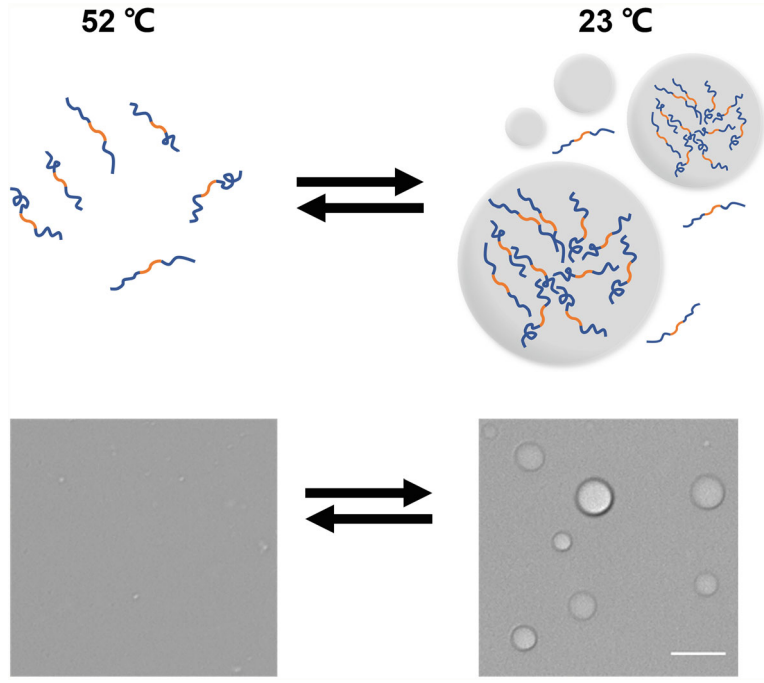

Fig. 3 *Temperature-dependent phase separation of RMR*. Scale bar: 5 μm. (Reproduced from Ref. [14] with permission from Nature Publishing Group)

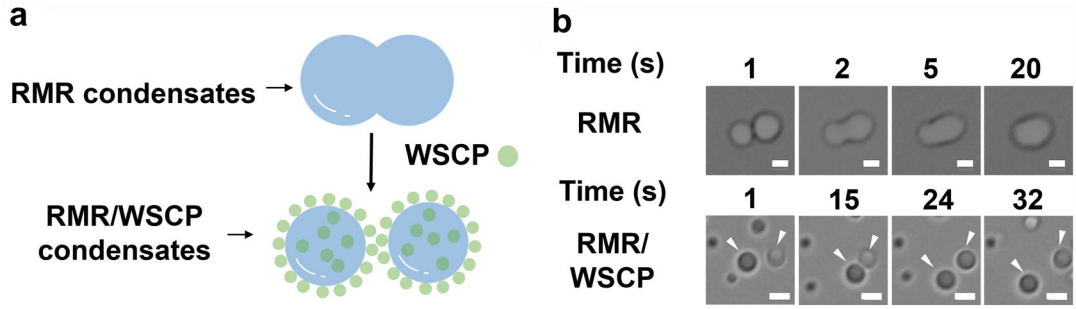

Fig. 4 *Modulation of RMR condensates with WSCP*. (**a**) Schematic illustration of the recruitment of WSCP into the condensates. (**b**) Representative images showing RMR condensates in the absence and presence of WSCP at 23 °C over time. WSCP decreases the surface tension of the RMR condensates, preventing the coalescence of two condensates. Scale bars: 1 μm. (Reproduced from Ref. [14] with permission from Nature Publishing Group)

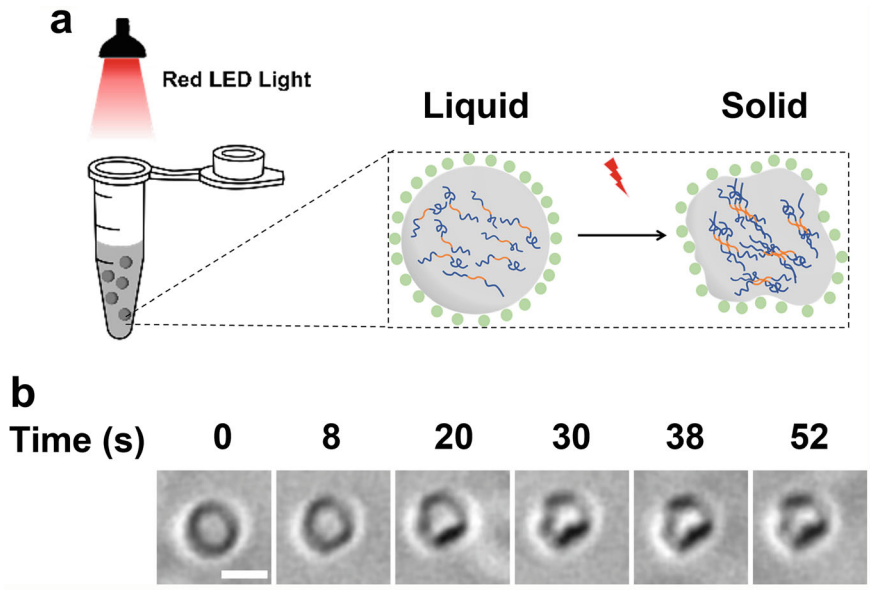

Fig. 5 *Red-light-induced liquid-to-solid phase transition of RMR/WSCP condensates*. (**a**) Schematic illustration of the red light-induced liquid-to-solid phase transition. (**b**) Representative images of a protein RMR/WSCP condensate at 23 °C under red light illumination, showing the condensate transitioning from liquid to solid over time. Scale bar: 1 μm. (Reproduced from Ref. [14] with permission from Nature Publishing Group)

3.6 Preparation of Spinach Extracts

1. Wash 500 g of fresh spinach leaves. Briefly heat the spinach leaves in boiling water for 1 min. Dry the spinach leaves using paper towels, followed by the addition of 800 mL of acetone. Stir the mixture at room temperature in the dark for 4 h.
2. Filter the resulting dark green supernatant using filter papers. Remove acetone using a rotary evaporator. Lyophilize the concentrated solution to obtain a black, hygroscopic solid.

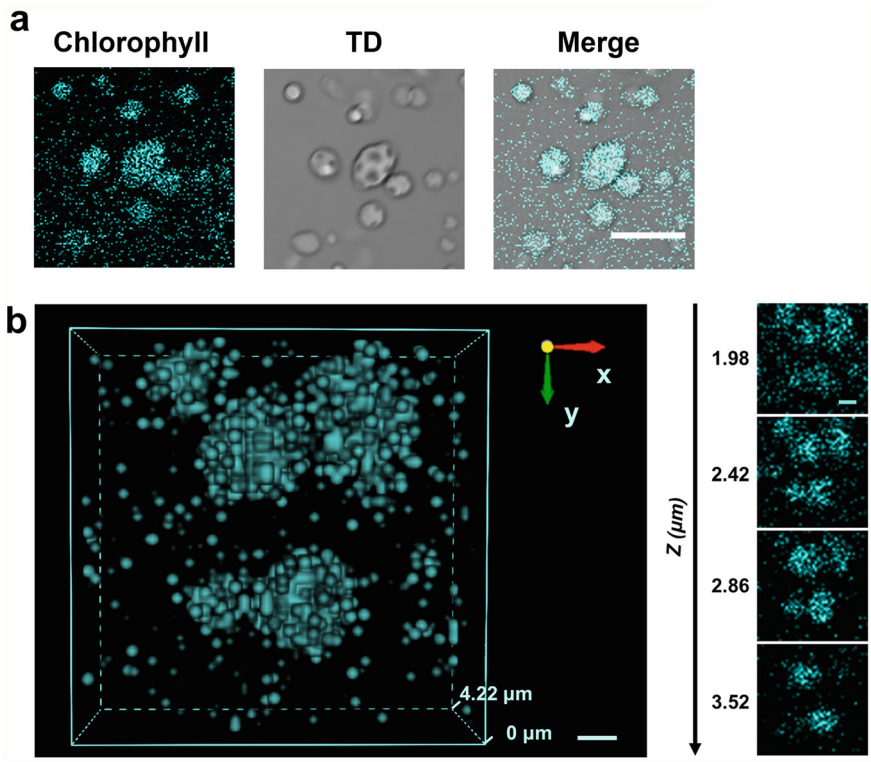

Fig. 6 *Representative confocal images of RMR/WSCP condensates.* (**a**) WSCP enrichment in RMR condensates. For fluorescence imaging, λ_{ex}, 405 nm. λ_{em}, 640 nm. *TD* transmitted detector image. Scale bar: 10 μm. (**b**) Representative 3D rendering and z-slice images of RMR/WSCP condensates. Scale bar: 1 μm. WSCPs are present on both the surface and interior of the RMR condensates. (Reproduced from Ref. [14] with permission from Nature Publishing Group)

3.7 Delivery of Chlorophyll into Mammalian Cells

1. Dissolve the freeze-dried spinach extract in DMSO to a concentration of 10 mg/mL. Filter the solution with a PTFE filter before use.
2. Add 50 μL spinach extract in DMSO to 2 mL DMEM supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin solution. Wash HEK293 cells with PBS in a 35-mm glass bottom dish. Add the prewarmed DMEM (37 °C) supplemented with the spinach extract to the cells (approximately 70% confluency). Incubate the cells under standard culturing conditions for 6 h.
3. After the 6-h incubation, wash the cells twice with PBS to remove any remaining spinach extract. Add fresh prewarmed DMEM to the cells. The cells are now ready for imaging or transfection (Fig. 7).

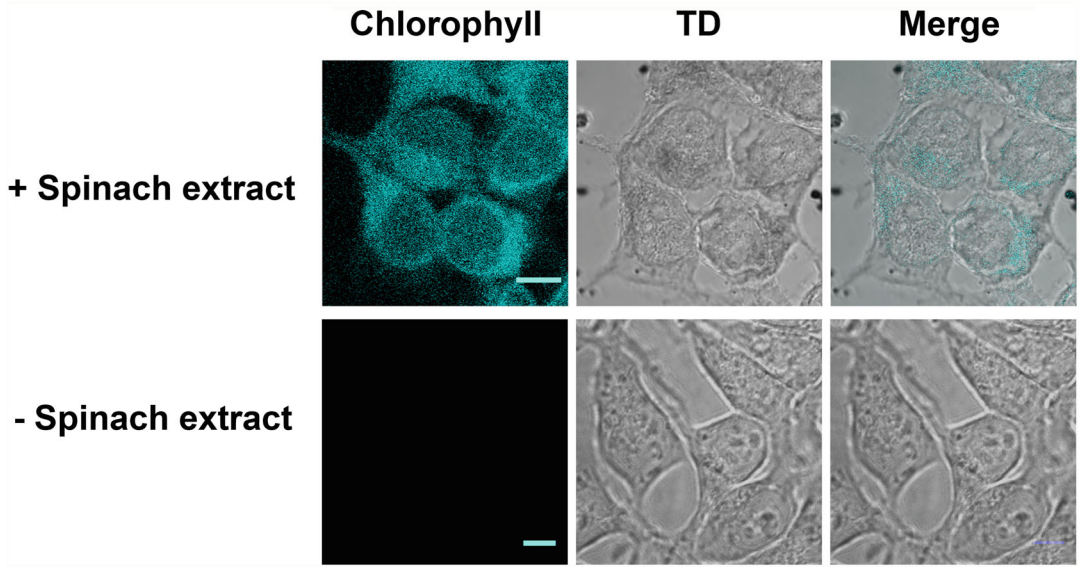

Fig. 7 *Delivery of chlorophyll into HEK293 cells.* The cells were treated with spinach extract (0.25 mg/mL) in DMEM for 6 h and imaged under standard cell culture conditions. Scale bar: 10 μm. (Adapted from Ref. [14] with permission from Nature Publishing Group)

3.8 Expression of Chlorophyll-Bound WSCP in Mammalian Cells

1. Dissolve the spinach extract in DMSO to a concentration of 1 mg/mL. Filter the spinach extract solution using a PTFE filter before use.
2. Add 40 μL of the spinach extract solution to 2 mL of DMEM supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin solution. Wash HEK293 cells with PBS in a glass-bottom dish. Add the prewarmed DMEM (37 °C) supplemented with the spinach extract to the cells (approximately 70% confluency). Incubate the cells under standard culturing conditions for 6 h.
3. Use the Lipofectamine 3000 reagent to transfect 1 μg of pEGFP::*WSCP-NLS-egfp* into the cells (*see* **Note 4**).
4. Culture the transfected HEK293 cells in the DMEM supplemented with the spinach extract for 14–16 h before imaging.
5. Image and determine the distribution of chlorophylls and WSCP inside the cells via fluorescence confocal microscopy (Fig. 8).

3.9 Production of RMR Condensates in Cellulo

1. Culture HEK293 cells in the DMEM supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin solution. Maintain the cells under a 5% CO_2 atmosphere at 37 °C [14]. Culture approximately 20,000 cells for transfection.
2. Add ~1 μg of pCAGGS::*RGG-mfp-3-RGG-mCherry* into the HEK293 cells in a glass-bottom dish. Use the Lipofectamine

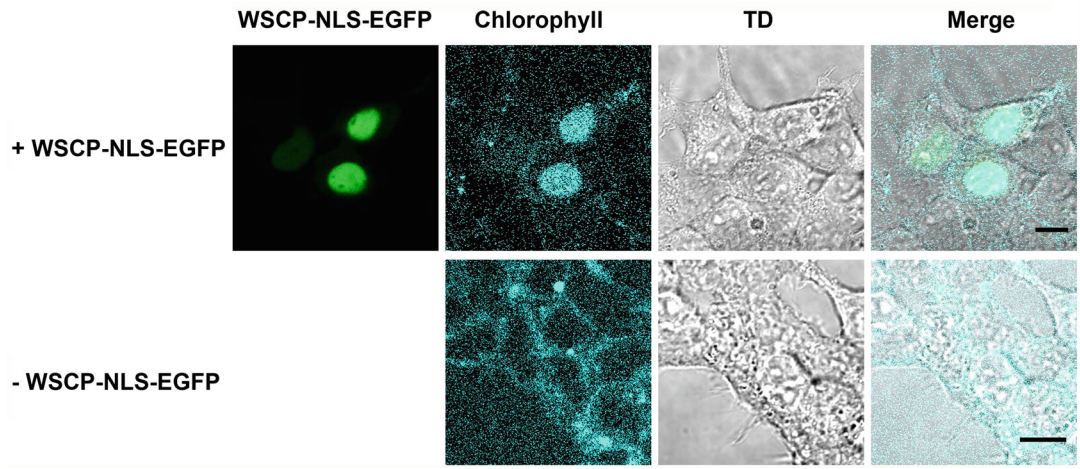

Fig. 8 *Colocalization of chlorophyll and WSCP in HEK293 cells*. Chlorophyll was predominantly co-localized with WSCP-NLS-EGFP in the nucleus, suggesting successful reconstitution of the WSCP/chlorophyll complex in living cells. In cells lacking WSCP-NLS expression, chlorophyll was mainly localized in the cytoplasm. *NLS* nuclear localization sequence. Scale bar: 10 μm

3000 reagent according to the manufacturer's protocol for transfection.

3. Culture the transfected HEK293 cells for 14–16 h before imaging.
4. Incubate the cells in an incubator during imaging. Image RMR-mCherry condensates using a fluorescence confocal microscope (Fig. 9).
5. Perform the Fluorescence Recovery After Photobleaching (FRAP) assay with the 561-nm laser from the confocal microscope (Fig. 10).

3.10 Photo-Induced Liquid-to-Solid Phase Transition in Cellulo

1. Transfect HEK293 cells with ~1 μg of pCAGGS::*RGG-mfp-3-RGG-mCherry* and ~1 μg of pEGFP::*WSCP-egfp*. Use the Lipofectamine 3000 reagent according to the manufacturer's instructions for transfection.
2. Culture the transfected HEK293 cells harboring chlorophylls for 14–16 h before imaging.
3. Use a confocal microscope to visualize and determine the localization of RMR-mCherry, WSCP-EGFP, and chlorophylls.
4. Irradiate the transfected HEK293 cells with the 405-nm laser beam from the confocal microscope for 5 min to induce the phase transition of RMR condensates in the cells (*see* **Note 5**).
5. Perform the FRAP assay using the 561-nm laser from the confocal microscope (Fig. 11) (*see* **Note 6**).

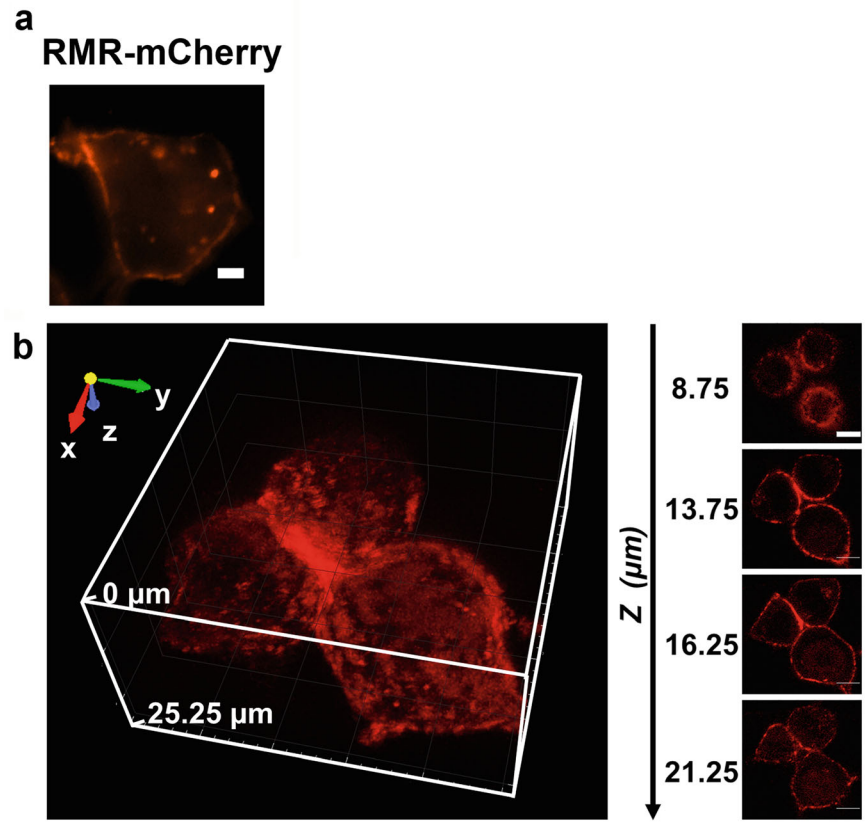

Fig. 9 *RMR phase separation in HEK293 cells.* (**a**) Fluorescent image of an HEK293 cell transfected with the gene encoding RGG-Mfp-3-RGG-mCherry (RMR-mCherry). Scale bar: 5 µm. (**b**) Representative 3D rendering and z-slice images of HEK293 cells producing RMR-mCherry. RMR is condensed not only in the cytoplasm but also along the cell membrane. Scale bar: 10 µm. (Reproduced from Ref. [14] with permission from Nature Publishing Group)

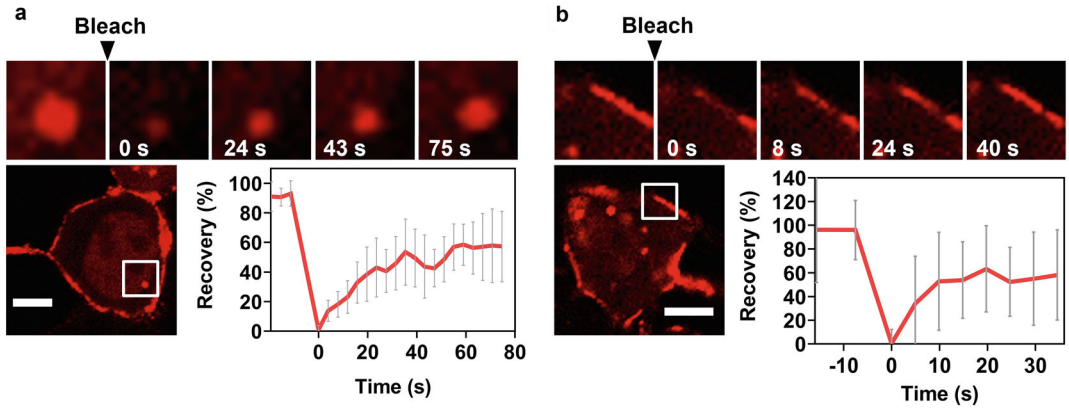

Fig. 10 *Fluorescence recovery after photobleaching (FRAP) assay of RMR condensates in the absence of WSCP in the cytoplasm* (**a**) *and along the cell membrane of HEK293 cells* (**b**). The images show the fluorescence of RMR condensates before and after photobleaching. The plots show the normalized fluorescence recovery after bleaching. Scale bar: 10 µm. (Reproduced from Ref. [14] with permission from Nature Publishing Group)

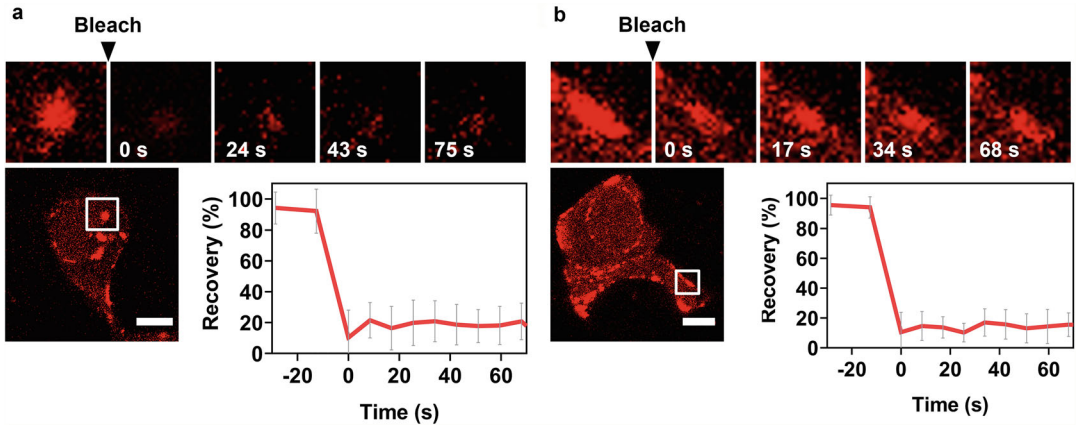

Fig. 11 *FRAP assays of RMR/WSCP condensates in the cytoplasm* (**a**) *and along the cell membrane of HEK293 cells* (**b**). The images show the fluorescence of RMR/WSCP condensates before and after photobleaching. The plots show the normalized fluorescence recovery after bleaching. After irradiation, compared to those in the absence of chlorophyll and WSCP (with ~60% fluorescence recovery) (Fig. 10), the condensates in the presence of WSCP-EGFP and chlorophyll barely recovered their fluorescence (<20%) after 1 min. Scale bar: 10 μm. (Reproduced from Ref. [14] with permission from Nature Publishing Group)

4 Notes

1. Set the FPLC at a mode of manual load and a flow rate of 5 mL/min. Keep A280 and pH monitoring "ON" and flow restrictor "OFF." Preprogram the FPLC by including an equilibration step with one column volume (CV) of the Lysis Buffer and 10 CVs of the Wash Buffer, and an elution step with 3 CVs of the Elution Buffer, linearly increased from 5% to 100%, and 2 CVs of the 100% Elution Buffer.
2. Measure the intensity of the LED light at 630 nm using the THORLABS Photometer. Ensure that the measured light intensity is less than the recommended threshold, 10 mW/cm^2.
3. Load the purified RMR protein onto a glass slide within a dual-beam optical tweezer system, the latter of which is equipped with an inverted microscope and an additional 532-nm laser source. Utilize the 532-nm laser to trigger the liquid-to-solid phase transition of protein condensates.
4. Nuclear localization sequence (NLS).
5. Use the THORLABS Photometer to determine the intensity of the laser beam before the photo-induced phase transition of RMR condensates in living cells.
6. Perform the FRAP assay to evaluate the material properties of RMR condensates in living cells [14]. Use the 561-nm laser

beam (100% power and 10-s irradiation) to quench the fluorescence. Quantify the fluorescence intensity using the NIS-Elements software.

Appendix

1. Amino acid sequence of RGG-Mfp-3-RGG.

MKGSSHHHHHHVEASESNQSNNGGSGNAALNRGGRY
VPPHLRGGDGGAAAAASAGGDDRRGGAGGGGYRRGGGN
SGGGGGGGYDRGYNDNRDDRDNRGGSGGYGRDRNYEDR
GYNGGGGGGGNRGYNNNRGGGGGGYNRQDRGDGGSSN
FSRGGYNNRDEGSDNRGSGRSYNNDRRDNGGDGVDGGG
GSELADYYGPKYGPPRRYGGGNYNRYGRRYGGYKGWNNG
WKRGRWGRKYYTSGGGGSLEESNQSNNGGSGNAALNRGG
RYVPPHLRGGDGGAAAAASAGGDDRRGGAGGGGYRRGG
NSGGGGGGGYDRGYNDNRDDRDNRGGSGGYGRDRNYE
DRGYNGGGGGGGNRGYNNNRGGGGGGYNRQDRGDGG
SSNFSRGGYNNRDEGSDNRGSGRSYNNDRRDNGGDGLE.

2. Amino acid sequence of LvWSCP.

INDEEPVKDTNGNPLKIETRYFIQPASDNNGGGLVPAN
VDLSHLCPLGIVRTSLPYQPGLPVTISTPSSSEGNDVLTNTN
IAITFDAPIWPCPSSKTWTVDSSSEEKYIITGGDPKSGESFFRI
EKYGNGKNTYKLVRYDNGEGKSVGSTKSLWGPALVLNDD
DDSDENAFPIKFREVDT.

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