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# Supplementary Information

## Glycation regulates phase separation by attenuating electrostatic interactions and increasing hydrophobic interactions

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## Experimental Materials and Methods

### Sample preparation

K9 and R9 peptides were purchased from SciLight Biotechnology, LLC; FITC-K9 and R9 peptides were purchased from GL Biochem (Shanghai) Ltd.; yeast total RNA was purchased from Solarbio; RNase-free water was purchased from Meilunbio; Methylglyoxal (MG) was purchased from Virtue-Clara; 1,6- hexanediol was purchased from Aladdin.

### Peptide glycation

K9 or R9 peptides were dissolved in PBS containing 100 mM MG and incubated at 37 ℃ for 6 days. After 6 days, the peptide was purified by RP-HPLC (Shimadzu, LC-6AD) with C18 column. A: 100% water +0.06% TFA, B: 20% water +80% acetonitrile +0.06% TFA. All solvents are RP-HPLC grade. The dried pure products were obtained by lyophilizing. The identification of all peptides was confirmed by low resolution ESI-MS (Thermo Scientific, Ultimate 3000 + MSQ Plus).

### Turbidity assay

Peptides and yeast total RNA was dissolved in DEPC-treated RNase-free water to get the stock solution. The concentration of peptide is 20 mM and the concentration of RNA is 1.2 mg/mL. During the experiment, various stock solution was diluted into DEPC-treated RNase-free water to the final concentration, mixed well and then 30 μL were taken into plastic 384-well plates (Corning, #3701) respectively, with 4 parallel wells set up in each group. Turbidity was measured immediately after droplet formation using Synergy 4 Plate Reader (BioTeck, Winooski, VT, USA) by absorption at 600 nm.

### Optical microscopy

Image from confocal microscopy was performed with an inverted Carl Zeiss LSM 780 microscope equipped with lasers of 488nm excitation. Images were acquired using a Zeiss 100× oil immersion lens. Stock solution was diluted into DEPC-treated RNase-free water to the final concentration, mixed well and then 30 μL were taken into glass bottom black wall 384 well plates (Cell-vis, #P384-1.5H-N) for imaging. For fluorescence imaging, FITC-labeled peptides and un-label peptides were used as 1:20 molar ratio.

### RFAP

Experiments were performed on a Carl Zeiss LSM 880 microscope equipped with a 100x oil immersion objective. Droplets label with FITC- were bleached by a 488 laser at 100% power for 30 iterations, and post bleaching images were taken at 2% laser power at rate of 1.032s. For each time point, at least three droplets were bleached either in each frame or in several frames. Before repetitive bleaching, 5 images were taken. The fluorescence emission intensity of the ROI was monitored at 510-589 nm.

### Glycation of peptides

Glycated peptides were prepared by incubating 5 mg of peptides with 100 mM MG in DEPC-treated RNase-free water at 37℃ for 5 days. The peptides were purified by RP-HPLC (Shimadzu,

LC-20AT) with preparative C18 column. The collected solutions with purified peptide were lyophilized to get peptide powder.

**Fluorescence analysis**

The peptide samples were diluted with PBS to 0.1 mg/ml, and 200ul of each sample was added to a 96 well glass bottom plate (Cell-vis, #P96-1.5H-N). The fluorescence analysis was scanned from 375 to 500 nm, after excitation at 340 nm by Synergy 4 Plate Reader (BioTeck, Winooski, VT, USA).

***E. coli* expression system**

The plasmids of GFP and short cationic peptide (GGSKKRKKRGGSKKRKKRGGSKKRKKR) were purchased from Tsingke Biotechnology Co., Ltd. Glycerol stocks of BL21(DE3) cells (Tiangen) transformed with each GFP variant were streaked onto LB agar plates with added kanamycin (50 μg/ml). A single colony was inoculated into sterilized LB media supplemented with kanamycin (50 μg/ml) and grown overnight at 37°C with shaking at 225 rpm. Cultures were induced with 1 mM isopropyl β-D−1-thiogalactopyranoside (IPTG, Gold Biotechnology) and then kept at 25 °C with shaking at 225 rpm for 24 h. For the glycation group, MG was added 8 hours before microscopic observation to a final concentration of 100 mM.

For fluorescence imaging, 30 μL were taken into glass bottom black wall 384 well plates (Cell-vis, #P384-1.5H-N) and imaging with an inverted Carl Zeiss LSM 780 microscope equipped with lasers of 488nm excitation. Images were acquired using a Zeiss 100× oil immersion lens.

Additional images are shown in Fig. S4A.

## Supplementary Figures

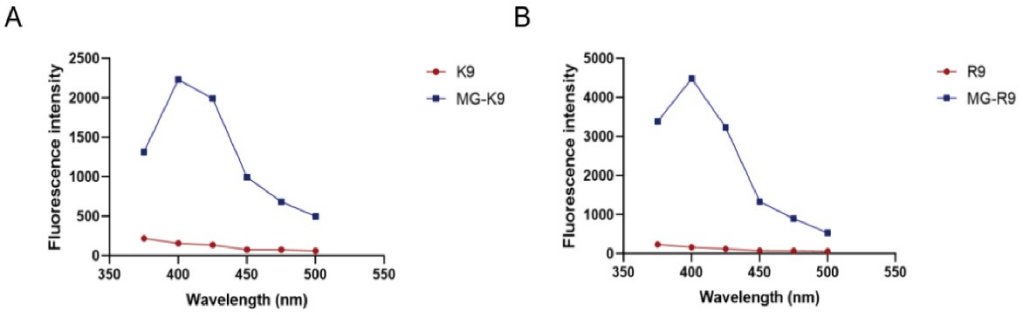


Figure S1 Fluorescence analysis of peptides glycation. A. Fluorescence analysis at Ex 340 nm and Em 375–500 nm of K9 and MG-K9. B. Fluorescence analysis at Ex 340 nm and Em 375–500 nm of R9 and MG-R9.

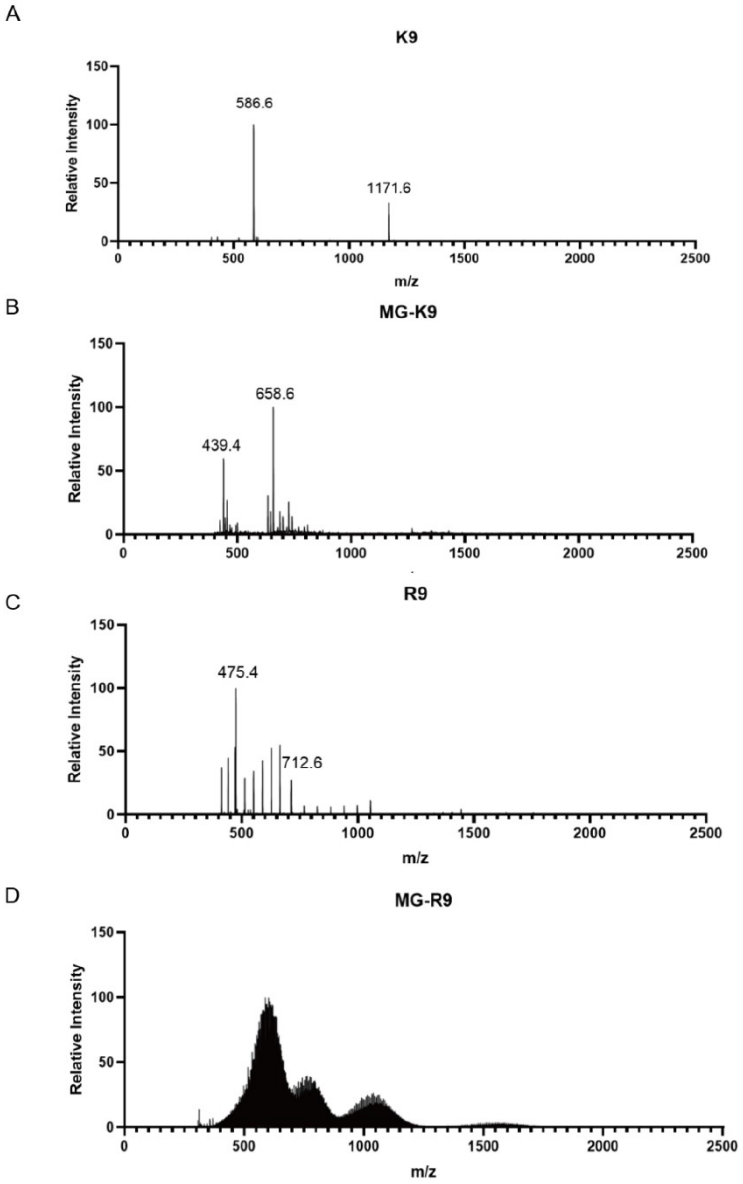

Figure S2 Characterization of the molecular weight of peptides. Mass spectrum of K9/MG-K9/R9/MG-R9 after deconvolution of ESI mass spectrum. A. K9 Calculated Mass: 1171.59; Experimental Mass: 1171.6. B. MG-K9 is a mixture of several glycation products, with approximately 4 modified side chains. C. R9 Calculated Mass: 1423.71; Experimental Mass: 1,423.2. D. MG-R9 is a mixture of several glycation products, with a molecular weight of approximately 2500-3000. About 9 side chains were modified.

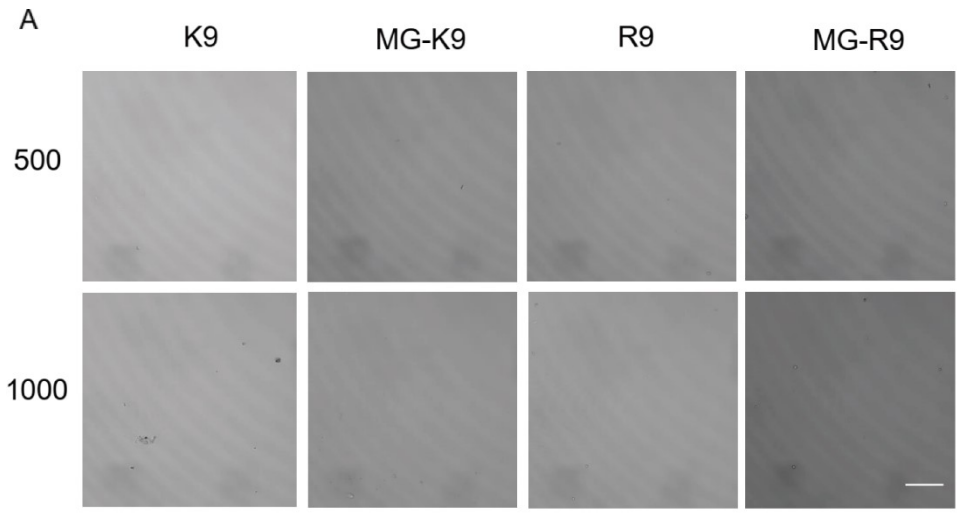


Figure S3 Bright field images of individual peptides at different salt concentrations. The concentration of peptide is 0.4 mM. Scale bar, 10 μm.

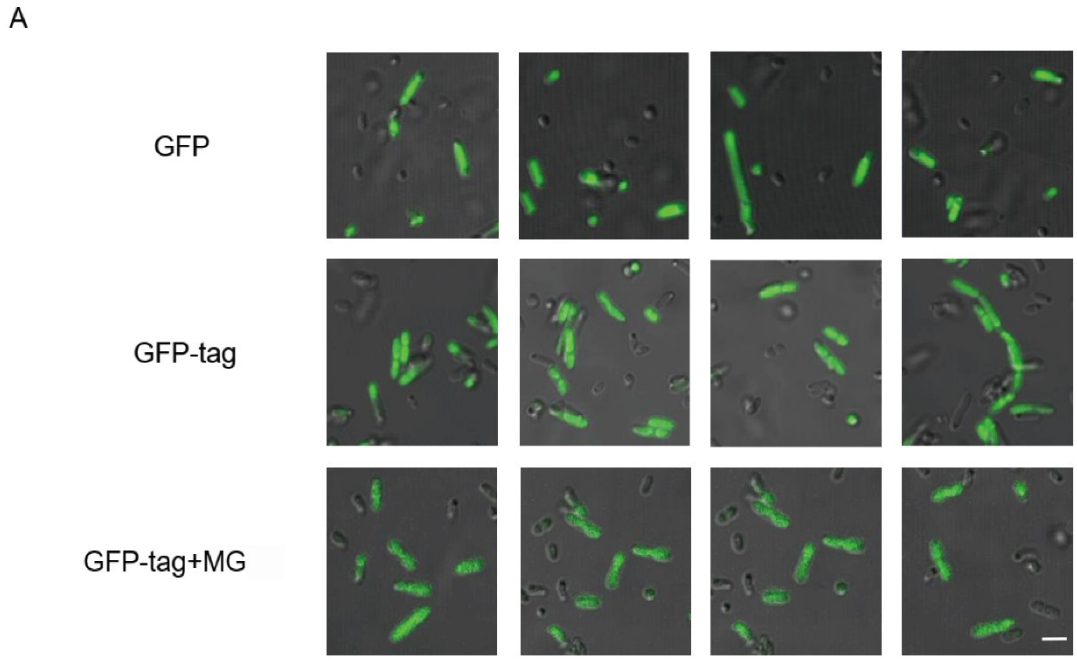


Figure S4 Additional photos of the intracellular system. Scale bar, 1 μm.