Supporting Information

Minimalist Design of an Intrinsically Disordered Protein-Mimicking Scaffold for an Artificial Membraneless Organelle

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Materials and Methods

No unexpected or unusually high safety hazards were encountered.

Materials

Dextran from *Leuconostoc* spp. (approximate MW: 6, 40, 550 and 2000 kDa), PEG 8000 (as the crowding agent) and Dextranase from *Chaetomium erraticum* were purchased from Sigma-Aldrich (USA). Model RNAs, including polyuridylic acid potassium salt (polyU, *ca.* 2.47-3.09 kb), polyadenylic acid potassium salt (polyA, *ca.* 0.6-4 kb), tRNA from baker’s yeast (*ca.* 0.071-0.084 kb), were purchased from Sigma-Aldrich (USA). Horseradish peroxidase (HRP) were purchased from Sigma-Aldrich (USA). THE RNA storage solution, RNaseZap RNase decontamination wipes, RNasesecure RNase inactivation reagent (for the treatment of solution) and nuclease-free water were purchased from Thermo Fisher Scientific (USA). Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) and hydrogen peroxide (3% solution) were purchased from Thermo Fisher Scientific (USA). Quant-iT RiboGreen RNA Assay Kit was purchased from Thermo Fisher Scientific (USA). FITC-PEG1000-SH (purity>95%) was purchased from Shanghai Ponsure Biotech (China). All the other chemicals were purchased from Sigma-Aldrich (USA) unless otherwise specified. Peptides were synthesized via solid-phase peptide synthesis method and purified (purity>95%) with high-performance liquid chromatography (HPLC) by Sangon Biotech (China). The identities of peptides were double confirmed by mass spectroscopy (Figure S2e) using mass spectrometer (Bruker Daltonics UltraflleXtreme MALDI TOF/TOF Mass-spectrometer, USA).

Methods

Quantification of DB of dextran
DBs of dextran were quantified from \(^1\)H NMR (on 500 MHz Varian NMR spectrometer, USA, dissolved at 10 mg/mL in deuterium oxide) by comparing integrations of branching peaks with main chain peak\(^1\).

**Synthesis of Dex-VS with desired DM**

Dex-VS was synthesized following an one-step Michael-addition click chemistry route\(^2\). 300 mg dextran was dissolved in 30 mL ultrapure H\(_2\)O (UPW). Under rigorous stirring, 120 μL NaOH (5M) was added to adjust the pH to alkaline condition ([OH\(^-\)] = 20 mM, pH=12.3). 669 μL DVS (1.2 times stoichiometrically excess to hydroxyl on dextran) was added quickly into solution. After certain reaction time, 126 μL HCl (5M) was quickly added (1.05 times stoichiometrically excess to NaOH) to adjust the pH to mild acidic condition (pH=3.0) to terminate the reaction, followed by dialysis against 3 L UPW using dialysis tubing (Spectra/Por Dialysis Membrane, Spectrum Labs, USA) for 5 days with 10 times UPW replacement. The MW cut-offs (MWCOs) for 6 kDa dextran and the others are 1 kDa and 3.5 kDa, respectively. Purified Dex-VS aqueous solution was lyophilized and stored in −20 °C for further usage.

DM, defined as number of conjugated VS groups divided by number of repeating units\(^2\), was quantified from \(^1\)H NMR by comparing integration of VS \(^1\)H peaks with integration of dextran \(^1\)H1 peak.

**Determination of DM of IPH to mimic IDPs**

DM of peptides of IPH was defined as:

\[
DM_{\text{peptides}} = \frac{\text{Number of repeating units modified with peptides}}{\text{Total number of repeating units}}
\]  
(S1)
Within the LCDs of IDPs, the patterning of RACs was quantified by average spacing of RAC ($\bar{L}$):

$$\bar{L} = \frac{X}{n} - 1 \quad (S2)$$

To mimic the patterning of RACs, DM of IPH was designed as:

$$DM = \frac{1}{\bar{L} + 1} \quad (S3)$$

where

$X$: Number of residues of LCD

$n$: Number of RACs harbored by LCD

**Synthesis of IPH**

The chemistry of synthesis is thiol-Michael addition reaction between thiol groups (from cysteine) and VS groups. NaCl (3 M) solution in 25 mL flask was pretreated for 30 min under N$_2$ flow, followed by peptides dissolution and 10 min N$_2$ flow treatment for potential disulfide bond reduction. Subsequently, Dex-VS was added and dissolved under rigorous stirring, followed by acidity adjustment to pH=6.0 for reaction initiation with minute amount of NaOH (5 M). N$_2$ flow was maintained for another 10 min, then the flask was sealed under stirring for 24 h-reaction. The materials were then transferred to dialysis tubing (MWCO=3.5 kDa) against 3 L UPW for 5 days with 10 times UPW replacement. After dialysis, the IPH aqueous solution was diluted for 4 times using UPW, followed by lyophilization and –20 °C storage for further usage. The structural identity was characterized with $^1$H NMR (dissolved at 10 mg/mL in deuterium oxide hereafter unless otherwise specified). DMs of peptides were quantified by comparing integrations of peptide characteristic peaks with integration of dextran $^1$H1 peak.
For a specific batch synthesis, 15 mg Dex-VS was dissolved at 2 mg/mL. The total molarity of peptides was 1.5 times stoichiometrically excess to [VS] of Dex-VS. The Y/R of IPH was controlled by tuning the molar feed ratio of reactant peptides ($FR_p$), namely,

$$FR_p = \frac{n_{CGGSYGYS}}{n_{CGGRGG}}$$

(S4)

Note that IPH* corresponds to the IPH that we are of the most interest, which mimics FUS protein in terms of triple aspects, namely, number of repeating units (by MW), motif patterning (by DM) and Y/R (by ratio of conjugated peptide), to the most extent. Dextran (40 kDa) was modified with VS groups at 37.9 % DM, followed by peptide conjugation at Y/R=1.03 (Figure S3a).

**Synthesis of IPH* with FITC labeling**

A two-step one-pot synthesis method was applied. 5 mL NaCl (3 M) aqueous solution in 10 mL flask and FITC-PEG1000-SH solution (in DMF at 5 mg/mL) was pretreated for 30 min under N$_2$ flow, followed by Dex-VS (10 mg) addition under rigorous stirring until complete dissolution (at 2 mg/mL). Subsequently, 90.6 μL FITC-PEG1000-SH solution (feeding fluorophore was 1.25 times stoichiometrically excess to IPH) was added very slowly (in 5 μL small aliquots), followed by pH adjustment to 6.0 using minute (around 0.15 μL) NaOH (5 M) and a 30-min reaction under rigorous stirring and N$_2$ flow. Peptides were then pre-mixed, added and dissolved, followed by pH adjustment to 6.0 using minute (around 0.15 μL) NaOH (5 M) and a 10-min reaction under rigorous stirring and N$_2$ flow. The materials were then transferred to dialysis tubing (MWCO=12-14 kDa) against 3 L UPW for 5 days with 10 times UPW replacement. After dialysis, the IPH aqueous solution was diluted for 4 times using UPW, followed by lyophilization and −20 °C storage until further usage. The structural identity was
characterized with $^1$H NMR and DMs of peptides were quantified by comparing integrations of peptide characteristic peaks with integration of dextran $^1$H1 peak. Dark condition was maintained for the whole synthesis, characterization and sample storage process. $^1$H NMR data was comparable with unlabeled IPH* (data not shown). The F/P (fluorophore/IPH) ratio was determined to be 0.461 by quantification of fluorescence intensity of fluorophores.

**LLPS formation from IPH**

For a prototypical preparation of IPH* physiological-mimicking solution, the stock solution (50 x) of IPH* was prepared by dissolving IPH* in UPW at 34.9 mg/mL (approximately 300 µM). IPH* stock solution was stored in −80 °C as single-use aliquots. For sample preparation, IPH* stock solution was heated to 70 °C and incubated for 2 min in sealed centrifuge tubes, followed by dilution in 25 °C intracellular physiology-mimicking buffer (IPM buffer, as the default buffer unless otherwise specified, containing 150 mM NaCl, 10 mM HEPES, 10 wt% PEG 8000, pH=7.4) at 0.697 mg/mL (approximately 6 µM), brief vortex mixing and 15-min incubation at 25 °C before characterization. For reference, intracellular concentration of FUS protein in HeLa cells$^3$ and Sf9 cells$^4$ ranges from 1 µM to 8 µM (0.0534 mg/mL to 0.427 mg/mL).

For the other IPHs with different structure, the stock (50x) concentration was used differently, whilst the rest of protocols are identical. For an IPH with different DM and Y/R, the stock (50 x) was prepared at 300 µM molarity. For an IPH with different MW, the stock (50 x) was prepared at 34.9 mg/mL mass concentration. Note that at physiology-mimicking mass concentrations (0.697 mg/mL), IPH with all MWs are considered as under dilute polymer solution regime, as critical concentration $c^*$ of dextran can be determined based on intrinsic
viscosity $^5 \eta \text{ via } c_\eta^* = \frac{2.5}{\eta}$ relationship$^6$. For dextran used in this paper, 0.697 mg/mL $\ll c_{\eta, \text{min}} = c_{\eta, \text{Dex-2000k}} = 41$ mg/mL.

For LLPS formation under different solvent conditions (salt, pH and crowding agent), the investigated parameter was modulated while the rest are fixed at physiology-mimicking conditions.

**Turbidimetry assay**

Turbidimetry assays were performed on microplate reader (Varioskan LUX multimode, Thermo Fisher Scientific, Finland), wherein 40 µL solution was loaded to 384-well plate (polystyrene, F-bottom, clear, non-binding, Greiner Bio-One, Germany). Absorbance at 600 nm (A600) was measured as the quantification of turbidity. Each individual sample preparation was triplicated. Turbidity for each well is taken by averaging 3 times of measurement with blank corrected.

**1,6-Hexanediol assay**

1,6-Hexanediol disruption-recovery assay was conducted for the characterization of lability of IPH assemblages. For 1,6-hexanediol disruption assay, 1,6-hexanediol was introduced as the last component to the IPH solution. For 1,6-hexanediol recovery assay, IPH, disrupted with 16 wt% 1,6-hexanediol, was transferred to the dialysis tubing (in 0.5-1 kDa MWCO tubing) against 3 L UPW for 5 days with 10 times UPW replacement. After dialysis, the IPH aqueous solution was diluted for 4 times using UPW, followed by lyophilization and sample dissolution in buffer (containing 150 mM NaCl, 10 mM HEPES, pH=7.4). We assume complete retention of PEG 8000 and IPH during the dialysis process owing to large size difference from MWCO.


**Microscopic imaging**

Imaging under room temperature was performed on confocal microscope (Leica TCS SP8, Germany) using 100x oil objective lens by loading 10 μL of sample solution to a confocal dish. Imaging under heated conditions was performed on Nikon C2 confocal microscope with 60x lens using 500 μL solution loaded onto a sealed confocal dish. All dishes were passivated with Pluronic F-127 (10 wt%) for 1 hour, prior to the experiment, to lower material adhesion unless otherwise specified.

**Fluorescence recovery after photobleaching (FRAP)**

FRAP experiments were performed on the laser scanning confocal microscope (Nikon C2, Japan) using NIS-Elements software. Photobleaching was performed using 488nm Argon laser with 50% intensity. For photobleaching within droplet, circular bleached spots with 2 μm diameter within ~6–8μm droplets were used for measurements, while for full droplet bleaching, entire size of the droplet was bleached. Fluorescence intensity changes over time following bleaching were recorded for 3 different regions of interest (ROIs, including bleached spot, reference, and background) at 1% laser intensity until fluorescent signals in bleached region recovered and reached an equilibrium state.

Data analysis was performed following reported protocol. Values were first background subtracted and corrected for any photofading during image acquisition:

$$I(t) = \frac{F(t)R_i}{F_iR(t)} \quad (S5)$$

where
\( F_i \): initial intensity of bleached ROI

\( F(t) \): intensity of bleached ROI at time \( t \)

\( R_i \): initial intensity of reference ROI

\( R(t) \): intensity of reference ROI at time \( t \)

\( I(t) \): corrected intensity of bleached ROI.

Full-scale normalization was performed on relative fluorescence intensity data, assigning minimum bleached intensity to 0 and pre-bleach intensity to 1.

\[
I_{\text{norm}}(t) = \frac{I(t) - I_0}{I_i - I_0} \quad (S6)
\]

\( I_0 \): corrected intensity of ROI immediately after bleaching

\( I_i \): corrected initial (pre-bleach) intensity of ROI

Normalized data was fitted to single exponential equation using MATLAB:

\[
I_{\text{norm}}(t) = A(1 - e^{-\frac{t}{\tau}}) \quad (S7)
\]

where \( \tau \) is the recovery time constant and \( A \) is constants, corresponding to amplitude of recovery. Half-time recovery timescale was further obtained using \( \tau \) value:

\[
t_{1/2} = \tau \cdot \ln 2 \quad (S8)
\]

The apparent diffusion coefficient (\( D_{\text{app}} \)) was obtained using Axelrod model equation for 2D-diffusion\(^8\):

\[
D_{\text{app}} = \frac{\gamma_D w^2}{4t_{1/2}} \quad (S9)
\]

With \( w \) being the radius of bleached region, and \( \gamma_D \) laser-beam shaped-dependent constant value, which equals 0.88 for uniform circular beam.
**Fusion from optical tweezer**

Controlled coalescence of suspended IPH* droplets were conducted using dual optical trap (m-Trap™, LUMICKS). Droplet sample was prepared at 6 µM of IPH and incubated for 15 mins under room temperature. Samples were loaded into 1.0 x 1.0 cm chamber (Gene Frame) fixed on a glass slide treated with 10% Pluronic-F. IPH* droplets were trapped with a 1064 nm laser at 20 % laser power, that was found to be optimal for stable trapping of our droplets. Trapping of the suspended droplets was achieved owing to a difference in refractive index between the IPH* droplets and buffer. After trapping, one droplet remained at stationary Trap-2 while another droplet on Trap-1 were set to move at a constant velocity of 0.13 µm/s until surfaces of both droplets were brought into contact. The Trap-1 movement was stopped once trapped droplets fused and relaxed back to a spherical share. Time series were recorded for each fusion event.

**Quantification of RNA concentration of stock**

The concentration of RNA stock solution was determined on NanoVue Plus spectrophotometer (Biochrom, USA). Measurement was triplicated while the average value was taken.

**Enrichment of RNA/protein from artificial MO**

Droplets from IPH were pre-formed and incubated for 15 min as aforementioned in ‘LLPS formation from IPH’ section. The polyU was first selected as the model RNA owing to its random coil structure⁹. For RNA enrichment, RNAs in the stock solution (c.a. 50x concentrated, in RNA storage solution) was pipetted into IPH solutions, followed by gentle pipette mixing and 15-min incubation. Methylene blue (MB), the staining reagent that binds to nucleic acids¹⁰,
was then incorporated last at 10 μM concentration and incubated for 15 min before imaging, given that MB per se shows no evident preferential partitioning in IPH* solution (Figure S8a). MB-stained RNA was visualized using diode laser line at 638 nm. Nuclease-free condition was maintained for the whole process of RNA-related experiments following technical bulletin provided by Thermo Fisher Scientific (USA). GFP was selected as the model protein owing to the negative charge at physiological pH and innate fluorescent property. For protein enrichment, GFP in the stock solution (c.a. 50x concentrated) was pipetted into IPH droplet solutions, followed by gentle pipette mixing and 15-min incubation before imaging. Samples were excited by a 488-nm laser to visualize partitioning behavior of GFP.

The quantification of enrichment of cargoes was performed based on the volume fraction determination of droplets. IPH* droplets were pre-formed in IPM buffer followed by loading cargoes at desired concentration, bringing the IPH concentration to 6 μM at a final volume of 200 μL. Droplets with loaded cargoes were incubated for 15 mins to ensure equilibrium. Condensed (droplet) phase was separated from the dispersed phase via centrifugation (21,000 x g, fixed 25 °C, 15 min) and concentration of cargoes in the dispersed phase ([Cargo]_{dis}) was determined by comparing with established standard curves (with blank corrected), whilst this method has been widely reported\textsuperscript{11–18}. The volume fractions of the condensed phase $\phi_{\text{con}}$ were estimated using 3-D rendering (Figure S9) of the sample images following Ghosh et al\textsuperscript{19}. We define dimensionless concentration ($DC$) in this partitioning system as:

$$ DC = \frac{[\text{Cargo}]}{[\text{Cargo}]_{\text{ctrl}}}. \quad (S10) $$

‘ctrl’ means the control system without any partitioning (namely, without IPH*).

Based on mass balance, $DC$ of cargoes in the condensed phase, $DC_{\text{con}}$, was determined by:

$$ DC_{\text{con}} = \frac{1-(1-\phi_{\text{con}})DC_{\text{dis}}}{\phi_{\text{con}}} \quad (S11) $$
and \textit{Enrichment} was determined as:

\[
\text{Enrichment} = \frac{DC_{\text{con}}}{DC_{\text{dis}}} \quad (S12)
\]

The experimental data of cargoes enriched, namely, GFP, polyU and HRP, were summarized as Table S1, which is comparable to the previous report\(^9,11,20,21\).

\textbf{Catalysis of reaction from HRP}

To investigate the possibility to carry out biochemical reactions inside droplets, enzymatic activity of HRP was evaluated. Amplex Red was selected as a probe, which undergoes HRP mediated oxidation in the presence of hydrogen peroxide (H\(_2\)O\(_2\)) substrate, forming resorufin with fluorescence generated. Reaction was performed by first loading 100 ng/ml HRP and 50 \(\mu\)M Amplex Red into preformed IPH* droplets in IPM buffer, followed by 15 mins incubation for equilibrium of the loaded components. Sample was then deposited onto a confocal dish, and area to monitor the reaction was located under the microscope. To initiate the reaction, 1 \(\mu\)L of 500 \(\mu\)M H\(_2\)O\(_2\) was added into the confocal dish without moving the sample and time lapse images were acquired immediately afterwards.

Imaging was performed on Nikon C2 scanning confocal microscope under excitation by 561 nm laser (0.35\% power) using a 60x oil immersion lens. Time series were recorded with an interval of 60 s right after addition of hydrogen peroxide at 25 °C.

For the quantification of fluorescence intensity change inside and outside of the droplet, images were analysed by Fiji software. Fluorescence signal change was recorded in different droplets (n=6) and in different regions of the exterior (n=6), obtained from one single experiment.

To determine the global reaction rate of HRP catalysis, time-dependant absorbance change of resorufin at 560 nm was recorded on a 384 well-plate. Reaction rate was determined by:
\( v_0 = \frac{\Delta A}{\Delta t \cdot \varepsilon \cdot d} \)  \hspace{1cm} (S13)

\( \frac{\Delta A}{\Delta t} \): change in absorbance at 560 nm over given time

\( \varepsilon \): extinction coefficient of resorufin product (54000 M\(^{-1}\) cm\(^{-1}\))

\( d \): pathlength of light (0.42 cm)

To determine the \( V_{\text{max}} \) in the condensed phase and dispersed phase, we used H\(_2\)O\(_2\) with a concentration of 500 μM which satisfies \([S] \gg K_M\) conditions\(^{22,23}\), and 1mM of Amplex Red. Change in fluorescence signal was traced under Nikon C2 scanning microscope using the same settings and quantified by Fiji software.

We compare \( V_{\text{max}} \) and \( k_{\text{cat}} \) of the condensed phase and dispersed phase in the initial stage of the reaction (Figure S10).

\[ v_0 = V_{\text{max}} = k_{\text{cat}} [E]_0 \]  \hspace{1cm} (S14)

\[ \frac{V_{\text{max,con}}}{V_{\text{max,dis}}} = \frac{(k_{\text{cat}}[E]_0)_{\text{con}}}{(k_{\text{cat}}[E]_0)_{\text{dis}}} = \frac{v_{0,\text{con}}}{v_{0,\text{dis}}} \]  \hspace{1cm} (S15)

\[ \frac{k_{\text{cat,con}}}{k_{\text{cat,dis}}} = \frac{V_{\text{max,con}}}{V_{\text{max,dis}}} \frac{[E]_0,\text{dis}}{[E]_0,\text{con}} \]  \hspace{1cm} (S16)
Supporting Figures

| a | MW [hD] | NMR | DB [%] | b | Reaction time [s] | NMR | DM [%] |
|---|---|---|---|---|---|---|---|
| 6 |  | | 5.15 | 240 | | 9.91 |
| 40 |  | | 4.90 | 720 | | 22.3 |
| 550 |  | | 5.13 | 1440 | | 37.9 |
| 2000 |  | | 5.08 | 2160 | | 50.2 |

| c | MW [hD] | Reaction time [s] | NMR | DM [%] |
|---|---|---|---|---|
| 6 | 1200 | | 39.7 | |
| 550 | 1504 | | 38.3 | |
| 2000 | 1470 | | 37.3 | |

* 2x D/D' molar excess was applied for this synthesis

(caption on next page)
**Figure S1. Characterization of dextran and Dex-VS.** (a) Quantification of DB of dextran from $^1$H NMR data. DBs were quantified by comparing the sum of integrations of peak B, peak C and peak D ($\delta=5.11, 5.18$ and $5.32$ ppm, respectively) with peak A ($\delta=4.98$ ppm). Peak A, peak B, peak C, and peak D corresponds to $\alpha$-1,6 linkage (Main chain), $\alpha$-1,2 linkage (1,2-Branching), 2,6-di-O-substituted glucopyranosyl unit and $\alpha$-1,3 linkage (1,3-Branching), respectively. $DB = \frac{\int (B+C+D)}{\int A}$. The results indicate DB=5% (r.e. $\leq$ 3%) for dextran with different MWs examined. The distribution of branching loci along the backbone was not studied in this paper. (b) Quantification of DMs of Dex40-VS from NMR data. (c) Quantification of DMs of Dex-VS with different MWs from NMR data. DMs were quantified by comparing the sum of integrations of peak E, peak F and peak G ($\delta = 6.3, 6.4$, and $6.9$ ppm, respectively, corresponding to double bonds of free VS) with peak A ($\delta=4.98$ ppm). $DM = \frac{\int (E+F+G)}{3 \int A}$. 
### Table a

| Protein | Length of full-length protein [residues] | Length of LCD [residues] | LCD [% of protein] |
|---------|-----------------------------------------|--------------------------|--------------------|
| FUS     | 526                                     | 214                      | 9.70               |

### Table b

| Protein | Length of full-length protein [residues] | Ty [residues] | Arg [residues] | Y/R |
|---------|------------------------------------------|--------------|---------------|-----|
| CELF4   | 485                                      | 14           | 17            | 0.826 |
| CSTF2   | 577                                      | 7            | 41            | 0.171 |
| CSTF2L  | 616                                      | 7            | 40            | 0.175 |
| DAZ1    | 744                                      | 48           | 29            | 1.95  |
| DAZ2    | 438                                      | 46           | 13            | 3.54  |
| DAZ3    | 438                                      | 46           | 13            | 2.92  |
| DAZ4    | 390                                      | 38           | 13            | 0.778 |
| DAZAP1  | 406                                      | 14           | 18            | 0.933 |
| ENSRY1  | 656                                      | 42           | 45            | 0.973 |
| FUS     | 526                                      | 36           | 37            | 0.938 |
| hnrNPA0 | 305                                      | 15           | 16            | 0.950 |
| hnrNPA1a| 320                                      | 12           | 24            | 0.760 |
| hnrNPA1b| 372                                      | 10           | 25            | 0.545 |
| hnrNPA2| 353                                      | 22           | 25            | 0.690 |
| hnrNPA3 | 378                                      | 22           | 29            | 0.799 |
| hnrNPA4 | 327                                      | 21           | 13            | 1.50  |
| hnrNPD1| 355                                      | 21           | 14            | 1.50  |
| hnrNPD2| 420                                      | 24           | 33            | 0.727 |
| hnrNPH1| 472                                      | 25           | 32            | 0.813 |
| hnrNPH2| 449                                      | 25           | 31            | 0.806 |
| hnrNPH3| 346                                      | 23           | 28            | 0.821 |
| PSPC1   | 523                                      | 6            | 62            | 0.18  |
| RBM14   | 669                                      | 47           | 41            | 1.15  |
| RBM33   | 1170                                     | 11           | 75            | 0.147 |
| SFPQ    | 707                                      | 14           | 60            | 0.233 |
| TAF15   | 589                                      | 54           | 56            | 0.964 |
| TDP43   | 414                                      | 6            | 20            | 0.490 |
| TIA1    | 386                                      | 17           | 13            | 1.208 |
| TIAL1   | 392                                      | 16           | 14            | 1.14  |

### Table c

| Protein | Length of full-length protein [residues] | Length of LCD [residues] | LCD [% of protein] |
|---------|------------------------------------------|--------------------------|--------------------|
| CGGSYSYS | 9                                      | [CH$_2$/CONH]$_2$CGGSYSYS-[CONH$_2$]$_2$ | 921 |
| CGGPRG  | 6                                      | [CH$_2$/CONH]$_2$CGGPRG-[CONH$_2$]$_2$ | 547 |
| CGGKL VYFA | 9                  | [CH$_2$/CONH]$_2$CGGKL VYFA-[CONH$_2$]$_2$ | 982 |

### Diagram e

- CGGSYSYS
- CGGPRG
- CGGKL VYFA

(caption on next page)
Figure S2. Design and identity characterization of oligopeptides. (a) Sequences of FUS protein. LCDs and LCMs are underlined and highlighted, respectively. (b) Determination of DM of peptides by corresponding LCM patterning. (c) Investigation of Y/R of IDPs. The highest and the lowest Y/R values are highlighted in red and green color, respectively. (d) Peptide sequences used in this research. N-terminal acetylation and C-terminal amidation were applied for eliminating associated charging effects. (e) Identification confirmation of peptides from MALDI mass spectroscopy data. CGGSYSGYS: Theoretical MW= 921 Da; Measured MW= 921 Da ([M – H^+] + Na^+]=943 Da; [M – H^+ + K^+]]=959 Da). CGGRGG: Theoretical MW= 547 Da; Measured MW= 547 Da ([M + H^+]= 548 Da). CGGKLVFFA: Theoretical MW= 982 Da; Measured MW=982 Da ([M – H^+ + Na^+]=1004 Da; [M – H^+ + K^+]]=1020 Da; [M_2 – H^+ + Na^+]=1984 Da). M_2 refers to dimers formed via disulfide bond linkages between cysteine residues.
Figure S3. Structural characterization of IPH. (a–d) $^1$H NMR of (a) IPH*; (b) IPH with different DMs; (c) IPH with different MWs; (d) IPH with different Y/R. Peak A ($\delta=4.98$ ppm) corresponds to dextran main chain $^1$H1. DM of CGGSYSYGYS was quantified by comparing integrations of peak H ($\delta=6.86$ ppm, corresponding to $^1$H3,5 of tyrosine) and peak I ($\delta=7.15$ ppm, corresponding to $^1$H2,6 of tyrosine) with peak A, namely, $DM_{CGGSYSYGYS} = \frac{\int (H+I)}{\int A}$. DM of CGGRGG was quantified by comparing integrations of peak J ($\delta=1.70$ ppm. corresponding to $^1$HG of arginine) and peak K, peak L ($\delta=1.79$ ppm and 1.89 ppm, respectively, corresponding to $^1$HB of arginine) with peak A, namely, $DM_{CGGRGG} = \frac{\int (J+K+L)}{4 \int A}$. The total DM of peptides conjugated, $DM_P$, was further compared with the total DM of VS along the dextran backbone ($DM_0$) and the conjugating efficiency ($C.E. = \frac{DM_P}{DM_0}$) indicate good data consistency. Note that for Y/R=$\infty$ sample of (d), DMSO-d$_6$ was used as the solvent, whilst peak A, peak H and peak I shift slightly to $\delta=4.69$, 6.62 and 6.97 ppm, respectively. The (approximate) MW of IPH was quantified from addition of MW of dextran backbone, VS linkage segment and peptide conjugated. (e) Dex-CGGKLVFFA for comparison. DM of CGGKLVFFA was quantified by comparing integrations of peak M, peak N and peak O (peak cluster, $\delta=0.7$-0.9 ppm, corresponding to $^1$HG of valine and $^1$HD of leucine) with peak A, namely, $DM_{CGGKLVFFA} = \frac{\int (M+N+O)}{12 \int A}$.
(caption on next page)
Figure S4. Microscopic images of compartmentalized droplets from IPH*. (a) Size and number of droplets are dependent of concentration and crowding agent conditions. (b) Incorporation of crowding agent can facilitate (no higher than 20 wt%) or hinder LLPS formation (higher than 20 wt%). (c) Droplet formation is dually responsive to ionic strength and pH. Note that for the condition of ‘NaCl 15 mM, pH 3.4’, nanoparticles smaller than 100 nm largely formed but generate no turbidity change. (d) Droplet formation could be disrupted by incorporation of 1,6-hexanediol (HDO), whilst removal of 1,6-hexanediol (HDO)could generate recovery of LLPS. (e) Different concentrations of IPH* solution exhibit LLPS with UCST phase behavior. Scale bars, 20 µm.
Figure S5. Imaging LLPS of IPH with different DM. (a) Turbidity change indicates occurrence of LLPS. (b) Microscale droplets formation. (c-e) Responsiveness of LLPS to ionic strength (c), pH (d) and temperature (e). (f) Reversibility of LLPS to 1,6-hexanediol (HDO). Scale bars, 20 µm.
Figure S6. Imaging LLPS of IPH with different MW. (a) Turbidity change indicates occurrence of LLPS. (b) Microscale droplets formation. (c–e) Responsiveness of LLPS to ionic strength (c), pH (d) and temperature (e). (f) Reversibility of LLPS to 1,6-hexanediol (HDO). Scale bar=20 µm. (g) Time-course cleavage of backbone linkage of IPH. Dextranase was loaded at different mass ratios to IPH, namely, Loading = \( \frac{m_{E,0}}{m_{S,0}} \). These data indicate multivalency is essential for both initiation and maintenance of LLPS. Scale bar=20 µm.
(caption on next page)
Figure S7. Imaging LLPS of IPH with different peptide composition. (a-f) Different Y/R ratio. Turbidity change indicates occurrence of LLPS (a). Microscale droplets formation (b). Responsiveness of LLPS to ionic strength (c), pH (d) and temperature (e). Reversibility of LLPS to 1,6-hexanediol (f). (g–i) Dextran-oligopeptide hybrids using different peptide sequences at DM=37.9 %: Dex40k-CGGKLVFFA (g), Dex40k-CGGNFGAFS (h) and Dex40k-CGGSGYDYS (i). Scale bars, 20 µm.
(caption on next page)
Figure S8. Complexation of RNA with artificial MOs. (a) Microscopic image of IPH* (6 μM, N/P ratio=1:0) and RNA solution (0.697 mg/mL, N/P ratio=1:∞). MB shows no evident partitioning in IPH* solution. No evident assembly forms for all the RNAs tested under microscope. (b) polyU incorporation modulates LLPS propensity of IPH* as indicated by turbidity test. (c–e) Microscopic image of IPH* complexation and preferential recruitment of polyU (c), polyA (d), tRNA (e). 10 μM methylene blue was incorporated for each set of experiments as the staining agent. Scale bars, 20 µm.
Figure S9. 3-D rendering. (a–d) 3-D rendering of IPH* (a) and IPH* loaded with GFP (b), polyU (c) and HRP (d). Scale bars, 20 µm.
Figure S10. Measuring $v_0$ in the condensed phase and dispersed phase when $[S] \gg K_M$. 500 μM of H$_2$O$_2$ was used. (a) Confocal fluorescence imaging. (b) Fluorescence intensity change in the initial stage. n=3. Scale bars, 20 μm.
Figure S11. Overall reaction rate enhancement modulated by extent of LLPS. (a, b) Modulating extent of LLPS (indicated by volume fraction of condensed phase) by changing IPH* concentration as shown by confocal imaging (a) and quantitative analysis (b). (c) Enhancement of reaction was modulated by the extent of LLPS of IPH*. n=3. Scale bars, 20 μm.
| No. | DM of peptides CGGSYSGYS and CGGRGG [%] | MW of dextran backbone [kDa] | Y/R | Note |
|-----|-------------------------------------|-----------------------------|-----|------|
| 1   | 37.9                                | 40                          | 1.03| IPH* |
| 2   | 9.91                                | 40                          | 1.01|      |
| 3   | 22.3                                | 40                          | 0.934|      |
| 4   | 50.2                                | 40                          | 0.997|      |
| 5   | 91.7                                | 40                          | 0.961|      |
| 6   | 39.7                                | 6                           | 0.949| IPH with different DM |
| 7   | 38.3                                | 550                         | 0.989| IPH with different MW |
| 8   | 37.3                                | 2000                        | 0.931|      |
| 9   | 37.9                                | 40                          | 0    | IPH with different Y/R |
| 10  | 37.9                                | 40                          | 0.268|      |
| 11  | 37.9                                | 40                          | 3.25 |      |
| 12  | 37.9                                | 40                          | 0    |      |

Figure S12. Summary of all IPHs studied in this paper.
Figure S13. Mixing of dextran-CGGSYSGY and dextran-CGGRGG with fluorescence labeling. Scale bars, 20 μm.
Figure S14. Estimation of enrichment of cargoes in IPH* droplets via fluorescence imaging. (a) Representative fluorescent images of enrichment of cargoes. (b) Estimated enrichment from fluorescence intensity. At least three representative images have been analyzed, and the ratio between fluorescence intensity in the condensed and the dispersed phase were calculated. The GFP, polyU and HRP cargoes were incorporated at 60 μg/mL, N/P ratio=1 and 0.1 mg/mL. Scale bars, 20 μm.
Supporting Table

Table S1. Quantification of enrichment of cargoes.

| Cargo | Loading (μg/mL) | Methods to determine $D_C$ | $D_C$ (μg/mL) | $\phi_{con}$ (%) | $D_C$ (μg/mL) | Enrichment |
|-------|----------------|-----------------------------|---------------|------------------|---------------|-------------|
| GFP   | 4              | Autofluorescence intensity assay | 67.2 ± 6.13   | 0.44 ± 0.12      | 7520 ± 1388   | 102 ± 29.8  |
| polyU | 120            | RiboGreen assay             | 12.8 ± 1.82   | 0.88 ± 0.325     | 9921 ± 205    | 716 ± 132   |
| HRP   | 0.1            | Amplex Red assay            | 55.9 ± 6.38   | 0.32 ± 0.026     | 13747 ± 2321  | 246 ± 65.5  |
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