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Supplementary information

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Biomolecular condensates with complex architectures via controlled nucleation

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38nt ssDNA

38 nucleotide (nt) long ssDNA and HPLC purified FITC- or Cy5-labeled 38nt long ssDNA were ordered at Integrated DNA Technologies (IDT) and dissolved in Milli-Q to reach a final concentration of 250 μM, of which 2.5 μM was either labelled with FITC or Cy5. Cy5 labelled DNA was used for figure 6 and Ext. Data Fig. 9. In other cases FITC labelled DNA was used. The ssDNA sequence is 5' TTTTTTTTTTCAGTCAGTCAGTCAGTCAGTCCATAAGG.

Amylose modification

Both the quaternized amylose (Q-am) and the carboxymethylated amylose (Cm-am) were prepared in accordance with previously published procedures [1]. Briefly, amylose was dissolved in aqueous NaOH. For Q-am, (3-chloro-2-hydroxypropyl)-trimethylammonium chloride solution (60%, *w/v*, in water) was added dropwise to the stirring reaction mixture, which was subsequently left to react overnight at 35°C. For Cm-am, chloroacetic acid was added and the reaction mixture was left to stir for 2 h at 70°C. Both were purified by precipitation into cold ethanol, followed by extensive dialysis against ultrapure water before lyophilization.

Cm-amylose-Cy3

For the synthesis of Cm-amylose-Cy3, first Cm-amylose was synthesized as described above, to which an azido group was introduced. The Cm-am was dissolved in 10 mM MES buffer, after which sulfo-NHS and EDC were added (Ext. Data Fig. **??**). The reaction was stirred at 30 rpm at room temperature for 20 minutes. 3-Azido-1-propanamine was dissolved in 100 mM KH_2PO_4, pH 8.1 and added to the sulfo-NHS-amylose, which was left to react overnight at 300 rpm. After extensive dialysis against demineralized water, lyophilization yielded white solids. For the labeling reaction, azido-Cm-amylose (12.3 mg, 1 equiv) was dissolved in 40 μL of Milli-Q, to which 10.3 μL of a dimethylsulfoxide (DMSO) solution of DBCO-Cy3 (10 mM, 1 equiv) was added. The reaction was left to react overnight at 300 rmp at 37°C. Extensive dialysis against 20% DMF was executed after which was switched to dialysis against demineralized water. Lyophilization was used to yield a fluffy solid (labeling efficiency was 0.05% of monomers, as determined by UV–vis spectroscopy).

Q-amylose-Cy5

Q-amylose-Cy5 was synthesized as described previously [2]. Briefly, first an azido functional group was introduced to the amylose in accordance with another previously published procedure [3], after which the same cationization protocol as described above was used to yield azido-Q-amylose. For the labeling reaction, azido-Q-amylose (5.3 mg, 0.38 μmol, 10 equiv) was dissolved in 40 μL of Milli-Q, to which 4.55 μL of a dimethylsulfoxide (DMSO) solution of DBCO-Cy5 (10 mg/mL, 0.038 μmol, 1 equiv) was added. The reaction was left to react overnight at 300 rmp at room temperature. Extensive dialysis against 20% DMSO was executed after which was switched to dialysis against demineralized water. Lyophilization was used to yield a glassy blue solid (reaction yield was $\sim$ 80% and the labeling efficiency was 0.14% of monomers, as determined by UV–vis spectroscopy).

Terpolymer

The poly(ethylene glycol)-poly-(caprolactone-γ-trimethylene carbonate)-poly-(glutamic acid) (PEG-PCLgTMC-PGlu) block terpolymer was synthesized as described previously [1]. Briefly, monomethoxy poly(ethyleneglycol) monomethyl ether 2kDa was used to initiate the ring-opening polymerization of ϵ-caprolactone and trimethylene carbonate. The terminal alcohol of this polymer

was subsequently modified by Steglich esterification with Boc-L-Phe-OH to yield a primary amine after TFA deprotection. The final poly(L-glutamic acid) block was introduced by the ring-opening polymerization of *N*-carboxyanhydride γ-benzyl L-glutamate, followed by hydrogenolysis.

Green Fluorescent Protein variants

Three Green Fluorescent Protein (GFP) variants were used; a super folder GFP (GFP), a super-charged GFP with net 30 negatively charged residues (-30GFP), and an eGFP coupled to a 20 nucleotide ssDNA strand (DNA-GFP). The proteins were expressed in *E. Coli* similar to previously described methods [4].

Name	Sequence
GFP	MVKMGASKGEELFTGVVPILVELDGDVNGHKFSVRGEGEG DATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP DHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFE GDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVYITAD KQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLL PDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDEL YKTLPETGENLYFQSGGSHHHHHH
-30GFP	MVKMGASKGEELFDGVVPILVELDGDVNGHEFSVRGEGEG DATEGELTLKFICTTGELPVPWPTLVTTLTYGVQCFSDYP DHMDQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFE GDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHDVYITAD KQENGIKAEFEIRHNVEDGSVQLADHYQQNTPIGDGPVLL PDDHYLSTESALSKDPNEDRDHMVLLEFVTAAGIDHGMDEL YKTLPETGENLYFQSGGSHHHHHH
DNA-GFP (GFP)	MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEG DATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP DHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE GDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMAD KQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLL PDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDEL YKGGC
DNA-GFP (DNA)	CCCTAGAGTGAGTCGTATGA-NH_2

DNA-GFP conjugation

First, the amine modified oligo was coupled to sulfo-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC). In this reaction, 10 μL of a 10 nmol oligo solution (dissolved in RNase free water) was added to 30 μL 1x PBS (pH 7.2) and 40 μL of 100 nmol Sulfo-SMCC (Thermo scientific, dissolved in DMSO). This reaction was stirred at 850 RPM for 2h at 20 °C. Excess Sulfo-SMCC was removed using two rounds of ethanol precipitation. 10% (v/v) 5 M NaCl and 300% (v/v) ice-cold ethanol were added to the SMCC-coupled oligos and incubated for 75 minutes at -30 °C. After incubation, the mixture was centrifuged at 19,000 xg for 30 min at 4 °C and the pellet was reconstituted in 1x PBS (pH 7.2). This procedure was repeated once more. After the final centrifugation the pellet was washed in 95% (v/v, in water) ice cold ethanol, centrifuged at 19,000 xg for 15 min and lyophilized.

For conjugation of eGFP to a SMCC-coupled oligo, eGFP was buffer exchanged to 100 mM Sodium Phosphate, 25 μM TCEP, pH 7 using a PD10 desalting column (GE Healthcare). Subsequently, the desalted eGFP was concentrated to a final concentration of 50 μM using Amicon 3 kDa MWCO centrifugal filters (Merck Millipore). 10 nmol lyophilized SMCC-coupled oligo was reconstituted in 40 μL 50 μM eGFP (2 nmol) resulting in a 5x excess of SMCC-oligo. The reaction was stirred at 850 RPM

for 3h at 20 °C. Purification of eGFP-oligo from excess oligo was performed on a micro-spin column with 200 μL Ni-NTA slurry. The conjugate was eluted with a 20 mM Tris-Cl buffer pH 7.9 with 500 mM imidazole and 500 mM NaCl. The elution fractions were collected and the conjugate concentration was quantified by measuring absorption at 488 nm.

References

1. Mason, A. F., Buddingh', B. C., Williams, D. S. & Van Hest, J. C. Hierarchical self-assembly of a copolymer-stabilized coacervate protocell. *Journal of the American Chemical Society* **139,** 17309–17312 (2017).
2. Van Stevendaal, M. H., Vasiukas, L., Yewdall, N. A., Mason, A. F. & van Hest, J. C. Engineering of biocompatible coacervate-based synthetic cells. *ACS Applied Materials & Interfaces* **13,** 7879–7889 (2021).
3. Tanaka, T., Nagai, H., Noguchi, M., Kobayashi, A. & Shoda, S.-i. One-step conversion of unprotected sugars to β-glycosyl azides using 2-chloroimidazolinium salt in aqueous solution. *Chemical communications,* 3378–3379 (2009).
4. Altenburg, W. J. *et al.* Programmed spatial organization of biomacromolecules into discrete, coacervate-based protocells. *Nature communications* **11,** 6282 (2020).