Supplementary Information for:

Pathway-Controlled Formation of

Mesostructured all-DNA Colloids and Superstructures

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Selected recurring abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ssDNA        | single-stranded DNA |
| dsDNA        | double-stranded DNA |
| GEP          | gel electrophoresis |
| RCA          | rolling circle amplification |
| dxTP         | Deoxynucleotide triphosphates (used as monomers for polymerization, x = A, T, G, C and U(Cy5 labelled) |
| bp           | base pair |
| kbp          | kilo base pair |
| CLSM         | confocal laser scanning microscopy |
| A,           | Polyadenine (Poly(deoxy-adenylic acid)) with a number average degree of polymerization, DP, of x. |

Materials

ssDNA oligomers were purchased from Integrated DNA Technologies (IDT) as summarized in Supplementary Table 1. The enzymes T₄ ligase (low concentration 2 U/μL and high concentration 20 U/μL), Exonuclease I (40 U/μL), Exonuclease III (200 U/μL), Inorganic pyrophosphatase (2 U/μL) and Φ₂₉ polymerase (10 U/μL) were purchased from Lucigen. The Terminal deoxynucleotinyl transferase (TdT, 30 U/μL) was bought from Promega. Deoxynucleotide triphosphate (dATP, dTTP, dGTP and dCTP) 100 μM 1 mL and dUTP-Cy5 1 mM 10 μL were purchased from Jena bioscience. Magnesium acetate tetrahydrate (MgAc₂), sodium chloride (NaCl), calcium chloride (CaCl₂), zinc chloride (ZnCl₂), manganese chloride tetrahydrate (MnCl₂), strontium chloride hexahydrate (SrCl₂), barium chloride dihydrate (BaCl₂), citric acid, sodium citrate, tris(2-carboxyethyl)phosphine hydrochloride, Tween 80, tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl and Trizma buffer substance pH=8), disodium ethylenediaminetetraacetate dehydrate (EDTA) and acetic acid were purchased (as bioreagent grade if available) from Sigma-Aldrich. Agarose Low EEO was purchased from AppliChem. SYBR gold and 50 bp and 1 kbp ladders were purchased from ThermoFisher Scientific. RotiSafe was purchased from Carl Roth. Poly(deoxy-adenylic acid) (250 nucleobases long) was purchased from Roche. Citrate-stabilized gold nanoparticles (radius 10 nm; concentration 6 nM) were purchased from Nanocomposix. Unless stated otherwise, the base of all solutions for particle preparation and assembly is a TE buffer that consists of 10 mM of Tris(hydroxymethyl)aminomethane (pH=8.0) and 1 mM of EDTA. DNA sequences are also stored frozen at -25 °C in TE buffer. Gel electrophoresis (GEP) were run using 2 wt% agarose gels in TAE buffer containing 40 mM of TRIS-HCl, 20 mM of acetic acid and 1 mM of EDTA. Depending on the DNA labelling in GEP (RotiSafe or SYBR gold added before or after performing GEP) the relative motion of the single-stranded RCA product can slightly differ compared to the double-stranded DNA molecular weight ladder. SYBR gold pre-staining was used if not denoted otherwise.
Supplementary Table 1. Sequences used, with their name, the sequence code used for ordering at IDT, the purification grade and modifications.

| Name                  | Sequence 5’→3’                                                                 | Purification | Modification   |
|-----------------------|--------------------------------------------------------------------------------|--------------|----------------|
| **Templates for RCA** |                                                                                   |              |                |
| Tp(A<sub>20</sub>-i)<sup>a</sup> | /5Phos/ATC TAT CCT AAT TTT TTT TTT TTT TTT TTT TGA ACC CGT AT                      | HPLC         | 5’-Phosphorylation |
| Tp(T<sub>20</sub>/j)<sup>a</sup> | /5Phos/ ATC CTC TAA AAT CAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA 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Supplementary Note 1. DNA synthesis

See Methods section for details on the experimental protocol.

Role of the pyrophosphatase during RCA: It is important to add pyrophosphatase in the mixture to avoid the formation of DNA/MgP₂O₇ nanoflowers as illustrated in Supplementary Fig. 1c,d. Note that while nanoflowers were initially thought to consist of pure ssDNA, they actually form through the complexation of ssDNA with magnesium pyrophosphate crystals, a by-product of the nucleoside triphosphate polymerization. Critically, these crystals prevent the rational use of the RCA products for material and self-assembly purpose, and must be avoided.

Direct ligation at high concentration to produce long ssDNA polymers: The strong base pairing between G₂₀ and C₂₀ prevents the RCA of the corresponding templates, a common problem for the enzymatic amplification of such sequences. To overcome this problem we synthesized p(G₂₀-l), p(C₂₀-l) and p(A₂₀-l) via ligation at high concentration (Supplementary Fig. 1e,f). To increase the content of long ligation products over small circular plasmids, the templates (G₂₀-l and C₂₀-l) and the corresponding ligation strands (l) are prepared at high concentration (i.e. 2 mM in TE buffer). The reaction is then run by mixing 4 μL of template and 4 μL of ligation strand, 1 μL of commercial 10 X ligase buffer and 0.5 μL of T₄ ligase (20 U/μL) and leaving it react overnight at room temperature before heat inactivation at 70 °C for 30 min. For comparison we also synthesized p(A₂₀-l) from A₂₀-l template based on the same protocol but with 100 μM starting concentration because ligation with A₂₀-l did not proceed at 800 μM.

Thermal cleavage to reduce the molecular weight: The very high molecular weight of the ssDNA synthesized by RCA makes it difficult to manipulate (see Supplementary Fig. 1i for an example of viscous thread formed during pipetting) and hampers reorganization kinetics at high temperature needed for the formation of particles (Supplementary Fig. 5a,b). We used heat-induced cleavage to reduce the molecular weight of the ssDNA products synthesized via RCA and facilitate their handling (Supplementary Fig. 1j). The effect of annealing p(A₂₀-l) product at 95 °C at 0.1 g/L in TE buffer for 5 to 60 minutes is presented Supplementary Fig. 1g,h. GEP shows a decrease of the average length with the time spent at 95 °C, as quantified via grey scale analysis. Similar effects are observable when p(T₂₀-j) and p(A₂₀-l-XL) are heated to 95 °C (Supplementary Fig. 1k,l). The approximate base number reported for the ssDNA p(A₂₀-l) corresponds to the double of the 50 bp and 1 kbp dsDNA ladder on the side. The thermal treatment dramatically facilitates the manipulation of the RCA products without significantly decreasing their multiblock nature (even after 60 min at 95 °C, the resulting product still contains several repeats of the initial template). The thermal treatment leads to starting materials with similar molecular weights (Supplementary Fig. 1m). We used this strategy to quantify the influence of the molecular weight on the cloud point temperature (Tₜ) in Fig. 2f (main manuscript), and in Supplementary Note 3. See also Supplementary Note 4 for polyadenine model materials with narrow molecular weight distribution.
Supplementary Figure 1: ssDNA synthesis via rolling circle amplification (RCA). (a) Scheme of the entire process and (b) corresponding GEP. A commercial oligomer (A) is circularized using a complementary ligation strand (red) and ligated using T4 ligase (B). Unligated products and ligation strands are then digested by Exonucleases I and III (C), and the cleaned templates are amplified using Φ29 polymerase (red). (c) SEM of “nanoflowers” forming in the absence of pyrophosphatase via complexation of ssDNA with precipitating pyrophosphates. This needs to be avoided by adding pyrophosphatase. (d) Macroscopic appearance of the reaction mixture after 24 h RCA in presence (left) or in absence (right) of pyrophosphatase, showing light scattering and particle formation if the pyrophosphatase is omitted (right). (e) GEP of the ligation product of G20-l, C20-l at 800 μM and (f), A20-l at 40 μM. (g) GEP of the time-dependent thermal cleavage of p(A20-l) at 95 °C in TE buffer together with the linear template p(A20-l). (h) Corresponding grey scale analysis of the gel bands associated with the corresponding base numbers as calculated from the DNA ladders. (i) Photograph of a filament formed between a pipet tip and the edge of an Eppendorf tube representative of the viscous behaviour of the RCA products before thermal cleavage (1 g/L). (j) Schematic representation of the thermal cleavage of the ssDNA RCA product at high temperature. (k) GEP of the of the time-dependent thermal cleavage of p(T20-j) at 95 °C in TE buffer together with the linear template p(T20-j). (l) GEP of the time-dependent thermal cleavage of p(A20-l-XL) at 95 °C in TE buffer together with the linear template p(A20-l-XL). (m) GEP of all starting materials for particle preparation indicates similar molecular weights for all materials. The red bars highlight ssDNA bands (i.e. p(A20-l) and p(T20-j)). Note that we purposely used RotiSafe dye.
for staining, which less efficiently stains ssDNA (p(A$_{20}$-i), p(T$_{20}$-j)), compared to partially double stranded ones (i.e. p(A$_{20}$-i-XL) and p(T$_{20}$-j-XL)), allowing to distinguish both species in this comparative analysis. GEPs are run in TAE buffer using either 2 wt% agarose gel and 6 V/cm for 90 min with SYBR gold pre-staining (b,g) or post-staining (e,f), similarly 0.8 wt% agarose gel, 7.5 V/cm and SYBR gold (k,l) or RotiSafe (m) pre-staining. The 50 bp ladder bands correspond to 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1000 base pairs, and the 1kbp ladder bands correspond to 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000 and 10000 base pairs from top to bottom.
Supplementary Note 2. LCST behaviour: Flocculation vs. coacervation

**General remark:** The lower critical solution temperature (i.e. LCST) is the minimum temperature in the phase diagram of a polymer/solvent system that undergoes phase separation/demixing upon heating (Supplementary Fig. 2a). The two-phase regime includes the binodal and the spinodal domains, which are metastable and unstable regions, respectively. The cloud points, \( T_{cp} \), are located on the binodal curve and correspond to the onset of demixing. LCST behaviour is a thermodynamic term and includes all systems presenting solubility/insolubility transitions (demixing) with increasing temperature, e.g. small molecules, neutral polymers and polyelectrolytes in a solvent, solvent mixture or salt-containing solvent.

Polymer solutions with LCST behaviour need to be distinguished into systems that (i) flocculate or (ii) form so-called coacervates.\(^{10-12}\) In the first case (flocculation), the polymer-rich phase expels most of the water during the phase-separation, resulting in solid precipitates with more fractal-like aggregation.\(^{13}\) In the second case, coacervation leads to water-rich polymer phases that retain some fluidity and which are prone to coalescence during ageing, ending up in spherical domains. PNIPAM, arguably the best known water-soluble polymer with LCST behaviour, belongs to the first class and shows flocculation. On the other hand elastin,\(^{14}\) poly(ionic liquids),\(^{15}\) and more generally polyelectrolytes with LCST behaviour tend to form coacervates.\(^{11,16}\)

The spherical shapes of our ssDNA particles obtained by the heating ramps indicate that the phase transition of purine-rich ssDNA leads to water-rich precipitates (coacervate class of LCST behaviour). We further performed a dedicated coalescence test to support this notion (Supplementary Fig. 2b-d). We use the thermodynamic nomenclature (LCST and cloud point) to promote interdisciplinary understanding.

**Coalescence test:** To test coalescence at high temperature, we performed the experiment depicted in Supplementary Fig. 2c. First, solid core/shell particles are formed by mixing \( p(A_{20-i}-XL) \) (0.2 g/L; non-fluorescent) and \( p(T_{20-k}-XL) \) (0.05 g/L) in TE buffer supplemented with 50 mM of MgAC\(_2\) (total volume 10 μL), and heating to 95 °C for 5 min with heating and cooling ramps of 3 °C/min. Please see the Methods for the details of particle formation.

Second, after the first particle formation process, 10 μL of \( p(A_{20-i}-XL)_{Cy5} \) (0.2 g/L, covalently labelled fluorescent ssDNA) and \( p(T_{20-k}-XL) \) (0.05 g/L) in TE buffer supplemented with 50 mM of MgAC\(_2\) are added to the 10 μL of already formed particles. A new heating ramp (95 °C for 5 min, 3 °C/min ramp speeds) is applied during which particles based on \( p(A_{20+i}-XL)_{Cy5} \) additionally form. After cooling, Janus particles containing domains of both \( p(A_{20-i}-XL) \) and \( p(A_{20+i}-XL)_{Cy5} \) as well as a shell of \( p(T_{20-k}-XL) \) that hybridizes onto these structures during cooling, are observed. Such Janus particles can only form via merging of primary particles, \( p(A_{20+i}-XL) \) and \( p(A_{20+i}-XL)_{Cy5} \). Note that, before CLSM, stoichiometric amounts of corresponding fluorescent barcode* oligomers (Atto488-1* and Atto647-k*) are added to the particle solution and left 1 h at room temperature to hybridize with the respective domains. Those lead to the green cores and red shells, while the covalently attached Cy5 is visible in blue.

**Experimental protocol for covalent dye labelling of the RCA product with Cy5:** The synthesis of the covalently labelled \( p(A_{20+i}-XL)_{Cy5} \) was done as follows: The synthesis protocol was kept exactly the same as described in Methods except that Cy5-dUTP was introduced in the mixture at a 1:10 ratio compared to the dTTPs. This leads to a random incorporation of Cy5-labled nucleobases instead of thymine.
**Supplementary Figure 2: LCST and coalescence test.** (a) Schematic representation of a phase diagram of a binary system presenting a LCST. The cloud point ($T_{cp}$) corresponds to the onset of phase-separation and is located on the binodal curve (blue). (b) Schematic representation of the covalent labelling of an RCA product using Cy5-dUTP in the reaction mixture. The Cy5-dUTPs randomly replace some thymine bases in the ssDNA strand. (c,d) Heating non-labelled p(A$_{20}$-i-XL)/p(T$_{20}$-k-XL) followed by another heating ramp after the introduction of labelled p(A$_{20}$-i-XL)$_{Cy5}$/p(T$_{20}$-k-XL)$_{Cy5}$, and subsequent staining with fluorescent dye-barcode* strands (Atto$_{488}$*-i, Atto$_{647}$*-k*) leads to the observation of few Janus cores particles. The CLSM shows particles where only half of the core is Cy5-labelled, which indicates coalescence events of particles formed by p(A$_{20}$-i-XL) and p(A$_{20}$-i-XL)$_{Cy5}$ at high temperature, and points to LCST behaviour with liquid coacervates.
Supplementary Note 3. Determination of the cloud point temperature (T_{cp})

See Methods section for details on the experimental protocol.

Effect of molecular weight: To evaluate the effect of molecular weight on the T_{cp} of p(A_{20-i}), we prepared four samples, one without thermal cleavage at 95 °C (Expected around 100,000 bases) and 3 thermally cleaved samples after 15 min (about 3000 bases), 30 min (about 1000 bases) and 60 min (about 300 bases) (Supplementary Fig. 1h). The T_{cp} was then determined using the protocol described in the Methods section. The data is presented in the main manuscript at Fig. 2f.

Additional information: Simple A_{20-l} sequences do not phase-separate, but ligated products, p(A_{20-l}), do, demonstrating that a certain length is required to trigger the phase transition (Supplementary Fig. 3a,b). This minimal length (between 50 and 100 nucleobases) also certainly depends on the detailed composition of the template. As seen Supplementary Fig. 3c, commercial pure Poly(deoxy-adenylic acid) (A_{250}) phase-separates (above 55 °C) which confirms that it is the homo-adenine run that drives the heat-induced phase-separation and rules out a decisive influence of the influence of the barcode domains (i, j, k, l) in the process. See additional data in Supplementary Note 4.

ssDNA with other nucleobases: Further UV-Vis spectra show that p(G_{20-l}) already phase-separates irreversibly in 10 mM of MgAc_2 (Supplementary Fig. 3d), while p(T_{20-j}) and p(C_{20-l}) remain soluble even in presence of 500 mM MgAc_2 (Supplementary Fig. 3e,f). This confirms specificity of the phase-separation to purine bases.

Nature of counterions: The heat-induced phase-separation requires specific counter ions. MgAc_2 and CaCl_2 induce heat-induced phase-separation of p(A_{20-i}) (Fig. 2 main text). Yet larger alkaline earth metals such as SrCl_2 or BaCl_2 (100 mM) do not yield any phase separation for p(A_{20-i}) in TE buffer (Supplementary Fig. 3g,h). On the contrary, transition metals such as such as ZnCl_2 (20 mM) or MnCl_2 (20 mM) already trigger phase-separation at room temperature of similar 0.06 g/L p(A_{20-i}) solutions in TE buffer (Supplementary Fig. 3i,j).
Supplementary Figure 3: Phase-separation depends on nucleobase and salt. The UV-Vis spectra at 25, 35, 45, 55, 65, 75 and 25 °C of (a) A20–l at 0.1 g/L in 100 mM MgAc2; (b), p(A20–l) at 0.06 g/L in 100 mM MgAc2; (c), commercial A350 at 0.1 g/L in 100 mM MgAc2; (d), p(G20–l) at 0.06 g/L in 10 mM MgAc2; (e), p(T20–l) at 0.06 g/L in 500 mM MgAc2; (f), p(C20–l) at 0.06 g/L in 500 mM MgAc2 show decisive influence on the length and nucleobase content of the ssDNA on the phase-separation. The comparison of the UV-Vis spectra of standard 0.06 g/L p(A20–l) before and after adding salt at either 25 or 75 °C show that (g) 100 mM SrCl2 and (h) 100 mM BaCl2 do not induce phase-separation while (i) 20 mM ZnCl2 or (j) 20 mM MnCl2 already lead to aggregation at 25 °C.
Supplementary Note 4. Polyadenine with controlled molecular weights and low dispersity: Molecular weight effects and hysteresis

**General remark:** Although RCA permits to easily and quickly produce large amounts of ssDNA, it does not allow to achieve samples with narrow molecular weight distribution (low dispersity). This may not be ideal for an exact evaluation of molecular weight-dependent effects, as higher molecular weights in broad distributions undergo phase-separation more easily (i.e. at lower $T_{cp}$). To address this problem, we (i) synthesized various homo-adenine polymers (polyadenine, A$_{x}$) of controlled molecular weight and with low dispersity using living/controlled terminal deoxynucleotidyl transferase (TdT)-mediated polymerization. In addition, we (ii) purchased solid-phase synthesized A$_{x}$ ultramers with 75, 100 and 150 adenine repeats (from IDT), and A$_{250}$ synthesized via TdT (from Roche).

**Living/controlled nucleotide polymerization using terminal deoxynucleotidyl transferase (TdT).** TdT catalyses the insertion of nucleotides at the 3’-end of a 5-10 bases primer (initiator). While there is no possibility to control the sequence, supplying a single nucleotide monomer (here dATP) results in single nucleobase ssDNA products, here polyadenine (A$_{x}$). Provided that the enzyme is introduced in excess compare to the primer, the polymerization runs in a controlled/living fashion, which results in narrowly dispersed products. The number-average degree of polymerization, DP, is determined by the ratio monomer/primer, which we used to make polyadenine between 100 and 2500 repeats (Supplementary Fig. 4a). GEP of the resulting products exhibits sharp bands (Supplementary Fig. 4b), and thus confirms drastically more narrowly dispersed samples compared to the RCA products synthesized above (see e.g. Supplementary Fig. 1). Shorter A$_{100}$, A$_{150}$, A$_{200}$ are merely shown to emphasize control over the reaction.

**Cloud points of narrowly distributed polyadenine with different DP.** The $T_{cpS}$ of different A$_{x}$ are plotted in Supplementary Fig. 4c. Note that we use the solid-phase synthesized samples (A$_{75}$, A$_{100}$, A$_{150}$) for lower molecular weights, while we use commercial A$_{250}$ (TdT) and our in-house synthesized A$_{500}$, A$_{1000}$, A$_{2000}$, A$_{2500}$ (TdT) for higher molecular weights. This allows a comprehensive comparison using different sources and uses the best accessible materials in terms of dispersity. Similar as for p(A$_{20}$-i) in Fig. 2 in the main manuscript, the $T_{cpS}$ of polyadenine ssDNA, A$_{x}$, decrease with increasing molecular weight, and show an upturn at low molecular weight. Interestingly, the $T_{cpS}$ of narrowly distributed A$_{x}$s are higher than polyydisperse p(A$_{20}$-i). This can however be simply explained by the polydisperse nature of RCA-produced p(A$_{20}$-i) and the presence of a fraction of long chains in p(A$_{20}$-i), which will phase-separate at temperatures much lower than the rest of the sample, which artificially decreases the $T_{cp}$s. Critically, this effect can be limited using well-defined A$_{x}$, promoting fundamental understanding. It also allows to narrow down the minimal base number needed for phase-separation. A$_{150}$ phase-separates clearly at 66 °C, while A$_{100}$ only phase-separates poorly at 70 °C. A$_{75}$ does not phase-separate below 80 °C (Supplementary Fig. 4d).

**Hysteresis behaviour:** LCST behaviours are known to feature hysteresis effects. ssDNA A$_{x}$s also display a hysteresis between heating and cooling (Supplementary Fig. 4d,e). Yet, there is no significant difference in hysteresis between samples ramped at 1 or 4 °C/min.

**Experimental protocols for living/controlled ssDNA synthesis via TdT:** The short primer (Trans-prim see Supplementary Table 1) is introduced at 0.2 μM final concentration in a reaction buffer containing 30 μL of reaction buffer 5x (Promega: 500 mM cacodylate buffer, 5 mM CoCl$_{2}$ and 0.5 mM DTT), and dATP (100 μM, 200 μM, 400 μM or 500 μM) according to target DP and supplemented with ultrapure water to reach 145 μL. After mixing, 5 μL of TdT (30 U/μL) are added leading to a final concentration of 1 U/μL. The reaction is run at 37 °C for 2 h (near full conversion) and quenched with the addition of 20 μL of EDTA solution at 200 mM. The products are purified by filtration with Amicon Ultracentrifugal filters with a 10 kDa cut-off (Merck Millipore) and rinsed 3 times using TE buffer in the same filter.

$T_{cpS}$ and hysteresis curves were measured on a Scandrop (Jena Analytics) with Peltier heating using ssDNA solutions of ca. 0.06 g/L diluted in TE buffer containing 50 mM of MgAc$_{2}$. Heating ramps were performed on 50 μL of solution in a 45 μL Quartz cuvette (Hellma Analytics) and heated at 1 °C/min (or 4 °C/min), the extinction at 600 nm was recorded every 30 seconds.
Supplementary Figure 4: Cloud point behaviour for Aₙ with controlled molecular weight and narrow molecular weight distribution. (a) Scheme for the synthesis of polyadenine ssDNA, Aₙ, via "living/controlled" TdT-mediated polymerization. (b) GEP of narrowly dispersed A₁₀₀, A₁₅₀, A₂₀₀, A₂₅₀, A₁₀₀₀, A₂₀₀₀, A₂₅₀₀ as synthesized by TdT-mediated polymerization. GEP was run in TAE buffer using 2 wt% agarose gel and 6 V/cm for 90 min, post-stained using SYBR. The narrow band in the centre of each lane (A₅₀₀ – A₂₅₀₀) corresponds to the actual distribution. The 50 bp ladder bands correspond to 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1000 base pairs, and the 1 kbp ladder bands correspond to 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000 and 10000 base pairs from top to bottom. (c) Evolution of the Tₕ of 0.06 g/L solutions of several commercial and synthetic Aₙ (50 mM MgAc₂). A₁₇₅ is not included as it does not phase-segregate till 80 °C. (d) Hysteresis in the extinction at 600 nm resulting from heating and cooling solutions of 0.06 g/L Aₙ (50 mM MgAc₂) at 1 °C/min. (e) Hysteresis in the extinction at 600 nm resulting from heating and cooling solutions of 0.06 g/L A₂₅₀ (50 mM MgAc₂) at 1 and 4 °C/min.
Supplementary Note 5. Particle preparation

See Methods section for details on the experimental protocol.

Formation mechanism of particles: The phase-separation occurs via binodal decomposition (nucleation and growth), and the formation of well-defined spherical structures indicates water-rich coacervate-type particles at high temperatures with low tendency of sedimentation, and sufficient dynamics for reorganization. Fusion of particles into well-defined larger spheres can occur for coacervate-type phase-segregated domains at higher temperatures, compelling to keep a short high temperature plateau and steep heating and cooling ramps.

Particle formation depends on molecular weight: The thermal reduction of the molecular weight is critical to obtain spherical particles instead of ill-defined aggregates (Supplementary Fig. 5a,b). For instance, thermal cleavage is necessary to observe the liquid core in p(A_{20-i})/p(T_{20-j}) structures, as the otherwise too high molecular weight of the entrapped ssDNA solution resembles a gel due to entanglements.

Stability and dissolution of particles: The particles are stable for weeks in their assembly buffer even if sedimenting (Supplementary Fig. 5i,j). It is also possible to transfer them into other buffer solutions NOT containing any Mg^{2+} (via centrifugation at 10 krcf for 3 min and redispersion) while keeping them intact at the condition that the salt concentration remains high enough (Supplementary Fig. 5c-h). For instance, p(A_{20-i})/p(T_{20-j}) core/shell particles (Supplementary Fig. 3c) remain stable in 100 mM of NaCl TE buffer (Supplementary Fig. 5e) but spontaneously dissolve at 10 mM of NaCl TE buffer (Supplementary Fig. 3g). The higher melting transition of the XL palindromic domain (T_m \approx 70 °C) stabilizes the p(A_{20-i-XL})/p(T_{20-k-XL}) even at low salt concentration of only 10 mM NaCl (Supplementary Fig. 5h).

Decreasing the ionic strength also makes the particles swell due to the increasing electrostatic repulsion p(A_{20-i})/p(T_{20-j}) core/shell particles are larger in 100 mM of NaCl TE buffer than in 50 mM MgAc_2 (Supplementary Fig. 5c,e) similarly p(A_{20-i-XL})/p(T_{20-k-XL}) swell strongly in 10 mM of NaCl TE buffer (Supplementary Fig. 5d,h).

In all cases, heating the particles to 95 °C in pure TE buffer or 100 mM NaCl leads to the disassembly of the structures. This is important to allow quantification of the degree of functionalization (e.g. with dye-barcode*) using scattering- and quenching-free fluorescence spectroscopy.
Supplementary Figure 5: Particle formation, stability and dissolution. (a-b) Thermal reduction of the molecular weight increases the reorganization dynamics and provides access to well defined particles. As visible on the CLSM, 0.2 g/L p(A20-i-XL) particles prepared in 50 mM MgAc2 (a) directly from RCA product without heat cleavage (very high molecular weight) form poorly defined structures while (b) after 15 min heat cleavage (lower molecular weight) they form well defined spheres. (c-h) Stability with buffer exchange: The CLSM show that 0.5 g/L p(A20-i)/p(T20-j) particles prepared (c) initially in 50 mM MgAc2 TE buffer and post-functionalized with Atto488-i* and Atto568-j* swell after transfer in (e) 100 mM NaCl TE buffer, and disassemble in (g) 10 mM NaCl TE buffer. Due to their higher stability 0.5 g/L p(A20-i-XL)/p(T20-k-XL) particles prepared (d) initially in 50 mM MgAc2 and post-functionalized with Atto488-i* and Atto647-k* remain stable both in (f) 100 mM NaCl TE buffer and in (h) 10 mM NaCl TE buffer, in which they nevertheless swell significantly. (i-j) CLSM of particles of p(A20-i-XL)/p(T20-k-XL) 0.3 g/L as prepared initially (i) and after 3 months at 4 °C (j) confirm stability.
Supplementary Note 6. Formation of hollow capsules

See Methods section for details on the experimental protocol.

Gel electrophoresis of p(A20-i) released during capsule formation. We prepared core/shell particles and capsules using the protocols described in Methods. After preparation, we centrifuged down the objects in dispersion (20 min; 10 000 g) and analysed the supernatant via GEP (together with the reference starting materials, Supplementary Fig. 6a).

A bicolour GEP allows to identify the nature of the ssDNA in the supernatant (Supplementary Fig. 6a). The image is formed by superposition of (i) the native image, where only the Atto488-i* fluorescence is visualized in blue, highlighting the presence of p(A20-i)/Atto488-i* and excess Atto488-i*, with (ii) a SYBR gold-stained image (post-staining), which visualizes all DNA in red.

The supernatant of the capsules (lane 6 from left) clearly contains free p(A20-i) which appears purple as a high molecular weight trail, because it is revealed both by Atto488-i* and SYBR gold staining. The same pattern is also visible in the premix before heating (lane 2 from left). Note the presence of unbound Atto488-i* (added purposefully in a slight excess) at the bottom of the gel. This clearly confirms release of core material (p(A20-i)) into solution. In contrast, core/shell particles prepared in absence of Atto488-i* do not release p(A20-i) ssDNA in the supernatant (lane 5 from left before post-staining with Atto488-i* and lane 4 after post-staining with Atto488-i*). One can only observe a faint red band at higher molecular weights (SYBR stain), that originates from traces of p(T20-j) ssDNA (shell material) remaining in solution after saturation of the shell in the core/shell microgels (lane 4/5).

Capsule formation depends on the presence of i* components. We also verified that the capsule formation is only related to the presence of barcode-complementary i* strand, and independent from the presence of fluorescent molecules (i.e. Atto488) bound to them. To do this we replaced the addition of fluorescent markers above (i.e. Atto488-i* and Atto568-j*) with 15 μM of i*, T20, A20 or j* oligomers as presented in Supplementary Fig. 6c-f, and compared them with standard particles formed without additional oligomers Supplementary Fig. 6b. For fluorescent imaging, all particles were dyed with SYBR Gold diluted at 1/1000. This experiment confirms that only i* leads to the formation of capsules (Supplementary Fig. 6c). T20 blocks the A20 sites at the interface of the phase separated p(A20-i) and prevents the formation of a continuous shell leading to the destruction of the particles (Supplementary Fig. 6f). On the other hand, A20 and j* have limited impact on the particles morphology (Supplementary Fig. 6d,e).
Supplementary Figure 6: Effect of small oligomers on capsule formation to reveal the decisive influence of adding barcode* ssDNA oligomers. (a) Bicolour GEP with 50 bp reference ladder on each side, showing (from left to right) (lane 2) premix for capsules (0.4 g/L p(A20)-i, 0.1 g/L p(T20)-j), 30 μM of Atto488-i* in MgAc2 50 mM and (lane 3) core/shell premix (identical to lane 2, but without Atto488-i*), (lane 4) supernatant of the core/shell particles supplemented with Atto488-i* (30 μM) and (lane 5) supernatant of the core/shell particles without Atto488-i*, and (lane 6) finally the supernatant of the capsules. The GEP image is a superposition of two images: Firstly, imaged without DNA-specific dyes where only Atto488-i* fluorescence is visible in blue, and, secondly imaged after staining with SYBR gold (red image) revealing all DNA present in the GEP. The presence of a purple band in the supernatant of the capsules (lane 6), but its absence in the supernatant of the core/shell particles (lane 4), confirms that p(A20-i) is released from the capsule when adding Atto488-i* before the temperature ramp. (b-f) The CLSM imaging of 0.2 g/L p(A20-i)/p(T20-j) particles prepared in 50 mM MgAc2 and post-stained with SYBR gold allow to probe the influence of dye-free barcode* oligomers and other controls. Compared to standard particles prepared (b) in the absence of i*, (c) the presence of 15 μM of i* leads to well-defined capsules, while (d) the presence of 15 μM of A20 only slightly or (e) 15 μM of j* barely modify the structure formed. On the contrary, (f) the presence of 15 μM of T20, blocks the recognition between p(A20-i) and p(T20-j) and prevents structure formation. (g) Z-stack CLSM imaging of a single capsule adsorbed on the glass coverslip (deformation at bottom) formed at 0.2 g/L p(A20-i)/p(T20-j) in presence of Atto488-i*/Atto568-j*. Top image shows a XY plane, while the two orthogonal slices depict XZ and YZ planes. (h) TEM image of a folded capsule and its grey scale analysis in (i). (j) For comparison, TEM image of a solid p(A20-i)/p(T20-j) particle of similar size. (k) Additional TEM image of a larger collapsed capsule.
Supplementary Note 7. Macroscopic assemblies

See Methods section for details on the experimental protocol.

Control for hybridization-driven assembly: We performed control experiments to evidence that controlled complementary interactions on the particles are needed to induce supra-particulate co-assembly: The controls include a mixture of two particle batches functionalized with a mixture of both strands (i*-R₁* , i*-R₁), as well as mixtures of two particle batches functionalized with the same modifier (either i*-R₁* or i*-R₁). Control experiments presented in Supplementary Fig. 7 (a-d) show p(A₂₀-i-XL) microgels formed at 0.17 g/L (40 μL) prepared as described above, splitted in 6 different batches (5 μL each) and functionalized with:

1) 0.5 μL of a mixture containing 10 μM of Atto₄₈₈-i*, 45 μM of i*-R₁* and 45 μM of i*-R₁,
2) 0.5 μL of a mixture containing 10 μM of Atto₆₄₇-i*, 45 μM of i*-R₁* and 45 μM of i*-R₁,
3) 0.5 μL of a mixture containing 10 μM of Atto₄₈₈-i* and 90 μM of i*-R₁*,
4) 0.5 μL of a mixture containing 10 μM of Atto₆₄₇-i* and 90 μM of i*-R₁*,
5) 0.5 μL of a mixture containing 10 μM of Atto₄₈₈-i* and 90 μM of i*-R₁*,
6) 0.5 μL of a mixture containing 10 μM of Atto₆₄₇-i* and 90 μM of i*-R₁*

The mixtures of batch (1) + (2); batch (3) + (4); and batch (5) + (6) shown in Supplementary Fig. 7a-c display no aggregation after 10 h shaking at room temperature. On the contrary, mixing batch (3) + (6) (positive control) induces the formation of supra-particulate aggregates thereby proving the necessity of having complementary surface strands on the individual particle batches to induce hybridization-driven co-assembly (Supplementary Fig. 7).

Cellular hydrogels: Cellular hydrogels are formed by two consecutive heating steps. A batch of p(A₂₀-i-XL)/p(T₂₀-k-XL) particles at 0.2 g/L was prepared as described above in 50 mM MgAC₂ TE buffer. The particles were functionalized with stoichiometric amounts of Atto₄₈₈-i* and Atto₆₄₇-k* left at room temperature for 1 hour. After centrifugation down to the bottom of the tube (10 krfc, 3 min) the tube is heated to 60 °C for 5 minutes and cooled back down to room temperature to induce stickiness between the particles (See Fig. 6e main text). To form the continuous gel the supernatant is removed from the sedimented particles (this can be done after a first 60 °C heating step or without preliminary heating with similar result but pipetting out the supernatant is more difficult in the latter case). It is important here to remove most of the liquid supernatant and to tightly close the tube before applying the second to 60 °C for 5 min (Supplementary Fig. 7e). Excessive heating leads to a progressive loss of the compartmentalized architecture as seen Supplementary Fig. 7f,g for gels heated to 70 °C and 80 °C respectively.
Supplementary Figure 7: Mixing non-complementary particles and effect of the temperature on cellular hydrogel formation.

(a-d) Control experiments for DNA driven self-assembly with: (a) Mixture of two particles (red, green) functionalized both with a mixture of complementary strands shows absence of aggregation due to intraparticle hybridization of R1*/R1: p(A20-i-XL) particles functionalized by Atto488-i*, i*-R1*, i*-R1 (ratio: 0.1 eq, 0.45 eq, 0.45 eq) mixed with of p(A20-i-XL) particles functionalized by Atto647-i*, i*-R1*, i*-R1 (ratio: 0.1 eq, 0.45 eq, 0.45 eq); (b) Mixture of two particles (red, green) functionalized both with R1 shows absence of aggregation due to lack of complementary interactions: p(A20-i-XL) particles functionalized by Atto488-i*, i*-R1 (ratio: 0.1 eq, 0.9 eq) mixed with p(A20-i-XL) particles functionalized by Atto647-i*, i*-R1 (ratio: 0.1 eq, 0.9 eq); (c) Mixture of two particles (red, green) functionalized both with R1* shows absence of aggregation due to lack of complementary interactions: p(A20-i-XL) particles functionalized by Atto488-i*, i*-R1* (ratio: 0.1 eq, 0.9 eq) mixed with p(A20-i-XL) particles functionalized by Atto647-i*, i*-R1* (ratio: 0.1 eq, 0.9 eq), and (d) the positive control, a mixture of two particles (red, green) functionalized with complementary R1 and R1* strands shows aggregation due to complementary interactions on the particles: p(A20-i-XL) particles functionalized by Atto488-i*, i*-R1 (ratio: 0.1 eq, 0.9 eq) mixed with p(A20-i-XL) particles functionalized by Atto647-i*, i*-R1* (ratio: 0.1 eq, 0.9 eq). (e-g) Heat-induced merging of sedimented p(A20-i-XL)/p(T20-k-XL) microgels into cellular hydrogels after removal of the supernatant and heating to (e) 60 °C, (f) 70 °C and (g) 80 °C. Excessive heat leads to loss of particle character.
Supplementary Note 8. Hybrid Au-NP/DNA structures

See Methods section for details on the experimental protocol.

**UV-Vis spectra:** Temperature-dependent UV-Vis spectra were recorded on a Jasco V650 spectrophotometer coupled with a Huber Petite Fleur thermocontroller. Typically, a volume of 50 μL of T\textsubscript{20} coated Au-NP solution at 10 nM in TE buffer supplemented with 50 mM of MgAc\textsubscript{2} (or the specified amount of corresponding divalent cation salt) is introduced in a 45 μL Quartz cuvette (Hellma Analytics). The extinction spectra are recorded between 400 and 900 nm initially at 25 °C and 5 min after reaching 75 °C. The TEM grid was prepared with the solution of T\textsubscript{20} coated Au-NP solution at 10 nM as obtained directly after functionalization.

Supplementary Figure 8: (a) UV-Vis spectra in 50 mM MgAc\textsubscript{2} TE buffer at 25 and 70 °C and (b) TEM image of 5 nM T\textsubscript{20}-coated Au-NPs. Au-T\textsubscript{20}-NPs remain dispersed at high temperature.
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