Supplementary Information

Constructing synthetic nuclear architectures via transcriptional condensates in a DNA protonucleus

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Affiliations

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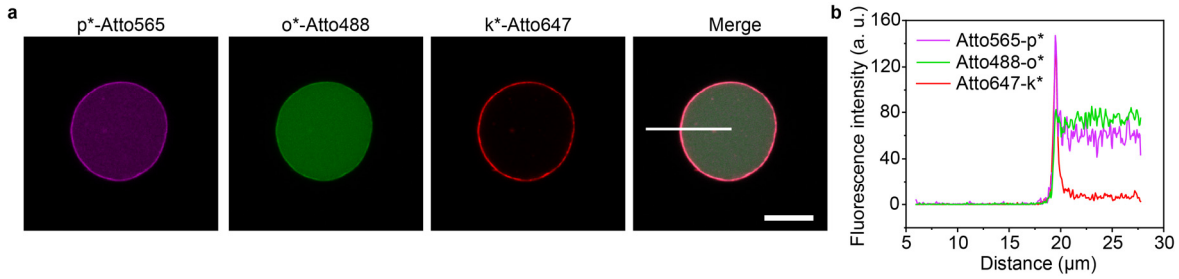
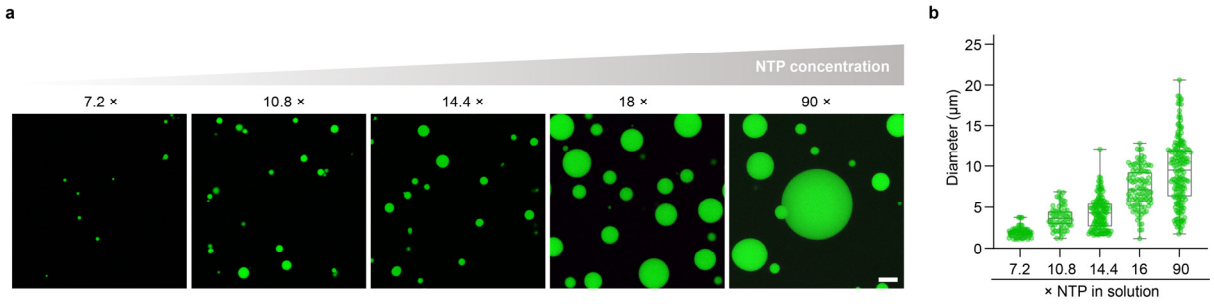
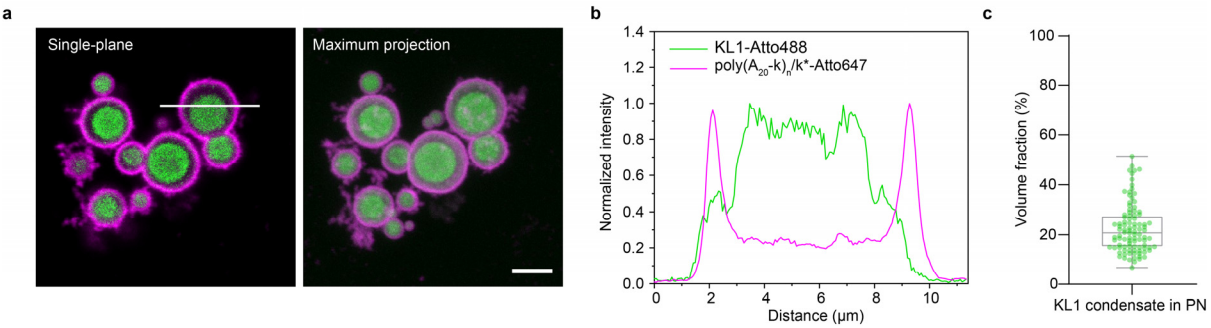


Supplementary Figure 1. Preparation of PNs containing promoter sequences (p) inside.
a, Representative CLSM images of the PNs containing different core (p and o) and shell (k) barcodes, labeled by their complementary ssDNA strands (k*-Atto647, p*-Atto565, and o*-Atto488). **b**, Fluorescence intensity profiles corresponding to the line segment analysis along the white line in (a) in three channels, showing a core-shell structure with poly(A_{20}-p)$_n$ and poly(A_{20}-o)$_n$ colocalized at the interior of the PN, and poly(T_{20}-k)$_n$ at the PN shell. Scale bar: 10 μm.



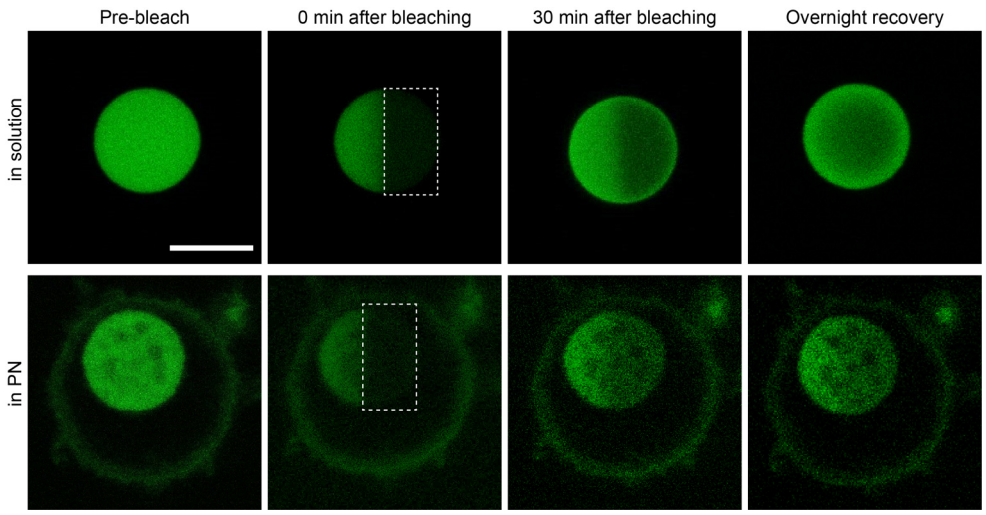
Supplementary Figure 2. Formation of transcriptional KL1 condensates in solution at different NTP concentrations.

a, Representative CLSM images of in-solution transcriptional KL1 condensates at various [NTP] : [T_{KL1}] ratios from 7.2 to 90, transcribed by promoter ssDNA ([T_{KL1}] : [p] = 1 : 1, 30 °C, 30 mM Mg^{2+}, 2.5 U/μL T7 RNAP). **b**, Diameter distribution at different NTP concentrations. In the box plot (b), the central line marks the median, the box represents the interquartile range (IQR) from Q1 (first quartile) to Q3 (third quartile), and the whiskers enclose all data points from the minimum to the maximum. This applies to all box plots shown in the Supplementary Information. Scale bar: 10 μm.

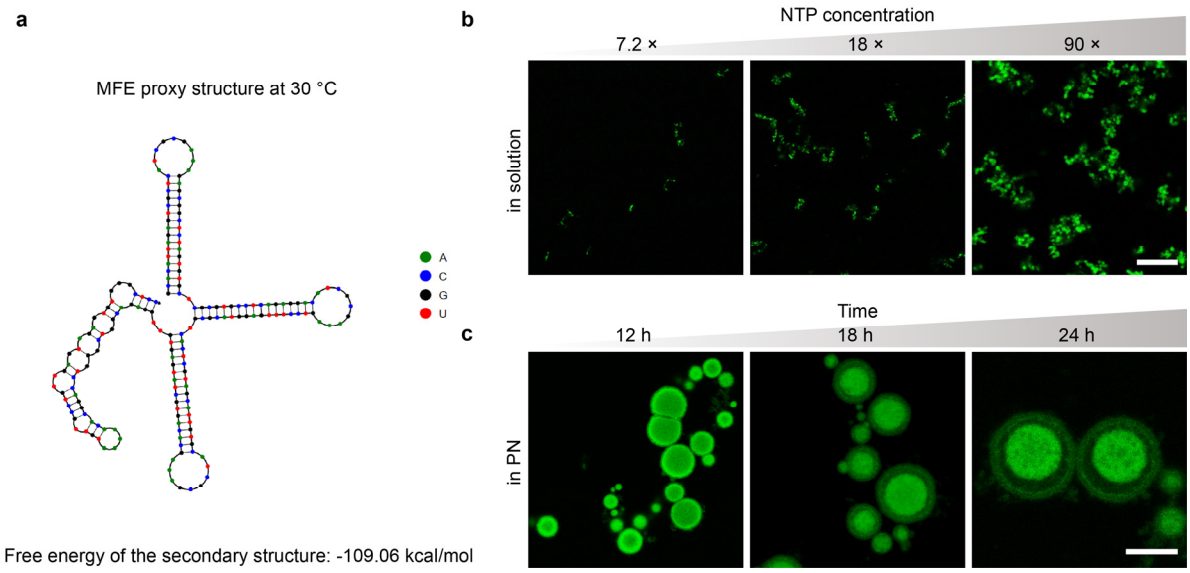


Supplementary Figure 3. Formation of single transcriptional KL1 condensates in PNs.

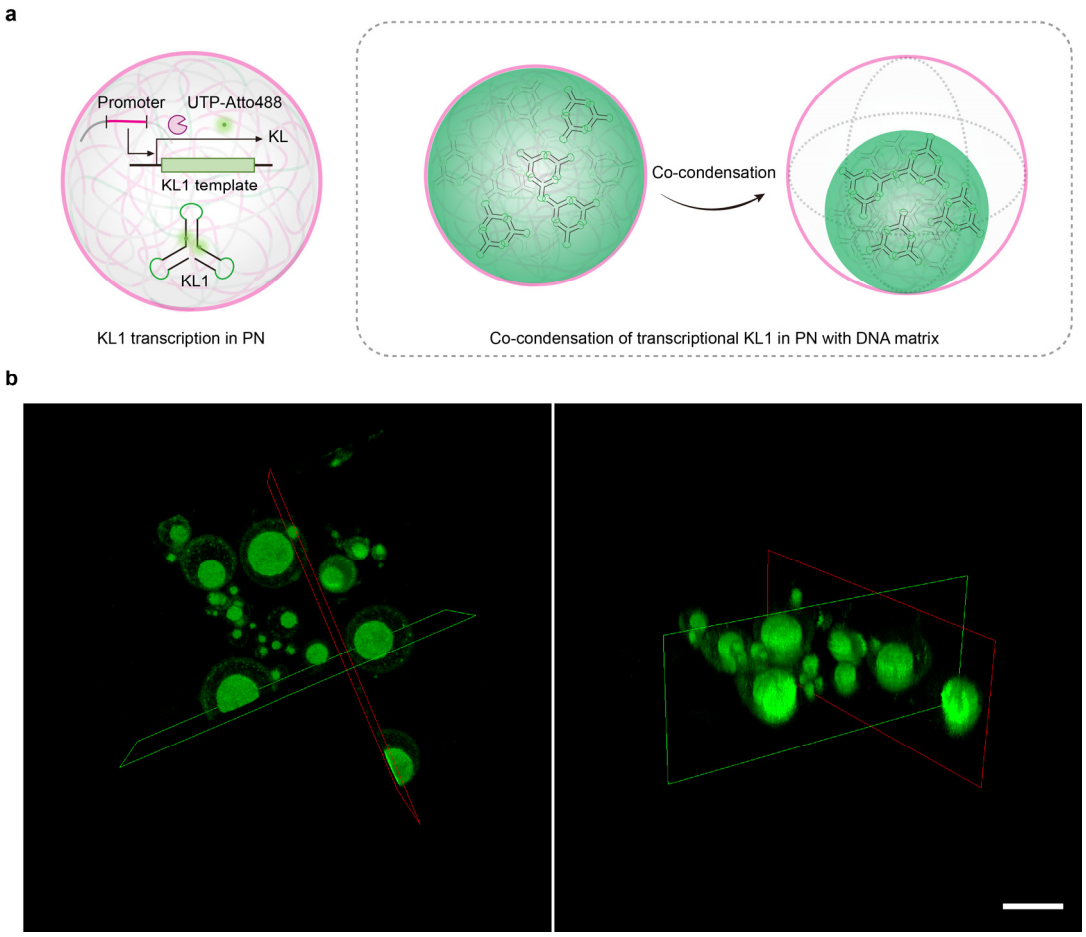
a, Representative single-plane CLSM image and maximum intensity projection of z-stacked CLSM images showing the formation of single KL1 condensates in each PN ([NTP] : $[T_{KL1}]$ = 3.6 : 1, 30 °C, 30 mM Mg^{2+}, 2.5 U/μL T7 RNAP, 18 h reaction). Green channel: KL1 condensate (labeled by UTP-Atto488); Magenta channel: PN shell (labeled by k*-Atto647). Scale bar: 5 μm. **b**, Normalized intensity profiles corresponding to the line segment analysis along the white line in (a) showing the distribution of KL1 condensates with a spongy structure, and the PN shell. **c**, Volume fraction of single transcriptional KL1 condensate relative to the host PNs. This figure relates to Fig. 2e in the main text.



Supplementary Figure 4. Half-bleaching experiment on KL1 condensates in solution and in PNs. Representative CLSM images showing the transcriptional KL1 condensates in solution and in PNs, before and after bleaching at different times. The bleached regions are indicated by the white dashed rectangle. Scale bar: 5 μm.

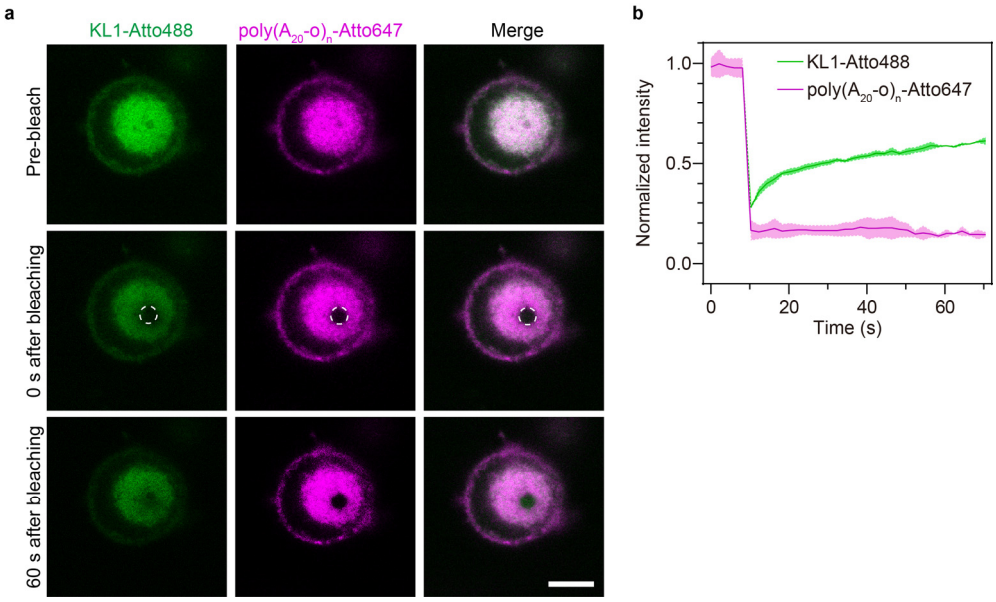


Supplementary Figure 5. Design and transcription of KL1-BrA in solution and in PNs.
a, NUPACK-simulated structure of KL1-BrA at 30 °C. **b**, Transcription and assembly of KL1-BrA in solution at varying [NTP] : [T_{KL1}] ratios from 7.2 to 90 ([T_{KL1}] : [p] = 1 : 1, 30 °C, 30 mM Mg^{2+}, 24 h reaction). **c**, Formation of transcriptional KL1-BrA condensates in PNs over 12-24 h ([NTP] : [T_{KL1}] : [p] = 3.6: 1 : 1, 30 °C, 30 mM Mg^{2+}, 2.5 U/μL T7 RNAP). Scale bars: 10 μm.



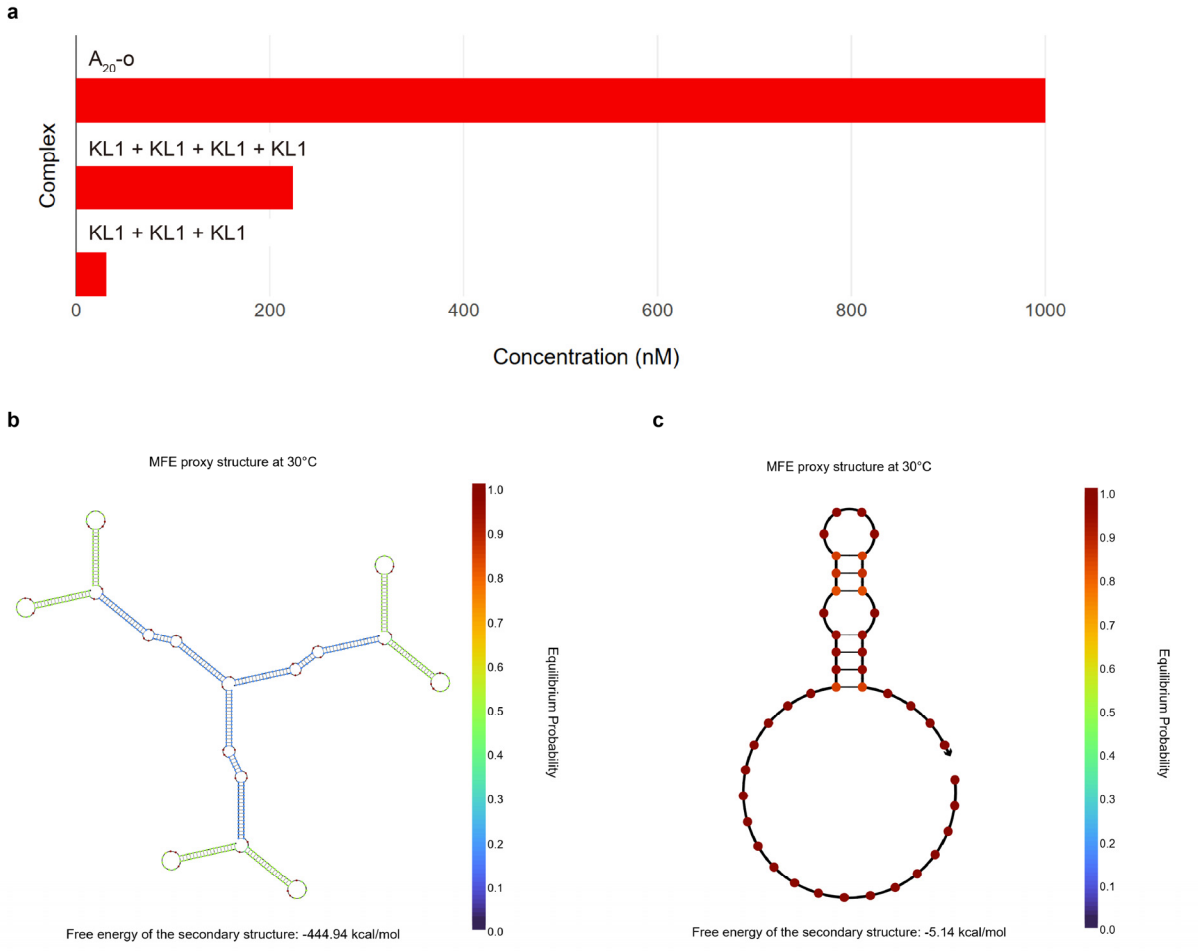
Supplementary Figure 6. Formation of transcriptional KL1-PN co-condensate deposited at the bottom of host PN.

a, Schematic illustration of KL1-PN co-condensation between KL1 transcripts and DNA matrix of the host PN induced by localized transcription of KL1 in the PN. **b**, Representative 3D CLSM images showing the top (left) and front (right) views of co-condensates in PNs. Scale bar: 10 μm.



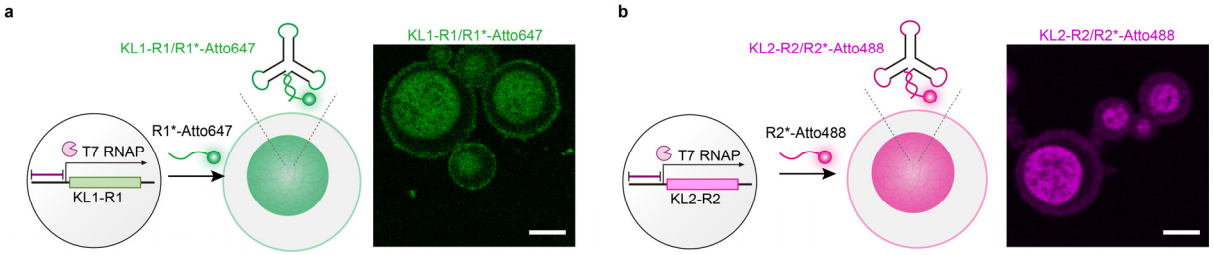
Supplementary Figure 7. Dynamic properties of KL1 phase and PN matrix phase in the KL1-PN co-condensate quantified by FRAP.

a, Representative CLSM images showing KL1 condensate phase (green channel) and PN matrix phase (magenta channel) of a co-condensate in PNs before and after bleaching at 30 ℃. The white dashed circles indicate the bleached regions. **b**, Normalized fluorescence recovery kinetics in the bleached regions in (a) for the KL1 phase and the PN matrix phase, quantifying the molecular diffusivity of co-condensates in PN at 30 ℃. Intensity values were normalized to pre-bleached levels. n = 3. Error areas represent standard deviation. Scale bar: 5 μm.



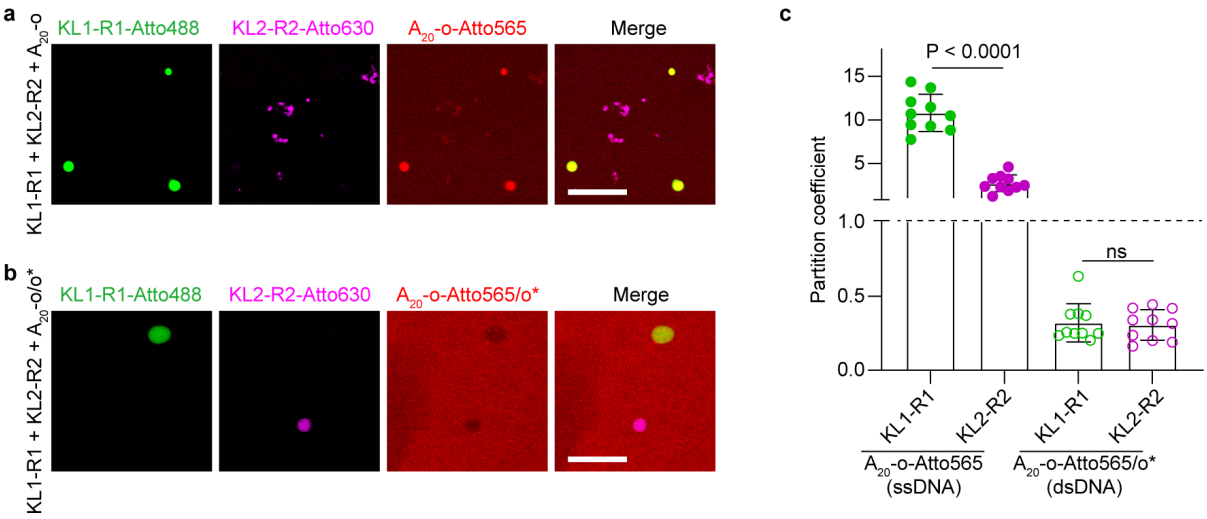
Supplementary Figure 8. NUPACK simulation showing the absence of specific interaction between A_{20}-o ssDNA and KL1 sequence.

a, Simulated complex structures formed by 1 μM A_{20}-o (ssDNA) and 1 μM KL1 (ssDNA) at 30 °C. **b**, NUPCK-simulated structures of KL1+ KL1+ KL1+ KL1. **c**, NUPACK-simulated structures of A_{20}-o. No complex structure between A_{20}-o and KL1 is formed. Note that all simulations are based on DNA sequences.



Supplementary Figure 9. Formation of KL1-R1 and KL2-R2 condensates in PNs.

a, Scheme and representative CLSM image showing the formation of single condensates in PNs by localized transcription of KL1-R1. The condensate is labeled by R1*-Atto647 (green channel). **b**, Scheme and representative CLSM image showing the formation of single condensates in PNs by localized transcription of KL2-R2. The condensate is labeled by R2*-Atto488 (magenta channel). 2.5 U/μL T7 RNAP, 30 mM Mg^{2+}, 30 ℃, [NTP] : [R1* or R2*] : [$T_{KL1\text{-}R1}$ or $T_{KL1\text{-}R1}$] : [p] = 3.6 : 3.6 : 1 : 1, 18 h reaction for both (a) and (b). Scale bars: 5 μm.



Supplementary Figure 10. Preferred partitioning of A_{20}-o ssDNA into KL1-R1 condensates and exclusion of A_{20}-o/o* dsDNA from both KL1-R1 and KL2-R2 condensates.

a, Representative CLSM images of mixed transcriptional KL1-R1 and KL2-R2 condensates in solution, with the addition of ssDNA A_{20}-o-Atto565 for 1 h. **b**, Representative CLSM images of mixed transcriptional KL1-R1 and KL2-R2 condensates in solution with the addition of dsDNA A_{20}-o-Atto565/o* for 1 h. In (a) and (b), both transcriptional condensates were initially prepared in separate reactions and subsequently mixed. ([NTP] : [$T_{KL1\text{-}R1}$ or $T_{KL2\text{-}R2}$] : [p] = 14.4 : 1 : 1, 30 ℃, 30 mM Mg^{2+}, 18 h reaction). KL1-R1 and KL2-R2 transcripts are labeled with 1 mol% UTP-Atto488 or UTP-Atto630, respectively. Green channel: KL1-R1 condensate; Magenta channel: KL2-R2 condensate; Red channel: A_{20}-o-Atto565 ssDNA in (a) and A_{20}-o-Atto565/o* dsDNA in (b). **c**, Partition coefficient of ssDNA A_{20}-o-Atto565 and dsDNA A_{20}-o-Atto565/o* in different KL condensates, quantified by normalizing their intensity in KL condensates to their intensity in solution. Error bars represent standard deviation. Scale bars: 10 μm.

Name		Sequence (5’ → 3’)	Purification	Modification	Supplier
Template and RCA	Tp(A_{20}-p)	/Phosphate/ATA GTG AGT CGT ATT ATT TTT TTT TTT TTT TTT TTT ATC CCT	HPLC	5’-Phosphorylation	Biomers
	Tp(A_{20}-o)	/Phosphate/TAC CTC AAT GCT TTT TTT TTT TTT TTT TTT TTG GGA GCA ACA A	HPLC	5’-Phosphorylation	Biomers
	Tp(T_{20}-k)	/Phosphate/ATC CTC TAA AAT CAA AAA AAA AAA AAA AAA AAA GTA AAA CCA CAC G	HPLC	5’-Phosphorylation	Biomers
	ligation-p	TAA TAC GAC TCA CTA TAG GGA T	HPLC	None	Biomers
	ligation-o	GCA TTG AGG TAT TGT TGC TCC CA	HPLC	None	Biomers
	ligation-k	TTT TAG AGG ATC GTG TGG TTT T	HPLC	None	Biomers
	primer-p	TAA TAC GAC TCA CTA TAG GG*A*T	Desalting	Phosphorothioated twice (*)	IDT
	primer-o	GCA TTG AGG TAT TGT TGC TC C*C*A	Desalting	Phosphorothioated twice (*)	IDT
	primer-k	TTT TAG AGG ATC GTG TGG TT*T*T	Desalting	Phosphorothioated twice (*)	IDT
Label	Atto565-p*	/ATTO565/ATC CCT ATA GTG AGT CGT ATTA	HPLC	5’-Atto565	Biomers
	Atto647-o*	/ATTO647N/TGG GAG CAA CAA TAC CTC AAT GC	HPLC	5’ Atto647N	Biomers
	Atto488-k*	/ATTO488/ AAA ACC ACA CGA TCC TCT AAA A	HPLC	5’ Atto488	Biomers
	Atto647-k*	/ATTO647N/ AAA ACC ACA CGA TCC TCT AAA A	HPLC	5’ Atto647N	Biomers
	Atto565-A_{20}-o	/Atto565/ AAA AAA AAA AAA AAA AAA AA GCA TTG AGG TAT TGT TGC TCCCA	HPLC	5’ Atto565	Biomers
	Atto647-A_{20}-o	/Atto647N/ AAA AAA AAA AAA AAA AAA AA GCA TTG AGG TAT TGT TGC TCCCA	HPLC	5’ Atto647N	Biomers

Supplementary Table 1. Oligomers for PNs and labels, with their names, sequences, purification methods, modifications, and suppliers.

Name		Sequence (5' → 3')	Purification	Modification	Supplier
Transcription	P (promoter)	TAA TAC GAC TCA CTA TAG GGA T	HPLC	None	Biomers
	Rep	/6-FAM/CTA CAT CCA CAT ACT A	HPLC	5'-6-FAM	Biomers
	Rep'	GTT AAT TAG TAT GTG GAT GTAG/BMN-Q1/	HPLC	3'-BMN-Q1	Biomers
	T_{Rep*} for plate reader	GTT AAT TAG TAT GTG GAT GTA GAT CCC TAT AGT GAG TCG TAT TA	HPLC	None	Biomers
	T_{x*} for CLSM	TTA GGA TAG ATA TAC GGG TTC ATC CCT ATA GTG AGT CGT ATT A	HPLC	None	Biomers
Kissing Loop	T_{KL1}	CACTCATAGCACTGTGCTTTCGCGATGCA CAATGCTACGAGTGACGCGTACCTCAAAG GACTTTCGCGATGTCCTCTGAGGCACGCG AGCACACTAGAGCCGCTCTTTCGCGATGA GCGACTCTAATGTGCATCCCTATAGTGAG TCGTATTA	HPLC	None	IDT
	T_{KL1}'	GCACATTAGAGTCGCTCATCGCGAAAGAG CGGCTCTAGTGTGCTCGCGTGCCTCAGAG GACATCGCGAAAGTCCTTTGAGGTACGCG TCACTCGTAGCATTGTGCATCGCGAAAGC ACAGTGCTATGAGTG	HPLC	None	IDT
	T_{KL1-R1}	TGGGGTCCTTACTCGTTCAGATGCACTCA TAGCACTGTGCTTTCGCGATGCACAATGC TACGAGTGACGCGTACCTCAAAGGACTTT CGCGATGTCCTCTGAGGCACGCGAGCACA CTAGAGCCGCTCTTTCGCGATGAGCGACT CTAATGTGCATCCCTATAGTGAGTCGTAT TA	HPLC	None	IDT
	T_{KL1-R1}'	GCACATTAGAGTCGCTCATCGCGAAAGAG CGGCTCTAGTGTGCTCGCGTGCCTCAGAG GACATCGCGAAAGTCCTTTGAGGTACGCG TCACTCGTAGCATTGTGCATCGCGAAAGC ACAGTGCTATGAGTGCATCTGAACGAGTA AGGACCCCA	HPLC	None	IDT
	T_{KL2-R2}	CTAACGCCTGTAAATAAGCCACCCACTCA TAGCACTGTGCTTGTCGACTGCACAATGC TACGAGTGACGCGTACCTCAAAGGACTTG TCGACTGTCCTCTGAGGCACGCGAGCACA CTAGAGCCGCTCTTGTCGACTGAGCGACT CTAATGTGCATCCCTATAGTGAGTCGTAT TA	HPLC	None	IDT
	T_{KL2-R2}'	GCACATTAGAGTCGCTCAGTCGACAAGAG CGGCTCTAGTGTGCTCGCGTGCCTCAGAG	HPLC	None	IDT

		GACAGTCGACAAGTCCTTTGAGGTACGCG TCACTCGTAGCATTGTGCAGTCGACAAGC ACAGTGCTATGAGTGGGTGGCTTATTTAC AGGCGTTAG			
	Atto647-R1*	/ATTO647N/TCC TTA CTCG	HPLC	5'-Atto647N	Biomers
	Atto488-R2*	/ATTO488/CGC CTG TAAA	HPLC	5'-Atto488	Biomers
	$T_{KL1\text{-}BrA}$	GGAGCCCACACTCTACTCAACAGGCAACA TTTTTGTGCCTGGACCCGACCGTCTCCAAC ACTCATAGCACTGTGCTTTCGCGATGCAC AATGCTACGAGTGACGCGTACCTCAAAGG ACTTTCGCGATGTCCTCTGAGGCACGCGA GCACACTAGAGCCGCTCTTTCGCGATGAG CGACTCTAATGTGCATCCCTATAGTGAGT CGTATTA	HPLC	None	IDT
	$T_{KL1\text{-}BrA}$'	GCACATTAGAGTCGCTCATCGCGAAAGAG CGGCTCTAGTGTGCTCGCGTGCCTCAGAG GACATCGCGAAAGTCCTTTGAGGTACGCG TCACTCGTAGCATTGTGCATCGCGAAAGC ACAGTGCTATGAGTGTTGGAGACGGTCGG GTCCAGGCACAAAAATGTTGCCTGTTGAG TAGAGTGTGGGCTCC	HPLC	None	IDT

Supplementary Table 2. Oligomers for kissing loop condensate transcription, with their names, sequences, purification methods, modifications, and suppliers. * Represents a fully complementary sequence; ' denotes a partially complementary sequence with a toehold.