Supporting Information for:

DNA affects the phenotype of fuel-dependent coacervate droplets

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Supporting Tables

Supporting Table S1. Two-sided Welch's t-test for independent samples: p values, t values and degrees of freedom for the maximum turbidity in the presence of different 30mer libraries

P values	**Control**	**n30**	**T**	**C**		**A-rich**
Control	x	0.31	0.019	0.018	0.57	0.01
T values	Control	n30	t	c	g	A-rich
Control	x	-1.25	-4.35	-4.44	-0.66	-4.78
Degrees of freedom	Control	n30	t	c	g	A-rich
Control	x	2.77	3.24	3.21	2.15	3.61

Supporting Table S2. Two-sided Welch's t-test for independent samples: p values, t values and degrees of freedom for the beginning of droplet dissolution for different 30mer libraries

P values	**Control**	**n30**	**T**	**C**	**G**	**A-rich**	**A30**
Control	x	0.23	0.52	0.37	0.47	0.10	0.0051
n30	0.23	x	0.52	0.64	0.16	0.58	0.003
A-rich	0.10	0.58	0.23	0.27	0.13	x	0.0009
T values	Control	n30	T	c	g	A-rich	A30
Control	x	-1.41	-0.71	-1.00	0.82	-2.53	-14
n30	1.41	x	0.71	0.5	1.81	-0.63	-12
A-rich	2.53	0.63	1.58	1.41	2.41	X	-34
Degrees of freedom	Control	n30	t	C	g	A-rich	A30
Control	x	4.00	4.00	3.94	3.17	2.44	2.0
n30	1.41	x	4.00	3.94	3.17	2.44	2.0
A-rich	2.44	2.44	2.44	2.56	2.14	x	2.0

Supporting Table S3. Two-sided Welch's t-test for independent samples: p values, t values and degrees of freedom for droplet lifetime for different 30mer libraries.

P values	**Control**	**n30**	**T**	**C**	**G**	**A-rich**	**A30**
Control	x	0.17	0.71	0.67	0.37	0.47	0.0037
n30	0.17	X	0.3	0.19	0.24	0.5	0.00001
A-rich	0.47	0.5	0.71	0.68	0.29	x	0.0052
T values	Control	n30	T	c	g	A-rich	A30
Control	x	-1.94	-0.40	-0.46	1.11	-0.80	-12.20
n30	1.94	X	1.34	1.77	1.64	0.80	-26.2
A-rich	0.80	-0.80	0.39	0.45	1.38	x	-10.69
Degrees of freedom	Control	n30	t	c	g	A-rich	A30
Control	x	2.33	3.99	3.74	2.24	3.99	2.33
n30	2.33	X	2.31	2.56	2.02	2.31	4.00
A-rich	3.99	2.31	4.00	3.67	2.26	x	2.31

Supporting Table S4: Two-sided Welch's t-test for independent samples: p values, t values and degrees of freedom for droplet lifetimes for different A-rich libraries

P values	**A30**	**A5N20A5**	**A10N10A10**	**N10A10N10**	**(A5N5)3**	**A7N16A7**	**N5A20N5**	**A10N20**
Control	0.0002	0.10	0.001	0.10	0.01	0.003	0.0007	0.0016
T values	A30	A5N20A5	A10N10A10	N10A10N10	(A5N5)3	A7N16A7	N5A20N5	A10N20
Control	-19.0	2.21	-11.0	-2.21	0.63	-8.00	-13.0	-10.0

Deg. of freedom	A30	A5N20A5	A10N10A10	N10A10N10	(A5N5)3	A7N16A7	N5A20N5	A10N20
Control	3.20	3.45	3.20	3.45	3.45	3.20	3.20	3.20

Supporting Table S5: Two-sided Welch's t-test for independent samples: p values, t values and degrees of freedom for droplet lifetimes for different G-rich libraries against N30.

P values	G8N14G8	G10A20
N30	0.0001	0.30
T values	G8N14G8	G10A20
N30	-103	1.34
Deg. of freedom	G8N14G8	G10A20
N30	2.00	2.21

Supporting Table S6: Two-sided Welch's t-test for independent samples: p values, t values and degrees of freedom for critical fuel concentration for different G-rich libraries against no DNA control.

P values	G8N14G8	G10A20
Control	0.52	0.07
T values	G8N14G8	G10A20
Control	0.71	-3.16
Deg. of freedom	G8N14G8	G10A20
Control	4.00	2.44

Supporting Table S7. Characterization of DNA libraries via HPLC.

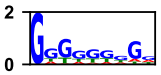
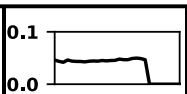
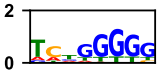
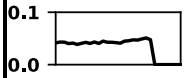
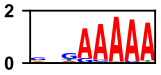
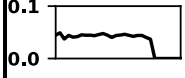
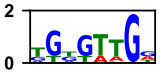
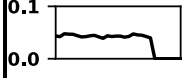
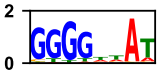
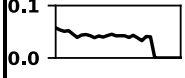
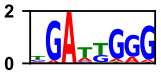
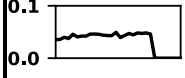
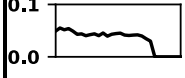
Name	retention time [min] @260nm	calibration value (mM/mAu)
N_{30}	5.71	1.50
A_{30}	10.01	1.19
$G_8N_{14}G_8$	5.20	1.07
A-rich	5.82	1.12
G-rich	5.16	1.39
T-rich	6.42	1.34
C-rich	5.17	1.88

Supporting Table S8: Characterization of peptides.

Name	Amino acid sequence	Mass calculated [g/mol]	Mass observed [g/mol]	Retention time* [min] at 220nm
Peptide	Ac-F(RG)$_3$D-OH	Mw = 962.05	$[MwH]^+$ 963.02 $[H_2Mw]^{2+}$ 482.12 $[H_3Mw]^{3+}$ 321.78	4.2
Peptide model	Ac-F(RG)$_3$N-NH_2	Mw=960.08	$[HMw]^+$ 960.49 $[H_2Mw]^{2+\cdot}$ 481.17 $[H_3Mw]^{3+}$ 321.16	4.2

*Gradient conditions: 6 min gradient 5%-98% ACN, total runtime 14 min

Supporting Table S9: List of all motifs enriched in Droplets compared to Supernatant. Sequence corresponds to the consensus sequence of the motif; $Logo_{Info}$ and $Logo_{Prob}$ are the motif logos with information content or nucleotide content as the y-axis; $Position_{Start}$ is the probability that a logo starts at that position; e-value is described in methods above.

Number	Sequence	$Logo_{Info}$	$Logo_{Prob}$	$Position_{Start}$	e-value
1	GGGGGGKGD				4.5e-320
2	AAAAAAWN				4.0e-096
3	TCTGGGGG				6.1e-029
4	GNGAAAAA				4.5e-016
5	TSTTTCCC				2.8e-010
6	TTGAGAGT				2.7e-009
7	GTYTGAWD				3.4e-009
8	TGKGTTGC				6.9e-009
9	GGGGWTAT				5.7e-008
10	TGATKGGG				2.3e-006
11	TCTTTCTS				7.2e-006
12	GTCCAGTCD				4.4e-005
13	GGGTTTTT				1-1e-002
14	AAAAKAAA				2.5e-002

Supporting Table S10: All nucleic acid sequences used in this study.

Name	Sequence (5'-3')
N_{30}	(N:0.25A, 0.25G, 0.25T, 0.25C) NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
A-rich	(N:0.76A, 0.08G, 0.08T, 0.08C) NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
G-rich	(N:0.08A, 0.76G, 0.08T, 0.08C) NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
T-rich	(N:0.08A, 0.08G, 0.76T, 0.08C) NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
C-rich	(N:0.08A, 0.08G, 0.08T, 0.76C) NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
A_{30}	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
$N_5A_{20}N_5$	(N:0.25A, 0.25G, 0.25T, 0.25C) NNNNNAAAAAAAAAAAAAAAAAAAANNNNN
$N_{10}A_{10}N_{10}$	(N:0.25A, 0.25G, 0.25T, 0.25C) NNNNNNNNNNAAAAAAAAAANNNNNNNNNN
$A_5N_{20}A_5$	(N:0.25A, 0.25G, 0.25T, 0.25C) AAAAANNNNNNNNNNNNNNNNNNNAAAAA
$A_7N_{16}A_7$	(N:0.25A, 0.25G, 0.25T, 0.25C) AAAAAAANNNNNNNNNNNNNNNNAAAAAAA
$A_{10}N_{10}A_{10}$	(N:0.25A, 0.25G, 0.25T, 0.25C) AAAAAAAAAANNNNNNNNNNAAAAAAAAAA
$A_{10}N_{20}$	(N:0.25A, 0.25G, 0.25T, 0.25C) AAAAAAAAAANNNNNNNNNNNNNNNNNNNN
$(A_5N_5)_3$	(N:0.25A, 0.25G, 0.25T, 0.25C) AAAAANNNNNAAAAANNNNNAAAAANNNNN
$G_8N_{14}G_8$	(N:0.25A, 0.25G, 0.25T, 0.25C) GGGGGGGGNNNNNNNNNNNNNNGGGGGGGG
$G_{10}A_{20}$	GGGGGGGGGGAAAAAAAAAAAAAAAAAA

Supporting Table S11: Diffusion coefficients and $t_{½}$ of measured FRAP recoveries in this study. Measurements have been performed in triplicate and the diffusion coefficients are the averages from these triplicates.

DNA sequence	$D_{peptide}$	$t_{1/2,peptide}$	$D_{poly\text{-}U}$	$t_{1/2,poly\text{-}U}$
N_{30}	3.3 (+/- 0.27)	0.43 (+/- 0.05)	0.024 (+/-0,003)	28 (+/- 8)
A_{30}	3.16 (+/- 0.12)	0.47 (+/- 0.05)	0.006 (+/- 0,0005)	146 (+/- 6)
no DNA	3.01 (+/- 0.27)	0.47(+/- 0.05)	0.033 (+/- 0,009)	21 (+/- 7)
$G_{10}A_{20}$	1.44 (+/- 0.47)	1.2 (+/- 0,5)	0.001 (+/- 0,0005)	760 (+/- 601)
$G_8N_{14}G_8$	1.36(+/- 0.22)	1.1 (+/- 0,2)	0.026 (+/-0,005)	37 (+/- 13)

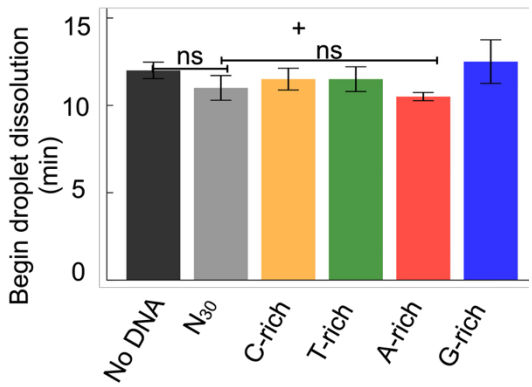


Fig. S1. Longevity of droplets in the presence of different biased randomer libraries. *Droplets were prepared according to standard active droplet sample preparation with 50 µM of DNA. Turbidity was observed as Absorbance at 600 nm on the UV VIS spectrometer for thirty minutes. For droplet longevity, we compare the time point at which the measured turbidity drops below 0.2 as the beginning of the droplet dissolution.*

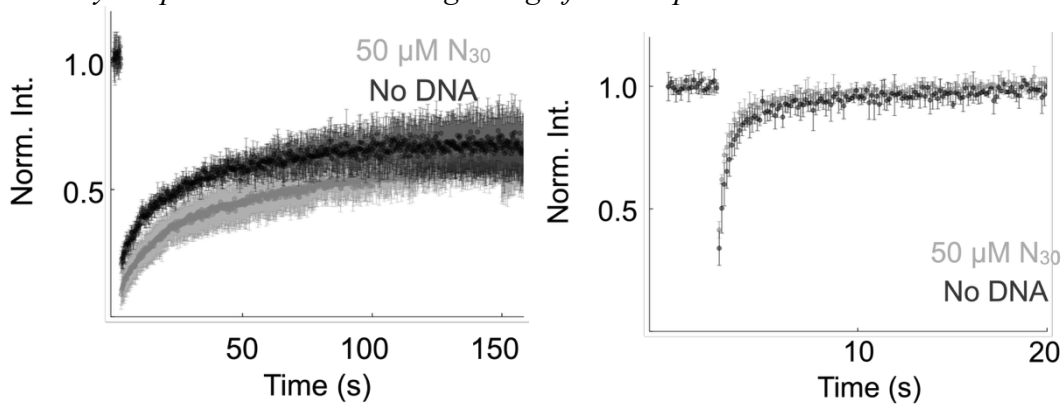


Fig. S2. Comparison of the precursor peptide and poly-U diffusivities with and without 50 µM randomer DNA. *Fluorescence recovery after photobleaching of fluorescently tagged precursor peptide NBD-G(RG)$_3$D-OH,(**A**) used at 1 mM concentration and poly-U (**B**) annealed with 500nM Cy5-A$_{15}$ RNA. Sample conditions were 20 mM peptide, 3 mM peptide model, 5.6 mM poly-U (no DNA Control) or 4.1 mM poly-U with 50 µM N30 (1.5 mM extra charge). The photobleached peptide was observed for 70 s, and the poly-U for 180 s. Error bars represent the standard deviation of 3 measurements.*

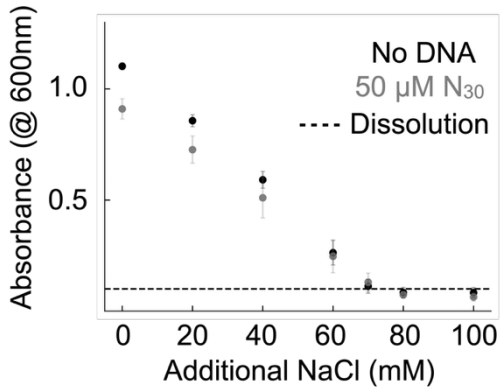


Fig. S3. Critical salt concentration in the presence of N_{30} DNA. *Static coacervate samples were titrated according to the standard static droplet protocol with and without 50 µM of random DNA library. 20 µL samples were titrated with concentrated NaCl solution, and the absorbance was measured on the UV VIS spectrometer after salt addition and mixing.*

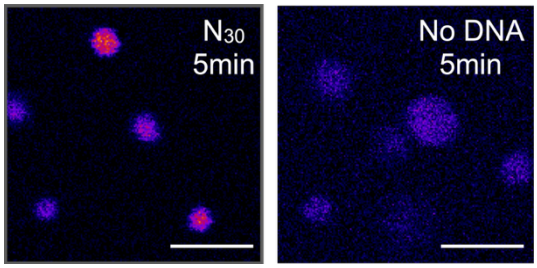


Fig. S4. Droplet morphology in the presence of N30. *Droplets were prepared according to standard conditions with 500 nM Sulforhodamine and observed under the confocal microscope using an IBIDI chamber coated with a 5 %PVA solution. Images were taken after five minutes, 20 µm above the glass.*

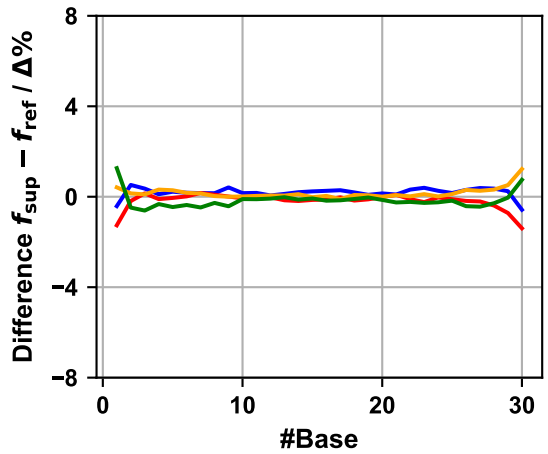


Fig. S5. Difference in nucleotide content between Supernatant and Reference. *Plotted is the nucleotide content per position of the Supernatant, subtracted by the nucleotide content per position of the Reference sample.*

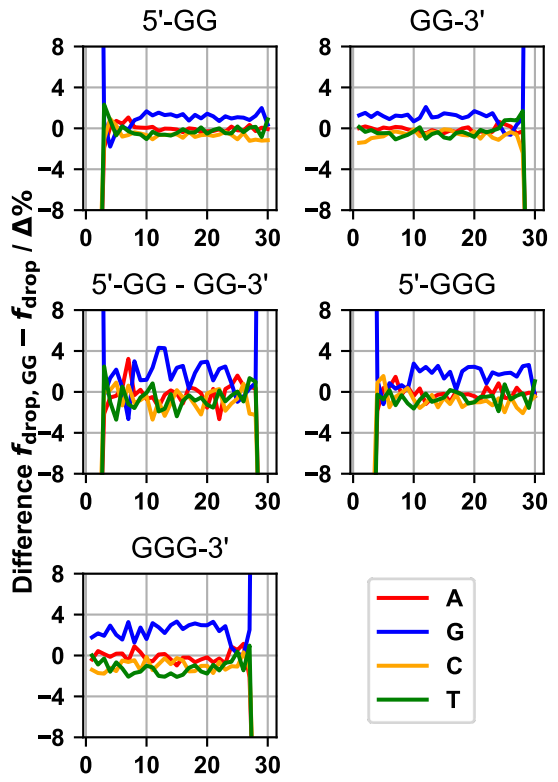


Fig. S6. Cooperative effect of multiple Gs at the terminus of sequences. *Sequences containing two or three consecutive G at the 5' or 3' terminus of a sequence have a higher probability of additional Gs in the same sequence. Plotted is the nucleotide content per position of sequences containing the defined number of Gs at the defined terminus in the droplet sample, subtracted by the nucleotide content of all sequences in the droplet sample.*

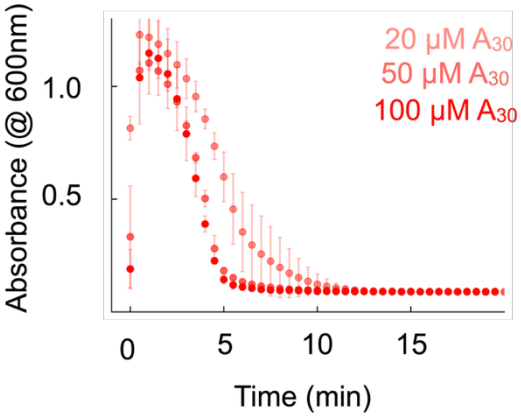


Fig. S7. Turbidity traces in the presence of different concentrations of A_{30} DNA. *Droplets were prepared according to standard conditions with 20, 50, or 100 µM of A_{30}. Turbidity was observed as Absorbance at 600 nm on the UV VIS spectrometer. Lifetime is determined at a threshold of 0.12.*

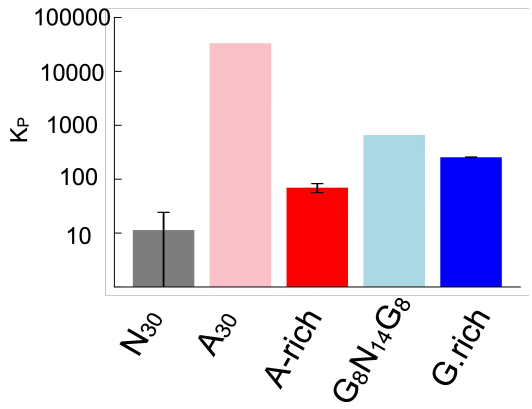


Fig. S8. Partitioning of A-rich and G-rich randomers compared to designed sequences. *DNA partitioning into the droplet phase was determined by preparing static droplets according to standard static droplet preparation, spinning down the samples, and measuring the DNA concentration in the supernatant using HPLC. The partition coefficient was then determined with the droplet volume for static droplets on the confocal microscope. Data is presented on a logarithmic scale. Error bars represent the average of three measurements.*

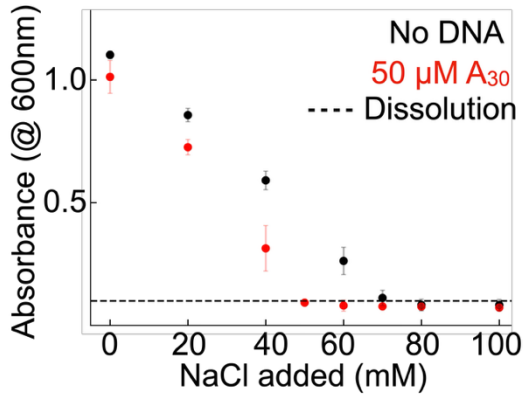


Fig. S9. Critical salt concentration *Titration of static coacervate samples prepared according to standard static droplet protocol with and without 50 µM of random DNA library. 20 µL samples were titrated with concentrated NaCl solution, and the absorbance was measured on the UV VIS spectrometer after salt addition and mixing.*

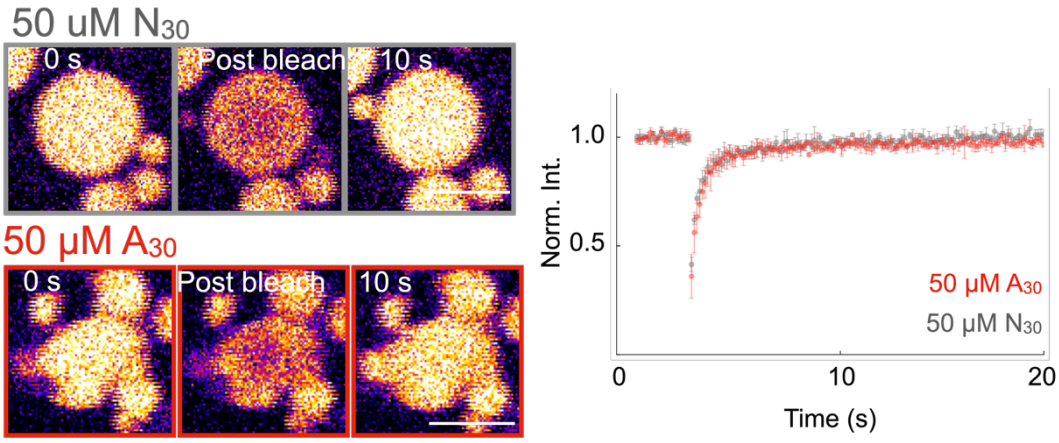


Figure S10: **Comparison of the precursor peptide diffusivities with 50 µM N_{30} or A_{30} DNA.** *Fluorescence recovery after photobleaching of fluorescently tagged precursor peptide NBD-G(RG)$_3$D-OH,(**A**) used at 1 mM concentration. Sample conditions were 20 mM precursor, 3 mM pseudo anhydride. 4.1 mM pU with 50 µM N_{30} or A_{30}. The photobleached peptide was observed with the confocal microscope for 70 s in total. Error bars represent the standard deviation of 3 measurements. Scalebars represent 10 µm.*

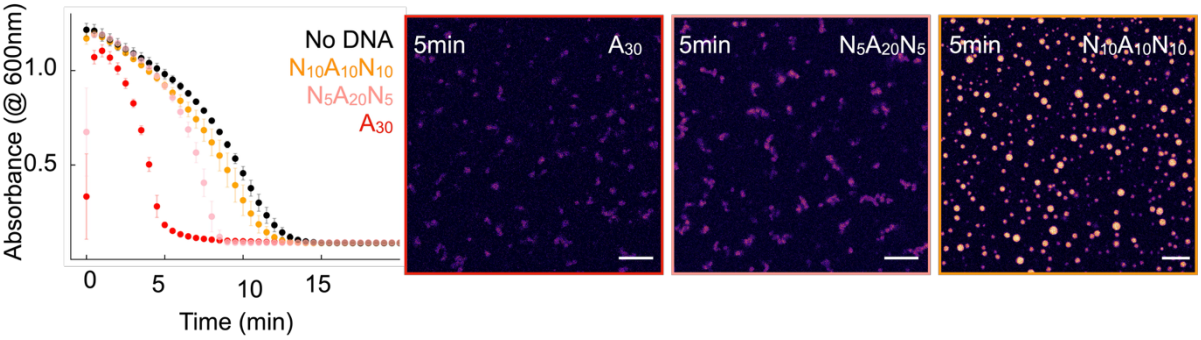


Figure S11: Droplet lifetime and morphology in the presence of central adenine stretches of increasing length. *Droplets were prepared according to standard conditions with 50 µM of DNA. Turbidity was observed as Absorbance at 600 nm on the UV VIS spectrometer. Lifetime is determined at a threshold of 0.12. Droplets were prepared according to standard conditions and imaged with 1 mM NBD-GRGRGRGD-OH on the glass after five minutes. Scalebars represent 10 µm.*

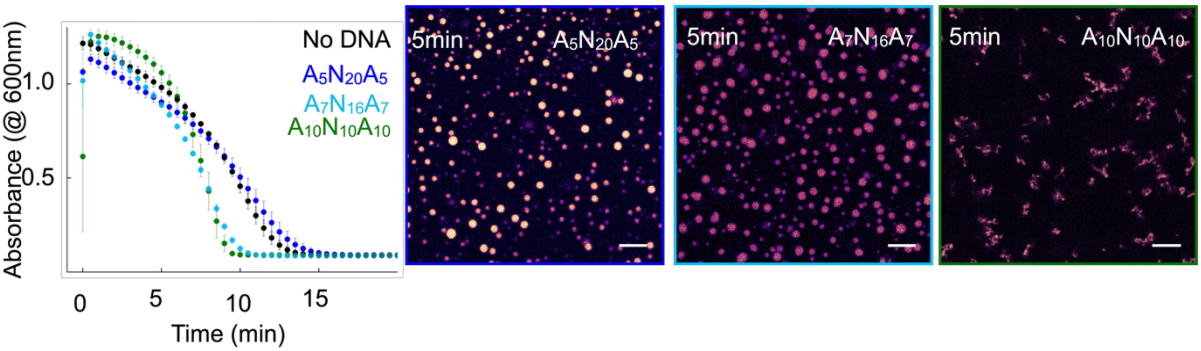


Figure S12: Droplet lifetime and morphology in the presence of terminal adenine stretches of increasing length. *Droplets were prepared according to standard conditions with 50 µM of DNA. Turbidity was observed as Absorbance at 600 nm on the UV VIS spectrometer.*

Lifetime is determined at a threshold of 0.12. Droplets were prepared according to standard conditions and imaged with 1 mM NBD-GRGRGRGD-OH on the glass after five minutes. Scalebars represent 10 µm.

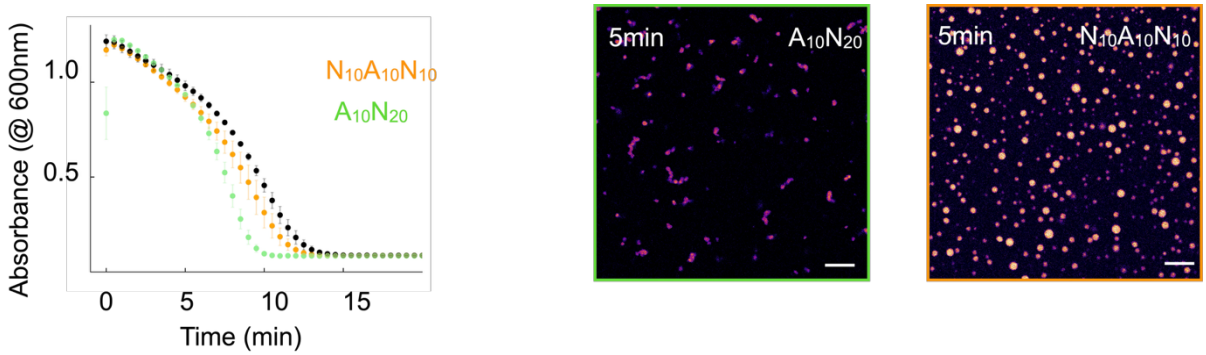


Figure S13. Droplet lifetime and morphology in the presence of central ten adenines compared to terminal ten adenines. *Droplets were prepared according to standard conditions with 50 µM of DNA. Turbidity was observed as Absorbance at 600 nm on the UV VIS spectrometer. Lifetime is determined at a threshold of 0.12. Droplets were prepared according to standard conditions and imaged with 1 mM NBD-GRGRGRGD-OH on the glass after five minutes. Scalebars represent 10 µm.*

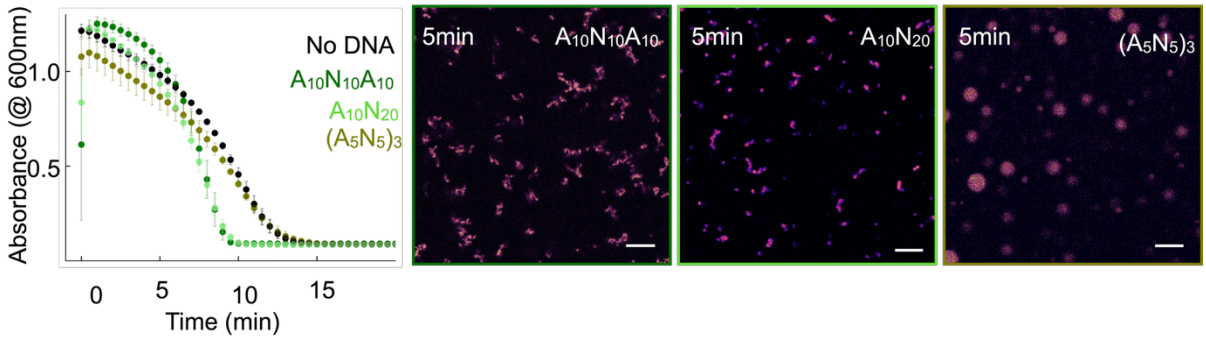


Figure S14: Figure S14. Droplet lifetime and morphology in the presence of differently distributed consecutive adenines. *We compare ten terminal adenines on one side to ten terminal adenines on both sides and fifteen distributed adenines in stretches of five. Scalebars represent 10 µm. Droplets were prepared according to standard conditions with 50 µM of DNA. Turbidity was observed as Absorbance at 600 nm on the UV VIS spectrometer. Lifetime is determined at a threshold of 0.12. Droplets were prepared according to standard conditions and imaged with 1 mM NBD-GRGRGRGD-OH on the glass after five minutes. Scalebars represent 10 µm.*

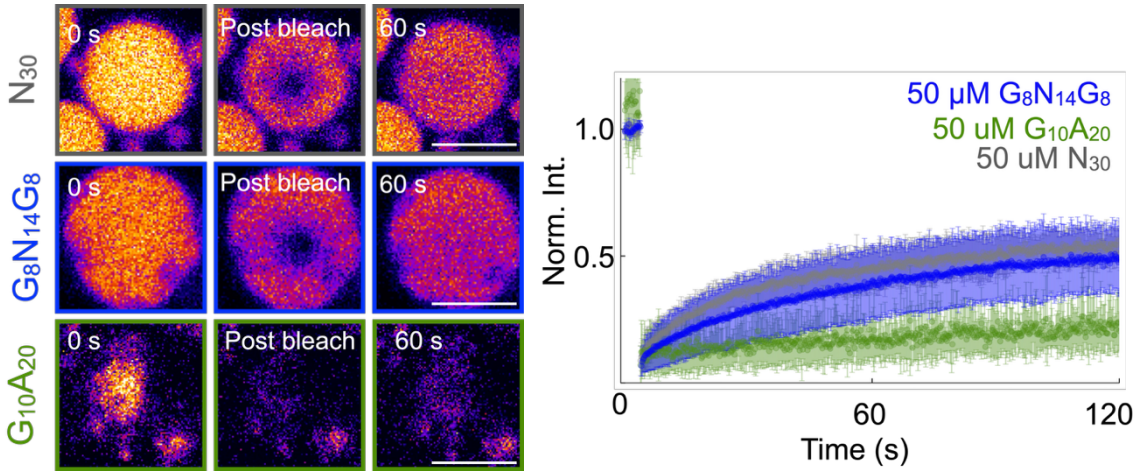
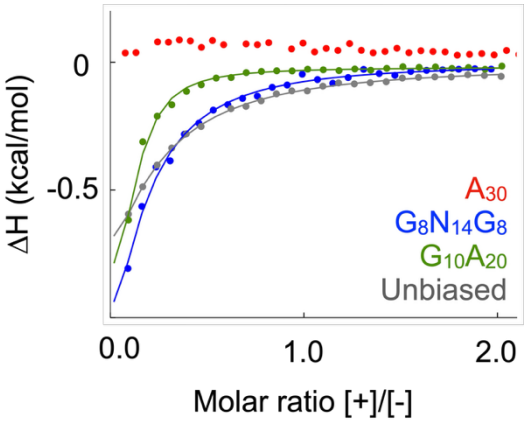


Fig. S16*:* Comparison of poly-U diffusivities with and without 50 µM A_{30} DNA. *Fluorescence recovery after photobleaching of Cy5-A_{15} RNA oligomer hybridized to poly-U used at 500 nM concentration. Sample conditions were 20 mM precursor, 3 mM pseudo anhydride, 4.1 mM poly-U with 50 µM $G_8N_{14}G_8$ or $G_{10}A_{20}$. The grey and blue solid lines represent the fit of the fluorescent recovery. The samples were observed for at least 120 s. Error bars represent the standard deviation of three measurements. Scalebars represent 10 µm.*



Sequence	Dissociation constant with AcF(RG)$_3$N-NH$_2$	ΔH (kcal/mol)
N_{30}	188 ± 44	-3.7 ± 0.7
$G_8N_{14}G_8$	113 ± 32	-3.5 ± 1.3
$G_{10}A_{20}$	45 ± 13	-2.0 ± 0.5
A_{30}	No binding	0.03± 0.04

Fig. S15. ITC measurements of peptide model with different DNA 30mers. ITC titrations of 500 µM of different DNA 30mers (charge concentration) with 5 mM of the peptide anhydride model peptide model (AcF(RG)$_3$N-NH$_2$) (charge concentration). Measurements are performed in triplicates.