SUPPLEMENTARY TABLES

Extended Data Table 1. Crystallographic statistics

	Lettuce–DFHBI-1T	Lettuce–DFHBI-1T Thallium I Dataset	Lettuce–DFHO	Lettuce–DFAME
Data collection*†				
Beamline	APS 24-ID-E	APS 24-ID-C	APS 24-ID-E	ALS 5.01
Wavelength (Å)	0.912	0.976	0.912	0.977
Space group	$P\ 2_12_12_1$	$P\ 2_12_12_1$	$P\ 2_12_12_1$	$P\ 2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	25.01 44.42 118.92	24.65 42.82 119.53	24.57 42.28 119.13	24.88 43.02 119.62
α, β, γ (°)	90 90 90	90 90 90	90 90 90	90 90 90
Resolution (Å)	59.46 - 2.5	119.53 - 2.27	119.13 - 2.01	59.81 - 2.90
R_{merge}	0.102 (0.287)	0.127 (1.223)	0.065 (2.844)	0.070 (0.269)
$CC_{1/2}$	0.998 (0.956)	0.996 (0.689)	0.998 (0.382)	0.994 (0.987)
$\langle I \rangle / \langle \sigma(I) \rangle$	13.9 (2.0)	12.6 (1.4)	15.6 (0.8)	20.0 (3.1)
Completeness (%)	97.63 (99.79)	99.8 (98.0)	99.5 (99.4)	99.91 (100.0)
Redundancy	10.2 (11.62)	12.1 (13.2)	12.5 (13.5)	11.46 (12.39)
Refinement				
Resolution (Å)	41.61 - 2.5 (2.59 - 2.5)	59.77 - 2.5 (2.59 - 2.5)	59.57 - 2.60 (2.69 - 2.60)	40.48 - 3.0 (3.10 - 3.0)
Unique reflections	4869 (473)	4773 (444)	4167 (409)	2870 (296)
R_{work} / R_{free}	0.225 / 0.268	0.212 / 0.251	0.214 / 0.265	0.228 / 0.291
No. atoms				
DNA	1153	1110	1091	1120
Ligand/ion	28	28	26	29
Water	17	29	18	16
B-factors (Å^2)				
DNA	59.77	61.69	72.26	53.07
Ligand/ion	49.64	53.25	60.41	44.76
Water	45.98	52.41	59.82	41.20
R.m.s. deviations				
Bond lengths (Å)	0.005	0.005	0.011	0.04
Bond angles (°)	0.78	0.83	1.23	0.66
Mean precision (Å)	0.42	0.39	0.44	0.41
PDB ID	8FHV	8FHX	8FHZ	8FI0

*One crystal was used for each dataset. †Values in parentheses are for highest resolution shell.

Lettuce C20G –	Lettuce C20T –	Lettuce C20T –	Lettuce C20T –

DFHO	DFHBI-1T	DFHO	DFAME
APS 24-ID-C	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C
1.105	1.105	1.105	1.105
$P\ 2_12_12_1$	$P\ 2_12_12_1$	$P\ 2_12_12_1$	$P\ 2_12_12_1$
25.81 46.99 120.98	24.84 44.56 119.59	24.57 43.10 117.64	25.02 43.95 120.10
90 90 90	90 90 90	90 90 90	90 90 90
120.98 - 2.35	41.76 - 2.70	43.10 - 2.80	60.05 - 2.40
0.154 (2.191)	0.174 (0.563)	0.164 (0.442)	0.113 (0.527)
0.985 (0.496)	0.998 (0.860)	0.996 (0.958)	0.998 (0.916)
9.6 (0.9)	10.7 (1.0)	22.8 (2.50)	19.3 (1.1)
99.9 (100)	99.0 (97.38)	98.63 (98.79)	98.48 (100.0)
12.3 (13.1)	10.5 (10.49)	9.15 (10.97)	11.6 (11.74)
43.81 - 2.6 (2.69 - 2.6)	35.73 - 3.0 (3.11 - 3.0)	34.77 - 2.90 (3.0 - 2.9)	41.28 - 2.8 (2.9 - 2.8)
4913 (461)	2926 (290)	3048 (289)	3540 (330)
0.219 / 0.271	0.244 / 0.287	0.244 / 0.289	0.217 / 0.283
1115	1154	1121	1117
25	27	25	28
19	9	16	16
73.46	61.25	45.18	43.08
55.77	53.09	44.16	41.47
65.43	50.07	36.13	37.01
0.006	0.004	0.005	0.006
0.77	0.70	0.68	0.75
0.49	0.24	0.38	0.23
8FI1	8FI2	8FI7	8FI8

*One crystal was used for each dataset. †Values in parentheses are for highest resolution shell.

Extended Data Table 2. Cryo-EM data collection and processing parameters

	Unliganded Lettuce
Microscope	Glacios
Voltage (keV)	200
Nominal Magnification	105,000x
Cumulative Exposure (e/Å^2)	52
Detector	Falcon 4
Pixel Size (Å/pix)	0.9
Defocus Range (μm)	(-0.8) – (-2.2)
Micrographs Used (no.)	3,327
Total Extracted Particles[1] (no.)	1,105,996
Refined Particles (no.)	55,288
Map Resolution[2] (Å)	6.5
EMDB ID	EMD-29329

1.Total extracted particles are defined as the particles obtained after Topaz picking.
2. Resolution was calculated based on the FSC-curve at the value of 0.143.

Extended Data Table 3. Pucker angle analysis

	Lettuce DNA aptamer (8 structures)			Deoxyribozyme 9DB1 [5]		Deoxyribozyme Dz36 [6]		*i*Spinach RNA aptamer [7]	
Position	Nucleotide	Angle mean	Standard dev.	Nucleotide	Angle	Nucleotide	Angle	Nucleotide	Angle
1	C	156	21.7	A	3	T	165	G	6
2	T	155	8.4	T	13	G	105	G	6
3	T	158	5.1	C	17	T	109	G	8
4	A	154	18.4	C	164	A	123	A	9
5	G	139	6.5	G	164	A	151	G	14
6	T	156	18.0	A	150	C	125	U	16
7	A	148	4.7	T	144	G	153	A	14
8	G	142	11.0	G	163	C	86	C	15
9	G	172	34.2	G	145	A	166	G	11
10	G	142	5.4	A	148	C	144	G	14
11	A	32	13.3	T	152	T	151	U	12
12	T	24	5.0	C	191	G	25	G	9
13	G	11	1.8	A	201	C	146	A	21
14	A	164	21.3	T	354	C	149	G	17
15	T	140	29.9	A	135	A	185	G	160
16	G	149	16.3	C	40	G	19	G	150
17	C	101	31.5	G	173	C	210	U	164
18	G	159	31.1	G	183	G	178	C	18
19	G	176	22.3	T	161	G	79	G	8
20	C, G or T	154	23.4	C	0	C	59	G	13
21	A	186	9.9	G	185	T	47	G	17
22	G	147	23.1	G	7	C	156	U	15
23	T	145	5.4	A	173	G	138	C	22
24	G	179	6.1	G	86	A	164	C	16
25	G	154	18.5	G	191	A	142	A	11
26	G	115	59.9	G	190	A	144	G	11
27	C	124	54.2	G	182	T	108	U	13
28	T	81	45.5	T	195	C	46	A	15
29	T	162	1.9	T	193	T	160	G	7
30	C	152	45.1	T	208	C	125	G	18
31	G	183	12.6	G	5	T	152	U	15
32	C	155	3.2	C	15	C	127	A	167
33	A	172	8.5	C	1	T	130	C	161
34	G	149	11.4	G	5	C	42	G	22
35	T	71	25.5	T	20	G	17	C	14
36	T	Disordered loop		T	134	T	31	C	10
37	C	Disordered loop		T	128	-	-	U	18

38	C	Disordered loop		A	51	-	-	A	11
39	T	150	32.9	A	32	-	-	C	13
40	G	158	23.5	G	353	-	-	U	14
41	C	192	7.6	T	359	-	-	G	23
42	G	16	11.2	G	21	-	-	U	133
43	A	188	14.7	C	19	-	-	U	28
44	G	153	18.9	C	147	-	-	G	25
45	G	183	9.0	-	-	-	-	A	168
46	G	104	35.2	-	-	-	-	G	17
47	G	57	38.5	-	-	-	-	U	164
48	A	158	13.6	-	-	-	-	A	161
49	C	130	31.4	-	-	-	-	G	72
50	T	164	11.6	-	-	-	-	A	154
51	A	159	17.9	-	-	-	-	G	162
52	A	135	11.0	-	-	-	-	U	163
53	G	163	20.5	-	-	-	-	G	21
54	-	-	-	-	-	-	-	U	39
55	-	-	-	-	-	-	-	G	1
56	-	-	-	-	-	-	-	G	14
57	-	-	-	-	-	-	-	G	14
58	-	-	-	-	-	-	-	C	11
59	-	-	-	-	-	-	-	U	22
60	-	-	-	-	-	-	-	C	14
61	-	-	-	-	-	-	-	C	14
62	-	-	-	-	-	-	-	G	18
63	-	-	-	-	-	-	-	U	357
64	-	-	-	-	-	-	-	A	11
65	-	-	-	-	-	-	-	C	352
66	-	-	-	-	-	-	-	U	354
67	-	-	-	-	-	-	-	C	18
68	-	-	-	-	-	-	-	C	20
69	-	-	-	-	-	-	-	C	16

Extended Data Table 4. Sequences of constructs used in this study

Name	Sequence, 5' to 3'
Lettuce w.t. - crystallization construct	CTTAGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGAGGGG ACTAAG
C20G - crystallization construct	CTTAGT AGGGATGATGCGGGAGTGGGCTTCGCAGTTCCTGCGAGGGG ACTAAG
C20T - crystallization construct	CTTAGT AGGGATGATGCGGTAGTGGGCTTCGCAGTTCCTGCGAGGGG ACTAAG
Lettuce w.t. - fluorescence experiments construct (f.c.) - modified P1 (4 nts)	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
Split Lettuce w.t. part 1	CTTAGT AGGGATGATGCGGCAGTGGGCTTCGCAG
Split Lettuce w.t. part 2	CTGCGAGGGG ACTAAG
A11G	CCGT AGGGGTGATGCGGAAGTGGGCTTCGCAGTTCCTGCGAGGGGACGG
A11C	CCGT AGGGCTGATGCGGAAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
A11T	CCGT AGGGTTGATGCGGAAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
G26A	CCGT AGGGATGATGCGGCAGTGGACTTCGCAGTTCCTGCGAGGGG ACGG
G26T	CCGT AGGGATGATGCGGCAGTGGTCTTCGCAGTTCCTGCGAGGGG ACGG
C17G and G26C	CCGT AGGGATGATGGGGCAGTGGCCTTCGCAGTTCCTGCGAGGGG ACGG
C17T and G26A	CCGT AGGGATGATGTGGCAGTGGACTTCGCAGTTCCTGCGAGGGG ACGG
C20G	CCGT AGGGATGATGCGGGAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
C20A	CCGT AGGGATGATGCGGAAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
C20T	CCGT AGGGATGATGCGGTAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
C20 deletion (del)	CCGT AGGGATGATGCGG/del/AGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
G18I*	CCGT AGGG ATGAT GC/I/GC AGTGG GCTTC GCAGT TCCTG CGAGG GG ACGG
C17>rC17, G18>rG18, G25>rG25, and G26>rG26*	CCGT AGGGATGATGrCrGGCAGTGrGrGCTTCGCAGTTCCTGCGAGGGG ACGG
A14T and T29A	CCGT AGGGATGTTGCGGCAGTGGGCTACGCAGTTCCTGCGAGGGG ACGG
A14G and T29C	CCGT AGGGATGGTGCGGCAGTGGGCTCCGCAGTTCCTGCGAGGGG ACGG
A14C and T29G	CCGT AGGGATGCTGCGGCAGTGGGCTGCGCAGTTCCTGCGAGGGG ACGG
T15A	CCGT AGGGATGAAGCGGCAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
T28A	CCGT AGGGATGATGCGGCAGTGGGCATCGCAGTTCCTGCGAGGGG ACGG
T15G and T28C	CCGT AGGGATGAGGCGGCAGTGGGCCTCGCAGTTCCTGCGAGGGG ACGG
T15C and T28G	CCGT AGGGATGACGCGGCAGTGGGCGTCGCAGTTCCTGCGAGGGG ACGG
T15G, T28C, A14T and T29A	CCGT AGGGATGTGGCGGCAGTGGGCCACGCAGTTCCTGCGAGGGG ACGG
A43 del	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCG/del/GGGG ACGG
A43G	CCGT AGGGATGATGCGGCAGTGG GCTTCGCAGTTCCTGCGGGGGG ACGG
A43C	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGCGGGG ACGG
A43T	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGTGGGG ACGG
G44 del	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGA/del/GGG ACGG
G44A	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGAAGGG ACGG
G44C	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGACGGG ACGG
G44T	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGATGGG ACGG

A7C	CCGT CGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
G8 del	CCGT A/del/GGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
G22C	CCGT AGGGATGATGCGGCACTGGGCTTCGCAGTTCCTGCGAGGGG ACGG
G47T	CCGT AGGGATGATGCGGCAGTGGGCTTCGCAGTTCCTGCGAGGGT ACGG
Minimization: 4 nts P1, 3 nts P2, and loop TTC	CCGT AGGGATGATGCGGCAGTGGGCTTCGCTTCGCGAGGGG ACGG
Minimization: 4 nts P1, 2 nts P2, and loop TTC	CCGT AGGGATGATGCGGCAGTGGGCTTCGTTCCGAGGGG ACGG
Minimization: 4 nts P1 and P2 del - just loop TTC	CCGT AGGGATGATGCGGCAGTGGGCTTTTCAGGGG ACGG
Circular permutation (C.P.) crystallization construct	CTGCG AGGGGACTAAGTTCCTTAGTAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: 4 nts $P1_{cp}$, and loop TTC	CTGCG AGGGGACGGTTCCCGTAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: 3 nts $P1_{cp}$, and loop TTC	CTGCG AGGGG ACGTTCCGT AGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: 2 nts $P1_{cp}$, and loop TTC	CTGCG AGGGGACTTCGTAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: 1 nt $P1_{cp}$, and loop TTC	CTGCG AGGGGATTCTAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: $P1_{cp}$ del - just loop TTC	CTGCG AGGGGTTCAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: $P1_{cp}$ del - just loop TC	CTGCG AGGGGTCAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: $P1_{cp}$ del - just loop T	CTGCG AGGGGTAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P.: $P1_{cp}$ and loop TTC del	CTGCG AGGGGAGGGATGATGCGGCAGTGGGCTT CGCAG
C.P. T15G, T28C, A14T and T29A: 4 nts $P1_{cp}$, and loop TTC	CTGCG AGGGGACGGTTCCCGTAGGGATGTGGCGGCAGTGGGCCA CGCAG
C.P. T15G, T28C, A14T and T29A: 1 nt $P1_{cp}$, and loop TTC	CTGCG AGGGGATTCTAGGGATGTGGCGGCAGTGGGCCA CGCAG
C.P. T15G, T28C, A14T and T29A: 4 nts $P2_{cp}$, and $P1_{cp}$ del - just loop T	CGCG AGGGGTAGGGATGTGGCGGCAGTGGGCCA CGCG
R-loop DNA Fwd with Lettuce C.P. T15>G, T28>C, A14>T and T29>A: 9 nts $P2_{cp}$ and $P1_{cp}$ del - just loop T (underlined)	CACTCCCAGAGTCGTGGCCGTAGGAAATCGCTTAAGATACACAGAATAT CCTCAGTCGAGGGGTAGGGATGTGGCGGCAGTGGGCCACGACTGAGG AATCTCTAGTCAAATTCCATCATCGTAGCGCTGAGGAATTTGGTGGACACTAGGA
R-loop DNA reverse complement	TCCTAGTGTCCACCAAATTCCTCAGCGCTACGATGATGGAATTTGACTAGAG ATTCCTCAGTCGTGGCCCACTGCCGCCACATCCCTACCCCTCGACTGAGGAT ATTCTGTGTATCTTAAGCGATTTCCTACGGCCACGACTCTGGGAGTG
R-loop Fwd primer	CACTCCCAGAGTCGTGGCCGTAGG
RNA used for R-loop formation	GGAAAUCGCUUAAGAUACACAGAAUAUCCUCAGUCGAGGGGUAGGGAUGUG GCGGCAGUGGGCCACGACUGAGGAAUCUCUAGUCAAAUUCCAUCAUCGU
RNA Lettuce w.t.	CUUAGUAGGGAUGAUGCGGCAGUGGGCUUCGCAGUUCCUGCGAGGGGACUAAG
RNA Split Lettuce part 2	CUGCGAGGGGACUAAG

* Custom synthesized by IDT.

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