One- and two-dimensional high-resolution NMR from flat surfaces

Supplementary Information

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I. Estimate of the quantity of oligonucleotides immobilized on a planar support

A. *Specific surface area of a planar support*

Specific surface area: s
Glass wafer thickness: c
The length and width of the slides are much greater than their thickness and can be neglected.
Density of cover slip: ρ

c = 0.15 mm
ρ = 2.4 g/cm^3

$s = 2/(\rho c)$ = **0.0056 m^2/g**

B. *Estimation for monolayer coverage*

Surface molecule density: d
Solid material packed inside rotor: $m_{\text{in,dry}}$
Moles of RNA: n

Approximate d = 1 molecule/nm^2
Approximate $m_{\text{in,dry}}$ = 30 mg

$n = sd(m_{\text{in,dry}})/(N_A)$ = **300 pmol**

This calculation assumed glass for the bulk material but n is independent of material (assuming fixed c, d) as any change in ρ is compensated by a proportional change in m.

This provides an estimate for the maximum amount of a heptaribonucleotide that can be deposited onto the surface without exceeding monolayer coverage, assuming (as was generally the case, esp. for sapphire) that the entire slip used for the deposition could be packed into the rotor for analysis. On this basis we deposited no more than 300 pmol of a given heptaribonucleotide oligonucleotide species onto the supports, corresponding to a maximum of 1.8 nmol of phosphodiester functional groups. For longer oligonucleotide sequences, lesser quantities were deposited in such a way that the 1.8 nmol functionality limit was not exceeded.

The amount of material for the ^{13}C reference sensitivity reference experiment is taken to be 30 mg of a solid of molar mass equal to 100 g/mol, leading to 300 µmol of material.

Typical routine solid-state NMR of organic solids is carried out primarily on ^{13}C at natural abundance, and this is the point of reference we use to calculate relative sensitivity, leading to the factor ~600000 found in the main text. If the direct comparison were made to ^{31}P spectroscopy of a bulk material, then this factor would be ~7000.

II. Azide functionalization of glass surfaces

To a solution of (3-chloropropyl)trimethoxysilane (730 mg, 3.66 mmol) in anhydrous DMF (2 mL), sodium azide (250 mg., 3.85 mmol) and sodium iodide (5.50 mg, 36.6 μmol) were added. The mixture was heated to 100 °C, and after being stirred for 16 h, it was cooled slowly to room temperature and diluted with dimethyl ether (100 mL). The organic mixture was washed with water (100 mL, 3 times) and brine (100 mL) and dried over anhydrous Na_2SO_4, and the solvent was removed in *vacuo*. The residue was distilled with Kugelrohr under Schlenk vacuum (approx.. 0.1 torr) at 110 °C to afford (3-azidopropyl)trimethoxysilane (416 mg, 55%) as a colorless liquid. Solution NMR:
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.68-0.71 (m, 2H), 1.68-1.74 (m, 2H), 3.27 (t, J = 6.9 Hz, 2H), 3.58 (s, 9H)
^{13}C NMR (125 MHz, $CDCl_3$): δ 6.31, 22.43, 50.57, 53.71 ^{15}N NMR (50 MHz, $CDCl_3$): δ 71.68, 210.32

To 500 mg of cleaned glass plates, a solution of (3-azidopropyl)trimethoxysilane in anhydrous toluene (10 mL, 0.05M) was added. The mixture was heated and gently stirred at 110 °C. After 16 h of reaction time, it was cooled down and filtered. The crude surface-modified plates were washed with methanol (20 mL 6 times) and dried under house vacuum (~20 torr) overnight to afford the azide-modified glass plates.

III. Preparation of samples for DNP-SENS

All preparation surfaces were thoroughly washed with ethanol and sample manipulations were exercised while wearing nitrile gloves in order to avoid human skin contact that can possibly catalyze backbone cleavage of the oligonucleotides.

For the borosilicate glass supports, several small plates, totaling roughly 80 mg, were crushed in an alumina mortar with an alumina pestle for about two minutes. An amount of this dry powder, of mass m_{dry}, was placed on a watch glass and the surface was impregnated with roughly 16 μL (mass m_{wet}) of the TEKPol/TCE solution using a spatula to distribute the radical containing solution over the powder. For the sapphire supports, a single disk weighing about 70 mg was crushed for around twenty to sixty seconds, of which roughly 55 mg of powder was generally recovered and impregnated with around 8 μL of the impregnating solution. For the fused silica (EPR tube) support, the lyophilized section of the tube was cleaved off, totaling 110 mg. This was crushed and 46.0 mg of this powder was impregnated with 9.2 μL of TCE. For samples **2B**, **2C**, **4B 0**, **4B 2**, and **4B 60**, the impregnating solution used was 12 mM TEKPol2/TCE, rather than 12 mM or 16 mM TEKPol/TCE.

Once impregnated, the sample was transferred and packed into a 3.2 mm sapphire NMR rotor over about one minute. The rapid transfer minimized evaporative loss of the TCE after the impregnation step. Once packed, the sapphire rotor was plugged with either a silicone or (usually) a polytetrafluoroethylene insert. The rotor was then capped with a zirconia drive tip and, if not immediately analyzed, stored inside a freezer at -30 °C for at least one night but no more than two weeks. Curiously, the absolute NMR sensitivity of samples stored in the freezer was consistently superior to that of samples which were analyzed immediately. Finally, while it is possible to machine 2.2 mm diameter disks to fill the rotor, leaving the flat surfaces intact, our simple hand-crushing procedure is unlikely to significantly disrupt the state of the oligonucleotides, and does not require preparation protocols specifically for analysis.

Sample	Support	Oligonucleotide Sequence	m_{dry}	m_{wet}	m_{in}	$m_{in,dry}$
1 S	Sapphire	dS(C_7)	62.1 mg	14.1 mg	65.1 mg	53.1 mg
1 FS	Fused silica	dS(C_7)	46.0 mg	14.4 mg	38.3 mg	29.2 mg
1 BG	Borosilicate glass	dS(C_7)	52.1 mg	18.9 mg	41.3 mg	30.3 mg
1D	Sapphire	r(GAAGAGAAGC)• dS(GCTTCTCTTC)	58.6 mg	11.5 mg	58.1 mg	48.6 mg
2A	Sapphire	r(A_{10})-dS(T_{10})	58.0 mg	11 mg	56.1 mg	47 mg
2B	Sapphire	*a*,*b*	60.3 mg	13.0 mg	56.9 mg	46.8 mg
2C	Sapphire	*a*	50.2 mg	10.4 mg	53.8 mg	44.6 mg
3 Na	Sapphire	dS(C_7)	62.1 mg	14.1 mg	65.1 mg	53.1 mg
3 Mg	Sapphire	dS(C_7)	49.5 mg	10.7 mg	49.1 mg	40.4 mg
4A Na	Borosilicate glass	r(UGCAUAU)	42.7 mg		43.7 mg	
4A Mg	Borosilicate glass	r(UGCAUAU)	63.6 mg	23.2 mg	41.8 mg	30.6 mg
4B 0	Sapphire	r(*x*UGCAUGU)	64.2 mg	12.0 mg	59.1 mg	49.8 mg
4B 2	Sapphire	r(*x*UGCAUGU)	55.0 mg	11.4 mg	50.5 mg	41.8 mg
4B 60	Sapphire	r(*x*UGCAUGU)	52.3 mg	10.7 mg	56.6 mg	47.0 mg

Table S1 | Samples used for DNP experiments. The concentration of TEKPol in TCE is 12 mM with the exception of samples 4A Na and 4A Mg, where it is 16 mM and 15 mM, respectively. Note that sample 3 Na is identical to sample 1S except that after the original analysis the rotor was stored at -30 °C for three days. Sequence *a*: d(ACAATCAGCTAAT*G*ACACTGCCTA). Sequence *b*: r(UGGCAGUGUCUUAGCUGGUUGU), also referred to as *hsa-miR34a-5P*. Label '*': Phosphorothioester linkage. Label '*x*': Terminal phosphorothioester tagged with dinitrobenzhydryl.

The mass of material packed inside the rotor, m_{in}, was determined by difference with the unpacked rotor. The mass of glass plates being used for the analysis, $m_{in,dry}$, is m_{in} scaled in proportion to the amount of dry material present after the incipient wetness impregnation step.

For the magnesium exchanged sample **4A Mg** shown in the main text Fig. 4, the plates of sample **4A Na** (as afforded by the procedure given in Methods) were soaked in an aqueous 1 M $MgCl_2$ solution for approximately 10 minutes. The plates were then transferred into a beaker containing pure water and swirled around. The magnesium soak and water rinse cycle were repeated two additional times. The plates were then placed into a beaker of methanol and were left soaking for several minutes. After observing the plates drying on a watch glass, the plates were dipped in methanol once more and the solvent removed quickly in a stream of dry nitrogen gas. For sample **3 Mg**, the oligonucleotide was prepared directly as the Mg^{2+} salt by adding 3 M $MgCl_2$ to the oligonucleotide solution prior to drying, bringing it to a final concentration of 0.3 M and precipitation was forced using three volumes of ethanol. The supernatant was discarded, and the disk was washed with 75% ethanol and dissolved in RNase free H_2O.

IV. NMR parameters

Relevant NMR parameters for all DNP enhanced NMR experiments are given in Tables S2 (CP CPMG) and S3 (CP PASS-PIETA). Note that the ordinary FID from CP was sometimes collected and used in the reconstruction for CPMG, leading to an additional "0.5 echo". When available, this FID was also used to calculate echo train enhancement, ε_{ETA}.

Sample	**1 S**	**1 S**	**1 FS**	**1 BS**	**1D**	**2A**	**2B**	**2C**	**4A Na**	**4A Mg**	**4B 0**	**4B 2**	**4B 60**
Temperature / K	100	100	100	100	100	100	100	100	118	120	100	100	100
Spin rate / Hz	12500	12500	12500	12500	12500	12500	15000	15000	10000	10000	14493	14993 15000	14993
Contact time / ms	0.8	0.8	0.8	0.8	0.8	0.8	1.2	1.2	0.63	0.63	1.2	1.2	1.2
^{1}H decoupling rf / kHz	113	113	113	113	107	104	100	100	100	100	100	100	100
^{1}H contact rf / kHz	80	80	80	80	80	78	80	80	72	71	80	80	80
X contact rf / kHz	65	65	65	65	65	65	93	93	66	70	92	92	92
X pulse rf / kHz	150	150	150	150	150	150	150	150	139	74	150	150	150
$T_{DNP}(^1H)$ / s	3.97	3.72	3.84	3.26	3.99 3.61	4.14	6.24	6.37 4.99			6.29	5.78 5.76	5.50
$\varepsilon_{DNP}(^1H)$ at 12.5 kHz MAS	504	503	377	181	470 400	460	330	330 360	150†	≳70†	330	360 370	370
ε_{ETA}		4.6					3.4	2.8	1.9	1.5	2.9	2.5	4.1
Total transients	14336	8576	20480	18432	3840	7808	20480	49664	38400	12960	10240	30720	9216
Acq. time per echo / ms	2.56	1.28	2.56	2.56	1.92	1.92	2.00	2.00	3.20	3.20	2.76	2.76 2.00	2.76
Number of echoes	14	30.5	14	14	32	32	40.5	40.5	60.5	30.5	30.5	30.5 40.5	30.5
Total aq. time / ms	35.84	39.04	35.84	35.84	61.44	61.44	81.00	81.00	193.6	97.6	84.18	84.18 81.00	84.18
Recycle delay /s	5	5	4.8	4	5	5.2	7.8	8.0 6.2	4	5	7.9	7.25	6.9
Total expt time /h	20.1	12.1	27.5	20.7	5.4	11.4	43.9	97.6	44.7	18.4	22.73	62.63	17.90

Table S2 | Experimental NMR parameters for the SENS optimized approach using DNP enhanced CP CPMG. Sample temperatures are approximate to within roughly 5 K. The proton decoupling program used during acquisition was SPINAL-64. The amplitude of the proton contact pulse was ramped from 90% to 100% of the amplitude listed. Columns with two entries in some cells indicate different parameters used in two separate experimental sessions on the same sample, with interim storage of the rotor at -30 °C (**1D, 4B 2**) or room temperature (**2C**). Sample **4B 60** was also left out for one night at room temperature in addition to time spent at -30 °C. The build-up time constant and DNP enhancement for the two sessions are reported separately, with the value recorded for the first session appearing above that of the second. The second CP CPMG experiment on sample **1 S** (not shown in a figure), recorded three days after the first (with interim storage of the rotor at -30 °C), had superior sensitivity to the first owing to a selection of CPMG parameters that favored sensitivity over spectral resolution, and illustrates that in such cases ε_{ETA} values exceeding four are possible under DNP SENS conditions. For sample 4B 2 data was collected at two slightly different spinning rates and acquisition conditions. This data was combined to produce the green spectrum plotted in Figure S9. †At 10 kHz MAS.

Sample	**1D**	**3 Na**	**3 Mg**
Temperature	100 K	100 K	100 K
Spin rate	3125 Hz	(3125.0 ± 0.8) Hz	3125 Hz
Contact time	630 µs	630 µs	630 µs
^{1}H pulse/ decoupling rf	107 kHz	113 kHz	115 kHz
^{1}H contact rf	80 kHz	80 kHz	81 kHz
X contact rf	75 kHz	75 kHz	kHz
X pulse rf	150 kHz	150 kHz	150 kHz
$T_{DNP}(^{1}H)$	3.71 s	3.86 s	2.35 s
$\varepsilon_{DNP}(^{1}H)$ at 12.5 kHz MAS	470	503	600
Transients	24	36	44
Phase dim size	64	64	64
Effective number of scans per PASS step	1536	2304	2816
PASS dim size	16	16	16
Acq. time per echo	1.28 ms	1.28 ms	1.28 ms
Number of echoes	30	28	28
Total aq. time	38.4 ms	35.84 ms	35.84 ms
Recycle delay	4.66 s	5 s	5 s
Total expt time	32.2 h	51.6 h	63.1 h

Table S3 | Experimental NMR parameters for the SENS optimized sideband separation approach using DNP enhanced CP PASS-PIETA. Sample temperatures are approximate to within roughly 5 K. The proton decoupling program used during acquisition was SPINAL-64. The amplitude of the proton contact pulse was ramped from 90% to 100% of the amplitude listed. Note that sample **3 Na** is identical to sample **1S** used for the CPMG experiments except that after the original analysis the rotor was stored at -30 °C for three days. For this sample the reported spin rate comes from a statistical analysis of the instantaneous spin rate recorded over the duration of the experiment. The sideband correlation spectrum for sample **1D** is given in Figure S5; due to gyrotron problems, some parts of this experiment were collected out of order, though all data was acquired in the same session. In this case the experiment time refers to the length of the experiment in the event it had completed without interruption.

V. NMR signal processing

Echoes were acquired inside each acquisition window with fully synchronous sampling. The center of the π pulses flanking the windows were rotor synchronized. Then the matching procedure involves first multiplying the amplitude of each data point comprising the k^{th} echo by the filter function

$$L(\tau_k) = \exp(-(\tau_k/T)^{\beta}) ,$$

where τ_k is the interval between excitation and the k^{th} echo top and the parameters T and β are estimated to match the decay of the CPMG envelope, followed by summation of the apodized echo train. The processing is completed after zero filling (except for PASS) and direct Gaussian apodization of the reconstructed echo. Details are reported in Table S4.

Sample	Experiment	Gaussian apodization (σ)	(T, β) for $L(\tau_k)$	S/N ratios	Normalized sensitivity
1 S	CP CPMG (first)	1 ms	(5 ms, 0.5)	28.5 (support) 19.3 (PS-DNA)	6.4 / √h 4.3 / √h
1 S	CP CPMG (second)	640 µs	(6.12 ms, 0.317)	75.9 (support) 47.4 (PS-DNA)	21.9 / √h 13.7 / √h
1 FS	CP CPMG	1 ms	(5 ms, 0.5)	17.2 (support) 23.9 (PF_6^- ?)	3.3 / √h 4.6 / √h
1 BS	CP CPMG	1 ms	(5 ms, 0.5)	18.1 (support) 6.3 (PS-DNA)	4.0 / √h 1.4 / √h
1D	CP CPMG	640 µs	(5.05 ms, 0.56)	118.1 (PO-RNA+support) 24.8 (29 ppm signal) 43.3 (PS-DNA)	50.7 / √h 10.7 / √h 18.6 / √h
1D	CP PASS-PIETA	None applied	(6 ms, 1)		
2A	CP CPMG	None applied	(5.05 ms, 0.56)	209.0 (PO-RNA+support) 73.4 (PS-DNA)	61.9 / √h 21.7 / √h
2B	CP CPMG	1 ms	(15 ms, 0.5)	388.2 (PO+support) 8.3 (PS)	58.6 / √h 1.3 / √h
2C	CP CPMG	1 ms	(15 ms, 0.5)	146.3 (PO+support) 5.4 (PS)	14.8 / √h 0.5 / √h
3 Na	CP PASS-PIETA	None applied (for ssb analysis)	(6 ms, 1)		
3 Mg	CP PASS-PIETA	None applied (for ssb analysis)	(6 ms, 1)		
4A Na	CP CPMG	1 ms	(5.02 ms, 0.543)	19.0	2.8 / √h
4A Mg	CP CPMG	1 ms	(5.02 ms, 0.543)	55.6	13.0 / √h
4B 0	CP CPMG	1 ms	(15 ms, 0.5)		
4B 2	CP CPMG	1 ms	(15 ms, 0.5)		
4B 60	CP CPMG	1 ms	(7.5 ms, 0.5)		

Table S4 | Signal processing parameters and achievable sensitivity for our DNP SENS approach. The signal-to-noise figures are given for the signals post-reconstruction and after further gaussian apodization of the signal envelope in the directly acquired dimension. The second CP CPMG experiment on sample **1 S** (not shown in a figure), recorded three days after the first, had superior sensitivity to the first owing to a selection of CPMG parameters that favored sensitivity over spectral resolution, and illustrates that in such cases ε_{ETA} values exceeding four are possible.

In Figure S4, the decay profiles were fit explicitly to an unnormalized version of the filter function given above. The parameters which describe the decay of the CPMG signal for the three major signals observed in that dataset are:

- 55 ppm: T = 13.2 ms, β = 0.44
- 29 ppm: T = 5.1 ms, β = 0.56
- 0 ppm: T = 12.2 ms, β = 0.45

VI. Control experiments

Figure S1 shows a selection of spectra from control experiments carried out in order to determine the nature of the signal near 0 ppm which appears in all DNP enhanced CP CPMG ^{31}P spectra of the three support systems discussed in this work. The polarizing agent on its own does not produce such a signal, nor do impregnated microcrystals of an organic solid, L-histidine monochloride monohydrate, prepared using the same procedure and grinding media (alumina mortar and pestle) used for the preparation of the immobilized oligonucleotide supports for DNP NMR analysis (though weak signals eventually appear for the TEKPol2 formulations, see section VIII). Nevertheless, a broad signal near 0 ppm is present when borosilicate glass is impregnated, in spite of a powerful surface cleaning treatment using a solution of ~2:1 H_2SO_4/H_2O_2. This signal is enhanced by DNP and clearly originates from the impregnated sample. Likewise, a similar signal is present when untreated sapphire cover slips are used instead of borosilicate glass cover slips. On the basis of these observations, we infer that a signal around 0 ppm, spanning roughly between 10 ppm and -15 ppm, results from phosphorous impurities embedded in the surface of the supports that are accessible to hyperpolarization by DNP SENS.

To qualitatively verify that sapphire-adsorbed oligonucleotides remain surface bound after TCE exposure, a sapphire cover slip was coated with 3'-black-hole-quencher-2 labelled d(C_7) (synthesized via 3'-BHQ-2 CPG, Glen Research) and exposed to TCE overnight. No removal of the dye-labelled DNA could be visually observed.

As given in the methods section, LA-ICPMS confirms the presence of phosphorous in the support materials:
Borosilicate glass, 20.9 ± 2.1 mg/kg; fused silica, 26 ± 5 mg/kg; sapphire, 8 ± 5 mg/kg.

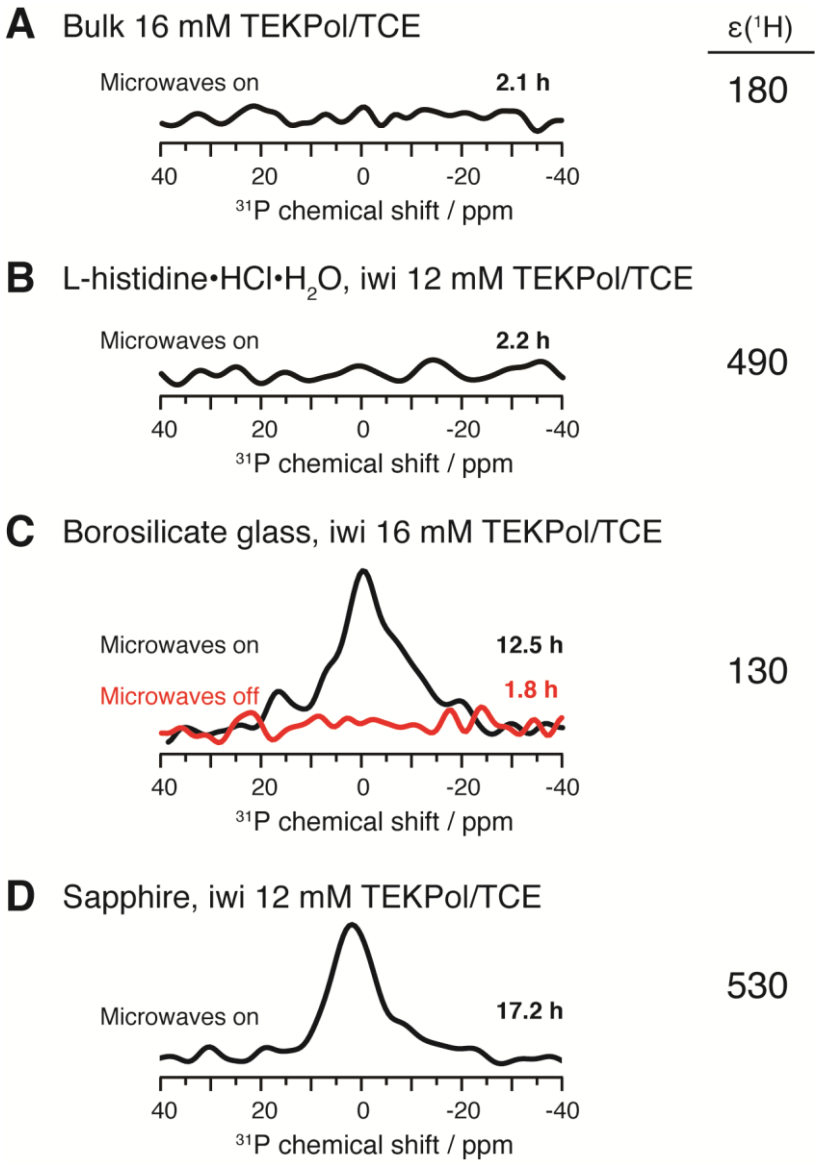

Figure S1 | Echo-reconstructed DNP CP CPMG ^{31}P NMR spectra from various control experiments on (A) a frozen solution of 16 mM TEKPol/TCE polarizing agent, (B) impregnated microcrystals of L-histidine monochloride monohydrate, (C) crushed borosilicate glass cover slips impregnated with the same 16 mM TEKPol/TCE solution whose spectra is shown in panel A, (D) impregnated crushed sapphire cover slips impregnated with the same 12 mM TEKPol/TCE solution whose spectra used for L-histidine hydrochloride monohydrate in panel C. The corresponding experiment with microwaves off (no DNP) is also shown for panel C. The experiment time is given toward the right of each spectrum. The right-hand column gives the solvent enhancements measured in each case.

VII. Spinning sideband analysis

Following IUPAC recommendations,[1] we have defined the principal axis system of the second-rank symmetric nuclear shielding tensor, **σ**, as the coordinate system where the matrix representation of **σ** is diagonal with principal components, σ_{ZZ}, σ_{YY}, and σ_{XX} ordered according to the Haeberlen convention such that

$$|\sigma_{ZZ}| \geq |\sigma_{XX}| \geq |\sigma_{YY}|,$$

subject to the additional condition that **σ** is traceless:

$$\sigma_{XX} + \sigma_{YY} + \sigma_{ZZ} = 0,$$

We further define the nuclear shielding anisotropy, ζ_σ, and asymmetry parameter, η_σ, according to

$$\zeta_\sigma = \sigma_{ZZ},$$

and

$$\eta_\sigma = (\sigma_{YY} - \sigma_{XX})/\sigma_{ZZ}.$$

ζ_σ measures the strength of the interaction. Its sign is significant; reversing the sign reflects the corresponding spectral pattern about its center of gravity. η_σ lies within the range [0,1]. The spectral pattern is symmetric about the center of gravity only when $\eta_\sigma = 1$ and is where the sign of ζ_σ is ambiguous.

The sideband amplitudes and phases can be calculated as a function of sideband order by taking the Fourier transform of a sideband generating function.[2] This function depends the sample rotation rate, rotation axis relative to the applied magnetic field, principal components of the shielding anisotropy, and the orientation of the tensor axis system relative to the sample holder (rotor) frame, as determined by the set of Euler angles (α,β,γ). In a crushed sample, a random distribution of orientations are present and the observed sideband manifold is given by a three-angle average over (α,β,γ).

The experimental sideband patterns shown in main text Fig. 3C were fit using a home built simulator coded in C. The three-angle integration over (α,β,γ) was first reduced to a two-angle integration over (α,β) after a simple analytic integration over γ. The two-angle integration was performed with Gaussian spherical quadrature utilizing a Levedev scheme for $L_{max} = 65$.[2] The fitting algorithm implemented a Markov chain biased walk to explore the $(\sigma_{ZZ}, \sigma_{YY})$ parameter space near an initial guess, using the Metropolis-Hastings criterion with the likelihood as merit function to judge whether or not the next randomly generated step will be accepted as an extension of the chain. The best fit and confidence intervals are then determined by a statistical analysis of the accepted samples. Generally, thousands of samples are required to fully explore a basin in the

χ^2 hypersurface and determine accurate statistics. Though the algorithm is inefficient, it generates statistics without assumptions regarding the shape of the χ^2 hypersurface.

The following figure shows relevant sideband profiles extracted for the PS DNA signals and the 29 ppm signal from Figures S5 and S8. Best fit simulations to the profiles analyzed using the aforementioned analysis program are overlaid, along with the best fit tensor parameters, ζ_σ and η_σ, with their corresponding approximate 95% confidence limits.

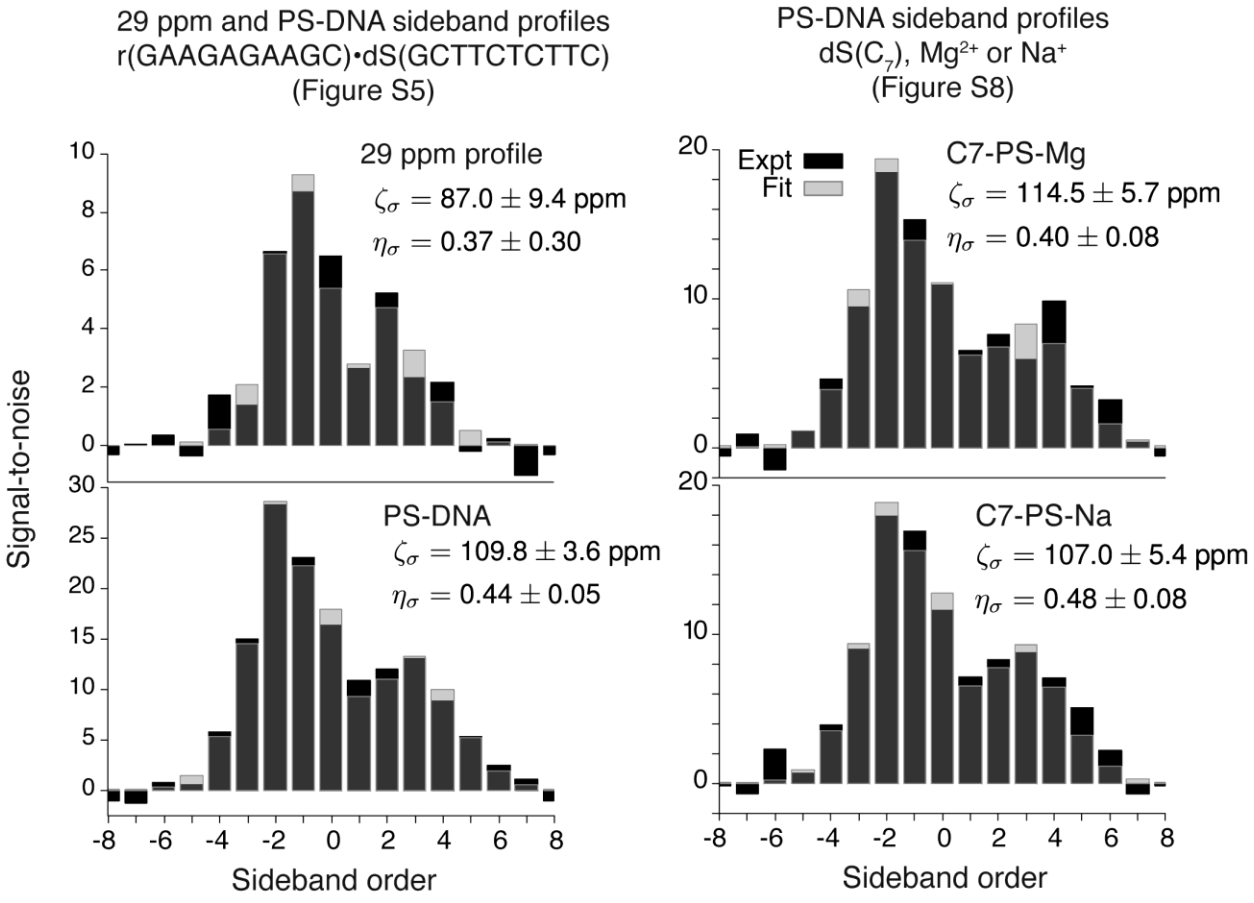

Figure S2 | Sideband profiles taken as cross-sections from the corresponding 2D datasets shown in Figures S5 and S8. The profiles were simulated and tensor parameters describing the profiles determined according to a single-site shielding model.

VIII. Phosphorous impurities in the wetting phase

As indicated in section VI, no ^{31}P signals are observable for L-histidine monochloride monohydrate impregnated with TEKPol/TCE. A control experiment was also run on L-histidine monochloride monohydrate impregnated with TEKPol2/TCE, which was used for experiments on samples **2B**, **2C**, **4B 0**, **4B 2**, and **4B 60**. In this case, weak signals are observed centered around 27 ppm, 2 ppm, and -19 ppm, as shown in Figure S3. This suggests a small amount of phosphorous containing impurities are present as contaminants in this impregnating solution.

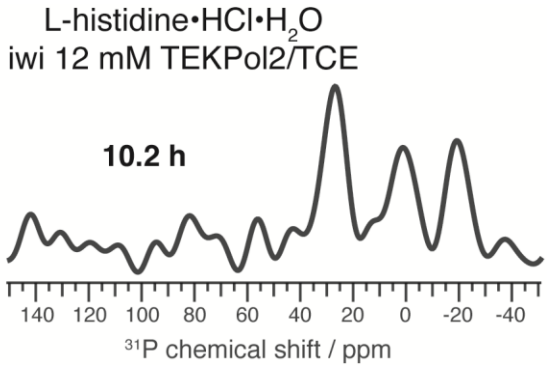

Figure S3 | Echo-reconstructed DNP CP CPMG ^{31}P NMR spectrum from microcrystals of L-histidine monochloride monohydrate impregnated with 12 mM TEKPol2/TCE, revealing traces of unidentified impurities. 5120 scans collected at a recycle delay of 7.1 s for a total experiment time of 10.2 h. The MAS rate was 12.5 kHz and the ^{1}H TCE enhancement was 520.

Another example is shown in Figure S4, which plots the one-dimensional reconstructed CPMG spectrum of the hybrid duplex r(GAAGAGAAGC)•dS(GCTTCTCTTC) above the two-dimensional contour plot obtained by stacking the NMR spectrum of each individual echo in the echo train. The one-dimensional NMR spectrum reveals a signal at 28 ppm that does not correspond to either the phosphodiester groups of the RNA strand nor the phosphorothioester signals of the DNA strand, both of which are also observed in the spectrum. We also see that the amplitude of the CPMG signal corresponding to this unidentified species decays far more rapidly than the unambiguously identifiable ^{31}P signals at –1 ppm and 55 ppm. The decay of CPMG signal depends upon a mixture of T_1 and T_2 relaxation processes and both relaxation rates would in general be enhanced by proximity to paramagnetic centers such as the TEKPol biradical used for DNP. This again suggests a species in the TCE solution and not a component of the duplex backbones.

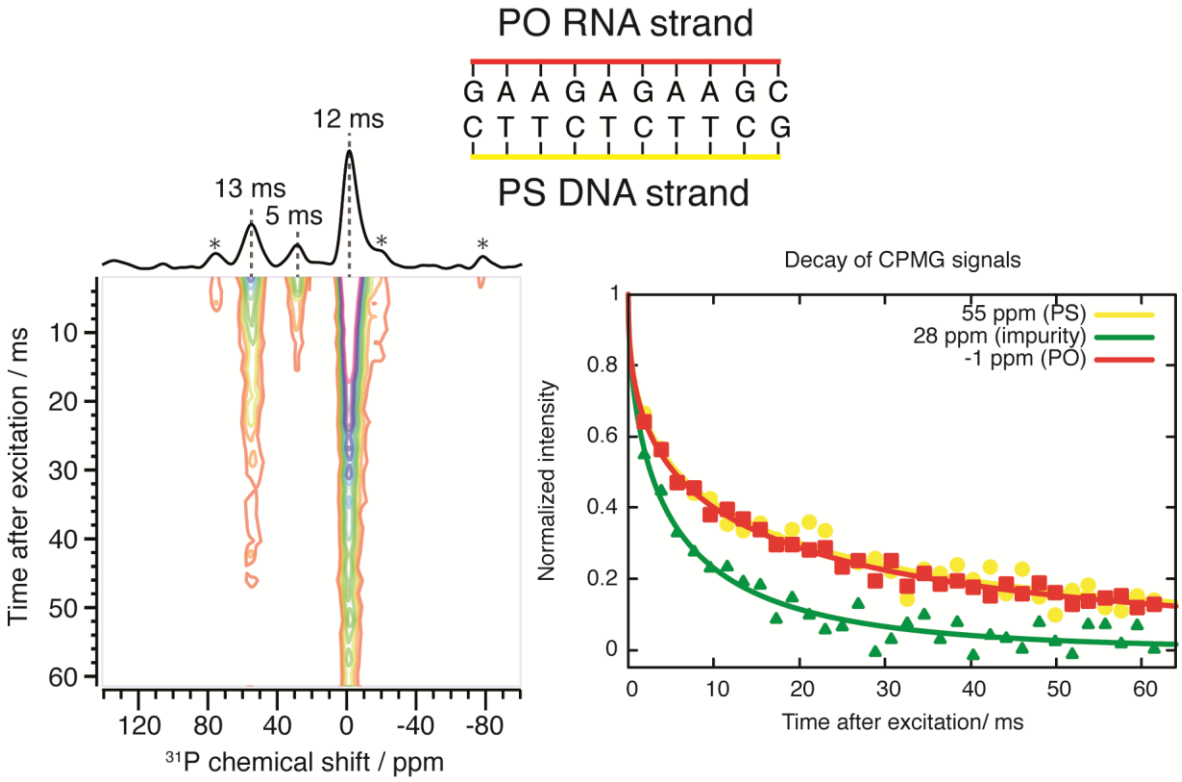

Figure S4 | *Left*: two-dimensional decay profile of DNP SENS CP-CPMG ^{31}P NMR spectra from 200 pmol of a hybrid oligonucleotide duplex with a phosphorothioated DNA strand deposited on a sapphire cover slip. Fitting the CPMG decay profile of each signal to a stretched exponential function gives a decay time constant which is reported above each component. The oligonucleotide sequence here is r(GAAGAGAAGC)•dS(GCTTCTCTTC) (sequences with 5'- to 3' directionality), modeled after the (non-thioated) duplex described by Conn and coworkers.[3] Asterisks indicate 12.5 kHz spinning sidebands. Experiment time: 5.4 h. *Right*: Normalized decay of CPMG signals for the three sites indicated in the left-hand spectrum.

Figure S5 shows the echo reconstructed 2D sideband separation spectrum of this hybrid duplex. We have also measured the shielding anisotropy for the 28 ppm signal observed in the r(GAAGAGAAGC)•dS(GCTTCTCTTC) sample **1D**. This signal has a significantly smaller anisotropy than the phosphorothioester signals, with ζ_σ = 90 ppm, and has a similar η_σ. As is the case for the isotropic chemical shift of this species, these values are outside the range of those previously reported for phosphomonoesters or phosphodiesters,[4] providing additional evidence that this signal does not originate from the backbones of the duplex strands.

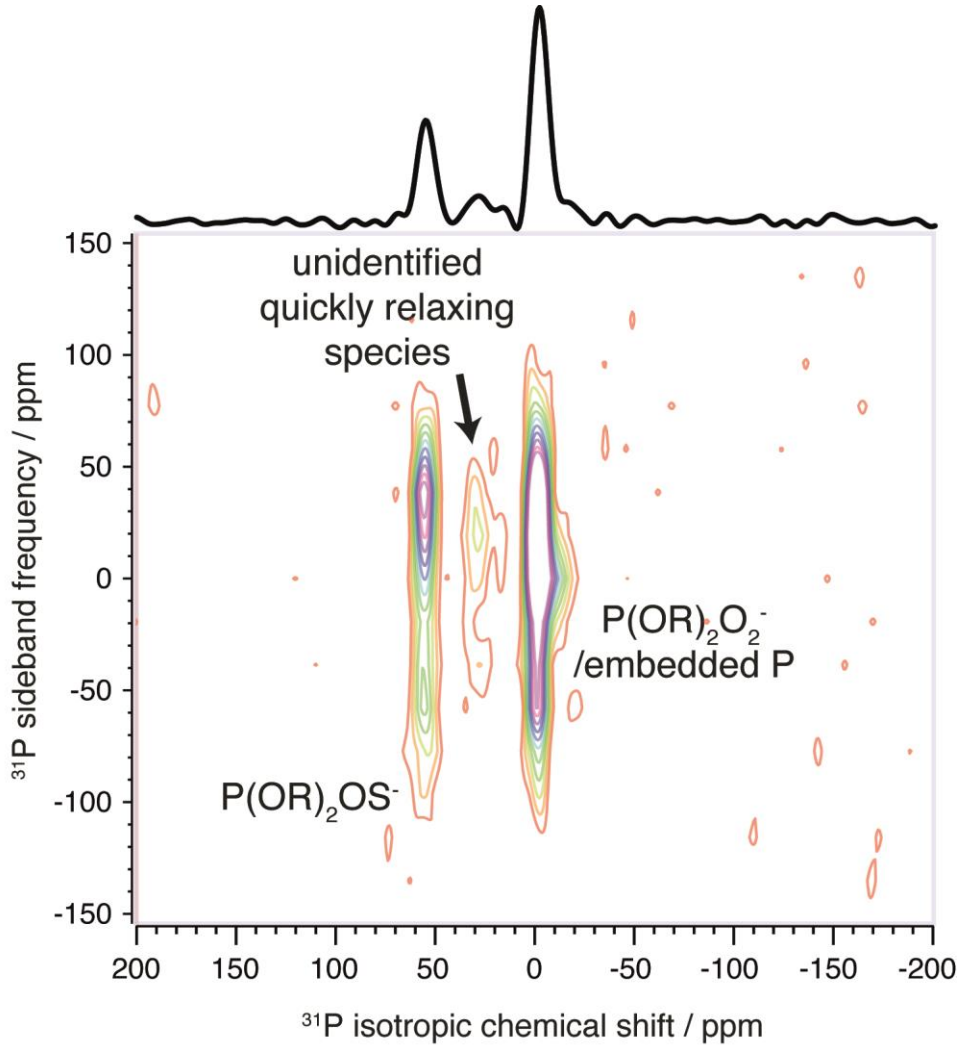

Figure S5 | Echo reconstructed 2D DNP SENS CP PASS-PIETA ^{31}P NMR spectra from 300 pmol of the hybrid oligonucleotide duplex r(GAAGAGAAGC)•dS(GCTTCTCTTC), showing the relatively small anisotropy of the weak signal near 28 ppm. The sum projection at the top presents the isotropic spectrum.

IX. Supplementary comparisons

Figure S6 shows the ^{31}P NMR spectra of the oligonucleotide duplex r(A_{10})-dS(T_{10}) shown in the main text Figure 2A along with a spectrum of the same sample at a higher signal-to-noise ratio.

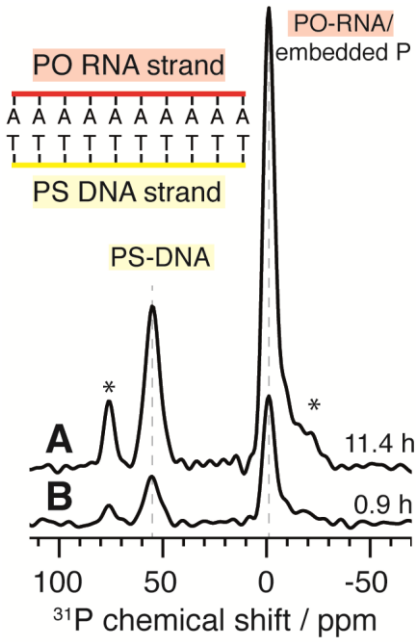

Figure S6 | Echo reconstructed 1D DNP SENS CP-CPMG ^{31}P spectra from 200 pmol of r(A_{10})-dS(T_{10}) duplex deposited on a sapphire cover slip. (A) Spectrum after 0.94 h of acquisition, giving a S/N ratio of 21 for the well-resolved phosphorothioester signal at 55 ppm. (B) The same spectrum after 11.4 h, plotted with the same response scale as A, giving an S/N ratio of 74 for the same signal. Spectra are scaled to have the same baseline noise. The solvent DNP enhancement factor is 460. Asterisks indicate 12.5 kHz spinning sidebands, with some overlap with signals originating from sapphire in the region around -20 ppm.

Figure S7 shows the ^{31}P NMR spectra of the DNA probe both in the presence of added miRNA binder (as in Figure 2B) and in the absence of binder. The PS signal of the DNA probe in the absence of binder apparently shifts to higher frequency by about 2 ppm, in alignment with the signal of the duplex of Figures 2A and S6.

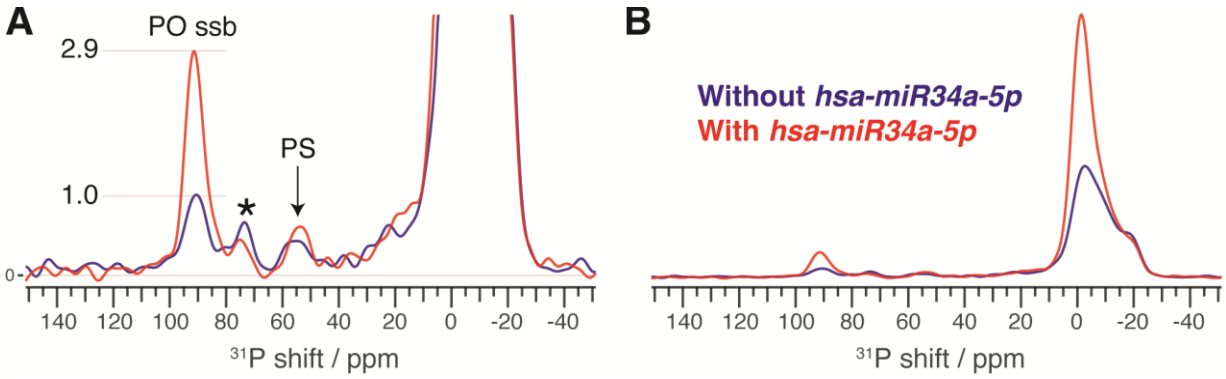

Figure S7 | Echo-reconstructed 1D DNP SENS CP-CPMG ^{31}P NMR spectra from 80 pmol of DNA probe d(ACAATCAGCTAAT*G*ACACTGCCTA), with phosphothioester groups between the positions labeled "*". (A) Spectra from the DNA probe both in the presence of 160 pmol of the nearly-complementary miRNA *hsa-miR34a-5p*, r(UGGCAGUGUCUUAGCUGGUUGU), (red) and in the absence of *hsa-miR34a-5p* (blue). The addition of *hsa-miR34a-5P* to the plate containing the DNA probe monolayer leads to an excess of PO signals, with the plate in the presence of binder containing 2.9 times the concentration of PO compared to the sample where the *hsa-miR34a-5p* is absent. As such, the normalization to the PO spinning sideband (PO ssb) at 90 ppm reflects this difference. On this scale, the PS signals should have similar intensity, as is observed. (B) Full vertical zoom of the spectra in panel A.

Figure S8 compares the reconstructred sheared 2D PASS spectra of dS(C_7) strands deposited on sapphire in their native sodium coordinated forms and after the magnesium soaking treatments described in Section III. No significant difference is observed between the signals at 55 ppm.

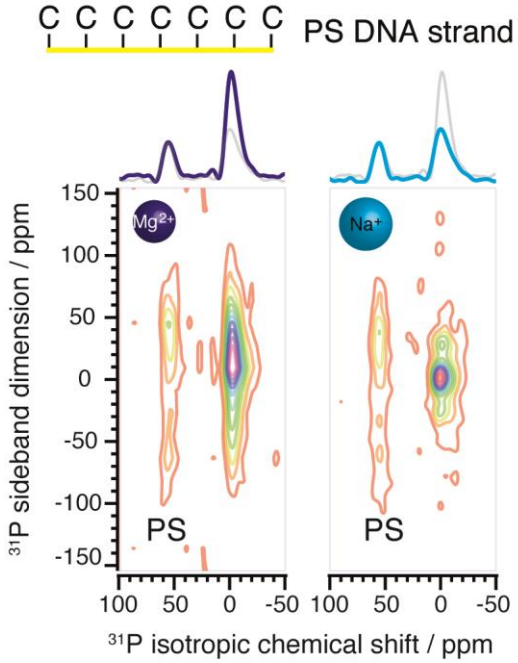

Figure S8. Echo-reconstructed 2D DNP SENS CP PASS-PIETA ^{31}P NMR spectra from 200 pmol of dS(C_7) strands deposited on sapphire. The sum projections at the top of each sheared 2D-PASS spectrum presents the isotropic spectrum. No significant shift differences are observed when the isotropic spectra are overlaid (faded gray lines). The vertical cross-sections give the sideband profile corresponding to a given isotropic frequency. The profiles at 55 ppm fit well to a single-site model of the nuclear shielding anisotropy, with parameters: ζ_σ = (114.5 ± 5.7) ppm, η_σ = 0.40 ± 0.08 for the Mg^{2+}-exchanged dS(C_7); ζ_σ = (107.0 ± 5.4) ppm, η_σ = 0.48 ± 0.08 for the Na^+-exchanged dS(C_7). The MAS rate was 3125 Hz. At 12.5 kHz MAS, the ^{1}H enhancement of the TCE impregnating the Mg^{2+}-exchanged dS(C_7) strands was 600.

Figure S9 takes the spectra of Figure 4B and adds a spectrum acquired from a disk that was exposed to UV irradiation for just two minutes (one minute per side). Since this new spectrum was reconstructed from data acquired in two sessions at two slightly different spinning rates and acquisition conditions the comparison is less clean than that of Figure 4B, which compares the extreme cases and where identical acquisition conditions were used.

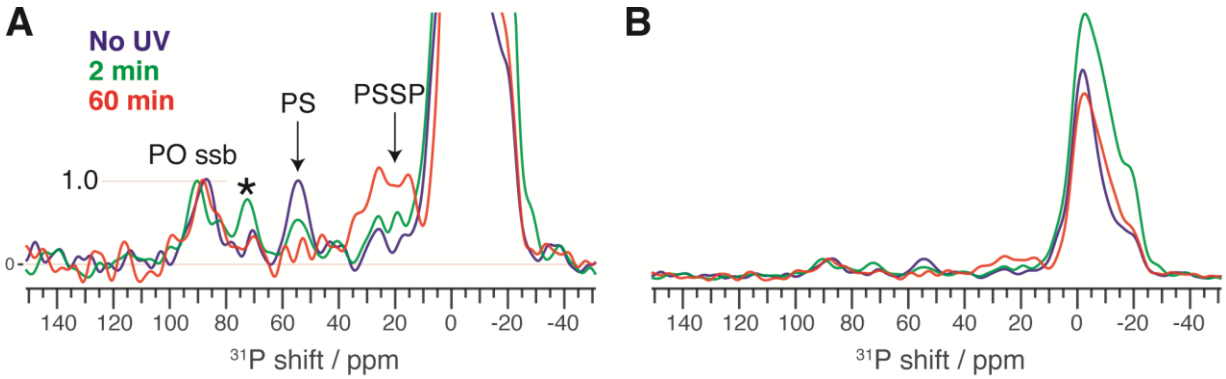

Figure S9 | Echo-reconstructed 1D DNP SENS CP-CPMG ^{31}P NMR spectra from 300 pmol of PS-DNB tagged oligonucleotide r(*x*UGCAUGU) deposited on sapphire disks and then exposed to different durations of UV irradiation. (A) Spectra of disks with no UV exposure (blue), 2 min of total exposure (1 min/side, green), and 60 min of total exposure (30 min/side, red). Spectra are normalized as described in the main text Figure 4B caption. (B) Full vertical zoom of the spectra in panel A.

Figure S10 shows a comparison of LC-MS data for r(*x*UGCAUGU) before and after 60 min UV irradiation in aqueous solution, suggesting dimerization of aqueous oligonucleotides.

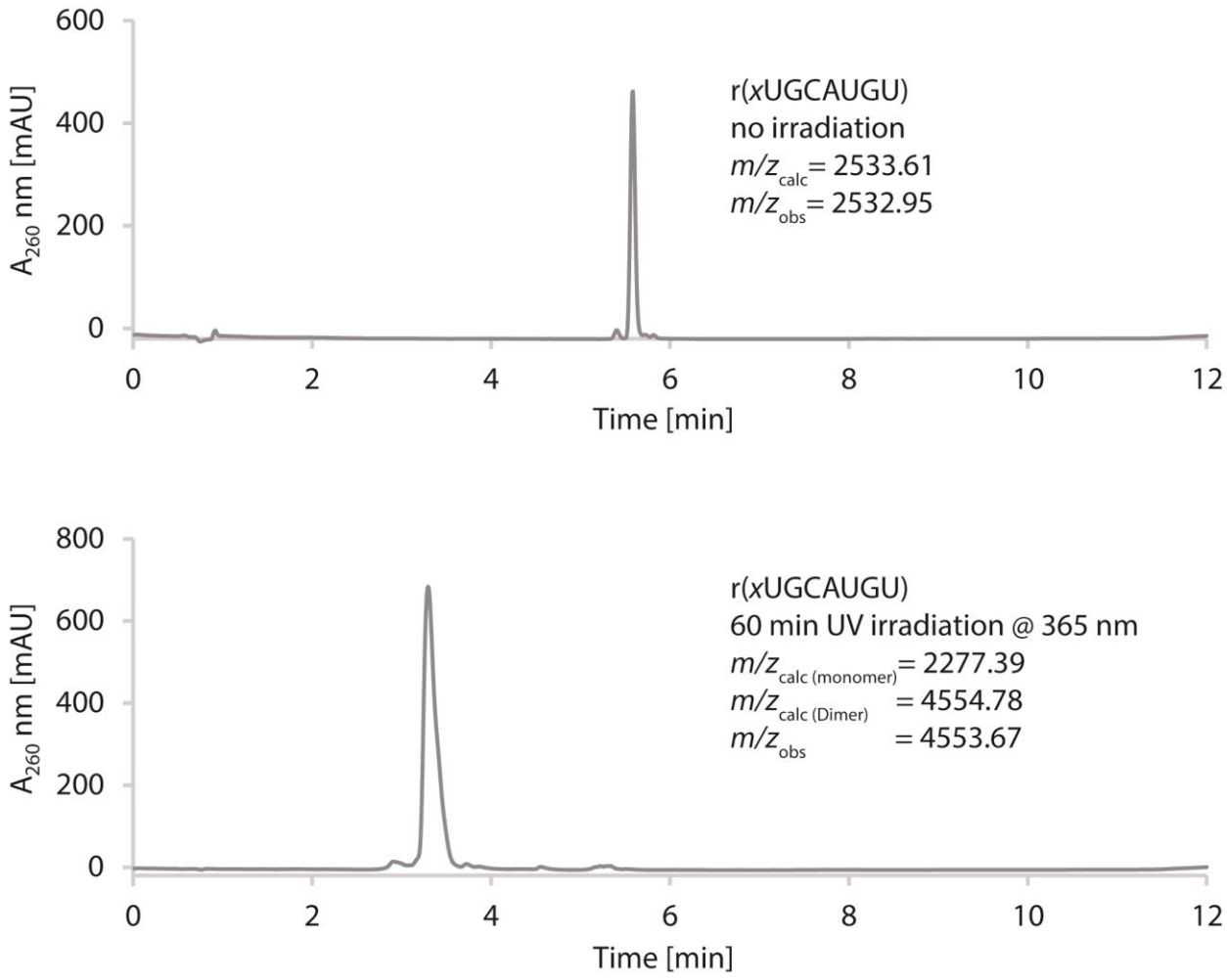

Figure S10 | LC-MS elution profiles of the 5'-PS-DNB capped RNA r(*x*UGCAUGU) before and after UV irradiation. Details about the elution are given in the Experimental section of the main text.

X. Additional references

1 Harris, R. K. *et al.* Further conventions for NMR shielding and chemical shifts IUPAC recommendations 2008. *Solid State Nuclear Magnetic Resonance* **33**, 41--56 (2008).

2 Edén, M. & Levitt, M. H. Computation of Orientational Averages in Solid-State NMR by Gaussian Spherical Quadrature. *Journal of Magnetic Resonance* **132**, 220--239 (1998).

3 Conn, G. L., Brown, T. & Leonard, G. A. The crystal structure of the RNA/DNA hybrid r(GAAGAGAAGC) • d(GCTTCTCTTC) shows significant differences to that found in solution. *Nucleic Acids Research* **27**, 555--561 (1999).

4 Pecoraro, V. L., Hermes, J. D. & Cleland, W. W. Stability constants of magnesium and cadmium complexes of adenine nucleotides and thionucleotides and rate constants for formation and dissociation of magnesium-ATP and magnesium-ADP. *Biochemistry* **23**, 5262--5271 (1984).