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Materials and Methods

Reagents: Tris-HCl (1.5 M, pH 8.8) buffer was purchased from Teknova (Hollister, CA). Tris/Borate/EDTA (TBE) buffer (10x) was purchased from Mediatech. (Manassas, VA). Photoinitiator 2,2-azobis[2-methyl-*N*-(2-hydroxyethyl) propionamide] (VA-086) was purchased from Wako Chemical (Richmond, VA). SYBR™ Gold Nucleic Acid Gel Stain (10,000x), Saline-Sodium Citrate (SSC) buffer (20x solution), and ULTRAhyb™ Ultrasensitive Hybridization Buffer were purchased from Thermo Fisher Scientific (USA). Gel Slick® solution and GelBond® PAG film were purchased from Lonza (Basel, Switzerland). Double-stranded RNA (dsRNA) ladder and the Gel Loading Dye (purple, 6x) were purchased from New England Biolabs (Ipswich, MA). Double-stranded DNA (dsDNA), single-stranded DNA (ssDNA) (unmodified and AlexFluor®647-modified) and single-stranded RNA (ssRNA) were purchased from Integrated DNA Technologies (Coralville, Iowa). Photomasks were designed with AutoCad student edition (Autodesk, San Rafael, CA) and printed with a Brother MFC-9320C digital color printer (Brother International Corporation, Bridgewater, NJ) on a transparent film (3M, St. Paul, MN). All the other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO), including urea, sodium chloride, sodium dodecyl sulfate (SDS), allylamine, succinimidyl-[4-(psoralen-8-yloxy)]-butyrate (SPB), dimethyl sulfoxide (DMSO), Triton™ X-100, TE buffer (100x), RNaseZAP™, solution of 30% (w/v) acrylamide/bis-acrylamide (29:1), and DEPC water (1 L, sterile, nuclease-free).

Fabrication of the psoralen-functionalized polyacrylamide gel: Psoralen-functionalized polyacrylamide gels were fabricated as a free-standing polyacrylamide gel (fsPAG).[1] Briefly, the precursor solution, which contained 12 %T(w/v) acrylamide, 2.7 %C(w/w) bis-acrylamide cross-linker, 0.2 %(w/v) VA-086 photo-initiator, 8 mM urea in ssDNA and ssRNA experiments, and allylated psoralen, was degassed for 5 min under house vacuum with sonication, and pipetted into the gap between Gelbond® and the glass cover slide. The height of the gap, which can be easily adjusted by spacers, defined the thickness of the gel (500 μm). Photo-polymerization was initiated by irradiating the precursor solution with UV light, allowing for a rapid gel fabrication; Custom-designed photomasks allowed for selective polymerization of the regions of interest. UV irradiation time was optimized for each psoralen concentration: for 0 mM, 0.03 mM, 0.3 mM, 0.6 mM, 0.9 mM, 1.5 mM, 3 mM and 6 mM of allylated psoralen, 345 s, 345 s, 370 s, 390 s, 410 s, 420 s, 595 s and 700 s exposure at 20 mW cm^{-2} (measured by OAI 308 UV intensity meter, OAI, San Jose, CA) was employed. A 390 nm long-pass filter (PN 39-426, Edmund Optics, Barrington, NJ) was used to prevent any potential decrease in capture activity of psoralen due to the UV activation during the gel fabrication process. After photo-polymerization, the resulting psoralen-functionalized gel was rinsed multiple times with ddH_2O to remove unreacted reagents as well as *N*-hydroxysuccinimide (NHS).

Polyacrylamide gel electrophoresis: The fabricated gel was soaked in 0.5x TBE buffer for 10 min, upon which a vacuum clean was applied to remove the residual buffer from the wells (2 mm x 2 mm). The sample solution, which contained 0.4 μl nucleic acid target (0.1 μg $μl^{-1}$ in TE buffer), 0.2 μl DNA loading dye (6x), 0.6 μl Triton X-100 (1 %(v/v)), and 0.58 mg Urea (only for ssDNA and ssRNA), was manually pipetted into the sample wells. Two electrode wicks (Crescent Chemical Company, Islandia, NY) wetted with 0.5x TBE buffer were aligned atop the gel at both ends, and in contact with the graphite electrodes that were placed above. The entire electrophoresis setup was housed in a home-made environmental chamber and connected to an external high-voltage power supplier (Bio-Rad Laboratories, Hercules, CA).[1a] The implement of this free-standing polyacrylamide gel electrophoresis (fsPAGE) effectively reduced the consumption of the reagents and eased the loading of the small-quantity samples. The electrophoresis was performed in 0.5x TBE buffer at an applied electric field of 40 V cm^{-1}.

Photo capture: After electrophoretically introduced into the psoralen-modified PA gel, the the nucleic acid targets were irradiated with the long-wavelength UV light (Hamamatsu Photonics, Japan; equipped with the 350 nm long-pass filter, Asahi Spectra USA Inc, Torrance, CA) for varied periods of time. The irradiation intensity was about 360 mW cm^{-2} at 365 nm. Gels were stained and scanned upon the capture to record the total amount of the targets, measured by the fluorescence intensity (I_0), as the reference for quantification of the capture efficiency. The uncaptured nucleic acids were washed away from the captured ones under a brief (~90 s) reversed electric field at 40 V cm^{-1}. The washed gel was scanned again to record the quantity of the captured targets, measured by the fluorescence intensity (I_c) (Figure S3). The capture efficiency (η) was defined as the ratio of I_c to I_0 : $\eta = I_c / I_0$.

Photo release: To evaluate the photo-release performance, the fsPAGE-separated dsRNA targets of respective lengths were irradiated with the long-wavelength UV (> 350 nm) for 60 s, separated from the uncaptured molecules via the briefly reversed electric field, and irradiated again with the short-wavelength UV (< 325 nm) for varied periods of time. The released nucleic acids were washed away from the unreleased ones under the briefly reversed electric field. The gel was then stained and scanned to record the fluorescence intensity from the unreleased samples (I_r) and the control sample (I_0) that was captured but not irradiated with the short-wavelength UV light (Figure S9). The ratio of I_r to I_0 was calculated as the relative fluorescence intensity to evaluate the release efficiency of the system.

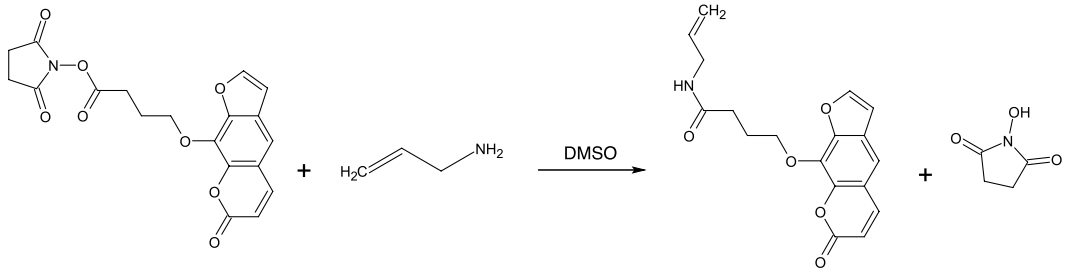
Hybridization: To investigate the impact of photo-capture on the hybridization capacity of the captured nucleic acids, the 40-nt ssDNA fragments were captured using the psoralen-functionalized polyacrylamide gel for varied irradiation time: 30 s, 60 s, 90 s. As the reference, the AlexFluor® 647-conjuagted ssDNA fragments were captured in parallel. The hybridization procedure was performed according to the protocol recommended by the manufacturer.[2] Briefly, upon washing off the uncaptured oligos with the reversed electric field, the gel with the captured targets was incubated with 4-μl complementary fragments (1 μg $μl^{-1}$, conjugated with AlexFluor®647 at the 5' end) in the ULTRAhyb™ Ultrasensitive Hybridization Buffer at 40 °C for 20 h. A 30 min pre-hybridization at 40 °C in the hybridization buffer was applied to the gel prior to the addition of the complementary fragments. The gel with the reference fragments was incubated without the complementary fragments in the hybridization buffer at 40 °C for 20 h. After hybridization, the

gels were washed twice in the washing buffer (2x SSC, 0.5 %(w/v) SDS) at 40 °C for 30 min, incubated in the 0.5x TBE buffer at room temperature for 20 min, and briefly washed under the reversed electric field before the imaging.

Imaging: To visualize the nucleic acid targets, a non-specific gel stain SYBR™ Gold was applied to the targets. For staining, the SYBR™ Gold was diluted 5,000x in 0.5x TBE buffer, and incubated with the gel slide on the rotator for 20 min in the dark. The stained gels were imaged using the laser scanner Typhoon Trio Imager (GE Healthcare Life Sciences, Pittsburgh, PA) at a scanning resolution of 50 μm.

Data quantification and analysis: Data quantification was performed with the custom Matlab scripts. Briefly, Gaussian fitting was implemented to calculate the quantity of the targets. The ratio of the quantity before and after the capture/release process was calculated to evaluate the capture/release efficiency. For data analysis, Spearman's coefficient was calculated for correlation analysis; Student's *t*-test was implemented on the paired samples to determine the statistical differences, for which 0.05 was adopted as the significance level.

Synthesis of allylated psoralen



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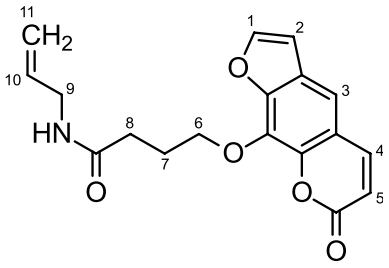
Scheme S1. Synthesis of the allylated psoralen via the NHS ester-amine reaction between succinimidyl-[4-(psoralen-8-yloxy)]butyrate and allylamine. Condition: RT, in the dark, overnight.

***N*-allyl-4-((7-oxo-7*H*-furo[3,2-*g*]chromen-9-yl)oxy)butanamide (1)**



The amine-reactive succinimidyl-[4-(psoralen-8-yloxy)]-butyrate (SPB) was coupled with allylamine to produce *N*-allyl-4-((7-oxo-7*H*-furo[3,2-*g*]chromen-9-yl)oxy)butanamide (allylated psoralen) and release *N*-hydroxysuccinimide (NHS). To 128 µmol of SPB in 2 ml of DMSO, equal molar of allylamine was added and allowed to react overnight under mixing using a tube inverter. For purification, the product was incubated with 25 mg QuadraPure® AK (50 to 90 mesh, Sigma-Aldrich, St. Louis, MO) on a tube inverter for 12 h to scavenge residual allylamine. The final product allylated psoralen was characterized by 1D ^{1}H NMR (Figure S1a), ^{1}H-^{13}C HSQC NMR (Figure S1b), and high-resolution electrospray ionization mass spectrometry (ESI-HRMS) (Figure S2). As expected, the peak at 8.02 in ^{1}H NMR spectrum disappeared after one drop of D_2O was added (Figure S1). The conversion of SPB to allylated psoralen was close to 100 %, since no SPB was detected in ^{1}H NMR and mass spectrometry. Allylated psoralen in DMSO was stored at -20 °C until use.

Molecular formula: $C_{18}H_{17}NO_5$

^{1}H NMR (800 MHz, DMSO-d_6, δ ppm): 1.98 (q, -CH_2), 2.34 (t, -CH_2), 3.68 (d, -CH_2 on D_2O exchange), 4.4 (t, -O-CH_2), 5.01 (d, ethylene-H), 5.75 (m, ethylene-H), 6.41 (d, coumarine-CH), 7.07 (d, benzofuran-CH), 7.67 (s, coumarine -CH), 8.02 (s, amide-NH), 8.09 (d, benzofuran-CH), 8.13 (d, coumarine-CH).

^{1}H-^{13}C HSQC NMR (800 MHz, DMSO-d_6, δ_H, δ_C, ppm): 1.98, 26.2 (q, 6.5 Hz, 7-CH_2), 2.34, 31.9 (t, 7.5 Hz, 8-CH_2), 3.68, 41.0 (d, 5.0 Hz, 9-CH_2 on D_2O exchange), 4.40, 73.0 (t, 6.8 Hz, 6-CH_2), 5.01-5.09, 114.8 (d, 17.4 Hz, 11-CH_2), 5.75, 136.1 (m, 10-CH), 6.41, 114.0 (d, 9.5 Hz, 5-CH), 7.07, 107.1 (d, 2.0 Hz, 2-CH), 7.67, 114.1 (s, 3-CH), 8.09, 145.1 (d, 2.0 Hz, 1-CH), 8.13, 145.1 (d, 9.5 Hz, 4-CH).

ESI-HRMS (m/z): Calculated for $[C_{18}H_{17}NO_5Na]^+$: 350.0999, found: 350.1002.

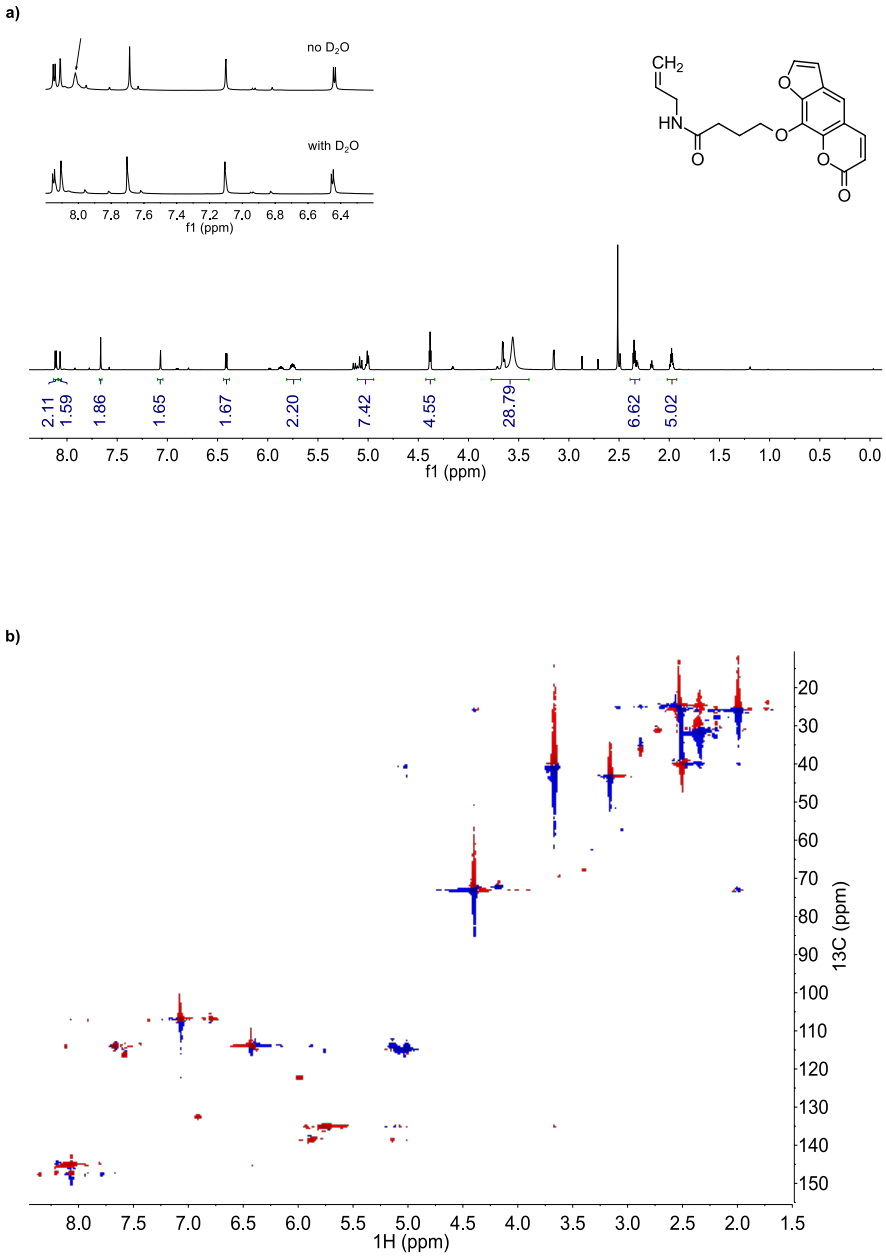


Figure S1. NMR spectra of allylated psoralen in DMSO-d_6. **a)** ^{1}H spectrum. The inset indicates the presence of the amide group in the allylated psoralen, where the peak at δ = 8.02 disappears upon the addition of 20 µl D_2O. **b)** ^{1}H-^{13}C HSQC spectrum.

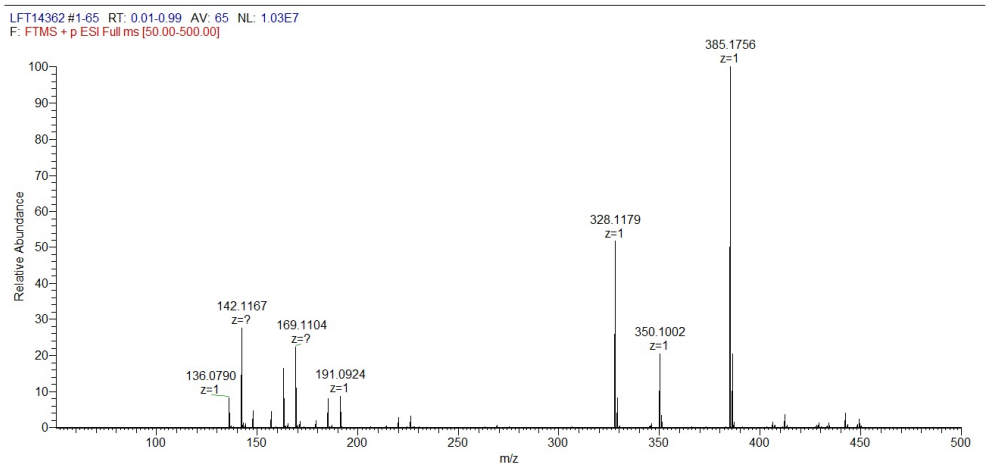


Figure S2. Mass Spectrum of allylated psoralen.

Capture performance of the hydrogel with varied psoralen concentrations

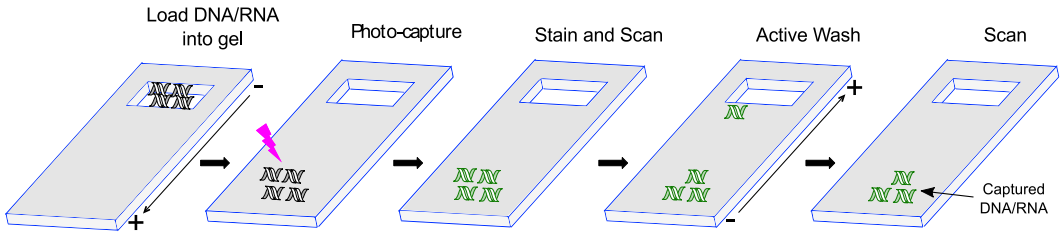


Figure S3. Schematic of the photo-capture procedures

Table S1. Sequence information of the oligonucleotide targets used in the experiments.

Target	Sequence (5'-3') or source[a]	Number percentage of thymidine or uridine (T or U) on a target molecule
100 nt ssDNA	ACG CCT TGC AGG TAT AAA ATG TCG TGT CGG CTT ACG TCT TGG CCT AAT CAG TAA ATA TAT CCT GGT CCA GCG ATC GGA CGT ACA AAA GCC CGG ACT GGT C	25%
100 nt ssRNA	ACG CCU UGC AGG UAU AAA AUG UCG UGU CGG CUU ACG UCU UGG CCU AAU CAG UAA AUA UAU CCU GGU CCA GCG AUC GGA CGU ACA AAA GCC CGG ACU GGU C	25%
100 bp dsDNA[b]	ACG CCT TGC AGG TAT AAA ATG TCG TGT CGG CTT ACG TCT TGG CCT AAT CAG TAA ATA TAT CCT GGT CCA GCG ATC GGA CGT ACA AAA GCC CGG ACT GGT C	50%
40 nt ssDNA	CAG ACT AAG CAT ACC CGG GTG TCG TGA GTC TAC ATC GAT T	25%
40 nt ssDNA[c]	AlexFluor®647- AAT CGA TGT AGA CTC ACG ACA CCC GGG TAT GCT TAG TCT G	25%
40 nt ssDNA[d]	AlexFluor®647- CAG ACT AAG CAT ACC CGG GTG TCG TGA GTC TAC ATC GAT T	25%
80 bp dsRNA	New England BioLabs®, N0363S	47.5%
150 bp dsRNA	New England BioLabs®, N0363S	49.3%
300 bp dsRNA	New England BioLabs®, N0363S	48.3%
500 bp dsRNA	New England BioLabs®, N0363S	49%

[a] The sequence of the dsRNA used in the experiments is proprietary information, therefore, only the percentage of the nucleobase is disclosed. [b] The complementary strand is not listed. [c] The complementary molecule used in the hybridization experiments. [d] The reference molecule used in the hybridization experiments.

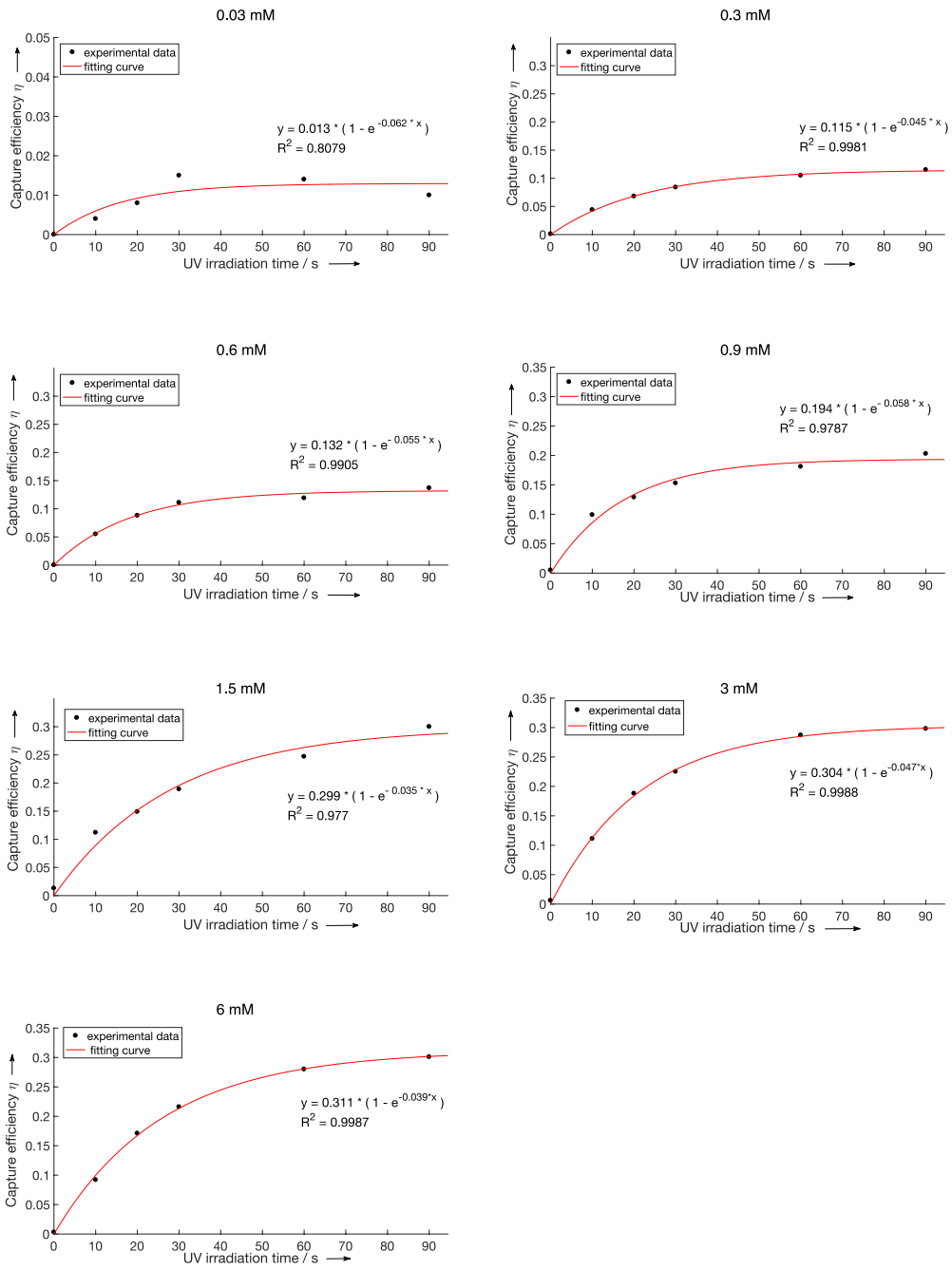


Figure S4. Experimental data and the fitting results of the capture efficiency for 100-bp dsDNA with different psoralen concentrations. The capture efficiency at each irradiation duration was the average of three replicates.

Capture performance for different nucleic acid targets

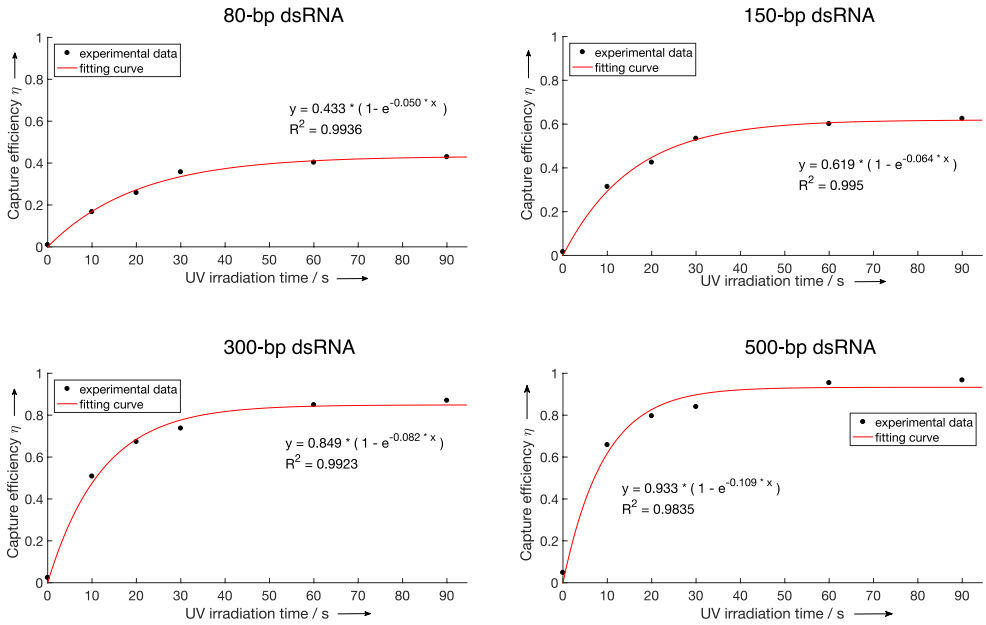


Figure S5. Experimental data and the fitting results of the capture efficiency for dsRNA of different lengths. The capture efficiency at each irradiation duration was the average of three replicates. Psoralen concentration: 3 mM.

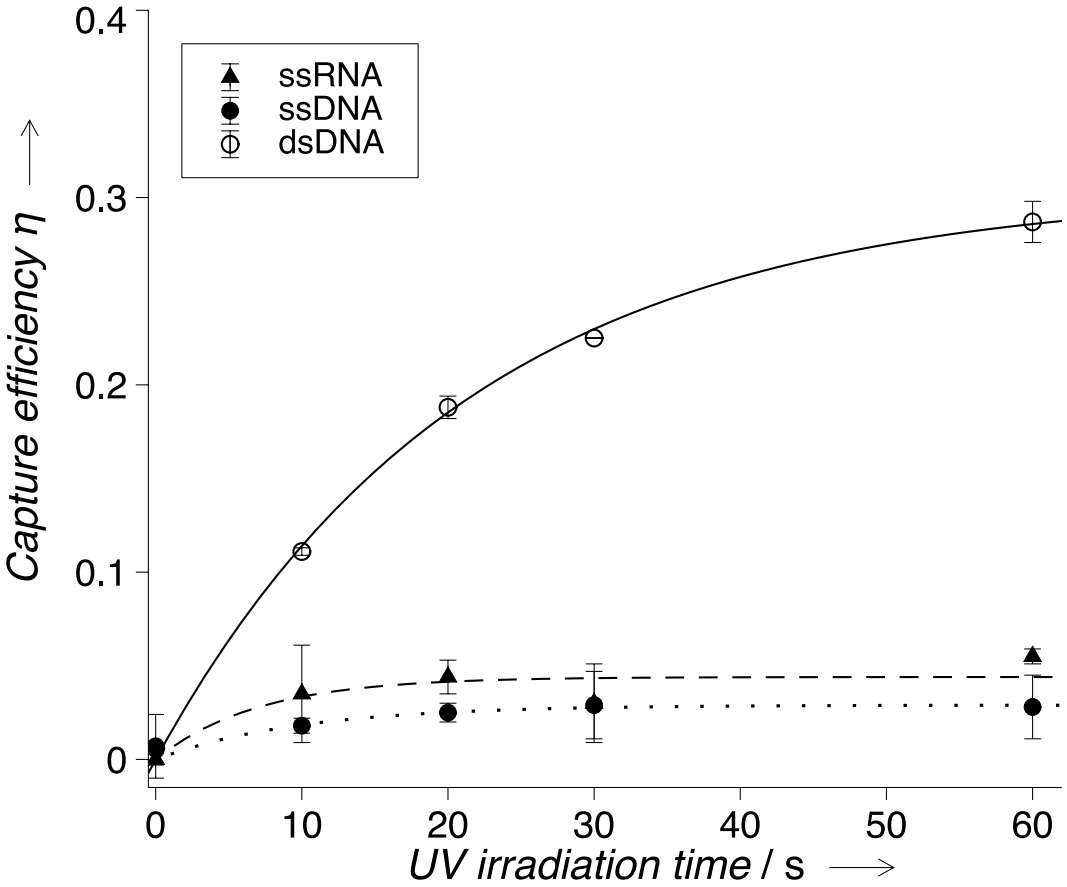


Figure S6. Capture performance for ssRNA, ssDNA and dsDNA of the same sequence (100 bp) in the psoralen-functionalized polyacrylamide gel. Psoralen concentration: 3 mM. The capture efficiency at each irradiation duration was the average of three replicates. Error bars: standard deviation. Connecting lines are the fitting to the experimental data. Fitting model: $\eta = \eta_0 (1 - e^{-kt})$.

Table S2. Capture efficiency[a] for ssDNA, dsDNA and ssRNA in polyacrylamide gels containing 3 mM allylated psoralen.

Capture time (s)	0	10	20	30	60	90	Steady-state capture efficiency η_0	Reaction rate constant k
ssDNA	0.007±0.017	0.018±0.004	0.025±0.005	0.029±0.018	0.028±0.017	0.073±0.029	0.029	0.101
dsDNA	0.006±0.001	0.111±0.002	0.188±0.006	0.225±0.000	0.287±0.011	0.298±0.003	0.304	0.047
ssRNA	0.000±0.003	0.035±0.026	0.044±0.009	0.030±0.021	0.055±0.004	0.081±0.008	0.044	0.143

[a] The capture efficiency value at each irradiation duration was the average from 3 replicates, and the error represents the standard deviation.

Hybridization to the captured oligonucleotides

To evaluate the effect of the UV-induced capture on the hybridization capacity of the target, we performed hybridization experiments with a photo-captured 40-nt ssDNA. We fluorescently labeled the complementary DNA strand at the 5' end to visualize the hybridized target, and utilized the 5' end fluorescently labeled target DNA as a reference to examine the hybridization completion level. The target DNA (unmodified ssDNA) and the reference DNA (fluorescently labeled ssDNA) were photo-captured in parallel under varied irradiation (> 350 nm) durations in the psoralen-functionalized polyacrylamide gel.

To ensure the legitimate use of AlexFluor®647-conjugated fragments as the reference in the hybridization evaluation, the capture efficiencies for AlexFluor®647-conjugated ssDNA and the unmodified ssDNA were examined under the same condition. The results revealed no evident difference in the capture efficiency between the fluorophore-conjugated and unmodified ssDNA fragments (Figure S7), indicating that the fluorescent modification to ssDNA fragments did not participate in the photo-cycloaddition reaction of psoralen and nucleobases. Therefore, AlexFluor®647-conjugated fragments can be used as the reference probe in hybridization experiments.

The captured target DNA was incubated with its complementary strand under hybridization conditions; the captured reference DNA was incubated under the same conditions with no complementary strands added. Fluorescence intensity was measured for the target and the reference upon photo-capture and the subsequent hybridization (Figure S8a). We observe a significant increase (Paired, two-tailed Student's *t*-test for target DNA before and after hybridization: * $p < 0.01$, ** $p < 0.001$) of the fluorescence intensity from the captured target after hybridization for all three irradiation durations (Figure S8b), indicating the occurrence of the hybridization for the captured ssDNA. Moreover, comparison of the fluorescence intensity between the target after hybridization and the reference reveals no significant difference (Paired, two-tailed Student's *t*-test for the target after hybridization and the reference) regardless of the UV capture time (Figure S8b), suggesting that photo-capture (< 90 s, > 350 nm of irradiation) does not inhibit hybridization.

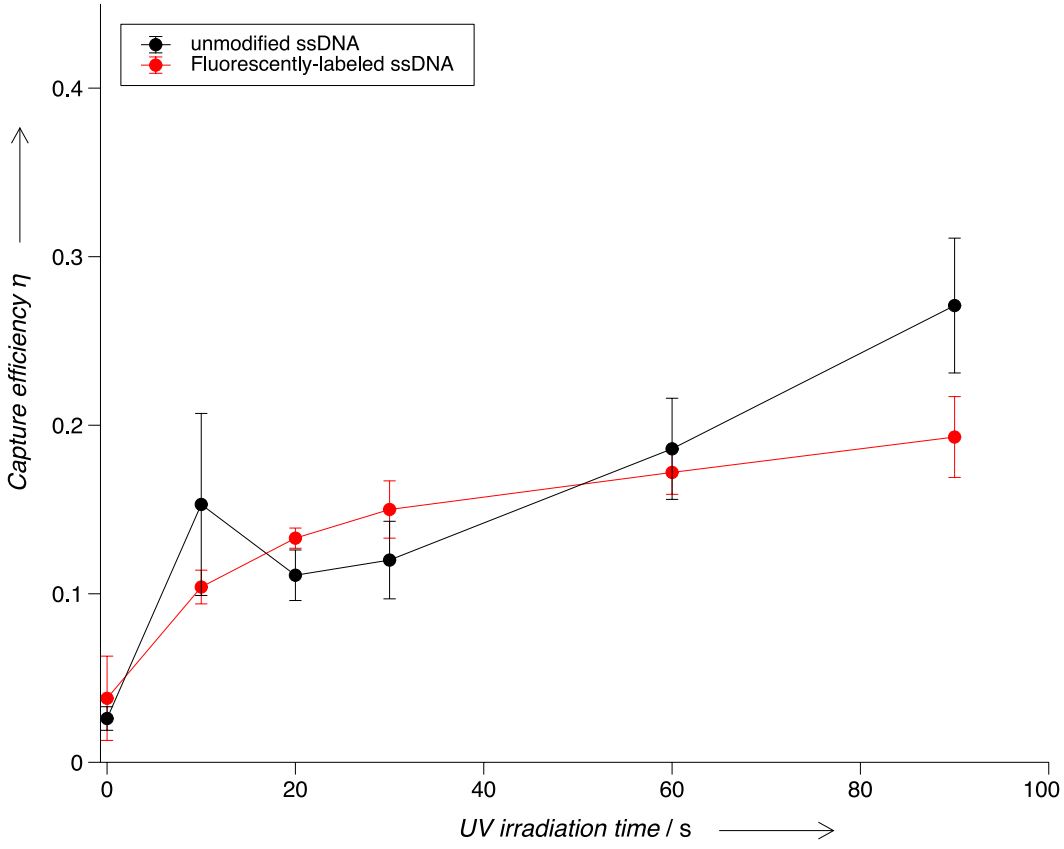
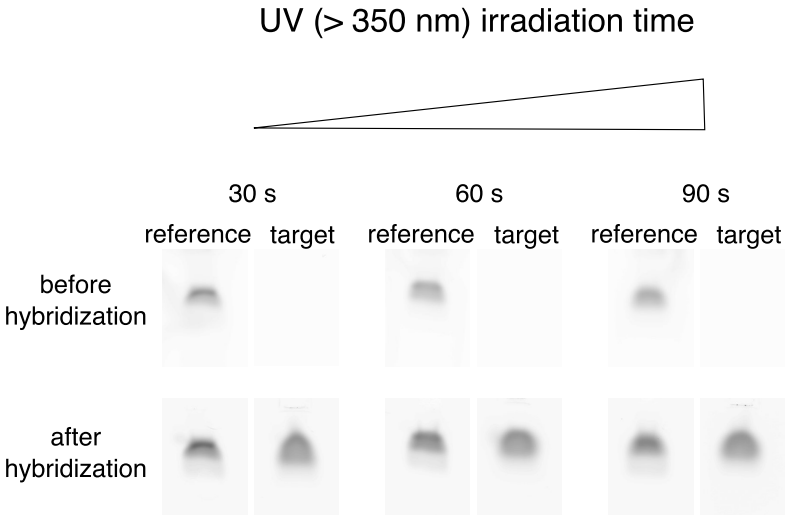


Figure S7. Capture efficiency for AlexFluor®647-conjugated ssDNA and unmodified ssDNA in the psoralen-modified polyacrylamide gel at different time intervals of UV irradiation. Psoralen concentration: 3 mM. DNA length: 40 nt. Stain: 2x SYBR™ Gold. Ex: 488 nm. Em: 520 nm BP 40 nm. PMT Gain: 450 V. Error bars are the standard deviations of three replicates.

a)



b)

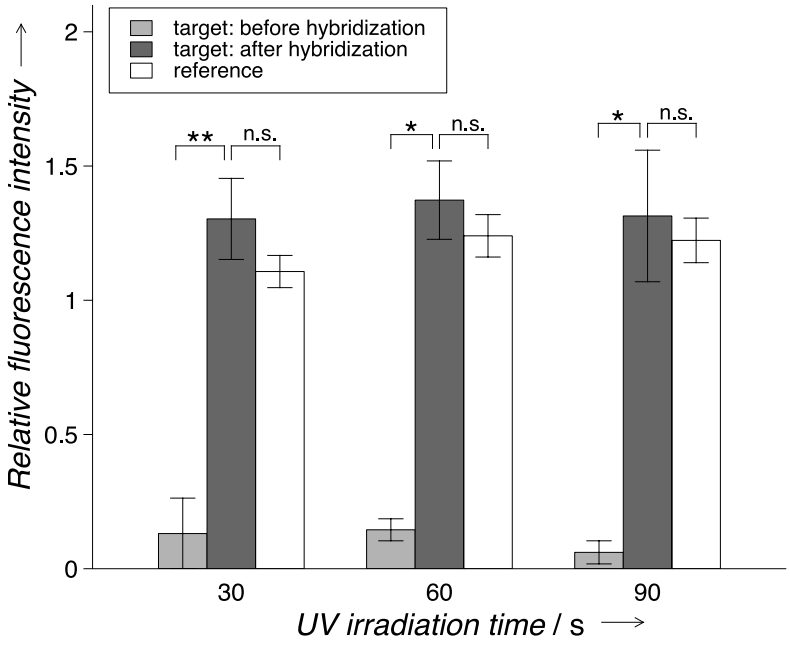


Figure S8. Hybridization to the photo-captured ssDNA (40 nt) irradiated with > 350 nm UV for varied durations. **a)** Fluorescence images of the photo-captured target DNA and the photo-captured reference DNA before and after hybridization. Capture scaffold: 12 %T polyacrylamide gel functionalized with 3 mM allylated psoralen. The complementary strand was fluorescently labeled with AlexFluor®647 at 5' end. The reference, which is the target DNA labeled with AlexFluor®647 at 5' end, was photo-captured and incubated in the hybridization solution (with no complementary strands) in parallel with the target. Hybridization condition: 40 °C, 20 h. Target DNA loading: 40 ng/well. Reference DNA loading: 40 ng/well. Complementary DNA loading: 10x amount of the target DNA. Ex: 633 nm. Em: 670 nm BP 30 nm. PMT Gain: 450 V. **b)** Comparison of the relative fluorescence intensity from the photo-captured target and the reference before and after hybridization for varied irradiation durations. The fluorescence intensity of the reference DNA before hybridization was referenced to calculate the relative fluorescence intensity. Paired, two-tailed Student's *t*-test: * $p < 0.01$, ** $p < 0.001$, n.s., no significant difference. n = 3 for each data point. Error bar: standard deviation.

Photo-release of the captured oligonucleotides

To release the captured dsRNA, the gels were irradiated by short-wavelength UV light (< 325 nm) for various time intervals, and visualized using SYBR™ Gold stain (Figure S9, S10). We notice that the maximum release of targets is less than 100 %, and the unreleased dsRNA level increases with the dsRNA length, in accordance with the relationship of the capture efficiency and target length for dsRNA. We hypothesize that in addition to the dissociation of psoralen-pyrimidine adducts at short-wavelength UV (< 325 nm), there may exist concurrent reactions responsible for the capture of released targets. Consistently, in non-modified polyacrylamide gels we observe a low level of dsRNA capture (η = ~20 %) under irradiation of the full-spectrum UV which contains irradiation wavelengths spanning from 280 nm to 410 nm (Table S4), but detected no capture under irradiation of > 350 nm UV (Figure S11). Moreover, this short-wavelength-UV-activated target-capture in non-modified polyacrylamide gels exhibits a time-dependent increase in capture efficiency for dsRNA of all lengths, and with 60 s full-spectrum irradiation, there has been a significant increase in capture efficiency compared to the results from the > 350 nm UV irradiation (Paired, two-tailed Student's *t*-test at 60 s for full-spectrum irradiation and > 350 nm irradiation: * $p < 0.05$, ** $p < 0.01$). Based on our experimental results and the fact that nucleic acids can crosslink with nitrocellulose and proteins under the 254 nm irradiation,[3] we reason that polyacrylamide gels can similarly crosslink with nucleic acids under short-wavelength UV irradiation. Another possible contributor to the observed target-capture under < 325 nm UV irradiation is the interaction of psoralen with the photo-excited nucleobases, which, distinct from the photo-cycloaddition at the long-wavelength irradiation, can occur on either double-bond or single-bond of the nucleobase depending on the intensity of the irradiation. Similar photo-capture between psoralen and the nucleotides under low-wavelength UV irradiation was also reported in free-solution experiments.[4]

Based on the analysis above, we applied a two-term exponential model, $\eta = e^{-k_1 t} + \eta_2(1 - e^{-k_2 t})$, to fit to the data from the release experiments, where the first term describes the release process, and the second term corresponds to the concurrent capture reactions (Table S3). Similar to the discussions in the characterization of the capture performance, we use the term 'steady-state' to refer to the state when the system's target-release level does not change with the increase of the irradiation duration. From the two-term exponential fitting model, we obtained the relative fluorescence intensity of the captured dsRNA at the steady state (η_2) and the release reaction rate (k_1) for all three dsRNA targets: η_2(500 bp) = 0.3478, k_1(500 bp) = 1.255; η_2(300 bp) = 0.2657, k_1(300 bp) = 1.405; η_2(150 bp) = 0.1612, k_1(150 bp) = 2.412. As η_2 for all three targets is well below 0.5, we reason that at the steady state, photo-release reaction dominates the concurrent photo-capture under low-wavelength UV irradiation.

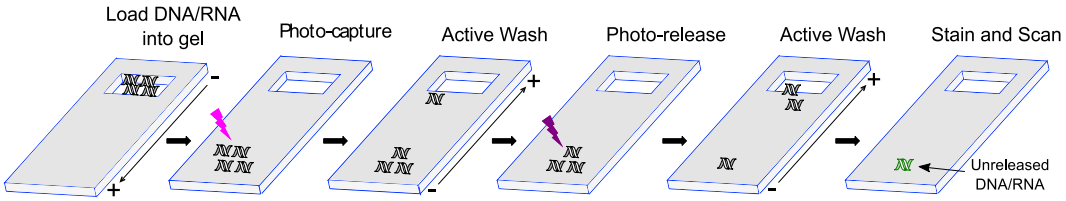


Figure S9. Schematic of the photo-release procedures.

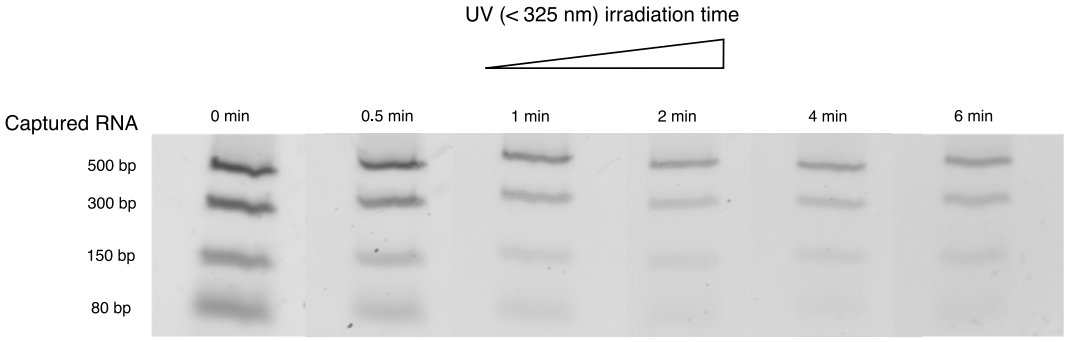


Figure S10. Representative fluorescence images of the psoralen-functionalized polyacrylamide gel after the photo-release of dsRNA targets. Psoralen concentration: 3 mM. Photo-capture condition: 1 min irradiation by > 350 nm UV light. Stain: 2x SYBR™ Gold. Ex: 488 nm. Em: 520 nm BP 40 nm. PMT Gain: 450 V.

Table S3. Fitting results to the photo-release experimental data[a] using a two-term exponential model, $\eta = e^{-k_1 t} + \eta_2(1 - e^{-k_2 t})$.

	k_1	η_2	k_2	R^2
500 bp	1.255	0.3478	0.5824	0.9977
300 bp	1.405	0.2657	0.5192	0.998
150 bp	2.412	0.1612	0.8492	0.9999

[a] Each data point was obtained by averaging over 4 replicates.

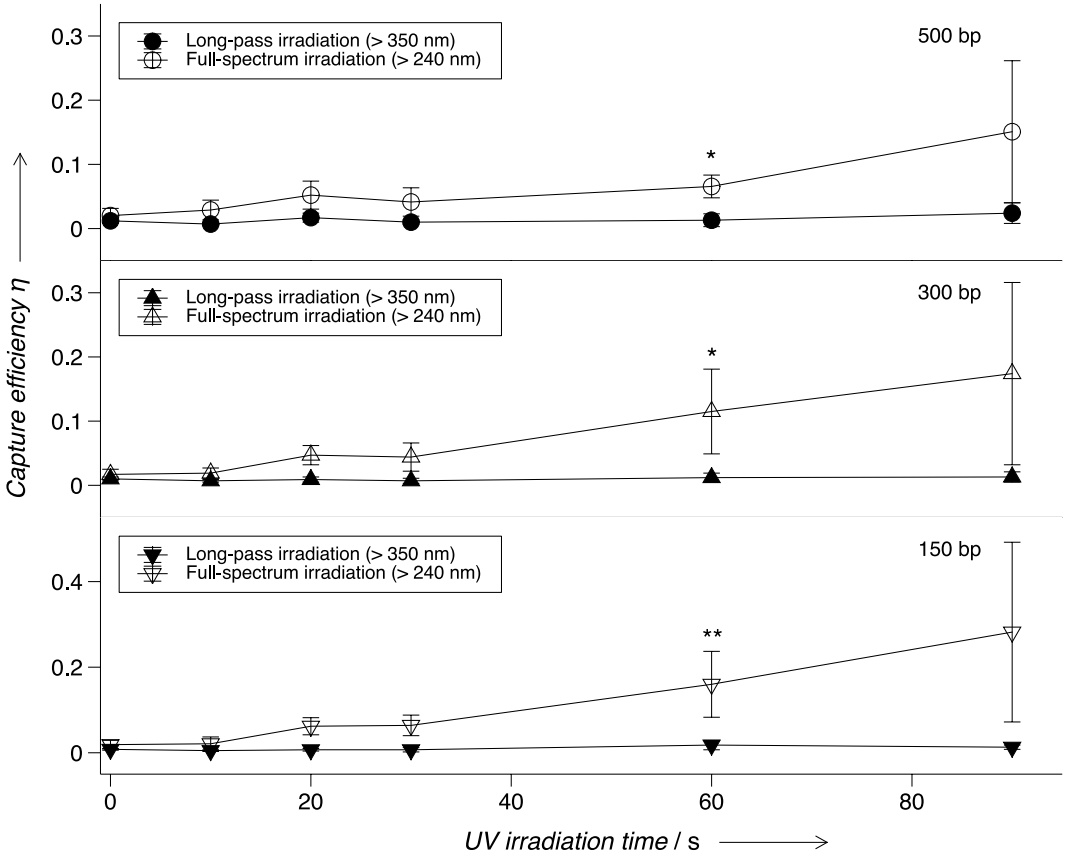


Figure S11. Comparison of the capture efficiency for dsRNA (150 bp to 500 bp) between full-spectrum UV irradiation and long-wavelength UV (> 350 nm) irradiation in the pure polyacrylamide gel, where no psoralen was added. Paired, two-tailed Student's *t*-test at 60 s: * $p < 0.05$, ** $p < 0.01$. n = 3 for each data point. Error bars: standard deviation.

Table S4. Peak distribution and intensity[a] of the UV irradiation used in photo-capture and photo-release experiments.

Wavelength (nm)	280	290	298	302	313	320	365	410
Relative intensity	0.1	0.12	0.41	0.39	0.8	0.11	1[b]	0.4

[a] Quantification was performed based on the information on the datasheet offered by the manufacturer. [b] The absolute intensity was ~360 mW cm^{-2} measured at 15 mm distance.

References

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Author Contributions

Y. Z. and A. E. H. designed the project. Y. Z. and P. P. Y. C. designed the chemical strategy for the psoralen-functionalized polyacrylamide gel. Y. Z. performed the chemical synthesis and device fabrication. Y. Z. designed and performed the system characterization experiments. Y. Z. wrote the Matlab scripts and did the data quantification and statistical analysis. All authors interpreted the results and wrote the manuscript.