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Supporting Information

Reversible Covalent Stabilization of Stacking Contacts in DNA Assemblies

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This PDF file includes:

Materials and Methods
Figures S1 to S9
References

Other Supplementary Material for this manuscript includes the following:

Table S1 with all DNA sequences as a separate Excel file

Materials and Methods

1. Synthesis of ^{CNV}K-modified DNA strands
The 3-cyanovinylcarbazole phosphoramidite (CAS: 1157899-72-3) was purchased from Glentham Life Science Ltd (GN8887). The coupling to the DNA strands was done by Eurofins MWG (Ebersberg, Germany). For the sequences see Table S1 in the separate excel file.

2. Folding of DNA origami switch object
The reaction mixtures contained the p8064 scaffold DNA at a concentration of 50 nM and oligonucleotide strands at 200 nM each. The folding buffer included 5 mM TRIS, 1 mM EDTA, 5 mM NaCl (pH 8) and 20 mM MgCl2. The reaction mixture was subjected to a thermal annealing ramp (15 min at 65°C; [58 – 55°C]; 90min/1°C) using TETRAD (MJ Research, now Biorad) thermal cycling devices. Oligonucleotides were purchased from Eurofins MWG (Ebersberg, Germany).

3. Purification of DNA origami switch object
After the folding reaction, the reaction product was purified using one round of PEG-precipitation (*1*). The resulting pellet was dissolved in folding buffer (5 mM TRIS, 1 mM EDTA, 5 mM NaCl) including 5 mM MgCl2. The final volume was chosen to get a monomer concentration of 5 nM if not otherwise stated. The samples were equilibrated at 30°C and 450 rpm overnight in a shaker incubator (Thermomix comfort from Eppendorf). All procedures were performed as previously described (*2*).

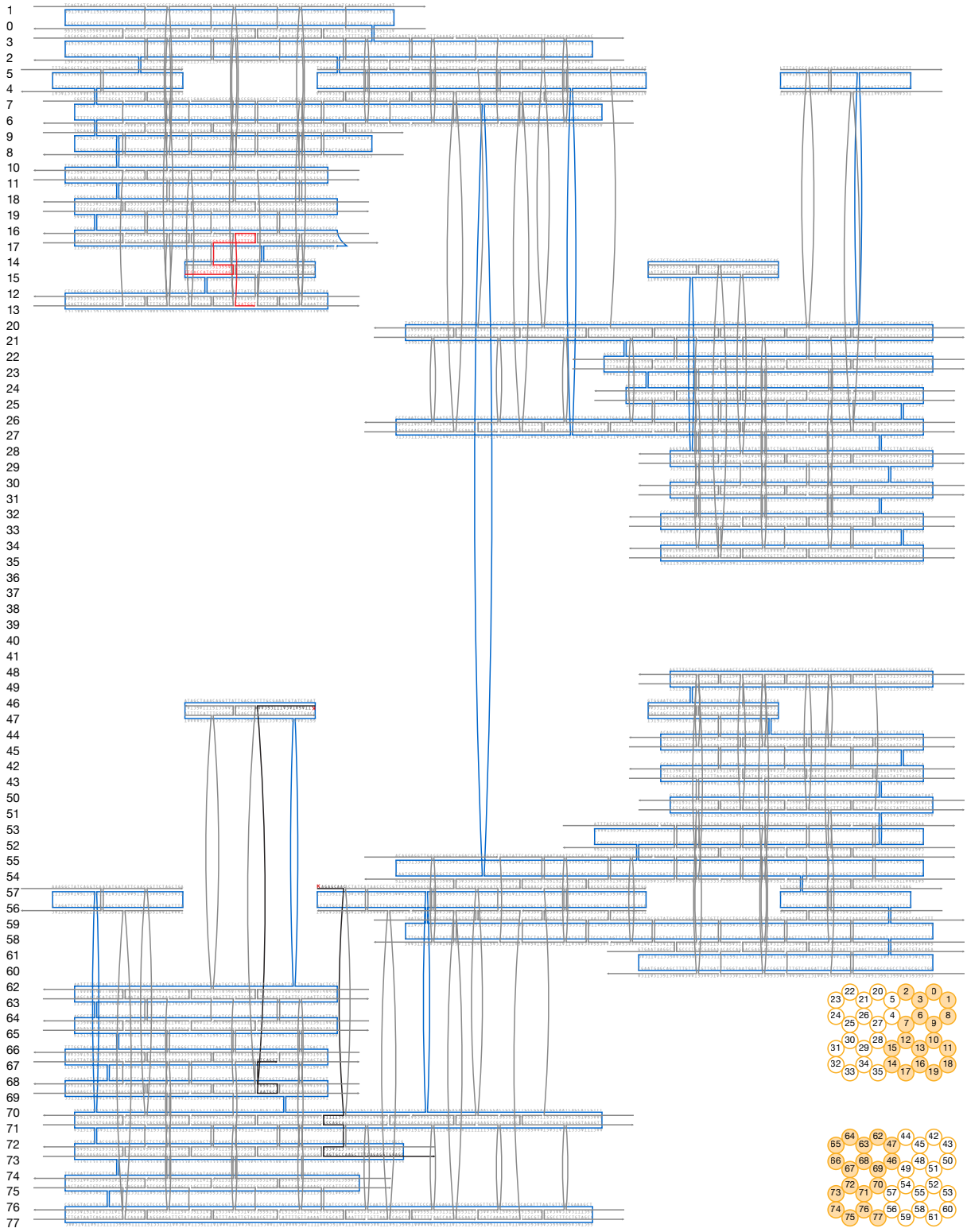
4. UV-irradiation
For UV-irradiation we used a 300W xenon light source (MAX-303 from Asahi Spectra) with a high transmission bandpass filter centered around 365 nm (XHQA365 from Asahi Spectra) or 310 nm (XHQA310 from Asahi Spectra). We used a light guide (Asahi Spectra) to couple the light into the sample by placing it directly on top of an 0.65 ml reaction tube. Samples were irradiated in folding buffer (5 mM TRIS, 1 mM EDTA, 5 mM NaCl) including 30 mM MgCl2, if not otherwise stated. To quantitatively evaluate the data obtained from gel electrophoresis, we determined the ratio between the intensity of the band corresponding to closed and crosslinked particles to the total band intensity of closed and open species. We define this ratio as the fraction of crosslinked switch particles.

5. Gel electrophoresis of DNA origami switch object
Samples were electrophoresed on 1.5% agarose gels containing 0.5xTBE and 5 mM MgCl2 for around 2 h at 90 V bias voltage in a gel box immersed in a water or ice bath, if not otherwise stated. Samples were loaded on the gel at a monomer concentration of approximately 5 nM. The electrophoresed agarose gels were scanned using a Typhoon FLA 9500 laser scanner (GE Healthcare) at a resolution of 25 µm/px in the Cy5-channel (635 nm). The resulting 16-bit tif-images were analyzed using ImageJ 1.440.

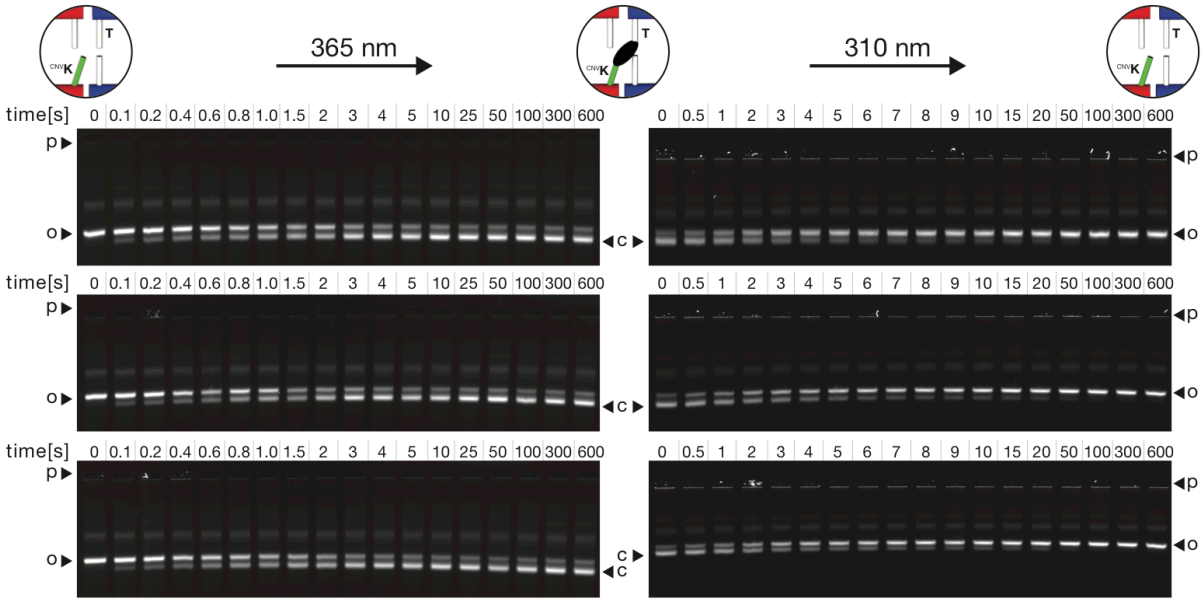
6. Negative-stain transmission electron microscopy (TEM): Preparation, acquisition and data processing
Samples were adsorbed on glow-discharged collodion-supported carbon-coated (10 nm) Cu400 TEM grids (in house production) and stained using a 2% aqueous uranyl formate solution containing 25 mM sodium hydroxide. Samples were incubated for 15-300 s depending on the buffer/solvent used. For samples dissolved in solvents including low concentrations of positively charged ions, we used higher monomer concentrations (100 nM) and longer incubation times. We used magnifications between 10.000x to 30.000x for acquiring the data.
Imaging was performed on different microscopes; see table below.

Microscope	Operating voltage (kV)	Camera	Data
Philips CM 100	100	AMT 4 megapixels CCD camera	All field-of-views
FEI Tecnai 120	120	Tietz TemCam-F416 (4k x 4k)	2D class average Figure 2c

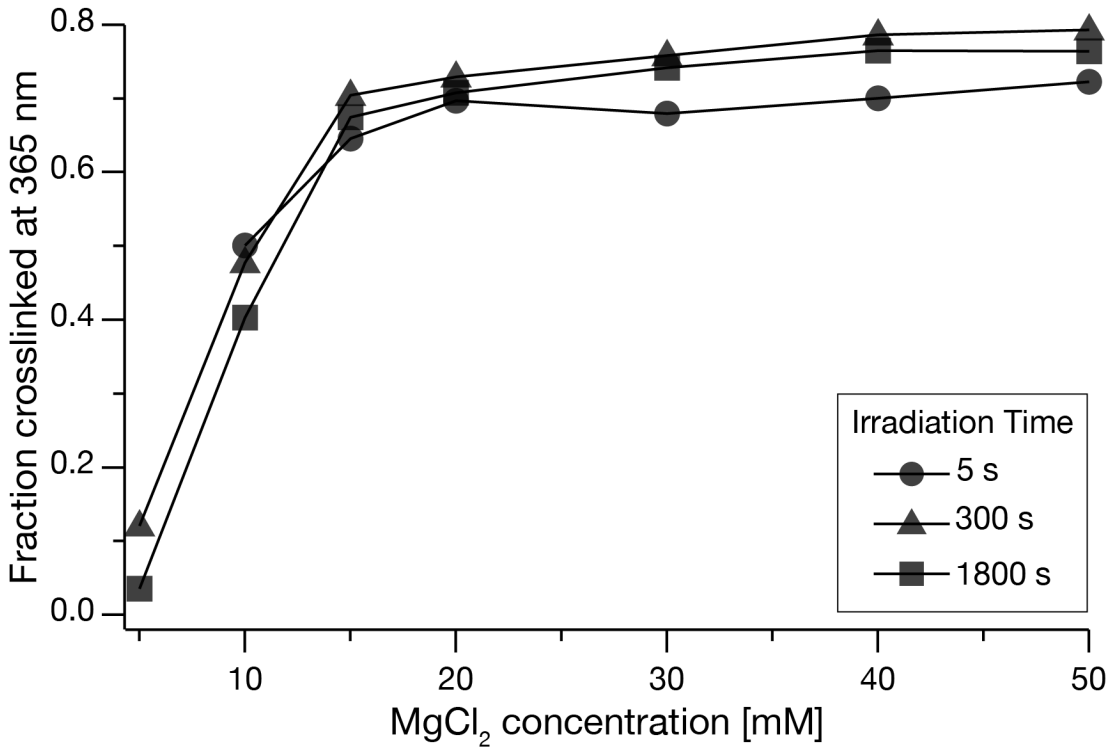
TEM micrographs used in the figures were high-pass filtered to remove long-range staining gradients, and the contrast was auto-leveled (Adobe Photoshop CS6).
For 2D image processing, libraries of individual particle micrographs were created by particle picking using the Relion-2 picking routine (*3*). Generation of average 2D particle micrographs was performed using Relion-2 (*3*). Typically, around 2000 individual particles were averaged.



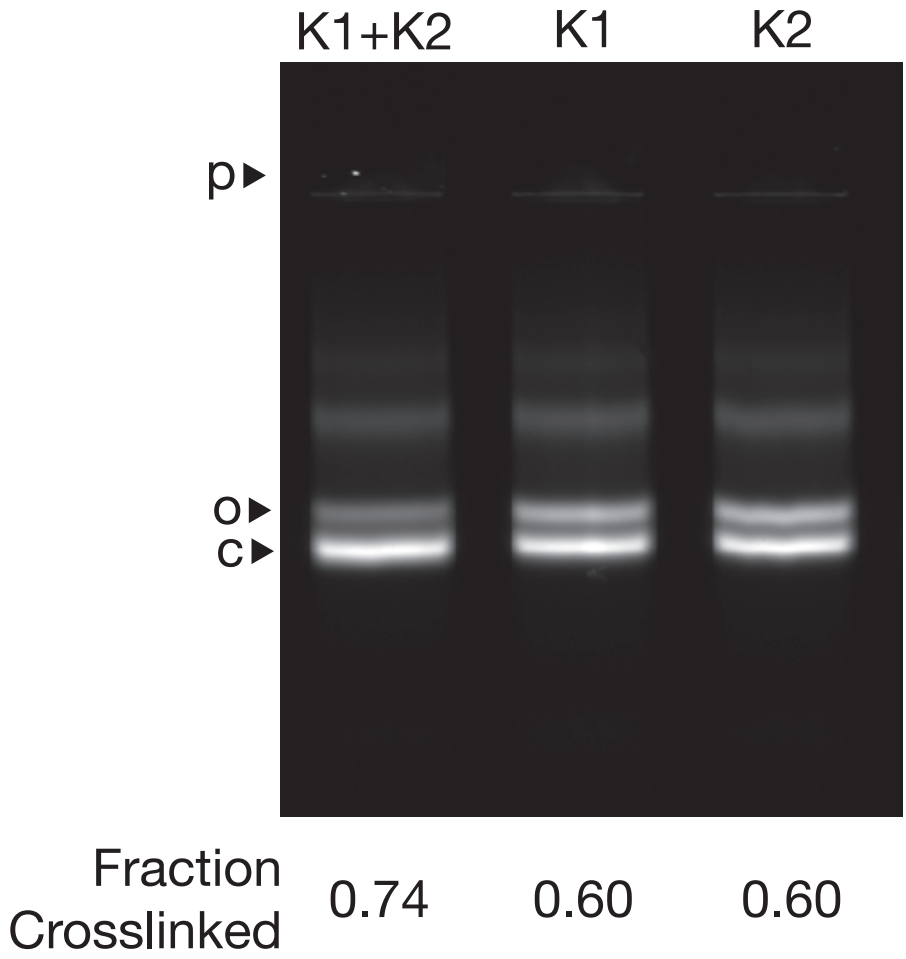
Supplementary Fig. 1 | Design diagram of the switch object prepared using caDNAno (*4*). Interfaces are passivated with poly-thymine overhangs. The two strands that we modified at the 5' end with the ^{cnv}K-moiety (labeled as 'K' in red) are labeled in black. The strand that is internally modified with the ATTO647n dye is labeled in red. Positions with more than one consecutive thymine in close vicinity to the stacking contact are indicated with green arrow heads. Inset lower right: Cross-section of the object designed in honeycomb lattice.



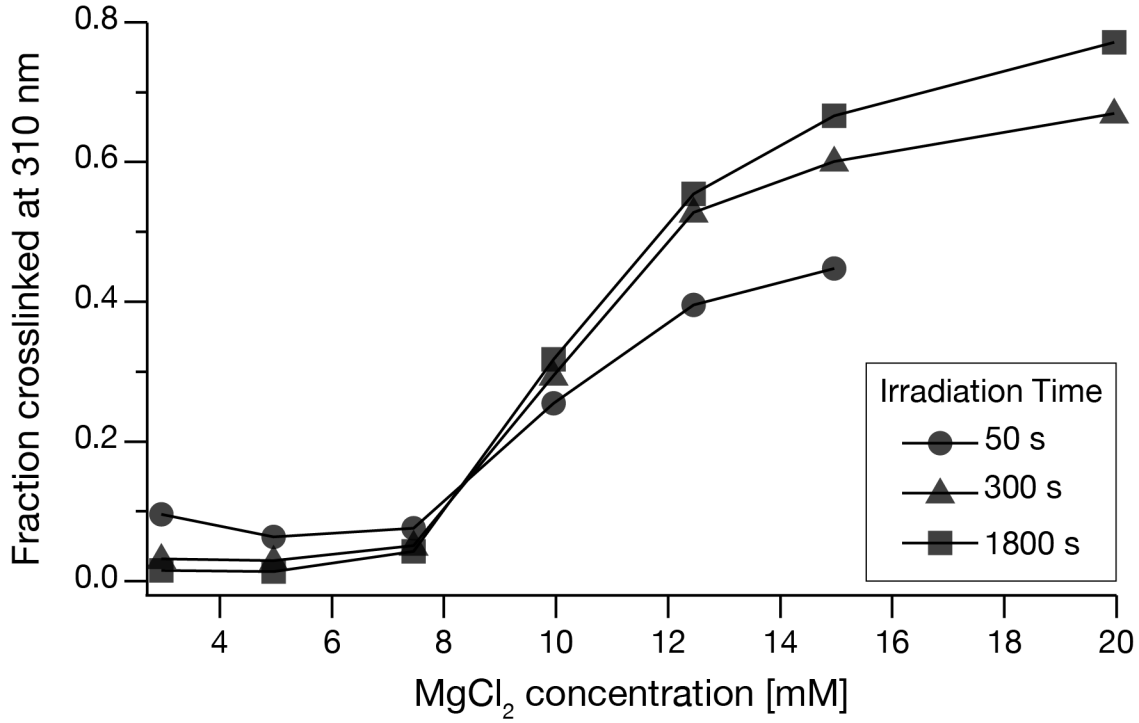
Supplementary Fig. 2 | Fluorescent images of 1.5% agarose gels laser-scanned in the Cy5-channel (635 nm). Time series of photo-crosslinking at 365 nm irradiation (left) and photo-cleavage at 310 nm irradiation (right). p: pocket, o: band corresponding to open (non-crosslinked) switch particles, c: band corresponding to closed (crosslinked) switch particles. The images of the gels are globally autoleveled.



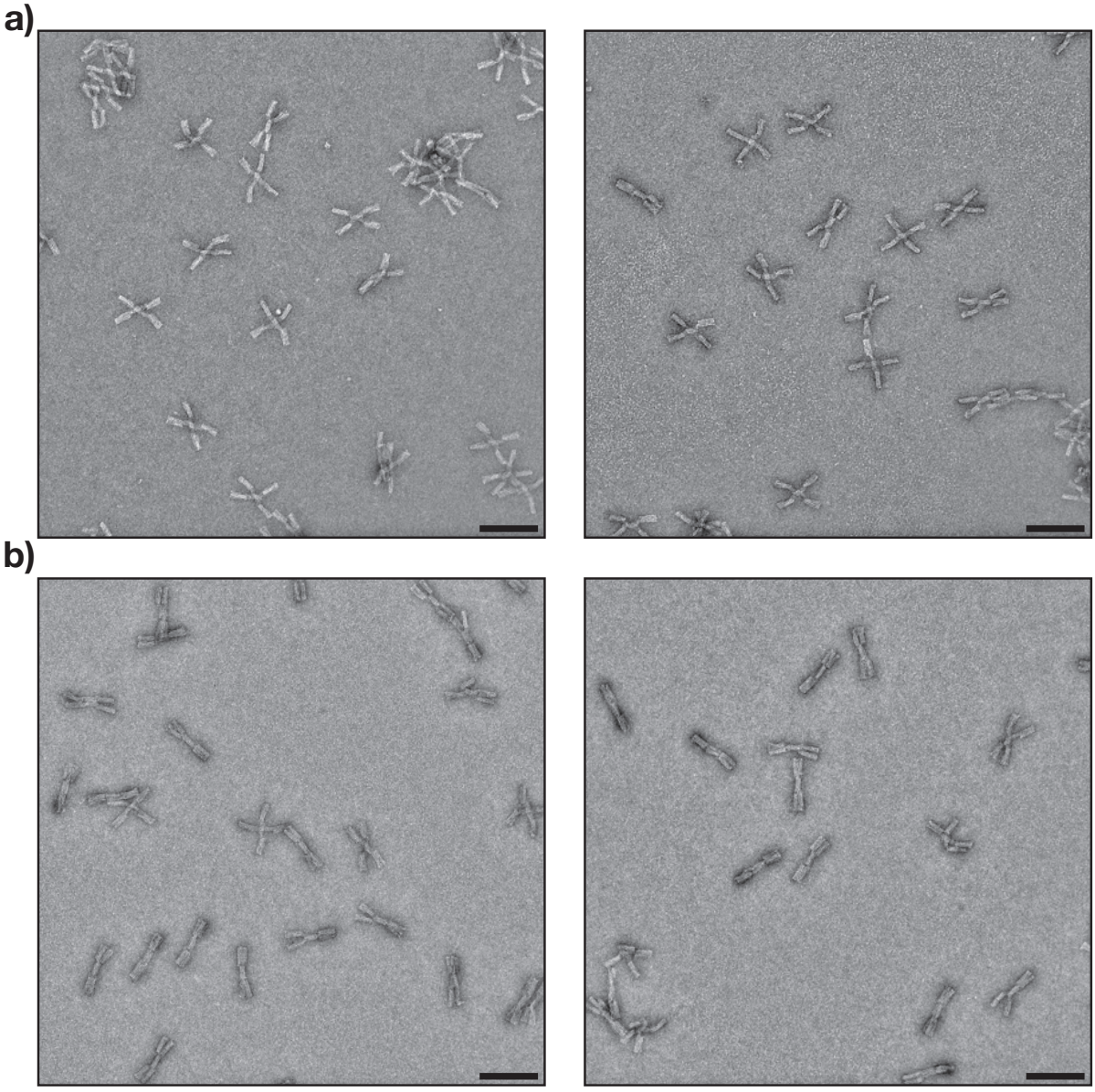
Supplementary Fig. 3 | Influence of the $MgCl_2$ concentration on photo-crosslinking for different irradiation times with 365 nm light. The fraction of crosslinked particles is computed as described in the methods section (4. UV-irradiation).



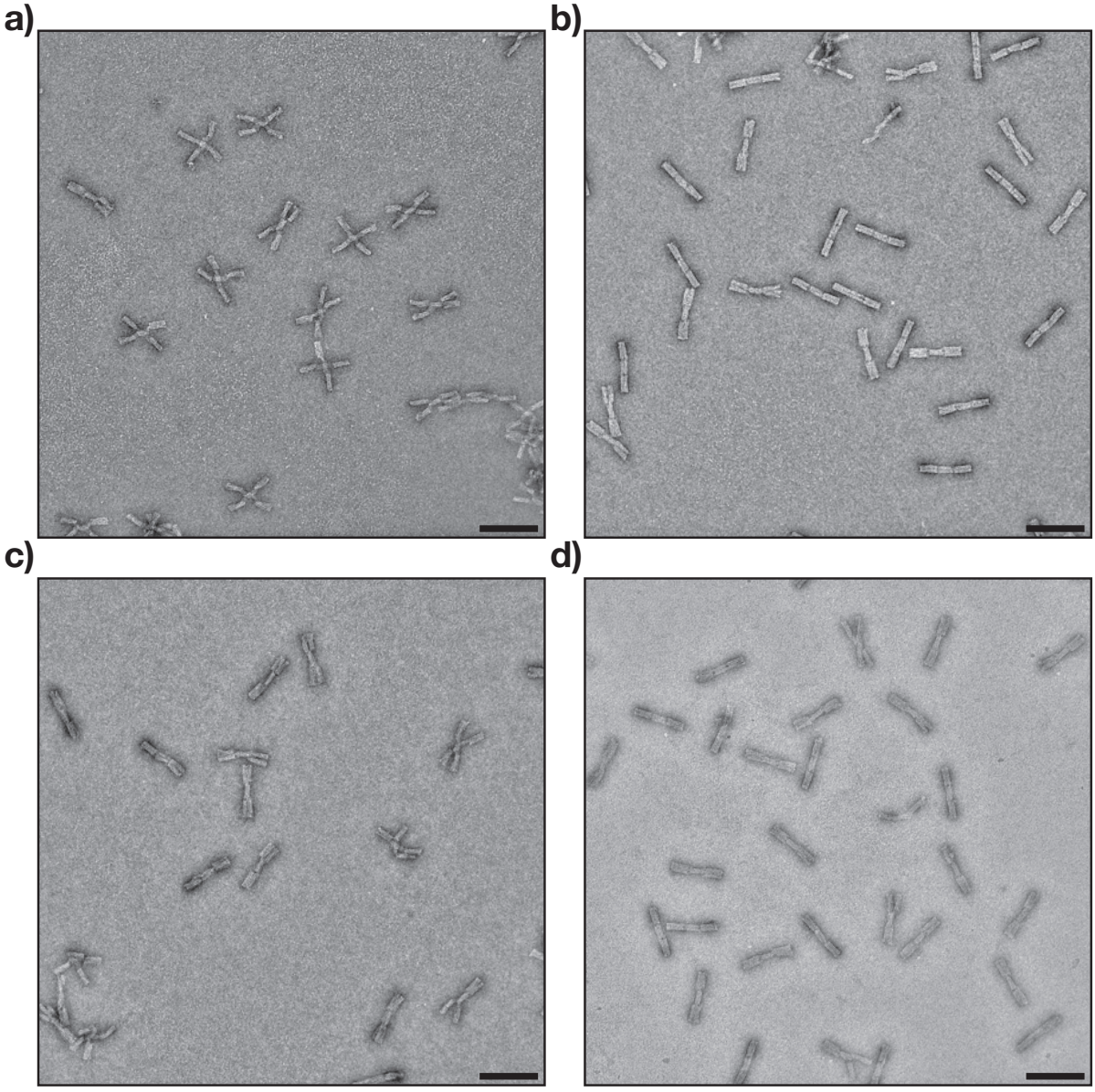
Supplementary Fig. 4 | Fluorescent image of a 1.5% agarose gel laser-scanned in the Cy5-channel (635 nm). Different variants of the DNA origami switch object were irradiated for 10 minutes with 365 nm light. The fraction of crosslinked particles is computed as described in the methods section (4. UV-irradiation). K1+K2: variant with both ^{cnv}K-modifications, K1: variant with only one of the two ^{cnv}K-modifications, K2: variant with the other ^{cnv}K-modification (Figure S1), p: pocket, o: band corresponding to open (non-crosslinked) switch particles, c: band corresponding to closed (crosslinked) switch particles. The image of the gel is globally autoleveled, and in addition, each lane was individually autoleveled.



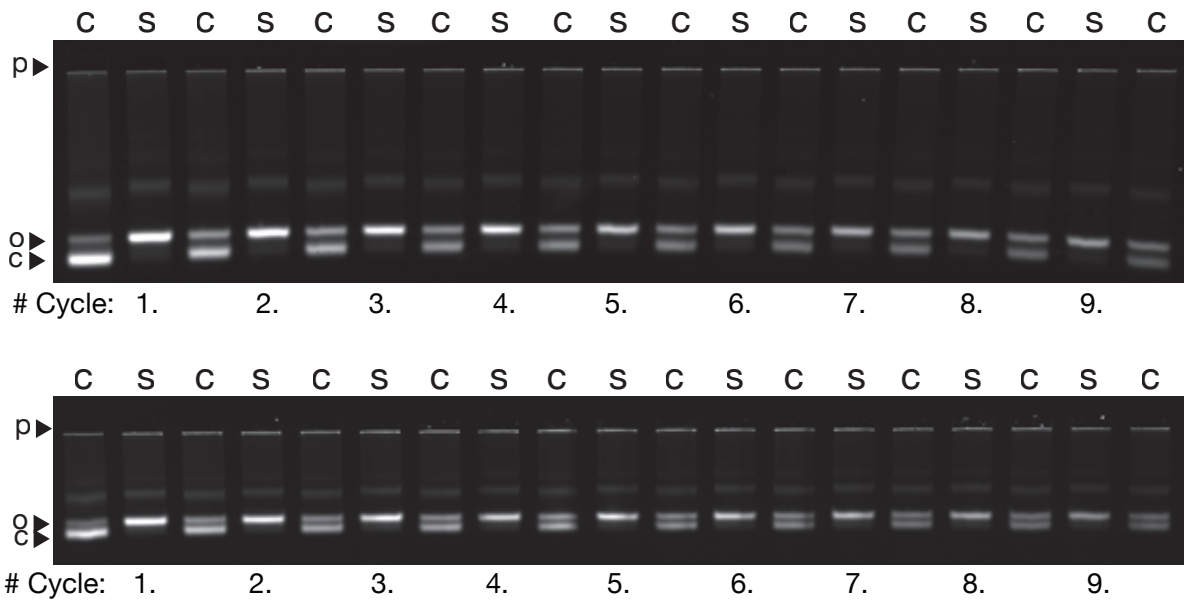
Supplementary Fig. 5 | Influence of the $MgCl_2$ concentration on photo-cleavage for different irradiation times with 310 nm light. The fraction of crosslinked particles is computed as described in the methods section (4. UV-irradiation).



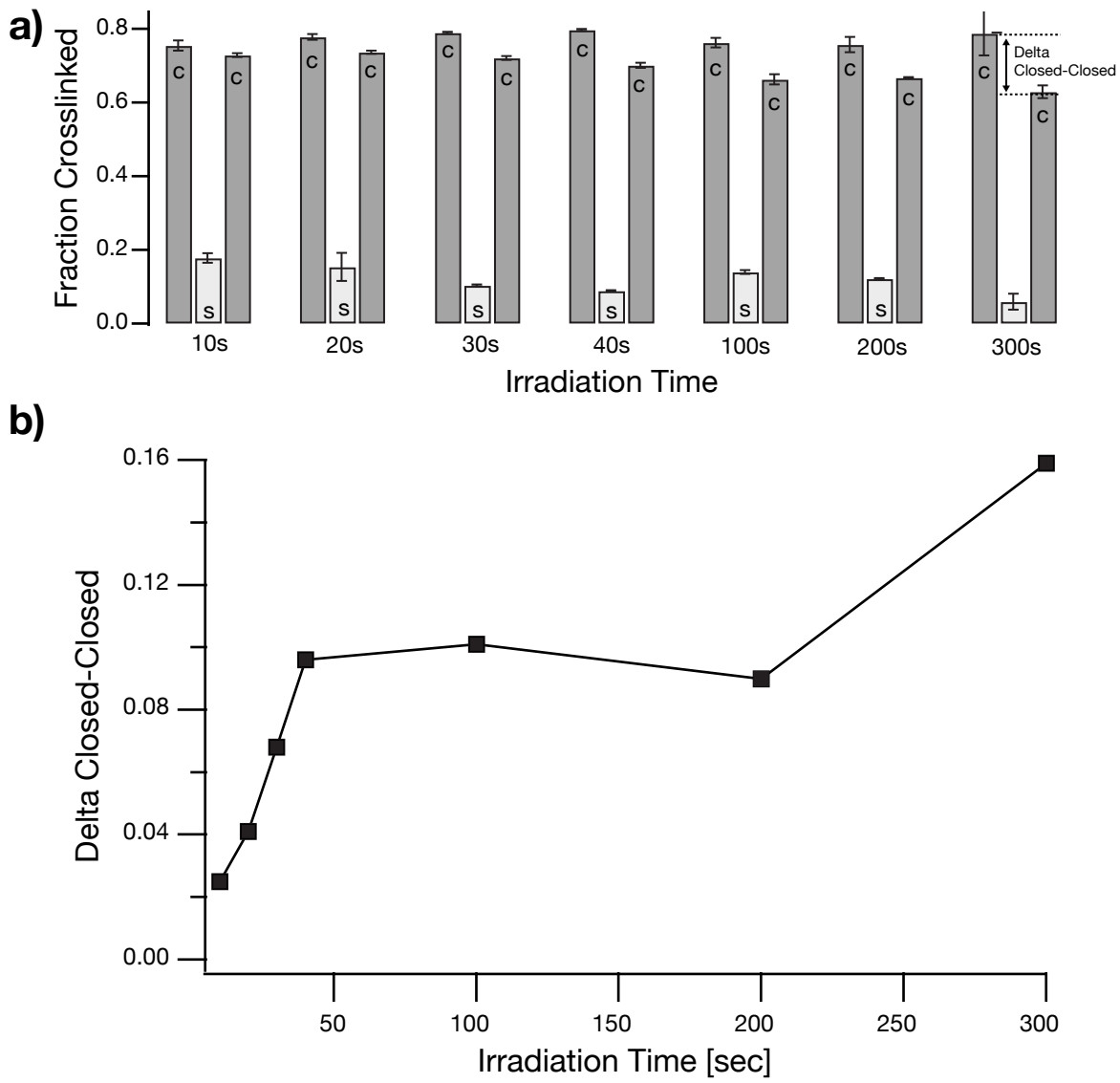
Supplementary Fig. 6 | Negative-stain TEM field-of-views of non-irradiated (non-crosslinked) switch particles a) and irradiated (crosslinked) switch particles b) in the presence of 5 mM $MgCl_2$. b) The sample was irradiated for 10 minutes with 365 nm light. Scale bars: 100 nm.



Supplementary Fig. 7 | Negative-stain TEM field-of-views for cycling the DNA origami switch object through crosslinked and non-crosslinked states as outlined in Figure 1 of the manuscript. a) Non-irradiated sample in the presence of 5 mM $MgCl_2$. b) Non-irradiated sample in the presence of 30 mM $MgCl_2$. c) Irradiated sample (5 minutes with 365 nm light) in the presence of 30 mM $MgCl_2$. d) Irradiated sample (5 minutes with 365 nm light) in the presence of 5 mM $MgCl_2$. Scale bars: 100 nm.



Supplementary Fig 8 | Fluorescent images of 1.5% agarose gels laser-scanned in the Cy5-channel (635 nm). Samples of nine successive cycles of photo-crosslinking and photo-cleavage were loaded on the gel. c: crosslinking with 365 nm light for 5 minutes, s: cleavage with 310 nm light for 5minutes (top labels). p: pocket, o: band corresponding to open (non-crosslinked) switch particles, c: band corresponding to closed (crosslinked) switch particles (left labels). The image of the gel is globally autoleveled.



Supplementary Fig. 9 | Influence of the irradiation time on the fraction of crosslinked switch particles for the first cycle. We performed the experiment in duplicate. a) Data points represent the mean and error bars the standard deviation. The fraction of crosslinked particles is computed as described in the methods section (4. UV-irradiation). c: crosslinking at 365 nm, s: cleavage at 310 nm. b) We computed the difference between the fraction of crosslinked particles for the first cycle as indicated in a) ('Delta Closed-Closed') and plotted it as a function of irradiation time.

Supplementary References

1. E. Stahl, T. G. Martin, F. Praetorius, H. Dietz, Facile and scalable preparation of pure and dense DNA origami solutions. *Angewandte Chemie* **53**, 12735-12740 (2014).
2. K. F. Wagenbauer *et al.*, How we make DNA origami. *Chembiochem : a European journal of chemical biology*, (2017).
3. D. Kimanius, B. O. Forsberg, S. H. Scheres, E. Lindahl, Accelerated cryo-EM structure determination with parallelisation using GPUs in RELION-2. *Elife* **5**, (2016).
4. S. M. Douglas *et al.*, Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic acids research* **37**, 5001-5006 (2009).