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Supplemental information

Photoswitchable single-stranded DNA-peptide coacervate formation as a dynamic system for reaction control

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Droplet Count Procedure

Images obtained from microscope observation were exported to TIF files and the following ImageJ macro (adapted from Grishagin, 2015) was used to obtain the droplet counts for all samples. Typical output images are as shown in **Figure S1D**. Output images were visually inspected to verify the validity of droplet counts.

```
macro "DropletCount--BrightField--Threshold--QD--BGSubtract" {

   //show prompt for selection of source directory
   dir = getDirectory("Choose source directory");

   //get the file list
   list = getFileList(dir);
   resultsFileName = "Droplet Counting Results (Threshold).txt"

   //make directory to store processed images
   File.makeDirectory(dir+"Processed Images");

   //hide all the details from user
   setBatchMode(true);

   //process every file...
   for (i=0; i<list.length; i++) {

            //...that has a .tif extension
            if (endsWith(toUpperCase(list[i]), ".TIF")) {

                     open(dir+list[i]);
                     fileNoExt = split(list[i], ".");

           //Subtract background
           run("Subtract Background...", "rolling=50 sliding");

           //Flatten image
            run("8-bit");
            setAutoThreshold("Default dark");

              //run("Threshold...")
            setThreshold(50, 255);
            run("Convert to Mask");

saveAs("Tiff", dir+"Processed Images"+File.separator+fileNoExt[0]+" Threshold");

                     //analyze and show as [Bare Outline]
run("Analyze Particles...", "size=0-100 pixel show=[Bare Outlines] clear");
saveAs("Tiff", dir+"Processed Images"+File.separator+fileNoExt[0]+" Outline");

                     //create overlay with original image and outline
   call("ij.plugin.frame.ThresholdAdjuster.setMode", "Red");
                     setAutoThreshold("Default");

                     open(dir+list[i]);
                     selectWindow(fileNoExt[0]+" Outline.tif");
run("Add Image...", "image="+"["+list[i]+"]"+" x=0 y=0 opacity=75");
                     run("Flatten");
saveAs("Tiff", dir+"Processed Images"+File.separator+fileNoExt[0]+" Outline Overlay");
                     close();
```

```
                        close();
                        close();

                        //analyze and show as [Overlay]
run("Analyze Particles...", " size=0-100 pixel show=Overlay clear summarize");
saveAs("Tiff", dir+"Processed Images"+File.separator+fileNoExt[0]+" Overlay");


                        //close all windows
                        selectWindow(fileNoExt[0]+" Overlay"+".tif");
                        close();
                }
    }

    //copy all relevant contents from the Summary window
    selectWindow("Summary");
    text = getInfo("window.contents");
    lines = split(text, "\n");

//create a text file with counting results, output cell count, total area and average size
    //copy the results into clipboard
    //reset string buffer
    String.resetBuffer;

    for (i=0; i<lines.length; i++) {

                if (i==0){
                        File.saveString("",dir+resultsFileName);
                        f = File.open(dir+resultsFileName);
                }

                labels = split(lines[i], "\t");
            labelNoExt = split(labels[0], ".");
            resultsList = newArray(labelNoExt[0]+"\t"+labels[1]+"\t"+labels[2]+"\t"+labels[3]);
                print(f,resultsList[0]);

//append another value to the string
                String.append(resultsList[0]+"\n");
    }

    File.close(f);

    //open the summary file, close the Summary window
    open(dir+resultsFileName);
    selectWindow("Summary");
    run("Close");

    //copy all values into the clipboard
    String.copy(String.buffer);

setBatchMode(false);

}
```

Figures and Schemes

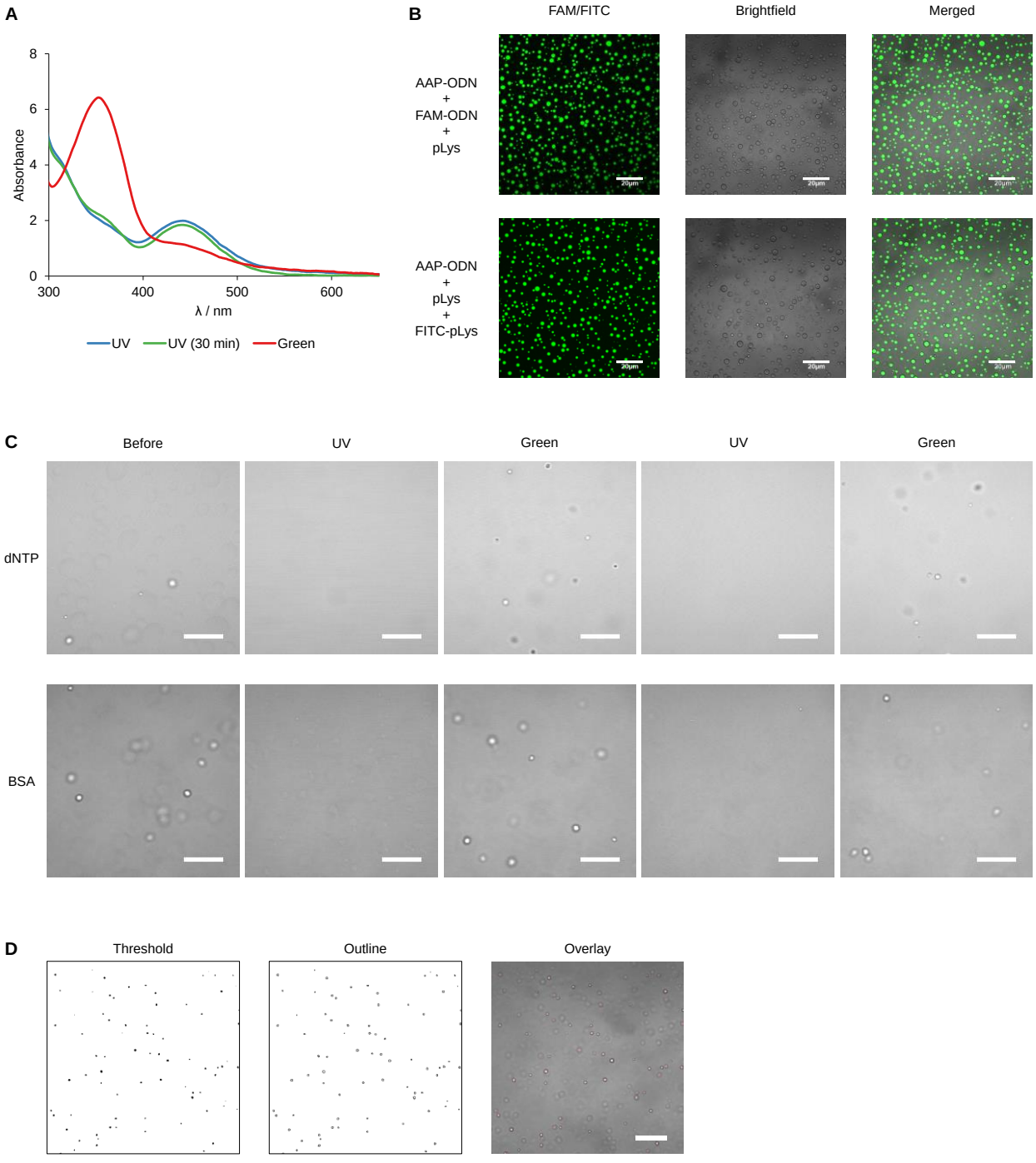


Figure S1. Characterization of photoswitchable droplets.

Related to Figure 1. (**A**) Absorption spectra of AAP-ODN droplets immediately following UV (365 min) and after 30 min incubation in the dark at room temperature, and after green light (520 nm) irradiation. (**B**) Fluorescence microscopy images of (AAP-ODN)-pLys droplets doped with FAM-ODN or FITC-pLys (scale bar: 20 µm). (**C**) Photoswitchable droplet formation in the presence of 160 µM dNTP and 1 µM BSA (66.5 kDa) (scale bar: 10 µm). (**D**) Processed microscopy images for droplet counting (scale bar: 20 µm).

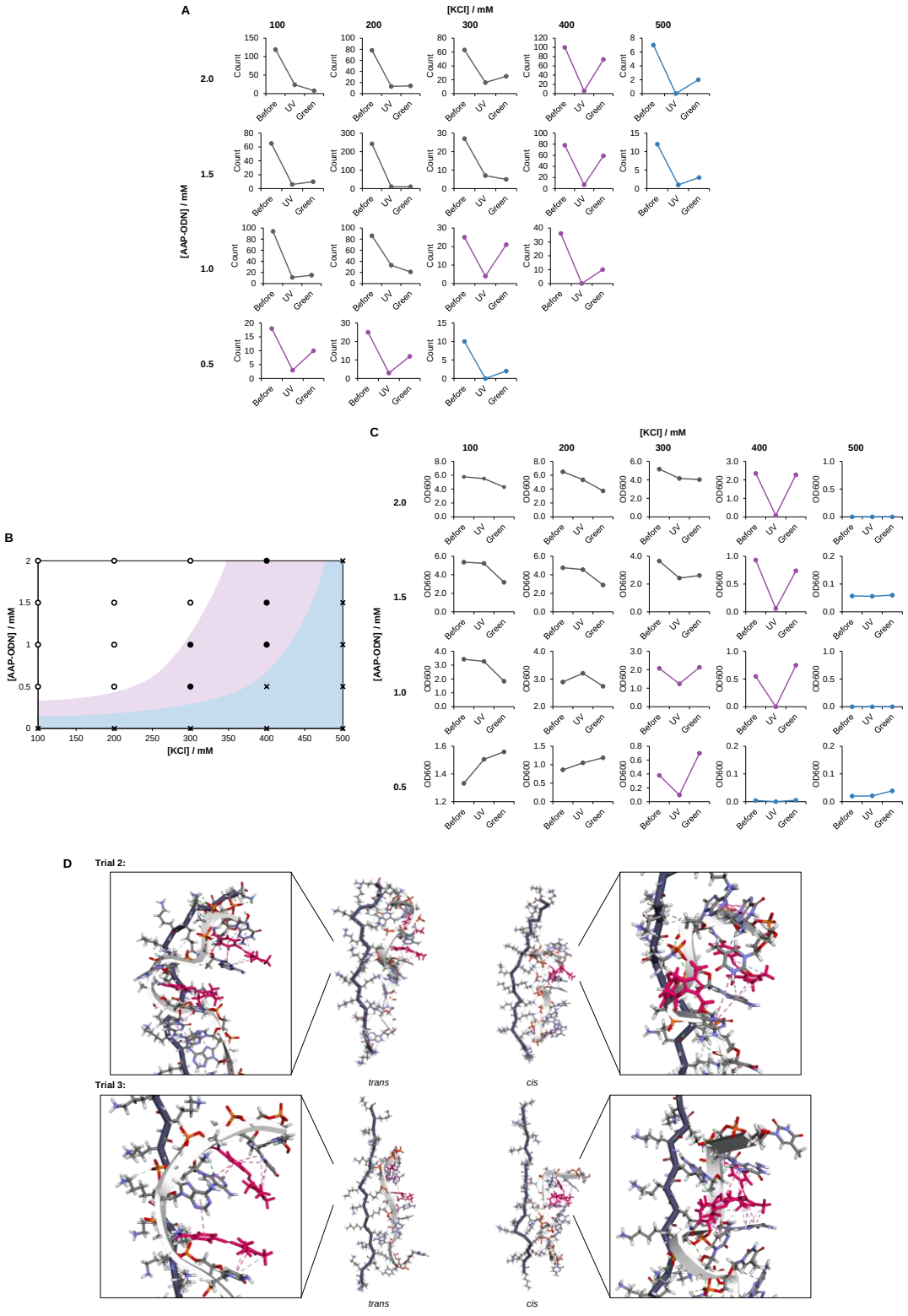


Figure S2. Photoswitchability of (AAP-ODN)-pLys droplets.

Related to Figures 2 and 3. (**A**) Droplet count profile of (AAP-ODN)-pLys samples. (**B**) Phase diagram based on turbidity data and (**C**) turbidity profile of (AAP-ODN)-pLys samples. (**D**) Energy minimum structures of *trans* and *cis*AAP-ODN with 20-mer pLys.

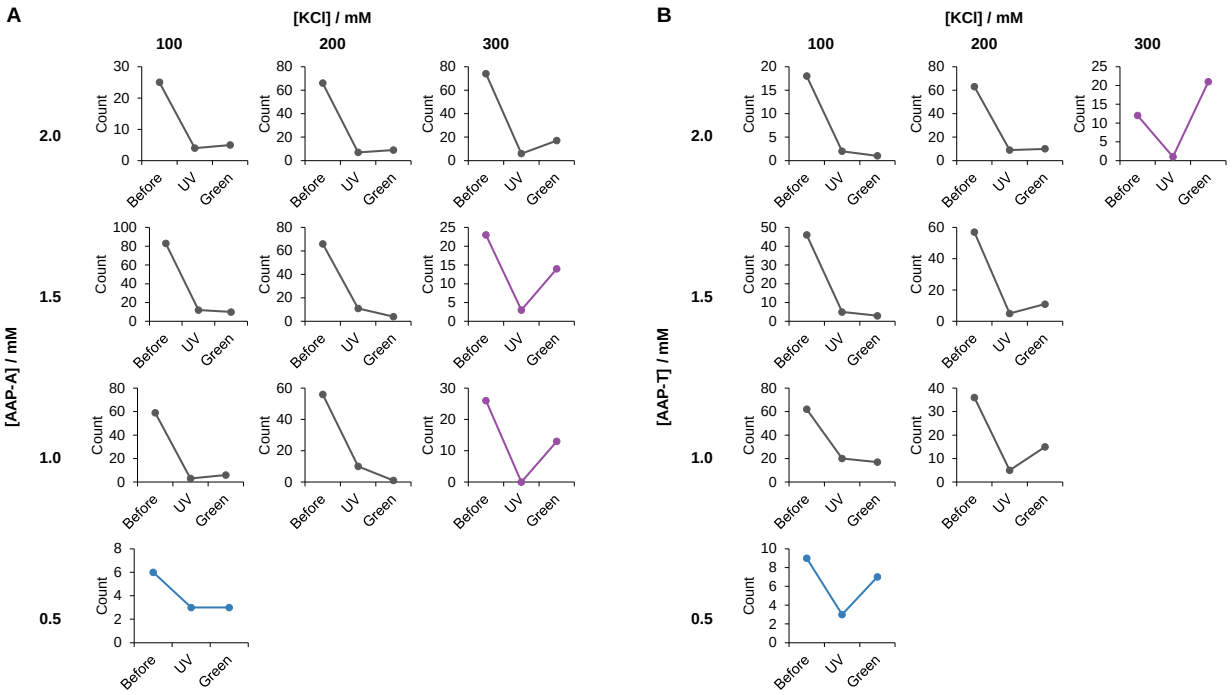
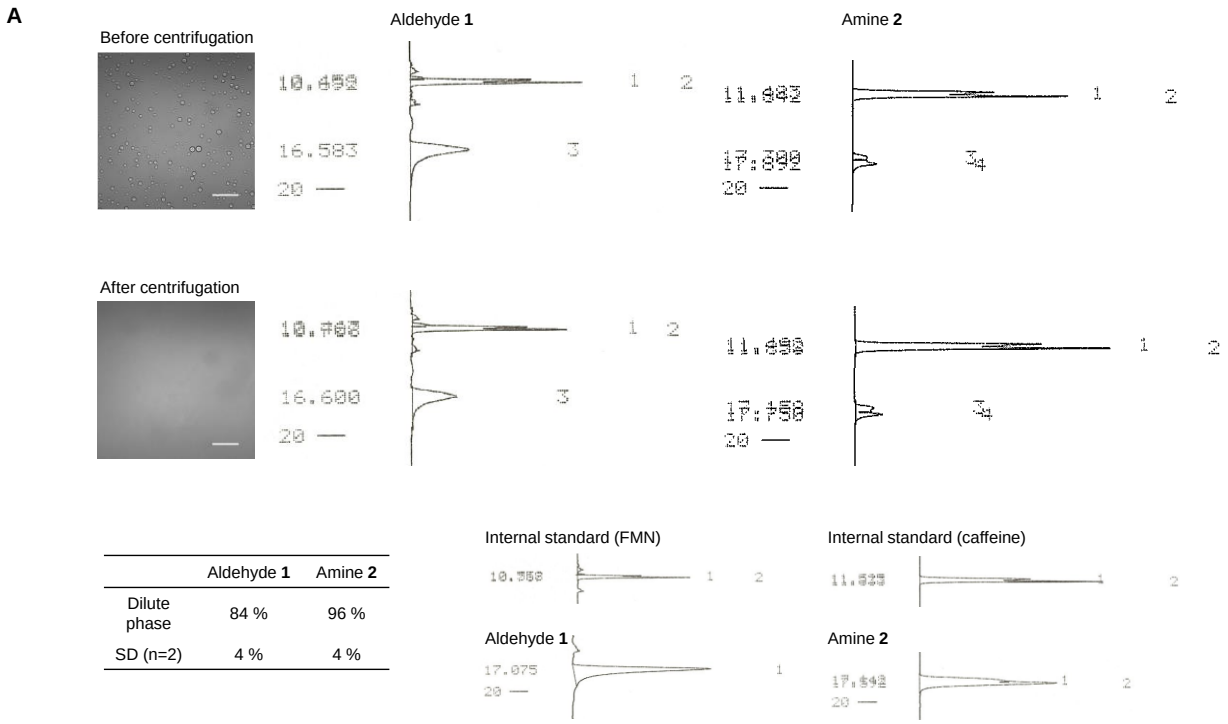


Figure S3. Photoswitchability of (AAP-A)-pLys and (AAP-T)-pLys droplets.

Related to Figure 2. Droplet count profile of (**A**) (AAP-A)-pLys and (**B**) (AAP-T)-pLys samples.



	Aldehyde **1**	Amine **2**
Dilute phase	84 %	96 %
SD (n=2)	4 %	4 %

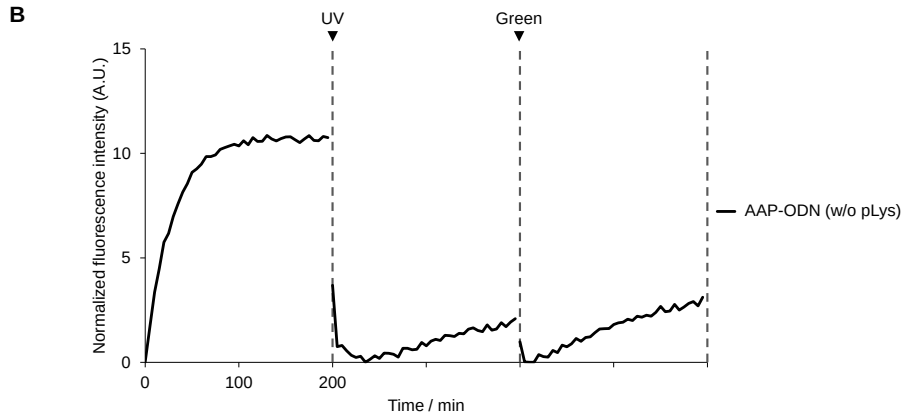


Figure S4. Photoswitchable control of an uncatalyzed condensation reaction.

Related to Figure 4. (**A**) Confocal microscope images of samples as well as HPLC charts of aldehyde **1** and amine **2** before and after centrifugation at 16000×g for 20 min. Riboflavin 5‘-monophosphate (FMN) and caffeine were added as internal standards for aldehyde **1** and amine **2** respectively. Detection was conducted at 340 nm for aldehyde **1** and 270 nm for amine **2**. Two independent experiments each were conducted for aldehyde **1** and amine **2** (scale bar: 10 µm). (**B**) Fluorescence trace of imine condensation with AAP-ODN and without pLys, before (k_{obs} = 0.16 min^{-1}) and after UV (365 nm) (k_{obs} = 0.012 min^{-1}) and green light (520 nm) (k_{obs} = 0.019 min^{-1}) irradiation. Minimum instensity for each iteration was adjusted to zero.

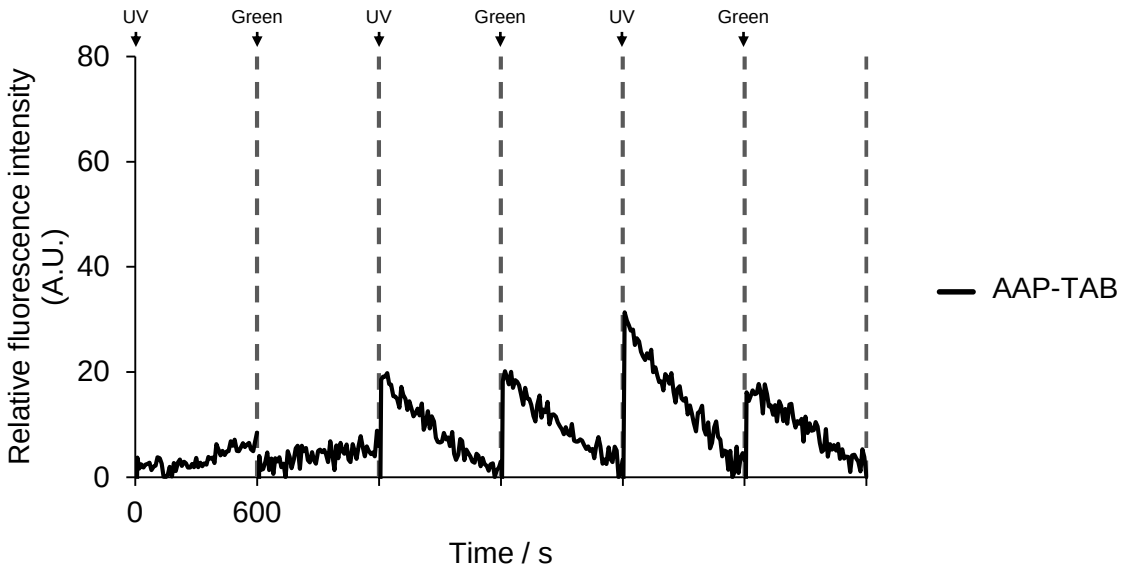
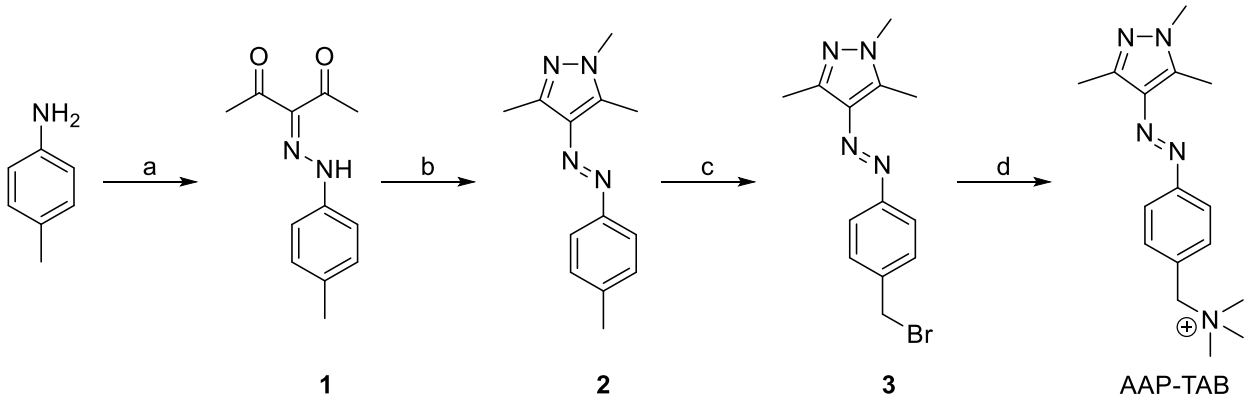


Figure S5. Photoswitchable control of a DNAzyme-catalysed oxidation reaction.

Related to Figure 5. Fluorescence trace of Amplex Red oxidation with AAP-TAB in the presence of QD-hemin complex. Minimum instensity for each iteration was adjusted to zero. A decrease in fluorescence intensity was observed for later iterations due to photobleaching of the accumulated resorufin product.



Scheme S1. Synthesis of AAP-TAB.

Related to Figure 1 and STAR Methods. Reagents and conditions: (a) $NaNO_2$, conc. HCl, AcOH, H_2O, 0 °C, 1 h then pentan-2,4-dione, EtOH, NaOAc, H_2O, r.t., 1 h; (b) *N*-methyl hydrazine, EtOH, 80 °C, 3 h; (c) NBS, AIBN, CCl_4, reflux, 4 h; (d) Me_3N, MeOH, EtOH, 50 °C, 3 days.

Tables

Table S1.

Related to Figures 1, 2, 5 and S1. Sequences of oligonucleotides, and MALDI-TOF-MS data as well as HPLC profiles of synthesized oligonucleotides.

Name	Sequence	Calcd.	Found	HPLC Profile
ODN	5’-TGA ACT AAC G-3'			
FAM-ODN	5’-FAM-TGA ACT AAC G-3'			
				Purchased
	5’-GGGTTGGG CGCGAAATCCG TGC-3’			
QD				
	3’-GGGTTGGG GCGCT TTAGGCACG-5’			
AAP-ODN	5’-TG**X** AA**X** CTA ACG-3’	3848.836	3848.741	
AAP-A	5’-AA**X** AA**X** AAA AAA-3’	3883.895	3883.371	
AAP-T	5’-TT**X** TT**X** TTT TTT-3’	3792.772	3791.443	

X = AAP

Table S2.

Related to Figure 3. Molecular dynamics NAMD protocol.

Run	Temperature / K	Time / ps
Heat	0 to 500	50
Equilibration	500	100
Production	500	950
Cool	500 to 20	50