Supporting Information

Controlling the Reversible Assembly of Liposomes through a Multistimuli Responsive Anchored DNA

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1. Materials
Chol-ONs were purchased from Biomers. The rest of ONs were purchased from Integrated DNA Nanotechnology (IDT). 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC) lipids were purchased from Sigma-Aldrich. SYTO® 9 (green fluorescent nucleic acid stain) was purchased from Thermo Fischer.

2. LUVs preparation
POPC was dissolved in chloroform to a lipid concentration of 200 mM. 0.1 mL of the lipid solution were added to a glass vial and the solvent removed with a rotary evaporator. Residual organic solvent was removed by using a vacuum pump for 60 minutes to give a flat lipid layer. Then 10 mL of a solution of 20 mM MgCl$_2$ buffered at pH=8 with 1xTE were added to the lipid film and the mixture was sonicated for 30 minutes at room temperature. 200 nm diameter LUVs were obtained by extruding the sonicated liposome mixture through 200 nm polycarbonate membranes (Nuclepore Track-Etched Membranes, Whatman) according to the protocols of Avanti Polar Lipids, Inc. The extruded LUVs were stored at 4 ºC and used within 1 week from preparation.[1]

3. Preparation and characterization of the employed AZO-ONs

| Name      | Sequence                      |
|-----------|-------------------------------|
| AZO-ON$_1$ | AZO-C$_{12}$-AAATATATTATGGCAAAAAATGTTCGACAGT |
| AZO-ON$_2$ | AZO-C$_{12}$-AAAGGCATCGTTGGAAAAATTTCCGGATCCA |
| AZO-ON$_3$ | AZO-C$_{12}$-AAAAAACCTCCCGGAGTCCGCTGCTGATCAAA |
| AZO-ON$_4$ | AZO-C$_{12}$-AAAAAACCGCTAAGCCACCTTTAGATCAAAA |

*Table S1.* Sequences of the investigated AZO-ONs. AZO-C$_{12}$ refers to the hydrophobic modification consisting of azobenzene residue attached to a C$_{12}$ chain as shown in the Figure 2a of the main text.
3.1. Preparation of AZO-ONs

3.1.1. Synthesis of AZO-NHS-ester

\[
\begin{align*}
\text{N=O} & \rightarrow \text{N=O} \\
\text{COO} & \rightarrow \text{N}
\end{align*}
\]

Synthesis was done activating 4-(phenylazo) benzoic acid with N-hydroxysuccinimide following a previously described procedure. [2]

3.1.2. Synthesis of AZO-ONs

\[
\begin{align*}
\text{N=O} & \rightarrow \text{CONH}-(\text{CH}_2)_12\text{-DNA}
\end{align*}
\]

The protocol for the synthesis of the AZO-ON\textsubscript{1} is described as a representative example. All AZO-ONs were synthesized following the same procedure starting from their corresponding amino modified ONs. Amino modified ON\textsubscript{1} (5’-NH\textsubscript{2}-C\textsubscript{12}-AAATATATTATGGCAAAAAATGTTCGACAGT = NH\textsubscript{2}-ON\textsubscript{1}) was used as the starting ON for the azobenzene coupling. Sodium hydrogen carbonate solution (0.2M, 100 μL) was added to a solution of the NH\textsubscript{2}-ON\textsubscript{1} (66.1 nmol) in H\textsubscript{2}O (100 μL). A solution of the previously prepared AZO-NHS-ester (3.6 μmol) dissolved in DMF (150 μL) was added over the solution containing the NH\textsubscript{2}-ON\textsubscript{1} and the mixture was stirred at 40 °C for 18 h. H\textsubscript{2}O (650 μL) was added to the reaction mixture. The mixture was purified using an Illustra NAP (Sephadex G-25) column. Samples were sent to HPLC and MALDI for purity analysis. HPLC utilised a semiprep XBridge C18 column, (2.5 μm, 10 x 2.5μm) with 200 μL loop. Eluent A: 5% Acetonitrile/ H\textsubscript{2}O. Eluent B: 70% Acetonitrile/ H\textsubscript{2}O. Both buffered with TEAAc (pH=7). Column was eluted with 5-50% B for 20 minutes followed by washing with 100% B. No peak corresponding to unreacted NH\textsubscript{2}-ON\textsubscript{1} is observed in the HPLC trace of AZO-ON\textsubscript{1} (Figure S1). This evidences that the reaction is quantitative so AZO-ON\textsubscript{1} was used without further purification.
3.1.3. Characterisation of AZO-ONs by MALDI

| Name    | Molecular Formula | Expected Mass | Found Mass |
|---------|-------------------|---------------|------------|
| AZO-ON$_1$ | C$_{332}$H$_{416}$N$_{125}$O$_{183}$P$_{31}$ | 10040.9       | 10039.5    |
| AZO-ON$_2$ | C$_{330}$H$_{414}$N$_{127}$O$_{183}$P$_{31}$ | 10042.9       | 10043.0    |
| AZO-ON$_3$ | C$_{328}$H$_{412}$N$_{125}$O$_{182}$P$_{31}$ | 9948.9        | 9948.0     |
| AZO-ON$_4$ | C$_{327}$H$_{412}$N$_{127}$O$_{182}$P$_{31}$ | 9924.9        | 9923.6     |

Table S2. Chemical formula, expected mass and found mass by MALDI of the different used AZO-ONs.
**Figure S2.** MALDI spectrum of AZO-ON$_1$

**Figure S3.** MALDI spectrum of AZO-ON$_2$
Figure S4. MALDI spectrum of AZO-ON₃.

Figure S5. MALDI spectrum of AZO-ON₄.
4. Optical density measurements (turbidity)
Optical density was measured at 650 nm (OD650) using UV-Vis spectrophotometry to investigate the formation of assemblies. \[3\]

**OD650 evolution with time:** OD650 of LUVs (1 mM POPC in 20 mM MgCl\(_2\) buffered at pH=8 with 1xTE) was monitored for ~30 minutes after the addition of ON.

**OD650 dependence on Mg\(^{2+}\) concentration:** OD 650 of LUVs (1 mM POPC in the solution containing the required Mg\(^{2+}\) concentration) was monitored for ~30 minutes after the addition of ON. The ON was added ~1min after having started measuring the OD650. The plotted OD650 data are the values obtained at time 30 min.

**OD650 dependence on DNA concentration:** OD650 of LUVs (1 mM POPC in 20 mM MgCl\(_2\) buffered at pH=8 with 1xTE) was measured after the addition of the required type of DNA. Titration was done by gradually increasing DNA concentration. After each addition the sample was carefully mixed with a micro pipette. The sample was left 5 minutes for equilibration before measuring the OD650.

5. LUVs assembly studies in cholesterol modified ONs

5.1. **OD650 evolution with time of LUVs incubated with chol-ON-T31**
No increase in turbidity (OD650) is detected when LUVs (1 mM) are incubated with 2µM of polyT Chol-ON (5’-Chol-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT). 

![Figure S6. OD650 of LUVs (1 mM) incubated with polyT Chol-ON (2 µM).](image-url)
5.2. Effect on LUVs produced by ON₁ modified with Chol in either 5’ (Chol-ON₁) or 3’ (ON₁-Chol)

5.2.1. Sequences

| Name       | Sequence                                      |
|------------|-----------------------------------------------|
| Chol-ON₁   | Chol-AAATATATTATGGCAAAAAATGTTCGACAGT          |
| ON₁-Chol   | AAATATATTATGGCAAAAAATGTTCGACAGT-Chol         |

Table S3. Sequences of the investigated Chol-ONs. Chol refers to the cholesterol-TEG modification attached to the oligos as indicated in the chemical structure of Figure 1a in the main text.

5.2.2. OD₆₅₀ of LUVs incubated with ON₁ with Chol modified in either 5’ (Chol-ON₁) or 3’ (ON₁-Chol) at different DNA concentrations

![Figure S7](image)

Figure S7. Evolution of OD₆₅₀ for LUVs incubated with increasing amounts of Chol-ON₁ (black) or ON₁-Chol (grey).
5.3. Size evolution of LUVs incubated with different concentrations of Chol-ON\textsubscript{1}:

The size of the LUVs was measured using a Malvern Zetasizer Nano ZS instrument. Each value is the average of 5 measurements. Concentration of POPC LUVs was 1 mM (in 20 mM MgCl\textsubscript{2} buffered at pH=8 with 1xTE) in all measurements.

An increase in the LUVs size is observed upon addition of increasing amounts of Chol-ON\textsubscript{1} (figure S8).

![Histograms showing the size distribution (diameter) of POPC LUVs incubated with different concentrations of Chol-ON\textsubscript{1} (from 0 to 1 µM).](image)

**Figure S8.** Histograms showing the size distribution (diameter) of POPC LUVs incubated with different concentrations of Chol-ON\textsubscript{1} (from 0 to 1 µM).
6. AZO-ON$_1$ isomerisation

The reversible isomerisation of AZO-ON$_1$ was studied by UV-Vis spectrophotometry. 100 μL of AZO-ON$_1$ (10 μM) in 20 mM MgCl$_2$ buffered at pH=8 with 1xTE was contained in a low volume quartz cuvette. An initial UV absorbance measurement was taken between 200-600 nm at 25°C. The sample was removed from the spectrometer under darkness and illuminated for 5 seconds at 365 nm (M365L2 Mounted LED, 700 mA, 190 mW, Thor Labs) before taking another measurement. The drop in absorbance at 330 nm is indicative of the trans to cis isomerisation (Figure S9a). For the reverse isomerisation experiments, the same process was used but with illumination at 420 nm (M420L3 Mounted LED, 100 mA, 750 mW, Thor Labs). An increase in the absorbance at 330 nm indicated cis to trans isomerisation (Figure S9b).

![Figure S9. Absorption spectra of AZO-ON$_1$ at different irradiation times at either 365 nm (a) or 420 nm (b).](image-url)
7. AZO-ON₁ interaction with lipids studied by confocal microscopy

We investigated the ability of AZO-ON₁ to interact with lipids by assessing their attachment to giant unilamellar vesicles (GUVs) using fluorescent confocal imaging. AZO-ON₁ was fluorescently labelled by hybridisation with its complementary ON carrying a Cy3 dye at its 5’ end (5’cy3-
ACTGTCGAACATTTTTTGCCATAATATTTT3’). POPC GUVs were prepared in 0.2 M sucrose via electroformation using the Vesicle Prep Pro unit (Nanion technologies) and a protocol previously described.[4] Fluorescent imaging was carried out on a Confocal Leica TCS SP5 microscope. Samples were excited at 514 nm. Images were processed using ImageJ. The fluorescence of the GUVs indicates that AZO-ON₁ is able to interact with GUVs (figure S10a, c). On the contrary, incubation of GUVs with ds cy3 NA-ON₁ (with no hydrophobic anchor) did not yield any fluorescent GUVs, evidencing the lack of interaction (figure S10e). In all cases the final concentration of DNA was 2 µM in a solution containing 20 mM MgCl₂ (1xTE).

![Figure S10](image)

*Figure S10.* Confocal images showing POPC GUVs incubated with ds cy3 AZO-ON₁ (a, b, c, d) and ds cy3 NA-ON₁ (e, f). Fluorescent images in (a), (c) and (e). Bright field images in (b), (d) and (f).
8. LUVs assembly studies in AZO modified ONs

8.1. Self-complementary fragments of the investigated AZO-ONs

| Name       | Sequence and self-complementary fragment[a]                                      | ΔG(Kcal/mol)[b] |
|------------|----------------------------------------------------------------------------------|-----------------|
| AZO-ON1    | AZO-C_{12}-AAATATATTATGGCAAAAAATGTTCGACAGT                                       | -6.81           |
| AZO-ON2    | AZO-C_{12}-AAAGGCATCGTTGGAAAAAAATTTTCGGATCCA                                     | -8.06           |
| AZO-ON3    | AZO-C_{12}-AAAAAAACTCCCGGAGTCGGCTGCTGATCAA                                       | -12.28          |
| AZO-ON4    | AZO-C_{12}-AAAAAAAAACGCTAAGCCACCTTTAGATCCAA[c]                                   |                 |

Table S4. Name and sequences of the investigated AZO-ONs. [a] The self-complementary fragment according to the analysis tool provided by NUPACK (http://www.nupack.org/) is shown in red. The introduced parameters for the analysis were: 20 mM Mg$^{2+}$, 50 mM Na$^+$, T= 25°C and 2 µM DNA concentration. [b] ΔG of the self-complementary fragment according to NUPACK. The introduced parameters for the analysis were: 20 mM Mg$^{2+}$, 50 mM Na$^+$, 25°C and 2 µM DNA concentration (considering only the self-complementary fragment). [c] No self-complementary fragment is identified by NUPACK using the previous parameters (20mM Mg$^{2+}$, 50 mM Na$^+$, 25°C and 2 µM DNA concentration).
8.2. OD650 of LUVs incubated with AZO-ON$_3$, AZO-ON$_2$, AZO-ON$_1$ and AZO-ON$_4$ at different DNA concentrations

The concentration of ON required to start LUVs assembly is lower for AZO-ON$_3$ than for the other studied AZO-ONs since it presents the strongest self-complementary fragment of the series (figure S11 blue data). AZO-ON$_4$ presents the weakest self-complementary fragment and hence the concentration required to start LUVs assembly is higher (figure S11 pink data). AZO-ON$_2$ and AZO-ON$_1$ present an intermediate behaviour as the stability of their self-complementary fragment is smaller than AZO-ON$_3$ but higher than AZO-ON$_4$ (figure S11 red and black data).

Figure S11. Evolution of OD650 for LUVs after the addition of increasing amounts of trans-AZO-ON$_1$ (black data), trans-AZO-ON$_2$ (red data), trans-AZO-ON$_3$ (blue data) or trans-AZO-ON$_4$ (pink data).
8.3. OD650 evolution with time of LUVs incubated with AZO-ON$_2$, AZO-ON$_3$, and AZO-ON$_4$ (trans and cis isomers) at the same DNA concentration (2 µM).

In all ONs, the cis isomers produce lower OD650 values and hence less LUVs assembly than the trans isomers. cis AZO-ON$_2$ and cis AZO-ON$_4$ show no LUVs assembly but some aggregation is observed in the case of cis AZO-ON$_3$ due to the stronger self-complementary fragment present in this ON. On the other hand, trans-AZO-ON$_4$ is not able to induce LUVs assembly at this DNA concentration as already described in section 8.2. Hence, in order to get a reversible light-driven LUVs assembly, it is important to have an ON with an intermediate stable self-complementary domain like AZO-ON$_1$ or AZO-ON$_2$.

Figure S12. Evolution of OD650 with time for LUVs after the addition of (a) AZO-ON$_4$, (b) AZO-ON$_2$, and (c) AZO-ON$_2$ in its trans (orange) or cis (blue) isomers at 2 µM DNA concentration.
9. Light-induced reversible assembly of LUVs observed by confocal microscopy

LUVs (1 mM) in 20 mM MgCl₂ buffered at pH=8 with 1xTE were mixed with \textit{trans} AZO-ON₁ (2 µM) and SYTO® 9 (2 µM). The samples were placed onto a glass slide sealed on the sides with a silicon chamber (sigma Aldrich) for imaging. Imaging was performed using a Confocal Leica TCS SP5 microscope. The sample was excited at 488 nm. Images were processed using ImageJ.

Isomerisation was performed by irradiating for 5 minutes the sample (illuminating directly above the glass slide) with LEDs with the appropriate wavelength. Isomerization \textit{trans-cis} was achieved by irradiation at 365 nm (M365L2 Mounted LED, 700 mA, 190 mW, Thor Labs) whereas \textit{cis-trans} was performed by irradiation at 420 nm (M420L3 Mounted LED, 100 mA, 750 mW, Thor Labs).

In Figure 2f the first two cycles are shown. In Figure S13 5 cycles of irradiation at 365 nm (blue arrows) and 420 nm (orange arrows) are presented.

\textbf{Figure S13.} Images obtained by confocal microscope revealing the reversible assembly of LUVs in the presence of AZO-ON₁.
10. LUVs assembly studies in ds DNA derivatives

10.1. OD650 of LUVs incubated with NA-ON1 at different DNA concentrations

OD650 of LUVs (1 mM) incubated with different concentrations of ds NA-ON1 was measured. As shown in Figure S14, OD650 value remains constant which indicates the lack of LUVs assembly when using ON with no hydrophobic anchor.

![Figure S14](image1.png)

**Figure S14.** OD650 of LUVs incubated with different concentrations of ds NA-ON1.

10.2. OD650 evolution with time of LUVs incubated with ds trans AZO-ON1 and with ds trans AZO-ON1 having a single base mismatch at the distal end.

![Figure S15](image2.png)

**Figure S15.** OD650 evolution with time of LUVs incubated with 1 µM of ds trans AZO-ON1 with (a) fully matched complementary sequence and (b), (c), (d) single mismatch at the side opposite the AZO modification.
11. Effect of temperature on LUVs (with no DNA)

11.1. Apparent absorbance at 260nm vs temperature of LUVs

The evolution of the absorbance at 260 nm vs temperature was recorded in samples containing LUVs (1 mM concentration of lipids in 20 mM MgCl₂ buffered at pH=8 with 1xTE). 5 heating-cooling cycles were recorded. Figure S16 shows the curves for the heating processes.

Note that although no sharp drop in app abs is detected a gradual decrease can be observed. The decrease in app abs of lipid vesicles has been previously reported by others.⁶ Lipid vesicles scatter less light when the temperature is increased and this is due to variation of refractive index, especially if not in the range of temperatures of the phase transition of the lipid (as in our specific case). The absorbance decrease with increasing temperature is therefore not due to vesicles aggregation that, in any case, would cause an increase of absorbance (unless serious precipitation starts to take place). To discard any possible ambiguity we have performed DLS measurements of the LUVs at different temperatures to prove that they do not aggregate or disaggregate with temperature (see Figure S17).

![Figure S16](image)

**Figure S16.** Apparent absorbance (260 nm) vs temperature (°C) of 5 subsequent heating cycles of samples containing LUVs (1 mM). In this measurement the heating rate was 5°C min⁻¹ and the cooling rate was 10°C min⁻¹.
11.2. DLS measurements on LUVs at different temperatures

DLS measurements were performed at 25°C, 50°C and 80°C on LUVs (1 mM POPC in 20 mM MgCl₂ buffered at pH=8 with 1xTE). As shown in Figure S17, increasing the temperature in LUVs does not produce any self-aggregation or disassembly.

*Figure S17.* Histograms showing the size distribution (diameter) of POPC LUVs at different temperatures (25°C, 55°C and 80°C).
12. DNA melting measurements

Evolution of the absorbance at 260 nm with temperature was measured by UV spectrophotometry. All measurements performed with LUVs were done at 1 mM concentration of lipids in 20 mM MgCl₂ buffered at pH=8 with 1xTE.

12.1. DNA melting measurements for ds trans AZO-ON₁ at different DNA concentrations in the presence of LUVs

The evolution of the apparent absorbance at 260 nm vs temperature was recorded in samples containing LUVs and ds trans AZO-ON₁ at different DNA concentrations. Up to 5 heating-cooling cycles were recorded. Figure S18 collects the curves for the heating processes that appear in different shades of blue (darker to lighter from 1<sup>st</sup> to 5<sup>th</sup> cycles).

![Graphs showing DNA melting measurements](image)

**Figure S18.** Apparent absorbance (260 nm) vs temperature (°C) of the subsequent heating cycles of samples containing LUVs (1 mM) and different concentrations of trans AZO ON₁. In these measurements the heating rate was 5°C min⁻¹ and the cooling rate was 10°C min⁻¹.
**12.2. DNA melting measurements for different ds AZO-ONs in the presence and absence of LUVs**

The melting temperature of different ds AZO-ONs (0.75 µM) in the presence or absence of LUVs (1 mM) was investigated by measuring the absorbance (260 nm) vs temperature of the mixtures. As shown in table S6, the melting temperatures obtained in the presence or absence of LUVs are very similar. This indicates that the LUVs assembly-disassembly process occurs at the melting temperature of the investigated ds DNA.

|                | Sequence of the two complementary strands forming the ds AZO-ONs | WITH NO LUVs | WITH LUVs |
|----------------|------------------------------------------------------------------|--------------|-----------|
| AZO-ON<sub>1</sub> | AZO-C<sub>12</sub>-AAATATATATGCGCCAAAAATGTTCGACAGTTCTGTCGAACTTTTTGGCCATAATAATATT | 75.3±0.3     | 76.0±0.7  |
| AZO-ON<sub>2</sub> | AZO-C<sub>12</sub>-AAAGGGATCGGAGGAAAAATTTCTGCGATCCAAGATCAGTTCCTTTTTTTTTT | 80.9±0.4     | 81.7±0.9  |
| AZO-ON<sub>3</sub> | AZO-C<sub>12</sub>-AAAGAACGCCGAGTCCGCTGCTGATCAAACCTCCGAGGACTCCCGAGTTCCTTTTTTT | 83.8±1.5     | 85.4±2.3  |
| AZO-ON<sub>4</sub> | AZO-C<sub>12</sub>-AAAGAACGCCGAGTCCGCTGCTGATCAAACCTCCGAGGACTCCCGAGTTCCTTTTTTT | 79.1±0.6     | 81.8±1.0  |

*Table S5.* Melting temperatures (°C) of different sequences of ds trans AZO-ONs (0.75 µM) in the absence and in the presence of LUVs (1 mM POPC, in 20 mM MgCl<sub>2</sub> buffered at pH=8 with 1xTE). The sequences of the complementary strands are shown in red. The reported values correspond to the average of three different experiments. Melting temperature on each experiment was calculated by averaging the melting temperature of 5 subsequent heating processes. In these measurements the heating rate was 5°C min<sup>-1</sup> and the cooling rate was 10°C min<sup>-1</sup>.
12.3. DNA melting measurements for ds AZO-ONs having different lengths in the ds fragment.

AZO-ON₁ was hybridised with complementary strands of different lengths (22, 14 and 11 NTS) (Table S7) and then incubated with LUVs to induce their assembly. The thermal disassembly process was measured by recording the evolution of the apparent absorbance at 260nm with temperature. Disassembly temperatures (first cooling ramp) of LUVs are reported in table S6. These temperatures match the melting temperature of the used dsDNA fragment, which evidences that the ds fragment is the main responsible for the assembly process. Importantly the difference in apparent absorbance given by LUVs disassembly is larger in amplitude than the observed for the normal melting curve of DNA (see Figure S19) which represents an advantage for sensing applications.

| Name | Sequence of the strands complementary to AZO-ON₁ (AZO-C₁₂-AAATATATTATGGCAAAAAATGTTCGACAGT) | [DNA] | WITH NO LUVs | WITH LUVs |
|------|-----------------------------------------------------------------|------|--------------|-----------|
| 22NTS | AZO-C₁₂-AAATATATTATGGCAAAAAATGTTCGACAGT CATTTTTTGCCATAATATTT | 1 µM | 60.3±3.2 | 61.0±1.2 |
| 14NTS | AZO-C₁₂-AAATATATTATGGCAAAAAATGTTCGACAGT GCCATAATATATTT | 2.5µM | 50.5±3.9 | 51.2±3.2 |
| 11NTS | AZO-C₁₂-AAATATATTATGGCAAAAAATGTTCGACAGT ATAAATATATTT | 3.5 µM | 33.1±3.6 | 38.0±5.3 |
| 11TNS B | AZO-C₁₂-AAATATATTATGGCAAAAAATGTTCGACAGT ACTGTGAAACA | 3.5 µM | 54.0±5.0 | 55.9±3.2 |

**Table S6.** Melting temperatures (°C) of the different dsDNAs formed by hybridisation of the trans AZO-ON₁ with complementary strands of different lengths in the absence and in the presence of LUVs (1 mM POPC, in 20 mM MgCl₂ buffered at pH=8 with 1xTE). The hybridised part in AZO-ON₁ is highlighted in red. The reported experimental values correspond to the derivative of the first heating scan fitted using a Gaussian curve. The given error is the sigma value obtained by this Gaussian fit. In these measurements the heating/cooling rate was 1°C min⁻¹.
**Figure S19.** Apparent absorbance (260nm) vs temperature (°C) of the first heating cycle of samples composed by ds DNAs resulting from the hybridisation of trans AZO-ON1 with complementary strands of different lengths in the absence (red curves) and in the presence (black curves) of LUVs (1 mM POPC, in 20 mM MgCl₂ buffered at pH=8 with 1xTE). The used concentrations are gathered in table S6. In these measurements the heating/cooling rate was 1°C min⁻¹.
12.4. DNA melting measurements for mutated ds AZO-ONs in the presence and absence of LUVs

LUVs were assembled by incubation with *trans* AZO-ON₁ hybridised with short complementary strands having no mutations or a single base mismatched (mutated strands). In order to show the versatility of our system we tried two different types of complementary strands: 14NTS strands (non-mutated and mutated) that are complementary to the fragment of the AZO-ON₁ close to its 5’ position and 13NTS strands (non-mutated and mutated) that are complementary to the fragment of the AZO-ON₁ close to its 3’ position. We then investigated the change of apparent absorbance at 260 nm with temperature by heating and subsequent cooling the sample at 1°C min⁻¹. Temperatures of LUVs disassembly (corresponding to the first heating ramp) are reported in tables S7 and S8. Importantly, the difference in apparent absorbance given by LUVs disassembly is larger in amplitude than the observed for the normal melting curve of DNA (see Figure S20 and S21) which represents an advantage for sensing applications.

12.4.1. 14NTS strands

| Name       | Sequence of the 14 NTS strands complementary to AZO-ON₁ (AZO-C₁₂-AAATATATTATGGCAAAAAATGTCGACAGT) | WITH NO LUVs | WITH LUVs |
|------------|------------------------------------------------------------------------------------------------|-------------|----------|
| Non Mutated | GCCATAATATATT                                                                 | 50.5±3.9    | 51.2±3.2 |
| Mut 1      | GCCATACTATATT                                                                 | 37.1±4.4    | 39.7±5.4 |
| Mut 2      | GCCATATTATATT                                                                 | 40.4±3.8    | 40.4±5.0 |
| Mut 3      | GCCATACTATATT                                                                 | 42.8±4.6    | 42.6±4.5 |

*Table S7.* Melting temperatures (°C) of the *ds* DNAs composed by the hybridisation of *trans* AZO-ON₁ with the non mutated and mutated (single mutation) strand of 14NTS in length in the absence and in the presence of LUVs (1 mM POPC, in 20 mM MgCl₂ buffered at pH=8 with 1xTE). The hybridised part in *trans* AZO-ON₁ is shown in red. The mutated bases are shown in blue. All measurements were done using a DNA concentration of 2.5 µM. The reported experimental values correspond to the derivative of the first heating scan fitted using a Gaussian curve. The given error is the sigma value obtained by this Gaussian fit. In these measurements the heating/cooling rate was 1°C min⁻¹.
Figure S20. Apparent absorbance (260 nm) vs temperature (°C) of the first heating cycle of samples composed by ds DNAs resulting from the hybridisation of trans AZO-ON$_1$ with the non-mutated and mutated (single mutation marked in red) strands of 14NTS shown in table S7 in the absence (red curves) and in the presence (black curves) of LUVs (1 mM POPC, in 20 mM MgCl$_2$ buffered at pH=8 with 1xTE). All measurements were done using a DNA concentration of 2.5 µM. In these measurements the heating/cooling rate was 1°C min$^{-1}$. 
12.4.2. 13NTS strands

| Name       | Sequence of the 13 NTS long strand complementary to AZO-ON1 (AZO-C_{12}AAATATATTATGGCAAAAATGTTCCGACAGT) | WITH NO LUVs | WITH LUVs |
|------------|--------------------------------------------------------------------------------------------------|--------------|-----------|
| Non Mutated | ACTGTCGAAACATT                                                                                   | 55.8±4.5     | 58.8±2.4  |
| Mut 1      | ACTGTCGAAACATT                                                                                   | 36.7±8.0     | 36.6±7.0  |
| Mut 2      | ACTGTTCGAAACATT                                                                                   | 35.2±10.5    | 36.3±7.3  |
| Mut 3      | ACTGTCGAAACATT                                                                                   | 35.3±17.4    | 36.1±7.2  |

Table S8. Melting temperatures (°C) of the ds DNAs composed by the hybridisation of trans AZO-ON1 with the non mutated and mutated (single mutation) strand of 13NTS in length in the absence and in the presence of LUVs (1 mM POPC, in 20 mM MgCl₂ buffered at pH=8 with 1xTE). The hybridised part in trans AZO-ON1 is shown in red. The mutated bases are shown in blue. All measurements were done using a DNA concentration of 2.5 µM. The reported values correspond to the maximum of the Gaussian fit performed to the derivative of the first heating scan (graphs in figure S21). The given error is the sigma value obtained by this Gaussian fit. In these measurements the heating/cooling rate was 1°C min⁻¹.
Figure S21. *Apparent* absorbance (260 nm) vs temperature (°C) of the first heating cycle of samples composed by *ds* DNAs resulting from the hybridisation of *trans* AZO-ON$_1$ with the non-mutated and mutated (single mutation marked in red) strands of 13NTS shown in table S8 in the absence (red curves) and in the presence (black curves) of LUVs (1 mM POPC, in 20 mM MgCl$_2$ buffered at pH=8 with 1xTE). All measurements were done using a DNA concentration of 2.5 µM. In these measurements the heating/cooling rate was 1°C min$^{-1}$. 
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