

Supporting Information

for

**Supramolecular Micelle-Based Nucleoapzymes for the Catalytic Oxidation of  
Dopamine to Aminochrome**

*by*

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## Materials

All chemicals and reagents were purchased from commercial suppliers and were used without further purification, unless otherwise noted. The 1-dodecyne, copper(I)iodide, tetrakis(triphenylphosphine)palladium(0), and diisopropylamine were purchased from Sigma-Aldrich and used as received; 5'-DMT-5-iodo deoxy uridine was obtained from Chemgenes. All lipidated oligonucleotides (ODNs) were synthesized using standard automated solid-phase phosphoramidite coupling methods on an ÄKTA oligopilot plus (GE Healthcare) DNA synthesizer. All solvents and reagents for oligonucleotide synthesis were purchased from Novabiochem (Merck, UK) and SAFC (Sigma-Aldrich, Netherlands). Solid supports (Primer Support™, 40 µmol/g) from GE Healthcare were used for the synthesis of DNA. The oligonucleotides were characterized by MALDI-TOF mass spectrometry using a 3-hydroxypicolinic acid matrix. Spectra were recorded on an ABI Voyager DE-PRO MALDI TOF (delayed extraction reflector) Biospectrometry Workstation mass spectrometer. The concentrations of the DNA were measured on a SpectraMax M2 spectrophotometer (Molecular Devices, USA) using 1 cm light-path quartz cuvette. Fluorescently labeled and unmodified oligonucleotides were purchased from Biomers.net in HPLC purification grade. <sup>1</sup>H-NMR and <sup>31</sup>P-NMR spectra were recorded on a Varian Mercury (400 MHz) NMR spectrometer at 25 °C. High-resolution mass spectra (HRMS) were recorded on an AEI MS-902 (EI<sup>+</sup>) instrument. Column chromatography was performed using silica gel 60 Å (200–400 Mesh).

## Sequences

The following sequences were prepared manually, using a C<sub>12</sub>-lipid modified 2'-deoxyuridine (T\*) residue as lipid anchor. In the lipoGQ sequences, the lipidated nucleotides were separated from the G-quadruplex by means of a spacer consisting of two thymine (T) units. The sequences were purified using RPC HPLC (column: Jupiter C4, 5 µm column, 4.6× 250 mm, Phenomenex. Gradient: 0–75% in 30 min from buffer A to B; buffer A: 100 mM Et<sub>3</sub>NH•OAc (pH 7.5) in ultrapure water and 5% MeCN, buffer B: 100% *iso*-propanol).

Bis-lipidated PS2.M (<sup>2</sup>lipoGQ,1):

5'-T\*T\*TTGGGTAGGGCGGGTTGGG

Tetra-lipidated PS2.M (<sup>4</sup>lipoGQ,2):

5'-T\*T\*TTGGGTAGGGCGGGTTGGGTTU\*U\*

Tetra-lipidated native DBA (lipoDBA,3):

5'-GTCTCTGTGTGC **GC**CAGAGACACTGTT\*T\*T\*T\*CAGATATGGGCC**AG**GCACAGAAT**GA**GGCCC

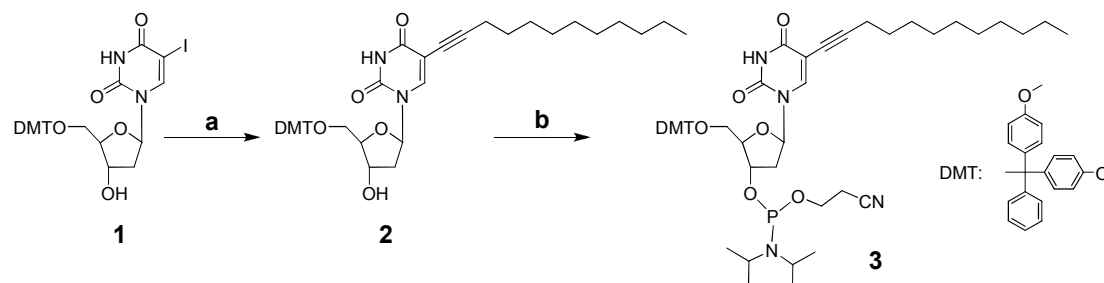
Tetra-lipidated mutated DBA (lipoDBA<sup>m</sup>, 4):

5'-GTCTCTGTGTGC **TT**CAGAGACACTGTT\*T\*T\*T\*CAGATATGGGCC**T**GCACAGAAT**TT**GGCCC

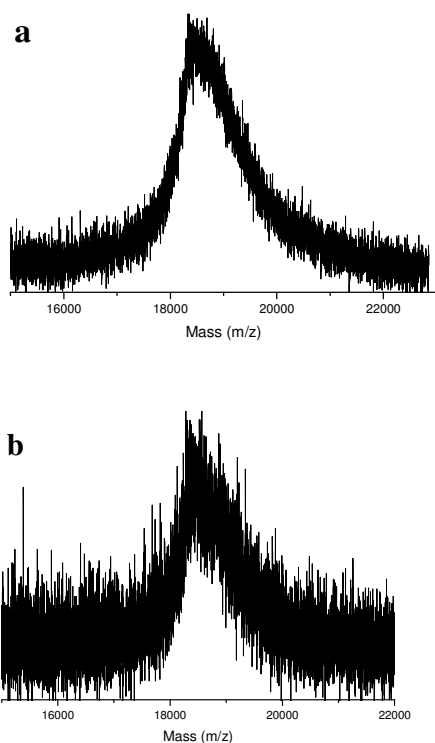
## Synthesis and characterization of the lipoDNA sequences

The modified 5-(dodec-1-ynyl)uracilphosphoramidite **3** was synthesized in two steps as previously reported in our group starting from **1** (Fig. S1).<sup>1</sup> The modified uracil phosphoramidite was dissolved in CH<sub>3</sub>CN to adjust the concentration to 0.15 M, in the presence of 3 Å molecular sieves. The prepared solution was directly connected to the DNA synthesizer. All oligonucleotides were synthesized in 10 µmol scale on a DNA synthesizer using standard β-cyanoethylphosphoramidite coupling chemistry. Deprotection and cleavage from the PS support was carried out by incubation in concentrated aqueous ammonium hydroxide solution for 5 h at 55 °C. Following deprotection, the oligonucleotides were purified by reverse-phase chromatography, using a C15 RESOURCE RPCTM 1 mL reverse phase column (GE Healthcare) through a custom gradient elution (A: 100 mM triethylammonium acetate (TEAAc)

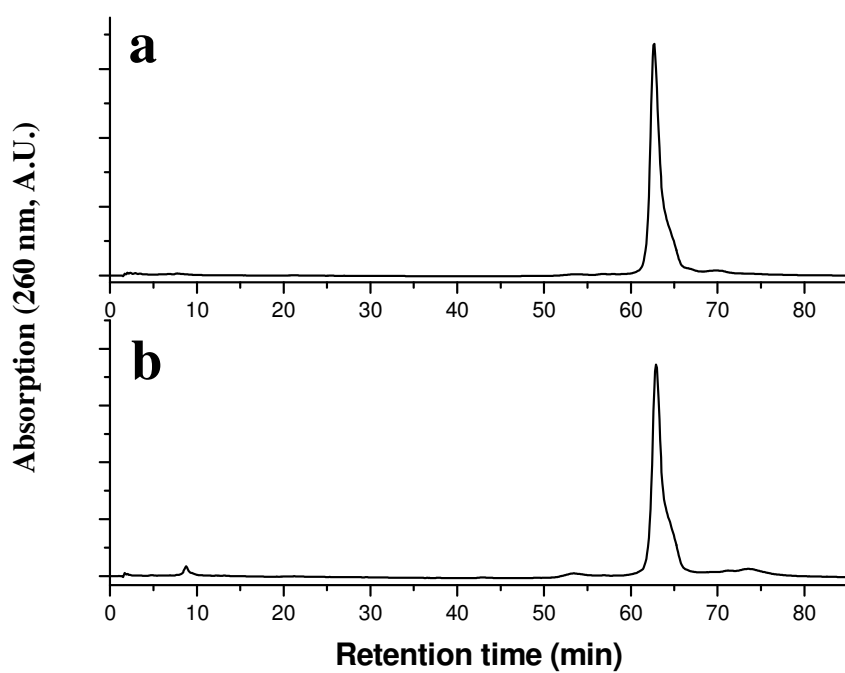
and 2.5% acetonitrile, B: 100 mMTEAAc and 65% acetonitrile). Fractions were desalted using centrifugal dialysis membranes (MWCO 3000, Sartorius Stedim). Oligonucleotide concentrations were determined by UV absorbance using extinction coefficients. Finally, the identity and purity of the oligonucleotides was confirmed by MALDI-TOF mass spectrometry and analytical anion exchange chromatography using a linear gradient elution, respectively (Fig. S2 and Fig.S3).



**Fig.S1.** Synthesis of 5-(dodec-1-ynyl) uracil phosphoramidite (T\*).



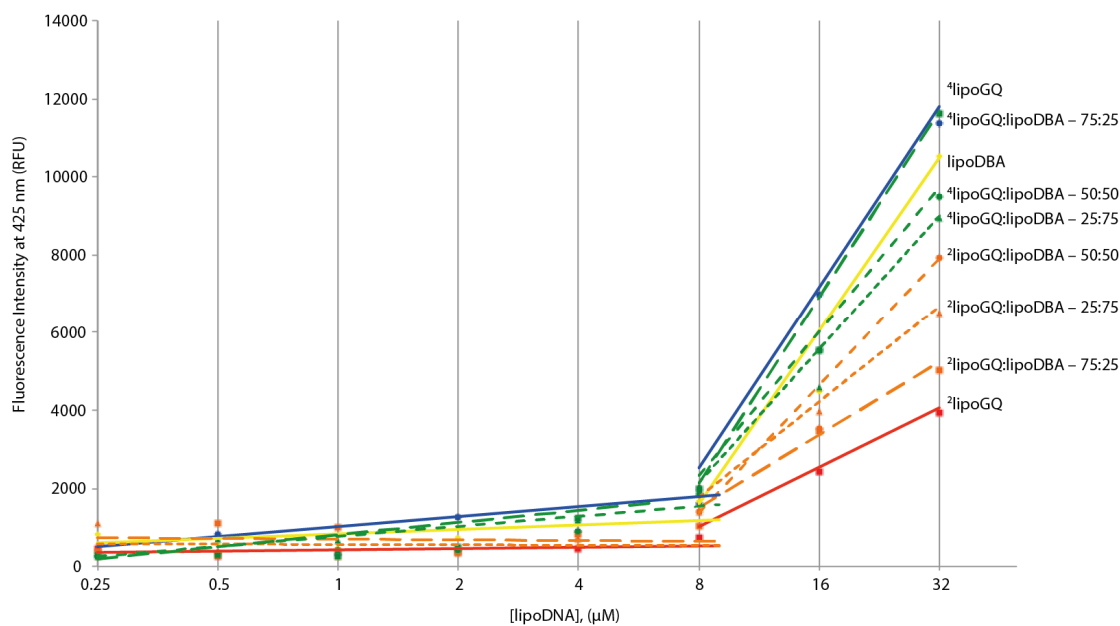
**Fig.S2.** MALDI-TOF spectra of: **(a)** lipoDBA (**3**) (calc. 18444 g/mol), and **(b)** lipoDBA<sup>m</sup> (**4**) (calc. 18391 g/mol).



**Fig.S3.** RP-HPLC chromatograms of: (a) lipoDBA (**3**), and (b) lipoDBA<sup>m</sup> (**4**). A linear gradient to 100 %B in 62.5 mL was used.

### Critical Micelle Concentration (CMC) Determination

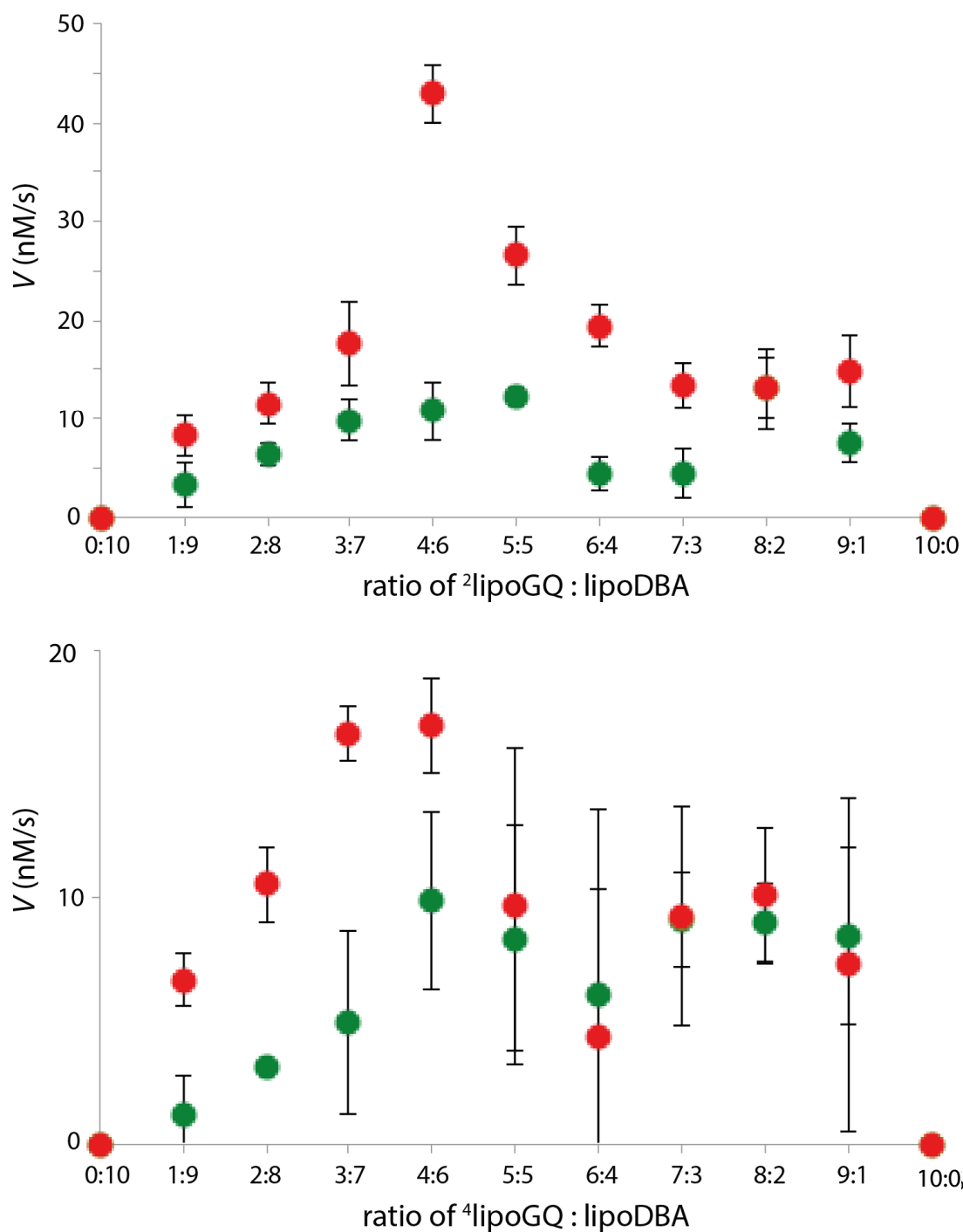
From a 1  $\mu\text{M}$  solution of 1,6-diphenyl-1,3,5-hexatriene (DPH) in acetone, 10  $\mu\text{L}$  (10 pmol) was placed in a black 96-well plate. The solvent was allowed to evaporate overnight. Meanwhile, the solutions containing various ratios of  $^2\text{lipoGQ}$  or  $^4\text{lipoGQ}$  and lipoDBA were prepared. The following ratios of GQ and DBA were used: 25:75, 50:50, 75:25 (% n/n), with the following concentrations: 0.25, 0.5, 1, 2, 4, 8, 16, 32  $\mu\text{M}$ . The solutions containing these ratios and concentrations of lipoDNA were thermally cycled (90  $^\circ\text{C}$ , 30 min,  $-1$   $^\circ\text{C}/2$  min until RT) to ensure proper micellization, and 100  $\mu\text{L}$  of each of the solutions was added to different wells containing dried DPH. The plate was incubated overnight, and the fluorescence spectra (375–500 nm,  $\lambda_{\text{ex}} = 350$  nm) were recorded in a microtiterplate reader (monochromator). The fluorescence maximum is plotted for each mixture and concentration; from this plot, another plot is prepared containing the concentration and fluorescence intensity at 425 nm. The concentration of lipoDNA at the intersection of the low fluorescence region with the high fluorescence region corresponds to the CMC. For all systems, a CMC of approximately 8  $\mu\text{M}$  was found; this was not significantly affected by the different ratios of GQ and DBA. Therefore, all catalytic studies were performed using 10  $\mu\text{M}$  of lipoDNA mixtures.



**Fig. S4.** CMC determination of the lipoDNA mixtures.

### **Oxidation of dopamine (1) to aminochrome (2) by means of lipoDNA micelles**

Micelles with the appropriate ratios of the two different lipidated DNA sequences were prepared as follows. Stock solutions of lipoDNA (100  $\mu\text{M}$ ) were prepared in the MES buffer (pH 5.5, 200 mM KCl, 2 mM  $\text{MgCl}_2$ ). From these stock solutions, a total of 10  $\mu\text{L}$  of lipoDNA from the two stock solutions was added to 69  $\mu\text{L}$  of the buffer; the total amount of lipoDNA was composed of the lipoGQ and lipoDBA sequences in order to reach the desired ratios. The resulting solution with 12.7  $\mu\text{M}$  lipoDNA was annealed as described above. Then, 1  $\mu\text{L}$  of a solution of hemin in DMSO was added (stock concentrations: 200, 400, 600, 800  $\mu\text{M}$  for the experiments of Fig. 2, and 100, 200, 300, 400, 500, 600, 700, 800, 900  $\mu\text{M}$  or 1 mM for the experiments of Fig. 3), formation of the hGQ unit was allowed to proceed for 1 hr. Correct formation of the hGQ DNAzyme unit was inferred from the presence of the Soret-band at 405 nm. Saturation kinetics curves were determined using micelles composed of lipoGQ:lipoDBA – 2:8, 4:6, 6:4, and 8:2 (% n/n). For this, to the solution of hemin/lipoDNA (80  $\mu\text{L}$ , 12.7  $\mu\text{M}$ ) was added dopamine (10  $\mu\text{L}$  of dopamine stock-solutions: 0.2, 0.4, 0.8, 1.5, 2.5, 5 mM). For determination of the optimal ratio of lipoGQ and lipoDBA, 10  $\mu\text{L}$  of dopamine (5 mM) was added. After this,  $\text{H}_2\text{O}_2$  (10  $\mu\text{L}$  from a stock-solution with a concentration of  $\text{H}_2\text{O}_2$  of 10 mM) was added, and formation of aminochrome (2) was determined by measuring the absorbance each well at 480 nm (values were corrected for baseline drifting by subtracting the absorbance at 800 nm; pathlength corrections were applied). For determination of the optimal ratio of lipoGQ and lipoDBA, the rates were determined at the saturation point, *i.e.* with 500  $\mu\text{M}$  dopamine (1).



**Fig. S5.** Rate of oxidation of dopamine to aminochrome in the presence of nucleopzyme micelles that were composed of different ratios of lipoGQ and lipoDBA. The red points show the rates obtained for the micelles that contained the native aptamer, i.e. lipoDBA (**3**); the green points correspond to the rates obtained for the micelles that contained the mutated aptamer, i.e. lipoDBAm (**4**).

## Reference

<sup>1</sup> M. Kwak, I. J. Minten, D.-M. Anaya, A. J. Musser, M. Brasch, R. J. M. Nolte, K. Müllen, J. J. L. M. Cornelissen, A. Herrmann, *J. Am. Chem. Soc.* **2010**, *132*, 7834.