

Supplementary Information

Phthalocyanine-DNA origami complexes with enhanced stability and optical properties

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1. Chemicals

All chemicals used were purchased from commercial sources and used as received without any further purification. The single-stranded scaffold derived from bacteriophage M13mp18 (7249 nucleotides long, type p7249) was obtained from Tilbit Nanosystems and the short single-stranded staple strands were bought from Integrated DNA Technologies. The 50× TAE Buffer (2 M tris(hydroxymethyl)aminomethane (Tris), 50 mM ethylenediaminetetraacetic acid (EDTA) and 1 M acetic acid for adjusting the pH to 8.0) was purchased from VWR Chemicals. Agarose was purchased from Thermo Fischer Scientific, 6× Gel Loading Dye Blue from New England Biolabs and heparin sodium salt (from porcine intestinal mucosa) DNase I (from bovine pancreas) DPBF and ethidium bromide from Sigma-Aldrich. The water used in all the experiment was MilliQ purified.

2. DNA origami (6HB)

2.1. Synthesis of DNA origami (6HB)

The design for the 6-helix bundle (6HB) DNA origami structure is adapted from Bui *et al.*¹ which also lists the 170 oligonucleotides staple strands needed. However, the quantum dots attachment strands in the design used in this study have been replaced with shorter staple strands to avoid overhangs in the structure.

The 6HB structure was folded as earlier described by Julin *et al.*² The 6HB was folded in a one-pot reaction by mixing the single stranded scaffold (type p7249) with 10 times excess of staple strands. The 6HB structure was folded using a folding buffer (FOB) consisting of 1× TAE (40 mM tris(hydroxymethyl)aminomethane (Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), and acetic acid for adjusting the pH to 8.0) and 12.5 mM MgCl₂. The amounts and final concentrations of the components are presented in Table S1. The DNA origami folding mixture was annealed in a G-storm G1 Thermal cycler using the following thermal folding ramp:

- The folding reaction is heated to 90 °C
- Annealed by cooling from 90 °C to 70 °C with a rate of 0.2 °C / 8 seconds
- Annealed by cooling from 70 °C to 60 °C with a rate of 0.1 °C / 8 seconds
- Annealed by cooling from 60 °C to 27 °C with a rate of 0.1 °C / 2 minutes
- Stored at 12 °C until the program is manually stopped

Table S1. The amounts and the concentrations (initial and final) of the components used for the folding of the 6HB structure.

COMPONENTS	VOLUME	C _{INITIAL}	C _{FINAL}
M13MP18 (TYPE P7249)	20 µL	100 nM	20 nM
STAPLE STRANDS	40 µL	500 nM	200 nM
FOLDING BUFFER	40 µL	2.5×	1×
TAE		2.5×	1×
MGCL ₂		31.25 mM	12.5 mM
TOTAL	100 µL		

2.2. Purification of DNA origami (6HB)

The folded DNA origami structures were purified by a poly(ethylene glycol) (PEG) purification method. The method was adapted from Stahl *et al.*³ Two folding reactions (*i.e.* 200 µL) were diluted in 600 µL of 1× FOB to obtain a starting volume of 800 µL. The solution was mixed thoroughly with 800 µL of PEG precipitation buffer (15 % PEG 8000 (w/v), 1× TAE and 505 mM NaCl), after which the mixture was centrifuged at 14000 rcf for 30 minutes at room temperature using an Eppendorf 5424R microcentrifuge. After the centrifugation, the supernatant was removed, and the DNA origami pellet was redissolved in

1× FOB. Depending on the required DNA concentration, the pellet was redissolved in different amount of 1× FOB to get final concentrations between 9 nM and 60 nM. The DNA origami solution was incubated at room temperature overnight to ensure complete dissolution of the pellet before refrigerated.

2.3. Concentration of DNA origami (6HB)

DNA origami concentration was measured *via* UV/Vis spectroscopy and determined using the Beer-Lambert law.

$$A_{260} = \epsilon C_{\text{DNA}} l$$

where A_{260} is the absorbance at 260 nm, ϵ is the molar extinction coefficient (for the 6HB structure $1.0 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$) and l is the path length through the solution in centimeters (0.05 cm). The absorbance was measured using a BioTek Eon Microplate spectrophotometer and a Take3™ micro-volume plate. A sample size of 2 μL was used for the measurements.

2.4. Agarose gel electrophoresis

Agarose gel electrophoresis was used to analyze the folded 6HB structures and to verify that the excess amounts of staple strands were removed during the PEG purification. A 2% (w/v) agarose gel was prepared by mixing 2 g of agarose into 90 mL of 1× TAE buffer and heated until a clear solution was obtained. After cooling it slightly, 10 mL of 110 mM MgCl_2 was added. The gel was stained with 80 μL of ethidium bromide (EtBr) solution (0.58 mg/mL) before being poured into the casting tray. For the DNA origami samples, 10 μL of DNA origami solution was used, whereas the scaffold reference sample was prepared by diluting 4 μL of the scaffold (type p7249, $c = 100 \text{ nM}$) in 6 μL of 1× FOB. The samples were mixed with 2 μL of Gel Loading Dye Blue (6×) and the whole sample of 12 μL was loaded into the gel pocket. The gel was run at a constant voltage of 90 V for 45 minutes using BioRad horizontal wide Mini-Sub GT electrophoresis system and a BioRad PowerPac™ Basic power supply. 1× TAE buffer containing 11 mM MgCl_2 was used as a running buffer and the gel electrophoresis chamber was kept on an ice bath for the run. The gel was visualized under UV light using a BioRad Gel Doc™ XR+ Documentation system.

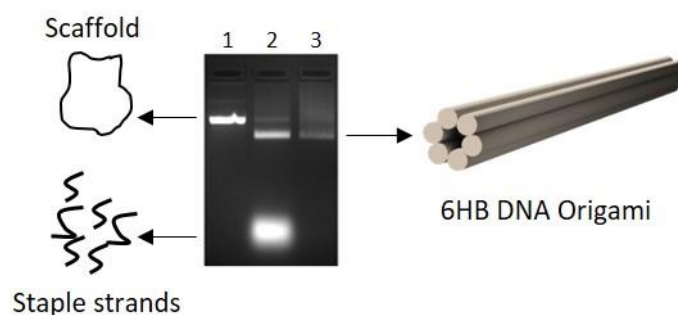


Figure S1. Qualitative analysis of 6HB using agarose gel electrophoresis. **1)** Scaffold (type p7249). **2)** Unpurified, but folded 6HB. **3)** PEG-purified 6HB.

2.5. Transmission electron microscopy (TEM)

The folded 6HB structures were imaged using transmission electron microscopy (TEM). The images were taken using a FEI Tecnai 12 Bio-Twin instrument operated at an acceleration voltage of 120 kV. Further, the images were processed using Gatan Digital Micrograph software.

The sample was negatively stained with uranyl formate (2 % (w/v)) containing 25 mM NaOH according to the method described by Castro *et al.*⁴ The sample was prepared by diluting the 6HB solution in 1× FOB to a final concentration of 3.75 nM. The TEM sample was prepared using plasma cleaned (20 seconds oxygen plasma flash using a Gatan Solarus) Formvar carbon coated copper grids (400 mesh, Electron Microscopy Sciences). 3 μL of the sample solution was applied on the carbon-coated side of the grid for 3-3.5 minutes. After the incubation, the excess solution was drained from the edge using filter paper.

First, the grid was immersed into a 5 μL droplet of uranyl formate solution, and excess liquid was directly drained off from the edge using filter paper, followed by a subsequent immersion into a 20 μL droplet of uranyl formate solution. The excess amount of stain was removed after 45 seconds from the edge of the grid using filter paper. After this process, the grid was left at room temperature for at least 30 minutes before imaging.

3. ZnPc

The synthesis of ZnPc (**1**) has been adapted from Li *et al.*⁵

4. Formation of 1-6HB complexes

4.1. Optical microscopy

Optical microscope images of the 1-6HB complexes were taken with a Leica DM4500 microscope equipped with cross-polarizers. The sample was prepared using a 3.7 nM 6HB solution to which ZnPc **1** (final concentration of 37.0 μM) was added at a 1-6HB ratio of 10 000. The formed complex was incubated for 20 minutes at room temperature after which they were refrigerated overnight. The following day the samples were imaged. 5 μL of the sample was deposited on a glass slide and covered with a cover slide stick with double-sided tape in order to preserve moisture and the structural features of the sample.

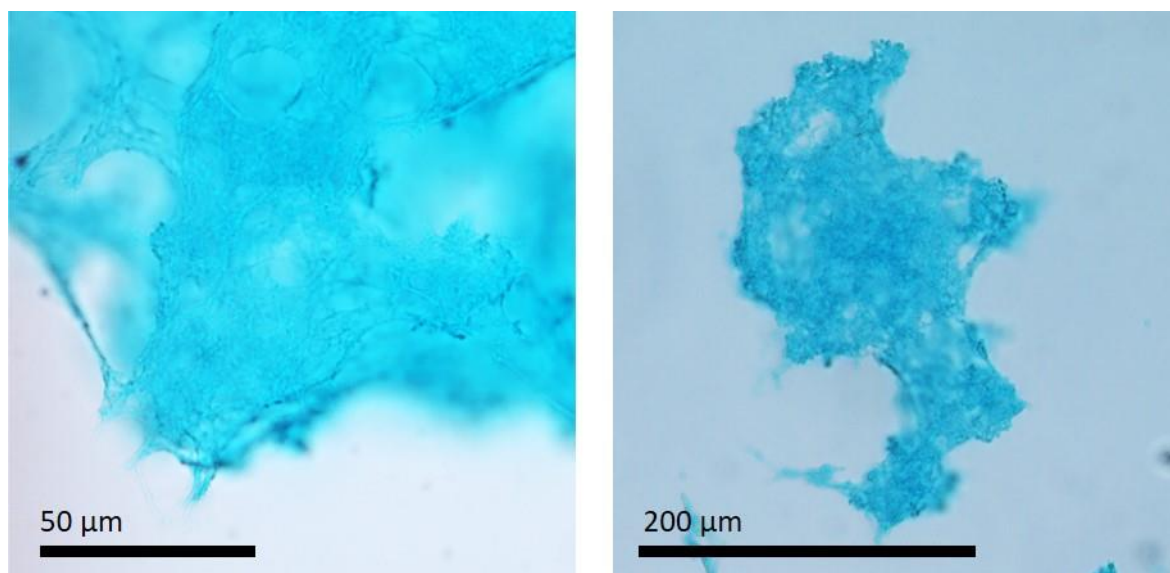


Figure S2. Optical microscope images of 1/6HB ([6HB] = 3.7 nM, [**1**] = 37 μM). As can be seen from the images, assembly of the 6HB structure and **1** yielded large blue mesh-like structures.

4.2. Electrophoretic mobility shift assay (EMSA)

The 1-6HB complexes were prepared by mixing the 6HB solution (final concentration of 4.93 nM) with increasing amounts of **1**, rendering 1/6HB ratios from 250 to 15 000. The mixtures were incubated at room temperature for 20 minutes. The total sample volume was 25 μL and 5 μL of gel loading dye (6 \times) was added before loading 28 μL of the total sample into the gel pockets. The agarose gel was prepared, ran and visualized using the protocol described in Section 2.4.

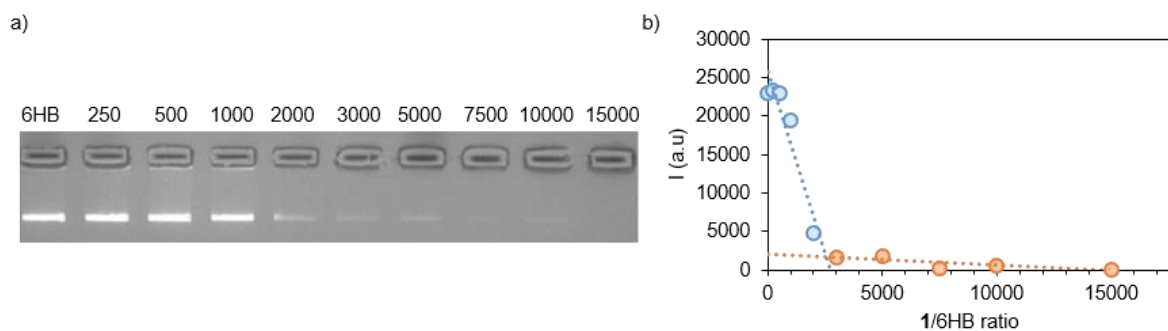


Figure S3. a) Agarose gel EMSA of 6HB (4 nM) titrated with increasing molar equivalents of **1**. b) Agarose gel EMSA intensities for different 1/6HB ratios. The gel intensity corresponding to the 1/6HB were linearly fitted to two series (First series: 0–2000, second series: 3000–15000), showing convergence at approximately 2 500 eq.

4.3. TEM

The images were taken using a FEI Tecnai 12 Bio-Twin instrument operated at an acceleration voltage of 120 kV. Further, the images were processed using Gatan Digital Micrograph software.

The sample was prepared by mixing the 6HB solution (final concentration of 1 nM) and the **1** (final concentration of 10 μ M) to obtain a 1/6HB ratio of 10 000, after which the sample was incubated at room temperature for 20 minutes. After the incubation, the TEM grids were prepared as described in Section 2.5.

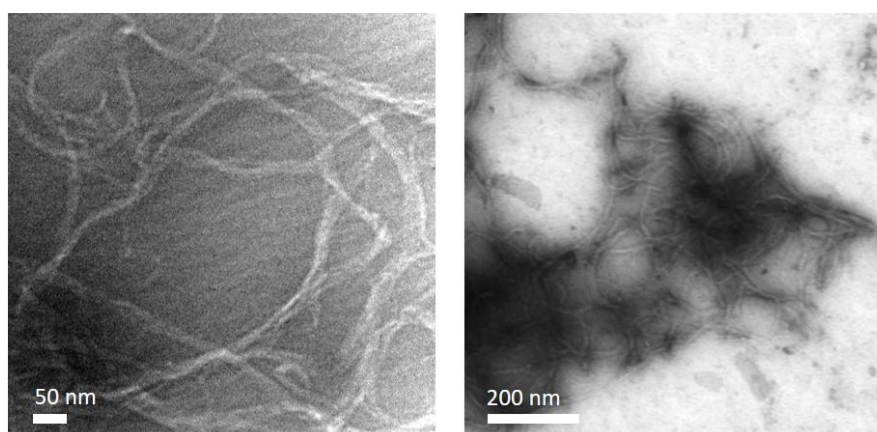


Figure S4. Additional TEM images of the ZnPc **1**-6HB complexes at a ratio of 10 000 ([6HB] = 1 nM, [**1**] = 10 μ M). As seen from the images, assembly of the 6HB with **1** resulted in large aggregates.

5. Effect of NaCl on the ZnPc-DNA origami complex

5.1. Optical microscopy

The effect of NaCl on the ZnPc **1**-6HB complexes were studied by optical microscope using a Leica DM4500 microscope equipped with cross-polarizers. The sample was prepared by mixing the 6HB solution (final concentration of 3.7 nM) with **1** (final concentration of 37.0 μ M) to obtain a 1/6HB ratio of 10 000. The samples were incubated at room temperature for 20 minutes and after the incubation, NaCl (final concentration of 50 mM) was added. The sample was then refrigerated overnight. The following day the samples were imaged. 5 μ L of the sample was deposited on a glass slide and covered with a cover slide stick with double-sided tape in order to preserve the moisture and structural features of the sample.

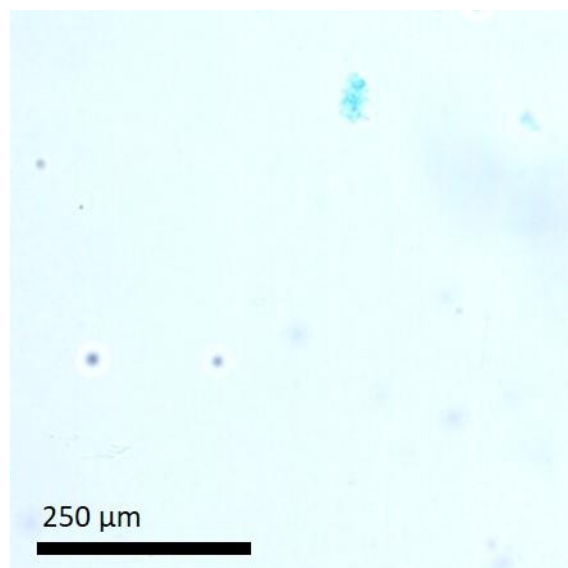


Figure S5 Optical microscope images of the ZnPc **1**-6HB complexes ([6HB] = 3.7 nM, [**1**] = 37 μM, NaCl = 50 mM). As can be seen from the images, large mesh-like structures dissociate when 50 mM NaCl is added.

5.2. EMSA

The **1**-6HB complexes were prepared by mixing the 6HB solution (final concentration of 3.0 nM) and the required amount of NaCl to prepare samples with final NaCl concentrations of 20, 50, 100, 200, 500 and 1000 mM. After that, **1** (final concentration of 30 μM) was added at a **1**/6HB ratio of 10 000 before the mixture was incubated at room temperature for 20 minutes. The total sample volume was 15 μL and 3 μL of gel loading dye (6×) was added before loading 18 μL of the total sample into the gel pockets. The agarose gel was prepared and ran using the protocol described in Section 2.4. The gel was visualized using a BioRad ChemiDoc™ MP Imaging system and the gel was excited at 532 nm (Alexa 546) and 633 nm (Alexa 647).

5.3. UV-Vis spectroscopy

The change in the absorbance spectra of **1** in the presence of 6HB structures and NaCl was studied with UV-Vis spectroscopy using a Cytation 3 plate reader (BioTek). The samples were initially prepared using 100 μL PCR tubes and then transferred to a clear flat-bottom 96-well plate. The ZnPc **1** concentration was kept constant at 2 μM, while the DNA origami concentration varied between the samples to obtain **1**/6HB ratios of 500, 1 000, 2 000 and 4 000, which correspond to final DNA origami concentrations of 4 nM, 2 nM, 1 nM and 0.5 nM, respectively. Samples without 6HB structures were also prepared as reference. First, the 6HB solution was diluted in 1× FOB to obtain the desired 6HB concentration, after which **1** was added. The samples were incubated for 20 minutes before NaCl was added in final concentrations of 50, 100, 200, 500 and 1000 mM. The samples were refrigerated overnight and before the measurements.

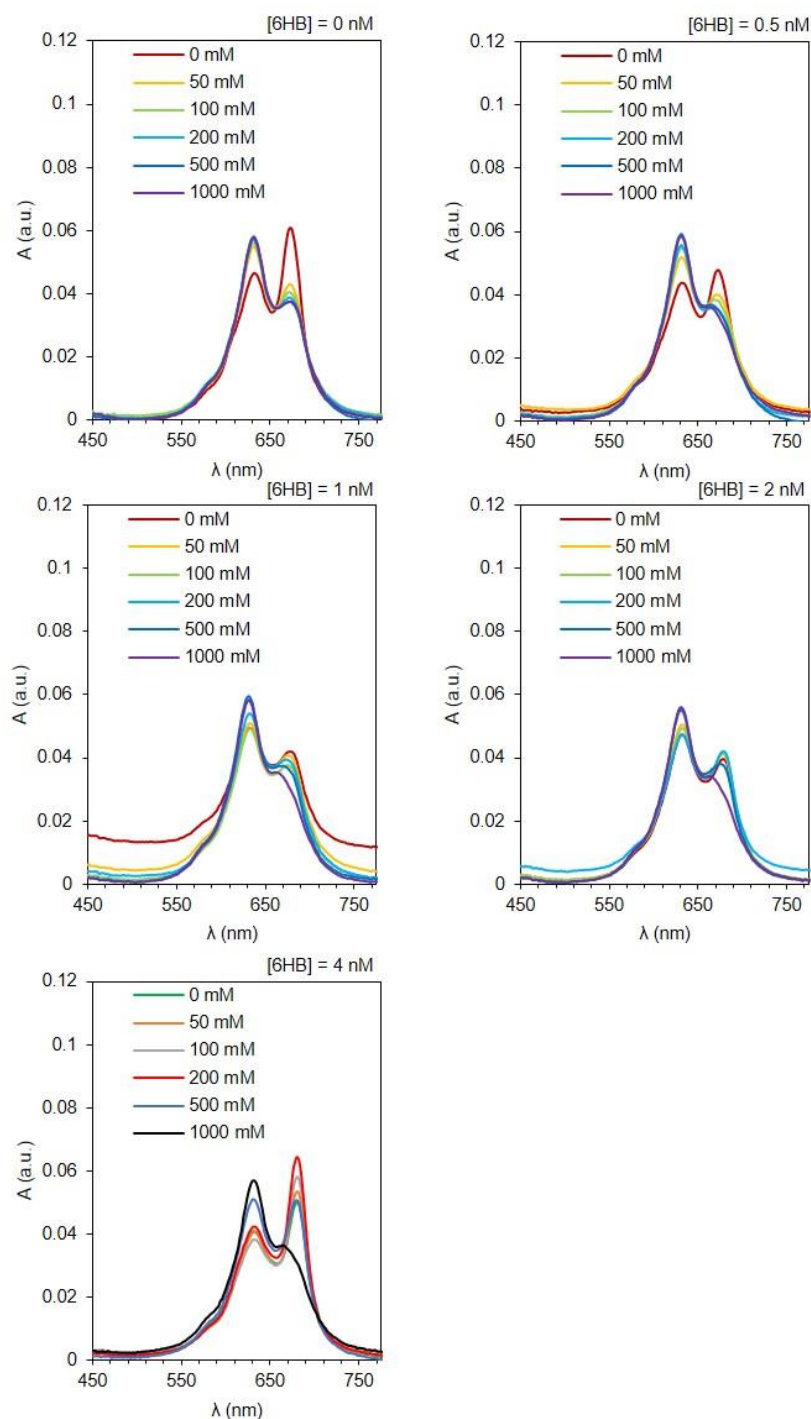


Figure S6. Absorption spectra of **1** (2 μ M) in the presence of 0, 0.5, 1, 2 and 4 nM of 6HB (1/6HB = 500, 1000, 2000 and 4000, respectively) at increasing amounts of NaCl (0, 50, 100, 200, 500 and 1000 mM).

5.4. TEM

The images were taken using a FEI Tecnai 12 Bio-Twin instrument or a JEOL JEM-2800 analytical HR-TEM operated at an acceleration voltage of 120 kV and 200 kV, respectively. The images were processed using Gatan Digital Micrograph software.

The samples were prepared by mixing the 6HB solution (final concentration of 4 nM) with 1x FOB and **1** (final concentration of 40 μ M) to obtain a **1**/6HB ratio of 10 000, after which the samples were incubated for 20 minutes. After the incubation, different amounts of NaCl was added to prepare samples with final NaCl concentrations of 50, 100, 200, 500, and 1000 mM. The samples were incubated for additional 20 minutes before the TEM grids were prepared as earlier described in Section 2.5.

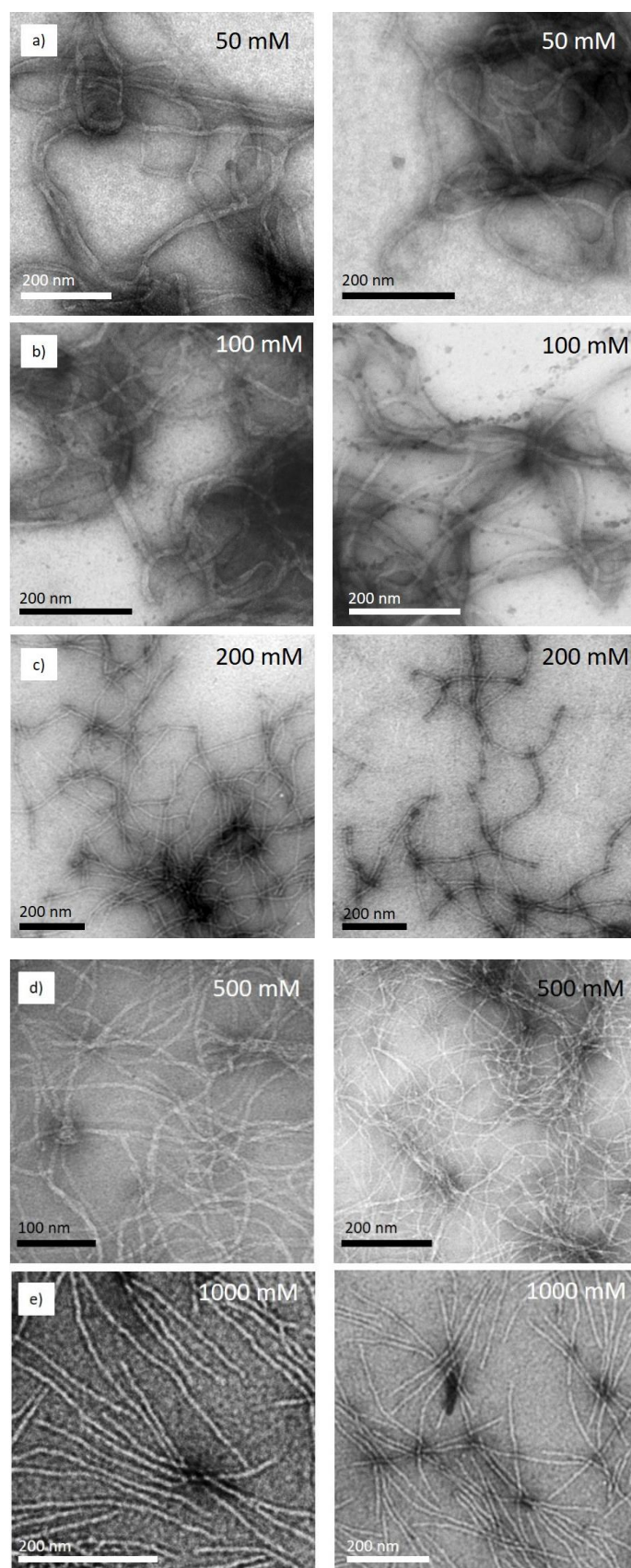


Figure S7. TEM images of the 1-6HB complexes in the presence of a) 50, b) 100, c) 200, d) 500 and e) 1000 mM of NaCl, respectively. Samples were negatively stained with uranyl formate (2 % (w/v)) before imaging.

6. ZnPc-DNA-origami photoactivity

Sample preparation: The aqueous Chremophor EL[®] (0.5% w/w) solution was prepared in milli-Q water and the NaCl concentration was adjusted by addition of 1 M NaCl solution. 100 μ L of a 750 μ M solution of 1,3-diphenylisobenzofuran (DPBF) in DMSO was added to this solution, and transferred to 1 cm-pathway disposable cuvettes, resulting in solutions with approximate absorptions of 1.5 units.

The **1-6HB** complexes were prepared separately and incubated for 20 minutes at room temperature before being added to the solution. The **1** concentration was kept constant at 0.8 μ M in order to obtain a ZnPc absorption of ca 0.1 units. Minimum ambient irradiation was ensured during the preparation.

Note that the van der Waals -based solubilizing effect of charge-neutral Chremophor EL[®] plays no significant role on the ZnPc **1-6HB** electrostatic interaction, as shown by a constant absorbance ratio between the composite in Chremophor EL[®] and in FOB (A_{Chrem} and A_{FOB} , respectively) measured at 684 nm (*i.e.* the wavelength of the deaggregated ZnPc fraction) observed at ZnPc **1/6HB** ratios of 500, 1000 and 2000 (Figure S8f). However, in absence of 6HB, the aqueous solubility of ZnPc **1** is anomalously enhanced, showing a three-fold increase in the $A_{\text{Chrem}}/A_{\text{FOB}}$ ratio. This is likely due to the absence of competing interaction with 6HB. Therefore, it is expected that the optical properties of ZnPc **1**, such as $^1\text{O}_2$ production (Figure S10d), are enhanced under these conditions.

Sample irradiation: Samples were irradiated for one-minute intervals with constant agitation, using a 120 LED handheld lamp (theLEDman, 40 mW/cm², $\lambda_{\text{exc}} = 660$ nm) at constant light fluence to achieve a decrease in DPBF absorption of about 3–4% in each cycle. Absorption spectra were recorded using a Cary 50 Conc spectrophotometer (Varian) and disposable 2.5 mL PMMA cuvettes (Brand). DPBF photodegradation was monitored at 417 nm. This methodology allows us to estimate and qualitatively compare the efficiency of different PS formulations.

Chremophor EL[®] effect:

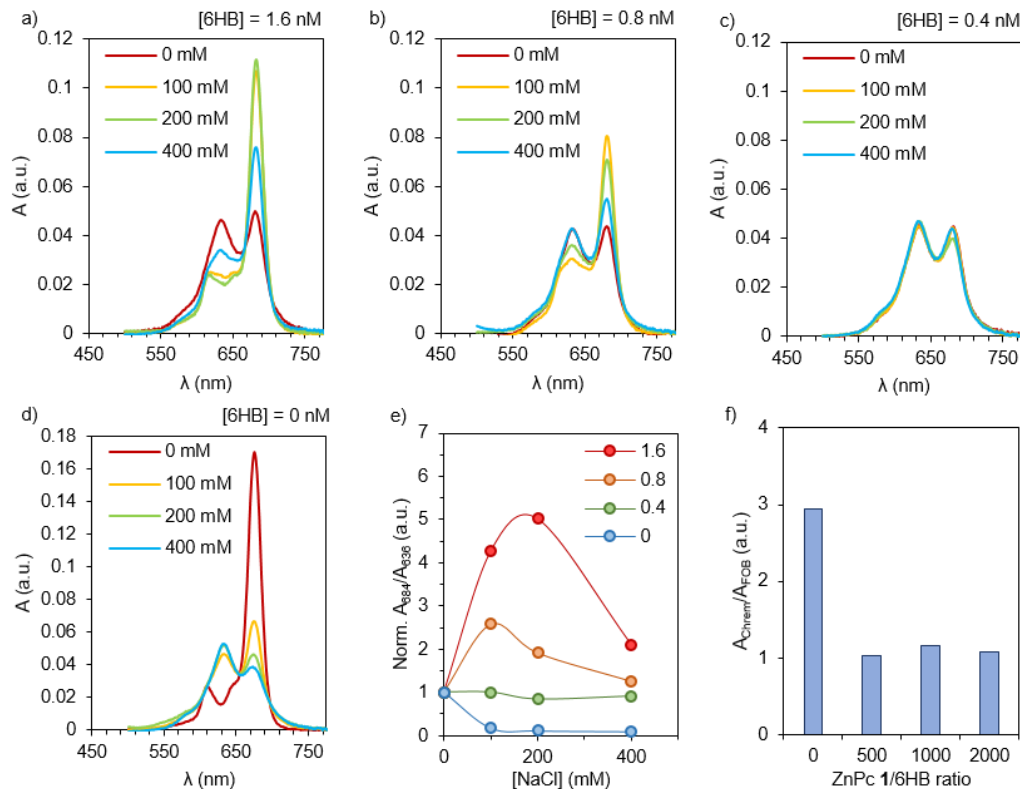


Figure S8. a-d) ZnPc absorption detail, showing the aggregated and non-aggregated bands at 636 and 684 nm, respectively. e) Normalized ratio between the aggregated and non-aggregated band, resembling a similar behavior as observed in the absence of Chremophor EL[®]. f) Ratio between the absorption at 684 nm in Chremophor EL[®] (A_{Chrem}) and FOB (A_{FOB}) at different ZnPc **1/6HB** ratios, in the absence of NaCl.

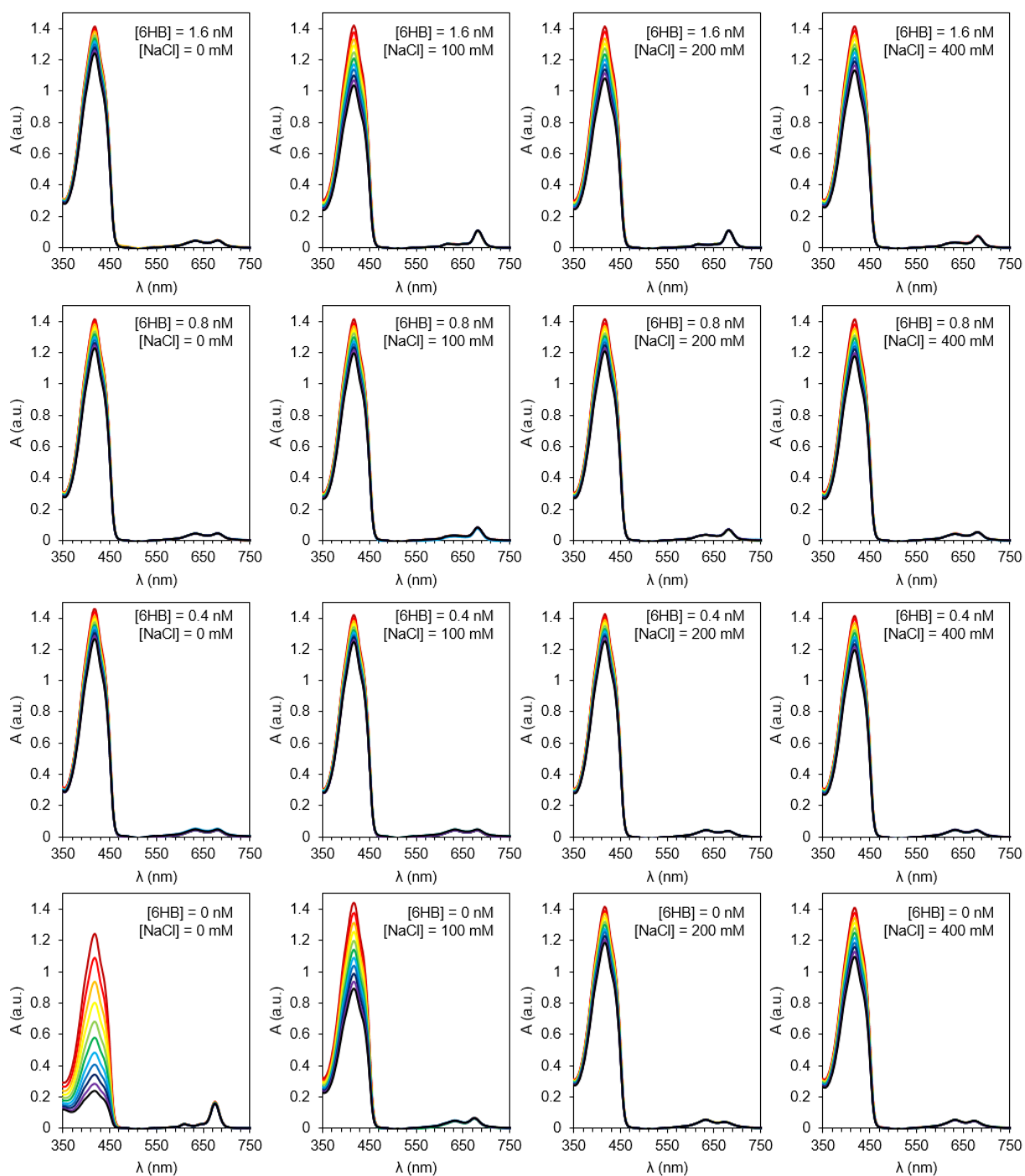


Figure S9. Absorption spectra of DPBF photodegradation recorded in 0.5% w/w Cremophor EL[®] in milli-Q water at different 6HB and NaCl concentrations. $[1] = 0.8 \mu\text{M}$.

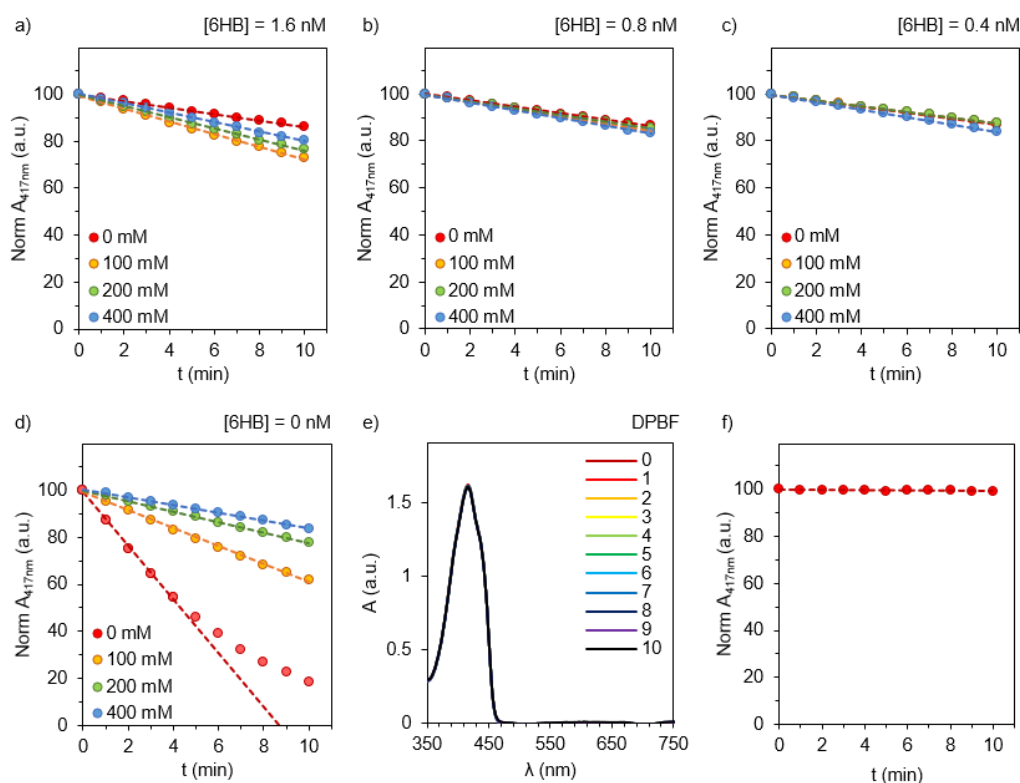


Figure S10. a-d) Normalized decrease in DPBF absorption monitored at 417 nm, after irradiation at different intervals, in presence of **1**-6HB complexes and NaCl concentrations. [**1**] = 0.8 μM . Note that **1** shows a high deaggregation effect due to the presence of Chremophor EL[®] in absence of 6HB and NaCl, showing abnormally high activity. Linear behavior was followed during the first four minutes of irradiation. e) DPBF stability control in absence of ZnPc at the same irradiation intervals, and f) the corresponding 417 nm absorption.

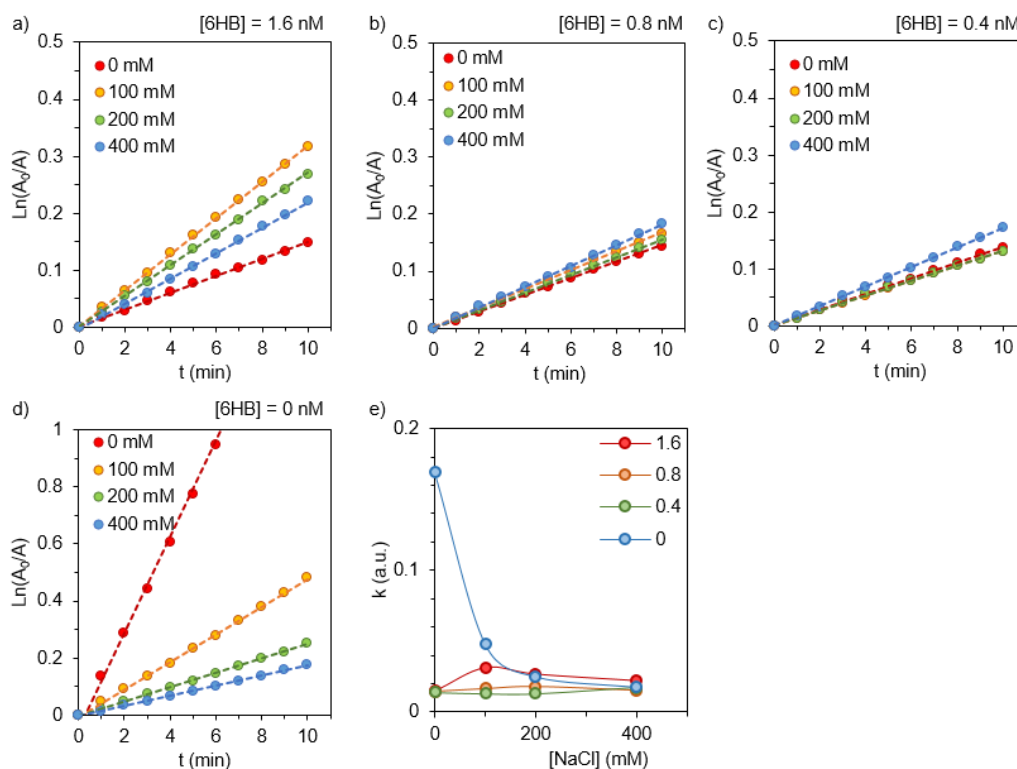


Figure S11. Normal logarithm of the ratio between the absorption at $t=0$ (A_0) and each absorption of DPBF at 417 nm, in presence of **1**-6HB complexes at different ratios and NaCl concentrations. [**1**] = 0.8 μM . e) Slope value of a plot of $\text{Ln}(A_0/A)$ versus irradiation time, at different NaCl and 6HB concentrations.

7. Photostability assay

Sample preparation: Six samples with a final volume of 75 μL were prepared by mixing 6HB (in FOB) with ZnPc **1** and NaCl (dissolved in FOB). Final concentrations: [6HB] = 2 nM; [ZnPc **1**] = 1 and 8 μM ; [NaCl] = 0, 100 and 200 mM. In addition, control samples consisting of 25 μL of 6HB (at $c = 2$ nM) were prepared.

Irradiation: 50 μL of the aforementioned samples were placed in a 96-well plate, and irradiated from above in the same conditions and light dose as in the photoactivity experiments (see Section 6). The remaining 25 μL were stored in the dark. After 10 minutes of irradiation, an aliquot of 25 μL was taken from each sample and stored in the dark. The remaining 25 μL aliquot was irradiated for additional 50 minutes.

All samples, including the controls, were treated with 350 μM of heparin sodium salt (*c.a.* charge ratio of 250, compared to the highest ZnPc charge concentration employed for the **1**/6HB = 2000) to release the 6HB. Loading dye was added, and 25 μL of each sample as well as control were analyzed with agarose gel EMSA (Figure S12).

Photodamage evaluation: It was observed that irradiation at typical therapeutical ZnPc concentrations (i.e. 1 μM or 500 eq.) led to neglectable photodamage, as full recovery of the 6HB band (Figure S12a-c, *top*) and no ZnPc signal neither at the 6HB band nor the well (Figure S12a-c, *middle*) was observed at any tested NaCl concentration. Therefore, we can conclude that the 6HB remains stable to photodamage in typical ZnPc and light-dose irradiation conditions.

As a positive control, photodamage was observed in the form of large non-mobile complexes only when the ZnPc concentration (8 μM) and light dose (60 minutes) exceeds the typical therapeutic concentration and dose (Figure S12a-c, *top and middle*, last well) by at least one order of magnitude.

The agarose gel was prepared, run and visualized as described in Section 2.4.

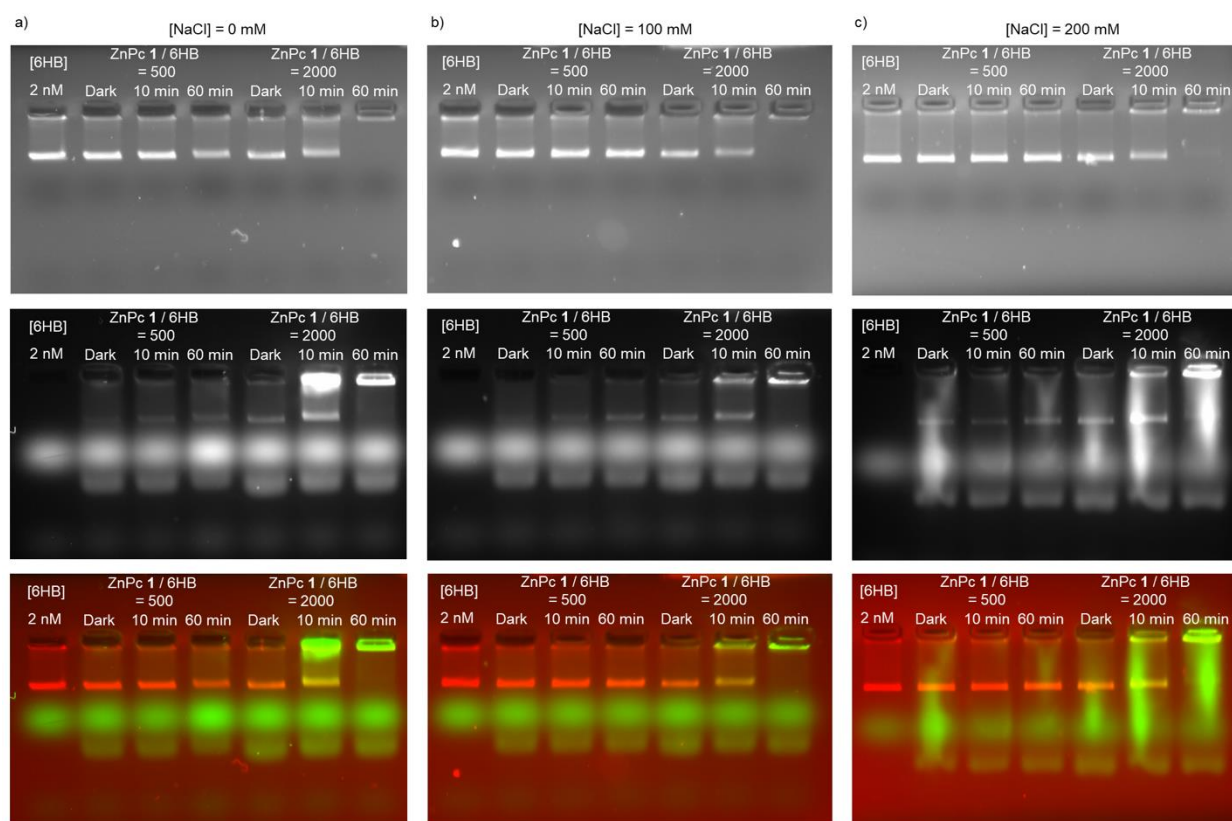


Figure S12. Agarose gel EMSA of 6HB (2 nM) at NaCl concentrations of 0 (a), 100 (b) and 200 mM (c). Each sample was irradiated for 0, 10 and 60 minutes in presence of ZnPc **1** at 1 and 8 μM (left, 500 eq; and right, 2000 eq., respectively). From top to bottom the gel was excited at 532 nm (Alexa 546), 633 nm (Alexa 647) and the overlapped image of both.

8. Enhanced protection of ZnPc-DNA origami complex

8.1. Incubation time determination of DNase I

The incubation time and concentration of DNase I required to completely digest the 6HB structure was studied using EMSA. The DNase I degradation of the 6HB structure results in short DNA fragments or oligonucleotides with higher electrophoretic mobility than the 6HB structure. The DNase I has reduced activity in NaCl solutions⁶ and therefore, different DNase I concentrations and incubation times have been tested.

For samples without NaCl, the 6HB structure (final concentration of 4.5 nM) was incubated with a constant DNase I concentration of 130 Kunitz Units/mL for different incubation times (2, 10, 30, 60 and 120 minutes). After the incubation, 3 μ L of gel loading dye (6 \times) was added. The M13mp18 scaffold strand (type p7249) was used as a reference sample after being diluted 2:3 in 1 \times FOB. The total volume added into the gel pocket was 16 μ L.

For samples with added NaCl, the 6HB (final concentration of 2.1 nM) was mixed with 1 \times FOB and NaCl to obtain a final concentration of 200 mM NaCl. The samples were incubated for 3 hours at room temperature with different amount of DNase I, ranging from 160 to 600 Kunitz U/mL. After the incubation, 5 μ L of gel loading dye (6 \times) was added. The total volume added into the gel pocket was 28 μ L.

The agarose gel was prepared, run and visualized as described in Section 2.4.

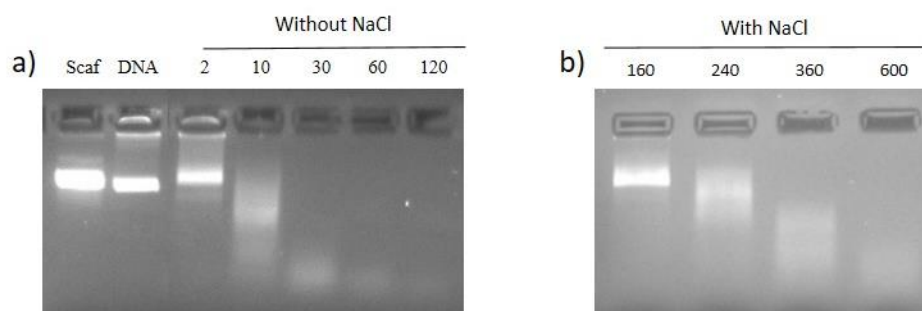


Figure S13. Agarose gel EMSA of 6HB for determining DNase I incubation time. **a)** 6HB (4.5 nM) without added NaCl, incubated with DNase I (concentration = 130 Kunitz Units/mL) for different times (expressed in minutes). **b)** 6HB (2.1 nM) with [NaCl] = 200 mM, incubated for 3 hours and titrated with increasing DNase I concentrations (expressed as Kunitz U/mL).

8.2. Determination of heparin concentration

To disassemble the 1-6HB complexes, heparin sodium salt was used. Heparin binds to **1**, which disassembles the 1-6HB complexes. The amount of heparin required for the disassembly is determined by charge concentrations. Based on previous studies⁷, which state that the average heparin disaccharide contains 2.7 sulfo groups, we approximated that the used 18 kDa heparin molecules contain on average 90 negative charges. **1** has nominally eight positive charges per molecule.

The decomplexation with heparin was studied using EMSA. Heparin was added after the formation of the 1-6HB complexes to disassemble the complexes and obtain free 6HB structures (seen in the gel as a band with the same mobility as the 6HB control sample). The 1-6HB complexes were prepared by mixing the 6HB solution (final concentration of 2.1 nM) with **1** (final concentration of 8.4 μ M) to obtain a ZnPc **1**/6HB ratio of 4 000. The mixture was incubated at room temperature for 20 minutes. After the incubation, different amount of heparin sodium salt was added to obtain final concentrations of 224, 597 and 1194 μ M, which corresponds to approximately 300, 800 and 1600 times higher charge concentration than the

ZnPc **1**. Control samples without **1** in which the heparin solution was added to 6HB solutions were also prepared.

The agarose gel for EMSA was prepared and ran using the protocol described earlier in Section 2.4. The gel was visualized using a BioRad ChemiDoc™ MP Imaging system and the gel was excited at 532 nm (Alexa 546) and 633 nm (Alexa 647).

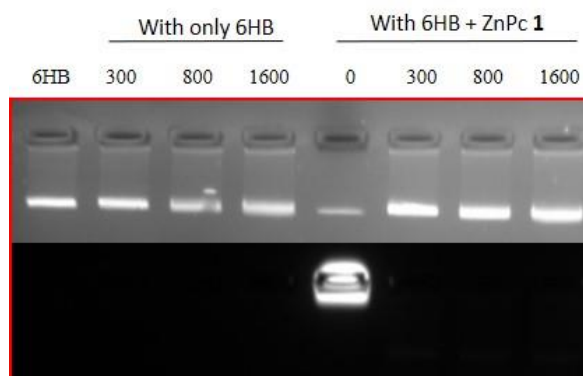


Figure S14. Agarose gel EMSA of 6HB (2.1 nM) and **1**-6HB ([6HB] = 2.1 nM, [**1**] = 8.4 μM) titrated with increasing molar equivalents of heparin sodium (based on charge concentration). The EMSA gels show the EtBr (top) and ZnPc (bottom) emission channels recorded at 532 and 633 nm, respectively.

8.3. DNase I degradation of the **1**-6HB complexes

EMSA was used to study whether the **1** protects the 6HB structures against digestion or not. Plain 6HB structures and the **1**-6HB complexes were incubated with DNase I and heparin sodium salt (800 times higher charge concentration than **1**) was added to disassemble the ZnPc **1**-6HB complexes.

The samples were prepared by mixing 6HB structures (final concentration of 2.1 nM) and **1** (final concentration of 8.4 μM) at a **1**/6HB ratio of 4 000. The samples were incubated for 20 minutes before NaCl and/or DNase I was added. The concentrations of DNase I selected for the samples with and without NaCl were 360 and 80 Kunitz U/mL, respectively. Initially, 6HB and **1** (final concentration = 8.4 μM) were incubated for 20 minutes.

For samples with NaCl: After the initial incubation, the **1**-6HB complexes were incubated with NaCl (final concentration of 200 mM) for additional 20 minutes and after which the complexes were incubated with DNase I (final concentration of 360 Kunitz U/mL) for 3 hours.

For samples without NaCl: After the initial incubation, the complexes were incubated with DNase I (final concentration of 80 Kunitz U/mL) for 30 minutes.

The interaction between the **1** and the DNase I digested 6HB structures was also studied. For this, the 6HB structure was initially degraded for 30 minutes or 3 hours (with and without NaCl, respectively), after which **1** was added to the sample.

For all samples, the total volume was 25 μL and 5 μL of (6×) gel loading dye was added. Immediately before the addition of the gel loading dye, heparin sodium salt (final concentration = 597 μM) was also added to selected samples. After the addition of heparin, 28 μL of the sample volume was loaded into the gel pocket.

The agarose gel for EMSA was prepared and ran using the protocol described earlier in Section 2.4. The gel was visualized using a BioRad ChemiDoc™ MP Imaging system and the gel was excited at 532 nm (Alexa 546) and 633 nm (Alexa 647).

9. References

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