Supporting Information

for

Spatially controlled clustering of nucleotide-stabilized vesicles

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A. Materials and methods

All commercially available reagents were used as received. Fluorophores 1,6-diphenyl-1,3,5-hexatriene (DPH), all the nucleotides, AgNO₃, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were procured from Sigma-Aldrich. Coumarin153 (C153) were obtained from Exciton. All the reagents were used without purification. The Zn(NO₃)₂-stock solution was standardized using EDTA following standard procedures. The stock solutions were prepared both by weight and UV-vis spectroscopy using the molar extinction coefficients: $\varepsilon_{259}(\text{AMP}) = 15400 \text{ M}^{-1}\text{cm}^{-1}$, $\varepsilon_{268}(\text{TMP}) = 9600 \text{ M}^{-1}\text{cm}^{-1}$, $\varepsilon_{253}(\text{GMP}) = 13700 \text{ M}^{-1}\text{cm}^{-1}$, $\varepsilon_{272}(\text{CMP}) = 8700 \text{ M}^{-1}\text{cm}^{-1}$. Surfactant C₁₆TACN•Zn²⁺ was synthesized and characterized as described in the literature (Reference 19 in the main manuscript).

Fluorescence measurements were done on a Varian Cary Eclipse fluorescence spectrophotometer also equipped with a thermostatted cell holder. UV-visible spectra were measured on a Varian Cary50 spectrophotometer equipped with thermostatted multiple cell holders. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano-S instrument. TEM images were recorded on a Jeol 300 PX electron microscope. First the grid was placed on a drop of sample solution for 1 min and then, for staining, it was placed on a drop of either uranyl acetate (2 %) solution for 30 s. The solvent was allowed to evaporate before imaging of the stained grid.

Confocal images were taken using a confocal laser scanning microscope (BX51WI-FV300-Olympus) coupled to a frequency doubled Ti:Sapphire femtosecond laser at 400 nm, 76 MHz (VerdiV5-Mira900-F Coherent). The laser beam was scanned on a 120x120 μm sample area with a 512x512 px resolution, using a 60x water immersion objective (UPLSAPO60xW-Olympus). A 435 nm longpass filter was used in front of the photomultiplier tube to collect the fluorescence of C153.

Optical microscopic images were captured using an optical setup, which comprised a microscope (Olympus BX60M) with a halogen lamp (100 W) and a 50X objective lens (LMPlanFLN 50X/0.5 BD ∞/0/FN26.5, Olympus). Images were recorded using a CCD camera attached to the optical microscope. Fluorescence images were taken using green excitation light.
B. GMP-templated formation of vesicles

Fluorescence response curves upon the addition of GMP at fixed surfactant concentration:

![Fluorescence response curve](image)

**Fig. S1** Fluorescence intensity at 428 nm as a function of the GMP concentration at a fixed surfactant concentration of 30 μM in the presence of DPH (2.0 μM) as the fluorescent probe; excitation wavelength = 355 nm, slit width (ex/em) = 5/10 nm. Experimental conditions: [HEPES] = 5 mM, pH 7.0, T = 25 °C.

**UV-Vis study in the absence and presence of surfactant:**

In addition to the experiments described in the manuscript, the selective binding affinity of GMP among all other nucleotides was also studied by UV-vis experiment. We have observed a decrease in the absorbance maxima of GMP’s UV-Vis spectrum in the presence of the surfactant C_{16}TACN-Zn^{2+} (30 μM) (Fig. S2). The decrease in the absorbance of GMP due to binding of metal ions or enzymes are reported elsewhere. [Reference 22 and 25 in the main manuscript] In this case, the decrease in absorbance is due to the binding of the nucleotides with surfactant. Aggregate formation was studied in more detail by titrating increasing amounts of GMP to a constant concentration of C_{16}TACN-Zn^{2+}. It is clear from both Fig. S2 and S3 that only the absorbance of GMP undergoes a substantial decrease in their absorbance maxima in the presence of 30 μM surfactant.
Fig. S2 UV-vis spectra of the nucleotides GMP in absence and presence of a constant surfactant (C_{16}TACN-Zn^{2+}) concentration (30 μM, below cac). Experimental Condition: T = 25 °C, HEPES buffer (pH 7.0, 5 mM).
Fig. S3 Difference in absorbance at the absorbance maxima of the GMP (at 253 nm) before and after binding with GMP as a function of GMP concentration using 30 μM concentration of surfactant. Experimental conditions: HEPES (5 mM, pH 7.0), T = 25 °C.
Fluorescence measurements using the C153 fluorophore:

**Fig. S4** Fluorescence intensity at 530 nm as a function of the amount of the GMP added to an aqueous buffer solution of surfactant C$_{16}$TACN•Zn$^{2+}$ (30 μM) in the presence of C153 as the fluorescence probe (final concentration = 2.5 μM). Excitation wavelength = 430 nm, Fluorescence slit width = 5/10 nm.
**DLS measurements in the presence of GMP:**

We analyzed the structure of the GMP-templated aggregates by DLS. We performed a series of DLS measurements of solutions containing a constant concentration of surfactant (30 mM) and varying concentrations of GMP (10–150 μM). Stable aggregates with a well-defined size of 31 ± 10 nm were obtained for GMP concentrations between 30 and 120 mM (Fig. 2e, f in the main manuscript and Fig. S5). At lower concentrations, no reproducible sizes could be measured, whereas at higher concentrations very large aggregates were observed, which tended to precipitate.

![DLS profiles](image)

**Fig. S5** Representative DLS profiles of nucleotide stabilized vesicles a) in terms of number, b) intensity in the presence of 60 and 120 μM of GMP at a fixed concentration of C16TACN•Zn²⁺ (30 μM). Experimental conditions: [HEPES] = 5 mM, pH 7, T = 25 °C.
Fluorescence microscopic images:

The presence of an aqueous interior in the aggregates was confirmed by encapsulating a watersoluble, cationic dye (rhodamine 6G) (Fig. S6). Considering that the apolar fluorescent probes DPH and C153 had also revealed the presence of a hydrophobic domain in these aggregates, we postulate that GMP templates the formation of vesicle-like aggregates, similar to that observed previously for ATP.

![Fluorescence microscopic images of a surfactant solution after dialysis without and with GMP (20 minutes after mixing). Samples contained C16TACN·Zn^{2+} (100 μM), Rhodamine 6G (5 μM) and GMP (100 μM). Experimental conditions: [HEPES] = 5 mM, pH 7.0, T = 25 °C. Here we have used cationic water-soluble dye Rhodamine 6G to confirm the presence of an aqueous interior in the GMP-induced aggregates. The use of a cationic dye excluded that the dye encapsulation was a result of electrostatic interactions with the (positively charged) surfactant. Here the pre-mixed surfactant+GMP+dye solution was dialysed with 30K cut-off membrane (Merck, Millipore) by centrifuging at 2500 rpm for 4 min. The retained solution (~50% volume) was used for imaging. The left and right images are from duplicate experiments.](image)

TEM images of the GMP-templated vesicles:
The TEM images revealed the formation of spherical aggregates with similar dimensions (Fig. 2c and Fig. S7, ESI).

Fig. S7 Additional TEM images of solutions containing (a-b) C16TACN·Zn2+ (30 μM) and GMP (45 μM) or (c-d) C16TACN·Zn2+ (30 μM) and GMP (60 μM). Staining was performed using 2% uranyl acetate in all cases.
C. Ag⁺-Induced hierarchical assembly of GMP-templated vesicle

Control UV-vis experiments without GMP:

Fig. S8 (a) UV-vis spectra of GMP as a function of the silver ion (Ag⁺) concentration in the absence of surfactant (30 μM) in aqueous HEPES buffer (5 mM, pH 7.0). (b) Plot of the absorbance maxima value (at 253 nm) as a function of Ag⁺ ion in presence and absence of surfactant under similar experimental conditions.

Fluorescence study:

A control fluorescence titration experiment with DPH revealed that the addition of AgNO₃ (in the absence of GMP) did not result in the formation of similar clusters in the absence of GMP.

Fig. S9 Fluorescence intensity at 428 nm (a.u.) as a function of the amount of the AgNO₃ added to aqueous buffer solutions of surfactant (30 μM) containing also DPH (1 μM).

Experimental conditions: [HEPES] = 5 mM, pH 7.0, T = 25 °C, Excitation wavelength = 355 nm, Slit width (ex/em) = 5/10 nm.
**Fig. S10** a) Fluorescence intensity at 428 nm (a.u.) as a function of the amount of the AgNO₃ added to aqueous buffer solutions of surfactant (30 μM) and GMP (45 μM) containing also DPH (1 μM). b) Fluorescence intensity at 428 nm (a.u.) as a function of time upon the addition of GMP (45 μM) and then Ag⁺ (45 μM) to an aqueous buffered solution of surfactant C₁₆TACN·Zn²⁺ (30 μM) containing DPH (1 μM). Experimental conditions: [HEPES] = 5 mM, pH 7.0, T = 25 °C, Excitation wavelength = 355 nm, Slit width (ex/em) = 5/10 nm.
Additional TEM images of the aggregated vesicles:

Fig. S11 TEM images of the aggregated vesicles with [C16TACN-Zn$^{2+}$] = 30 μM and [GMP] = 45 μM and [Ag$^{+}$] = 30 μM (a,b) with staining and (c,d) without staining. (scale bar = 500 nm for a,c and d, 200 nm for b).
Confocal Laser Scanning Microscopy Study:

**Fig. S12 (a-e)** Confocal images over time showing the appearance of aggregated vesicles due to addition of Ag⁺. Samples were prepared using C16TACN·Zn²⁺ (50 μM), C153 (2.5 μM), GMP (50 μM) and [Ag⁺] = 150 μM.
No specific structures were observed in the absence of either GMP or C_{16}TACN·Zn^{2+} (Fig. S13).

**Fig. S13** Confocal images taken 30 min in a sample containing only surfactant + Ag⁺ (without GMP) and only GMP+ Ag⁺ (without surfactant) in separate experiments. Samples were prepared using C_{16}TACN·Zn^{2+} (100 μM), C153 (2.5 μM), GMP (100 μM) and [Ag⁺] = 300 μM.
Control experiment in presence of other nucleotides (AMP, TMP and CMP):

**Fig. S14** Confocal image without GMP but in presence of C\textsubscript{16}TACN-Zn\textsuperscript{2+} (100 μM) and Ag\textsuperscript{+} (300 μM) and other mononucleotides AMP/TMP/CMP (300 μM). Experimental condition: C153 (2.5 μM), [HEPES] = 5 mM (pH 7), images were taken 1 hr after mixing.
D. Local aggregation of vesicles near Ag-patch

Experimental set up:
An Ag-film of nearly 5 mm diameter was formed on a glass slide (by using 10 µl of 0.5 M glucose and 3 µl of Tollens’s reagent having 0.1 M Ag⁺). After formation of the Ag-film, it was washed thoroughly with a stream of mQ water to clean the surface. Then, it was dried by flowing air and also under vacuum for 6 h. Next a hybridization chamber (20 mm of diameter and 0.6 mm height) was placed on it. After that, GMP-stabilized vesicle solution mixed with 0.1 mM H₂O₂ was injected inside the chamber and was sealed (to prevent any evaporation and other external turbulence). Then, images were taken at different distance away from the Ag-film under optical microscope (at 50x zoom).

Control experiment results:
The presence of Nile red also permitted imaging by fluorescence, which confirmed that aggregates only formed in close proximity of the Ag patch. Importantly, no aggregation was observed in the absence of H₂O₂, indicating that the oxidation of Ag is essential to trigger the process (Fig. S17). Other control experiments under similar experimental conditions, in the presence of H₂O₂ but in the absence of either GMP or surfactant, also did not result in the formation of any structure (Fig. S18 and S19). The observation of similar aggregates in the entire hybridization chamber upon the co-injection of GMP-stabilized vesicles and Ag⁺ confirmed that the observed objects indeed result from Ag⁺-induced clustering (Fig. S20–S23 and supporting Video S3).

Additional optical and fluorescence microscopic images near Ag patch

Fig. S15 Optical microscopic images of the aggregated vesicle (a) 0.5-1 mm, (b) 1-2 mm, (c) >3 mm away from the Ag-patch. (x-axis of the all the images = 152.4 µm).
Experimental condition: [C₁₆TACN·Zn²⁺] = 250 µM, [GMP] = 250 µM, [H₂O₂] = 100 µM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.
**Fig. S16** Fluorescence microscope images at close (0.5-1 mm) and different distance away (1-2 mm and 2-3 mm) from the Ag-patch. Left side of the image contains the Ag-patch. Nile red (5 μM) was used as the hydrophobic fluorescence probe. (x-axis of all images = 152.4 μm). Experimental conditions: [C_{16}TACN-Zn^{2+}] = 250 μM, [GMP] = 250 μM, [H_{2}O_{2}] = 100 μM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.

**Control experiment without H_{2}O_{2}:**
In the absence of H_{2}O_{2}, formation of Ag^{+} ions near Ag-patch is not possible and, consequently no aggregates are formed near the Ag-patch.

**Fig. S17** Optical microscopic images of the GMP-stabilized vesicle near the Ag-patch (at 0.5-1 mm) in the absence of H_{2}O_{2}. (x-axis of the all the images = 152.4 μm).
Experimental conditions: [C_{16}TACN-Zn^{2+}] = 250 μM, [GMP] = 250 μM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.
Control experiment with only surfactant in presence of H$_2$O$_2$:
In the absence of GMP, no vesicle are formed and thus, even in the presence of H$_2$O$_2$ -which results in formation of Ag$^+$ ions - no structure was observed near the Ag-patch.

Fig. S18 Optical microscopic images of the only surfactant near the Ag-patch (at 0.5-1 mm) in the presence of H$_2$O$_2$. (x-axis of the all the images = 152.4 μm).
Experimental conditions: [C$_{16}$TACN-Zn$^{2+}$] = 250 μM, [H$_2$O$_2$] = 100 μM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.

Control experiment with only buffer and GMP in presence of H$_2$O$_2$:

No structure was observed without surfactant and GMP near the Ag-patch in the presence of H$_2$O$_2$.

Fig. S19 Optical microscopic images with only buffer near the Ag-patch (at 0.5-1 mm) in the presence of H$_2$O$_2$. (x-axis of the all the images = 152.4 μm).
Experimental conditions: [H$_2$O$_2$] = 100 μM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.
Images of GMP-stabilized vesicles in absence and presence of H$_2$O$_2$ without Ag-patch:

In the absence of the Ag-patch no aggregated structures were observed independent of whether H$_2$O$_2$ was present. It shows that under the experimental condition only H$_2$O$_2$ does not cause aggregation of the vesicle in the absence of the Ag-patch.

Fig. S20 Optical microscopic images of the GMP-stabilized vesicle without any Ag-patch (a,b) in absence of H$_2$O$_2$ and (c,d) in presence of H$_2$O$_2$ (x-axis of the all the images = 152.4 μm).

Experimental conditions: $[C_{16}\text{TACN-Zn}^{2+}] = 250\ \mu\text{M}, [\text{GMP}] = 250\ \mu\text{M}, [\text{HEPES}] = 5\ \text{mM (pH 7.0)}, T = 25\ ^\circ\text{C}.$
Fig. S21 Fluorescence microscopic images of the GMP-stabilized vesicle without any Ag-patch (x-axis of the all the images = 152.4 μm). Nile red (5 μM) was used as the hydrophobic fluorescence probe. Experimental conditions: [C₁₆TACN·Zn²⁺] = 250 μM, [GMP] = 250 μM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.

Images of aggregated GMP-stabilized vesicles in presence of AgNO₃:

Fig. S22 Optical microscopic images of the aggregated GMP-stabilized vesicle in the presence of AgNO₃. (x-axis of the all the images = 152.4 μm). Experimental conditions: [C₁₆TACN·Zn²⁺] = 250 μM, [GMP] = 250 μM, [AgNO₃] = 250 μM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.
**Fig. S23** Fluorescence microscopic images of the aggregated GMP-stabilized vesicle in the presence of AgNO$_3$. (x-axis of the all the images = 152.4 μm). Nile red (5 μM) was used as the hydrophobic fluorescence probe. Experimental conditions: [C$_{16}$TACN·Zn$^{2+}$] = 250 μM, [GMP] = 250 μM, [AgNO$_3$] = 250 μM, [HEPES] = 5 mM (pH 7.0), T = 25 °C.
Representative set of fluorescence microscopic images for four Ag-patch system:

**Fig. S24** Representative fluorescence microscope images near the Ag patch (position 1-4 as described in the scheme) and in the center (position 5). Nile red (5 μM) was used as the hydrophobic fluorescence probe. (x-axis of all images = 152.4 μm). Experimental conditions: \([\text{C}_{16}\text{TACN-Zn}^{2+}] = 250 \mu\text{M}, [\text{GMP}] = 250 \mu\text{M}, [\text{H}_2\text{O}_2] = 100 \mu\text{M}, [\text{HEPES}] = 5 \text{mM} \text{ (pH 7.0)}, T = 25 ^\circ\text{C}.\)
E. Movie Caption

Supporting Video S1.

Aggregation of GMP-stabilized vesicles after addition of AgNO₃.

This movie shows the time-dependent formation of aggregated vesicles (as fluorescence dots) after addition of AgNO₃. AgNO₃ was added 2 min after starting the imaging experiment. Initially, no apparent changes seem to occur for a period of around 12 minutes after AgNO₃ addition and only objects corresponding to the vesicles are detected. Then a strong increase in the number of objects as well as their aggregation into large structures with micrometer-sized dimensions. The fluorescence dots arise due to encapsulation of the hydrophobic probe (C153) in the hydrophobic bilayer region of the vesicles, which causes an increase in intensity of multiple orders of magnitude. Conditions: HEPES buffer (5 mM, pH 7), [C₁₆TACN-Zn²⁺] = 100 μM, [GMP] = 100 μM, [AgNO₃] = 300 μM, [C153] = 2.5 μM. Movie 1 is captured by confocal laser scanning microscopy at 1 frame per 3 sec. Total experiment time is 40 min. The video has been sped up by putting 10 frame per sec (overall speeding up 30x). Each side of the frame is 120 μm.

Supporting Video S2.

Aggregation of GMP-stabilized vesicles near Ag-surface observed under optical microscope.

This movie shows the aggregated vesicles only near Ag surface (within 1 mm away from the Ag surface) upon introduction of GMP-stabilized vesicle in the hybridization chamber along with H₂O₂ (0.1 mM). Here the video has been taken 5 min after injection of the vesicle solution into the hybridization chamber (under 20x objective lens). The x-axis of the frame is 384.8 μm. Here top of the screen contains the Ag-patch. We observe the aggregated structure only near the patch and as we scan down the screen the number of the particle significantly reduced. It suggests the aggregated structure of micrometer dimension which can only be detected under this condition can only be formed near Ag patch under our experimental condition. Conditions: HEPES buffer (5 mM, pH 7), [C₁₆TACN-Zn²⁺] = 100 μM, [GMP] = 100 μM, [H₂O₂] = 100 μM. Total experiment time is 30 sec (video is real time).

Supporting Video S3.

Visual detection of the Ag⁺ mediated preformed aggregated vesicles under optical microscope.

This movie shows the visible appearance of the aggregated micrometer sized vesicles formed by addition of AgNO₃ in GMP-templated vesicle (under 20x objective lens). The x-axis of the frame is 384.8 μm. Here aqueous AgNO₃ solution was added in GMP stabilized vesicle and then shaken gently and the movie was taken about 15 min after the addition. Conditions: HEPES buffer (5 mM, pH 7), [C₁₆TACN-Zn²⁺] = 100 μM, [GMP] = 100 μM, [AgNO₃] = 300 μM. Total experiment time is 10 sec (video is real time).