# Supporting Information to

# Entrapment of Metal Nanoparticles in Polymer Stomatocytes

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*Stomatocyte, hybrid polymersomes, shape transformation, platinum nanoparticles, confinement, nucleation, template*

**Content**

## 1. Materials

All reagents and chemicals were used as received unless otherwise indicated. Styrene was distilled prior to use to remove the inhibitor. Anisole and *N,N,N',N",N"*-pentamethyl-diethylenetriamine (PMDETA) were distilled prior to use. Ultra pure MilliQ water was obtained with a Labconco Water Pro PS purification system (18.2 MΩ) and was used for the self-assembly of polymersomes and for the dialysis experiments. Spectra/Por® Dialysis Membrane MWCO: 12-14,000 was used for dialysis of polymersomes and their shape transformation into stomatocytes. Ultrafree-MC centrifugal filters 0.22 $\mu$m were purchased from Millipore. Pluronic F127 (Mn ~ 12500), polyvinylpyrrolidone (Mn ~ 10000), polyacrylic acid (sodium salt) Mw 2100 and potassium tetrachloroplatinate (II) 99.9% were purchased from Sigma-Aldrich. L (+) ascorbic acid was purchased from Acros Organics and used as received.

## 2. Instrumentation

NMR spectra were recorded on a Varian Inova 400 spectrometer with $CDCl_3$ as a solvent and TMS as internal standard. Molecular weights of the block copolymers were measured on a Shimadzu Prominence GPC system equipped with a PL gel 5 μm mixed D column (Polymer Laboratories) and differential refractive index and UV (254 nm) detectors. THF was used as an eluent with a flow rate of 1 mL/min. Polystyrene standards in the range of 580 to 377,400 Da were used for calibration. **Dynamic light scattering (DLS)** experiments were performed on a Malvern Zetasizer Nano S equipped with a He-Ne (633 nm, 4 mW) laser and an Avalanche photodiode detector at an angle of 173 °. All DLS data were processed using a Dispersion Technology Software (Malvern Instruments). Ultrasonication for the synthesis of the nanoparticles was performed on a VWR Ultrasonic Cleaner Model 75D.

**Scanning electron microscopy** (SEM) was performed on a JEOL 2300 microscope operated at an acceleration voltage of 3 kV. For each experiment 15 μl of polymersome solution was air-dried on silicon wafer and placed on a conductive tape and attached to an aluminum sample block. The sample was then sputtered with a Au/Pd alloy to give a thickness of ~ 10 nm before visualization in the instrument.

**Transmission electron microscopy** (TEM) was performed on a JEOL 1010 microscope equipped with a CCD camera operating at an acceleration voltage of 60 kV. Sample specimens were prepared by placing a drop of the solution on a carbon-coated Cu grid (200 mesh, EM science) and subsequent air-drying. Each size distribution histogram was constructed using more than 70 particles. Processing and analysis of the TEM images was performed with ImageJ,[4,5] a program developed by the NIH and available as public domain software at http://rsbweb.nih.gov/ij/.

**Cryogenic transmission electron microscopy** (Cryo-TEM) was performed on a Tecnai $G^2$ T20 Twin transmission electron microscope equipped with TWIN objective lens, CCD digital camera, a LaB6 emitter at an acceleration voltage 20-200kV and a Jeol JEM2100 equipped with a GATAN US4000 digital camera and a GATAN cryo-holder. FEI **Vitrobot**™ Mark IV, a fully automated vitrification robot was used for the plunge freezing of the aqueous sample specimens.

### 3. Synthesis of amphiphilic block-copolymer $PEG_{45}$-*b*-$PS_{175-300}$

Amphiphilic block-copolymers containing polyethylene glycol as hydrophilic and polystyrene as hydrophobic units of different lengths were synthesized by atom-transfer living radical polymerization (ATRP). The poly(ethylene glycol) macroinitiator with a degree of polymerization (DP) = 45 and the block copolymer PEG-*b*-PS were synthesized according to a method reported previously.[1] The size of the hydrophobic polystyrene block was adjusted to be in the range of 175 to 300 for polymersome formation. All block copolymers were characterized by $^1$H NMR and GPC to evaluate the molecular weight and the size distribution. All samples showed narrow size distribution with a polydispersity index of PDI: 1.05 - 1.08.

### 4. Shape transformations of polymersomes into stomatocytes

In a typical experiment poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{177}$ (20 mg) was dissolved in a mixture of THF and 1,4-dioxane (2 mL, 1.6/0.4 ratio) in a 15 ml capped vial equipped with a magnetic stirrer and closed with a rubber septum. The solution was stirred for 30 min at room temperature to allow complete dissolution of the polymer. 2 mL of MiliQ water was then added to the organic phase with vigorous stirring (900 rpm) via a syringe pump using a 5 ml syringe equipped with a steel needle. The syringe pump was set up for an addition rate of 1ml/h. The solution turned cloudy after the addition of 0.5 ml of water. After complete addition of water the colloidal mixture was transferred in a dialysis bag and dialyzed against water. The dialysis water was replaced after 1 h followed by frequent changes for 48 h. The organic

mixture 80% THF / 20% dioxane used for polymer dissolution generated stomatocytes with a narrow opening of less then 5 nm.

### 5. Synthesis of platinum nanoparticles with tailored size and shape

Platinum nanoparticles were obtained at room temperature using either sonication[2] or hydrogen reduction methods[3]. These methods were selected because they are compatible with the polymeric stomatocytes and provide large concentrations of platinum nanoparticles with tailored shape and size depending on conditions of formation. The control of the size and shape of these structures is strongly dependent on the nature and the concentration of the platinum salt, the nature and concentration of the capping agents (surfactant or polymer), as well as the time allowed for nucleation and growth (Table S1).

***Synthesis of platinum nanoparticles via the sonication method***

We used potassium tetrachloroplatinate as the platinum source, Pluronic 127 ($PEO_{100}PPO_{65}PEO_{100}$), PVP (polyvinyl pyrrolidone) and $PEG_{3k}$-$(NH_2)_2$ as capping agents while ascorbic acid or hydrogen was used as the reducing agent.

**Pluronic capped Pt-NP** (size ~20 nm)

Pluronic F-127 (Mn ~ 12600) capping agent (10 mg) was dissolved in 1 ml of a 20 mM solution of $K_2PtCl_4$ aged for at least 24 h. An amount of 17.6 mg of ascorbic acid dissolved in 1ml of milliQ water was added at once to the platinum salt solution and the vial was sonicated for 10 min at room temperature. After 10 min, the colour of the solution changed from light reddish to colloidal black indicating the formation of colloidal particles. Dendritic platinum nanoparticles of size ~20 nm were obtained. The nanoparticle solution was centrifuged for 20 min at 12000 rpm and washed twice with clean miliQ water to remove the excess of the capping agent. After the final wash, the freshly prepared platinum nanoparticles were redispersed in water and sonicated for 1 min prior to use. Complete reduction of the platinum salt and formation of the platinum nanoparticles was observed by UV and TEM analysis.

**PVP capped Pt-NP** (sizes from 20-100 nm)

The PVP (Mn~10000) capping agent (20 mg) was dissolved in 2 ml of a 20 mM solution of $K_2PtCl_4$ aged for at least 24 hrs. An amount of 35 mg of ascorbic acid dissolved in 1ml of MilliQ water was added at once to the platinum salt solution and the vial was sonicated for 30, 40, and 60 min at room temperature to generate PVP capped platinum nanoparticles of sizes between ~20, 40, to 80 nm. When the temperature of the sonication bath was increased to 45 ºC larger nanoparticles of ~ 100 nm were obtained. Two centrifugation/washing cycles were performed to remove the excess of the capping agent before further use

of the particles for the entrapment experiments. After the final wash, freshly prepared platinum nanoparticles were redispersed in MiliQ water and sonicate for 1 min prior to use. The same procedure was used for the **$NH_2$-PEG3k-$NH_2$ capped Pt-NP** for a sonication time of 20 min generating porous nanoparticles of ~ 46 nm size.

***Synthesis of platinum nanoparticles via the hydrogen reduction method***

**PAA capped platinum nanoparticles**

An amount of 105 mg of $K_2PtCl_4$ was dissolved in 250 ml of MiliQ water, to which 0.2 ml of 0.1 M sodium polyacrylate (PAA, Mw ~ 2100) was added at once with stirring. A rubber septum was attached to the vessel which was subsequently degassed by bubbling Ar gas through the solution for 20 min. High flow rate $H_2$ gas was then bubbled through the solution for 5 min to proceed the reduction of the platinum ions. The reaction vessel was then completely sealed and the solution was left standing overnight without stirring. After 12 h, the solution turned dark and the absorption spectrum showed the formation of colloidal Pt. Two centrifugation/washing cycles at 5000 rpm were performed to remove the excess of the capping agent that remained in solution.

**6. Entrapment of platinum nanoparticles in stomatocytes *via in situ* method**

***In situ* formed Pluronic capped Pt-Np in stomatocytes**

**Pluronic F-127 capped platinum nanoparticles - mushroom-like structures on stomatocytes**

An amount of 44 mg of ascorbic acid dissolved in 2.5 ml of stomatocyte solution (80% THF opening) was added to 2.5 ml of a 20 mM aqueous solution of $K_2PtCl_4$ containing 25 mg Pluronic F127. The mixture was gently stirred for 15 min followed by sonication for 10 min at room temperature. The reddish solution changed colour to black indicating the formation of colloidal platinum as indicated by UV. The colloidal solution was filtered through 0.2 micron filters and centrifuged 4 times at 1000 rpm for 10 min after which it was purified over a short Sephadex G-200 column. The change in the order of addition of reagents ie. stomatocytes mixed with the reducing agent and added to the $K_2PtCl_4$ solution instead of adding the reducing agent over the platinum salt containing the capping agent; generated nucleation of platinum in semi sphere "mushroom-like" structures onto stomatocytes as observed by TEM.

**Pluronic F-127 capped platinum nanoparticles. Dendritic-like structures on stomatocytes**

In a typical procedure, 2.5 ml of ascorbic acid solution (44 mg of AA dissolved in MilliQ water) was added to 2.5 ml of a 20 mM aqueous solution of $K_2PtCl_4$ containing 25 mg Pluronic F127 followed by addition of 2.5 ml stomatocyte solution (80% THF protocol – 5 nm opening). The mixture was gently stirred for 15 min followed by sonication for 10 min at room temperature. The reddish solution changed the colour to black indicating the formation of colloidal platinum as demonstrated by UV-VIS. The colloidal solution

was filtered through 0.2 micron filters and centrifuged 5 times at 1000 rpm for 10 min followed by purification on a short Sephadex G-200 column. Dendritic platinum nanoparticles of ~ 20 nm size nucleating on the stomatocyte membrane were observed by TEM. Since similar structures were observed without stomatocytes, we used the same order of addition for the in situ formation of other nanoparticles, i.e. reducing agent added to a solution of platinum salt and the capping agent and finally the addition of stomatocytes to the mixture. Observation: When the platinum salt was aged for more than a month, smaller platinum nanoparticles (15-17 nm) were observed nucleating on the membrane of the stomatocytes. The order of addition of stomatocytes was found to play an important role in the final outcome of the shape of the nanoparticles.

**PVP capped platinum nanoparticles. Formation of larger structures inside the stomatocytes. Templating role of the stomach.**

In a typical procedure, 2.5 ml of ascorbic acid solution (44 mg of AA dissolved in MilliQ water) was added to 2.5 ml of 20 mM aqueous solution of $K_2PtCl_4$ containing 25 mg PVP followed by addition of 2.5 ml stomatocyte solution (70% THF opening). The mixture was gently stirred for 30 min followed by sonication for 60 min at room temperature. The reddish solution changed colour to black indicating the formation of colloidal platinum as demonstrated by UV spectra. PVP nanoparticles formation required longer sonication time as compared to Pluronic F-127. No interaction of the PVP capped nanoparticles with the stomatocyte membrane could be detected by TEM, instead larger structures were observed inside the stomach as compared to the surrounding solution.

**PAA (polyacrylic acid) capped platinum nanoparticles - Cubic structures on stomatocytes**

An amount of 10.37 mg of $K_2PtCI_4$ was dissolved in 24 ml of MiliQ water, to which 0.2 ml of an aqueous 0.1 M sodium polyacrylate solution (PAA, Mw ~ 2100) was added followed by 1 ml of Stomatocyte solution (70% THF protocol, 20 nm opening), which was introduced at once with stirring. A rubber septum was attached to the vessel and the solution was degassed by bubbling Ar gas through the solution for 20 min. High flow rate $H_2$ gas was subsequently bubbled through the solution for 11 min to induce the reduction of the platinum ions. The reaction vessel was completely sealed and the solution was left standing overnight without stirring. After 12 hrs, the solution turned dark and the absorption spectrum showed the formation of colloidal Pt. TEM analysis showed the nucleation of cubic platinum nanoparticles on the stomatocyte membrane with formation of cubic platinum nanoparticles of smaller size than the ones formed in solution.

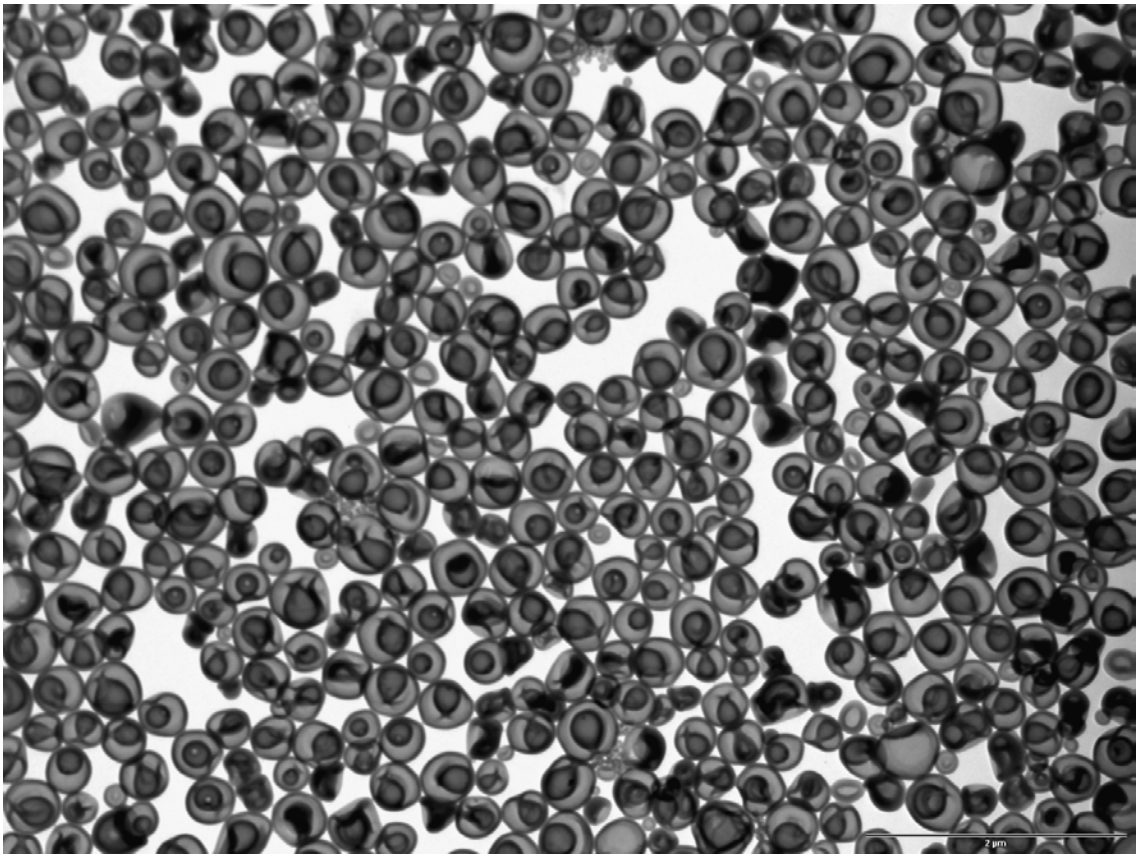


**Figure SF1.** Stomatocytes obtained from polymersomes assembled from $PEG_{44}$-b-$PS_{177}$ dissolved in 80% THF and 20% dioxane by the addition of 50% water.

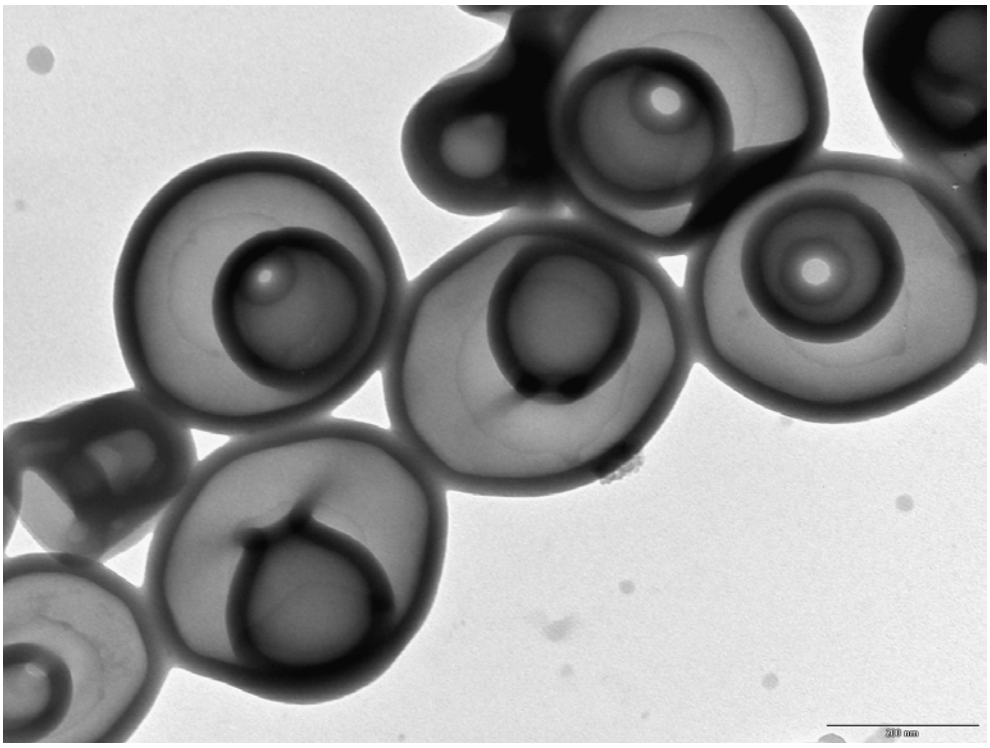


**Figure SF2.** Stomatocytes obtained from polymersomes assembled from $PEG_{44}$-b-$PS_{177}$ dissolved in 70% THF and 30% dioxane by the addition of 50% water.

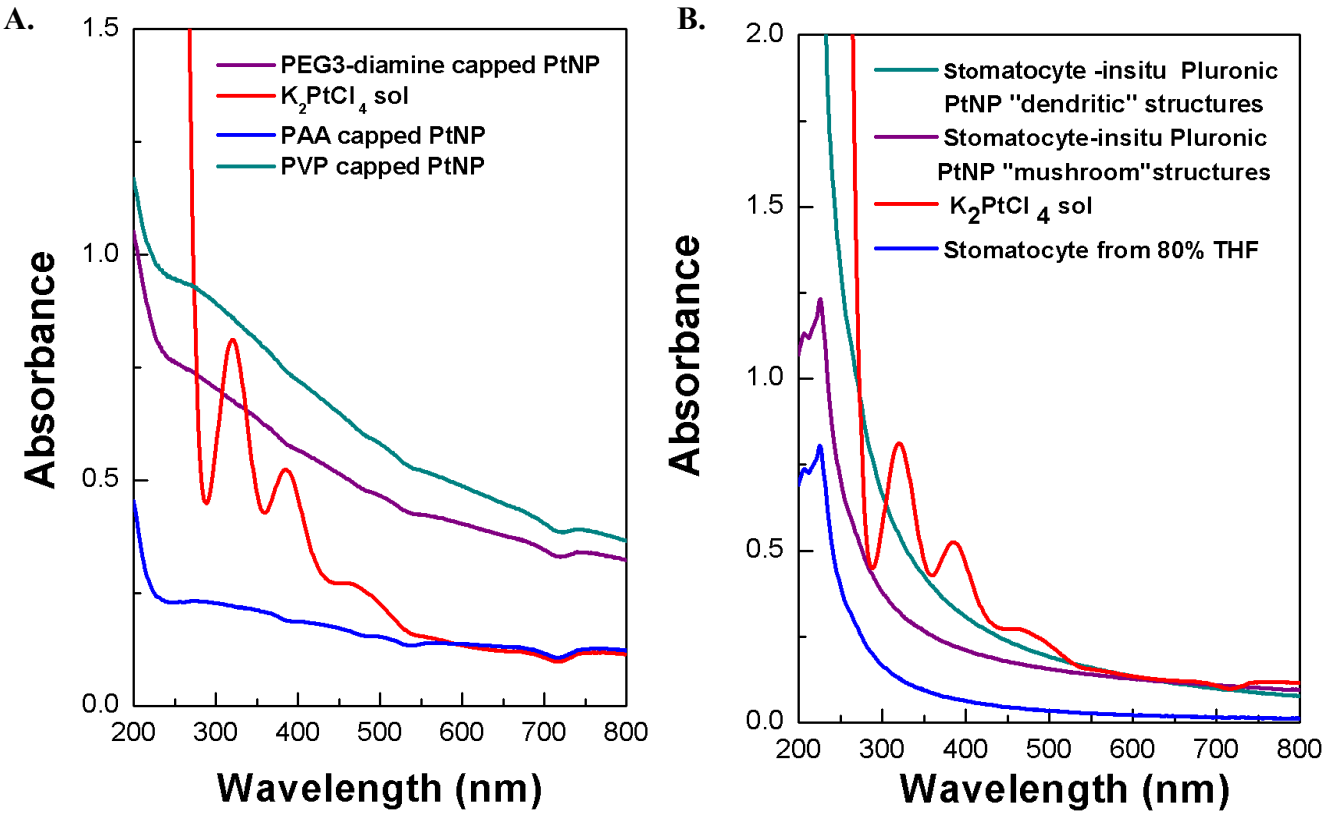



**Figure SF3. A.** Overlays of the UV-Vis spectra of PVP, PAA and $PEG_{3k}$-diamine capped platinum nanoparticles and the solution of potassium tetrachloroplatinate (II). **B.** Overlays of the UV-Vis spectra of stomatocytes formed using the 80% THF procedure against stomatocytes containing the *insitu* formed "mushroom"-like or dendritic like platinum nanoparticles prepared using the sonication method (see figure 3 in the paper). The nanoparticles nucleate either on the surface in mushroom-like structures, when the stomatocytes were mixed with the reducing agent and added to the potassium tetrachloroplatinate solution or completely cover the surface of the stomatocytes as "dendritic"-like structures when the stomatocytes where added after the solution of the platinum salt containing Pluronic F-127 was mixed with the reducing agent (nucleation already started). Both overlays with the UV-VIS of potassium tetrachloroplatinate (II) show complete consumption and reduction of the platinum salt to platinum (0).

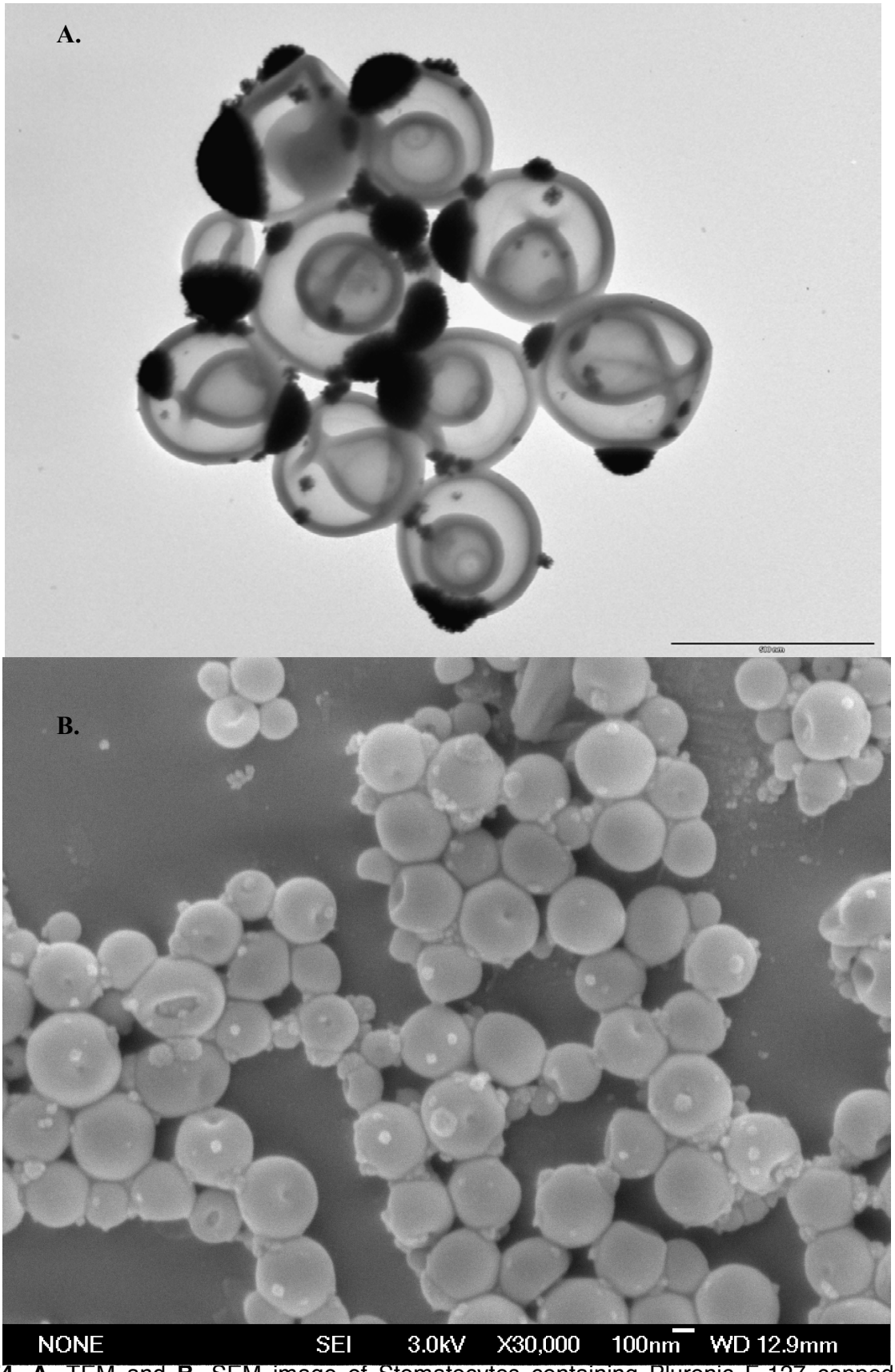



**Figure SF4. A.** TEM and **B.** SEM image of Stomatocytes containing Pluronic F-127 capped platinum nanoparticles formed in situ by the sonication method. Nucleation occurs mainly on the surface with formation of "mushroom like" nanoparticles on the membrane. These structures were only observed when the stomatocytes were mixed with the reducing agent and added to the potassium tetrachloroplatinate solution.

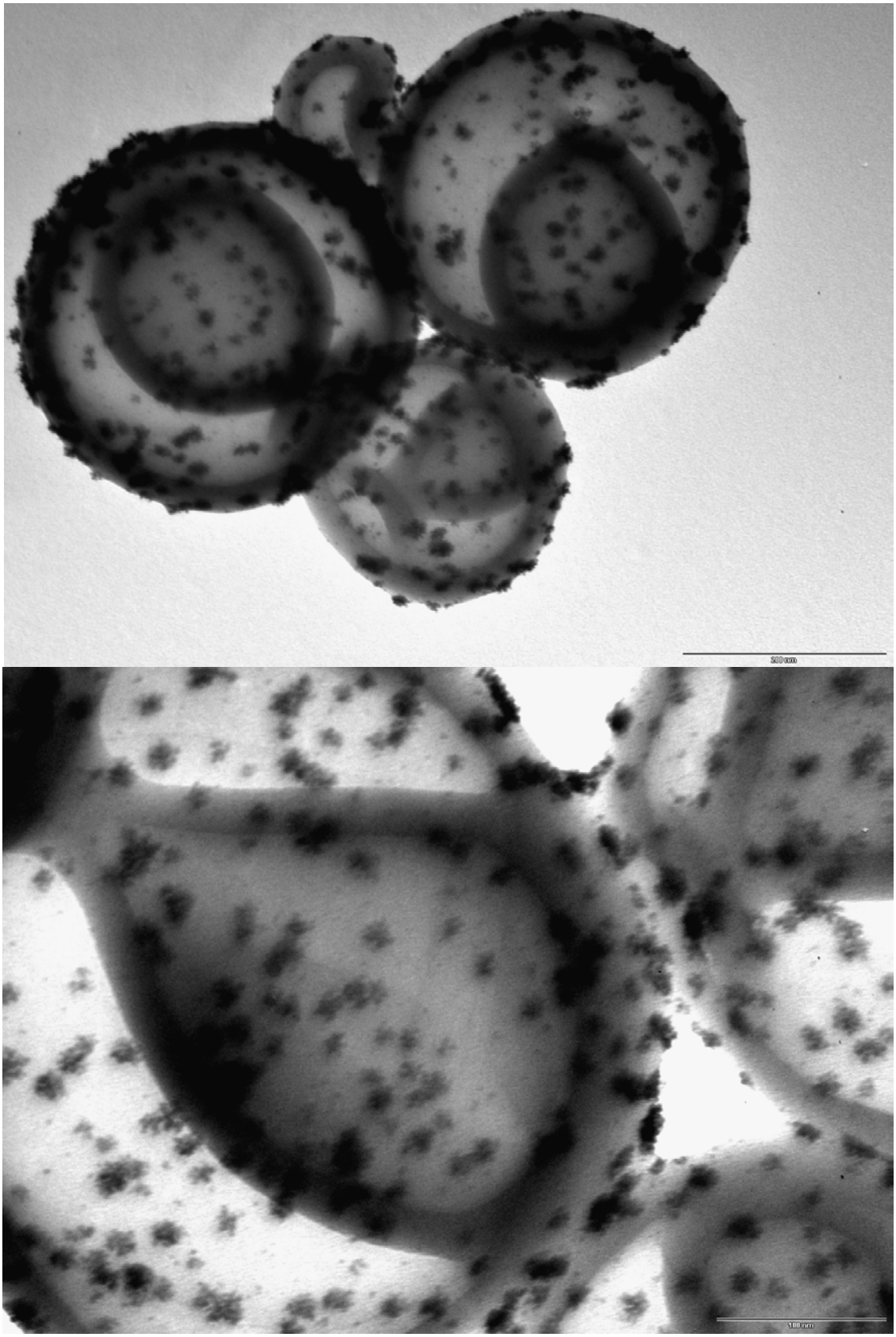
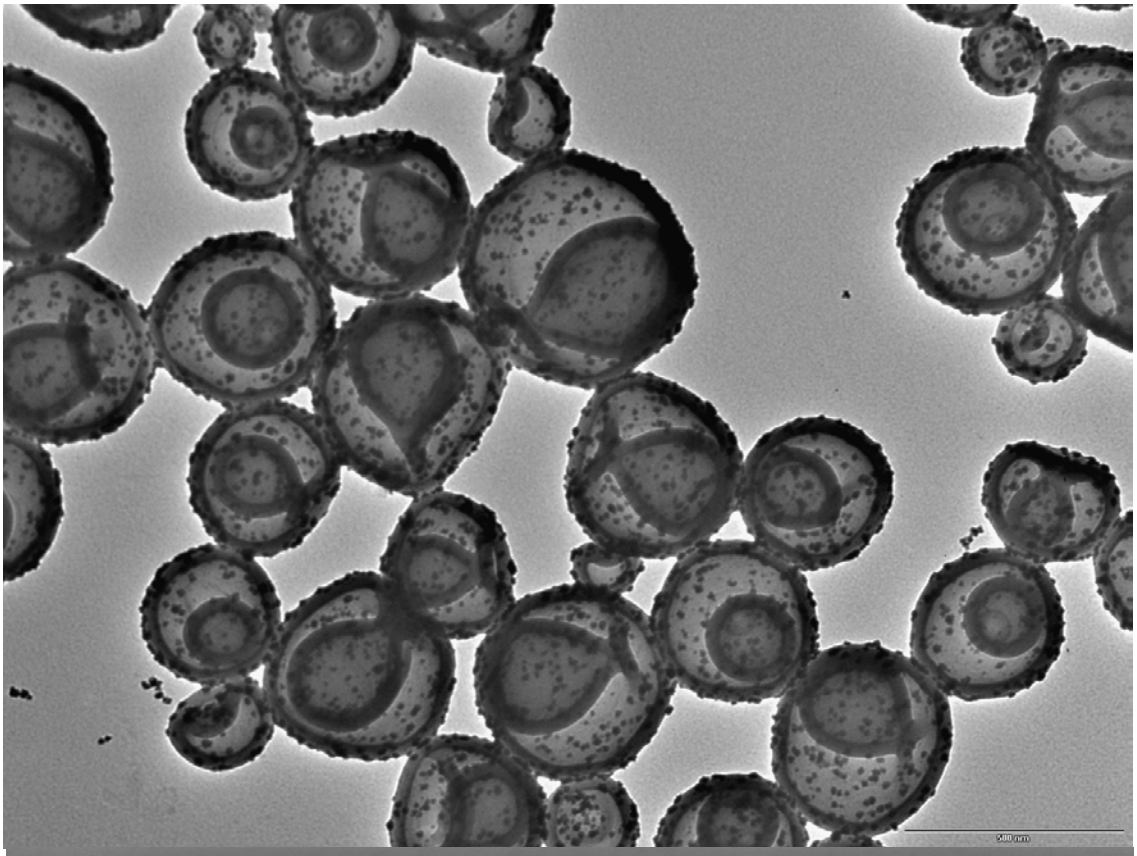


**Figure SF5.** TEM image of Stomatocytes containing Pluronic F-127 capped platinum nanoparticles formed *insitu* by the sonication method. Nucleation occurs mainly on the surface with formation of dendritic nanoparticles on the membrane. These structures were observed only when the stomatocytes were added after the solution of the platinum salt containing Pluronic F-127 was mixed with the reducing agent (nucleation already started).

**A.**



**B.**

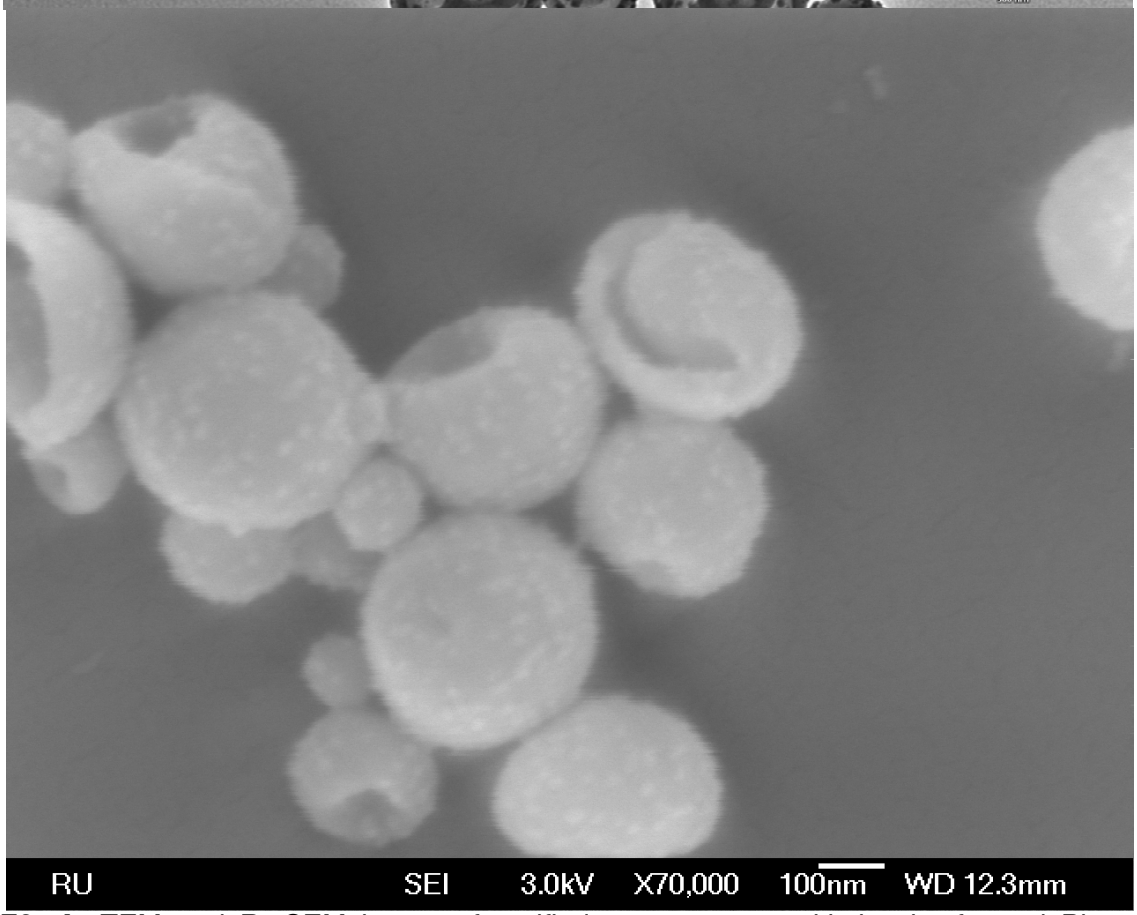
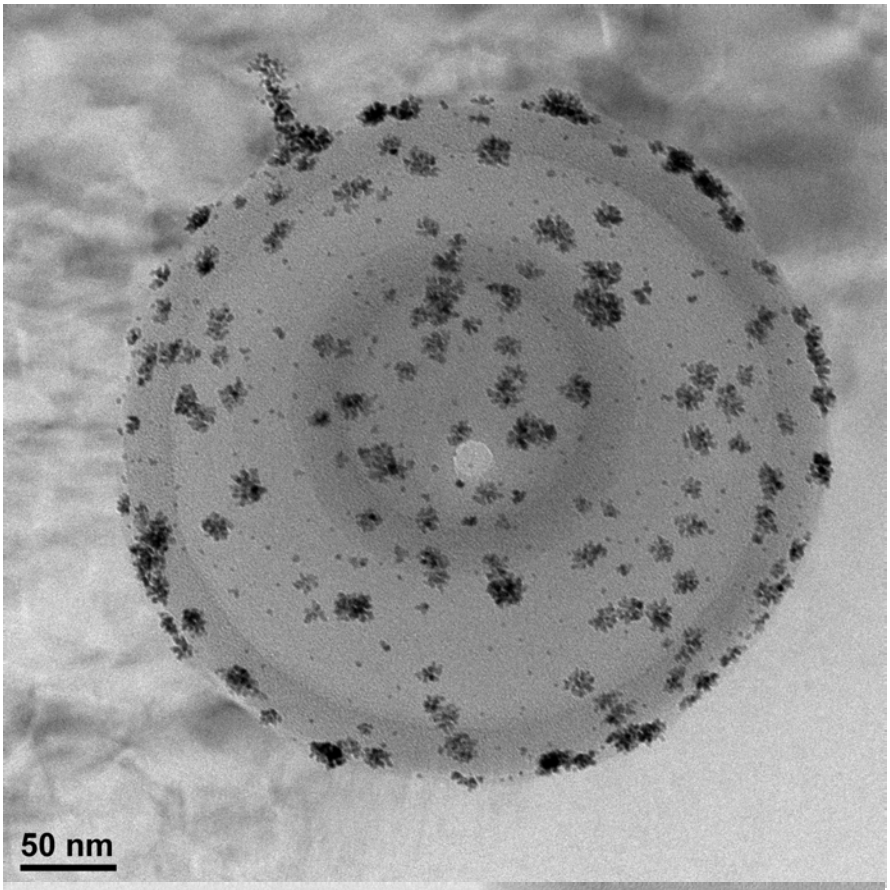



**Figure SF6. A.** TEM and **B.** SEM image of purified stomatocytes with in situ formed Pluronic F-127 capped platinum nanoparticles demonstrating that the nucleation of the nanoparticles occurs on the membrane.

**A.**




**B.**

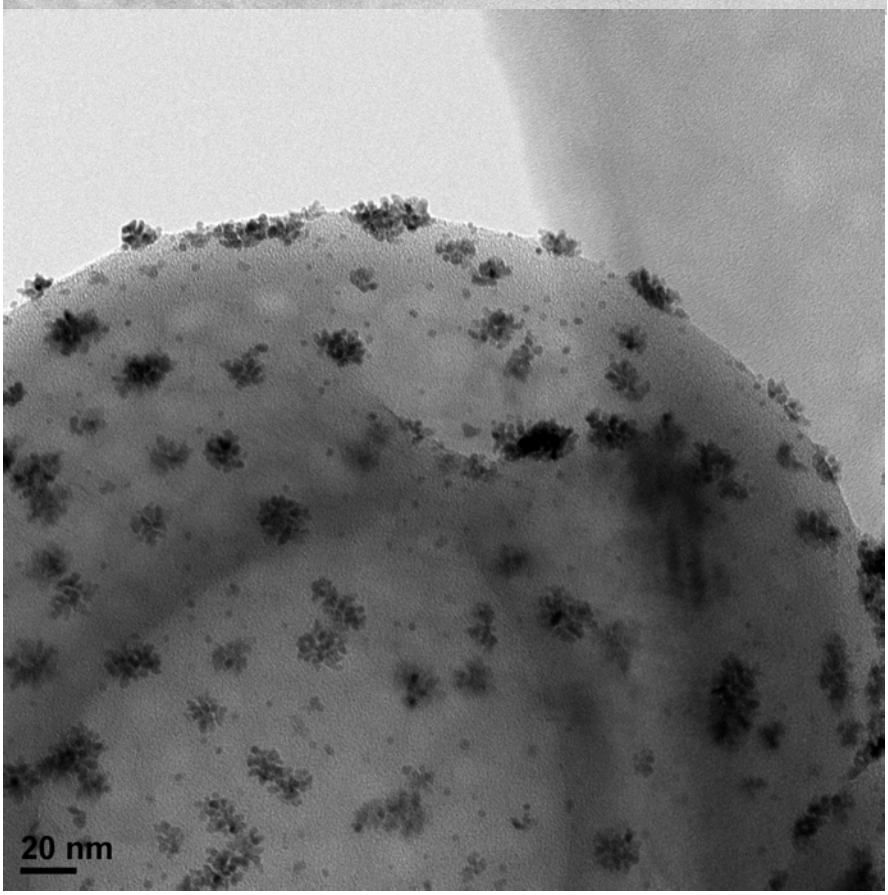



**Figure SF7.** Cryo-TEM image of purified stomatocytes with in situ formed Pluronic F-127 capped platinum nanoparticles demonstrating that the nucleation of the nanoparticles occurs on the membrane **A.** top view and **B**. side view.

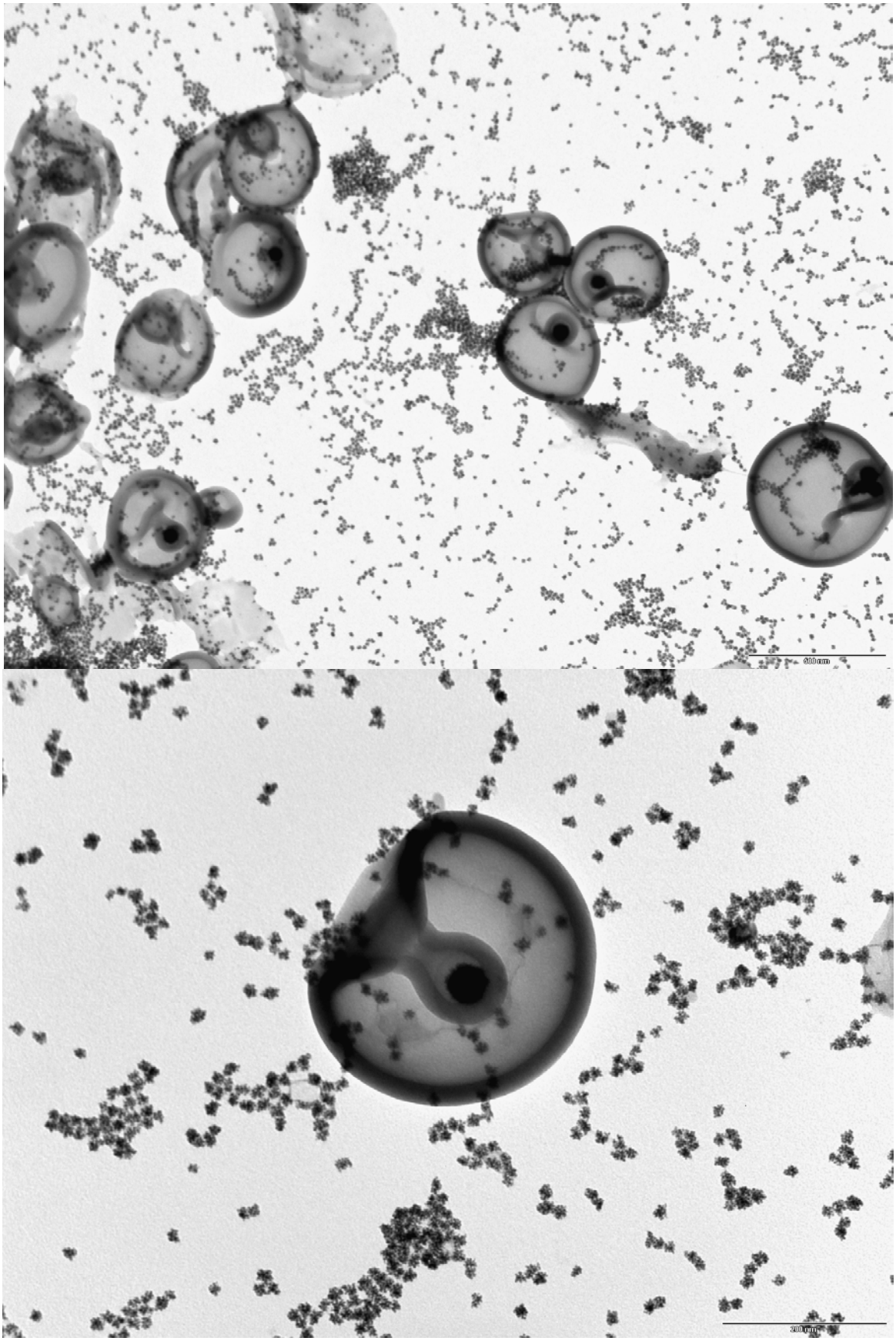


**Figure SF8.** TEM images of PVP capped platinum nanoparticles formed in situ by the sonication method The nanocavities of the stomatocytes contains one large platinum nanoparticle. No interaction with the stomatocyte membrane is observed.

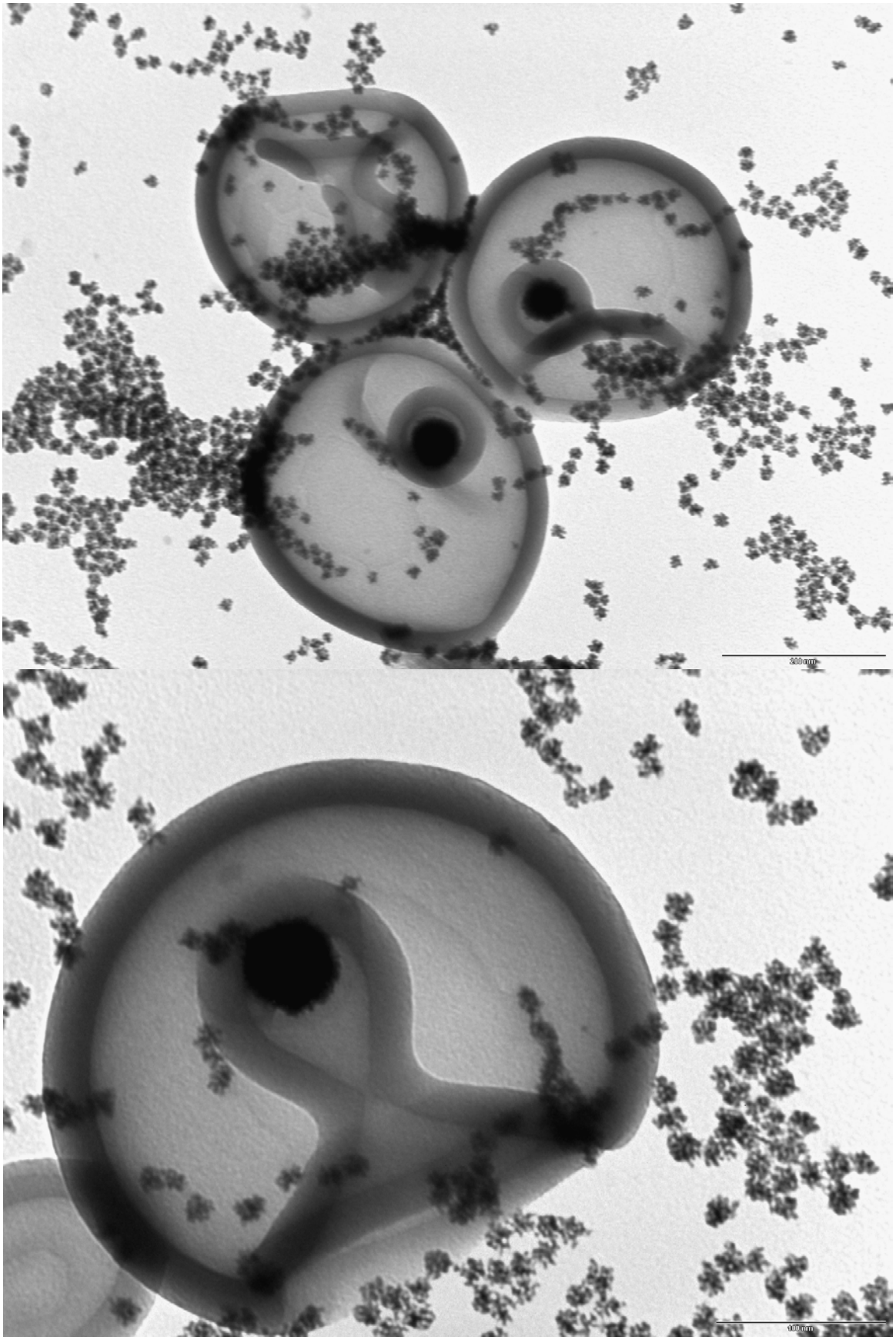


**Figure SF9.** TEM images of PVP capped platinum nanoparticles formed in situ by the sonication method. One large nanoparticle nucleates inside the stomatocyte structure. No interaction with the stomatocyte membrane is observed.

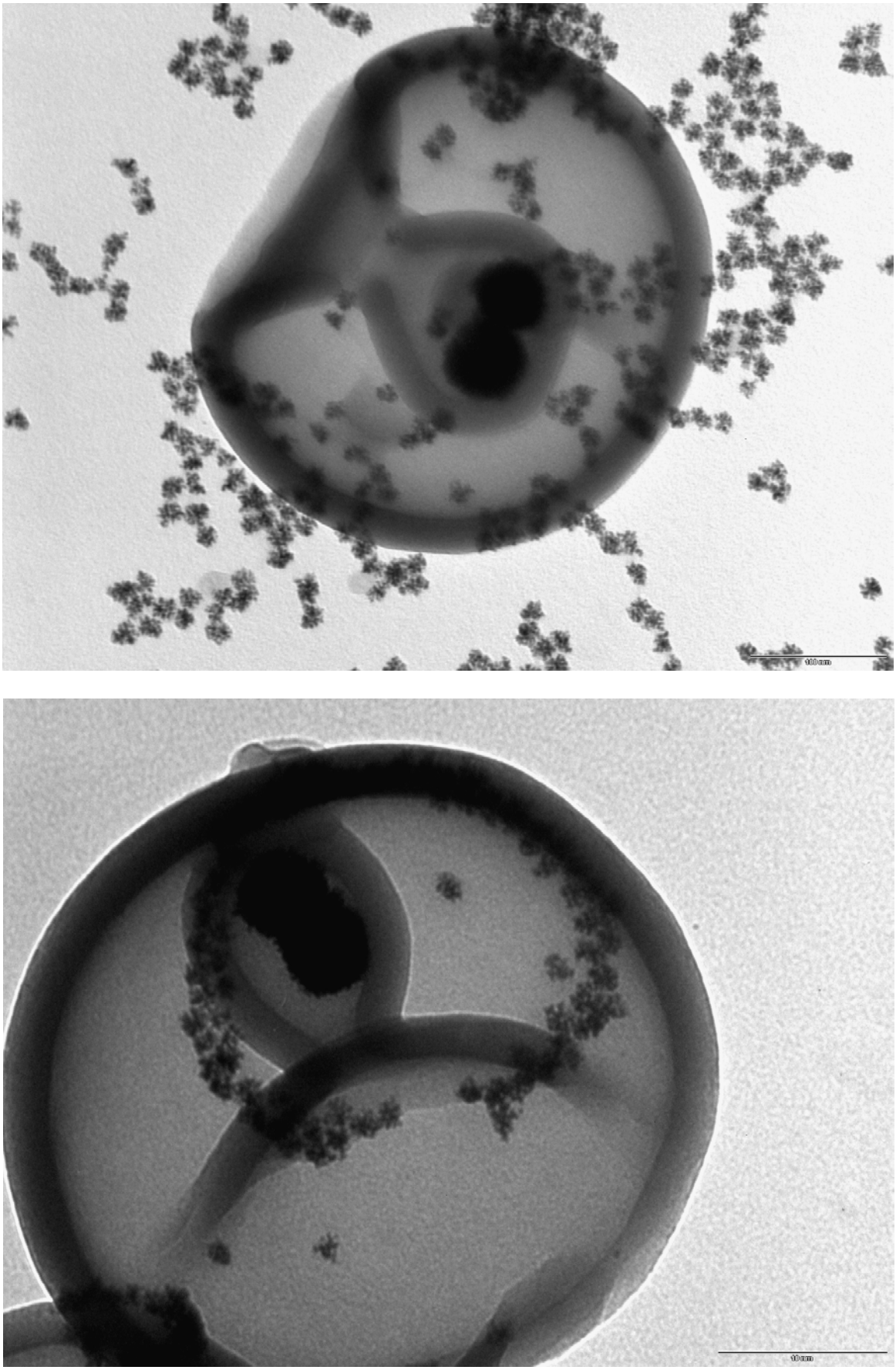


**Figure SF10** TEM images of PVP capped platinum nanoparticles formed in situ by the sonication method. No interaction with the stomatocyte membrane is observed. Two large nanoparticles can be seen inside the stomach.

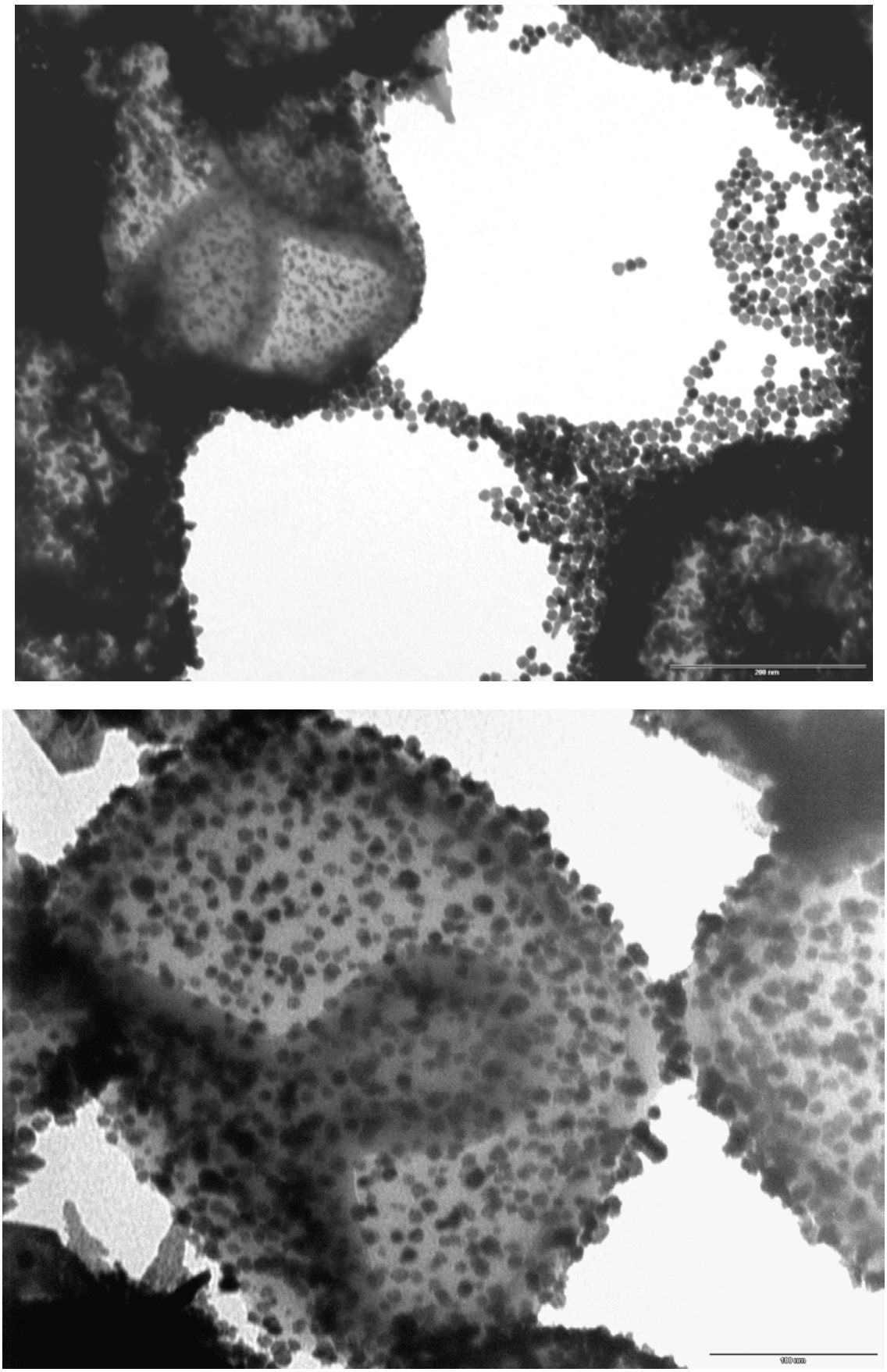


**Figure SF11** TEM images of PAA capped platinum nanoparticles formed in situ by the hydrogen reduction method. Cubic nanoparticles nucleate on the surface of the stomatocytes.

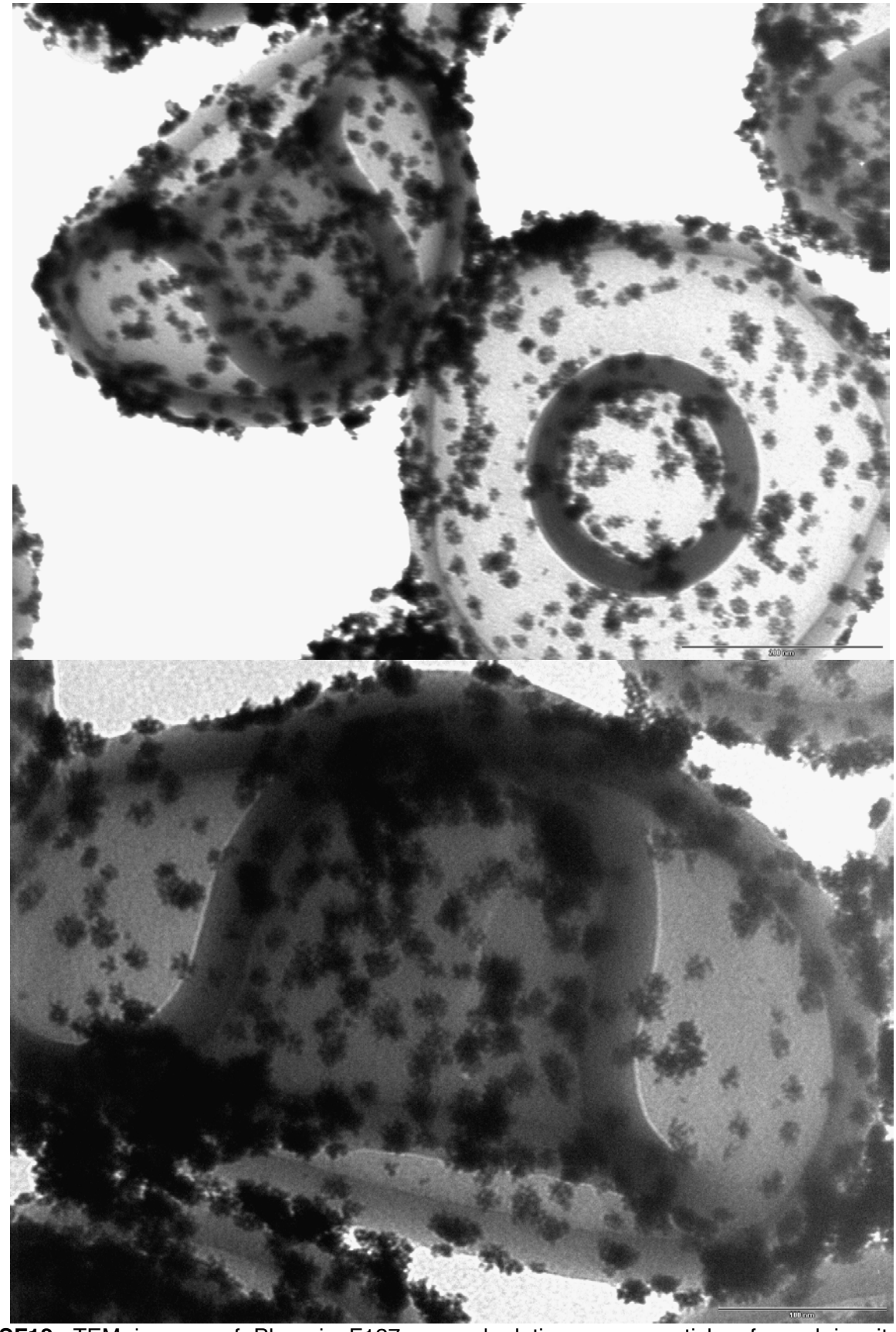


**Figure SF12.** TEM images of Pluronic F127 capped platinum nanoparticles formed in situ by the sonication method in 150 nm opening stomatocytes. Strong interaction of the PtNP with the stomatocyte membrane is observed. Nanoparticles can be seen nucleating onto the outer membrane and the inner folded stomatocyte membrane.

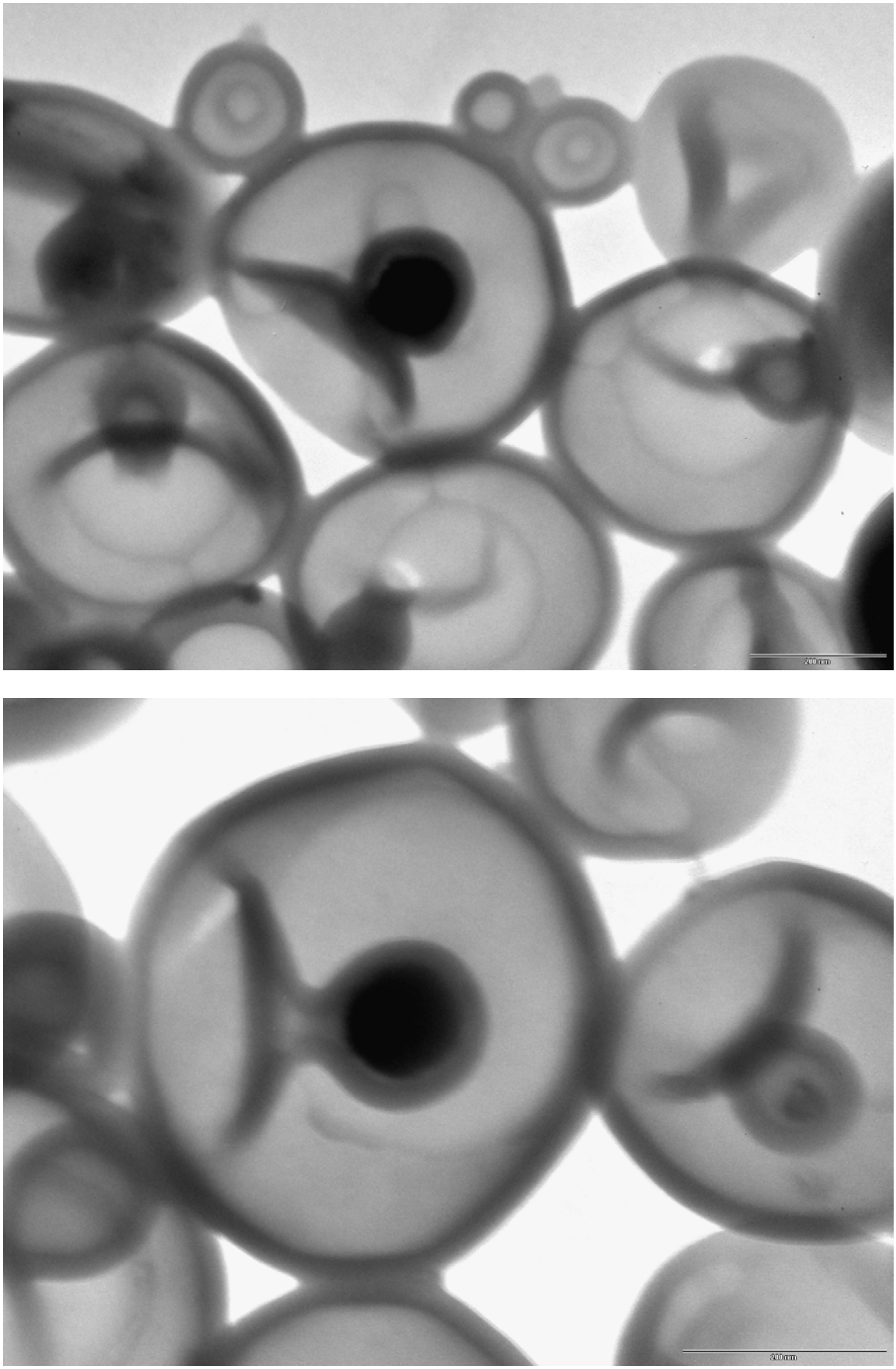


**Figure SF13.** TEM images of 100 nm PVP capped platinum nanoparticles entrapped inside the stomatocyte stomach during the shape transformation. Only one particle is engulfed inside the stomach while the size and the shape of the stomach is templated by the large nanoparticle.

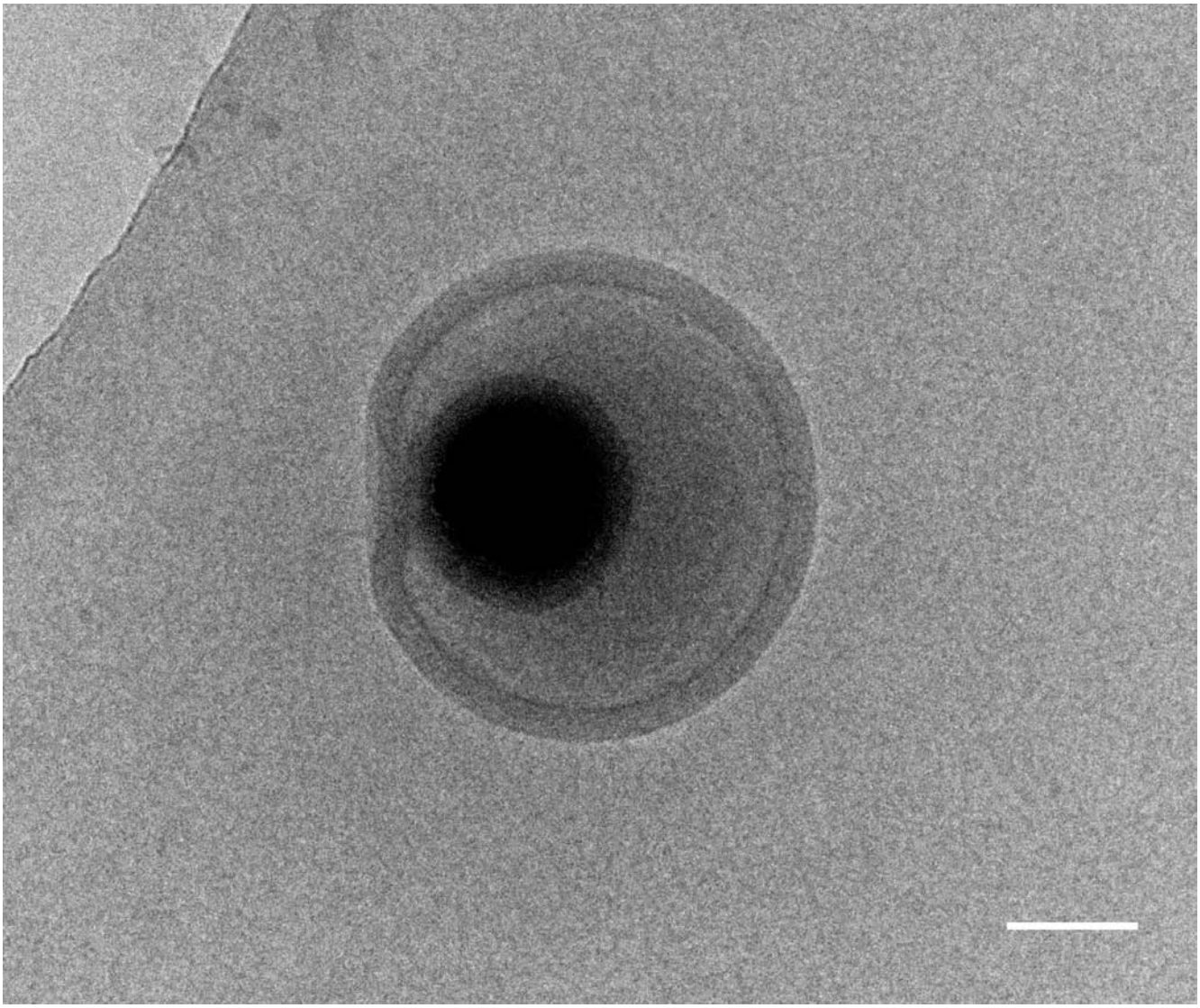


**Figure SF14.** Cryo-TEM image of a PVP capped platinum nanoparticle entrapped inside the stomatocyte stomach during the shape transformation. Only one particle is engulfed inside the stomach.

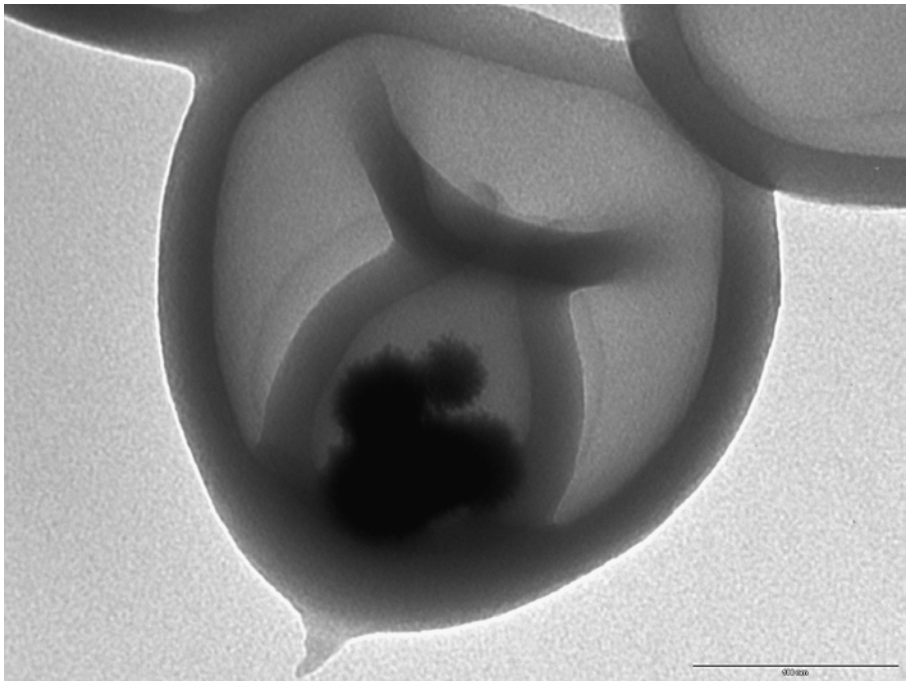


**Figure SF15.** TEM image of 50 nm PVP capped platinum nanoparticles entrapped inside the stomatocyte stomach during the shape transformation. Multiple platinum nanoparticles are engulfed inside the stomach.

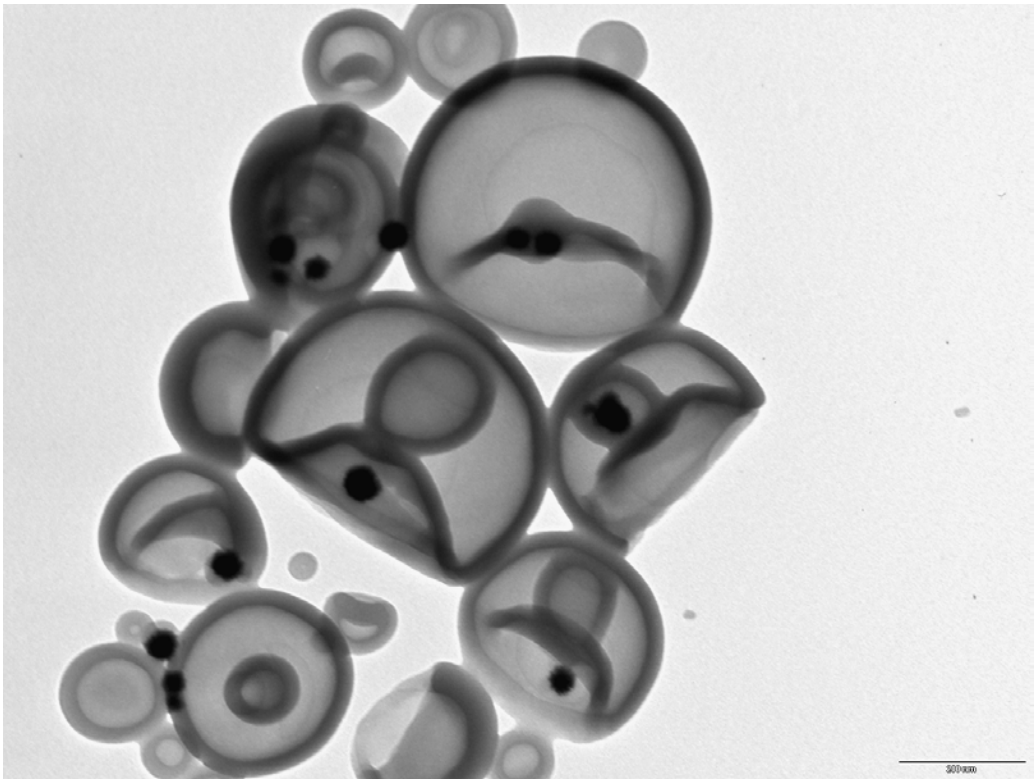


**Figure SF16.** TEM image of $PEG_{3k}$-diamine capped platinum nanoparticles entrapped inside the stomatocyte stomach during the shape transformation.

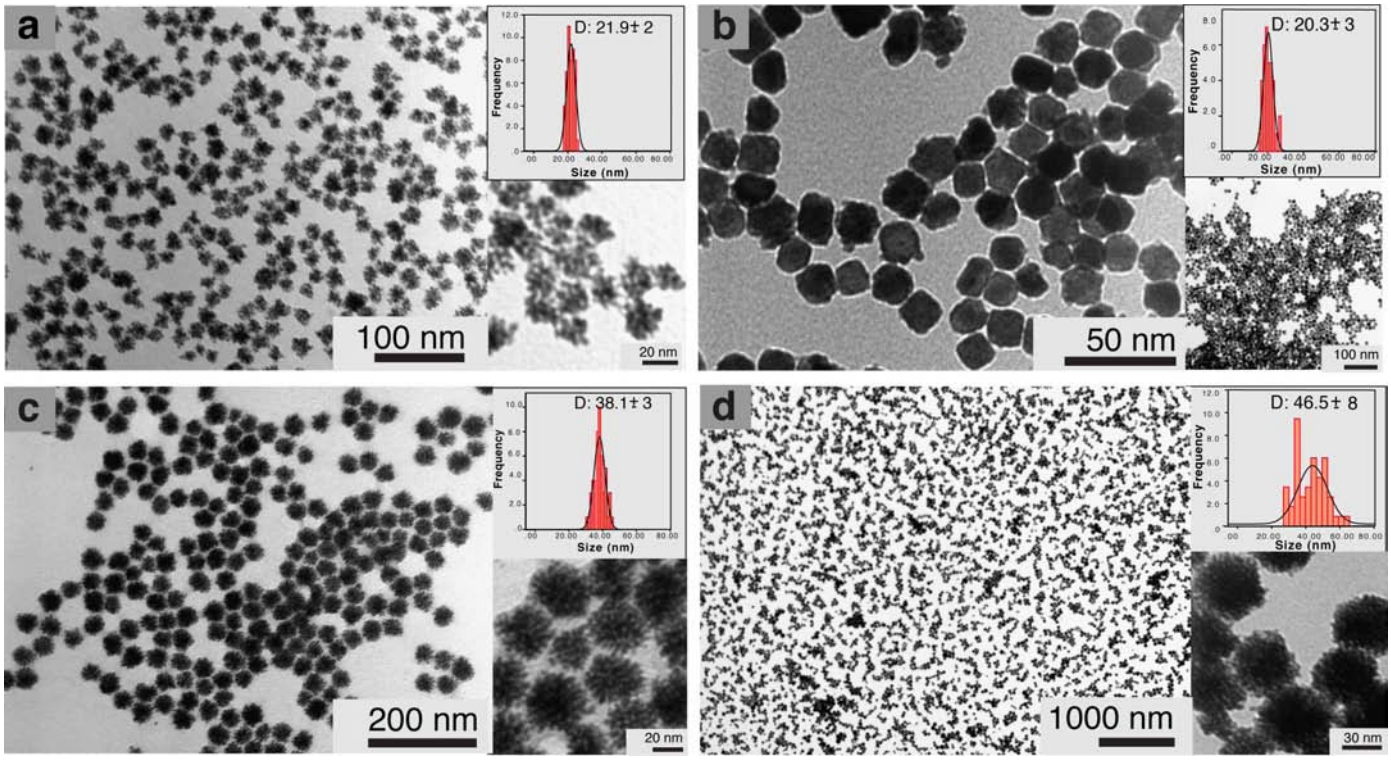



**Figure SF17. TEM of platinum nanoparticles with controlled shape and size**. **a**, TEM image of Pluronic F-127 capped Platinum nanoparticles obtained by the sonication method **b**, TEM of PAA capped platinum nanoparticles obtained by the hydrogen reduction method, **c**, TEM of PVP capped Platinum nanoparticles obtained by the sonication method. **d**, TEM of $PEG_{3k}$-$(NH_2)_2$ capped platinum nanoparticles obtained by the sonication method. Insets show the size distribution histograms and the detailed structures of the nanoparticles.

## References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