Supplementary information for:

Elucidating the anomalous membrane permeability of Ag(I), Cu(II), Zn(II) and Au(III) towards new nanoreactor strategies for synthesizing metal nanoparticles

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S1. Synthesis of AuNPs at 55°C

AuNPs were synthesised using 100 nm liposomes entrapping 200 mM HEPES as nanoreactors. In brief, a mixture of 10 mM liposome and 3.6 mM Au^{3+} was stirred at 55°C for 2 hours. The liposome preparation, cryo-TEM imaging and image analysis was conducted as described in the materials and method section of the main manuscript.

The outcome of the synthesis is AuNPs entrapped inside the liposomes (Fig. S1 b-d), however, larger AuNPs are also formed outside the liposomes. The latter may be explained by increased nucleation rate outside the liposomes and increased leakage of HEPES from the liposomes at 55°C. Still, a good correlation between the AuNP and liposome size is obtained (Fig. 1S a), which indicate nucleation rate limited growth of particles inside the liposomes. The latter was also observed for AuNPs formed at 40°C.

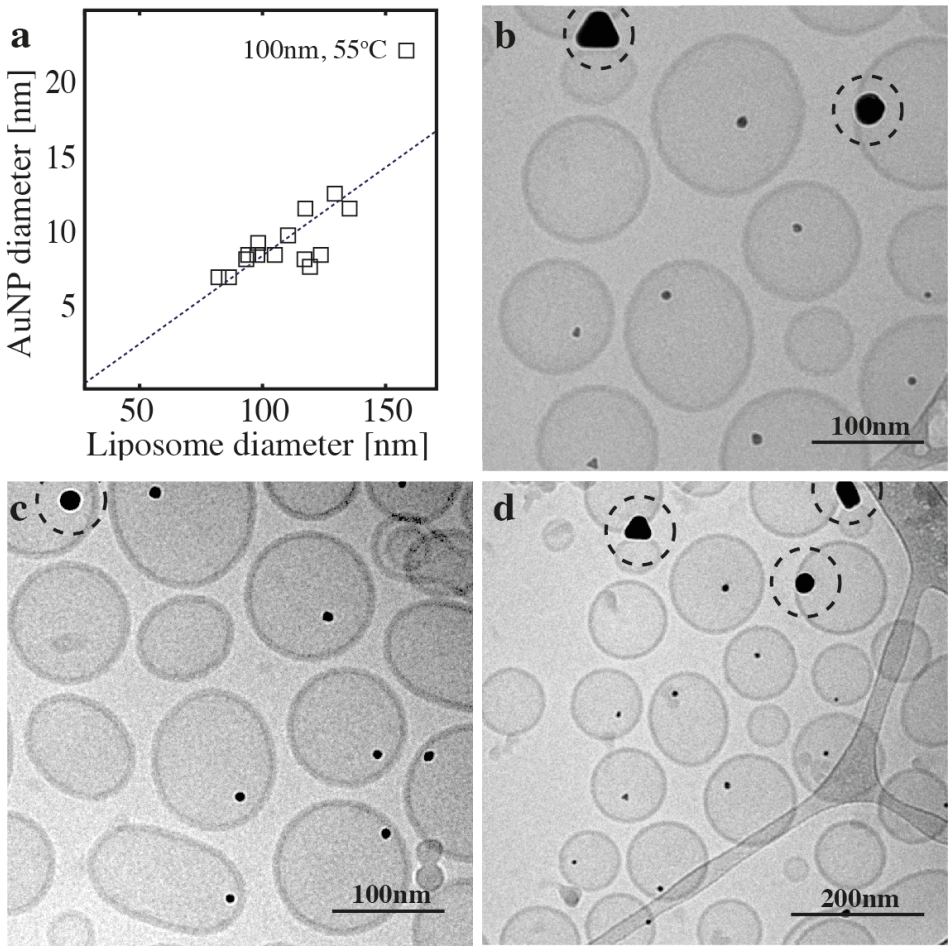

Figure S1: AuNP nanoreactor synthesis conducted at 55°C. Liposome - AuNP size correlation (a) and cryo-TEM images (b-d) of liposome nanoreactors entrapping AuNPs. The dashed circles (b-d) indicate larger AuNPs formed outside the liposomes.

S2. Analysis of AuNPs synthesized at RT, 40°C and 55°C

AuNPs were synthesised using 102 nm liposomes (PDI 0.02) entrapping 200 mM HEPES as nanoreactors. In brief, a mixture of 10 mM liposome and Au^{3+} was stirred at either room temperature (24-26°C), 40°C or 55°C. Au^{3+} was added in three portions. 1.2 mM was added initially, extra 1.2 mM after 30 min incubation and the final 1.2 mM was added upon 60 min incubation yielding a total of 3.6 mM Au^{3+}. Images of the reaction mixture and UV-VIS spectra were recorded as function of time and are presented in figure S2. UV-VIS were conducted using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific). The reaction was allowed to proceed for 3h after which the liposomes were stored at 5°C until Cryo-TEM analysis. For cryo-TEM analysis, 3 µL of sample was placed on a glow discharged lacy carbon 300 mesh

copper TEM grid (Ted Pella, Inc.), blotted, and plunge frozen in liquid ethane using a FEI Vitrobot Mark IV. The grids were imaged using a FEI Tecnai G2 T20 transmission electron microscope operated at 200 keV in low dose mode with a FEI High-Sensitive 4k x 4k Eagle camera located at the Core Facility for Integrated Microscopy, Faculty of Health and Medical Sciences, University of Copenhagen.

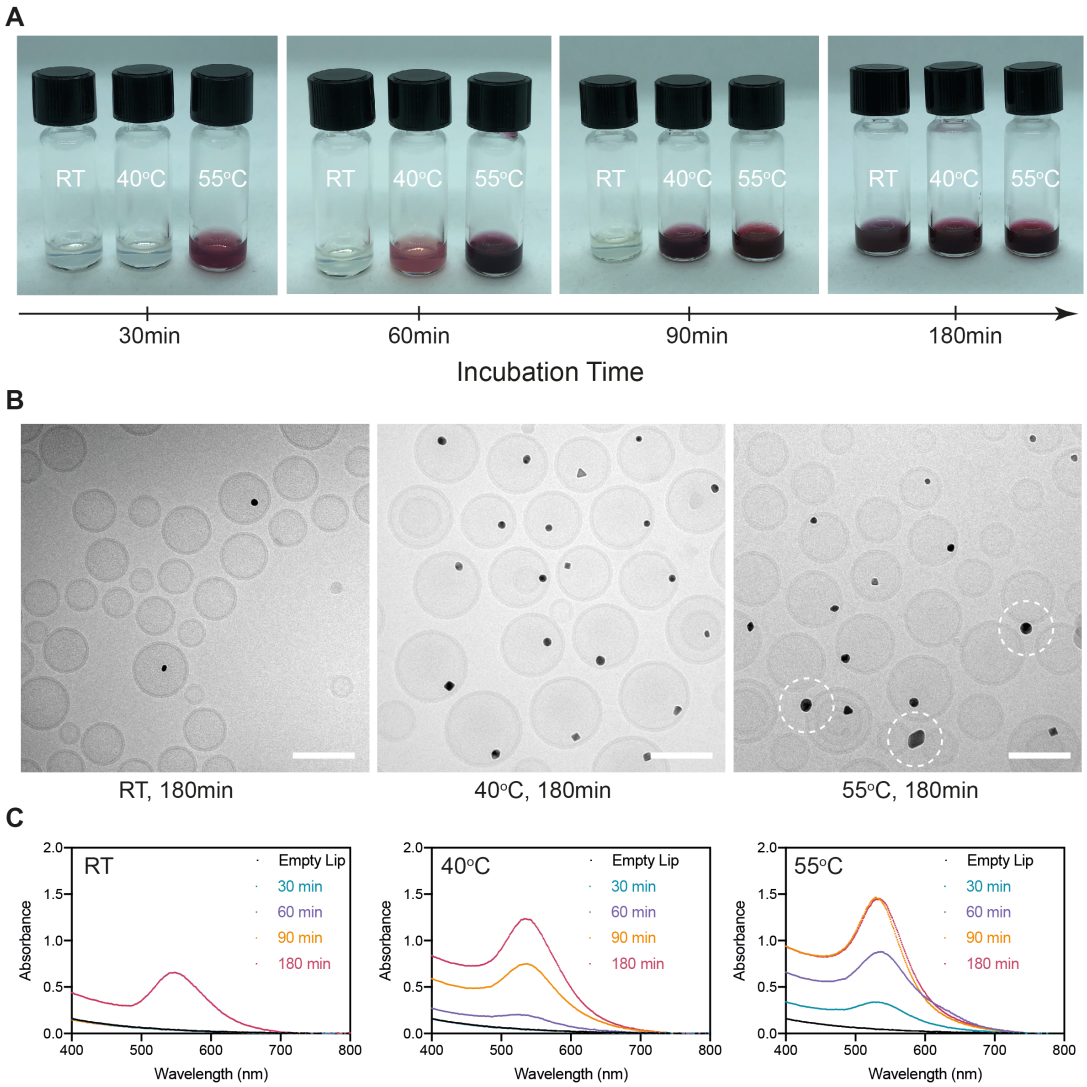

Figure S2: Images of reaction mixtures (A), cryo-TEM images (B) and UV-VIS spectra (C) of AuNP synthesis conducted at room temperature (RT), 40ºC or 55ºC. The dashed circle in inset B highlight AuNPs that reside outside a liposome nanoreactor. The scalebar in B corresponds to 100 nm.

The outcome shown in figure S2 is a gradual change in colour as function of time for all reaction mixtures. The reaction proceeds slower at RT, and few AuNPs are shown

to form inside the liposomes (Figure S2B). The reaction proceeds with the highest rate at 55°C, however AuNPs are also formed outside the liposomes. At 40°C, intermediate reaction speed is observed and AuNPs are predominantly formed inside the liposomes.

S3. High resolution TEM and TEM-EDS of AuNPs synthesized at 40°C

TEM and TEM-EDS analysis were performed on the AuNP liposome sample prepared at 40°C. Preparation of the sample is described in S2. For TEM-EDS analysis, 3 µL of sample was placed on a glow discharged 400 mesh nickel TEM grids with an ultra-thin carbon film (Electron Microscopy Sciences) and allowed to adsorb. After 10 seconds the excess solution was wicked away using filter paper. The grids were rinsed twice on droplets of milliQ water with the excess water removed via filter paper between rinse steps. The grids were then allowed to air dry prior to imaging. Transmission electron microscopy was performed using a FEI Tecnai G2 T20 transmission electron microscope operated at 200 keV located at the Center for Electron Nanoscopy at the Technical University of Denmark. Images were acquired using a TVIPS-XF416 CMOS camera and EDS spectra were acquired using an Oxford X-Max 80T x-ray detector.

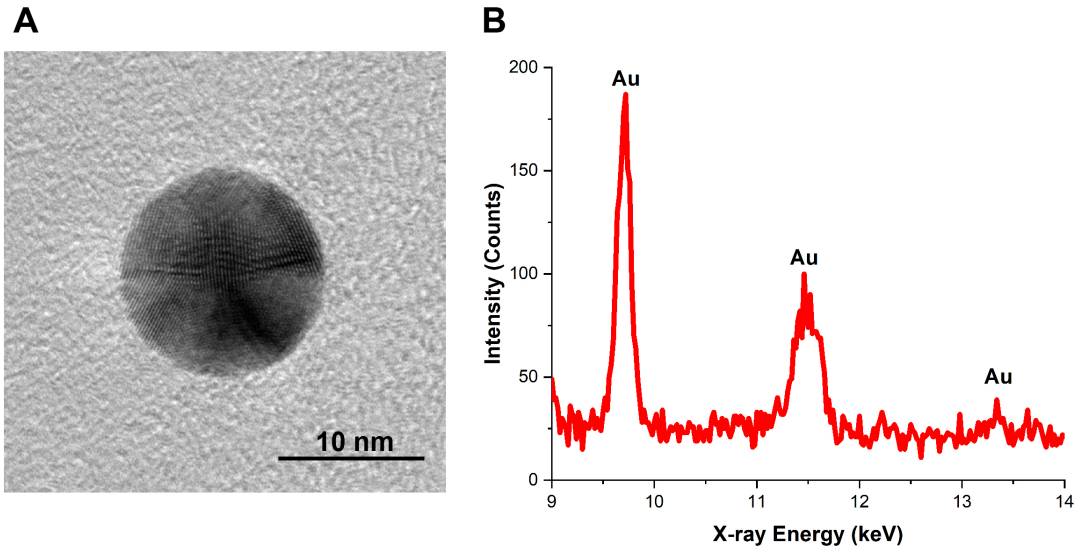

Figure S3: (A) High resolution phase contrast TEM image of a Au nanoparticle with lattice fringes corresponding to the 111 and 200 lattice spacings for Au, 0.235 and 0.2035 nm respectively. (B) Energy dispersive x-ray spectrum from (A) showing the characteristic x-ray peaks for Au at 9.7, 11.4, and 13.4 keV verifying that the nanoparticle is comprised of Au.