

SUPPLEMENTARY INFORMATION

Engineering Biomolecular Systems: Controlling the Self-Assembly of Gelatin to form Ultra-Small Bioactive Nanomaterials

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*Supporting 3D spheroid reconstruction video (Sv) can be accessed as a separate file (.PPTX)

SUPPORTING FIGURES BELOW:

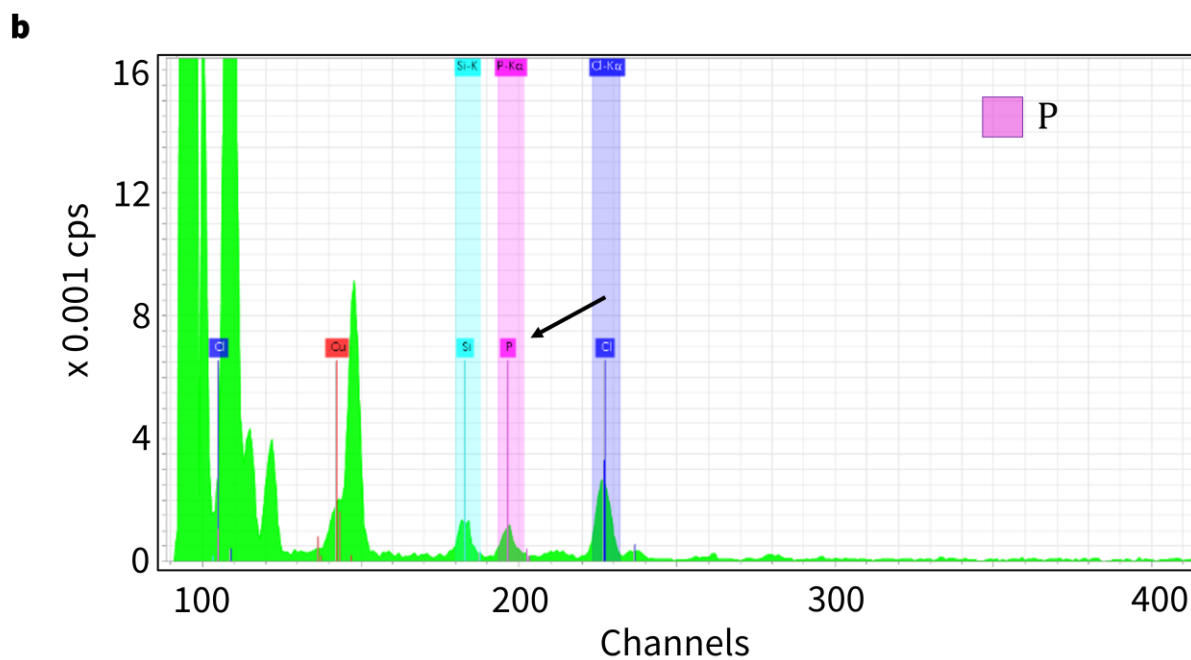
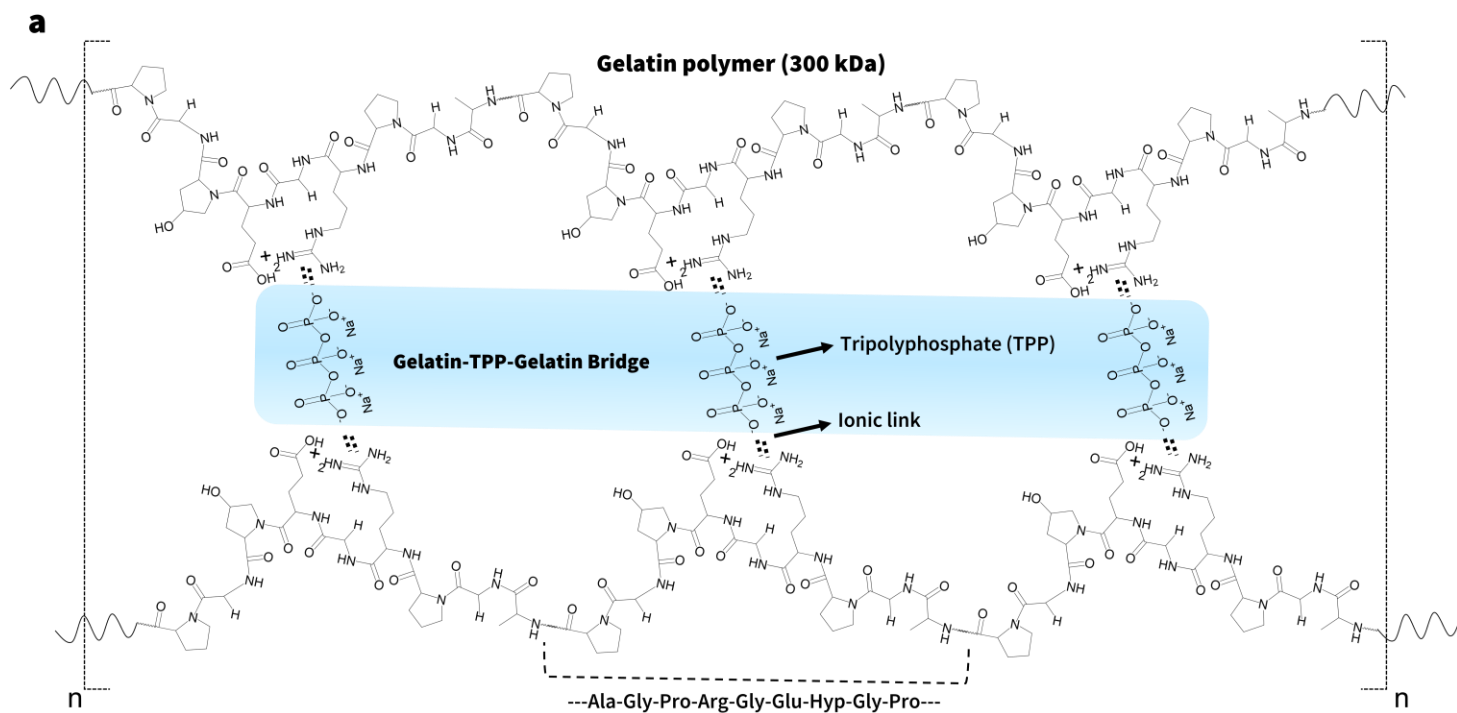


Figure S1. (a) Molecular assembly of Gelatin-TPP-Gelatin bridge, and (b) phosphorus analysis for G^x analyzed using STEM-EDS showing a phosphorus-peak indicating presence of TPP in G^x .

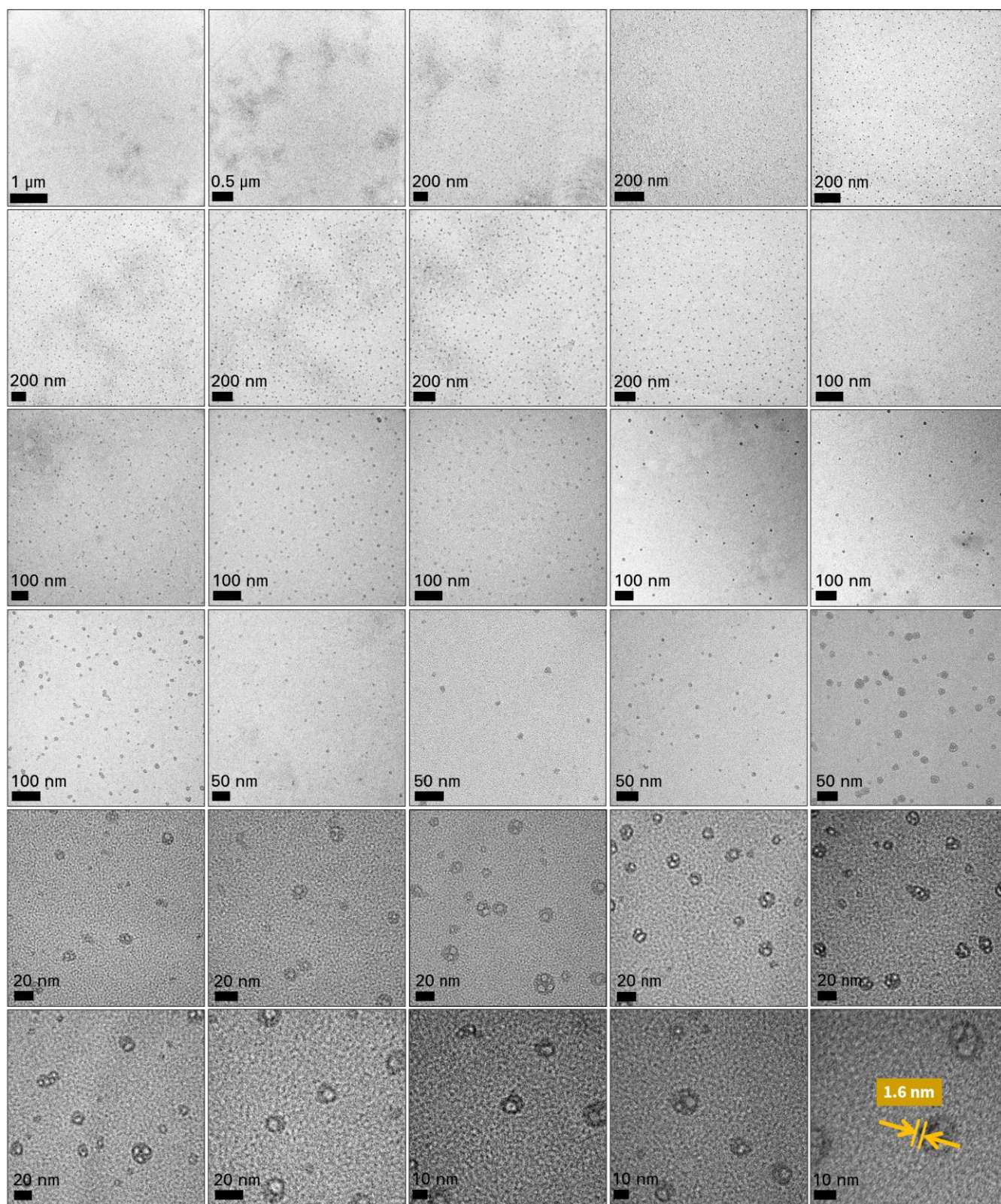
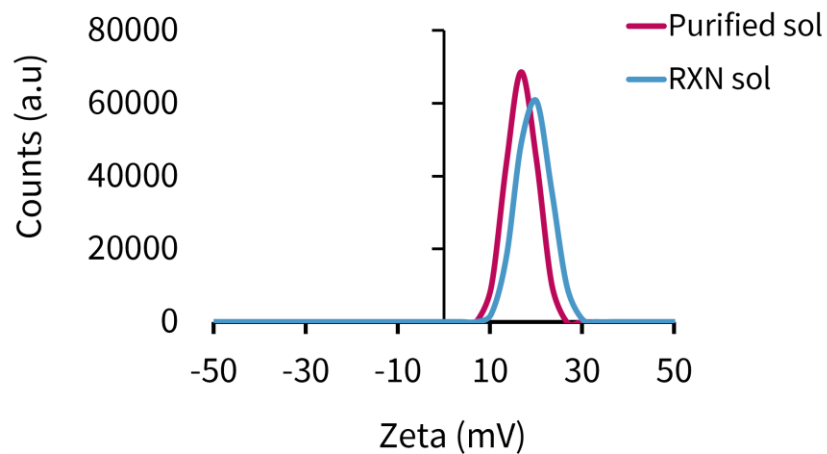


Figure S2. HR-TEM images of G^x at multiple magnifications from synthesized batches indicating homogenous spherical nanoparticles. Particles exhibited polyhedral geometry with a ~ 1.6 nm layer thickness.

G^X - TPP Zeta



Sample	Zeta (mV)	SE
Purified sol	19.67	1.78
RXN sol	14.30	1.60

Figure S3. Zeta potential spectra of G^X from the reaction solution and purified resuspended solution.

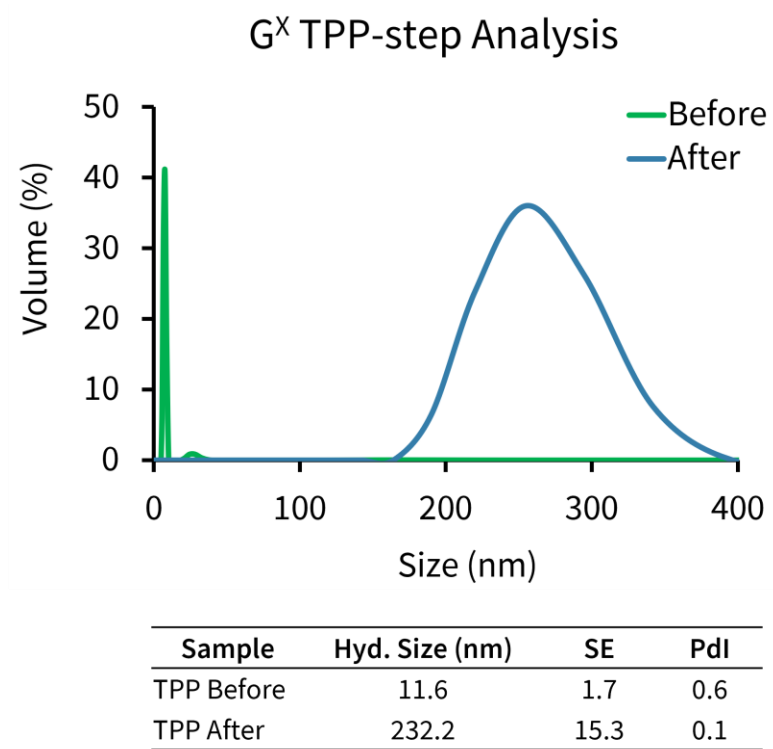
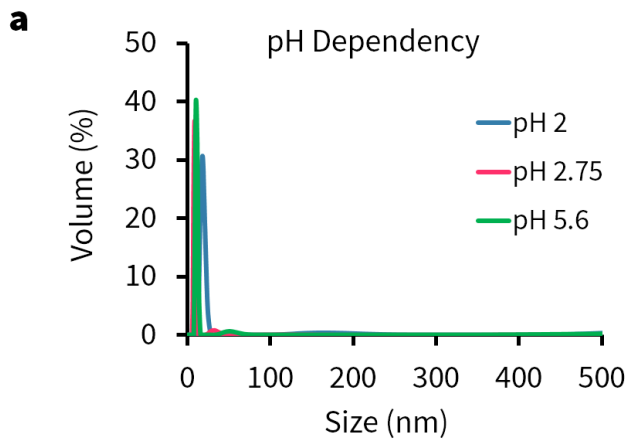
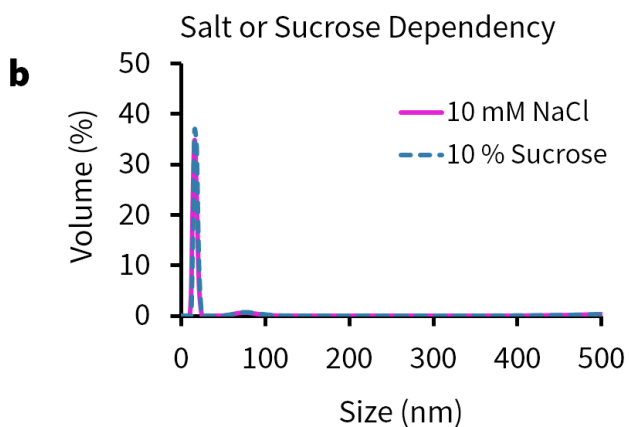


Figure S4. Effect on G^x formation by the addition of TPP before (early) or after (late) addition of nanoprecipitant.



Sample	Hyd. Size (nm)	Nanoprecipitant (ml)	Observation
pH 2	16.2	15.0	Whitish Translucent
pH 2.75	9.9	10.0	Whitish Opaque
pH 5.6	8.0	2.0	*Glassy

*pH 5.6 Reaction batch did not require reheating



Sample	Hyd. Size (nm)	SE	PdI	Observation
10 mM NaCl	13.7	0.9	0.6	No difference
10 % Sucrose	16.4	2.0	0.6	No difference

Figure S5. Effect on G^x formation with varying (a) reaction-pH or (b) addition of NaCl/Sucrose for gelatin stabilization.

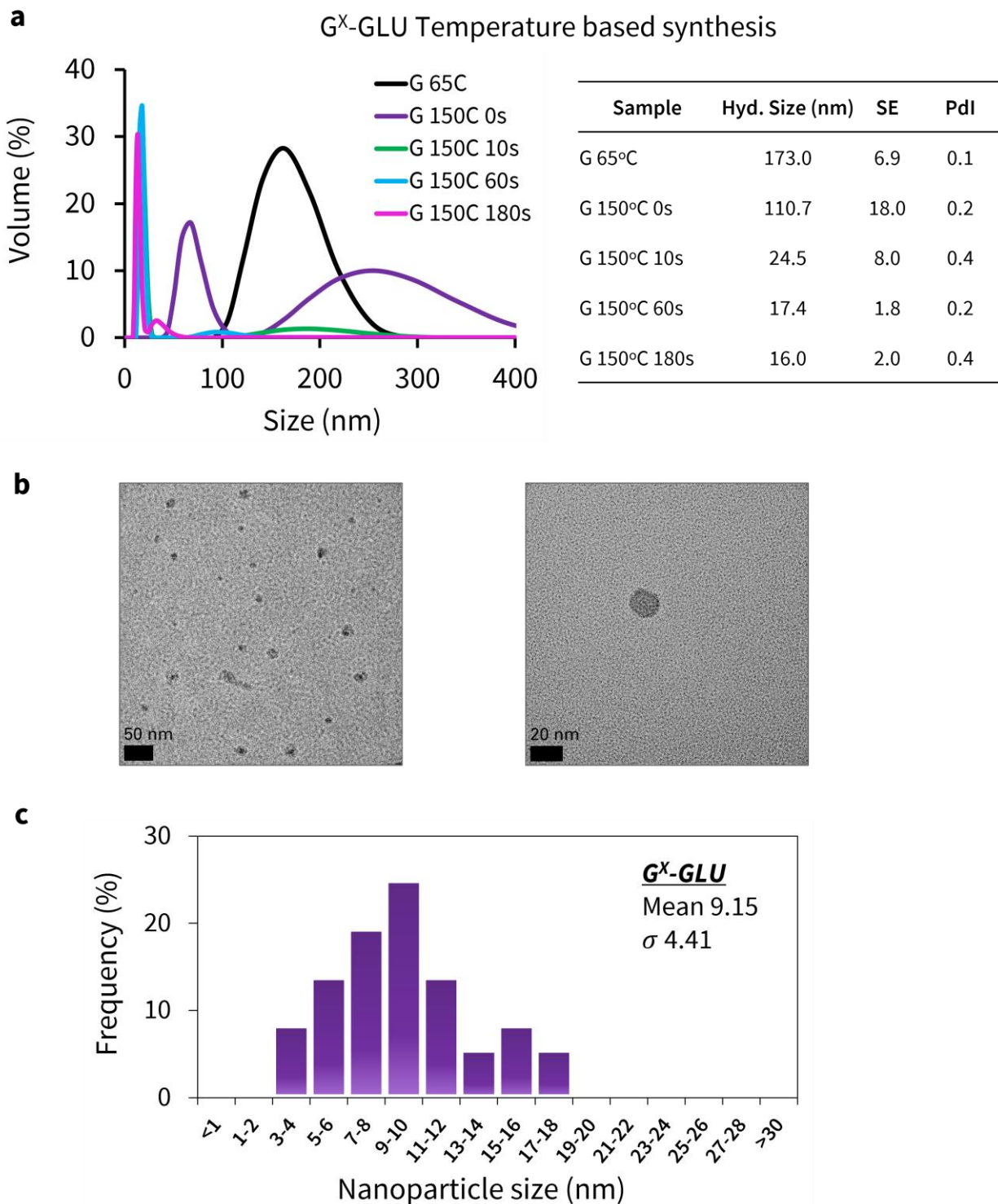


Figure S6. (a) Effect on G^X formation by varying reaction-temperature with glutaraldehyde (GLU) as crosslinker. In this reaction, ethanol:acetone mixture (1:10 v/v) was added to the RBF (25 mlh^{-1}) containing acidified gel sol until a white opaque colloid formed. The solution was then heated to 150°C and the solution turned to a translucent whitish solution. At this stage, GLU ($25 \mu\text{l}$; 25% v/v) mixed with ethanol ($175 \mu\text{l}$) was added dropwise and the reaction was stirred (900 RPM ; 55°C) until 18 h. (b) HR-TEM images showed formation of non-uniform $\sim 10 \text{ nm}$ nanoparticles. (c) Corresponding size-distribution histogram.

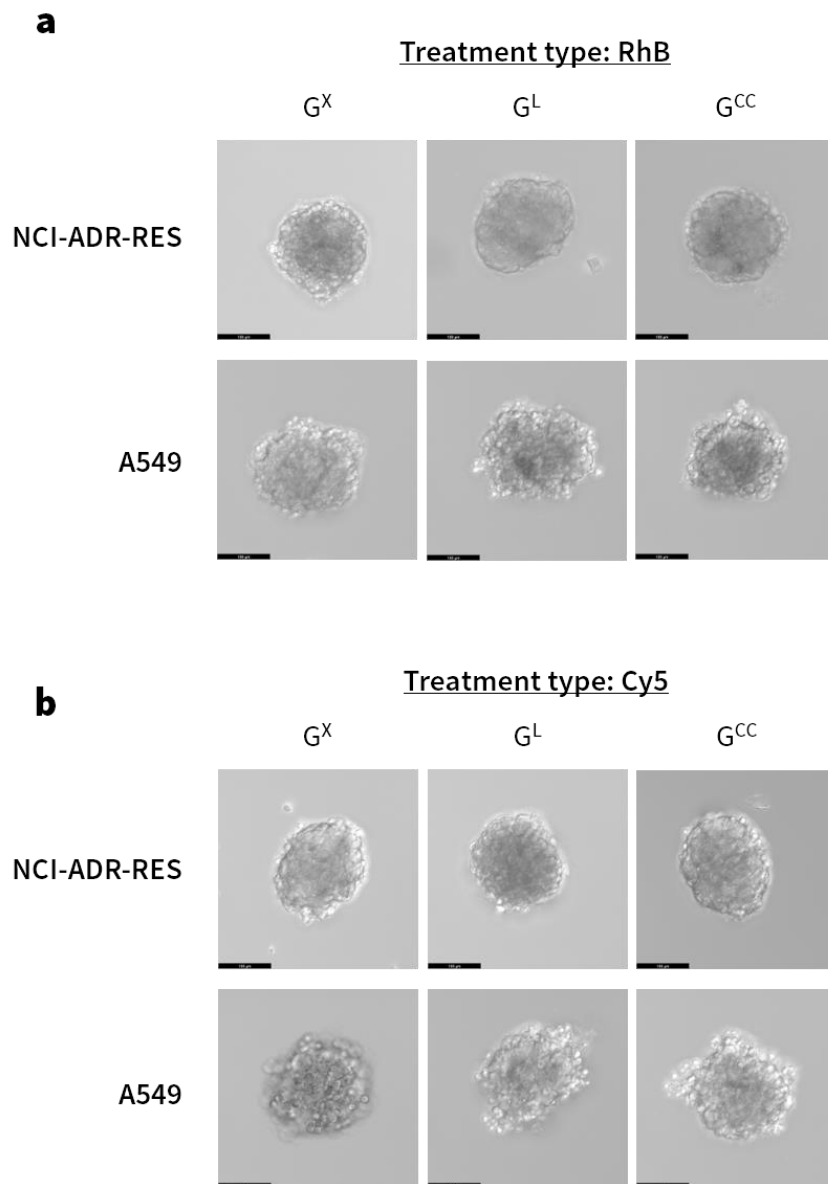
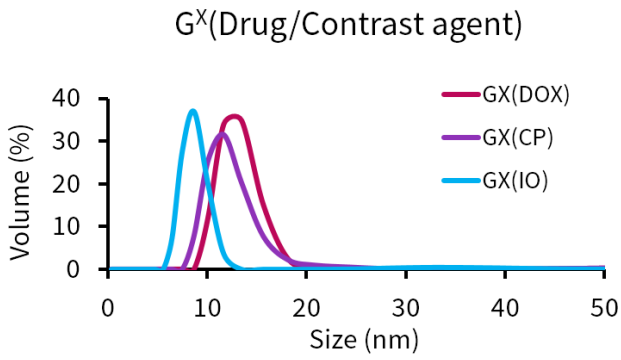


Figure S7. Representative bright-field images for NCI-ADR-RES and A549 3D tumor spheroids treated with G^X , G^L and G^{CC} tagged with (a) RhB or (b) Cy5 for ~6h. Results show negligible difference to spheroid morphology indicating minimal cytotoxicity.



Sample	Hyd. Size (nm)	SE	Pdl
G^X (DOX)	12.5	1.8	0.8
G^X (CP)	18.9	7.3	0.5
G^X (IO)	7.9	0.6	0.8

Sample	Zeta (mV)	SE
G^X (DOX)	22.5	2.3
G^X (CP)	19.9	1.7
G^X (IO)	25.3	0.1

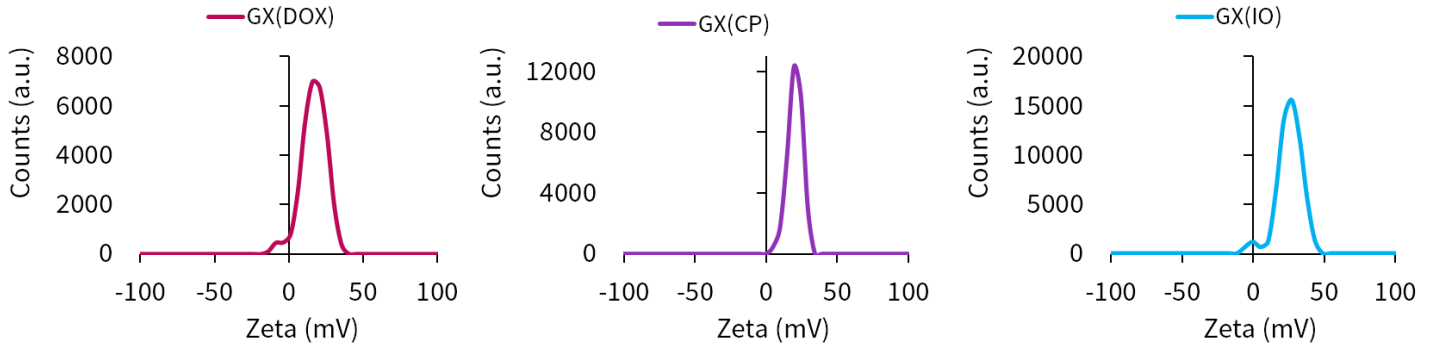


Figure S8. Particle size and zeta potential spectra for drug/ contrast-agent encapsulated G^X constructs.

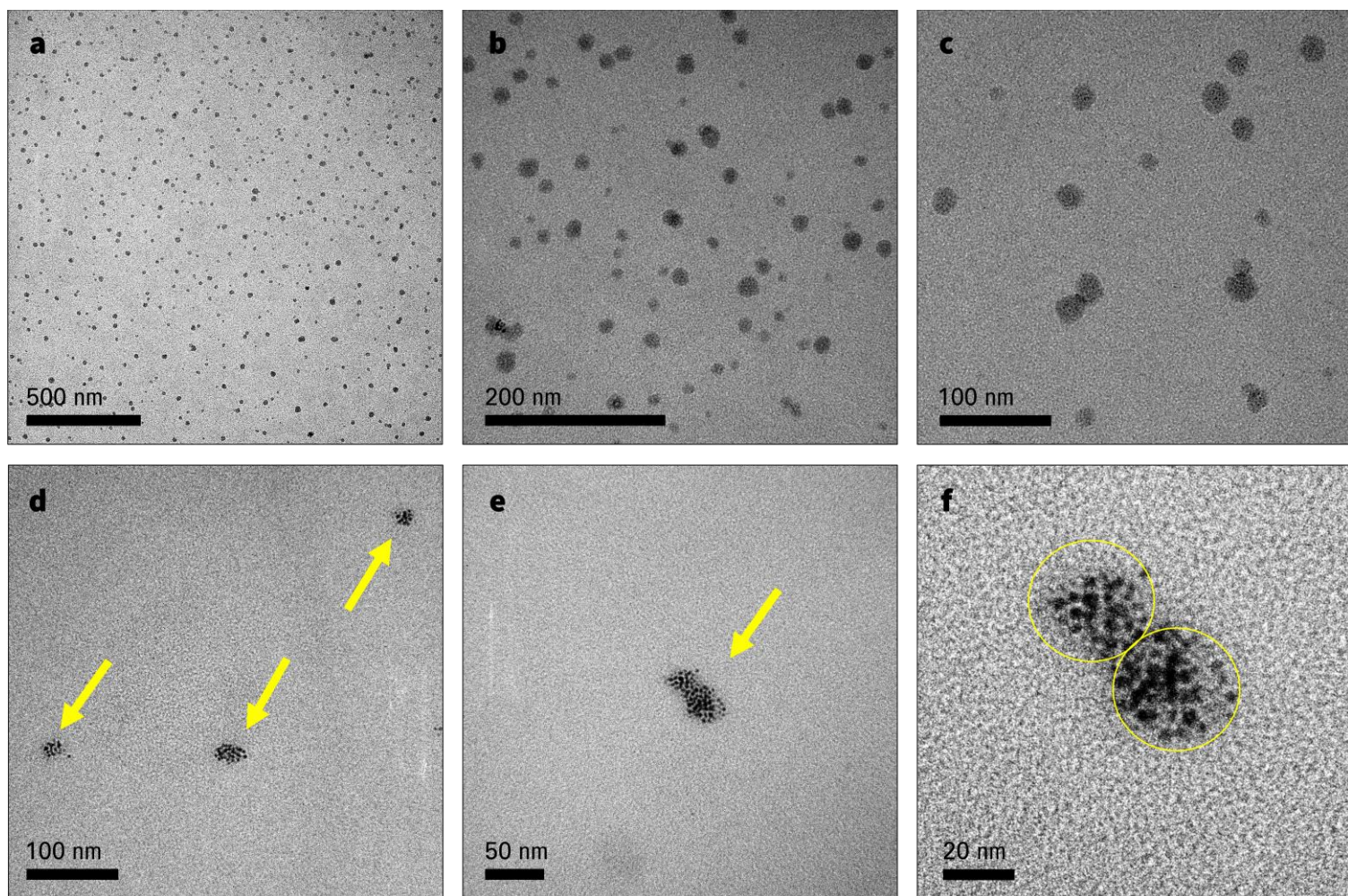
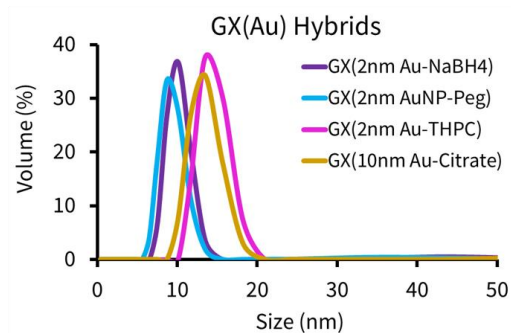
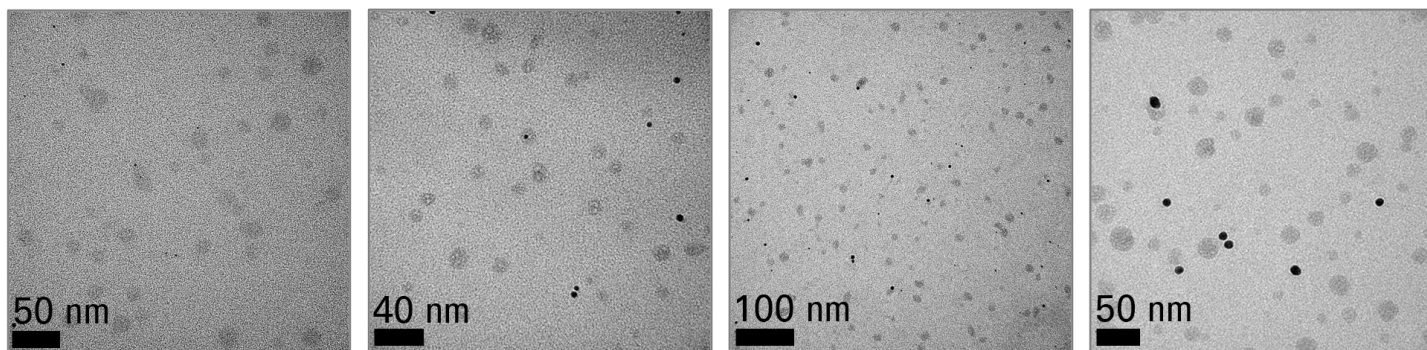


Figure S9. Supporting HR-TEM images for $G^x(\text{AuNP})$ hybrid nanocomposites formed by encapsulating ultra-small 2 nm Au-DTDTPA coated nanoparticles within G^x .

a

Sample	Hyd. Size (nm)	SE	Pdl
G ^X (2nm Au-NaBH ₄)	10.0	0.5	0.9
G ^X (2nm AuNP-Peg)	12.6	2.3	0.8
G ^X (2nm Au-THPC)	13.4	0.9	0.6
G ^X (5-10nm Au-Citrate)	17.5	4.3	0.8

Sample	Zeta (mV)	SE	Pdl
G ^X (2 nm Au-NaBH ₄)	27.00	1.47	0.9
G ^X (2 nm AuNP-Peg)	27.13	0.68	0.8
G ^X (2 nm Au-THPC)	26.20	0.24	0.6
G ^X (5-10 nm Au-Citrate)	25.70	0.68	0.8

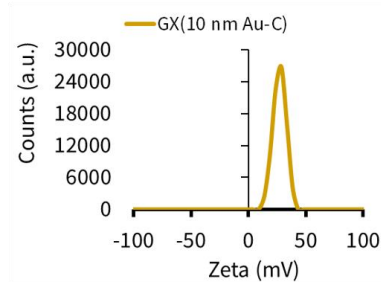
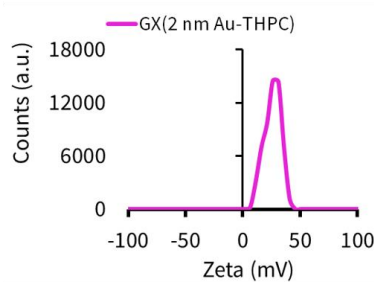
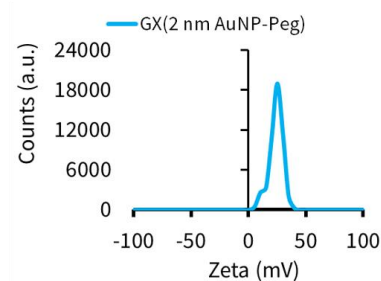
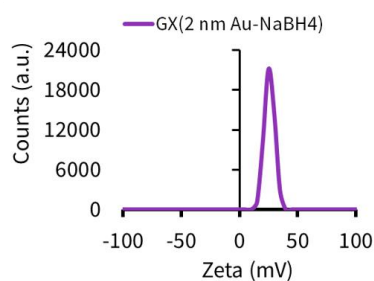
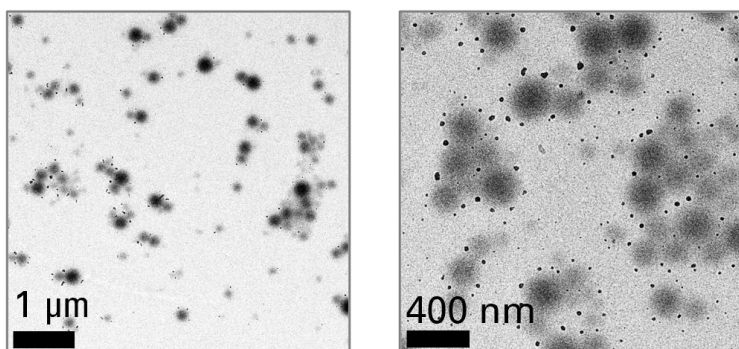
**b**

Figure S10. HR-TEM, Particle size and Zeta potential data for (a) alternative G^X(AuNP) hybrid nanostructures and (b) HR-TEM images showing tunability of G^{CC}-G^X-Satellite hybrid nanocomposites using addition of higher amount of glutaraldehyde.

Temp Restabilization

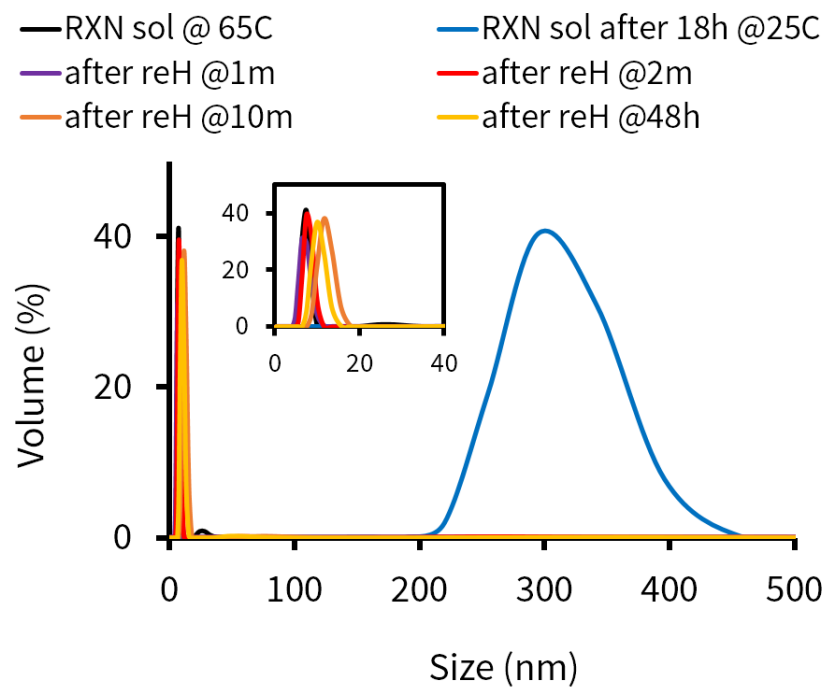
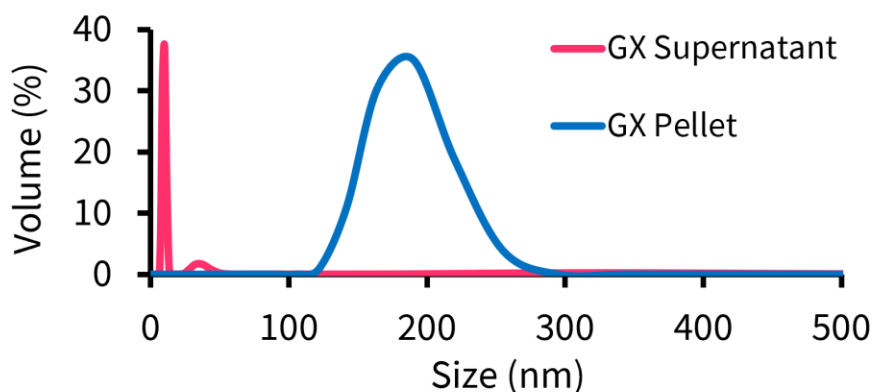


Figure S11. Particle size data after reheating cooled reaction solution for monitoring hydrodynamic size stability.

a

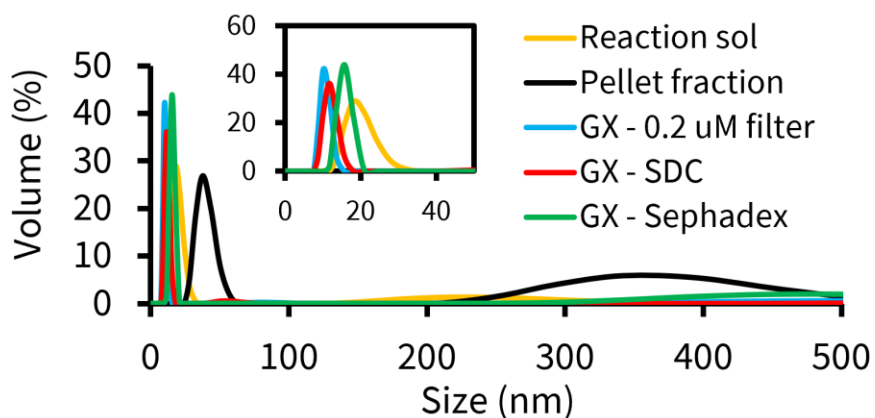
G^X Reaction solution fractions



Sample	Hyd. Size (nm)	SE	PdI
GX Supernatant	11.7	0.8	0.4
GX Pellet	180.6	1.1	0.03

b

G^X TPP Purification



Sample	Hyd. Size (nm)	SE	PdI
Reaction sol (supernatant)	16.3	4.2	0.5
Pellet fraction	35.5	4.5	0.8
G ^X - 0.2 μ M filter	21.7	6.5	0.6
G ^X - Sucrose-DC	10.8	0.3	0.3
G ^X - Sephadex	15.8	1.09	0.5

Figure S12. DLS spectra for (a) centrifuged reaction solution after isolating G^X to remove trace larger-sized particles found in pellet (b) purification of G^X through filter, sucrose-density centrifugation (SDC) or Sepharose column.