Supplementary Information for *In situ* transmission electron microscopy observations of $CaCO_3$ crystallization onto polysaccharide-coated nanoparticles

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1. Supplementary Figures

Fig. S1 Attenuated total reflectance (ATR) spectra of SiO_2-NH_3^+ nanoparticles (**upper**), chitosan-coated nanoparticles (**middle**), heparin-coated nanoparticles (**lower**). The polysaccharide-containing systems exhibit two peaks over 2750 – 3000 cm^{-1}, indicative of the crosslinking material (glutaraldehyde). Many of the signals from the polysaccharides cannot be confidently distinguished from the SiO_2 nanoparticles or noise. For example, polysaccharide C-N bonds likely introduce the shoulder in the peak ~1100 cm^{-1}, and C=N bonds formed between glutaraldehyde and the polysaccharides appear ~1600 cm^{-1}.[1-3]

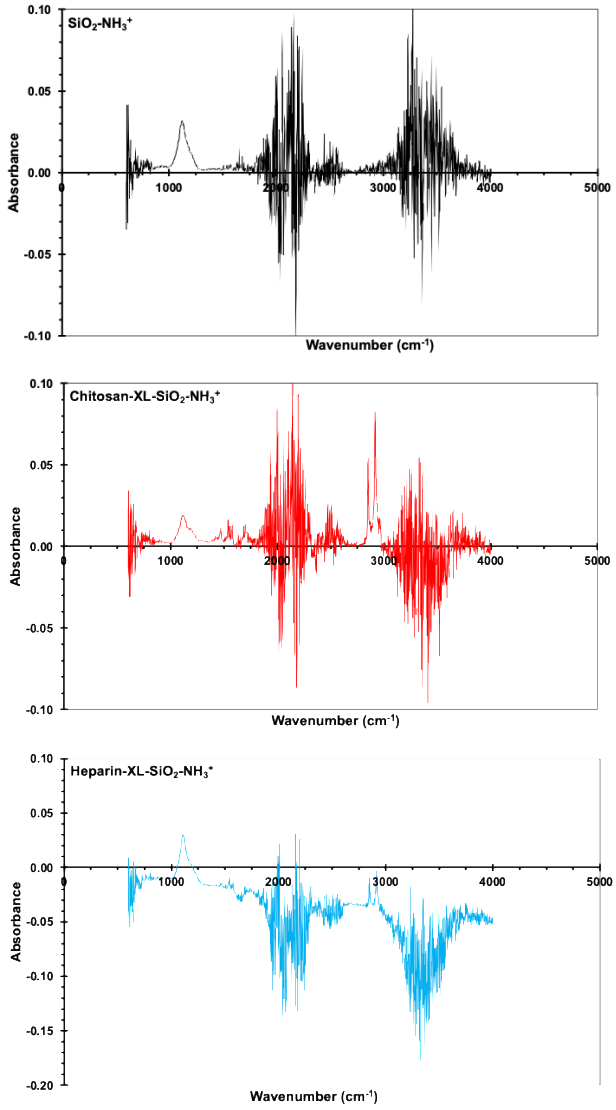
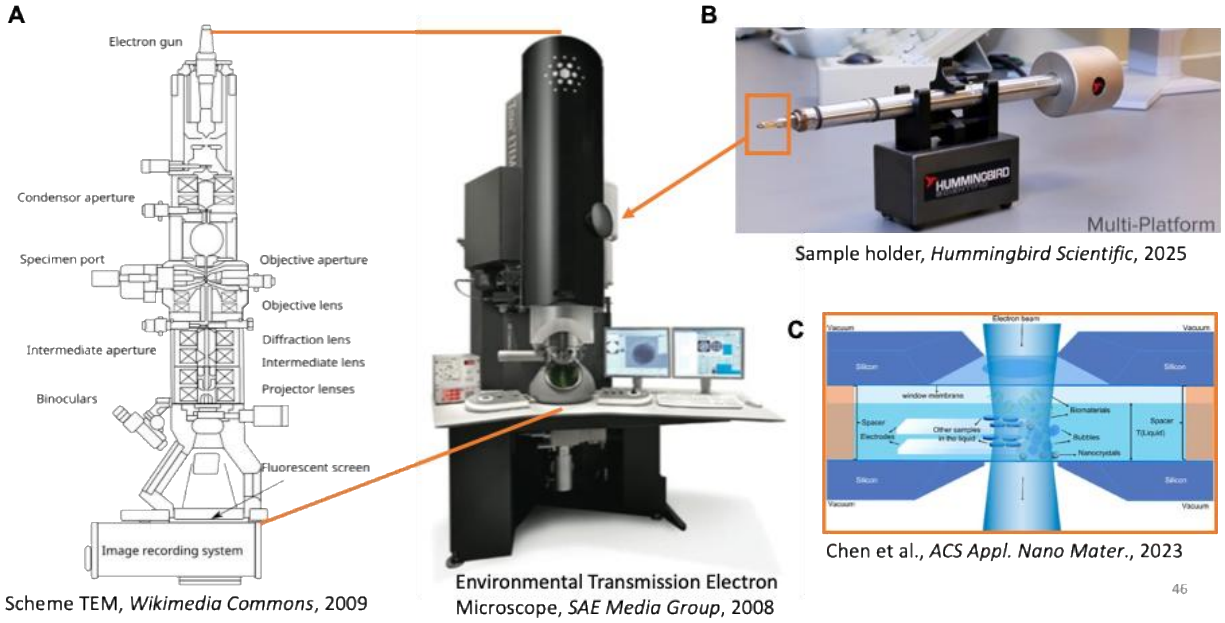


Fig. S2: Representation of the *in situ* LP-TEM experimental setup. (**A**) Transmission electron microscope components and example image of a Thermo Fisher Scientific field emission Titan ETEM. The instrument is approximately 10 ft tall. (**B**) The sample holder with the liquid cell composed of 2.6 mm^2 chips. (**C**) Schematic of a typical liquid cell during an experiment. (**D**) Example image from PNNL. Dr. Biao Jin observing aggregated SiO_2 nanoparticles via TEM.



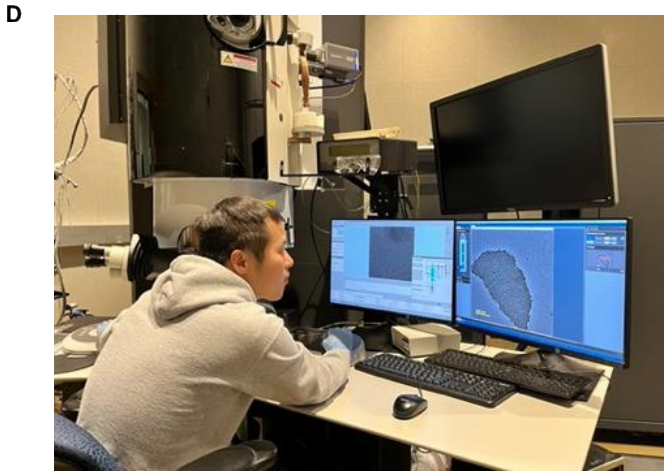


Figure S3: Selected area electron diffraction (SAED, **left**) could not discern if the particles were crystalline or amorphous material. Vaterite and calcite were observed *ex situ* (**right**).

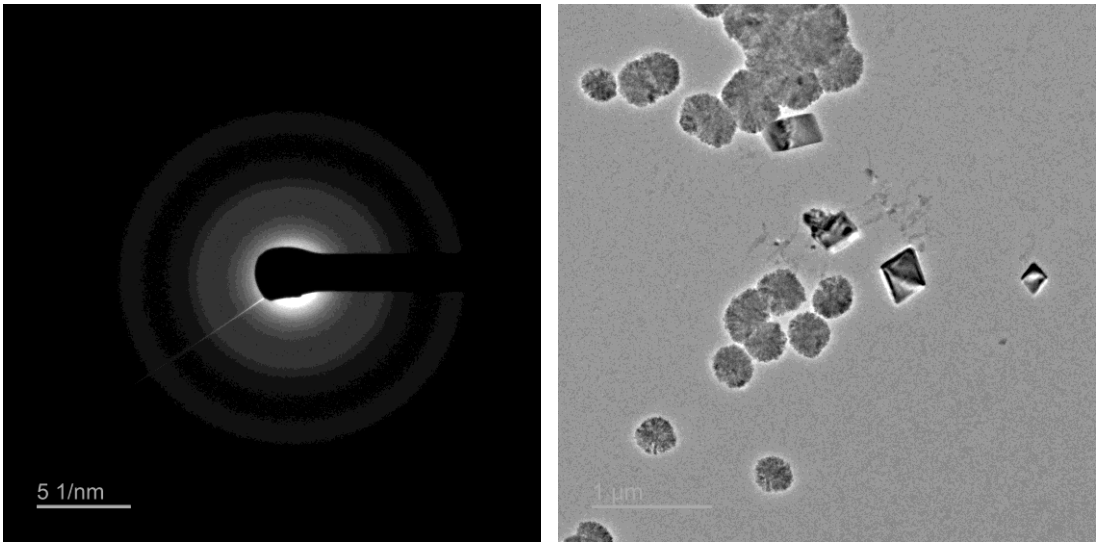


Fig. S4. *In situ* LP-TEM images from the three heparin experiments demonstrate the reproducibility of the findings.

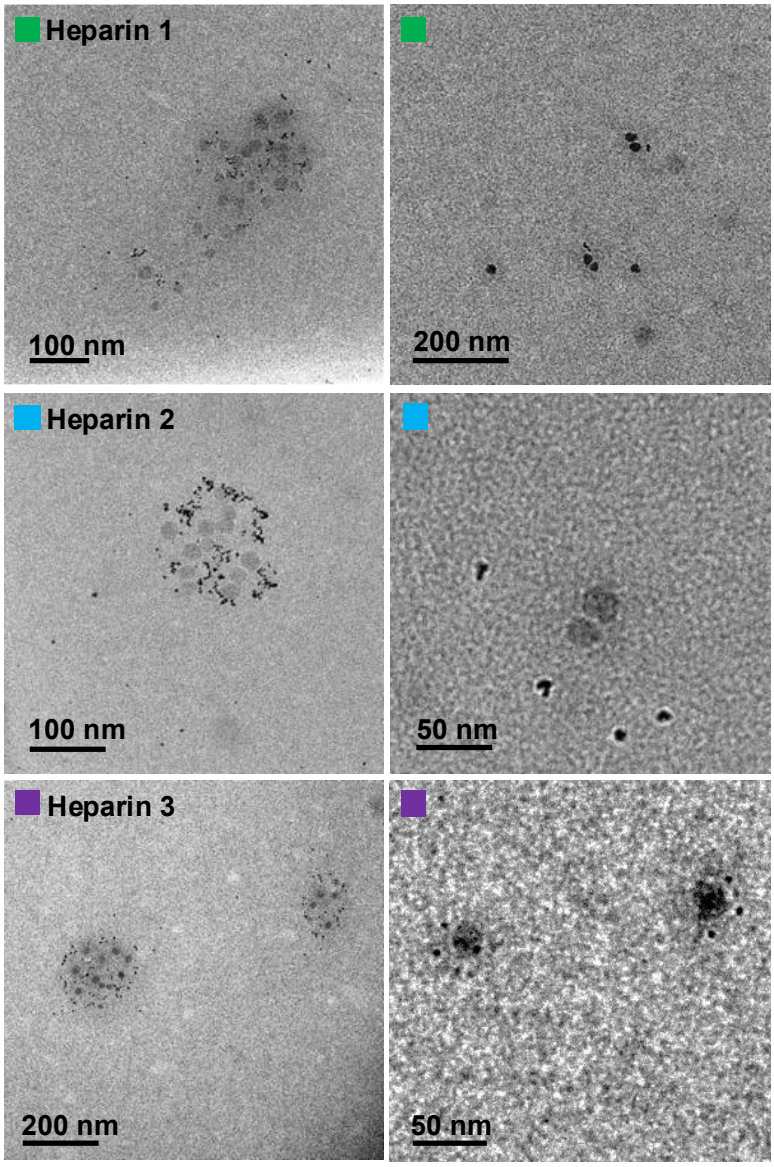
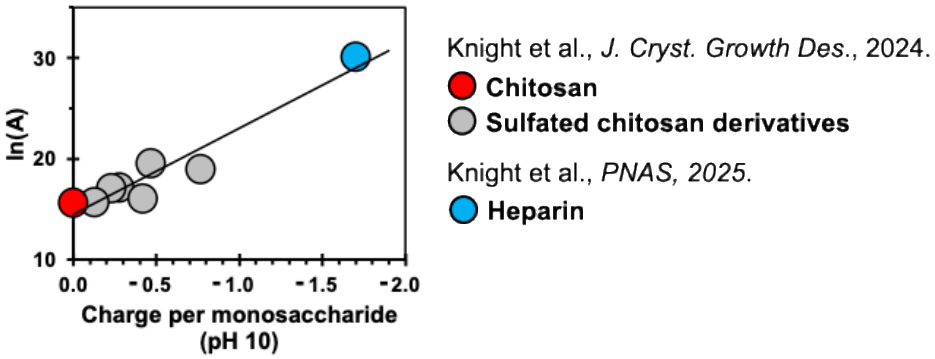


Fig. S5. Reported measurements of calcite nucleation kinetics show the kinetic prefactor (ln(A)) increases with increasing net charge of the polysaccharide. The chitosan and heparin materials in this TEM study are the same as those investigated in Knight et al., 2024 and 2025,[4, 5] respectively. To the best of our knowledge, the ln(A) for $CaCO_3$ nucleation onto aminated silica nanoparticles has not been measured and hence is unavailable for comparison.



2. Supplementary experiments using different solution conditions

2.1 Crosslinked polysaccharides without SiO_2 nanoparticles

Chitosan and heparin were dissolved and crosslinked as described in **Sect. 2.2**) For these TEM experiments, 0.1 mL 2% of the crosslinked polysaccharide solution was applied to the spacer chip instead of nanoparticle solution (**Sect. 2.3**). Globules of crosslinked polysaccharide were visible in the TEM liquid cell (**Fig. S6**). After 30 minutes, no $CaCO_3$ nucleation was observed in the chitosan system, and only 1-2 crystals were observed in the heparin system (**Fig. S6**). One explanation for this result is that the polysaccharides were absorbing Ca^{2+} (heparin) or possibly HCO_3^- (chitosan), decreasing local supersaturation and leading to a nucleation rate governed by counterion diffusion into the globule. Similar results were reported for $CaCO_3$ nucleation in the presence of polystyrene sulfonate by Smeets et al.[6]

2.2 High ionic strength (IS = 0.6 M)

A previous kinetic study of $CaCO_3$ nucleation in the presence of chitosan and alginate demonstrates a dependence of the energy barrier to $CaCO_3$ nucleation on ionic strength (IS).[7, 8] Complementary molecular dynamics simulations suggest both polysaccharide properties (e.g., charge) and ionic strength modifies the distances of Ca^{2+} and HCO_3^- ions from the polysaccharide-water interface to change the probability of finding Ca^{2+}-HCO_3^- in a localized environment (0.5 – 2 nm).[7] To test the influence of solution ionic strength on the distance of $CaCO_3$ nucleation from the interface, we performed additional TEM experiments using the aminated silica nanoparticles, chitosan-coated, and heparin-coated nanoparticles at high ionic strength. Experiments were conducted as described in **Sect. 2.3** with the NaCl concentration of the $CaCl_2$ solution adjusted to 1.165 mM, resulting in a final ionic strength of 600 mM in the TEM liquid cell.

In the control, SiO_2-NH_3^+ system at high ionic strength conditions, we observed aggregates of smaller $CaCO_3$ crystals in bulk solution without association with the nanoparticles (**Fig. S7**). In the presence of chitosan and heparin, nanoparticle functionalization appeared to fail. Plausible explanations for the failed results at higher ionic strength conditions are slower crosslinking with glutaraldehyde and decreases in SiO_2-NH_3^+ nanoparticle stability.[9, 10] Dark globules, like those in **Fig. S6**, were observed and were not correlated to or surrounding the

SiO_2-NH_3^+ nanoparticles. Instead, the $CaCO_3$ particles nucleated in the bulk solution of the chitosan system, suggesting the coating may have been released to the bulk solution. In the heparin system, little to no nucleation was observed, and there appeared to be some solution phase separation as evidenced by regions of high and low mass density in the bulk solution (**Fig. S7**).

Fig. S6. $CaCO_3$ nucleation in the presence of crosslinked polysaccharides without aminated silica nanoparticles. Chitosan (**left**) and heparin (**middle**) results are qualitatively like those reported by Smeets et al.[6] for polystyrene sulfonate (**right**).

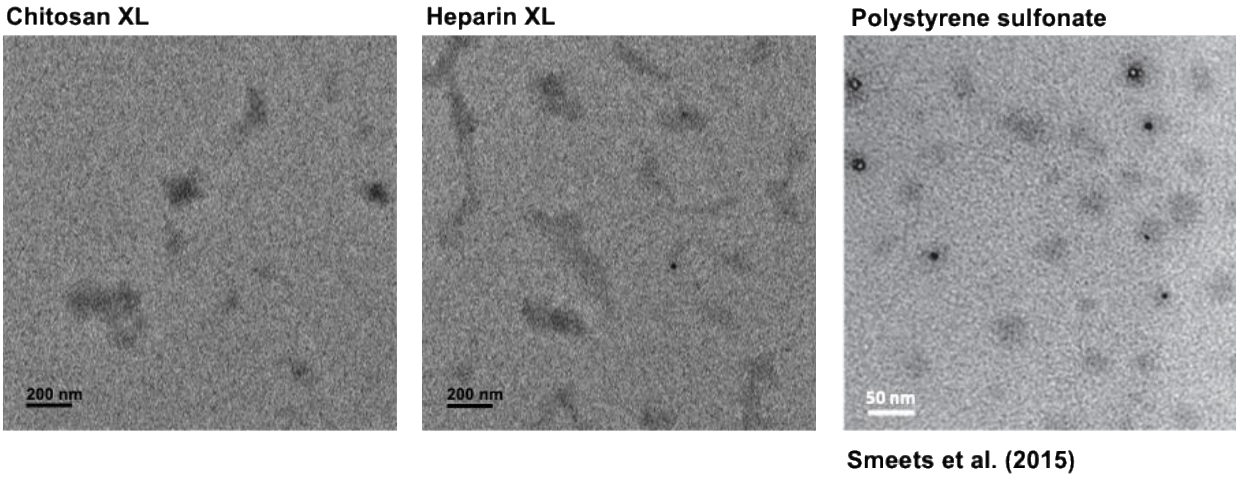
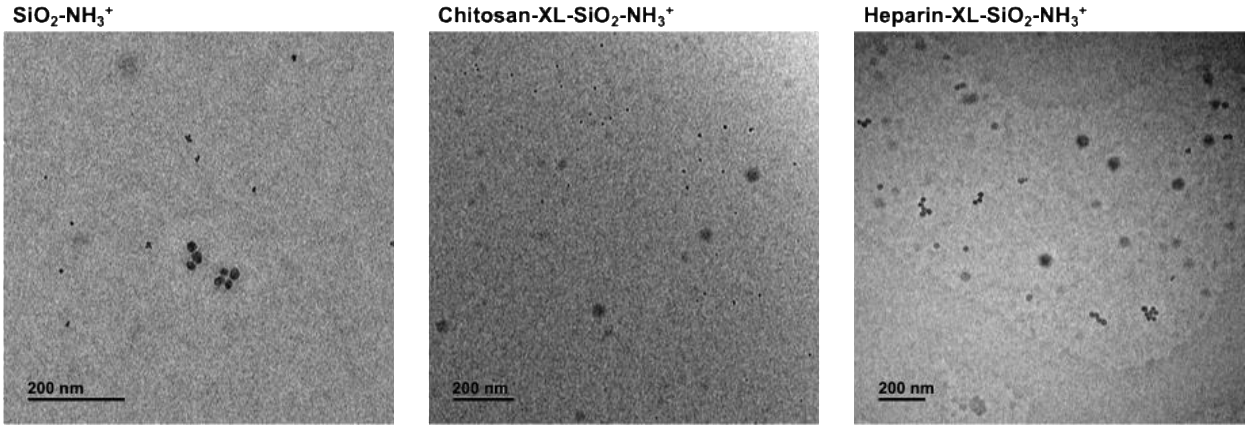


Fig. S7: $CaCO_3$ nucleation in high ionic strength solutions (IS = 0.6 M) in the presence of aminated silica nanoparticles (**left**, control). The high ionic strength conditions inhibited the crosslinking interactions and thus nanoparticle functionalization with chitosan (**middle**) or heparin (**right**).



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