Electronic Supplementary Material (ESI) for Soft Matter.

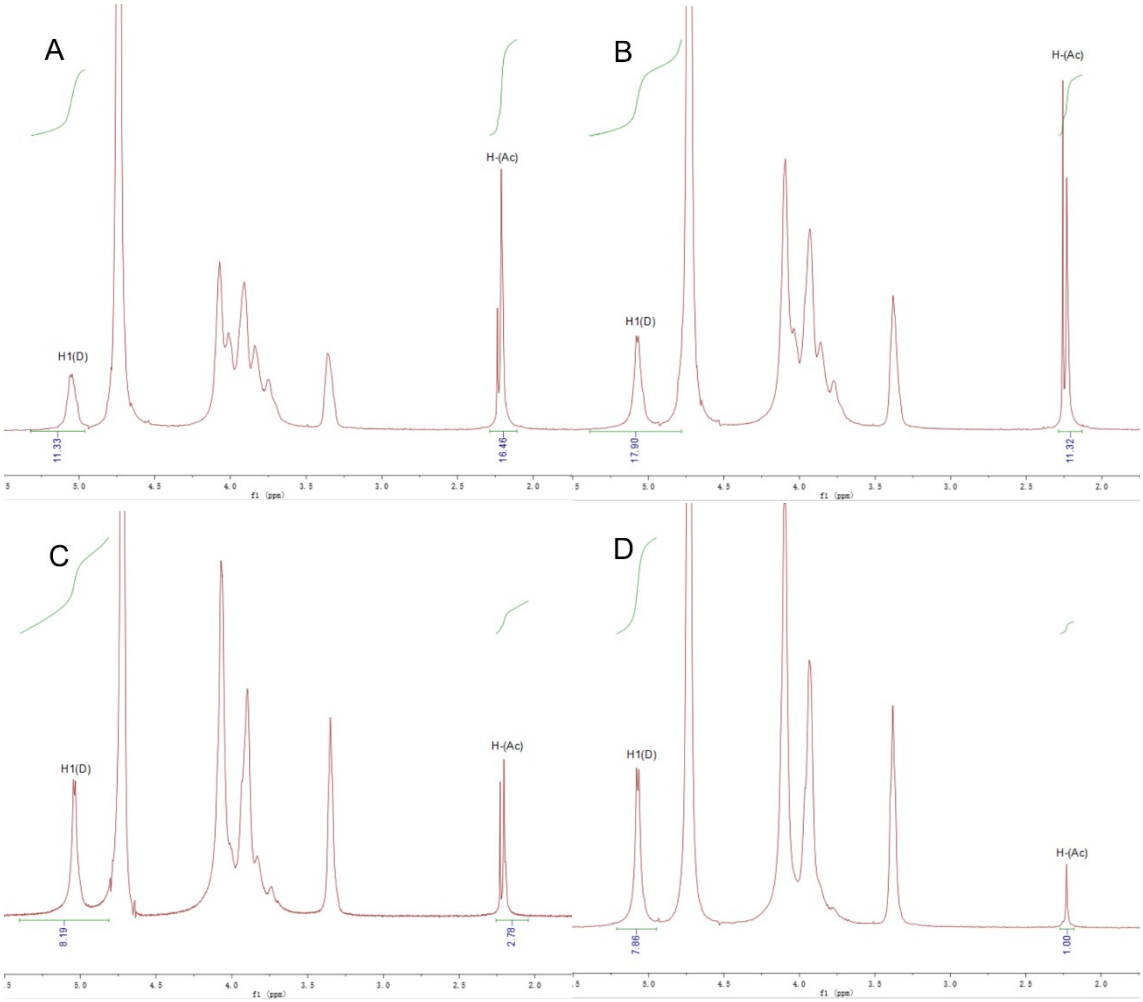
Ionically Crosslinked Polyelectrolyte Nanoparticle Formation Mechanisms: The Significance of Mixing

Yu Yuan, Yan Huang*

Determine the degree of deacetylation of chitosan

Ten mg of chitosan was dissolved in 1.96 ml of D_2O and 0.04 ml of DCl stirring at room temperature for about half an hour to ensure complete dissolution. The ^{1}HMR spectrum of different samples of chitosan were acquired at 50℃ (AVANCE Ⅲ 500) (Figure 1). The solvent (HOD) proton resonates at 4.67ppm. The DDA was calculated using integrals of the peak of proton H1 of deacetylated monomer (H1-D) and of the peak of the three protons of acetyl group (H-Ac):

$$DDA(\%) = \left(\frac{H1D}{H1D + {HAc}/{3}}\right) \times 100\%$$



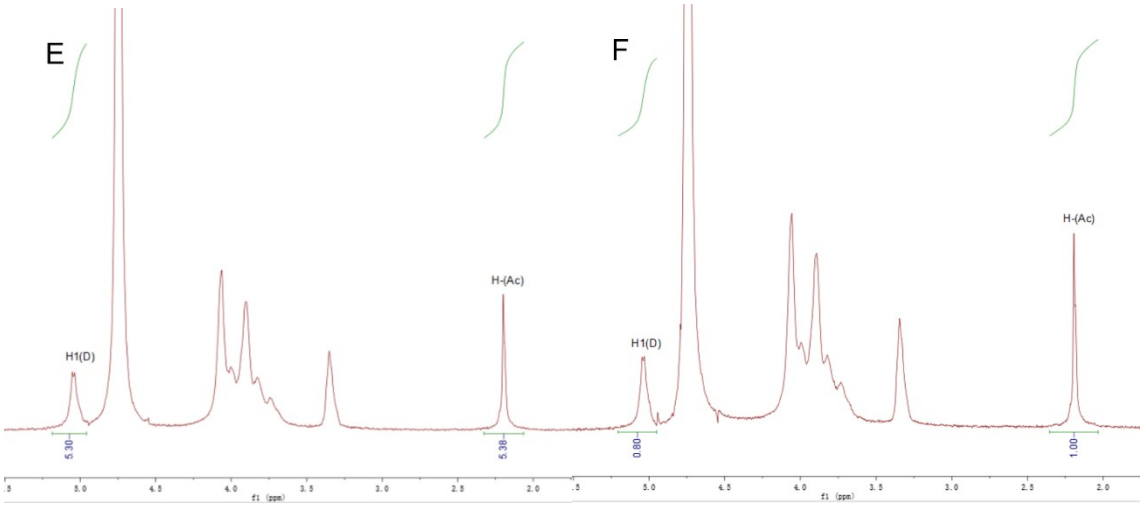


Figure S1. the spectrum of different samples of chitosan at 50℃. (A)LMW chitosan with 67% DD; (B)LMW chitosan with 82% DD; (C)LMW chitosan with 90% DD; (D)LMW chitosan with 96% DD; (E)MMW chitosan with 74% DD; (F)HMW chitosan with 70% DD

The device of preparation nanoparticles through FNC and dropwise addition

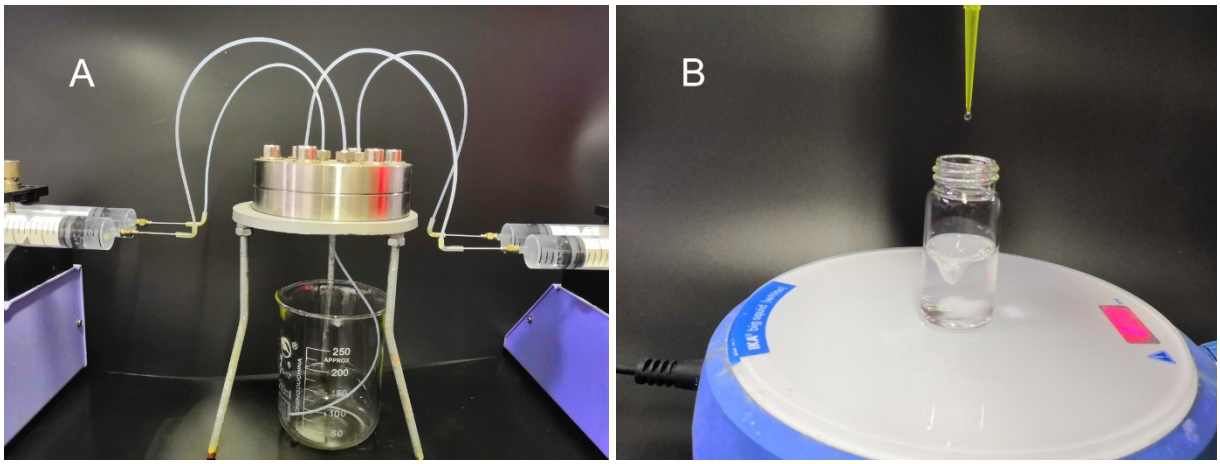


Figure S2. (A) multi-inlet vortex mixer and digital programmable syringe pumps used for preparing nanoparticles through FNC; (B) preparing nanoparticles through dropwise addition at 800 rpm inside a 20 mL scintillation vial.

Effect of mixing on chitosan/TPP nanoparticles

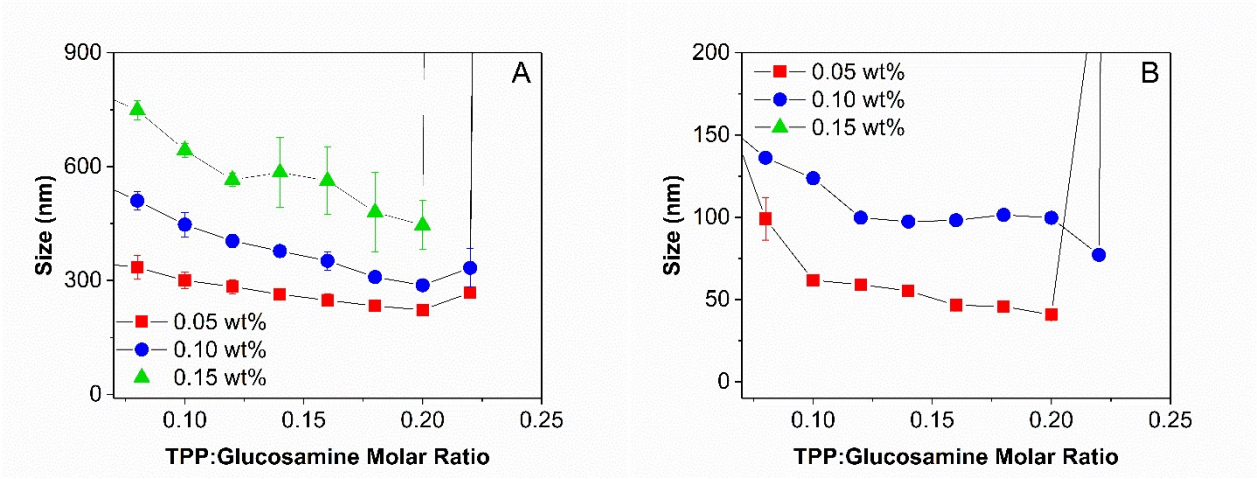


Figure S3. The effect of TPP:glucosamine molar ratio on the size of chitosan/TPP nanoparticles prepared at different chitosan concentrations and pH 4.0 by (A) dropwise addition (B) flash nanocomplexation.

The PDI of chitosan/TPP nanoparticles prepared through dropwise addition was about 0.5 until the stoichiometric point, after which the PDI significantly increased because of the coagulation and sedimentation of particles (Figure S4). The PDI of chitosan/TPP nanoparticles prepared in 0.15 wt% final chitosan solution increased at TPP:glucosamine molar ratios of 0.16:1, which may be due to the high viscosity of chitosan solution reduced the mixing efficiency and formed polydisperse particles. The PDI of chitosan/TPP nanoparticles prepared through FNC had a similar trend as the particles prepared through dropwise addition, which was inconsistent with previous report,[1] where the PDI of particles prepared through FNC was smaller than that of particles prepared through dropwise addition. This may be due to the different chitosan concentrations, TPP:glucosamine molar ratios and mixers used in this study.

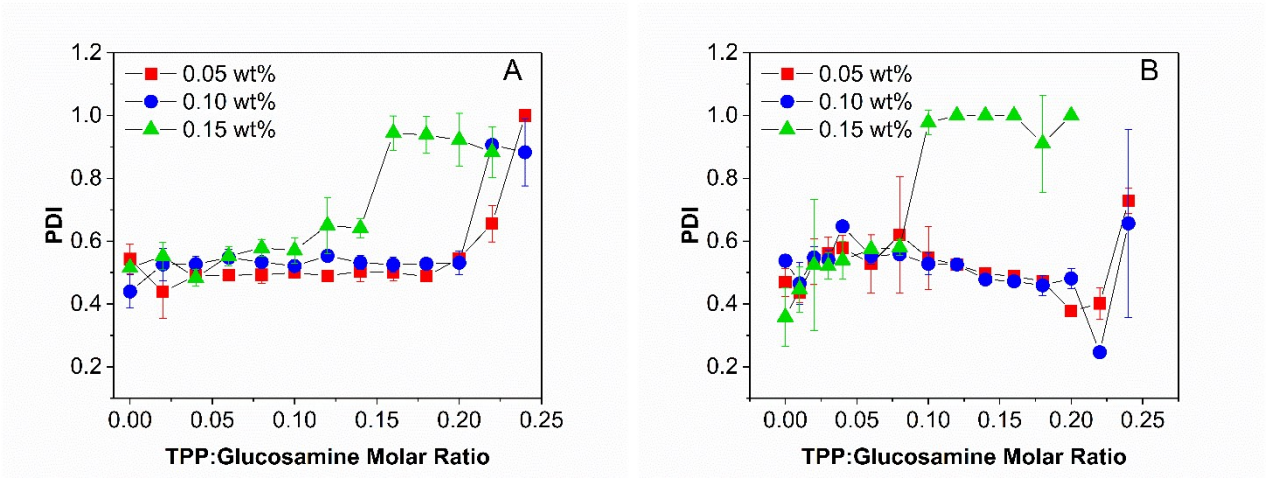
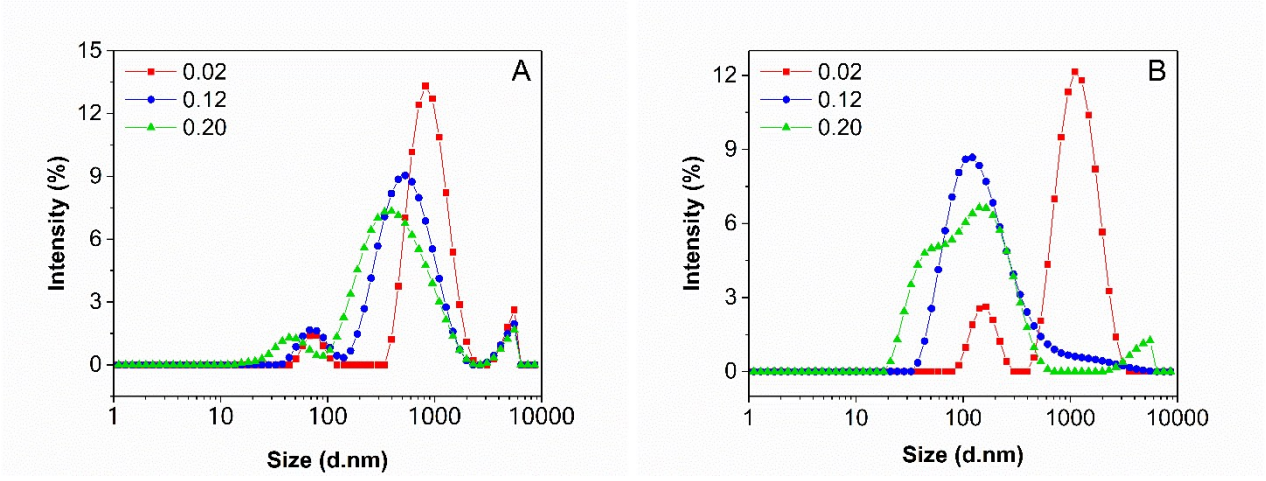


Figure S4. The effect of TPP:glucosamine molar ratio on the PDI of chitosan/TPP nanoparticles prepared at different chitosan concentrations and pH 4.0 by (A) dropwise addition (B) FNC

The size distribution of chitosan/TPP nanoparticles prepared through dropwise addition and FNC was showed in figure S5. The nanoparticles prepared by dropwise addition were significantly larger then those prepared by FNC at the same TPP:glucosamine molar ratio. Furthermore, the size distributions of nanoparticles prepared by dropwise addition yielded aggregate peaks in the 1-10 μm range, which indicated the aggregation of nanoparticles due to the inhomogeneous mixing and local supersaturation, while the nanoparticles prepared by FNC at 0.12 TPP:glucosamine molar ratio was more monodispersed.



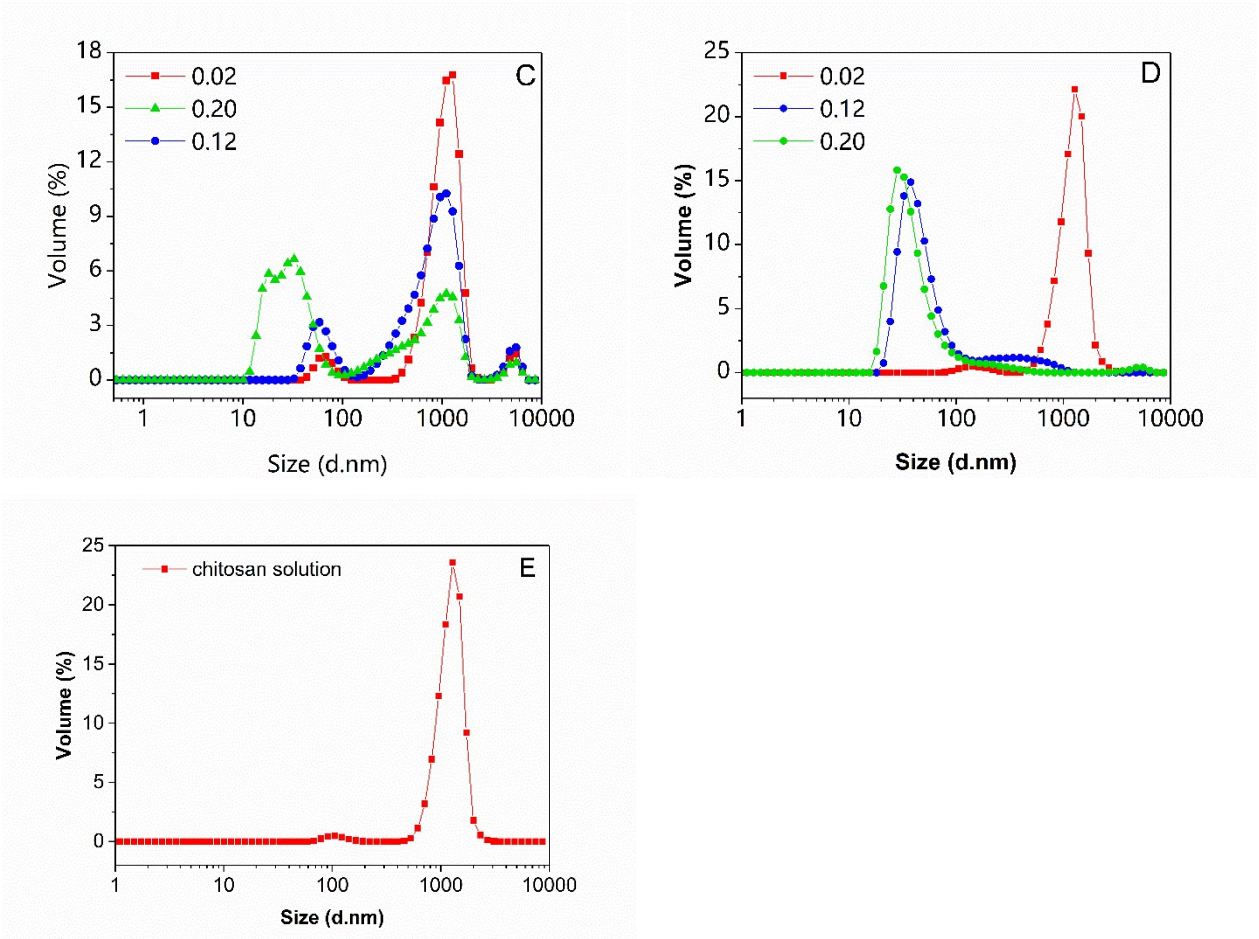


Figure S5. (A) intensity-weighted size distributions of nanoparticles prepared by dropwise addition; (B) intensity-weighted size distributions of nanoparticles prepared by flash nanocomplexation; (C) volume-weighted size distributions of nanoparticles prepared by dropwise addition; (D) volume-weighted size distributions of nanoparticles prepared by flash nanocomplexation in 0.1wt% chitosan at pH 4.0; (E) volume-weighted size distributions of 0.1wt% chitosan solution at pH 4.0.

The effect of mixing on size of other ionically crosslinked polyelectrolyte colloids

The light scattering intensity of the chitosan and alginate mixture prepared by FNC was almost the same as the light scattering intensity of alginate solution plus chitosan solutions at corresponding concentrations until the critical glucosamine:carboxyl molar ratio of 0.09:1. Hence, the slight increase in light scattering intensity in the chitosan/alginate mixtures at low glucosamine:carboxylate ratios prepared by FNC was likely due to the increasing polymer concentrations.

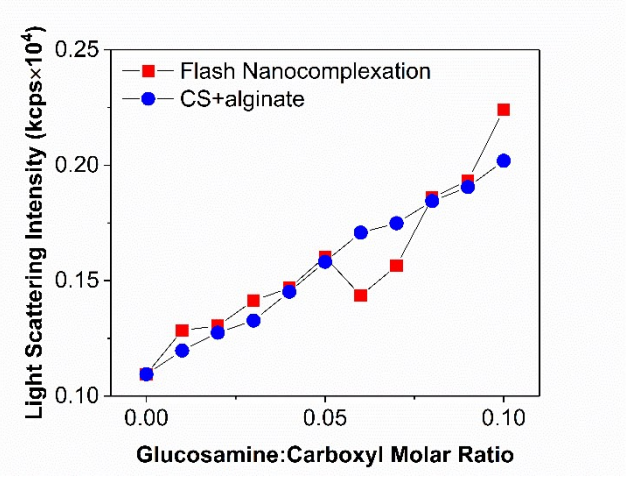


figure S6. The comparation of the light scattering intensity between the mixture of chitosan/alginate solution mixed by FNC and alginate solution plus different chitosan solutions.

The size of PAH/TPP and PAH/PPi nanoparticles prepared through FNC was significantly smaller than those prepared through dropwise addition and those in previous report[2], which has similar trend to the chitosan/TPP nanoparticles prepared in different methods. While the size of chitosan/alginate nanoparticles prepared through FNC were almost identical to those prepared through dropwise but coagulated at lower chitosan:alginate ratios likely due to the high polymer concentration and viscosity.

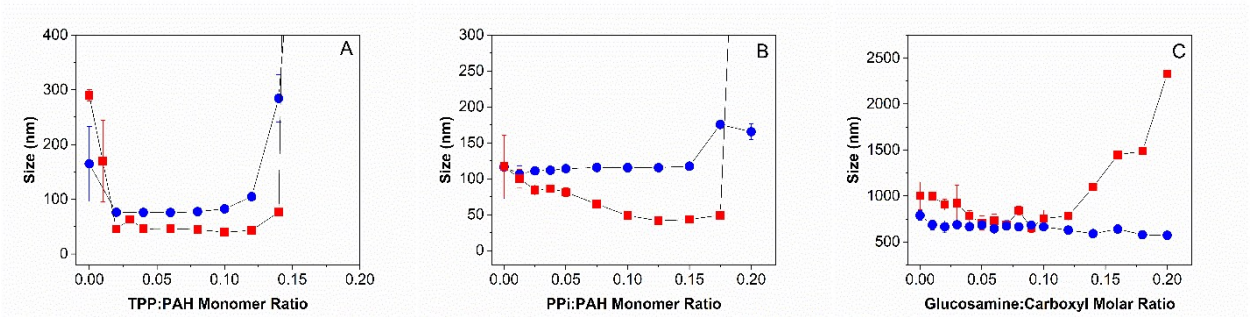


Figure S7. The comparison between the size of (A) PAH/TPP (B) PAH/PPi and (C) chitosan/alginate complexation by (●) dropwise addition and (■) FNC.

References

1 Z. He, J. L. Santos, H. Tian, H. Huang, Y. Hu, L. Liu, K. W. Leong, Y. Chen and H. Q. Mao, *Biomaterials*, 2017, **130**, 28-41.

2 Y. Huang and Y. Lapitsky, *J. Phys. Chem. B*, 2013, **117**, 9548-9557.