Impact of Chitosan Molecular Weight and Attached Non-Interactive Chains on the Formation of α-Lactalbumin Nanogel Particles

Juan Du, Young-Hee Cho, Ryan Murphy, and Owen Griffith Jones

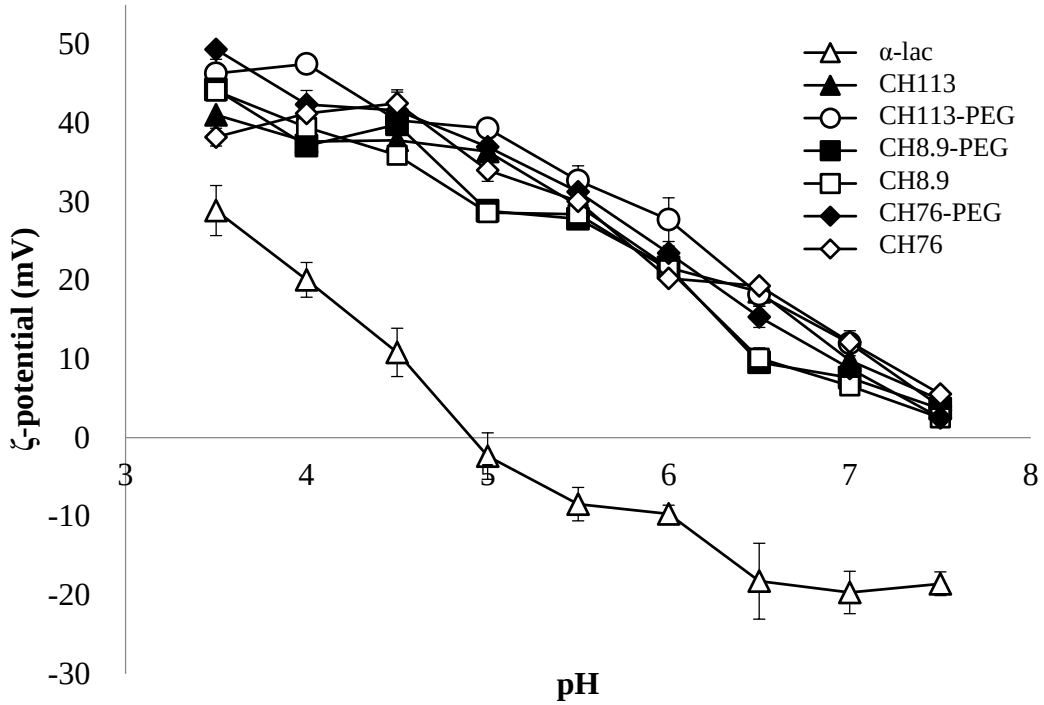

Figure S1. ζ-Potential of α-lactalbumin, chitosan (CH), or chitosan-*graft*-PEG (CH-PEG) as a function of solution pH.

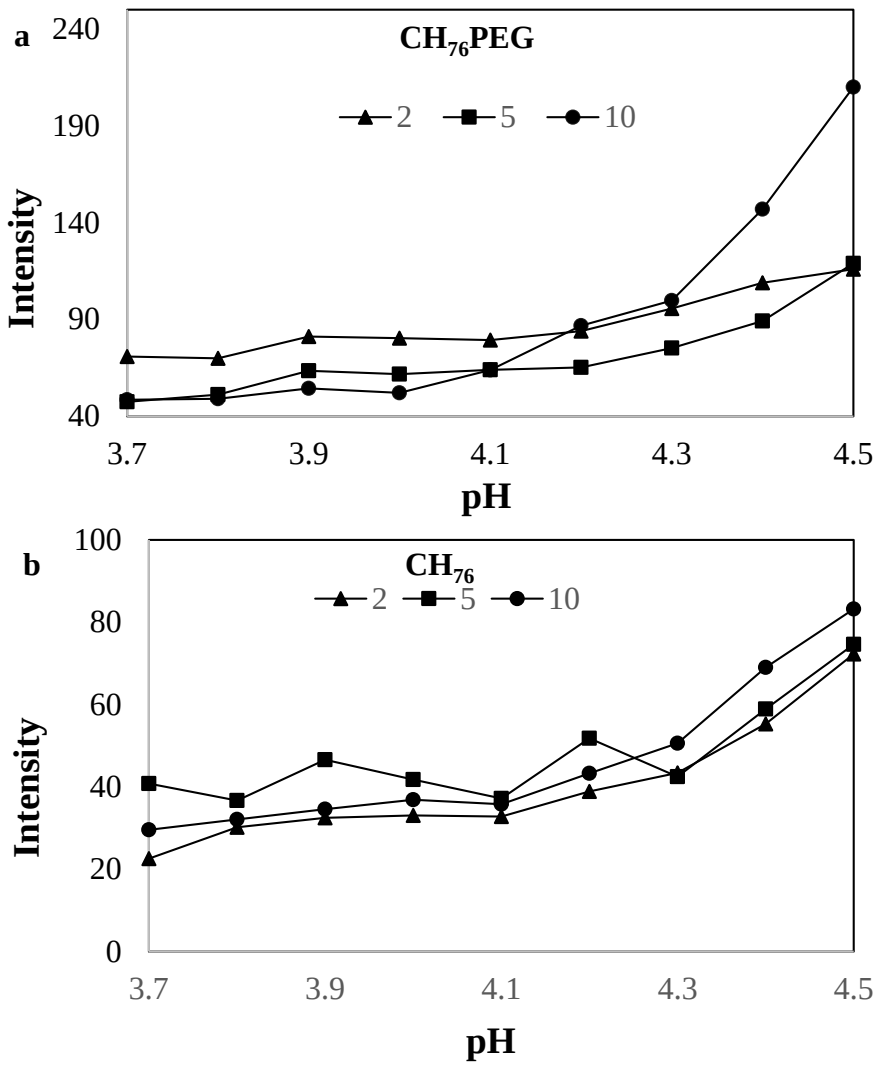

Figure S2. Intensity of scattered light at 90 degree scattering angle for mixtures of α-lactalbumin and (**a**) CH_{76}PEG or (**b**) CH_{76} at different *r*-value as a function of solution pH.

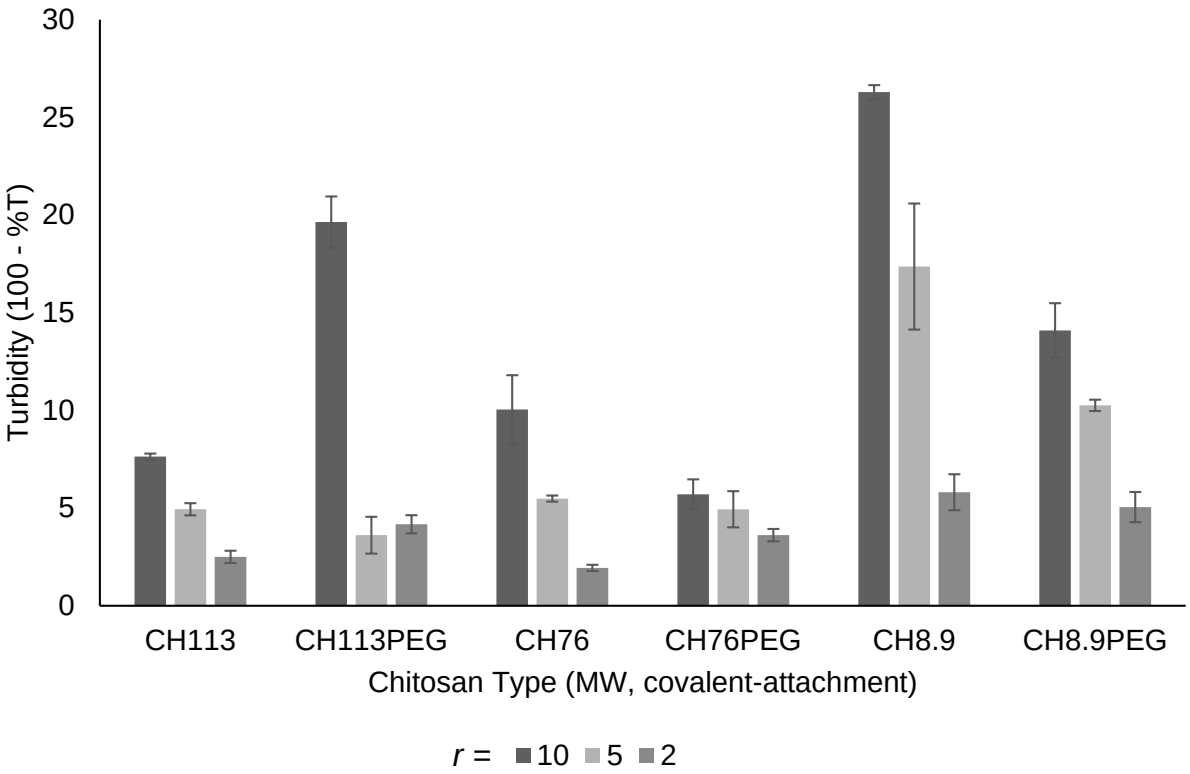

Figure S3. Effect of *r*-value on turbidity of α-lac complexes heated at pH 4.8 for mixtures with CH_{113}, CH_{76}, $CH_{8.9}$, CH_{113}PEG, CH_{76}PEG, or $CH_{8.9}$PEG.

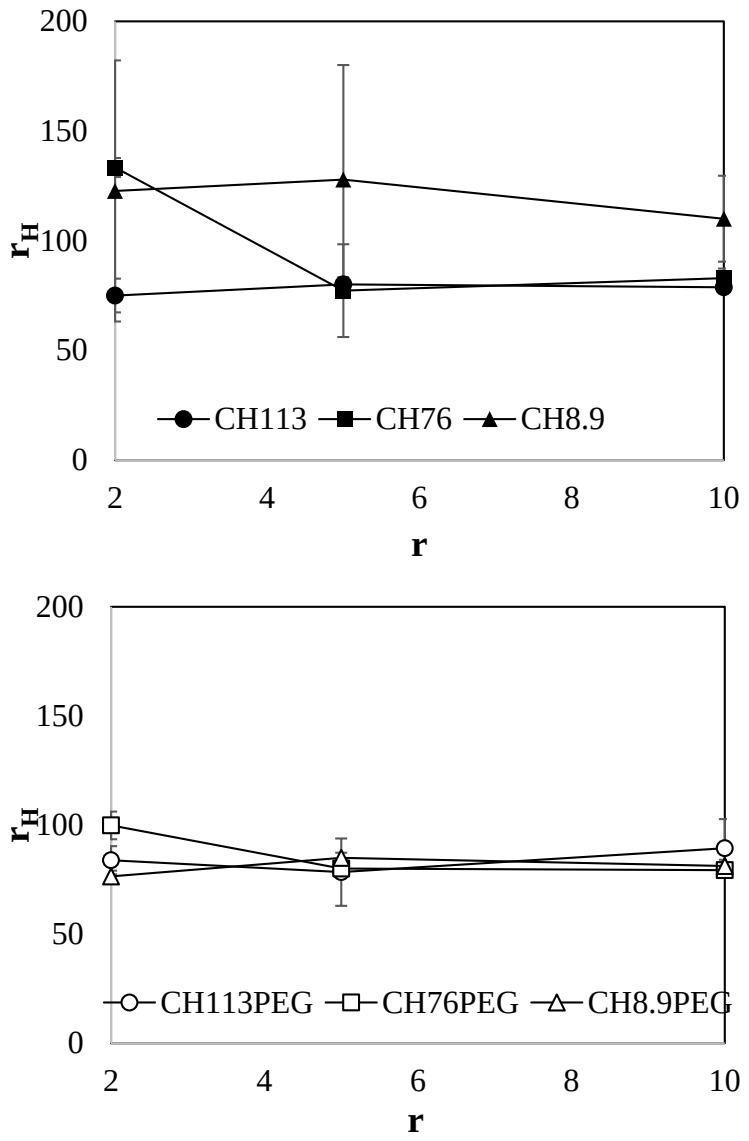

Figure S4 Effect of *r*-value on hydrodynamic radii of detected colloids within heated mixtures of α-lactalbumin and (**a**) CH or (**b**) CH-PEG of different molecular weight at pH 4.8.

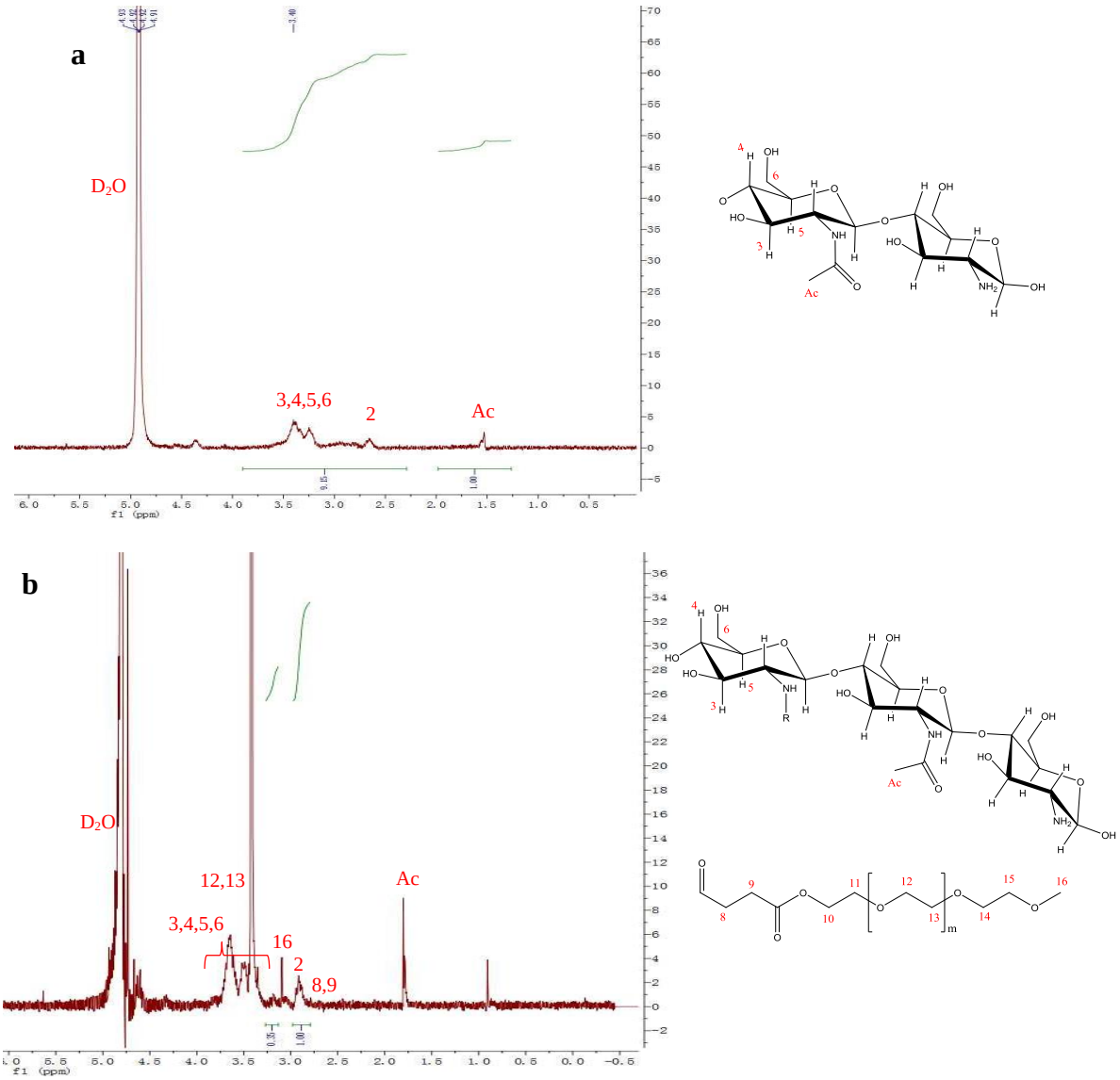

Figure S5. 1D Proton NMR spectra of (**a**) CH_{113} and (**b**) $CH_{113}PEG$ in 8% DCl/D_2O (*v*/*v*) at concentrations of 5 mg/mL. Schematics of the representative chemical structures and peak assignments are given on the right.

Table S1. Turbidity of heated α-lac/CH_7PEG mixtures at different pH and *r*-value 1 day after preparation and 14 days after preparation.

r	pH	Day 1	Day 14
		100-T%	100-T%
5	4.3	1.37	0
	4.8	5.59	2.95
	5.3	10.26	6.67
	5.8	15.67	10.26
2	4.3	1.60	0
	4.8	3.39	1.37
	5.3	6.46	2.50
	5.8	7.53	3.62

Table S1 shows that turbidity of heated mixtures decreased ~30%–60% after 2 weeks of storage (data at pH 4.3 was below noise threshold), possibly due to sedimentation of the largest nanogels in the suspensions and/or separation of dust or bubbles from sample tubes during storage. However, the general trend of turbidity between samples remained identical. This implied that mixtures with higher turbidity on day 1 possessed nanogels of larger average size and/or number compared to sample mixtures of lower turbidity, and this relation persisted following storage so that the same sample mixtures possessed nanogels of larger average size and/or number after 2 weeks.