




Specific Ion Effects of Trivalent Cations on the Structure and Charging State of β -Lactoglobulin Adsorption Layers

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Present by Sam Sokhuoy

(Soft-matter optical spectroscopy, R1020, 2022/07/02)

Specific Ion Effects of Trivalent Cations on the Structure and Charging State of β -Lactoglobulin Adsorption Layers

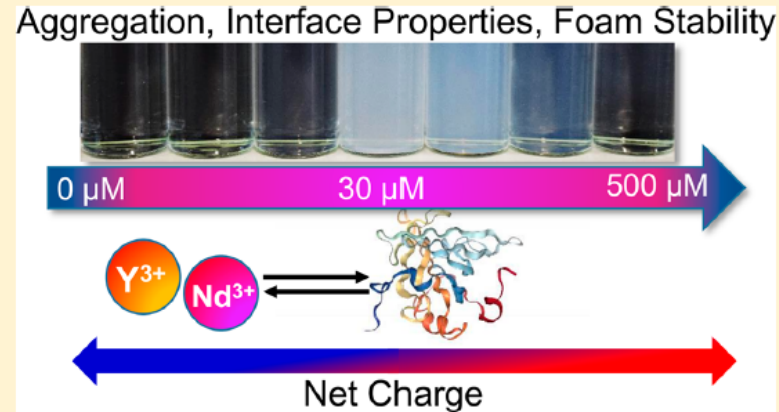
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Supporting Information

ABSTRACT: The properties of proteins at interfaces are important to many processes as well as in soft matter materials such as aqueous foam. Particularly, the protein interfacial behavior is strongly linked to different factors like the solution pH or the presence of electrolytes. Here, the nature of the electrolyte ions can significantly modify the interfacial properties of proteins. Therefore, molecular level studies on interfacial structures and charging states are needed. In this work, we addressed the effects of Y^{3+} and Nd^{3+} cations on the adsorption of the whey protein β -lactoglobulin (BLG) at air–water interfaces as the function of electrolyte concentration. Both cations caused very similar but dramatic changes at the interface and in the bulk solution. Here, measurements of the

electrophoretic mobility and with vibrational sum-frequency generation (SFG) spectroscopy were applied and consistently showed a reversal of the BLG net charge at remarkably low ion concentrations of 30 (bulk) and 40 (interface) μM of Y^{3+} or Nd^{3+} for a BLG concentration of 15 μM . SFG spectra of carboxylate stretching vibrations from Asp or Glu residues of interfacial BLG showed significant changes in the resonance frequency, which we associate to specific and efficient binding of Y^{3+} or Nd^{3+} ions to the proteins carboxylate groups. Characteristic reentrant condensation for BLG moieties with bound trivalent ions was found in a broad concentration range around the point of zero net charge. The highest colloidal stability of BLG was found for ion concentrations <20 μM and >50 μM . Investigations on macroscopic foams from BLG solutions revealed the existence of structure–property relations between the interfacial charging state and the foam stability. In fact, a minimum in foam stability at 20 μM ion concentration was found when the interfacial net charge was negligible. At this concentration, we propose that the persistent BLG molecules and weakly charged BLG aggregates drive foam stability, while outside the bulk reentrant zone the electrostatic disjoining pressure inside foam lamellae dominates foam stability. Our results provide new information on the charge reversal at the liquid–gas interface of protein/ion dispersions. Therefore, we see our findings as an important step in the clarification of reentrant condensation effects at interfaces and their relevance to foam stability.

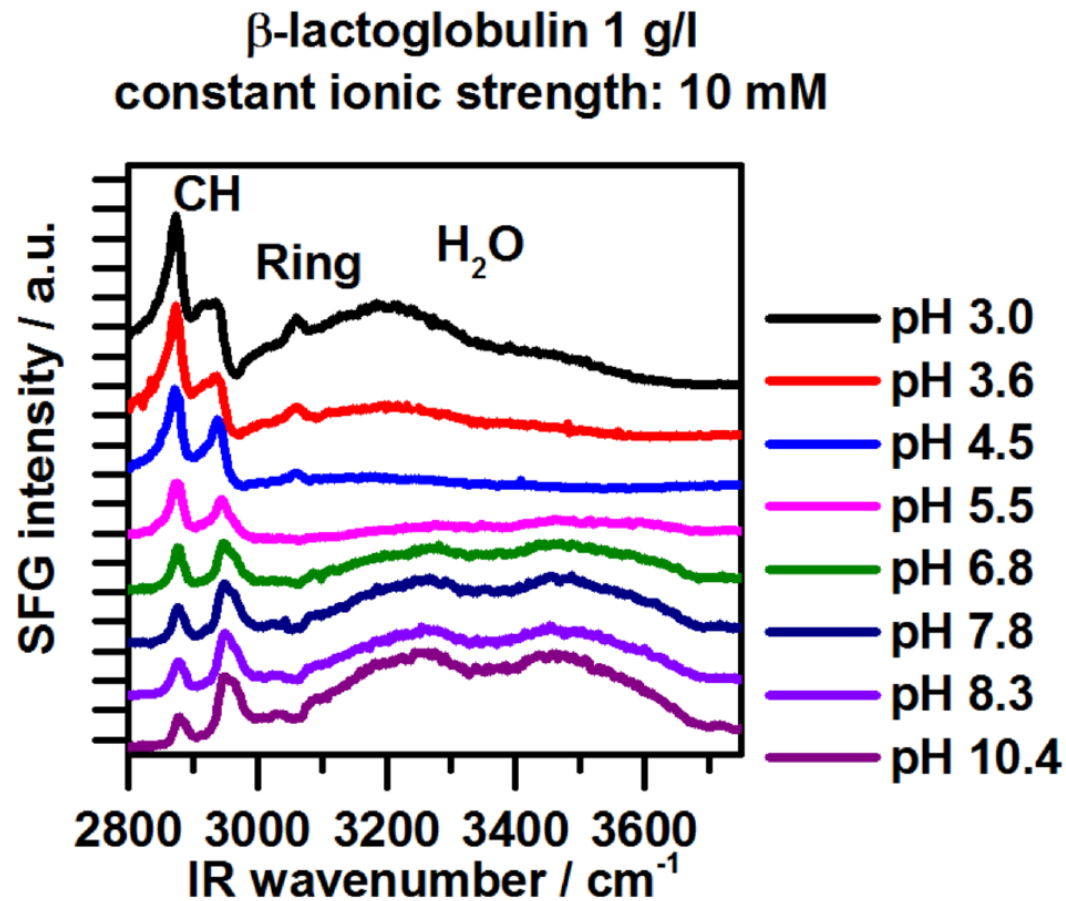


Main idea

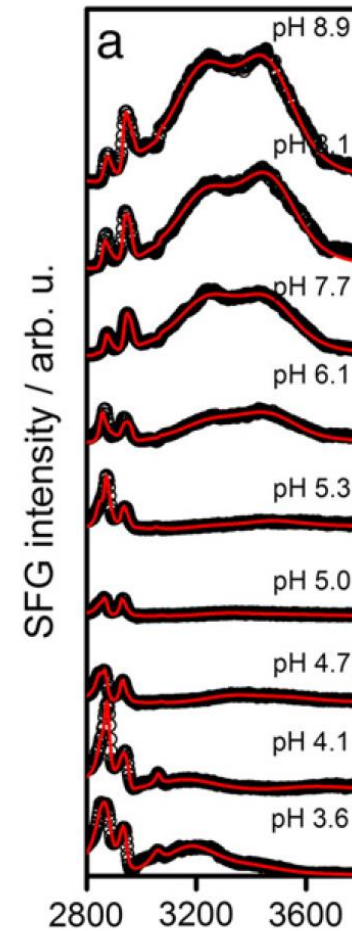
-  Trivalent salt induced charged inversion of BLG from negatively charged to positively charged
-  Trivalent salts effect the stability of BLG foam
-  30 μM of trivalent salts screening the BLG in the bulk and 40 μM at the surface

□ Previous talks

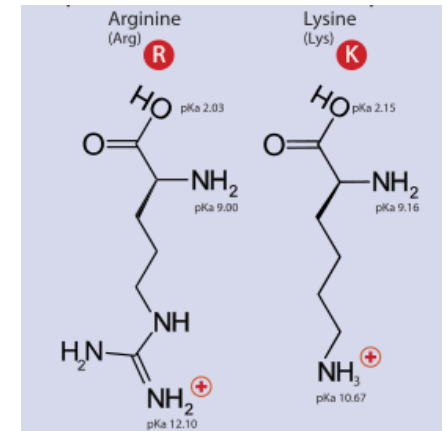
□ Effect of pH: Increase the pH reduce the charge reversal from positive to negative.



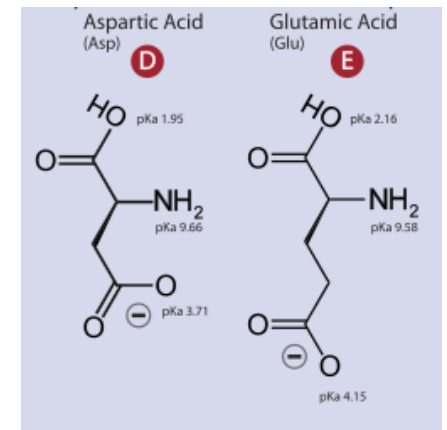
Langmuir 2019, 35, 5004–5012



□ Positive amino acids



□ Negative amino acids

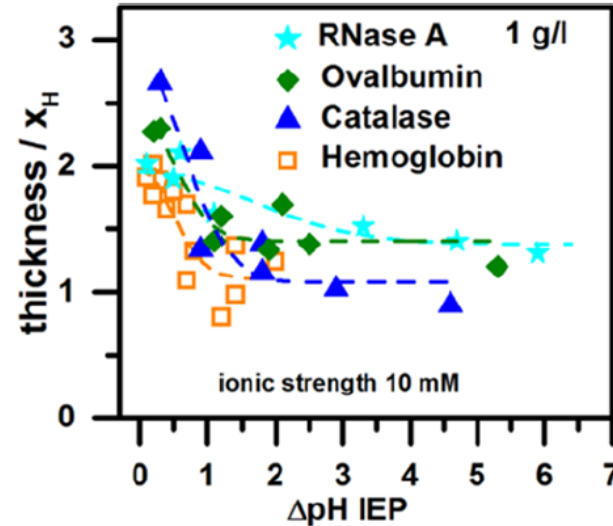
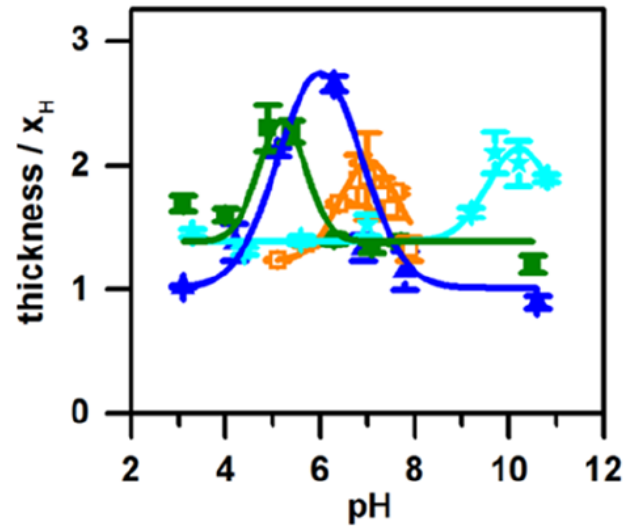
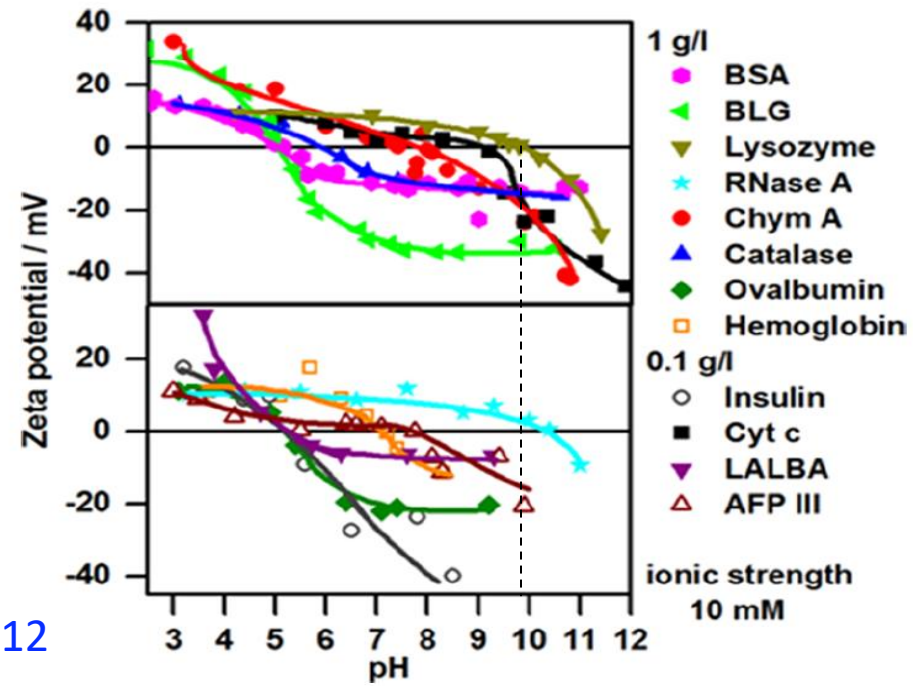


<https://doi.org/10.1016/j.cocis.2014.03.008>

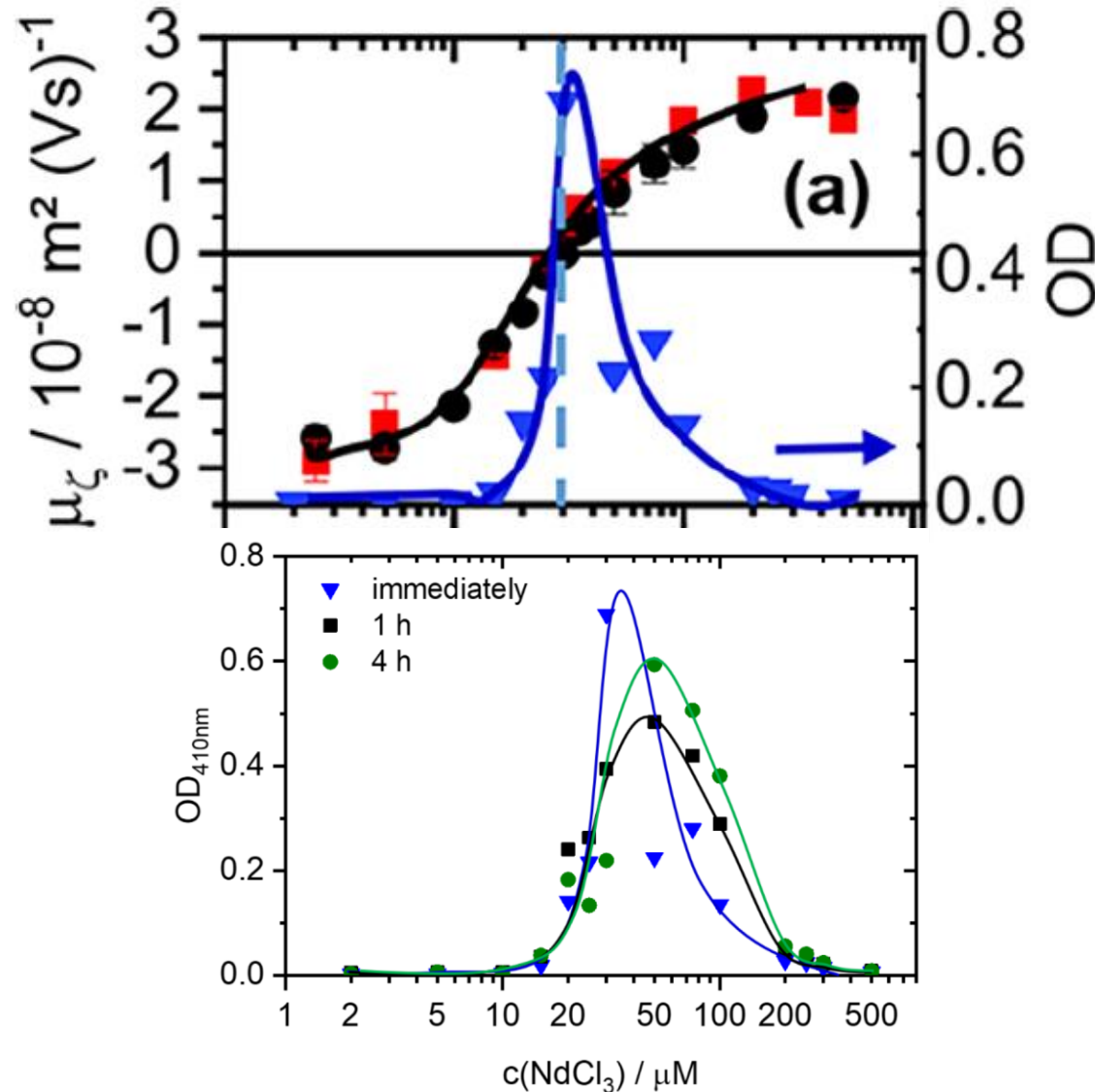
□ Previous talks

□ Using Zeta potential and ellipsometry experiment

Langmuir 2019, 35, 5004–5012



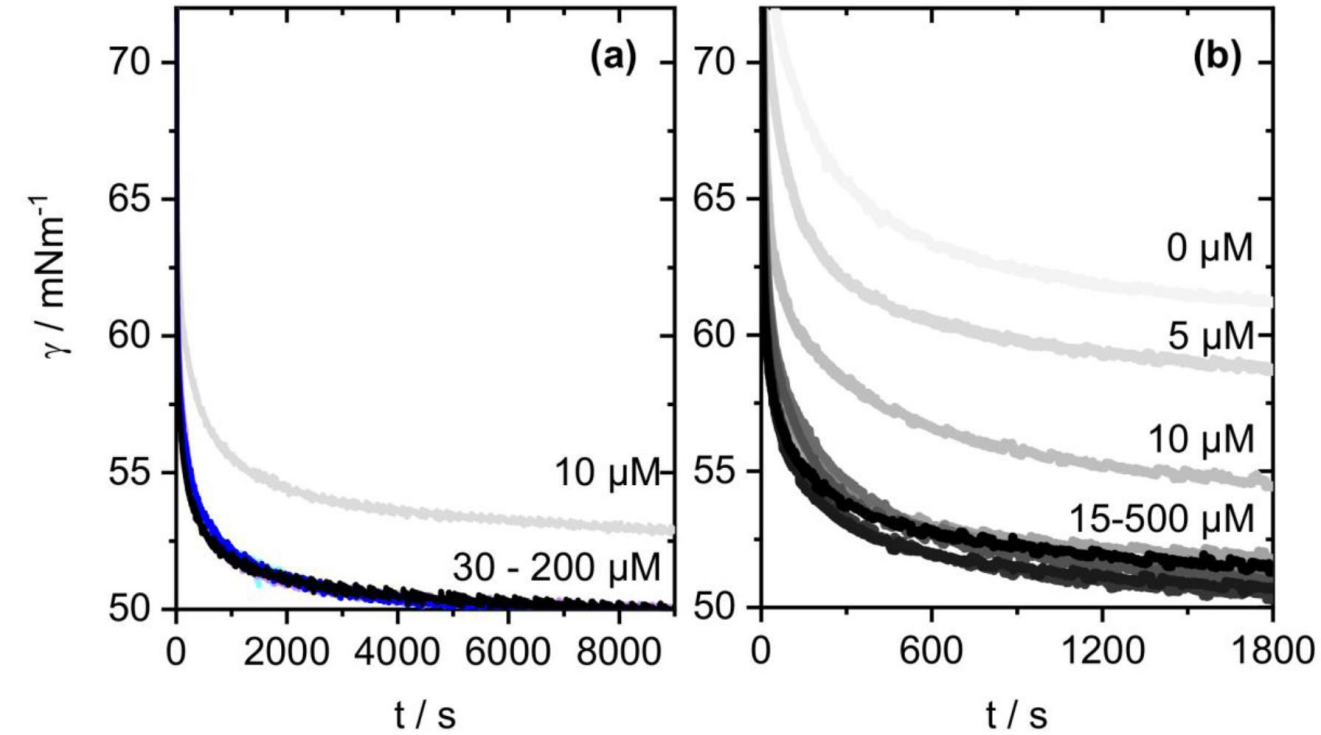
□ Electrophoretic Mobility μ_z (in the bulk) and Optical Density (OD)



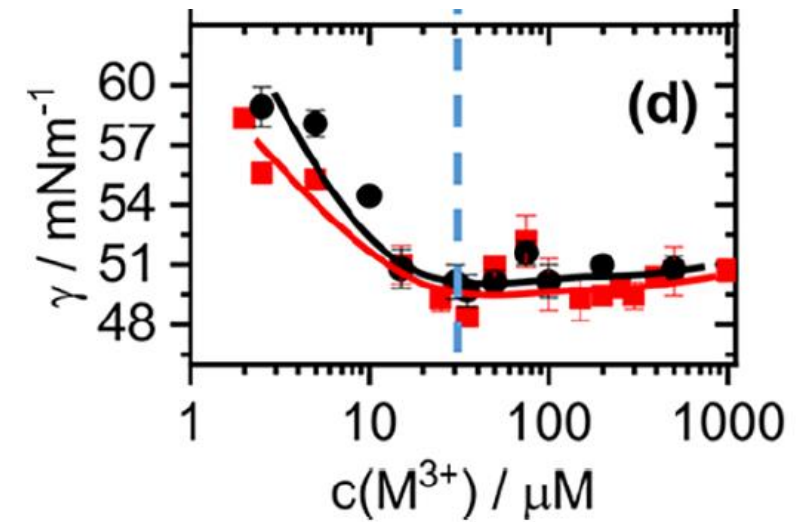
- Electrophoretic Mobility μ_z changing from negative to positive when increasing YCl_3 (black) and NdCl_3 (red)
- 30 μM of trivalent makes the zero charge BLG in the bulk
- High OD (OD at 410 nm) at 30 μM (zero charge, most aggregate at zero charge)
- OD changing with time during 1 and 4 hours, changing from 30 to 50 μM to colloidal stable solution.

□ Surface tension experiment

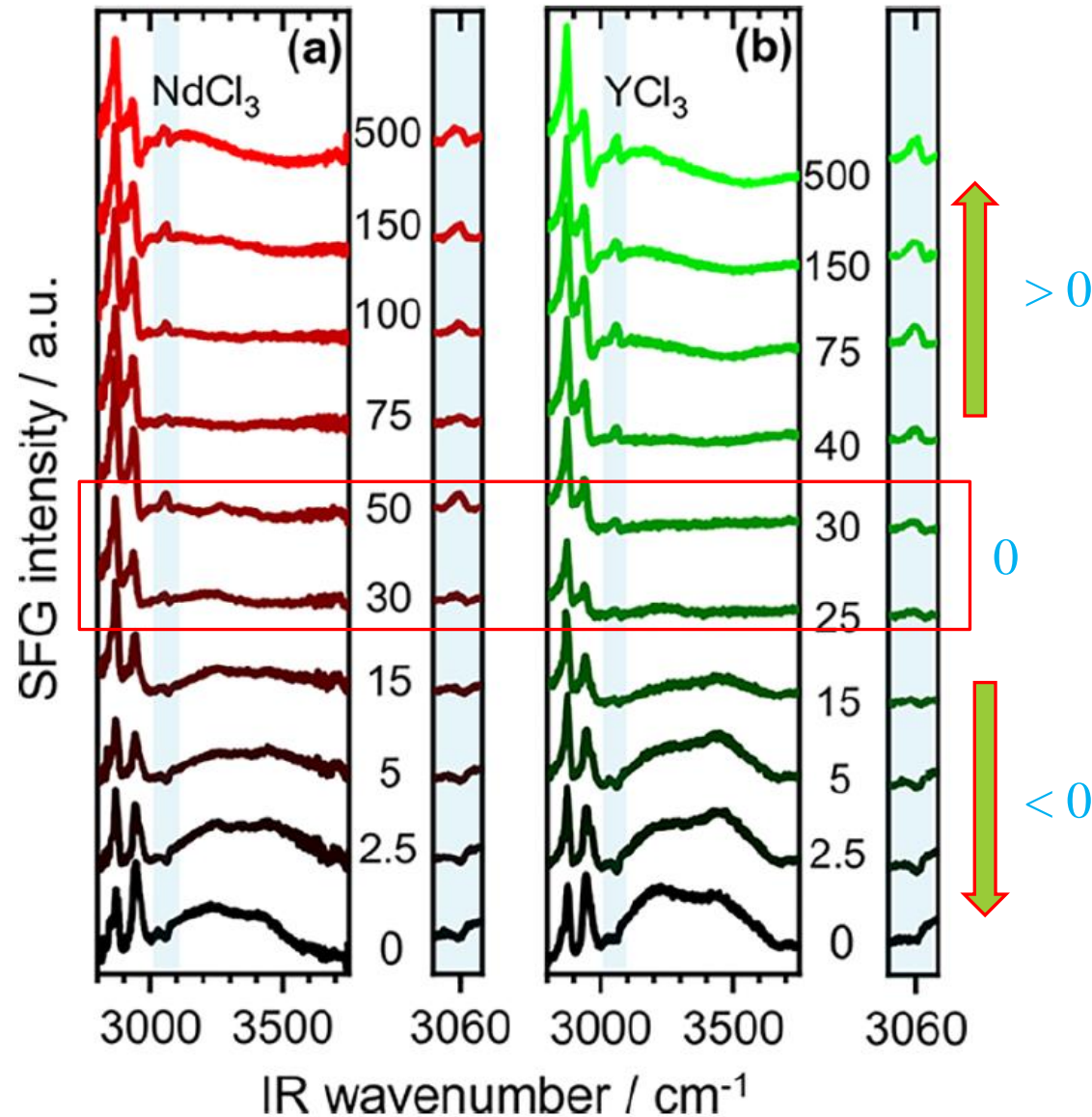
2. Dynamic surface tension



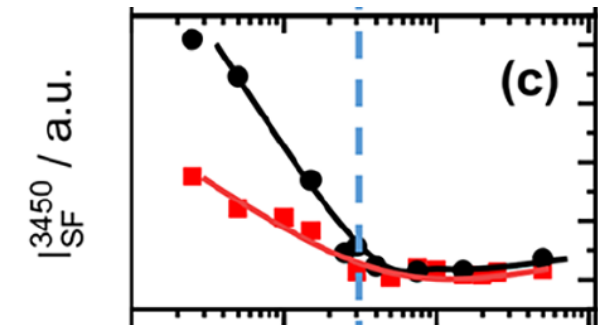
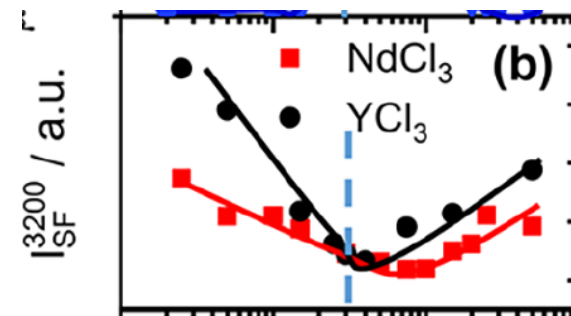
- Surface tension was taken after 30 min (required)
- At 0 μM salt, surface tension of BLG is $\sim 61.3 \pm 0.5$ mN/m at 15 μM
- Increasing salt concentrations decreased the surface pressure due to surface excess is enhanced.



□ Trivalent salts - BLG mixture

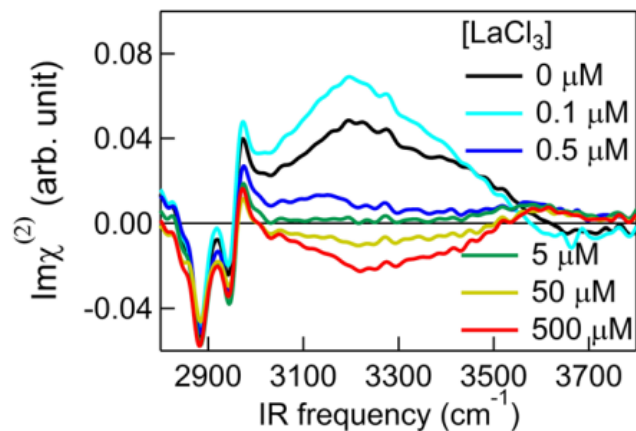


- Without salt BLG is negatively charged (pH~6.5)
- For $< 40 \mu\text{M}$: trivalent salts decrease the OH intensities and C-H of aromatic ring (3060 cm^{-1}) is in negative dip
- For $> 40 \mu\text{M}$: trivalent salts increase the OH intensities and C-H of aromatic ring (3060 cm^{-1}) is in positive peak
- The similar behavior between NdCl_3 and YCl_3
- Charge reversal after adding more trivalent salts into the solution (from negatively charged to positively charged)

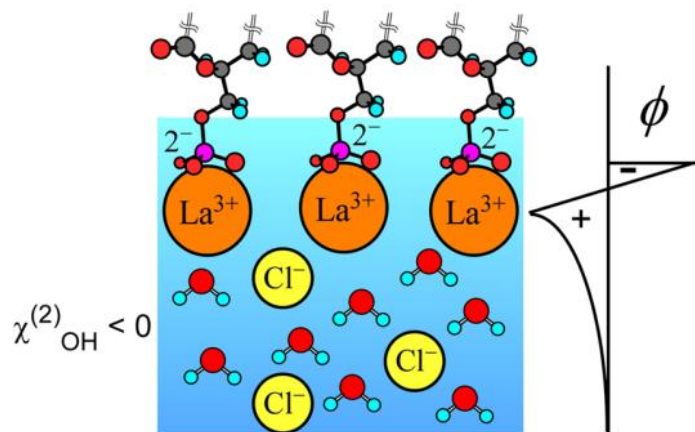


Charge reversal behavior

DMPA-LaCl₃ from Tahara's group

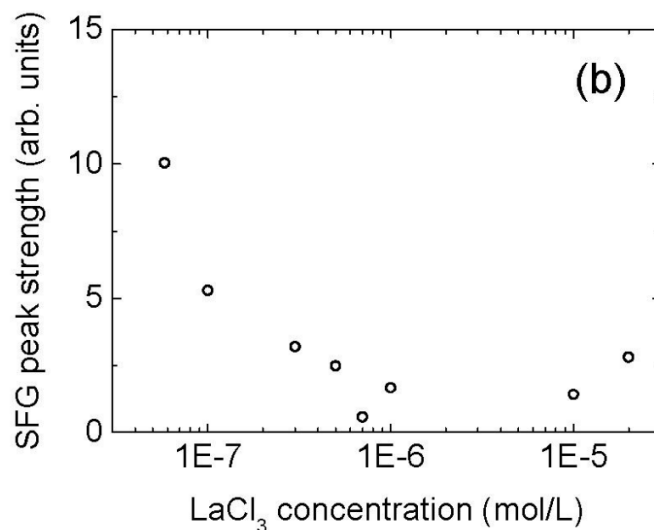
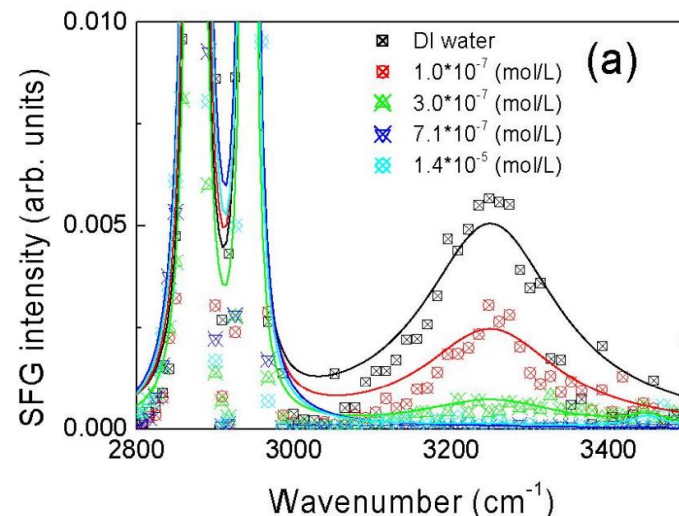


(a) Phosphate



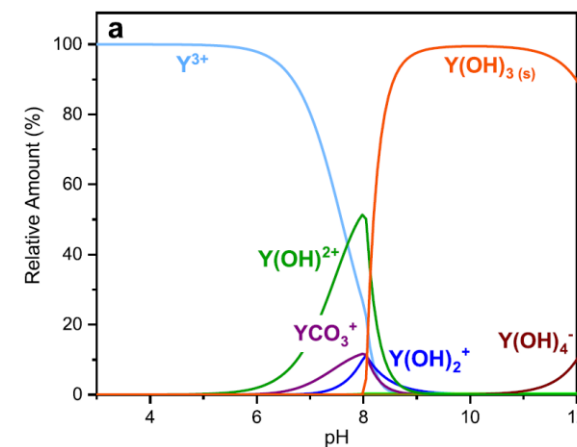
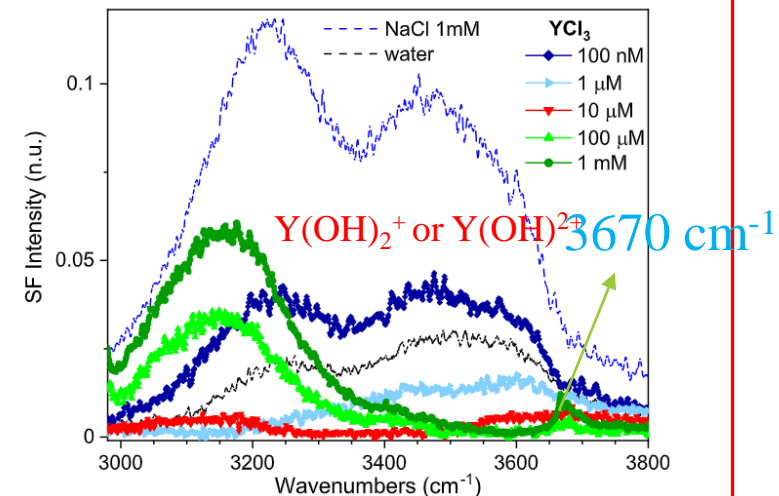
J. Chem. Phys. 149, 024703 (2018)

DMPA-LaCl₃ from our group



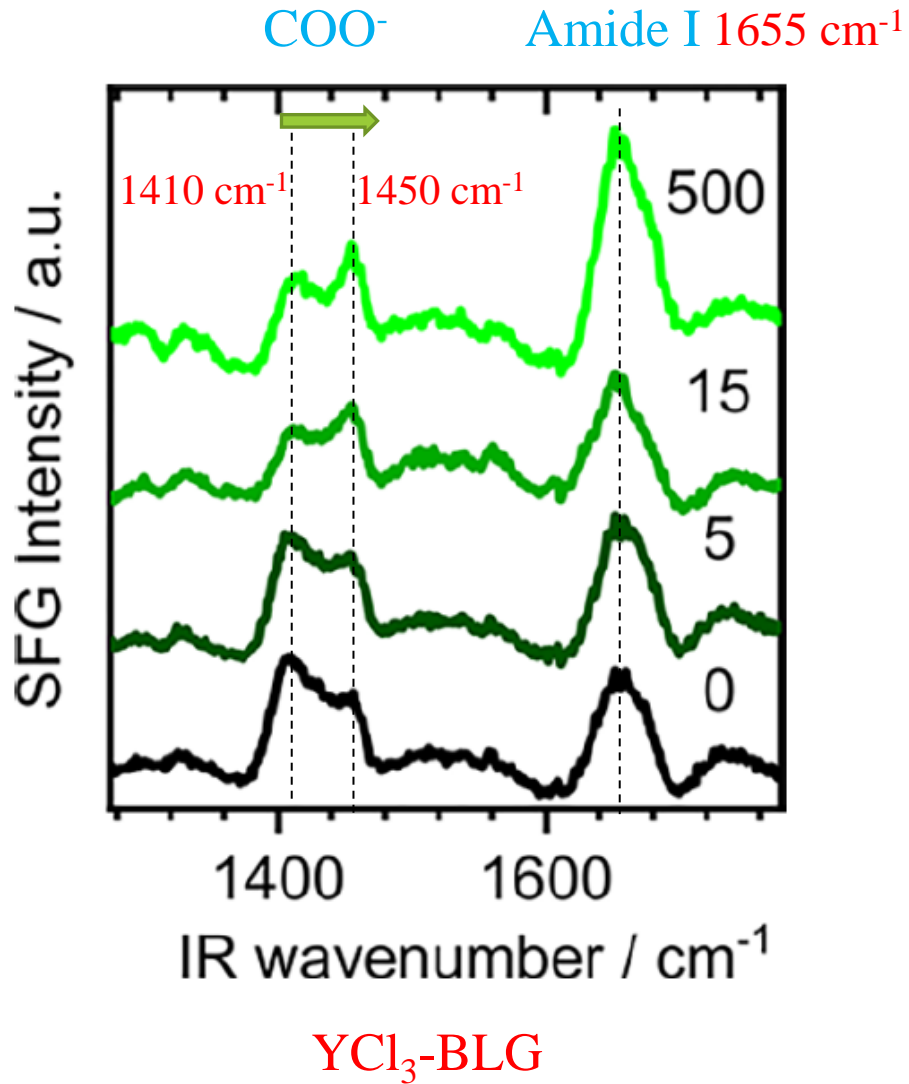
<https://doi.org/10.3938/jkps.53.2320>

AA-YCl₃ from Erictyrode's group

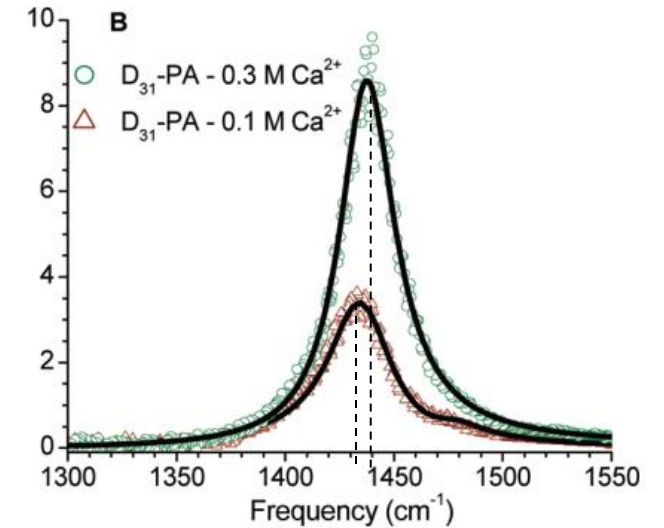
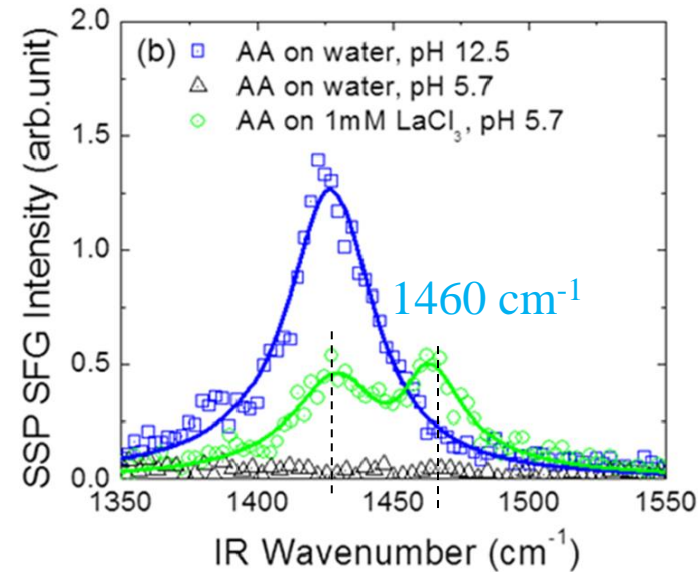


<https://doi.org/10.1016/j.jcis.2021.10.052>

Amide I and carboxylic headgroup region



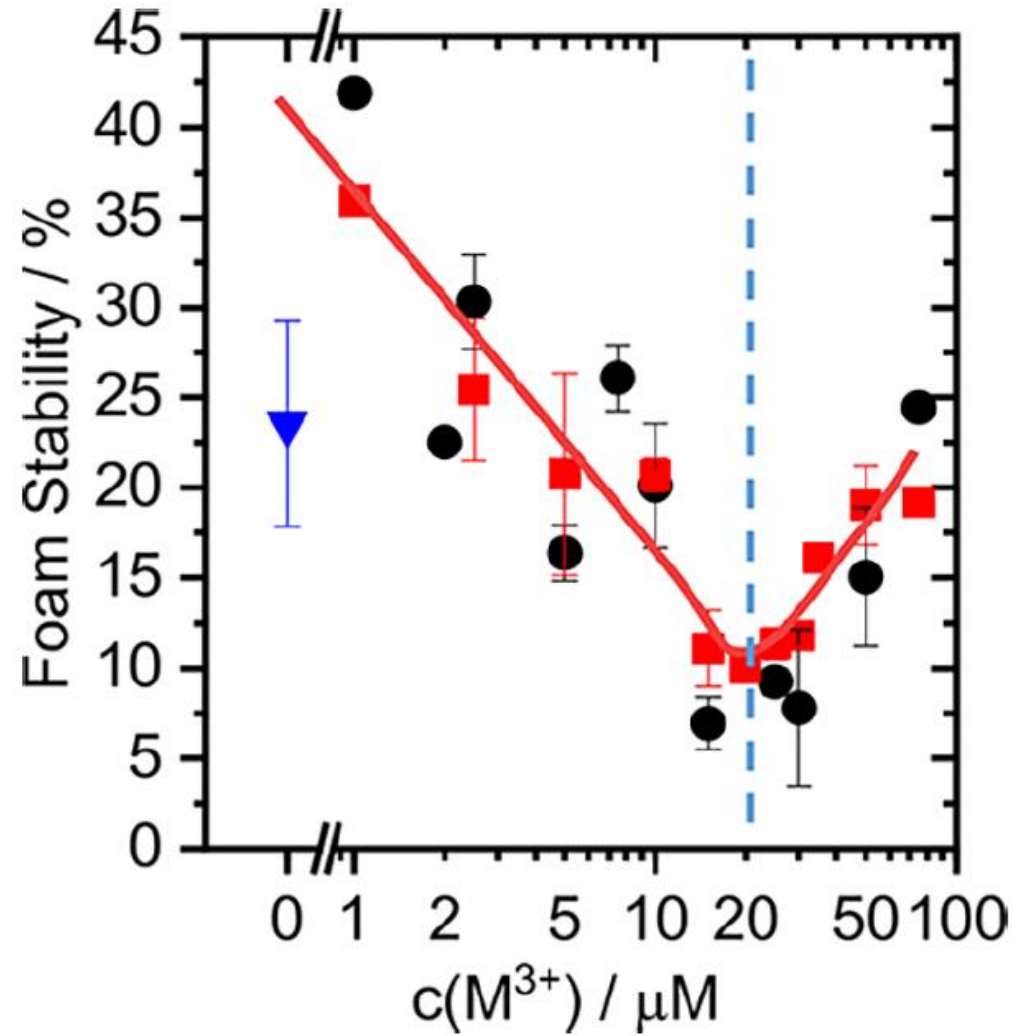
- Carboxylate band originates from Glu and Asp amino acid
- Increasing YCl₃ concentration has split the COO⁻ into two peaks (1410 and 1450 cm⁻¹)
- $C_{\text{ion}}/C_p \sim 2$ or 3 responded to 2 or 3 of Y³⁺ or Nd³⁺ with 1 BLG molecule?
- Increasing Amide I intensity peak due to surface excess of BLG moieties at the interface
- The shape is similar which mentioned no significant unfolding of secondary structure.



J. Chem. Phys. 149, 163304 (2018)

J. Phys. Chem. B 2010, 114, 51, 17068–17076

□ Foam stability



- Increasing concentration of YCl_3 (black) and $NdCl_3$ (red) decreased the stability of the BLG foam
- At 20 μM , it reached minimum the foam stability
- The foam stability is poor at near zero charged (30 and 40 μM , in the bulk and interface, respectively)

we define here as follows: $FS = (h_{30 \text{ min}}/h_{\text{max}}) \times 100$. Here, h_{max} is the initial foam height after the foaming process was completed and $h_{30 \text{ min}}$ is the foam height after a waiting time of 30 min.

□ Photographs of BLG solutions at different concentration of trivalent salt

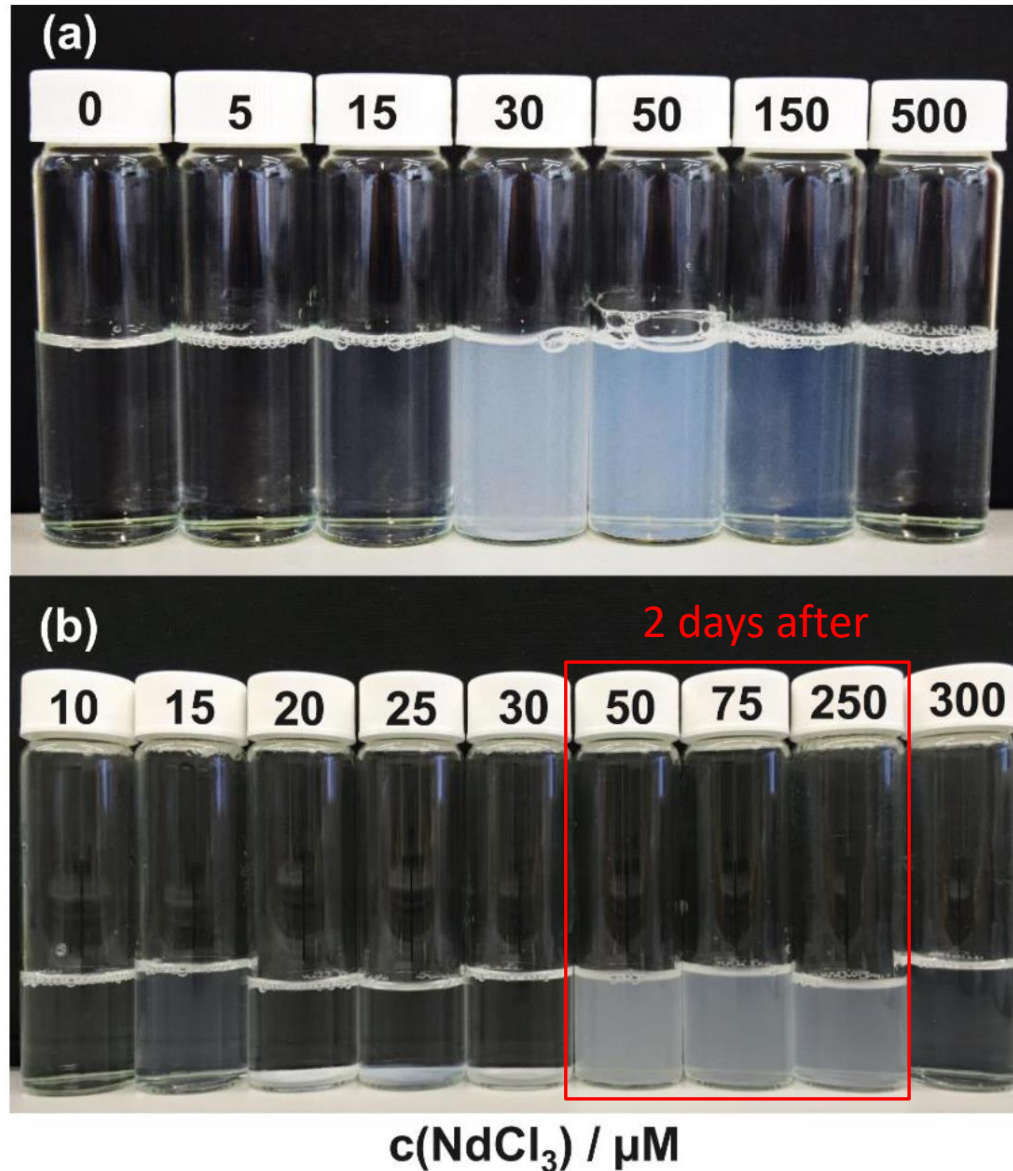


Figure S2 Photographs $15 \mu\text{M}$ β -lactoglobulin solutions with different concentrations of NdCl_3 (as indicated in the figure). (a) Presents a photograph that was taken directly after mixing the protein solutions with the necessary aliquots of NdCl_3 , while (b) presents a photograph of the same solutions but after 48 h aggregation and sedimentation. Solutions of $15 \mu\text{M}$ BLG with NdCl_3 concentrations between 50 and $250 \mu\text{M}$ stay colloidal stable for at least 48 h, but obviously sedimentation can be observed at NdCl_3 concentrations around the point of zero net charge ($\sim 30 \mu\text{M}$, see main text).

Specific effects of Ca^{2+} ions and molecular structure of β -lactoglobulin interfacial layers that drive macroscopic foam stability†

Soft Matter, 2016, 12, 5995

□ Main idea

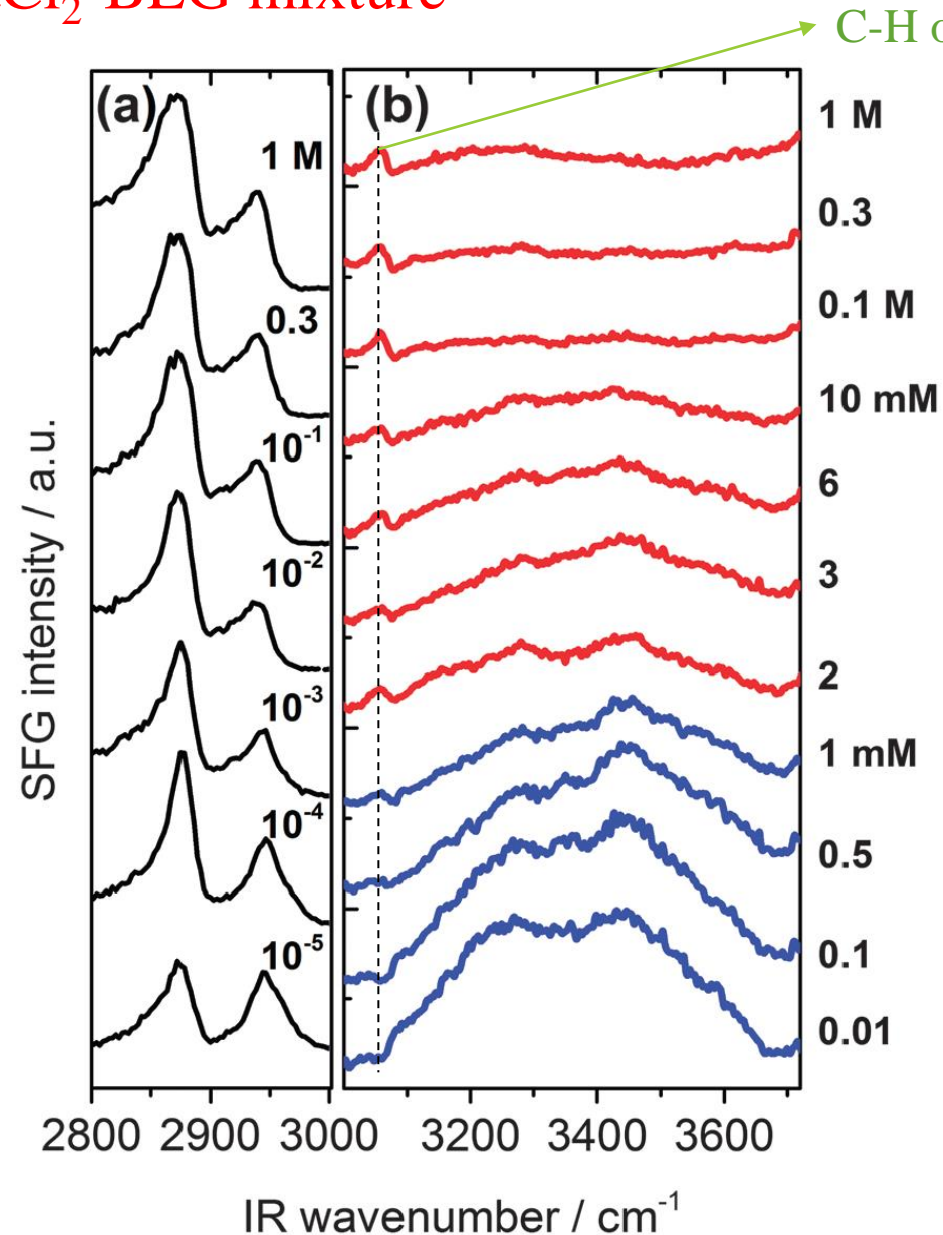
Björn Braunschweig,^{*abcd} Felix Schulze-Zachau,^{acd} Eva Nagel,^a Kathrin Engelhardt,^{‡a} Stefan Stoyanov,^e Georgi Gochev,^{ef} Khr. Khristov,^e Elena Mileva,^e Dotchi Exerowa,^e Reinhard Miller^f and Wolfgang Peukert^{abcd}

β -Lactoglobulin (BLG) adsorption layers at air–water interfaces were studied *in situ* with vibrational sum-frequency generation (SFG), tensiometry, surface dilatational rheology and ellipsometry as a function of bulk Ca^{2+} concentration. The relation between the interfacial molecular structure of adsorbed BLG and the interactions with the supporting electrolyte is additionally addressed on higher length scales along the foam hierarchy – from the ubiquitous air–water interface through thin foam films to macroscopic foam. For concentrations <1 mM, a strong decrease in SFG intensity from O–H stretching bands and a slight increase in layer thickness and surface pressure are observed. A further increase in Ca^{2+} concentrations above 1 mM causes an apparent change in the polarity of aromatic C–H stretching vibrations from interfacial BLG which we associate to a charge reversal at the interface. Foam film measurements show formation of common black films at Ca^{2+} concentrations above 1 mM due to considerable decrease of the stabilizing electrostatic disjoining pressure. These observations also correlate with a minimum in macroscopic foam stability. For concentrations >30 mM Ca^{2+} , micrographs of foam films show clear signatures of aggregates which tend to increase the stability of foam films. Here, the interfacial layers have a higher surface dilatational elasticity. In fact, macroscopic foams formed from BLG dilutions with high Ca^{2+} concentrations where aggregates and interfacial layers with higher elasticity are found, showed the highest stability with much smaller bubble sizes.

□ Charge inversion happened when $> 2\text{mM}$ of CaCl_2 in BLG solution

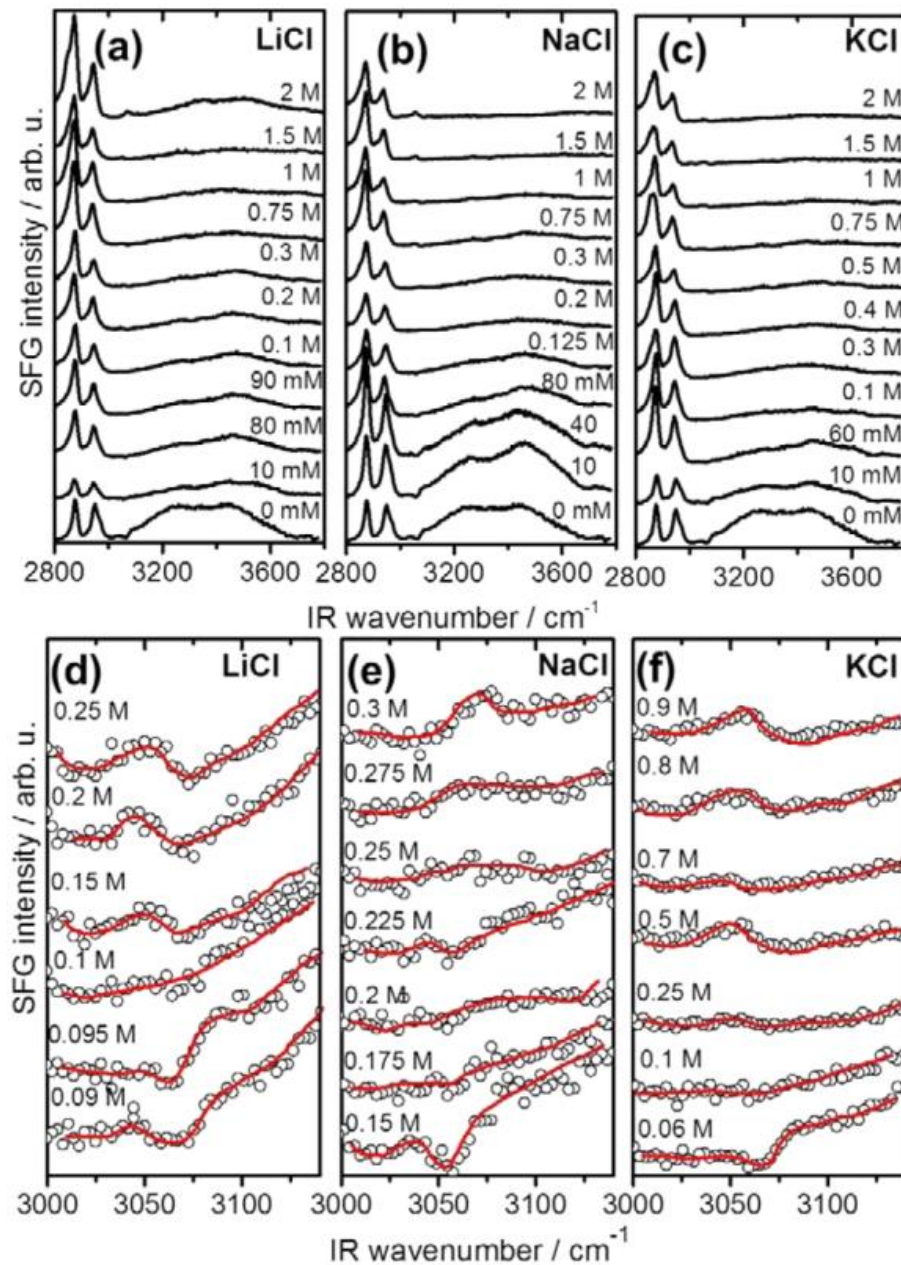
□ C-H of aromatic ring change the sign from negative to positive

❖ CaCl_2 -BLG mixture



- ❖ At 0 mM CaCl_2 , BLG is negatively charged
- ❖ < 2 mM CaCl_2 , decrease OH intensities
- ❖ > 2 mM CaCl_2 , CH of aromatic ring is changing the sign from negative peak to positive peak which indicated the charged inversion
- ❖ > 100 mM CaCl_2 , enhance the OH again
- ❖ CH_3 slightly changed due to interfere with OH but overall CH is very similar

□ Monovalent salts- BLG



below and in the Supporting Information. At salt concentrations of 2 M, the intensities of O–H bands decrease to negligible values for NaCl and KCl solutions, while there is a weak but noticeable increase in O–H intensity when the LiCl concentration is increased from 1.5 to 2 M.

. *Phys. Chem. B* 2015, 119, 17, 5505–5517