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# Rheology of protein-stabilised emulsion gels envisioned as composite networks.

## 1 - Comparison of pure droplet gels and protein gels

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### Abstract

#### *Hypothesis*

Protein-stabilised emulsion gels can be studied in the theoretical framework of colloidal gels, because both protein assemblies and droplets may be considered as soft colloids. These particles differ in their nature, size and softness, and these differences may have an influence on the rheological properties of the gels they form.

#### *Experiments*

Pure gels made of milk proteins (sodium caseinate), or of sub-micron protein-stabilised droplets, were prepared by slow acidification of suspensions at various concentrations. Their microstructure was characterised, their viscoelasticity, both in the linear and non-linear regime, and their frequency dependence were measured, and the behaviour of the two types of gels was compared.

#### *Findings*

Protein gels and droplet gels were found to have broadly similar microstructure and rheological properties when compared at fixed volume fraction, a parameter derived from the study of the viscosity of the suspensions

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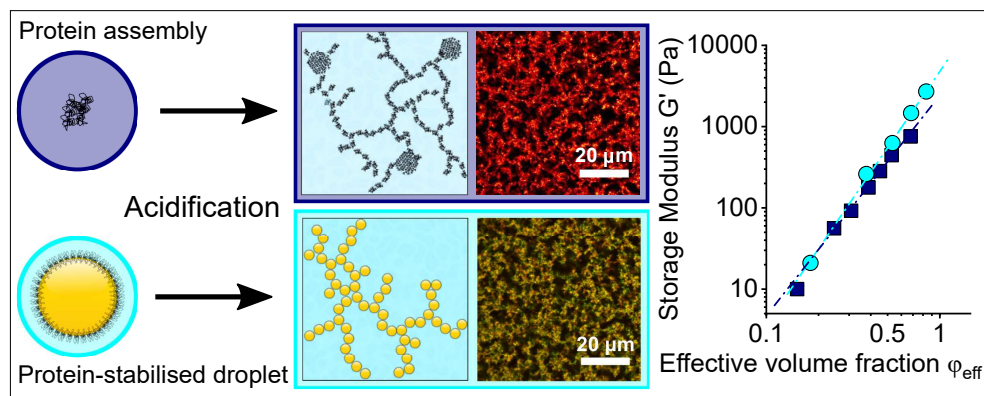
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formed by proteins and by droplets. The viscoelasticity displayed a power law behaviour in concentration, as did the storage modulus in frequency. Additionally, strain hardening was found to occur at low concentration. These behaviours differed slightly between protein gels and droplet gels, showing that some specific properties of the primary colloidal particles play a role in the development of the rheological properties of the gels.

*Keywords:* Colloidal gel, Rheology, Emulsion, Sodium caseinate, Viscoelasticity, Protein-stabilized droplet, Microstructure

## Graphical abstract



## 1. Introduction

Emulsion and protein gels form the basis of many food products, such as yoghurt, soft cheese or tofu, where the flocculation of a vegetable or animal milk leads to the formation of a soft solid via aggregation of proteins and fat droplets. This process has been used for millenia in traditional cooking, but a deep understanding of the mechanisms of the physical transformation occurring in these systems only came in recent decades with the study of colloidal gels [1, 2, 3]. While much effort has been spent in correlating the structure formation and the gel properties with the interparticle interactions [4], there is yet to be a full understanding of food-based colloidal gels, both in terms of fundamental science and of specific applications.

The gels of interest here typically exhibit a fractal microstructure that can be described by a fractal dimension  $D_f$  [5]. This fractal microstructure affects their mechanical properties [6, 7]. The storage modulus  $G'$  of the gels also typically shows a power-law variation with the volume fraction  $\phi$  [8, 9]:

$$G' \sim \phi^A \tag{1}$$

12 It has been shown that the exponent  $A$  can be related to the fractal dimension  
 13 of the gels, with the relationship depending on the gelation regime. For gels  
 14 formed via diffusion limited cluster aggregation, generally at low volume  
 15 fractions, it was found that  $A = (3 + D_b)/(3 - D_f)$ , where  $D_b$  is defined as  
 16 the bond (or backbone) dimension of the network [4, 3]. At higher volume  
 17 fractions, the links between clusters are weaker and  $A = 1/(3 - D_f)$  [10]. A  
 18 general model, as suggested by Wu and Morbidelli, is  $A = (1 + (2 + D_b)(1 -$   
 19  $\epsilon))/(3 - D_f)$ , where  $\epsilon \in [0; 1]$  depends on the type of regime [9].

20 This theoretical framework for colloidal gels can be applied not only to  
 21 model attractive hard spheres, but also to protein and emulsion gels, and in  
 22 particular to casein systems [5, 11, 12].

23 Caseins are the most common proteins in cow's milk. They have at-  
 24 tracted considerable attention for the last 40 years, mainly because of their  
 25 widespread use as food ingredients in numerous commercial products (pro-  
 26 cessed cheese, ice-cream, coffee whiteners, cream liqueur, etc). In this study,  
 27 sodium caseinate, which is derived from the caseins in milk, was used both  
 28 as gelling agent and as emulsifier.

29 Sodium caseinate, when suspended in water, forms naturally-occurring  
 30 aggregates, that are thought to be elongated with a length around 20 nm [13,  
 31 14, 15]. The surface of these aggregates is charged negatively at neutral pH,  
 32 and electrostatic repulsion is an important condition for their stability [16].  
 33 When such suspensions are acidified, the decrease in electrostatic repulsion  
 34 causes the aggregation of proteins that, if slow and rather homogeneous,  
 35 leads to the formation of a gel [17, 12, 18, 19, 20].

36 Previous work using confocal microscopy has highlighted their fractal  
 37 structure, which was found to be dependent on the pH, ageing time and  
 38 addition of other components [21, 22, 6, 23, 24, 25]. A power-law dependence  
 39 of the viscoelasticity on concentration of acid casein gels, using both native  
 40 casein and sodium caseinate, has been observed in previous studies [7, 18],  
 41 which was attributed to their fractal nature [12, 5]. In addition, the frequency  
 42 dependence of the gels has been characterised [12, 7, 26]. Finally, the brittle

43 fracture of casein gels has also been studied from a fundamental perspective  
44 [27, 28].

45 Besides gel formation, sodium caseinate is widely used to form oil-in-  
46 water emulsions [29, 30, 31]. Typically, during emulsification, the proteins  
47 do not completely adsorb at the interface, leaving a residual fraction of pro-  
48 tein suspended in the continuous phase after emulsification [32, 33], and the  
49 resulting emulsion is thus a mixture of droplets and of un-adsorbed proteins  
50 [34]. Consequently, the distinction is made here between protein-stabilised  
51 emulsions, and purified droplet suspensions, from which the fraction of un-  
52 adsorbed proteins was removed.

53 As with caseinate gels, the acidification of sodium caseinate-stabilised  
54 emulsions, and of purified droplet suspensions, leads to the formation of  
55 fractal gels called *emulsion gels* [16], and of pure droplet gels respectively.  
56 For pure caseinate-stabilised droplet gels, the nature of the interactions at  
57 play during gelation is the same as for caseinate gels, as the droplets become  
58 attractive at the isoelectric point of the protein.

59 Emulsion gels have been studied in the past and compared to protein gels  
60 [12, 11, 16], and they have been shown to present a similar fractal structure  
61 [35]. However, the properties of pure droplet gels have not been clearly inves-  
62 tigated, in that the systems studied have invariably contained both droplets  
63 and free protein. This has made it extremely difficult to draw a consis-  
64 tent comparison of protein assemblies and protein-stabilised droplets as gel-  
65 forming particles. Investigating the pure components - droplets and proteins  
66 - would enable a consistent comparison of their behaviours and understand-  
67 ing of their mixtures, that would be relevant from both a fundamental and  
68 a technological point of view.

69 To this end, the present study investigates the similarities and differ-  
70 ences between caseinate gels and pure caseinate-stabilised droplet gels over a  
71 wide range of concentrations. It focuses more specifically on the microstruc-  
72 ture and on key rheological features of these systems, namely the linear and  
73 non-linear viscoelasticity and the frequency dependence, as well as on their  
74 variations with the concentration in colloidal species.

75 This comparison between caseinate gels and droplet gels draws on the re-  
76 sults of a previous study of the viscosity of the suspensions that are used to  
77 prepare these gels [34]. It was shown that both droplets and protein assem-  
78 blies can be studied in the framework developed for soft colloidal particles  
79 [36]. Consequently, their concentrations can be scaled by the effective volume  
80 fraction  $\phi_{eff}$ , which can reach high values due to the possible compression,

81 interpenetration and deformation of soft colloids [37, 38]. It is demonstrated  
82 in the present study how the same concentration scaling can be used to un-  
83 derstand the behaviour of both sodium caseinate and droplet gels.

## 84 **2. Materials & Methods**

### 85 *2.1. Preparation of protein and droplet suspensions*

86 Suspensions of pure sodium caseinate (hydrodynamic radius 11 nm) and  
87 of pure sodium caseinate-stabilised droplets (hydrodynamic radius 110 nm)  
88 were prepared at a range of concentrations, as described previously [34]. The  
89 procedure is given in detail in Section 1.1 of the supplementary information  
90 for completeness. These suspensions were then used as sols for the prepara-  
91 tion of acid-induced gels.

92 In the following, concentrations of both the protein and droplet samples  
93 are given in terms of the effective volume fraction  $\phi_{eff}$ . This was determined  
94 from intrinsic viscosity measurements on dilute samples as detailed in Ref.  
95 [34]. As such the weight concentration is related to  $\phi_{eff}$  by a simple fac-  
96 tor, which was found to be  $(2.2 \pm 0.1) \text{ mL g}^{-1}$  and  $(8.5 \pm 0.2) \text{ mL g}^{-1}$  for the  
97 droplet and protein suspensions respectively. The use of this parameter is  
98 discussed in detail in Ref. [34].

### 99 *2.2. Preparation of protein and droplet gels*

100 The decrease in pH required for the gelation of the suspensions of sodium  
101 caseinate and of pure sodium-caseinate stabilised droplets to occur was achieved  
102 by the slow hydrolysis of glucono  $\delta$ -lactone (Roquette), as detailed in Section  
103 1.2 of the supplementary material.

### 104 *2.3. Laser scanning confocal microscopy*

105 The gels were imaged using laser scanning confocal microscopy, here a  
106 setup based on an LSM 780 microscope on inverted Axio observer (Zeiss).  
107 It has to be noted that the resolution of confocal microscopy (limited to  
108  $\approx 200 \text{ nm}$  by light diffraction) does not allow imaging of the single protein  
109 aggregates, or single droplets. Instead, the lengthscale accessible by this  
110 imaging technique corresponds to the structure over a few colloidal particles,  
111 and is thus suitable for the description of colloidal aggregation and gelation.

112 *2.3.1. Protocol for imaging of gels*

113 Rhodamine B (Sigma Aldrich) and Bodipy 493/503 (Molecular Probes)  
 114 were added to the samples of protein and droplet suspensions, that were then  
 115 mixed with glucono  $\delta$ -lactone, as detailed in Section 1.3 of the supplementary  
 116 material.

117 *2.4. Image analysis*

118 The image analysis of 2D micrographs was performed using the image  
 119 processing software ImageJ [39].

120 The Fast Fourier Transform (FFT) analysis was applied to the image  
 121 after Hanning windowing. The image in the Fourier space was then radially  
 122 averaged to obtain the spectrum  $I(q)$ . The wave vector  $q$  represents a spatial  
 123 frequency, it is a function of the distance from the centre in the Fourier space  
 124 and of the image size, and is expressed in  $\mu\text{m}^{-1}$ .

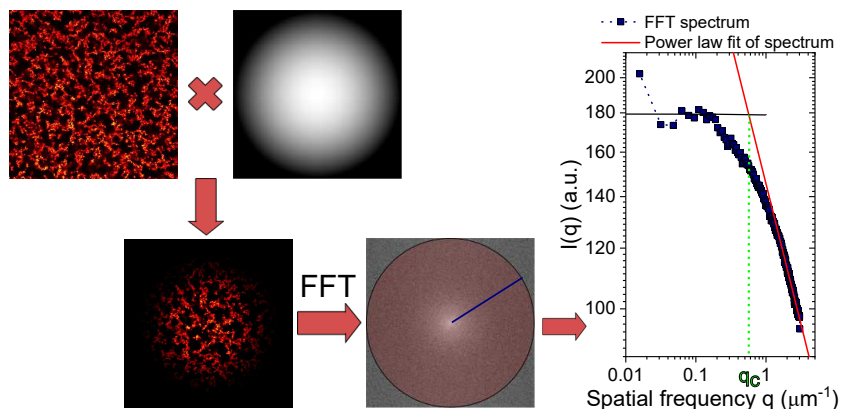


Figure 1: Protocol for the analysis of a micrograph.

The image is multiplied by a Hanning window of the same size before the Fast Fourier Transform is calculated. The spectrum  $I(q)$  is obtained using the plugin Radial Profile to perform a radial average of the Fourier transform.

The decrease of the spectrum  $I(q)$  is then fitted by a power law, linear in double logarithmic scale. Its intersection with the plateau defines the critical spatial frequency  $q_c$ .

125 The variations of  $I(q)$  can be described by several parameters. The po-  
 126 sition of the shoulder  $q_c$  was chosen in this study as critical wave vector,  
 127 because it can be estimated in a reproducible way by fitting the power law  
 128 decrease of the peak, as opposed to the top of the peak that is slightly flat-  
 129 tened. The determination of the position of the shoulder  $q_c$  is illustrated in

130 Figure 1. This value was then used to estimate the critical lengthscale of the  
131 network in the real space  $L_C = 2\pi/q_c$ .

### 132 *2.5. Rheological measurements*

133 Oscillatory rheology measurements were performed using a stress-controlled  
134 MCR 502 rheometer (Anton Paar) and a Couette geometry (17 mm diame-  
135 ter profiled bob and cup CC17-P6, inner diameter 16.66 mm, outer diameter  
136 18.08 mm yielding a 0.71 mm tool gap, gap length 25 mm). To avoid slip at  
137 the wall during shearing, profiled bob and cup (serration width 1.5 mm, ser-  
138 ration depth 0.5 mm) were selected as measurement tools. The temperature  
139 was set by a Peltier cell at 35 °C during the entire measurement sequence.  
140 The operating temperature was chosen to ensure that the gelation occurs in  
141 the time scale of thousands of seconds for all the samples studied here. To  
142 prevent evaporation, a thin layer of silicon oil of low viscosity (10 cSt) was  
143 deposited on the surface of the sample.

144 The measurements were started immediately after mixing of the sample  
145 with glucono  $\delta$ - lactone and subsequent loading in the instrument. As repre-  
146 sented in Figure 2, the measurements consisted of 4 steps, detailed in Section  
147 1.4 of the supplementary information, for which the strain was chosen to stay  
148 in the linear viscoelastic region at steps 1, 2 and 4.

149 For each sample, 3 measurements of the same batch of sample were per-  
150 formed and the values of each data point were averaged.

## 151 **3. Results & Discussion**

152 The comparison of pure caseinate-stabilised droplet gels and caseinate  
153 gels was performed by studying each type of system over a wide range of  
154 concentrations, scaled by the effective volume fractions  $\phi_{eff}$ . This extensive  
155 characterisation of each type of system ensured that the similarities and  
156 differences observed derived from the intrinsic differences in size, structure  
157 and softness between caseinate assemblies and caseinate-stabilised droplets.  
158 This precaution distinguishes the present study from previous comparisons  
159 of emulsion gels and protein gels [40, 12] and is the key to the progress made  
160 here.

### 161 *3.1. Microstructure of gels: colloidal species and volume fraction*

162 Confocal microscopy is a commonly used technique to observe the struc-  
163 ture of colloidal gels at the micron scale [41, 42, 43] that makes possible



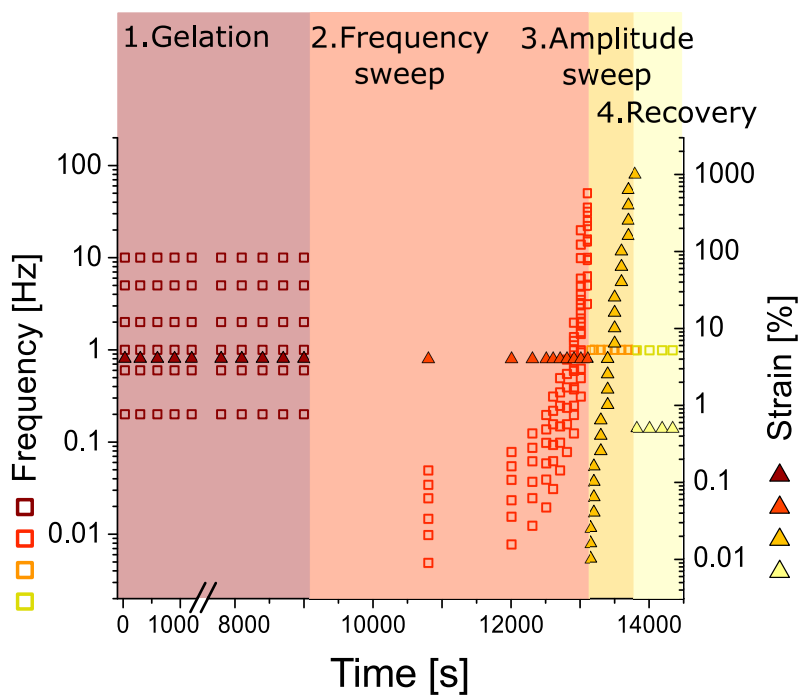


Figure 2: Illustration of the measuring sequence for the oscillatory rheometry of the emulsion gels, detailed in the Methods section. Frequency (open squares) and strain amplitude (filled triangles) of the oscillatory shear vary with time in the 4 steps of the measurement. The multiwave mode was activated at steps 1 and 2, leading so several signal frequencies were used simultaneously. At the time  $t = 0$  s, the glucono  $\delta$ -lactone was added to the sols.

164 the comparison of this structure for gels of different composition and volume  
165 fraction. Here, gels that were prepared by acidifying suspensions of either  
166 sodium caseinate or pure caseinate-stabilised droplets, at different concentra-  
167 tions, are imaged and compared. The micrographs of caseinate and droplet  
168 gels, together with their characteristic lengthscale  $L_C$  are presented in Fig-  
169 ure 3.

170 As can be seen, the micrographs are similar for protein and droplet gels,  
171 especially at lower volume fraction. Indeed, in both cases, the fractal struc-  
172 ture typical of colloidal gels is present, with interconnected networks of par-  
173 ticle aggregates (in colour) and water-filled pores (in black). At high concen-  
174 trations, these networks are denser in particles, with the pores of the droplet  
175 gels appearing to be smaller than for the protein gels.

176 In addition, the characteristic lengthscales  $L_C$  are of the same order of  
177 magnitude for the two components, and their values range between  $5\ \mu\text{m}$   
178 and  $20\ \mu\text{m}$  for all the gels presented here. The variation of  $L_C$  as a function  
179 of the volume fraction cannot be interpreted quantitatively because of the  
180 significant noise in the data. This is partially related to the fact that the  
181 features picked up by the Fast Fourier Transform are probably a combination  
182 of the size of the aggregates and the size of the pores.

183 Thus, although the individual droplets are one order of magnitude larger  
184 than the individual protein assemblies, the gels formed by these two types of  
185 colloidal particles present a very similar fractal structure at a given effective  
186 volume fraction  $\phi_{eff}$ . It would also be interesting to perform a more thorough  
187 investigation of the dependence of the characteristic length scale  $L_C$  of the  
188 network on the effective volume fraction  $\phi_{eff}$ , by using higher quality confocal  
189 micrographs and a more precise image analysis technique, for example texture  
190 analysis microscopy [44].

### 191 *3.2. Rheological study of droplet gels and protein gels*

192 In order to investigate further the comparison between protein gels and  
193 droplet gels, it is interesting to characterise their rheological behaviour. As  
194 detailed in Figure 2, the viscoelastic moduli,  $G'$  and  $G''$ , are first compared  
195 at fixed frequency, strain and time after gelation for gels of different compo-  
196 sitions. Then the dependences of  $G'$  and  $G''$  on the frequency are presented.  
197 Finally, the non-linear viscoelasticity of the gels is considered.

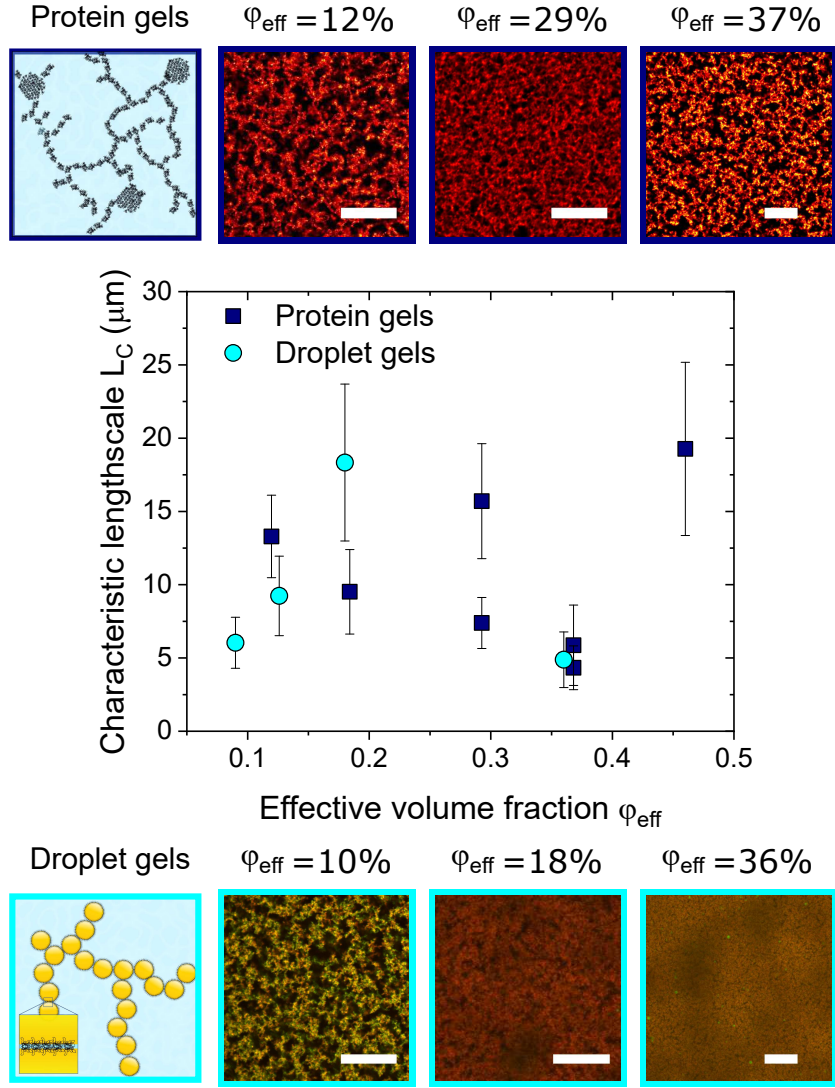


Figure 3: Micrographs ( $100 \mu\text{m} \times 100 \mu\text{m}$ ) of aged acid-induced gels formed from suspensions of: (top) sodium caseinate, and of (bottom) caseinate-stabilised droplets, at different volume fractions  $\phi_{\text{eff}}$ . The scale bars are  $30 \mu\text{m}$  long. The inset in the cartoon representing the droplet gels shows the interactions between the caseinate adsorbed at the oil-water interface. The graph presents the characteristic lengthscale  $L_C$  of the gels, as a function of the volume fraction  $\phi_{\text{eff}}$ , for caseinate gels (squares, navy blue) and caseinate-stabilised droplet gels (circles, cyan).

For each point,  $L_C$  was obtained by performing a FFT of one micrograph and extracting the position of the peak in the spectrum  $I(q)$ , as described in Figure 1. The inaccuracy of this determination is indicated by the error bar. Where two points are presented for one concentration, they correspond to different micrographs of similar samples.

198 *3.2.1. Linear viscoelasticity of gels*

199 The gelation of sodium caseinate and sodium caseinate-stabilised droplets  
200 is presented in Figure 4(a). As with previous studies on colloidal gels, these  
201 systems do not reach an equilibrium state, but go through rearrangements of  
202 their network upon ageing [45, 46]. To compare the viscoelasticity of the gels  
203 at similar ageing state, it is possible to superimpose the gelation curves by  
204 using horizontal and vertical shifts in logarithmic scale [26, 47, 48], as can be  
205 seen in Figure 4(b). The horizontal and vertical shift factors  $\alpha_t$  and  $\alpha_{G'}$ , and  
206 the protocol used to determine them, can be found in Figure S3 and Section  
207 2 of the supplementary material.

208 The viscoelastic behaviour of gels was arbitrarily compared at  $t/\alpha_t = 1.4$ .  
209 This value was chosen because it is the highest reached by all the gels studied,  
210 even those with a very slow gelation. Because the kinetics that determine  
211  $\alpha_t$  remain the same post gelation, the rise in elastic modulus  $G'$  with  $t/\alpha_t$   
212 is similar for all samples. Thus using  $G'$  at constant  $t/\alpha_t$  is appropriate for  
213 comparison of different concentrations. The elastic modulus  $G'$  and the loss  
214 modulus  $G''$  of the two types of gels at  $t/\alpha_t = 1.4$ , measured at 1 Hz, are  
215 presented in Figure 4 as functions of their effective volume fraction  $\phi_{eff}$ .  
216 In addition, the phase angle  $\delta = \arctan(G''/G')$ , indicating the viscoelastic  
217 character of the gels, is found to be significantly different for each sort of gels,  
218 with  $\delta_{prot}$  varying between  $21^\circ$  and  $24^\circ$ , and  $\delta_{drop}$  between  $13^\circ$  and  $17^\circ$ . The  
219 higher phase angle found for protein gels indicates that their behaviour is  
220 slightly shifted towards the viscous materials on the spectrum of viscoelastic  
221 behaviour.

222 As can be seen in Figure 4, sodium caseinate and sodium caseinate-  
223 stabilised droplets form gels of very similar viscoelasticity when scaled by  
224 the volume fraction. More precisely, the storage and loss moduli of droplet  
225 gels are slightly higher, at a given volume fraction, than those of protein gels.  
226 The similarity of the viscoelasticity of the two types of gels can be related to  
227 their similar microstructure, as observed in Figure 3.

228 Our result differs significantly from a previous study on caseinate-stabilised  
229 emulsion gels [12]. Although the concentrations chosen for the comparison  
230 were arbitrary in Ref. [12], it was shown that emulsion gels had a similar mod-  
231 ulus to a protein gel with a threefold increase in protein concentration, and  
232 the authors thus concluded that emulsions form gels with a higher viscoelas-  
233 ticity than protein gels. It is thought that this discrepancy arises mostly from  
234 the choice of parameter to describe the composition of these systems. Indeed,

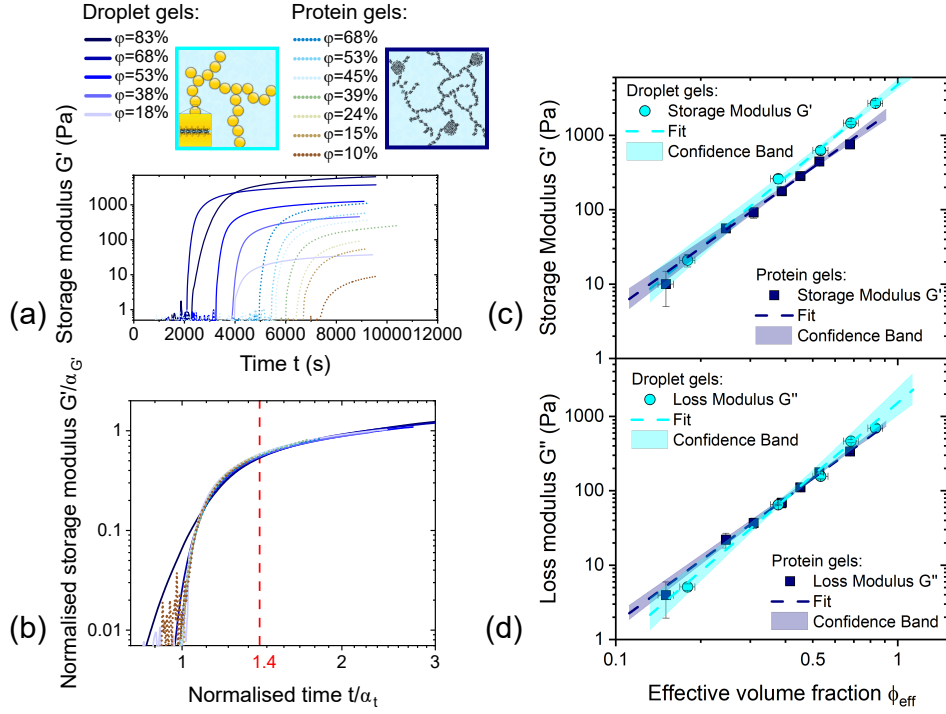


Figure 4: Left panel: (a) Storage moduli  $G'$  upon formation of droplet gels and protein gels, (b) Master curve for the formation of the colloidal gels, the horizontal ( $\alpha_t$ ) and vertical ( $\alpha_{G'}$ ) shift factors are presented in Figure S3. Right panel: Storage ( $G'$ , (c)) and loss ( $G''$ , (d)) moduli at 1 Hz of protein-stabilised droplet gels (circles, cyan) and of protein gels (squares, navy blue) at  $t/\alpha_t = 1.4$  as functions of the effective volume fraction of the gel  $\phi_{eff}$ . A fit (Equation 2) of each type of system was performed and the model (parameters listed in Table 1) as well as the 95% confidence band are displayed on each graph.

The horizontal error bars arise from error propagation upon calculation of the volume fraction, as detailed in Section 3 of the supplementary material, while the vertical error bars arise from the uncertainties in determining the shift factors  $\alpha_{G'}$  and  $\alpha_t$ .

235 the storage modulus can be presented as a function of either the protein con-  
 236 centration, or of the weight concentration of each colloidal particle, leading  
 237 to large differences between protein gels and droplet gels, but in opposite  
 238 directions, as illustrated in Figure S4 of the supplementary material.

239 More generally, the weight concentration is unlikely to be a relevant pa-  
 240 rameter to compare gels made of colloidal particles of a very different nature,  
 241 such as caseinate assemblies and droplets - the former being water-swollen  
 242 and soft, while the latter are filled with oil and more rigid. The same is true  
 243 for the use of the protein concentration, as shown in Ref. [12]. Instead, we  
 244 argue here that a more appropriate scaling to use for comparing protein gels  
 245 and droplet gels is the volume fraction, despite its definition being non-trivial  
 246 for complex colloidal particles [34].

247 Consequently, we find that there is little difference between the two types  
 248 of gels, provided that the comparison is drawn between samples at the same  
 249 effective volume fraction  $\phi_{eff}$ . Furthermore, the variation of the viscoelas-  
 250 ticity with the volume fraction for the protein gels and the droplet gels can  
 251 be quantified by using a fit to a power law, as discussed below.

*Power-law increase with volume fraction.* As can be seen in Figure 4, the  
 variations of both storage  $G'$  and loss  $G''$  moduli as functions of effective  
 volume fraction can be described as a power law for the two types of gels:

$$G(\phi_{eff}) = G_{0,\phi} \times \phi_{eff}^{\alpha} \quad (2)$$

252 Where the pre-factor of the power-law  $G_{0,\phi}$  and the exponent  $\alpha$  are two pa-  
 253 rameters to be determined. The values found by fitting  $G'(\phi_{eff})$  and  $G''(\phi_{eff})$   
 254 with Equation 2 are summarised in Table 1.

Table 1: Parameters for Equation 2 to fit viscoelasticity of gels at 1 Hz displayed in Figure 4

Gel type	Storage modulus $G'$		Loss modulus $G''$	
	$G'_{0,\phi}$	$\alpha$	$G''_{0,\phi}$	$\alpha$
Droplet gels	$(4.78 \pm 0.22)$ kPa	$3.1 \pm 0.1$	$(1.52 \pm 0.21)$ kPa	$3.2 \pm 0.1$
Protein gels	$(2.42 \pm 0.19)$ kPa	$2.7 \pm 0.1$	$(1.01 \pm 0.03)$ kPa	$2.8 \pm 0.1$

255 This power-law dependence of the viscoelasticity of sodium caseinate gels  
 256 is in good correspondence with previous studies on casein gels [6, 7, 18, 26,  
 257 24]. The value of the exponent for sodium caseinate varies significantly with  
 258 temperature, as it was found that  $\alpha = 2.57$  at 30 °C and  $\alpha = 3.73$  at 50 °C

259 [26]. The value found here for gels formed at 35 °C is thus in good agree-  
260 ment with these results. In addition, no data is available on the rheological  
261 properties of acid-induced droplet gels.

262 The power law dependence of the elastic modulus  $G'$  is a feature of fractal  
263 colloidal gels, as previously observed experimentally and numerically [5, 8,  
264 49, 50, 51, 52], that can be related to the fractal dimension  $D_f$ . However,  
265 the large range of volume fractions for the gels presented here makes such  
266 analysis impractical, in the absence of additional characterisation of these  
267 networks..

268 The study of the gel moduli as a function of their composition, described  
269 both by the nature of the elementary particles and by their volume fraction,  
270 thus offers some information on the mechanical properties of caseinate gels  
271 and caseinate-stabilised droplet gels. The behaviour of the two types of gels  
272 is very similar and reminiscent of those of more model colloidal gels. In  
273 addition to this static view of protein and emulsion gels, it is important to  
274 compare their dynamic properties.

### 275 3.2.2. Frequency dependence of gels

276 The moduli of the newly formed gels were then measured over a wide  
277 range of frequency. This measurement of the frequency dependence makes it  
278 possible to probe the dynamics of the gels. Because these exhibit a solid be-  
279 haviour in the linear viscoelastic range, this aspect is limited to fluctuations  
280 within the gel network, for example rearrangement of the particle bonds, re-  
281 laxation of the stress bearing strands, or motion of non-stress bearing strands  
282 like dangling chains.

283 *Comparison between protein gel and droplet gel.* In order to compare simi-  
284 lar gels of proteins and of protein-stabilised droplets, gels of equal volume  
285 fraction ( $\phi_{eff} = 0.53$ ) are displayed in Figure 5 (a).

286 Both the protein gel and the droplet gel exhibit an increase of their vis-  
287 coelasticity with the angular frequency  $\omega$ , in agreement with previous studies  
288 on colloidal gels [7, 26, 12, 53]. The storage modulus  $G'$  increases moder-  
289 ately for the two types of gels, while the loss modulus  $G''$  also rises with  $\omega$ ,  
290 but with a slightly different behaviour for protein gels and droplet gels. The  
291 increase of  $G''$  is at odds with the frequency dependence of dilute colloidal  
292 gels, for which a decrease of  $G''$  was observed [50], but is in good correspon-  
293 dence with the computed linear viscoelasticity of a similar system [53]. This  
294 behaviour may indicate the presence of a relaxation process that is visible in

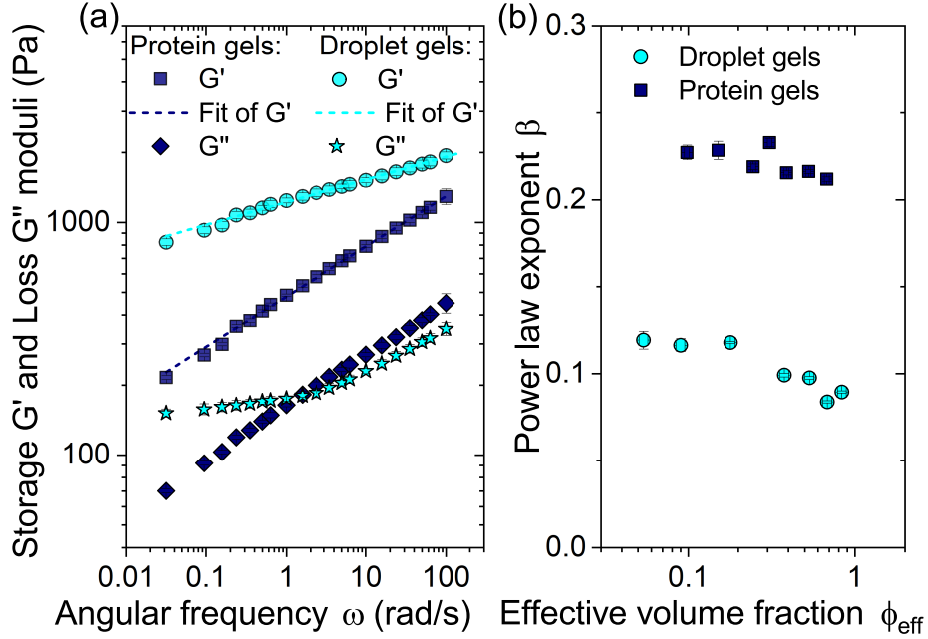


Figure 5: (a) Comparison of the frequency dependence for protein gels (sodium caseinate:  $\phi_{eff} = 53\%$ , in navy blue) and droplet gels (caseinate-stabilised oil droplets:  $\phi_{eff} = 53\%$ , in cyan). Storage modulus  $G'$  and loss modulus  $G''$  are represented as functions of the angular frequency  $\omega$ .  $G'$  was fitted with a power law for both types of samples, and the fitting parameters can be found in Table 2.

(b) Comparison of frequency dependence for protein gels (squares, navy blue) and protein-stabilised droplets (circles, cyan): power-law exponent  $\beta$ , obtained by fitting  $G' = f(\omega)$  with Equation 5, as a function of the effective volume fraction  $\phi_{eff}$ .

295 the frequency range covered at low concentration, but which moves to much  
 296 lower frequencies at higher concentrations, and so becomes invisible.

297 In addition, as can be seen in Figure 5 (a), the viscoelastic response  
 298 of the two types of gels differ slightly. Indeed, the protein gel displays a  
 299 higher dependence on frequency than the droplet gel, as both storage and  
 300 loss moduli increase faster with the angular frequency than for the droplet  
 301 gel. Another noticeable difference is the non-monotonic behaviour of the loss  
 302 modulus  $G''$  for droplets gels. This behaviour may be an indication of a  
 303 relaxation of droplet networks, that would be absent for protein gels in this  
 304 range of frequency, but an extended spectrum would be required to definitely  
 305 identify a possible peak.



In order to quantify the difference in variation of the storage modulus  $G'$  with the angular frequency  $\omega$  for the two types of gels, its behaviour can be modelled by a power law [54, 55]:

$$G' = G'_{0,\omega} \left( \frac{\omega}{\omega_\beta} \right)^\beta \quad (3)$$

306 Where  $G'_{0,\omega}$  and  $\beta$  are two empirical parameters to be determined, and  $\omega_\beta =$   
 307  $1.00 \text{ rad s}^{-1}$  is used for dimensional purposes.

308 The frequency dependence of the protein gel and droplet gel of effective  
 309 volume fraction  $\phi_{eff} = 53\%$  is thus fitted as displayed in Figure 5 (a), and  
 310 the values of the empirical parameters for can be found in Table 2.

Table 2: Frequency dependence of gels: parameters from using Equation 3 to fit the variation of the storage modulus  $G'$  with the angular frequency  $\omega$  of protein gel and droplet gel of effective volume fraction  $\phi_{eff} = 53\%$  displayed in Figure 5.

Gel type	$G'_{0,\omega}$	$\beta$
Protein gels	0.5 kPa	0.22
Droplet gels	1.2 kPa	0.10

311 The value of the exponent  $\beta$  for caseinate gels is slightly higher than  
 312 in previous studies. Indeed, for acid-induced casein gels at  $30^\circ\text{C}$ ,  $\beta$  was  
 313 measured to be 0.15 [7, 26, 12]. This discrepancy may arise from a difference  
 314 of pH of the gels studied, a parameter which was shown to have a strong  
 315 influence on the frequency dependence of such systems [12].

316 No comparable data could be found for the frequency dependence of gels  
 317 made of pure protein-stabilised droplets, but the comparison between protein  
 318 gels and gels of mixtures of proteins and droplets was performed and appears  
 319 to be system-dependent. On one hand, the exponent  $\beta$  was found to be  
 320 identical for acid-induced gels of caseinate emulsions and for caseinate gels,  
 321 i.e. 0.15 [12]. On the other hand, for heat-set gels and emulsion gels prepared  
 322 with  $\beta$ -lactoglobulin, the slope  $\beta$  was found to be three times higher for  
 323 protein gels than for emulsion gels [11]. This discrepancy is believed to  
 324 result from the nature of the bonds between particles in these two types of  
 325 gels: heat-set gels form more transient bonds than acid-induced gels, making  
 326 for more mobile structures.

327 *Influence of the volume fraction on the frequency dependence.* This analysis  
 328 of the frequency dependence can be extended to gels at all concentrations,

329 as the curves display a similar power-law variation. These can be found  
330 in Figure S6 of the supplementary information. The empirical model in  
331 Equation 3 was thus applied to the gels of droplets and proteins prepared at  
332 different volume fractions, and the resulting value of the power law exponent  
333 for all the gels is displayed in Figure 5 (b).

334 As can be observed, there is little influence of the volume fraction on  
335 the variation of the elasticity of the gels with the frequency. This indicates  
336 that over the range of concentrations studied, the dynamical behaviour of  
337 the gels is the same. By contrast, the viscosity of the suspensions increases  
338 dramatically over the same range of volume fraction, as discussed in a previ-  
339 ous study [34]. The negligible variations of the frequency dependence of the  
340 gels seem to indicate that there is no change in regime due to the crowding  
341 of the colloidal particles in the solid state, and the gels formed by proteins  
342 and droplets suspensions are similar in that respect.

343 Consequently, the difference in dynamic behaviour between protein gels  
344 and droplet gels observed in Figure 5 (a) is consistent over the range of  
345 volume fractions explored here, with the exponent  $\beta$  being larger for protein  
346 gels than for droplet gels. This seems to indicate that caseinate gels have  
347 more internal fluctuations than droplet gels regardless of their concentration  
348 [55].

349 Furthermore, the non-monotonic behaviour of the loss modulus  $G''$  with  
350 the frequency  $\omega$  observed for the droplet gel in Figure 5 (a) is also consistent  
351 over the range of volume fractions, as can be found in Figure S5. This is  
352 better visualised by looking at the phase angle of the gels, as presented in  
353 Figure S6. In contrast, the phase angle of all the protein gels studied is  
354 constant with frequency. The physical mechanism underlying this behaviour  
355 is not known but it represents an additional significant difference in the  
356 frequency dependence of droplet gels compared to protein gels.

357 Finally, these results of the linear viscoelasticity of colloidal gels can be  
358 compared with another sort of arrested state of colloidal particles, such as  
359 glasses of soft colloids like microgels [56]. For such systems, it was observed  
360 that at moderately high volume fraction, the glasses display a slow increase  
361 in elastic modulus  $G'$  with the frequency, associated with some mobility of  
362 the particles in an entropic glass. By contrast, at higher volume fraction,  
363 the particles are completely jammed and  $G'$  is constant over the range of  
364 frequency explored [57]. The fact that this frequency-independent regime  
365 is not reached here seems to indicate that the acid-induced gels studied are  
366 quite dynamic, rather than completely arrested, and that this is more the

367 case for protein gels than for droplet gels. Interestingly, this result is true  
368 over the range of volume fraction studied here, even for gels that are very  
369 concentrated.

### 370 *3.2.3. Strain dependence of the gels*

371 The oscillatory strain sweep performed on the protein and droplet gels  
372 after formation and frequency sweep, as shown in Figure 2 allows the study  
373 of the variations of the storage modulus with the amplitude of the strain  
374 oscillation. The typical strain behaviour of the gel is represented in Figure  
375 S7 , together with the definition and the values of the critical strain  $\gamma_c$ . To  
376 highlight the differences in strain response for all gels, this parameter was  
377 used to normalise the strain response of the gels and  $G'$  was divided by  
378 its value in the linear regime. The resulting normalised curves presenting  
379 the non-linear viscoelastic behaviour pure gels of proteins and of protein-  
380 stabilised droplets at different concentrations are displayed in Figure 6.

381 As can be seen, the nature of the non-linear regime varies with the type  
382 of gel formed and its volume fraction in proteins or droplets. The behaviour  
383 of gels at each concentration range is discussed separately below.

384 First, for gels prepared with suspensions of moderately low volume frac-  
385 tion of both proteins and protein-stabilised droplets, an increase of the nor-  
386 malised storage modulus  $G'/G'_0$  is observed when larger shear amplitudes  
387 are applied. This phenomenon is known as strain stiffening, and this result  
388 is in correspondence with previous studies of low-concentration gels, both  
389 experimental [51] and computational [58, 59]. Using, in one case, ultrasonic  
390 imaging and, in the other, simulations of the topology of the gel networks,  
391 it was found that this behaviour could be related to irreversible stretching  
392 and reorientation of the gel branches. This behaviour was also shown to be  
393 very dependent on the structure of the network, and hence on the volume  
394 fraction of the gel. The sparser the gel, the more structural heterogeneities  
395 make possible the redistribution of the stress before failure of the material,  
396 while denser gels are more homogeneous and thus lead to a quicker breaking  
397 of bonds in the absence of reorganisation of the network.

398 The strain stiffening is more pronounced, and is present on a wider range  
399 of volume fraction, for gels made of protein-stabilised droplets than for pro-  
400 tein gels. Because strain stiffening is related to the structural heterogeneities  
401 within the network, this result may indicate that the proteins form gels that  
402 are overall more homogeneous than the droplet gels at low volume fraction,  
403 making these networks less prone to stress redistribution. The decrease in

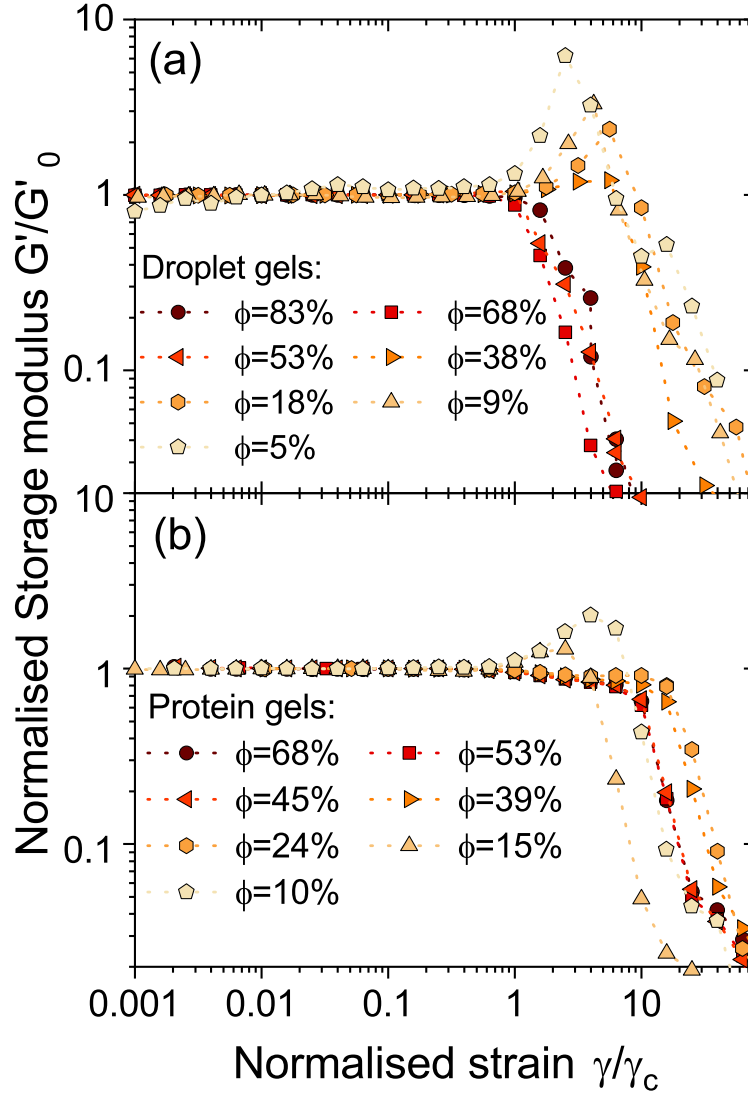


Figure 6: Storage modulus  $G'$  normalised by its value in the linear regime  $G'_0$  as a function of the oscillatory strain amplitude  $\gamma$  normalised by its value at the onset of the non-linear regime  $\gamma_c$ . (a) Sodium caseinate-stabilised droplet gels at several volume fractions  $\phi$ , (b) Sodium caseinate gels at several volume fractions  $\phi$ . Each curve is the average of 3 measurements, but for clarity the error bars are not represented here.

404 strain stiffening with the volume fraction for the two types of gels studied  
405 here is also in agreement with this phenomenological explanation. As no  
406 difference in the homogeneity of the gels is visible in the micrographs in Fig-  
407 ure 3, it can only be hypothesised that the difference is at smaller length  
408 scales.

409 In addition, for gels at higher concentrations, the protein gels show a  
410 slight softening in the non-linear regime, over one order of magnitude of  
411 strain amplitude, before fracture of the material. This effect is absent in the  
412 gels made of protein-stabilised droplets, where concentrated gels break at the  
413 end of the linear regime. This difference in the stress-bearing behaviour of  
414 concentrated gels may arise from structural differences between the networks;  
415 which is similar to more dilute gels. Indeed, it seems that the breakage of  
416 some bonds in the dense protein gels is not critical to the elasticity of the  
417 overall network, and leads only to a moderate decrease of  $G'$  as the shear  
418 amplitude increases. On the other hand, for the dense droplet gels, the  
419 immediate drop in elasticity seems to indicate that the integrity of the whole  
420 structure is degraded upon application of a critical shear stress  $\sigma_c$ .

421 This suspected difference in the structure of the two networks would  
422 thus possibly explain the different non-linear behaviours for protein gels and  
423 droplet gels. To test this hypothesis however would require imaging each of  
424 the gel samples over a wide range of lengthscales to quantify the structural  
425 heterogeneity not only over the scale of the fractal clusters, but also over the  
426 scale of the stress-bearing backbone.

427 Finally, a common feature of all the protein and droplet gels is the fracture  
428 of the material at very high shear, indicated by the decrease in their elasticity.  
429 The subsequent application of low-amplitude oscillatory shear on the gels led  
430 to no time-dependent recovery of the viscoelasticity, as presented in Figure  
431 S8, which indicates that the gel structure was irreversibly damaged. This  
432 result is in agreement with an extensive study on the fracture of caseinate  
433 gels [28].

#### 434 4. Conclusion

435 The full sequence of rheological measurement presented in Figure 2 and  
436 confocal imaging allowed a thorough characterisation of protein gels and  
437 droplet gels by their microstructure, linear and non-linear viscoelasticity,  
438 and frequency dependence. As the two types of gels are made with colloidal  
439 particles of different nature, their behaviour was characterised over a wide

440 range of volume fraction, in order to discriminate the intrinsic differences  
441 between the gels. Thus, in addition to the relevance of droplet gels to food  
442 products like yogurt, this comparison also yields fundamental insights into  
443 the nature of the gels.

444 The first notable result is the similar properties of the two types of gels  
445 as a function of volume fraction  $\phi_{eff}$ , derived from the viscosity of semi-  
446 dilute suspensions [34]. This result is significant, as the differences seen  
447 in the gel properties of proteins and protein stabilised emulsions that have  
448 been observed previously [40, 12] are accounted for by a careful choice of the  
449 composition parameter. The approximation of the effective volume fraction  
450  $\phi_{eff}$  held for the gels studied here, despite the complex structure of the  
451 primary colloidal particles, caseinate assemblies in one case and caseinate-  
452 coated oil droplets in the other case.

453 When comparing the behaviour of protein gels and droplet gels in more  
454 detail, some differences appear between the two types of system. First, at  
455 fixed volume fraction, the droplet gels present a slightly higher elasticity  
456 than protein gels, as can be seen by the slightly higher value for the storage  
457 modulus  $G'$  and the lower phase angle. Then, the viscoelasticity of protein  
458 gels is more frequency-dependent than for droplet gels, as both the storage  $G'$   
459 and the loss  $G''$  moduli vary more with frequency. The phase angle of droplet  
460 gels also displays a non-monotonic behaviour with frequency that is not seen  
461 for the protein gels. Finally, if the two types of gels at low concentrations  
462 display strain stiffening at moderate shear amplitude, this behaviour is more  
463 marked for the droplet gels, while for concentrated gels, the non-linear regime  
464 is more extended for protein gels than for droplet gels.

465 These minor differences seem to indicate that the theoretical framework  
466 of colloidal gels may not be sufficient for an entirely accurate description of  
467 casein gels and casein-stabilised droplet gels. It may thus be necessary to take  
468 into account some system-specific characteristics. It is possible that droplets  
469 and protein assemblies have a different inter-particle interaction, as it is be-  
470 lieved that the single proteins adsorb at the surface of the droplets upon  
471 emulsification, and these proteins change conformation as the hydrophobic  
472 parts of their chains are anchored in the oil. Another possible explanation  
473 is that the size difference between protein assemblies and droplets leads to a  
474 different mobility of these two colloidal elements during gelation, which could  
475 be the reason for a discrepancy of microstructure and consequently of rheo-  
476 logical behaviour. Finally, it is possible that a role is played by the softness  
477 of the particles, as the protein assemblies are soft and water-swollen, while

478 the droplets have an incompressible oil core below the soft layer of adsorbed  
479 proteins.

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