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## The presence of RNA cargo is suspected to modify the surface hydrophobicity of the MS2 phage

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1 **The presence of RNA cargo is suspected to modify the surface**  
2 **hydrophobicity of the MS2 phage**

3

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17

18 **Abstract**

19 The surface hydrophobicity of native or engineered non-enveloped viruses and  
20 virus-like particles (VLPs) is a key parameter regulating their fate in living and artificial  
21 aqueous systems. Its modulation is mainly depending on the structure and  
22 environment of the particles. Nevertheless, unexplained variations have been reported  
23 between structurally similar viruses and with pH. This indicates that some modulating  
24 factors of their hydrophobicity remain to be identified. Herein we investigate the  
25 potential involvement of RNA cargo in the MS2 phage used as non-enveloped RNA  
26 virus model, by examining the SDS-induced electrophoretic mobility shift (SEMS)  
27 determined for native MS2 virions and corresponding RNA-free VLPs at various pH.  
28 Interestingly, the SEMS of VLPs was larger and more variable from pH 5 to 9 compared  
29 to native virions. These observations are discussed in term of RNA-dependent  
30 changes in surface hydrophobicity, suggesting that RNA cargo may be a major  
31 modulator/regulator of this viral parameter.

32

33 **Keywords**

34 Surface hydrophobicity; non-enveloped virus; virus-like particle; protein-based  
35 nanocapsules; bionanoparticles; RNA cargo; coat protein; pH.

36

## 37 **Introduction**

38 Non-enveloped viruses, virus-like particles (VLPs), and other protein-based  
39 nanocapsules (PNCs) are promising candidates for the development of  
40 nanobiotechnologies. Examples include the use of native viruses that infect bacteria  
41 (*i.e.* bacteriophages; also known as phages) to combat multidrug-resistant strains  
42 (Kortright et al., 2019), virus-inspired platforms that display antigenic epitopes on their  
43 surface for vaccines (Lee et al., 2016), or nanocarriers designed as nucleic acid  
44 delivery vehicles for gene therapy (Giacca & Zacchigna, 2012). The success of  
45 applications is depending on the ability of particles to reach their target.

46 The ionic charge and hydrophobicity are the main physicochemical surface  
47 properties of viral particles and PNCs influencing their adsorption on biotic and abiotic  
48 substrates, which participates in the regulation of their fate in living or artificial aqueous  
49 systems. Therefore, fundamental knowledge about these surface properties and their  
50 variability must be acquired. Some advances have been provided concerning the  
51 surface hydrophobicity of considered particles thanks to model viruses like phages and  
52 developments of innovative techniques designed to estimate this parameter. It has  
53 been notably evaluated by monitoring the elution of virions in hydrophobic interaction  
54 chromatography (Heldt et al., 2017), their adsorption at the air/water interface  
55 (Hämmerling et al., 2017a, 2017b; Nguyen et al., 2011) and onto hydrophobic surfaces  
56 (Armanious et al., 2016; Dika et al., 2013; Hartard et al., 2021). It has also been  
57 explored by using the fluorescence properties of hydrophobic molecular probes (Brié  
58 et al., 2016; Heldt et al., 2017), from single-particle chemical force microscopy (Mi &  
59 Heldt, 2020) and surface tension measurements of water deposited onto surfaces  
60 adsorbed virions (Samandougou et al., 2021; Shi and Tarabara, 2018), or from *in silico*  
61 approaches (Armanious et al., 2016; Heldt et al., 2017; Meder et al., 2013). In this field,

62 we recently described a new method named “SDS-induced Electrophoretic Mobility  
63 Shift Assay” (SEMSA) (Bastin et al., 2022). The SEMSA consists of labelling the  
64 hydrophobic domains on the capsids with sodium dodecyl-sulfate (SDS) through non-  
65 polar interactions of the lipophilic tail of SDS molecules, exposing in their stead the  
66 negatively charged hydrophilic head (Sautrey et al., 2018). This increases the negative  
67 charge density of viral particles, altering their electrophoretic mobility measured by  
68 capillary zone electrophoresis (CZE). Such a shift can thus be interpreted in term of  
69 surface hydrophobicity, which is accurately quantified by subtracting the native from  
70 the SDS-modified electrophoretic mobility of particles, to define an “hydrophobic index”  
71 ( $H$ ) increasing with their surface hydrophobicity. All data show that the surface  
72 hydrophobicity of viral particles is depending on the capsid surface exposure of  
73 hydrophobic amino acids, considering the 3D structure and organization of coat  
74 proteins. Concerning the effect of the viral environment such as pH, we showed by  
75 SEMSA that the  $H=f(\text{pH})$  profile of several model viruses, namely the F-specific RNA  
76 phages MS2, Q $\beta$  and GA, is characterized by a phage-specific pH unit (called “pivotal  
77 pH”) where the  $H$  value changes suddenly (Bastin et al., 2022). We interpreted this  
78 effect as changes in surface hydrophobicity, supported by similar observations  
79 reported elsewhere (Hämmerling et al., 2017a). However, no studies have been  
80 specifically devoted to assessing the impact of other particle components besides coat  
81 proteins and some environmental parameters.

82 Herein, we focused our study on the RNA phage MS2 as a model to explore the  
83 potential impact of RNA cargo on the surface hydrophobicity of non-enveloped RNA  
84 viruses and RNA-loaded PNCs. We determined and compared the  $H$  values as a  
85 function of pH determined by SEMSA for native MS2 virions and analogous VLPs  
86 lacking the RNA component. The results show that the RNA cargo is a major modulator

87 and regulator of the  $H=f(\text{pH})$  profile of MS2 phages, which can be interpreted as  
88 changes in surface hydrophobicity. This implies that potential relationships between  
89 surface hydrophobicity and RNA content of non-enveloped viruses, VLPs, and more  
90 generally RNA-loaded PNCs should be considered to predict their behavior in aqueous  
91 systems towards optimized applications.

## 92 **Materials and methods**

### 93 *Chemical and reagents*

94 Phosphoric acid solution  $\geq 85\%$ , phosphate salts, sodium chloride, dimethyl  
95 sulfoxide (DMSO), sodium dodecyl-sulfate (SDS) and other reagents in analytical  
96 grade were from Sigma Aldrich (Saint Louis, USA). Amicon® Ultra-0.5 centrifugal filter  
97 devices (100 kDa cutoff) were from Merck (Darmstadt, Germany). Ultrapure water was  
98 obtained in a Purelab Ultra MKII system from Elga (Le Plessis Robinson, France). All  
99 solutions were filtered through a 0.22  $\mu\text{m}$  membrane (Alltech, Templeuve, France) prior  
100 to use. All experiments were performed at least in triplicate.

### 101 *Production of MS2 phages and MS2 VLPs*

102 The MS2 phages were produced, purified and enumerated as we previously  
103 described (Bastin et al., 2020). The typical concentration of purified phages in 1 mM  
104 PBS (13.7 mM NaCl, 0.27 mM KCl, 1.0 mM  $\text{Na}_2\text{HPO}_4$ , 0.18 mM  $\text{KH}_2\text{PO}_4$ , pH 7.4) was  
105  $5 \times 10^{13}$  PFU/mL. The MS2 stock suspensions were stored at 4°C and away from light  
106 until use. They were used as starting material to prepare the MS2 VLPs by hydrolysis  
107 of the RNA cargo (Hooker et al., 2004), using Amicon® ultracentrifugal filter devices  
108 as described elsewhere for particle purification (Nguyen et al., 2011) with minor  
109 modifications in the protocol. Briefly, 10  $\mu\text{L}$  of MS2 stock suspension were diluted in  
110 440  $\mu\text{L}$  of phosphate saline solution (100 mM  $\text{Na}_2\text{HPO}_4$  + 100 mM NaCl) previously

111 buffered to pH 11.8 by adding the adequate volume of 1 M NaOH solution, washed  
112 twice with the same solution using 100 kDa Amicon® Ultra-0.5 centrifugal filter devices  
113 following the manufacturer's instructions, and incubated for 4h at room temperature  
114 away from light. The medium was then neutralized by two successive washes with low  
115 ionic strength phosphate solution (10 mM KH<sub>2</sub>PO<sub>4</sub>) previously buffered to pH 7.4 by  
116 adding the adequate volume of 0.1 M NaOH solution. The native MS2 phages were  
117 subjected to the same procedure but under neutral conditions for the first two washes  
118 and incubation (100 mM NaH<sub>2</sub>PO<sub>4</sub> + 100 mM NaCl solution buffered to pH 7.4) where  
119 RNA hydrolysis is not promoted. The final volume of neutral- and alkaline-treated  
120 samples was ~20 µL, which corresponds to a ~2-fold dilution of the initial 10 µL of MS2  
121 stock suspension. In order to normalize dilution and salt composition of all samples for  
122 all experiments, the neutral- and alkaline-treated samples were 2-fold diluted in 20 mM  
123 NaCl solution while the untreated MS2 stock suspension was 4-fold diluted in 13.3 mM  
124 NaCl solution.

#### 125 *Dynamic light scattering (DLS)*

126 Normalized untreated MS2 stock, neutral- and alkaline-treated samples were  
127 diluted 10 times in 1 mM PBS. The average hydrodynamic diameter of particles in each  
128 sample was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS  
129 instrument (Malvern Instruments) as we previously described (Dika et al., 2013).

#### 130 *Capillary zone electrophoresis (CZE) and SDS-induced electrophoretic mobility shift* 131 *assay (SEMSA)*

132 CZE experiments were performed on a PA 800 *Plus* Pharmaceutical Analysis  
133 System apparatus equipped with a diode array UV detector operating between 200  
134 and 300 nm for the acquisition of UV spectra of separate peaks (SCIEX) as we  
135 previously described (Bastin et al., 2022). The DMSO (neutral marker) then native or

136 VLP MS2 samples were successively injected at the inlet side of the capillary in the  
137 hydrodynamic forward mode, both sides of the capillary were immersed in the  
138 background electrolyte, and 10 kV voltage was applied (normal polarity). In this  
139 configuration, MS2 virions or VLPs migrate more slowly than DMSO, and the more  
140 their negative charge density, the longer is their migration time.

141 The hydrophobic index ( $H$ ) of MS2 phages ( $H_{phage}$ ) and analogous VLPs ( $H_{VLP}$ )  
142 were determined from CZE experiments for different pH as we previously described  
143 (Bastin et al., 2022). Briefly, samples (10  $\mu$ L) were 2-fold diluted in 20 mM NaCl or 20  
144 mM SDS solution (10  $\mu$ L) prior injection in the CZE system, and analyzed by using a  
145 background electrolyte (10 mM ionic strength phosphate buffer at appropriate pH)  
146 supplemented with 10 mM NaCl or 10 mM SDS, respectively. The SDS-induced shift  
147 in the electrophoretic mobility of the particles for each pH was exploited to determine  
148 the  $H_{phage}=f(pH)$  and  $H_{VLP}=f(pH)$  profiles.

#### 149 *Statistical analysis of data*

150 The statistical comparisons ( $p$ -value) used in text to evaluate the significance of the  
151 difference between two sets of  $H$  values were performed with GraphPad InStat v3.06  
152 software from GraphPad Prism Software (San Diego, USA), using two-way ANOVA  
153 test followed by Bonferroni's posttest. The  $p$ -values  $<0.05$  correspond to significant  
154 differences and the  $p$ -values  $<0.001$  correspond to highly significant differences, while  
155  $p$ -values  $>0.05$  correspond to non-significant differences.

## 156 **Results and discussion**

157 The virions of MS2 phage consist of a genomic ssRNA cargo enclosed in a 28 nm  
158 capsid made from 178 copies of a single coat protein (CP) in three quasi-equivalent  
159 conformations A, B and C organized as 89 A/B or C/C dimers (Golmohammadi et al.,

160 1993). A unique copy of the A-protein (AP), involved in host recognition and infection,  
161 is also incorporated (instead of hypothetical 90<sup>th</sup> dimer of CPs) to complete the capsid  
162 arranged in a  $T=3$  icosahedral lattice (Dent et al., 2013). Purified MS2 phages were  
163 used as starting material to prepare MS2 VLPs by alkaline hydrolysis of the RNA cargo  
164 (Hooker et al., 2004). The same procedure has been also successfully used to  
165 generate VLPs derived from the nucleocapsid of hepatitis B virus (Strods et al., 2015).  
166 The MS2 phages were parallelly subjected to the procedure under neutral conditions  
167 to obtain native virions similarly as VLPs. First, analytical controls were performed to  
168 ensure that MS2 VLPs were properly generated. As illustrated in Figure 1a, dynamic  
169 light scattering (DLS) analyses confirmed that the nanoparticle structures were  
170 preserved after neutral or alkaline treatment without aggregation (hydrodynamic  
171 diameters of about 30 nm with satisfactory polydispersity of  $0.12 \pm 0.06$  and  $0.12 \pm$   
172  $0.01$  for neutral- and alkaline-treated samples, respectively). However, the size  
173 distribution of alkaline-treated samples (red) was slightly shifted towards larger  
174 hydrodynamic diameters. This is consistent with previously published studies using  
175 such treatments (Strods et al., 2015). Capillary zone electrophoresis (CZE) equipped  
176 with a diode array UV detector (200-300 nm) was also employed for analytical purpose  
177 (Kenndler and Blaas, 2001). Representative UV spectra of particles extracted from the  
178 CZE analyses of MS2 stock suspension, neutral-treated and alkaline-treated samples  
179 as well as the corresponding electropherograms are given in Figure 1b and 1c,  
180 respectively. These data provide substantial information on the RNA content, recovery,  
181 and surface ionic charge density of particles after treatment. The UV spectra obtained  
182 for the neutral-treated samples (blue) were unchanged in comparison to the untreated  
183 MS2 stock suspension (grey), both characterized by a maximum absorbance peak  
184 located near 260 nm wavelength which is specific for RNA-containing samples

185 (Porterfield and Zlotnick, 2010). As expected, the maximum absorbance peak  
186 observed for the alkaline-treated samples (red) was shifted to 280 nm wavelength,  
187 which is characteristic for RNA-free MS2 particles (Hooker et al., 2004). Also, the  
188 260/280 nm absorbance ratio determined for the neutral-treated samples (blue) was  
189 1.88 while that of the alkaline-treated samples (red) was 0.78. These are typical values  
190 for samples containing both RNA and proteins or samples containing only proteins  
191 (Porterfield and Zlotnick, 2010; Sommer et al., 2003), which confirms the presence of  
192 encapsulated RNA in neutral-treated samples or its removal in alkaline-treated  
193 samples, respectively (Armanious et al., 2016; Wigginton et al., 2012). As illustrated in  
194 Figure 1c, the migration peak for native MS2 phages (blue) appeared similar in terms  
195 of peak intensity and position as the peak detected for untreated MS2 stock suspension  
196 (grey), indicating a good recovery (87%, estimated from the maximum absorbance at  
197 200 nm on the corresponding UV spectra given in Figure 1b) without change in the  
198 surface ionic charge density of the particles after applying the ultrafiltration procedure.  
199 The migration peak detected for the MS2 VLPs (Figure 1c red) was slightly enlarged  
200 and shifted toward lower migration times compared to the one detected for native MS2  
201 phages (Figure 1c blue), indicating a little heterogeneity and global decrease in the  
202 anionic charge density of the particles after RNA clearance. This effect agrees with our  
203 previous works showing the mild impact of the RNA cargo on the surface ionic charge  
204 of MS2 virions (Langlet et al., 2008). The peak broadening leads to an apparent  
205 decrease in peak intensity for MS2 VLPs (Figure 1c), but the maximum absorbance at  
206 200 nm indicates a VLPs recovery of 78% from the starting MS2 stock suspension  
207 (Figure 1b), also consistent with the literature (Hooker et al., 2004). Together, these  
208 analytical controls confirm the preservation of chemical composition and structure for  
209 native MS2 phages after neutral treatment, as well as slight structural changes

210 accompanying efficient degradation/removal of a large amount of the genomic RNA  
211 cargo after alkaline treatment.

212 To estimate and compare the apparent surface hydrophobicity as a function of pH  
213 between native MS2 phages and MS2 VLPs, the corresponding  $H_{phage}$  and  $H_{VLP}$  values  
214 were determined by SEMSA from pH 5 to 9. The SDS-induced shift in the  
215 electrophoretic mobility of particles for each pH, illustrated in Figure 2a for pH 9, was  
216 exploited to determine the  $H_{phage}=f(pH)$  and  $H_{VLP}=f(pH)$  profiles given in Figure 2b. It  
217 can be observed in Figure 2b that native MS2 phages (blue) displayed a very similar  
218 profile as we already published (Bastin et al., 2022), with relatively low and stable  $H_{phage}$   
219 values from pH 9 to 7 ( $\sim 0.13 \pm 0.01$ ,  $p$ -values  $>0.05$  between each point) and a  
220 characteristic  $H_{phage}$ -jump from pH 7 to 5 reaching  $0.55 \pm 0.16$  at pH 5 ( $p$ -value  $<0.05$   
221 between pH 7 and 6,  $p$ -value  $<0.001$  between pH 6 and 5). Concerning MS2 VLPs  
222 (red), all the  $H_{VLP}$  values (e.g.  $1.03 \pm 0.06$  at pH 8) were strongly higher than the  $H_{phage}$   
223 values of native phages ( $0.13 \pm 0.03$  at pH 8,  $p$ -value  $<0.001$ ), indicating that presence  
224 of the RNA genome decreases the SDS-induced shift in the electrophoretic mobility of  
225 MS2 virions. To give the reader with a relative scale, the  $H$  value at pH 8 we previously  
226 reported for the highly hydrophobic phage GA was  $2.62 \pm 0.31$  (Bastin et al., 2022).  
227 The SEMSA results provided here for native MS2 virions and corresponding VLPs can  
228 thus be interpreted for modulation in surface hydrophobicity of particles. Such results  
229 and their interpretation are supported by the deviations in adsorption kinetics at the  
230 air/water interface and onto gold-chemisorbed dodecanthiol monolayers, i.e. at  
231 hydrophobic/hydrophilic interfaces, previously observed from native MS2 virions to  
232 MS2 VLPs prepared by alkaline treatment of the former (Armanious et al., 2016;  
233 Nguyen et al., 2011). It is currently accepted that the surface hydrophobicity of viral  
234 particles depends on the exposition and distribution of hydrophobic amino acids on the

235 outer surface of the proteinaceous capsid (Armanious et al., 2016; Heldt et al., 2017;  
236 Meder et al., 2013). This implies that the variations in hydrophobicity we measured by  
237 SEMSA after alkaline treatment should rely on some changes in these structural  
238 parameters affecting the SDS accessibility of hydrophobic domains on MS2 VLPs. In  
239 other words, alkaline treatment of native MS2 virions altered the hydration sphere of  
240 particles, as supported by slight variations in hydrodynamic diameter and  
241 electrophoretic mobility measurements here (Figure 1a and 1c), and elsewhere  
242 reported (Armanious et al., 2016; Nguyen et al., 2011; Strods et al., 2015; Wigginton  
243 et al., 2012). Assuming that most of the genomic RNA was cleared after alkaline  
244 treatment (or severely degraded), as shown here (Figure 1b) and in earlier studies  
245 (Armanious et al., 2016; Hooker et al., 2004; Nguyen et al., 2011; Wigginton et al.,  
246 2012), the question is: what component(s) of the viral shell is(are) specifically affected  
247 by RNA removal to modulate the capsid surface hydrophobicity, and by what  
248 mechanism(s)? The SEMSA procedure only provides information on the bulk  
249 hydrophobicity of particles surface without distinction between capsid components  
250 namely CPs and the AP for MS2 phage. However, although the AP is present in a  
251 single copy per capsid, it covers a large area of the outer surface impacting significantly  
252 the three-dimensional structure of the viral capsid (Dai et al., 2017; Koning et al., 2016;  
253 Kuzmanovic et al., 2006), and could thus influence the overall physicochemical surface  
254 properties of MS2 particles. Conservation of the AP in term of amount and structure  
255 after RNA release from native MS2 virions could be explored by protein and single  
256 particle analysis techniques, e.g. mass spectrometry (Bastin et al., 2020; Wigginton et  
257 al., 2012), or cryogenic electron microscopy (cryo-EM) (Luque and Castón, 2020), in  
258 order to assess its contribution to the effects observed in our hands. Recent progress  
259 in solving the atomic structure of whole MS2 virions and recombinant VLPs (lacking

260 the AP and genomic RNA cargo) can help us to strengthen the discussion. It has been  
261 notably shown that the RNA cargo inside wild-type MS2 virions adopts an asymmetric  
262 tertiary structure made from about sixty interconnected stem-loops, where the majority  
263 bind CPs and the AP from inside the capsid (Dai et al., 2017; Dent et al., 2013; Koning  
264 et al., 2016). Similar complex network between the genomic RNA and the capsid inner  
265 surface has also been observed in human viruses like hepatitis E virus (Xing et al.,  
266 2010). The capsid of MS2 phage (and probably that of other viruses) is thus  
267 asymmetric, the RNA-capsid binding regions being predominantly localized on one  
268 side containing the AP. In contrast, MS2 VLPs made from 180 copies of recombinant  
269 wild-type CP only and assembled in absence of the genomic RNA template are  
270 perfectly symmetric (de Martín Garrido et al., 2020). Interestingly, such VLPs exhibit  
271 symmetry variability between normal  $T=3$  icosahedrons with an approximate diameter  
272 of 28 nm and  $T=4$  variants (for which a fourth quasi-equivalent conformation of the CP  
273 has been observed) with a maximal diameter of ~33 nm. This is consistent with the  
274 hydrodynamic diameter measurements here shown in Figure 1a and those reported in  
275 another study (Strods et al., 2015). Hence, we propose that a transition may occur in  
276 native MS2 virions from the RNA-modeled asymmetric  $T=3$  towards a mixture of  
277 symmetric  $T=3$  and  $T=4$  configurations upon removal of the genomic RNA template,  
278 leading to changes in the outer exposure of CPs and/or the AP that interacted with the  
279 removed RNA cargo. As we observed by SEMSA, the consequence would be a  
280 modulation in the SDS accessibility of hydrophobic domains localized on the affected  
281 region of capsids. This possible structural dynamics of MS2 particles after RNA  
282 removal will deserve further analysis by high resolution imaging like cryo-EM.

283 In addition to the comparison of hydrophobicity under specific conditions, the pH-  
284 dependent dynamics in  $H$  values displayed in Figure 2b for MS2 VLPs (red) and native

285 MS2 phages (blue) are significantly different. While the  $H_{VLP}$  and  $H_{phage}$  values  
286 decrease similarly from pH 5 to 6, the  $H_{VLP}$  value increases from pH 6 to 7 (from  $1.05$   
287  $\pm 0.04$  to  $1.17 \pm 0.05$ ,  $p$ -value  $<0.05$ ) whereas the  $H_{phage}$  value continues to decrease  
288 (from  $0.28 \pm 0.11$  to  $0.12 \pm 0.02$ ,  $p$ -value  $<0.05$ ). Then the  $H_{VLP}$  value stays constant  
289 until pH 7.5, decreases between pH 7.5 and pH 8 (from  $1.18 \pm 0.05$  to  $1.03 \pm 0.06$ ,  $p$ -  
290 value  $<0.05$ ) and keeps until pH 9, while the  $H_{phage}$  value stays constant from pH 7 to  
291 pH 9. The similar increase in  $H_{VLP}$  and  $H_{phage}$  values from neutral to acidic pH is easily  
292 attributable to approaching the isoelectric pH ( $\sim 4$ ) of MS2 particles (Dika et al., 2011),  
293 i.e. when the electrical surface charge of particles tends towards zero and they become  
294 more hydrophobic (Bastin et al., 2022; Dika et al., 2013, 2011). However, the variability  
295 in  $H_{VLP}$  values between neutral and alkaline pH cannot be explained in this way  
296 because the surface charge density of MS2 particles remains stable over the pH range  
297 7-9 (Bastin et al., 2022; Dika et al., 2011). Based on the molecular flexibility of the MS2  
298 capsid discussed above, the observation of several breaks in the  $H_{VLP}=f(\text{pH})$  profile  
299 (i.e. from pH 6.5 to 7 and from pH 7.5 to 8) may rather be interpreted as pH-induced  
300 conformational rearrangements in CPs and/or the AP affecting the accessibility of  
301 external hydrophobic domains to the SDS probe. Such a structural dynamic has indeed  
302 been demonstrated in norovirus (structurally close to the MS2 phage) where, by  
303 twisting the flexible hinge connecting the CP dimers as pH increases (the precise  
304 mechanism is not yet clear), the protruding domain of CPs reversibly rotates with  
305 respect to the shell domain, which modify transiently the solvent accessibility of nearby  
306 secondary structures containing hydrophobic residues (Song et al., 2020).  
307 Interestingly, high variability in  $H$  values with significant  $H$ -jumps was only observed  
308 here for the MS2 VLPs, but not for native virions. These results support our proposal  
309 for greater capsid flexibility in absence of genomic RNA, affecting the hydrophobicity

310 variability of MS2 particles. Hence, we suggest that genomic RNA cargo in MS2 virions  
311 is involved in the modulation of this physicochemical parameter and its regulation in  
312 response to pH changes, by playing the role of a molecular anchor stabilizing the  
313 assembly and arrangement of CP dimers (Ni et al., 1995) and leading to a specific map  
314 of solvent accessibility over the capsid surface.

315 The previous hypotheses based on SEMSA results will need to be confirmed by  
316 complementary hydrophobicity measurement approaches and *in silico* estimations  
317 (Armanious et al., 2016; Heldt et al., 2017), as well as by structural characterizations  
318 using cryo-EM or Raman spectroscopy (Luque and Castón, 2020; Němeček and  
319 Thomas, 2009). Moreover, the specific role of CPs or the AP is missing which can be  
320 explored in future works by adding recombinant MS2 VLPs in the experimental setup.  
321 But they are still important for applications because they implicate that the surface  
322 hydrophobicity of RNA-loaded viral particles cannot be deduced from the  
323 corresponding VLPs devoid of known RNA cargo, that it can vary unpredictably  
324 according to the solution's chemistry, and that it must thus be systematically  
325 characterized.

326

## 327 **Conclusion**

328 The data reported here show that presence of the RNA core in the MS2 phage  
329 affects strongly the SDS-induced shift in the electrophoretic mobility of particles. As  
330 discussed, these results can be interpreted as changes in apparent surface  
331 hydrophobicity according to the solvent accessibility of hydrophobic domains over the  
332 capsid surface, that is modulated by RNA-dependent arrangement of capsid proteins  
333 from the inside to the outside of particles. Moreover, this effect varies differently with  
334 pH between native MS2 virions and corresponding VLPs, supporting that RNA cargo

335 is also involved in the regulation of the pH-dependent variability of surface  
336 hydrophobicity of MS2 virions. Thus, this early work needs to be followed by further  
337 investigations to clarify the mechanism at the molecular level. Being of purely  
338 physicochemical origin, similar variability should also exist in other native or  
339 engineered non-enveloped RNA viruses, and more generally in RNA-loaded PNCs  
340 where the cargo interacts with coat proteins. Consequently, the hydrophobicity  
341 phenotype of such particles must be further investigated for a comprehensive  
342 understanding of their diffusion in aqueous systems toward optimized/new applications  
343 in medicine and for the environment.

344

345 **CRedit authors contribution statement: Guillaume Bastin:** Conceptualization,  
346 Formal analysis, Visualization, Writing – Original Draft, Visualization. **Evelyne**  
347 **Schvoerer:** Conceptualization, Writing – Review & Editing **Christophe Gantzer:**  
348 Writing – Review & Editing. **Guillaume Sautrey:** Conceptualization, Methodology,  
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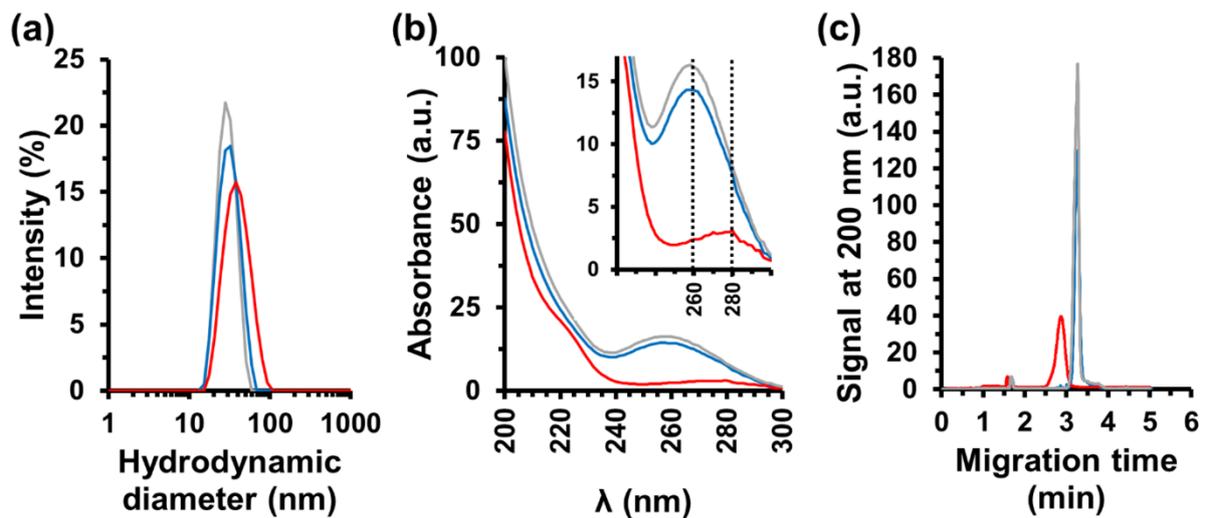
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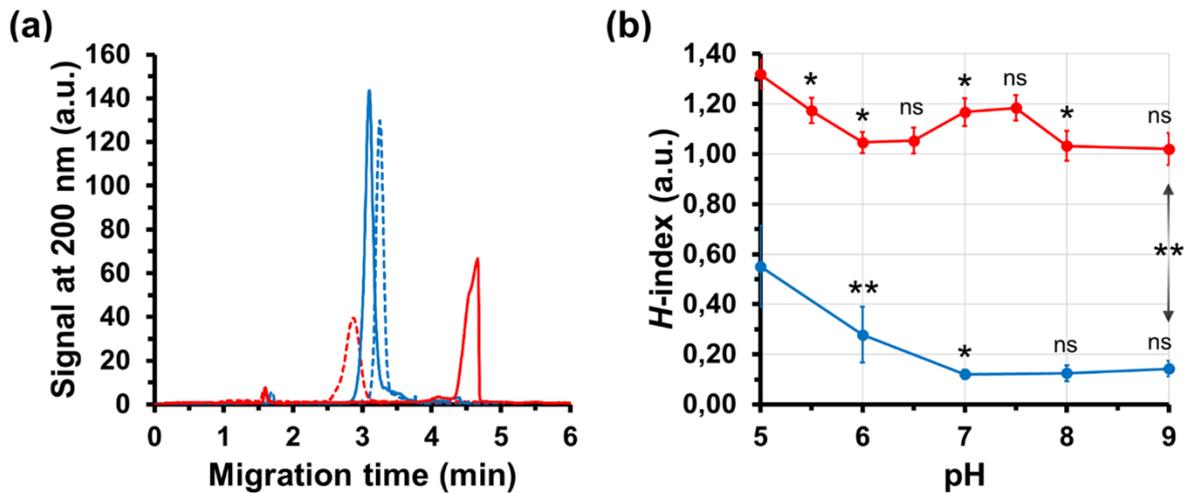
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483 **Figure 1.** Dynamic light scattering (DLS) and Capillary zone electrophoresis (CZE)-UV  
 484 analysis of native MS2 phages and VLPs. (a) Size distribution of particles in MS2 stock  
 485 (grey), neutral-treated (blue) and alkaline-treated (red) samples. (b) Representative  
 486 UV spectra of MS2 stock (grey), neutral-treated (blue) and alkaline-treated (red)  
 487 samples, with enlargement near the 260-280 nm region. Data normalized according to  
 488 the absorbance at 200 nm for MS2 stock samples (100%) (c) Representative  
 489 electropherograms (detection wavelength 200 nm) obtained for MS2 stock (grey),  
 490 neutral-treated (blue) and alkaline-treated (red) samples (background electrolyte pH 9  
 491 supplemented with 10 mM NaCl).

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494 **Figure 2.** Apparent surface hydrophobicity of native MS2 phages and VLPs. (a)  
 495 Representative electropherograms obtained by capillary zone electrophoresis (CZE)  
 496 with SDS-free (dotted line) or SDS-containing (solid line) background electrolyte at pH  
 497 9 for native MS2 phages (blue) and VLPs (red). A shift of elution peaks to higher  
 498 migration times indicates an increase in the negative charge density of SDS-labeled  
 499 viral particles. (b)  $H=f(\text{pH})$  profiles of native MS2 phages (blue) and VLPs (red). Error  
 500 bars are SD. From pH 5 to pH 9, each set of values was statistically compared to the  
 501 previous one: ns means not significant ( $p$ -values  $>0.05$ ); \* means significant ( $p$ -value  
 502  $<0.05$ ); \*\* means highly significant ( $p$ -value  $<0.001$ ). All experimental details related to  
 503 the CZE analyses (a) and the  $H$  determination (b) are given elsewhere (Bastin et al.,  
 504 2022).

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