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1 **Fluorescent magnetosomes for controlled and repetitive drug release**  
2 **under the application of an alternating magnetic field under conditions of**  
3 **limited temperature increase (< 2.5 °C).**

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22

23 **ABSTRACT:** Therapeutic substances bound to nanoparticles have been shown to dissociate following  
24 excitation by various external sources of energies or chemical disturbance, resulting in controllable and  
25 efficient antitumor activity. Bioconjugation is used to produce magnetosomes associated with  
26 Rhodamine B (RhB), whose fluorescence is partially quenched by the presence of the iron oxide and  
27 becomes strongly enhanced when RhB dissociates from the magnetosomes under the application of an  
28 alternating magnetic field. This novel approach enables to simultaneously release a RhB model  
29 molecule and to monitor such mechanism by fluorescence. The dissociating mechanism of RhB is  
30 highlighted by exposing a suspension of fluorescent magnetosomes to an alternating magnetic field, by  
31 magnetically isolating the supernate of this suspension, and by showing fluorescence enhancement of  
32 the supernate. Furthermore, to approach *in vivo* conditions, fluorescent magnetosomes are mixed with  
33 tissue or introduced in mouse brain and exposed to the alternating magnetic field. Most interestingly, the  
34 percentages of RhB dissociation measured at the beginning of magnetic excitation ( $\Delta R/\delta t$ ) or 600  
35 seconds afterwards ( $R_{600s}$ ) are  $\Delta R/\delta t \sim 0.13\%$  and  $R_{600s} \sim 50\%$  under conditions of limited temperature  
36 increases ( $<2.5\text{ }^{\circ}\text{C}$ ), larger values than those of  $\Delta R/\delta t \sim 0.02\text{-}0.11\%$  and  $R_{600s} \sim 13\%$ , estimated at  
37 temperatures above  $2.5\text{ }^{\circ}\text{C}$ . Furthermore, when magnetic excitations are repeated two to five times,  
38 temperature increase becomes undetectable, but RhB dissociation continues to occur up to the fifth  
39 magnetic excitation. Since large heating temperatures may be damaging for tissues, this study paves the  
40 way towards the development of a safe theranostic dissociating nano-probe operating at low  
41 temperature.

42

43 **KEYWORDS:** magnetosomes, magnetotactic bacteria, probe, thermometer, fluorescence,  
44 luminescence, nano-thermometry, fluorescent nanoparticles, nanotechnology.

45 **ABBREVIATIONS:** MCR400: magnetosomes produced by magnetotactic bacteria cultivated in the  
46 presence of 400  $\mu\text{M}$  of rhodamine B introduced in the bacterial growth medium followed by  
47 magnetosome extraction from these bacteria; MC: magnetosomes produced by magnetotactic bacteria  
48 cultivated without rhodamine B introduced in the bacterial growth medium followed by magnetosome  
49 extraction from these bacteria; FACS: Fluorescence activated cell sorting; AMF: alternating magnetic  
50 field; Ca: calcein acetoxymethyl ester; RhB: rhodamine B

51 **DEFINITION:** Magnetosome mineral core: Magnetosomes studied here are composed of an iron oxide  
52 mineral core essentially composed of crystallized iron oxide surrounded by a layer containing biological  
53 material (LPS, proteins, lipids) and rhodamine B. For a schematic picture of these magnetosomes, see  
54 scheme 2.

55

## 56 INTRODUCTION

57 Antitumor activity can be reached with nanoparticles introduced to tumors and exposed to an external  
58 source of energy, either producing a temperature increase, [1-5], or the release and activation of a  
59 therapeutic substance, [6]. The first method of treatment is usually designated as magnetic  
60 hyperthermia. It led to preclinical efficacy on various cancer types, including prostate, [7], glioblastoma,  
61 [8-10], head and neck, [11], melanoma, [12], and to a 6 months increase in survival among patients  
62 bearing glioblastoma compared with the standard of care, [13, 14]. It also appears to be safer than  
63 conventional thermotherapies such as ultrasounds since it yields antitumor activity at more moderate  
64 heating temperatures, *i.e.* 41-46 °C, [15-17], compared with temperatures larger than 60 °C for high  
65 intensity ultrasound treatments, [18]. To strengthen safety even more, drugs could be released from  
66 nanoparticles under magnetic excitation in the absence of a significant temperature increase. Chemical  
67 synthesis methods have been used to fabricate dissociating nano-probes, [19-21], but it appears that they  
68 often require high temperatures to reach efficient dissociation, possibly due to the relatively strong  
69 bonds between the nanoparticles and the therapeutic substance that can't easily be broken at low  
70 temperatures.

71 In this manuscript, we describe the synthesis method of a dissociating nano-probe operating in  
72 conditions of limited temperature increase ( $< 2.5$  °C). This nano-probe is further made fluorescent to  
73 monitor the dissociation. For that, we introduce in the growth medium of magnetotactic bacteria a  
74 fluorescent substance mimicking a therapeutic agent (RhB), [22], we wait for these bacteria to  
75 incorporate RhB in the magnetosomes, and we finally extract fluorescent magnetosomes designated as  
76 MCR400 from these bacteria. We show that RhB is sufficiently strongly bound to the magnetosomes to  
77 enable MCR400 to maintain their fluorescence following their isolation and re-suspension in water. To  
78 examine if MCR400 can be used as intracellular probe, fluorescent magnetosomes are then brought into  
79 contact with cancer cells and their internalization, cytotoxicity, and fluorescent properties are studied.  
80 The operating mechanism of the MCR400 probe is deciphered by exposing a MCR400 suspension to an

81 alternating magnetic field (AMF), by magnetically separating from bacterial debris the supernate of this  
82 suspension in which RhB has potentially been released, and by measuring its fluorescence intensity. To  
83 approach real magnetic hyperthermia treatment conditions, MCR400 are either mixed with brain tissue  
84 or introduced in a mouse brain and exposed one to five times to an AMF. MCR400 concentration and  
85 environment are varied to modulate the magnitude of temperature increases. The initial rate and quantity  
86 of RhB released are deduced from the variation of the MCR400 fluorescence intensity, measured at the  
87 beginning of magnetic excitation and 600 seconds afterwards, as a function of various heating  
88 temperatures.

## 89 **RESULTS AND DISCUSSION**

90 **Properties of whole fluorescent magnetotactic bacteria.** In the absence of a compound that can  
91 permeabilize the membrane of gram-negative bacteria such as PEI, it is difficult to introduce fluorescent  
92 substances inside such bacteria during their growth, [23]. For this reason, it was usually attempted to  
93 stain bacteria with RhB following their amplification. It either yielded very weak fluorescence, [24], or  
94 more significant fluorescence, [25], depending on the use (or not) of a specific protocol to bind RhB to  
95 bacterial membranes. By using magnetotactic bacteria, we were able to trigger a truly unusual behavior,  
96 *i.e.* incorporate RhB in these bacteria by simply adding RhB to their growth medium, which in turn  
97 seems to have changed their metabolism since both whole bacteria and magnetosomes became larger in  
98 the presence of RhB as explained below. The fluorescence of magnetotactic bacteria cultivated in the  
99 presence of 400  $\mu\text{M}$  of RhB was observed by optical microscopy. Whereas whole magnetotactic  
100 bacteria cultivated without RhB appeared non-fluorescent under optical microscopy observation, [26],  
101 those grown in the presence of 400  $\mu\text{M}$  of RhB displayed a strong intracellular fluorescent signal (Fig.  
102 1). This behavior was further supported by flow cytometer measurements. Indeed, the FL3-H signal,  
103 which is proportional to RhB fluorescence, is lower than 10 a.u. for bacteria grown without RhB  
104 indicating that the bacteria are non-fluorescent according to a criterion of the flow cytometer. It  
105 increases to a value larger than 10 a.u. for magnetotactic bacteria cultivated in the presence of RhB,

106 yielding 54% of fluorescent bacteria according to this criterion (Fig. 2(a) and table S1). To examine  
107 possible changes in bacterial morphologies induced by RhB, we analyzed the cloud of points  
108 representing the side scatter height (SSC-H) signal as a function of the forward scatter height (FSC-H)  
109 signal. Between bacteria grown in the absence and presence of RhB, Figs. 2(b) and 2(c) show that the  
110 FSC-H and SSC-H signals increase from 20-200 a.u. up to 200-10000 a.u. and from 30-300 a.u. up to  
111 200-10000 a.u., respectively. Such behavior may be associated with an increase in size of magnetotactic  
112 bacteria and magnetosome chains, [27], when RhB is added to the growth medium. It may arise from  
113 the chelation of iron by RhB that could simultaneously increase the amount of iron and RhB introduced  
114 in magnetotactic bacteria. On the one hand, chelation could result in enhanced stress applied on these  
115 bacteria or to change in their metabolism, which would modify their size, [28]. On the other hand, it  
116 could favor the formation of larger magnetosomes or longer magnetosome chains due to more iron  
117 introduced in these bacteria, [28, 29]. This behavior is further confirmed by comparing the histograms in  
118 size of MC and MCR400 presented in Figs. S2(a) and S2(b) deduced from transmission electron  
119 microscopy images of bacteria cultivated in the absence (Fig. S3(a)) or presence (Fig. S3(b)) of RhB. In  
120 the absence of RhB, Fig. S2(a) shows that magnetosome sizes with maximum frequency are 22 nm for  
121 small magnetosomes (between 0 and 30 nm) and 40 nm for large magnetosomes (between 30 and 60  
122 nm). In the presence of RhB, these two sizes increase to 30 and 52 nm, respectively (Fig. S2(b)), [29].

123 **Properties of MCR400 extracted from magnetotactic bacteria.** For the magnetic hyperthermia  
124 treatment of tumor, it is difficult to use whole magnetotactic bacteria, due to the too low magnetosome  
125 concentration in a bacterial suspension that does not enable sufficient heat production under AMF  
126 application, [30]. To enable their potential further use in magnetic hyperthermia, magnetosomes were  
127 therefore extracted from fluorescent magnetotactic bacteria. We first analyzed whether the fluorescence  
128 of RhB which was observed in the bacterial cells as underlined in the previous section, was maintained  
129 following extraction. The FT-IR spectrum of magnetosomes extracted from fluorescent magnetotactic  
130 bacteria is presented in Fig. 3(a). It shows the superposition of two series of peaks. The first series of

131 peaks is also present in RhB free magnetosomes MC (in blue), *i.e.* amide I at  $1650\text{ cm}^{-1}$ , amide II at  
132  $1530\text{ cm}^{-1}$ , lipopolysaccharides (LPS) or phospholipids at  $1050\text{ cm}^{-1}$  and at  $1250\text{ cm}^{-1}$ , maghemite iron  
133 oxide at  $580\text{ cm}^{-1}$ , Fig. 3(a), [31]. The second series of peaks is due to RhB (in red), *i.e.* peaks at  $1700$   
134  $\text{cm}^{-1}$ ,  $1600\text{ cm}^{-1}$ ,  $1475\text{ cm}^{-1}$ ,  $1250\text{ cm}^{-1}$ ,  $1175\text{ cm}^{-1}$ ,  $1125\text{ cm}^{-1}$ ,  $675\text{ cm}^{-1}$ , Fig. 3(c), [32]. These results  
135 suggest that in MCR400, RhB is either adsorbed at magnetosome surface or complexed with  
136 magnetosomes, possibly due to the carboxylic acid function of RhB that can interact with the FeOH  
137 groups at magnetosome surface, [33]. Further proofs of the association of RhB to the magnetosomes in  
138 MCR400 are provided by the absorption spectrum of a MCR400 suspension that displays a shoulder at  
139  $550\text{ nm}$  (Fig. 3(d)), a wavelength corresponding to maximum absorption by free RhB (Fig. 3(f)) and to  
140 the absence of an absorption signal from MC at this wavelength (Fig. 3(e)). Moreover, the fluorescence  
141 spectrum of the MCR400 suspension excited at  $405\text{ nm}$ , displays a peak at  $569\text{ nm}$  (Fig. 3(g)), which is  
142 absent in the spectrum of the MC suspension excited in the same conditions as MCR400 (Fig. 3(h)) and  
143 is positioned at a slightly lower wavelength than that of  $576\text{ nm}$  observed for free RhB (Fig. 3(i)). This  
144 wavelength shift is possibly due to interactions between RhB molecules and the magnetosome surface in  
145 MCR400, as previously reported for fluorescent molecules associated to other types of nanoparticles,  
146 [34].

147 **Cytotoxicity, fluorescence, and internalization properties of MCR400 in the presence of cancer**  
148 **cells.** In a previous study, we showed that MCR400 incubation with Zymozan-activated murine  
149 peritoneal macrophages leads to enhanced fluorescence intensity, a behavior which was attributed to the  
150 capture of MCR400 by lysosome following their cellular internalization leading to RhB dissociation  
151 from the magnetosomes at acidic pH, [35]. To be able to use MCR400 as an intracellular fluorescent  
152 probe, MCR400 shall not only maintain a high level of fluorescence but also yield limited cytotoxicity.  
153 In this study, MCR400 were therefore incubated with MDA-MB-231 cells during 6 hours in the  
154 presence (or not) of Calcein AM. The Calcein AM fluorescence emission intensity (FL1-H), which is  
155 proportional to the number of viable cells, [36], was observed to increase from 2 for cells alone to ~



156 1000 for cells in the presence of Calcein AM both with and without MCR400 (Figure 4(a)), indicating  
157 that ~97% of cells are viable following MCR400 incubation (table S1). MCR400 therefore do not  
158 induce any significant cytotoxicity towards these cancer cells. This result is consistent with the absence  
159 of RhB cytotoxicity below a certain concentration threshold, [37]. Furthermore, between cells alone and  
160 cells incubated with MCR400, the fluorescence signal of RhB (FL3-H) and its associated percentage of  
161 fluorescent cells increase from 1 to 55 (Fig. 4(b)) and from 0% to 99% (table S1), respectively.  
162 Therefore, almost all cancer cells become fluorescent following their incubation with MCR400. Finally,  
163 to determine if MCR400 internalize in these cancer cells, two types of epi-fluorescence microscopy  
164 images of MCR400 incubated with cancer cells were taken, either in the absence (Fig. 4(c)) or presence  
165 (Fig. 4 (d)) of RhB emission. The superposition of Figs. 4 (c) and 4 (d), presented in Fig. 4(e), shows  
166 that RhB molecules fluoresce where MDA-MB231 cells are located, which suggests that MCR400 have  
167 penetrated inside MDA-MB-231 cells without losing their fluorescence properties. The other possible  
168 location of MCR400 at cell surface, which may also be considered by analyzing Fig. 4(d), appears  
169 unlikely since Fig. 4(c) doesn't show any magnetosome aggregates at this location. MCR400 enhanced  
170 fluorescence intensity following cellular internalization was observed with cancer cells (MDA-MB-231)  
171 and macrophages, [38], making this behavior likely to occur for different cell types. We can conclude  
172 that MCR400 may be used as intracellular fluorescent probe, which could have potential diagnostic and  
173 therapeutic applications both *in vitro* and *in vivo*.

174 **Operating mechanism of the MCR400 probe studied in suspension.** We further examined the  
175 operating mechanism of the MCR400 probe by suspending MCR400 in water at an iron oxide  
176 concentration of 1 mg<sub>Fe</sub>/mL and by applying an AMF of 25 mT and 198 kHz during 1200 seconds that  
177 produced a temperature increase of 7 °C (Fig. 5(a)). This yielded conditions in terms of AMF  
178 parameters and temperature that are typical to those reached during magnetic hyperthermia, [15, 16].  
179 During magnetic excitation, Fig. 5(b) shows that the fluorescence intensity of this suspension remains  
180 unchanged, possibly due to fluorescence quenching by the iron oxide of the magnetosome mineral core,

181 which could limit RhB emission, [39]. By contrast, the fluorescence intensity of the supernate of this  
182 suspension, which was magnetically separated from the magnetosome mineral cores, increased during  
183 the first 100 seconds of the magnetic excitation from 1 to 19 a.u. and then stabilized at 18.5-19.5 a.u.  
184 between 100 and 1200 seconds (Fig. 5(b)). Such behavior may not come from the variation of the  
185 intrinsic RhB fluorescence, which decreases with increasing temperature, [40]. Instead, it may be due to  
186 the dissociation of RhB from the magnetosomes under magnetic excitation, which could lead to  
187 fluorescence de-quenching. Furthermore, the emission wavelength, corresponding to the maximum  
188 fluorescence intensity for an excitation at 405 nm, is observed to increase from 569 nm for MCR400 to  
189 576 nm for the supernate of MCR400. In fact, RhB molecules may either interact with the  
190 magnetosomes in the MCR400 suspension leading to an emission wavelength below that of free RhB or  
191 may produce such interactions in the supernate of this suspension due to the diffusion of RhB molecules  
192 away from the magnetosomes, yielding for the supernate the same emission wavelength as that of free  
193 RhB, (Fig. S4(b)). We have highlighted a mechanism by which RhB molecules initially interact with the  
194 iron oxide of the magnetosome mineral, resulting in fluorescence quenching. Following AMF  
195 application, RhB molecules are released from the magnetosomes, leading to fluorescence intensity  
196 increase, which could also be designated as fluorescence de-quenching. Such mechanism could  
197 potentially be used to control the release of a drug under the application of an AMF provided RhB is  
198 replaced by a therapeutic substance.

199 ***Ex vivo* release of RhB from magnetosome minerals in the presence of a temperature increase**  
200 **comprised between 2.5 °C and 10 °C.** To complement studies in solution and come closer to the real  
201 conditions of treatment, we have introduced 2 µl of a suspension containing 40 or 400 µg of MCR400 at  
202 a depth of 1 to 3 mm in a mouse brain extracted from a dead mouse (Scheme 3) that we have exposed to  
203 an alternating magnetic field of frequency 200 kHz and strength 25 mT for 30 minutes. Figs. 6(a) and  
204 6(b) show that these conditions yield a temperature increase lying within the range of 2.5-10 °C, a  
205 similar temperature increase than that reached in a magnetic hyperthermia treatment, which was  
206 previously determined as ~ 3-10 °C from preclinical studies carried out with magnetosomes, [15], [16].

207 Furthermore, the influence of the position of the excitation/detection fiber on MCR400 fluorescence  
208 properties was studied in sample 1 by mixing homogenously 40  $\mu\text{g}$  of MCR400 with brain tissue and by  
209 positioning this fiber at different heights of 1 to 3 mm above the tissue. The various fiber heights led to  
210 similar values of MCR400 fluorescence intensity (Fig. 7(a)), indicating that the distance between the  
211 fiber end and the magnetosome region (Scheme 3) is not an important parameter in this distance range.  
212 Between samples 1 and 2, we increased the amount of MCR400 from 40  $\mu\text{g}$  (sample 1) to 400  $\mu\text{g}$   
213 (sample 2), resulting in enhanced heat production and diffusion outside of the injection volume, two  
214 behaviors that compensated each other to yield in both cases a similar value of fluorescence intensity,  
215  $\Delta F_{600s} \sim 125$  a.u., and percentage of RhB released from the magnetosomes,  $R_{600sec} \sim 12\%$ , measured 600  
216 seconds following magnetic excitation (Figs. 6(c) to 6(f)).

217 Given that after a certain time of magnetic excitation (600 seconds), the fluorescence intensity and  
218 percentage of released RhB appear to reach saturation and to become independent of temperature and  
219 conditions of excitation, we have examined another parameter, *i.e.* the initial rate of released RhB,  
220  $\Delta R/\delta t$ , which takes place just after activation of the magnetic excitation. It appears to be larger and less  
221 influenced by MCR400 interactions with its surrounding environment than rates of release estimated at  
222 later stages, hence possibly being a controllable parameter to trigger a therapeutic activity. Most  
223 interestingly, between samples 1 and 2,  $\Delta R/\delta t$  increases from 0.02 %/sec. to 0.11 %/sec (Figs. 7(c) and  
224 7(d)). On the one hand, such sharp transition could be due to  $\Delta T/\delta t$  increase between samples 1 and 2,  
225 reaching a certain threshold value of  $\sim 0.008\text{-}0.12$   $^{\circ}\text{C}/\text{sec}$ . (Figs. 7(c) and 7(d)). However, this  
226 hypothesis seems unlikely since Fig. 7 shows that temperature variations influence rather weakly  
227 fluorescence intensity and RhB release. On the other hand, it could come from more brain tissue  
228 material surrounding the nanoparticles in sample 1 than in sample 2, which could more strongly prevent  
229 efficient RhB diffusion. In fact, in sample 1 brain tissue material could have adsorbed at MCR400  
230 surface or fill MCR400 environment when MCR400 were mixed with tissue, while in sample 2  
231 MCR400 could mainly be surrounded by brain liquid and much less by brain tissue materials as was

232 observed during MCR400 injection in mouse brain. The optimal conditions for efficient release of RhB  
233 from magnetosomes and for the monitoring of such mechanism therefore seem to be an environment  
234 that does not prevent RhB diffusion away from the magnetosomes, a sufficiently large initial  
235 temperature gradient, and the measurement of fluorescence variations carried out at the beginning of  
236 magnetic excitation. For *in vivo* or clinical applications, these results imply that magnetosomes could be  
237 used to release a drug provided RhB is replaced by a therapeutic substance. Heat and pharmacological  
238 treatments could hence potentially be combined during magnetic hyperthermia by using a drug, which  
239 would diffuse away from the magnetosomes and thus be activated following magnetic excitation.

240 **Repetitive *ex vivo* release of RhB from magnetosomes under magnetic excitation in the absence of**  
241 **significant temperature increase (< 2.5 °C).** Previous studies have reported antitumor activity when  
242 magnetic nanoparticles were mixed with tumor cells or introduced to tumors and exposed to an AMF  
243 without inducing any measurable temperature increase, [15]. Such behavior was either explained by  
244 mechanical disruption of tumor cells induced by AMF application, [41], or to an indirect mechanism of  
245 tumor destruction, possibly involving immune cells, such as natural killer, T cells or polynuclear  
246 neutrophils, [15], which could eradicate unheated tumor cells. In order to control such mechanism, an  
247 immunogenic substance could be bound to nanoparticles and be progressively released from them under  
248 AMF application, as recently suggested for lipopolysaccharide (LPS) dissociating from magnetosomes  
249 under magnetic excitation, possibly leading to the activation of the immune system against the tumor,  
250 [15]. Most interestingly, such activation could be repeated by re-applying the AMF, [15, 16]. Here, by  
251 using RhB as a model molecule that mimics the behavior of a therapeutic substance, we examined if  
252 such a substance could repetitively dissociate from the magnetosomes in the absence of any significant  
253 temperature increase over several AMF applications. For that, we administered 40 µg of MCR400 at a  
254 depth of 3 mm in a mouse brain (Scheme 3) that we exposed to five successive magnetic sessions (MS1  
255 to MS5) during which an AMF of 25 mT and 200 kHz was applied for 30 minutes. Whereas MS1  
256 produced a limited temperature increase of 2.4 °C, MS2 to MS5 did not induce any detectable change in

257 temperature (Fig. 8(a), Fig. 9(c)), presumably due to magnetosome diffusion away from the exposed  
258 area. Most interestingly, in the absence of any detectable temperature increase (MS2 to MS5), although  
259 the four different parameters  $F_{600s}$ ,  $\Delta F/\delta t$ ,  $R_{600s}$ ,  $\Delta R/\delta t$ , decreased during increasing number of magnetic  
260 sessions (Figs. 9(a) and 9(b)), they remained significant, indicating that RhB could be repetitively  
261 released from the magnetosomes and that such release could be monitored by measuring the parameters  
262  $F_{600s}$  and  $\Delta F/\delta t$ . This result differs from previous studies which suggested that significant temperature  
263 increase was necessary to trigger drug release from nanoparticles, [42-44]. However, to our knowledge  
264 such studies did not consider the change in nanoparticle environment or nanoparticle-nanoparticle  
265 interactions with increasing temperature, which could strongly influence and possibly hinder efficient  
266 drug release. Our finding is interesting for medical applications since it indicates that efficient drug  
267 release could be induced by AMF application in the absence of a detectable temperature increase, hence  
268 using safer conditions than the high temperatures usually reported to be required for triggering an  
269 efficient release mechanism, which could be damaging towards healthy tissues and dangerous for the  
270 patient.

## 271 **CONCLUSION:**

272 In this study, we describe an original method of fabrication of a theranostic fluorescent nano-probe, in  
273 which magnetotactic bacteria are cultivated in the presence of RhB followed by magnetosome  
274 extraction from these bacteria, yielding a suspension of MCR400. Magnetosomes thus made are shown  
275 to maintain their fluorescence properties following internalization in cancer cells, showing their  
276 potential use as intracellular theranostic probes. When a MCR400 suspension is exposed to an AMF, it  
277 produces enhanced fluorescence intensity of its supernate, indicating the release of RhB from the  
278 magnetosomes. The operation of this probe is further highlighted in conditions that are close to those  
279 reached in a magnetic hyperthermia treatment, *i.e.* for 40  $\mu\text{g}$  or 400  $\mu\text{g}$  of MCR400 mixed with tissue or  
280 introduced in a mouse brain and exposed to the AMF. Indeed, with increasing duration of the magnetic  
281 excitation, they lead to an enhancement of MCR400 fluorescence intensity and hence also of RhB

282 release. Moreover, we demonstrate that we can control such release mechanism by monitoring it  
283 through fluorescent measurements, and by adjusting the parameters of the magnetic excitation, the  
284 temperature, as well as MCR400 environment and concentration.

285 To summarize, we have shown that:

286 i) It is possible to release a substance from a nano-probe and to monitor the dissociation by  
287 fluorescence.

288 ii) The fluorescence, which is initially quenched by the iron oxide, becomes enhanced following  
289 dissociation, making this nano-probe more sensitive than other fluorescent nanoparticles operating  
290 under conditions of fluorescence intensity decrease, [45].

291 iii) Provided the fluorescent substance (Rhodamine B) is replaced by a therapeutic drug, this nano-  
292 probe could also be used as a therapeutic drug operating in conditions of limited temperature increase (<  
293 2.5 °C), hence providing safer treatment conditions than conventional thermotherapies working at  
294 higher temperatures.

295 iv) The release of a therapeutic substance could be repeated by re-applying the alternating, hence  
296 possibly enabling a therapeutic activity to be triggered repetitively.

297 The field of drug released from nanoparticles, which is rapidly expanding, [46, 47], should benefit from  
298 the new approach presented in this study.

## 299 **MATERIALS AND METHODS**

300 **Culture of magnetotactic bacteria in the presence (or not) of RhB.** *Magnetospirillum magneticum*  
301 AMB-1 magnetotactic bacteria were obtained from ATCC (ATCC 700274) and grown under micro-  
302 aerophile conditions at 30 °C in a growth medium slightly different from the MSGM medium (ATCC  
303 medium 1653). In one liter, this medium contained 0.68 g of monobasic potassium phosphate, 0.85 g of  
304 sodium succinate, 0.57 g of sodium tartrate, 0.083 g of sodium acetate, 225 µl of 0.2% resazurin, 0.17 g  
305 of sodium nitrate, 0.04 g of L-ascorbic acid, 2 ml of a 10 mM iron quinate solution, 10 ml of a solution  
306 of Woolf vitamins and 5 ml of a solution of Woolf minerals. While RhB was not introduced in the

307 bacterial growth medium to synthesize non-fluorescent magnetosomes (MC), 400  $\mu$ M of RhB were  
308 introduced in this medium to synthesize fluorescent magnetosomes (MCR400). The pH of the culture  
309 medium was adjusted to 6.85 using a 1M sodium hydroxide solution. The bacteria were collected during  
310 the stationary phase and concentrated using a tangential flow filtration column (mPES, 500 kDa) with a  
311 flow rate of 950 mL/min and then washed 5 times for 30 minutes with a solution of saline phosphate  
312 buffer at pH 7.4 (137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.76 mM KH<sub>2</sub>PO<sub>4</sub>). Bacteria were  
313 collected by centrifugation at 4000 rpm for 1 hour, the supernatant was removed and the bacteria were  
314 re-suspended in 50 mM Tris-HCl buffer solution at pH 7.4 and diluted to yield an optical density of 5 at  
315 600 nm.

316 **Preparation of MC and MCR400 in suspension.** Suspensions of magnetotactic bacteria cultivated in  
317 the presence (or not) of 400  $\mu$ M RhB were lysed, magnetosomes were extracted from whole  
318 magnetotactic bacteria, bacterial suspensions were sonicated at 30 W at 5 °C during 60 minutes with  
319 pulses of 2 sec. and an interval between pulses of 1 sec. Following sonication, magnetosome chains  
320 were magnetically isolated from cellular debris using a neodymium magnet. The supernate containing  
321 the cellular debris was removed and fluorescent (MCR400) or non-fluorescent (MC) magnetosome  
322 chains were washed five times magnetically with a 50 mM Tris-HCl buffer solution at pH 7.4 and then  
323 fifteen times with Millipore® water. They were finally re-suspended in Millipore® sterile water to  
324 obtain suspensions of MC and MCR400.

325 **Preparation of MC or MCR400 brought into contact with MDA-MB-231 cells.** MDA-MB-231 cells  
326 were purchased from the American Type Culture Collections (ATCC). They were cultivated in  
327 Dulbecco's modified Eagle's medium (DMEM) supplement, which contained 10% fetal calf serum  
328 (FCS), 2 mM l-glutamine, 1 mM sodium pyruvate, and 50 U/ml streptomycin, which were purchased  
329 from Life Technologies Inc. The cells were then incubated during 6 hours with suspensions of MC and  
330 MCR400 at an amount of 63  $\mu$ g in iron oxide and then studied by flow cytometry or epifluorescence.

331 **Preparation of MC or MCR400 mixed with brain tissue or introduced in a mouse brain.** To  
332 prepare MC and MCR400 mixed in brain tissue, 2  $\mu$ l of a MC or MCR400 suspension at a concentration

333 of 20 mg/mL in iron oxide were mixed homogenously with mouse brain tissue contained in a 2 mm<sup>3</sup>  
334 volume. To obtain MC and MCR400 introduced in mouse brains, 2 or 20 µl of a MC or MCR400  
335 suspension at a concentration of 20 mg/mL in iron oxide were introduced at a depth of 1, 2 and 3 mm of  
336 a mouse brain extracted from a dead mouse.

337 **Magnetic treatment of MC and MCR400 in suspension, mixed with brain tissue, or introduced in**  
338 **a mouse brain.** 300 µl of MC and MCR400 dispersed in suspensions at 3.6 mg/mL in iron oxide were  
339 exposed to one magnetic session (MS). MC and MCR400 mixed in brain tissue or introduced in a  
340 mouse brain as described above were also exposed to 1 MS. Each MS consisted in the application of an  
341 alternating magnetic field (AMF) of 25 mT and 200 kHz for 1200 to 20000 seconds.

342 **Optical microscopy imaging of whole magnetotactic bacteria.** 10 µl of a suspension of whole  
343 magnetotactic bacteria were deposited on a microscope slide and observed with an inverted phase-  
344 contrast microscope (Zeiss Primovert) using an oil immersion objective (100 X, Ph1/0.4) with a LD  
345 condenser 0.4 (working distance: 55 mm).

346 **Epifluorescence microscopy of cells brought into contact with MCR400.** We imaged MDA-MB-231  
347 cells incubated in the presence of MCR400 to determine if MCR400 were internalized inside these cells.  
348 We used the same conditions of incubation as for flow cytometer measurements except that we fixed  
349 cells using 5% PFA. We used an epi-fluorescence microscope with an oil immersion objective x64 for  
350 optical imaging with/without RhB detection.

351 **Flow cytometer measurements on whole magnetotactic bacteria.** We used a flow cytometer to  
352 compare the properties of magnetotactic bacteria cultivated in the absence and presence of 400 µM  
353 RhB. Before flow cytometer measurements, the bacteria were washed 5 times with deionized water. for  
354 each wash, the suspension was placed against a magnet, the supernate was removed, and replaced by  
355 deionized water. 500 µl of suspensions containing magnetotactic bacteria cultivated with/without RhB  
356 were then mixed with Water Millipore® and introduced into a flow cytometer. The study was carried  
357 out using 50 000 bacteria for each condition. The flow cytometer, a FACS-calibur, was equipped with a  
358 laser of excitation wavelength 488 nm and enabled to measure various parameters such as size,



359 granularity and fluorescence of magnetotactic bacteria (or cancer cells see below). These parameters  
360 were analyzed simultaneously by means of an optical filter set, which decomposes the light emitted by  
361 the fluorochromes and directs each light signal to a different photomultiplier to record it. The detected  
362 optical signals were then converted into electrical signals and then stored and analyzed in digital values.  
363 This light-matter interaction also led to the diffusion of light by bacteria. These scattering signals were  
364 collected along an axial axis (FSC-H: Forward Scattering or axial diffusion) and a lateral axis (SSC-H:  
365 Side scattering). The FSC-H signal is proportional to bacterial sizes whereas the SSC-H signal gives  
366 information on the intracellular aspect or content of magnetotactic bacteria, *i.e.* on their granularity. The  
367 luminescence signal of RhB was also collected through a FL3-H channel at emission wavelengths  
368 between 560 nm and 627 nm. According to the flow cytometer specifications, a FL3-H signal lower and  
369 larger than the threshold of 10 a.u. corresponds to the absence and presence of RhB fluorescence,  
370 respectively. The flow cytometer also estimated the percentage of fluorescent bacteria.

371 **Flow cytometer measurements on MDA-MB231 cells.** We used the same flow cytometer as described  
372 above to study the viability and fluorescent properties of MDA-MB-231 cancer cells incubated in the  
373 presence of MC and MCR400. For that, 500 000 MDA-MB-231 cells contained in a petri dish of 2 mL  
374 were incubated during 6 hours with 31.5  $\mu\text{g/mL}$  in iron oxide of MC or MCR400. Then, a cell viability  
375 probe, calcein acetoxymethyl ester (Ca), which becomes luminescent in the presence of living cells, was  
376 added to the petri dish and the resulting assemblies were collected after trypsinization and centrifugation  
377 in tube for flow cytometer measurements. The flow cytometer detected either the fluorescence of Ca  
378 (FL1-H) at 530 nm or that of RhB at 585 nm (FL3-H). According to the flow cytometer specifications,  
379 FL1-H and FL3-H signals are either lower or larger than a threshold of 10, corresponding to the  
380 threshold value above which cells are viable for FL1-H or fluorescent for FL3-H. The flow cytometer  
381 also estimated the percentages of viable and fluorescent cells.

382 **FT-IR characterization of MC, MCR400, and RhB.** Suspensions at pH 7 containing MC, RhB and  
383 MCR400 with a concentration of 1 mg/mL in iron oxide were lyophilized and integrated in a KBr

384 matrix. Their Fourier Transform infra-red (FT-IR) absorption spectra were recorded using a Nicolet FT-  
385 IR model 380 with resolution of  $0.5 \text{ cm}^{-1}$  and a number of scans of 30.

386 **Transmission electron microscopy of whole bacteria.** Electron transmission microscopy images of  
387 whole bacteria, MC and MCR400 were obtained with a JEM-2100 from JEOL. For that, 5  $\mu\text{l}$  of a  
388 suspension of whole bacteria, MC, and MCR400, were deposited on top of a carbon grid and dried. Size  
389 distributions of magnetosomes MC and MCR400, were measured using 300 magnetosomes and plotted  
390 in histograms.

391 **Absorption and fluorescence measurements of MC, MCR400, and RhB suspended or mixed in**  
392 **water.** The absorption spectra of MC and MCR400 (suspensions containing 40  $\mu\text{g}$  in iron oxide of  
393 nanoparticles contained in 1 mL of water) or of RhB (solution containing 4  $\mu\text{g}$  of RhB in 1 mL of water)  
394 were recorded between 350 nm and 700 nm using a Varian Cary 3E UV-Vis spectrophotometer. The  
395 fluorescence spectra of MC and MCR400 (170  $\mu\text{g}$  in iron oxide of nanoparticles contained in 800 $\mu\text{L}$  of  
396 water) or of RhB (0.4 $\mu\text{g}$  of RhB in 800  $\mu\text{L}$  of water) were recorder on a Aminco-Bowman 2  
397 spectrofluorimeter (Edison, NJ). Fluorescence intensity was recorded between 550 and 650 nm under  
398 excitation at 405 nm.

399 **Fluorescence measurements of MCR400 suspended in water in the presence of a magnetic**  
400 **treatment.** 1 mg/mL in iron oxide of MCR400 were mixed in 100  $\mu\text{L}$  of water and exposed to one MS,  
401 we measured the fluorescence intensity of this suspension and of its supernate, which was magnetically  
402 isolated from the magnetosomes using a Neodymium magnet of 0.6 T. We measured the fluorescence  
403 intensity of the MCR400 suspension and of its supernate at different times following magnetic  
404 excitation (0, 100, 200, 300, 600, 900 or 1200 seconds). Fluorescence intensity was measured at 576 nm  
405 for an excitation at 555 nm using a Fluoroskan Ascent (Thermo Scientific) microplate spectrometer.

406 **Fluorescence measurements of MCR400 mixed with brain tissue or introduced in a mouse brain,**  
407 **using a bi-fiber set-up, in the presence of a magnetic treatment.** For MCR400 and MC mixed in  
408 brain tissue or introduced in mouse brain as described above, samples were excited by a pulsed diode  
409 laser emitting at 405 nm with a mean power of 1 mW and a repetition rates of 40 MHz from PicoQuant

410 (GmbH, Berlin, Germany). A bi-fibered configuration was employed for excitation and collection  
411 positioned 3 mm above the surface of the brain or mixed issue. The fibers used for excitation and  
412 collection had a core diameter of 200  $\mu\text{m}$  and 365  $\mu\text{m}$ , respectively, with a numerical aperture of 0.22.  
413 The spatial resolution was 500  $\mu\text{m}$ . Collected fluorescence signal was sent toward a computer controlled  
414 cooled spectrometer (Ocean optics QP600-1-UV-VIS) for spectroscopic analysis. For fluorescence  
415 detection, the fiber was positioned 1, 2, or 3 mm, above the lamella for the sample containing MCR400  
416 mixed with brain tissue while it was fixed at a height of 3 mm above the lamella for the sample  
417 containing MCR400 introduced in mouse brain at a depth of 1, 2, or 3 mm. In this way, the distance  
418 between the fiber end and the center of the samples was the same for a height of 1, 2, or 3 mm in mixed  
419 tissue than for a depth of 1, 2, or 3 mm in mouse brain.

420 **Temperature measurements of MCR400 in suspensions mixed with brain tissue or introduced in a**  
421 **mouse brain during a magnetic treatment.** For studies in brain tissues and *ex vivo*, spatial temperature  
422 distribution in the region containing MCR400 was measured using an Easir 2 thermographic infrared  
423 camera positioned 20 cm above the tissues or brain. The plotted temperatures correspond to the highest  
424 temperature measured at each time point within the heated surface of  $\sim 3 \text{ mm}^2$ . A microprobe  
425 thermocouple (IT-18, Physitemp, Clifton, USA) was inserted inside the magnetosome suspensions and  
426 employed for measurements in solution using a Thermes USB (Physitemp) thermometer. The spatial  
427 resolutions of the infrared camera and of the thermocouple were 660  $\mu\text{m}$  and 600  $\mu\text{m}$ , respectively.

428 **Temperature parameters  $\Delta T_{600\text{sec.}}$ ,  $\Delta T/\delta t$ , and deduction of magnetosome biodistribution**  
429 **properties from the values of  $\Delta T/\delta t$ .**  $\Delta T_{600\text{sec.}}$  and  $\Delta T/\delta t$  correspond to the temperature increase  
430 measured 600 seconds following the beginning of magnetic excitation and to the initial slope of the  
431 temperature variation with time, measured at the beginning of magnetic excitation, respectively. For  
432 MCR400 mixed in tissue or introduced in mouse brain, we could deduce MCR400 biodistribution  
433 properties from the different values  $\Delta T/\delta t$  by using the relation between the magnetosome specific  
434 absorption rate (SAR),  $\Delta T/\delta t$ , and the magnetosome concentration,  $C_{\text{mag}}$ , which is given by:

435  $SAR=C_v(\Delta T/\delta t)/C_{mag}$ , where  $C_v$  is the specific heat of water. Assuming that  $C_v$  and SAR remain  
436 unchanged between the different samples, we deduced that the percentage of MCR400 in the injection  
437 volume is proportional to  $(\Delta T/\delta t)_2/(\Delta T/\delta t)_1$ , where  $(\Delta T/\delta t)_2$  and  $(\Delta T/\delta t)_1$  are the initial slopes of the  
438 temperature variation, measured when MCR400 occupy part or 100% of the injection volume,  
439 respectively.

440 **Fluorescence measurement of RhB introduced in mouse brain or mixed with brain tissue, using a**  
441 **bi-fiber set-up, in the absence of magnetic treatment.** We followed the same protocols of preparation  
442 as for MCR400 mixed with brain tissue or introduced in mouse brain. On the one hand, different  
443 quantity of RhB (between 1 ng and 40 ng) were mixed with 2 mm<sup>3</sup> of brain tissue and deposited  
444 between a blade and a lamella. On the other hand, 2  $\mu$ l containing different quantity of RhB dissolved in  
445 water (between 1 ng and 100 ng) were introduced at different depth (1, 2, and 3 mm) of a brain  
446 extracted from a dead mouse. The fluorescence of the two types of samples was excited and detected  
447 using the same bi-fiber set-up and excitation/detection conditions as for MCR400. The fluorescence  
448 intensity of free RhB was then plotted as a function of the RhB concentration in these different  
449 conditions.

450 **Fluorescence parameters,  $\Delta F_{600sec}$ . and  $\Delta F/\delta t$ .**  $\Delta F_{600sec}$ . corresponds to the fluorescence intensity  
451 increase measured 600 seconds following the beginning of magnetic excitation. The measurement time  
452 of 600 seconds was selected to be sufficiently long to enable attainment of a stationary state while  
453 avoiding fluorescence quenching and possible random MCR400 interactions occurring beyond 600  
454 seconds (Fig. 6(c)).  $\Delta F/\delta t$  corresponds to the initial slope of the fluorescence intensity variation with  
455 time, measured at the beginning of magnetic excitation, respectively.

456 **Parameters measuring the release of RhB from magnetosomes ( $R$ ,  $R_{600sec}$ . and  $\Delta R/\delta t$ ) for MCR400**  
457 **mixed with brain tissue or introduced in a mouse brain and exposed to a magnetic treatment.** The  
458 percentage of RhB released from the magnetosomes was estimated from the value of the fluorescence  
459 intensity of RhB released from the magnetosomes in MCR400 (Fig. 6(c), 6(d), 8(b)), using the formula:

460  $R=100*(Q_{RhBR}/Q_{RhBT})$ , where  $Q_{RhBR}$  and  $Q_{RhBT}$  are the quantities of RhB released from the  
461 magnetosomes under magnetic excitation and  $Q_{RhBT}$  is the total quantity of RhB associated to the  
462 magnetosomes before magnetic excitation.  $Q_{RhBR}$  was estimated by measuring  $\Delta F = F_{RhBt} - F_{RhBt_0}$ , where  
463  $F_{RhBt}$  and  $F_{RhBt_0}$  are the fluorescence intensities of RhB during magnetic excitation (time t) and at the  
464 beginning of magnetic excitation ( $t_0$ ) deduced from Figs. 6(c), 6(d) and 8(b). We have then measured  
465 and plotted the variation of the fluorescence intensity of free RhB as a function of the quantity of RhB  
466 quantity, for RhB mixed with tissue (Fig. S5(a)) or introduced in a mouse brain (Fig. S5(b)). Given that  
467  $\Delta F$  corresponds to the quantity of RhB dissociated from the magnetosomes, we have deduced  $Q_{RhBR}$  by  
468 estimating the quantity of RhB corresponding to  $\Delta F$  values in Figs. S5(a) and S5(b).  $Q_{RhBT}$  was  
469 estimated by dissolving 40 or 400  $\mu\text{g}$  of MCR400 at pH 0.84. Following this treatment, the fluorescence  
470 spectrum of MCR400 shows a peak at 582 nm (Fig. S4(a)), a similar emission wavelength as that of free  
471 RhB (Fig. S4(b)), indicating that RhB has dissociated from the magnetosomes. Furthermore, given the  
472 value of the maximum fluorescence intensity of this peak of 0.56 a.u. (Fig. S4(a)) as well as the relation  
473 between RhB fluorescence intensity and RhB concentration (Fig. S4(c)), we deduced that this treatment  
474 induces the dissociation of  $\sim 750$  nmol/L of RhB from the magnetosomes.  $Q_{RhBT}$  was then deduced as  
475 84  $\mu\text{g}$  and 840  $\mu\text{g}$  for the samples containing 40  $\mu\text{g}$  and 400  $\mu\text{g}$  of MCR400, respectively.  $R_{600\text{sec.}}$  and  
476  $\Delta R/\delta t$  represent the RhB release rate measured 600 seconds following magnetic excitation and the  
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619

620 **SCHEME:**

621 **Scheme 1:** Schematic diagrams showing the steps involved in the preparations of chains of  
622 magnetosomes isolated from magnetotactic bacteria and associated to RhB (MCR400). In this  
623 preparation, AMB-1 magnetotactic bacteria were first cultivated in a growth medium containing 400  
624  $\mu\text{M}$  of RhB and incubated during 14 days at 30 °C under these conditions. Chains of magnetosomes  
625 associated with RhB have then been isolated from these bacteria to yield MCR400.

626 **Scheme 2:** Schematic diagrams showing the operating mechanism of the MCR400 probe. Under the  
627 application of an alternating magnetic field, RhB dissociates from the magnetosome mineral core  
628 yielding enhanced fluorescence intensity and fluorescence wavelength shift.

629 **Scheme 3:** (a), Schematic diagram showing the bi-fiber set-up used to excite 2  $\mu\text{l}$  or 20  $\mu\text{l}$  of a MCR400  
630 suspension at a concentration of 20 mg/mL in iron oxide introduced in a brain extracted from a dead  
631 mouse at different depth of -1 mm, -2 mm, or - 3 mm, estimated from the mouse brain surface, where  
632 the bi-fiber is positioned at 4 mm (\*), 5 mm (\*\*), or 6 mm (\*\*\*) above the center of the region  
633 containing the magnetosomes. (b), Schematic diagram showing the bi-fiber set-up used to excite 2  $\mu\text{l}$  of  
634 a MCR400 suspension at a concentration of 20 mg/mL in iron oxide mixed with brain tissue, where the  
635 center of the bi-fiber is positioned at a distance of 4 mm (1), 5 mm (2), or 6 mm (3) from the center of  
636 the tissue.

637

638 **FIGURES:**

639 **Figure 1:** Optical image of living AMB-1 magnetotactic bacteria cultivated in the presence of 400  $\mu\text{M}$   
640 of RhB. After 7 days of growth of magnetotactic bacteria in the presence of 400  $\mu\text{M}$  of RhB, an aliquot  
641 of 7  $\mu\text{l}$  was taken, deposited on top of a lamella, and observed under optical microscope using an oil  
642 objective.

643 **Figure 2: (a),** Number of bacteria counted with the flow cytometer as a function of the FL3-H  
644 fluorescence signal, measuring RhB fluorescence intensity, for magnetotactic bacteria cultivated in the  
645 absence of RhB and in the presence of 400  $\mu\text{M}$  of RhB. The black line plotted between  $10^1$  and  $10^4$   
646 delineates the region where bacteria are luminescent. Cloud of points representing the lateral scattering  
647 signal (SSC-H) as a function of the forward scattering signal (FSC-H), measured by flow cytometry, of  
648 magnetotactic bacteria cultivated in the absence of RhB (b), or in the presence of 400  $\mu\text{M}$  RhB, (c). The  
649 values of the SSC-H, FSC-H, and FL3-H signals are expressed in arbitrary units.

650 **Figure 3:** FT-IR spectra of chains of magnetosomes extracted from magnetotactic bacteria cultivated in  
651 the presence of 400  $\mu\text{M}$  RhB (MCR400), (a), of chains of magnetosomes extracted from magnetotactic  
652 bacteria cultivated in the absence of RhB (MC), (b), of free RhB, (c). Absorption spectra of 1 mL of  
653 suspensions containing 40  $\mu\text{g}$  of MCR400, (d), 40  $\mu\text{g}$  of MC, (e), 4  $\mu\text{g}$  of free RhB, (f). Fluorescence  
654 spectra, recorded for an excitation wavelength of 405 nm, of 800  $\mu\text{L}$  suspensions containing 170  $\mu\text{g}$  of  
655 MCR400, (g), 170  $\mu\text{g}$  of MC, (h), 0.4  $\mu\text{g}$  of free RhB, (i).

656 **Figure 4:** (a) Number of cells counted with a flow cytometer (FACS-Calibur) as a function of FL1-H  
657 signal, measuring Ca fluorescence, for MDA-MB-231 alone (blue histogram), MDA-MB-231 cells in  
658 the presence of Ca (blue line), MDA-MB-231 cells in the presence of Ca and fluorescent chains of  
659 magnetosomes MCR400 (red line); (b) Number of cells counted with a flow cytometer (FACS-Calibur)  
660 as a function of FL3-H signal, measuring RhB fluorescence, for MDA-MB-231 cells in the presence of  
661 Ca (Blue line), MDA-MB-231 cells in the presence of Ca and MCR400 (red line); (c), Optical image,

662 recorded in transmission without rhodamine B fluorescence, of MDA-MB-231 cells incubated with  
663 MCR400 during 6 hours. (d), Optical epi-fluorescence image, measuring rhodamine B fluorescence, of  
664 MDA-MB-231 cells incubated with MCR400 during 6 hours; (e), Merge of (c) and (d).

665 **Figure 5:** (a), for a 100  $\mu$ L of suspension of MCR400 at an iron oxide concentration of 1 mg/mL  
666 exposed to an AMF of 25 mT and 198 kHz during 1200 seconds, variation of temperature of this  
667 suspension as a function of time measured using a thermocouple. (b), For the same MCR400 suspension  
668 and treatment as in (a), variation of the fluorescence intensity of the MCR400 suspension and of its  
669 supernate, which was magnetically from the magnetosomes. The fluorescence was excited at 405 nm. In  
670 (b), the initial fluorescence intensity at 0 second is comprised between 0 and 0.4 a.u. (the error bar can't  
671 be seen in the graph for this point).

672 **Figure 6:** Fluorescence analysis of the MCR400 nano-probe in conditions where the application of an  
673 AMF on MCR400 yields a temperature increase of more than 3  $^{\circ}$ C. For 2  $\mu$ l of a suspension of  
674 MCR400 at a concentration of 20 mg/mL in iron oxide mixed with brain tissue and exposed to an AMF  
675 of 200 kHz and 25 mT during 30 minutes, variation of the temperature, (a), fluorescence intensity (c),  
676 and percentage of RhB released from the magnetosome mineral core, (e), as a function of time. For 20  
677  $\mu$ l of a suspension of MCR400 at 20 mg/mL in iron oxide introduced in a mouse brain, variation of the  
678 temperature (b), fluorescence intensity, (d), and percentage of RhB released from the magnetosome  
679 mineral core, (f), as a function of time. The fluorescence intensity was measured at 580 nm for an  
680 excitation at 405 nm. The bi-fiber set-up used for these measurements was such that the distance  
681 between the center of the magnetosome regions and the fiber end was 4 mm.

682 **Figure 7:** Fluorescence analysis of the MCR400 nano-probe in conditions where the application of an  
683 AMF on MCR400 yields a temperature increase of more than 2.5  $^{\circ}$ C. For 2  $\mu$ l of MCR400 at 20 mg/mL  
684 mixed with brain tissue (40  $\mu$ g of MCR400 mixed with brain tissue) or 20  $\mu$ l of MCR400 at 20 mg/mL  
685 introduced in a mouse brain (400  $\mu$ g of MCR400 in brain), fluorescence ( $\text{Fluorescence}_{600s}$ ), (a), or

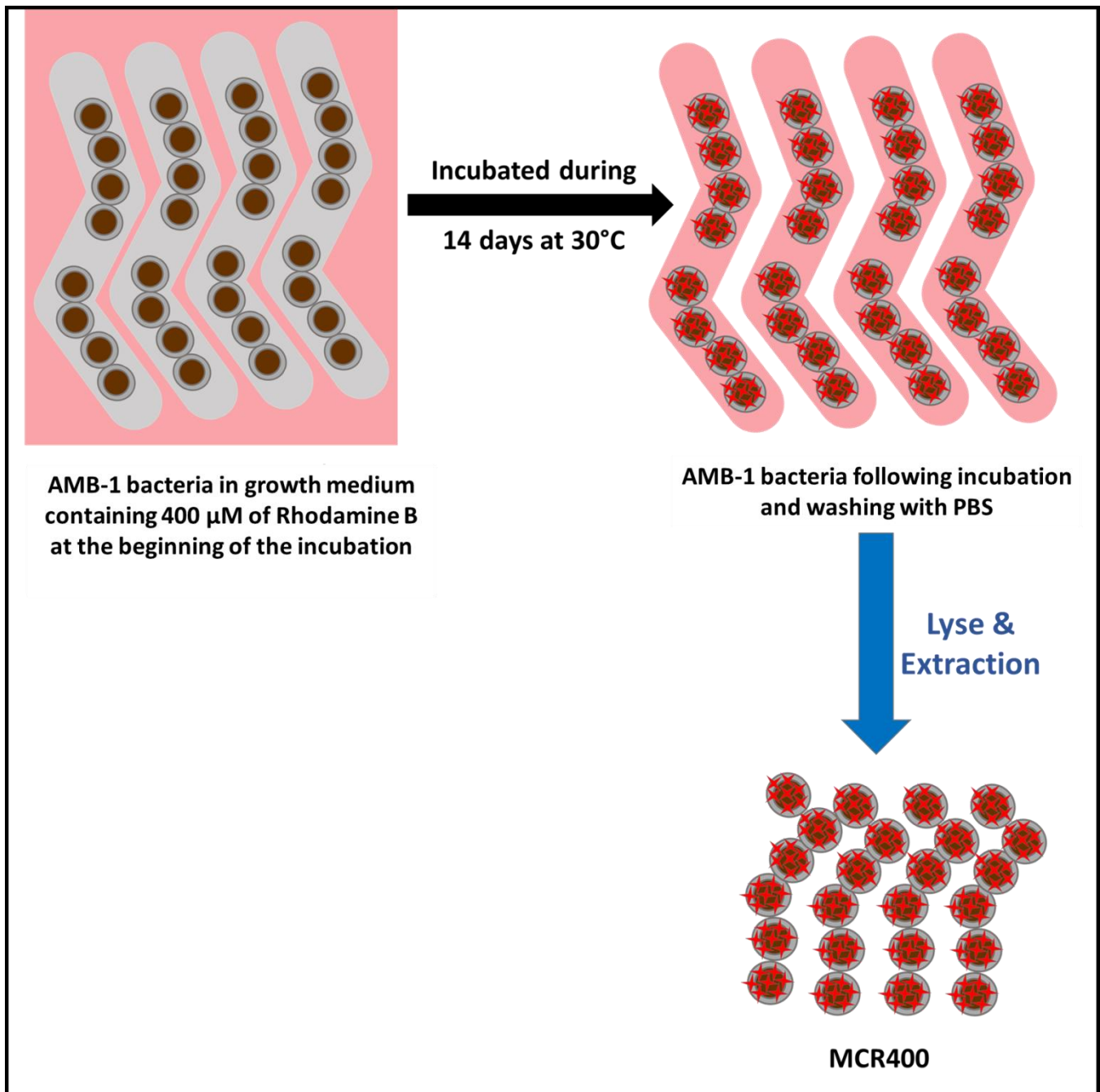
686 percentage of RhB released from the magnetosome mineral core ( $R_{600s}$ ), (b), measured 600 seconds after  
687 the application of an AMF of 200 kHz and 25 mT, as a function of  $\Delta T_{600s}$ , where  $\Delta T_{600s}$  is the difference  
688 in temperature between the initial temperature at the beginning of magnetic excitation and the  
689 temperature measured after 600 seconds of magnetic excitation. For the same samples and treatments as  
690 in (a) and (b), initial slope of the variation of fluorescence with time ( $\Delta F/\delta t$ ), (c), and initial slope of the  
691 variation of the percentage of RhB released from the magnetosome mineral core with time ( $\Delta R/\delta t$ ), (d),  
692 as a function of the initial slope of the temperature variation with time ( $\Delta T/\delta t$ ). The fluorescence  
693 intensity was measured at 580 nm for an excitation at 405 nm. The bi-fiber set-up used for these  
694 measurements was such that the distance between the center of the magnetosome regions and the fiber  
695 end was 4 mm. In (a) to (d), the dotted lines are guides to the eyes, the numbers 1, 2, 3 represent the  
696 positions of the fiber 1, 2, and 3 mm, above the mouse head, while, \*, \*\*, and \*\*\*, represent the  
697 injection depths of 1 mm, 2 mm, and 3 mm (Scheme 3).

698 **Figure 8:** Fluorescence analysis of the MCR400 nano-probe in conditions where the application of an  
699 AMF on MCR400 yields a temperature increase of less than 2.5 °C. For 2  $\mu$ l of a suspension of  
700 MCR400 at a concentration of 20 mg/mL in iron oxide introduced in a mouse brain at a depth of 3 mm  
701 and exposed to several magnetic sessions (MS1 to MS5) during which an AMF of 200 kHz and 25 mT  
702 during 30 minutes was applied for 2000 seconds. Variation of temperature, (a), fluorescence intensity  
703 (b), and percentage of RhB released from the magnetosomes, (c), as a function of time. The  
704 fluorescence intensity was measured at 580 nm for an excitation at 405 nm. The bi-fiber set-up used for  
705 these measurements was such that the distance between the center of the magnetosome regions and the  
706 fiber end was 4 mm.

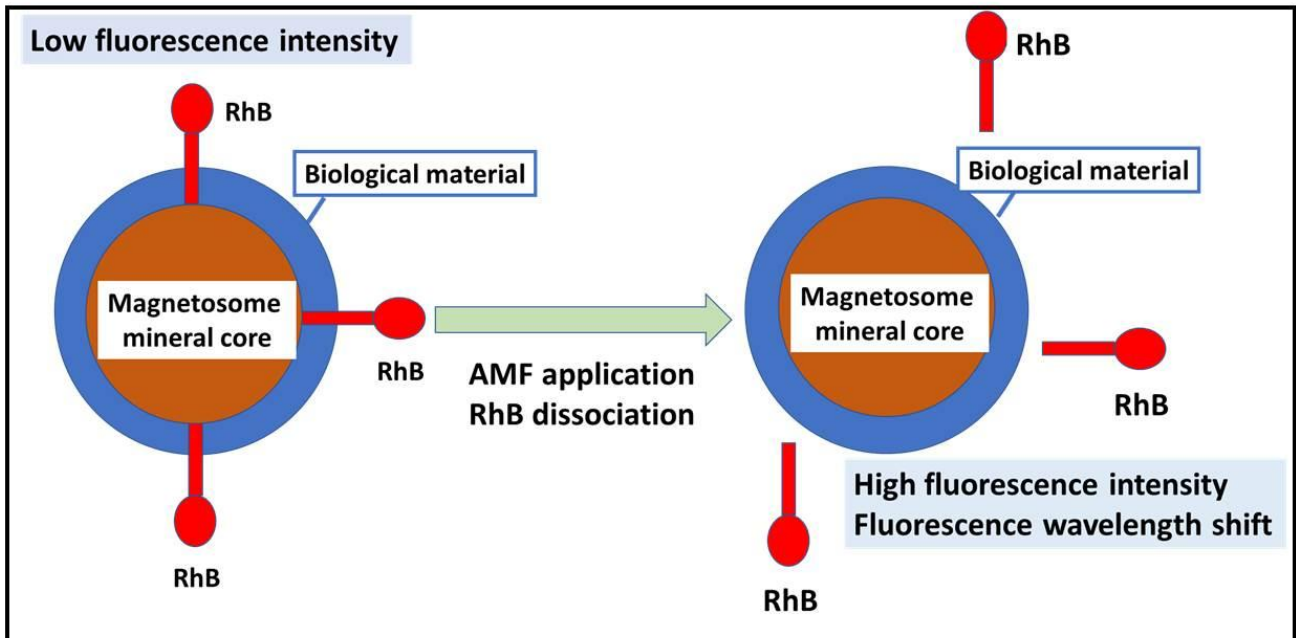
707 **Figure 9: MCR400 fluorescence properties** for 20  $\mu$ l of a MCR400 suspension at 20 mg/mL  
708 introduced in a mouse brain at a depth of 3 mm and exposed to several magnetic sessions (MS1 to MS5)  
709 during which an AMF of 200 kHz and 25 mT during 30 minutes was applied for 2000 seconds. (a),  
710 fluorescence intensity measured 600 seconds after magnetic excitation,  $F_{600sec.}$ , and slope at the origin of

711 the fluorescence variation with time,  $\Delta F/\delta t$  for the various MS. (b), Percentage of released rhodamine B  
712 measured 600 seconds following magnetic excitation,  $R_{600\text{sec.}}$ , and slope at the origin of the variation of  
713 the percentage of released rhodamine B,  $\Delta R/\delta t$ , during the various MS. (c), Variation of temperature  
714 measured 600 seconds following magnetic excitation,  $\Delta T$ , and slope at the origin of the temperature  
715 variation,  $\Delta T/\delta t$ , during the various MS.

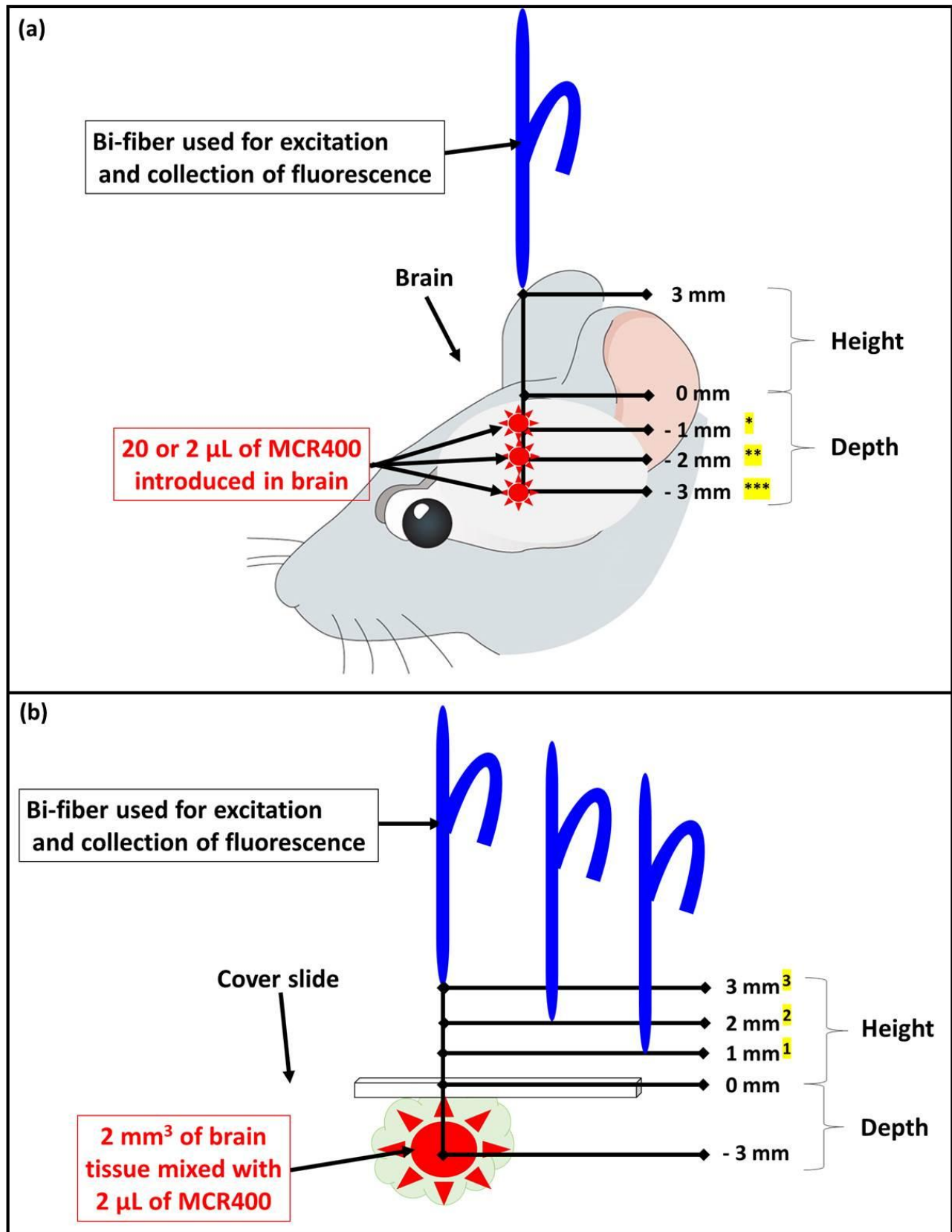




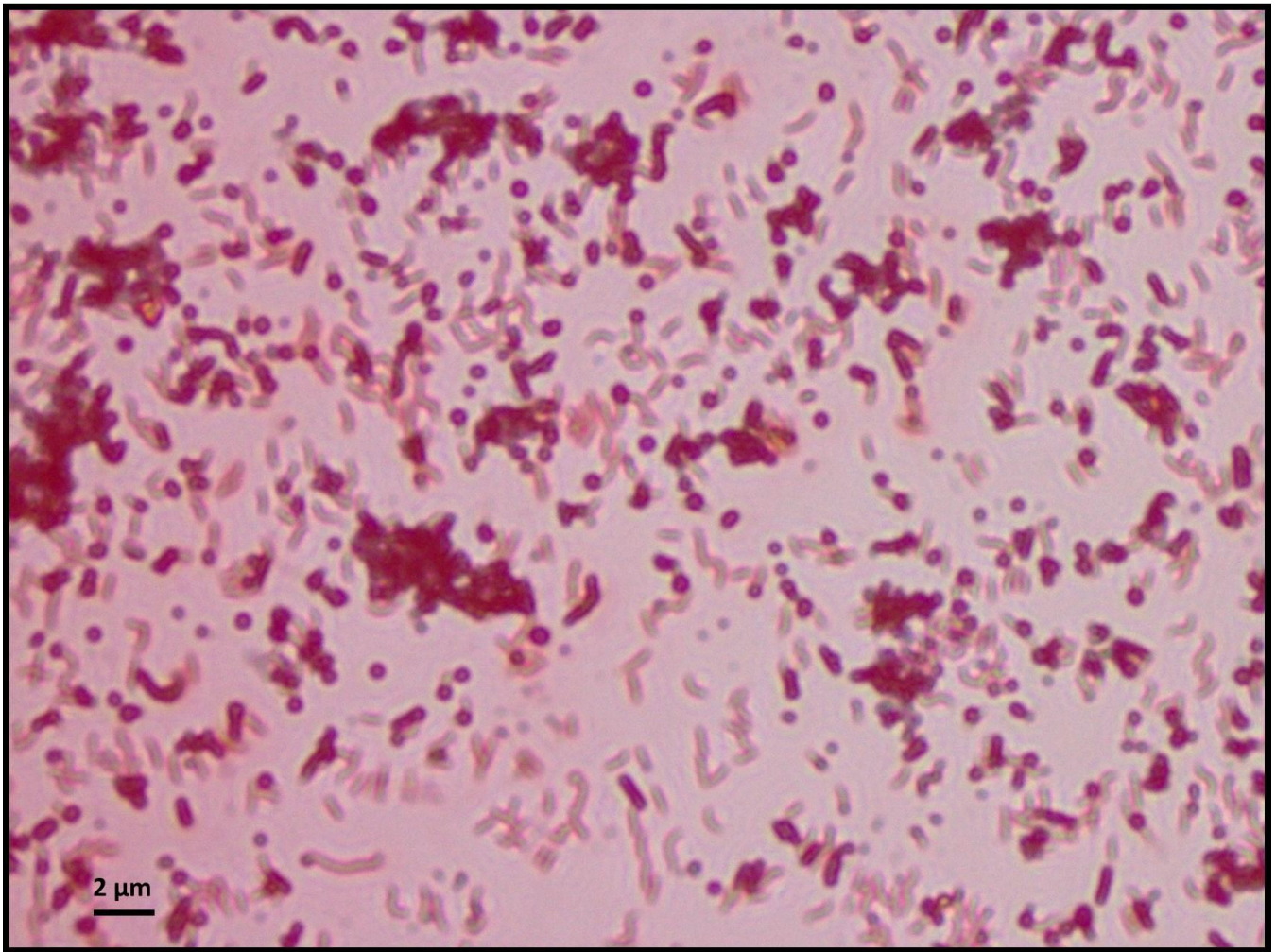
**Scheme 1**



**Scheme 2**

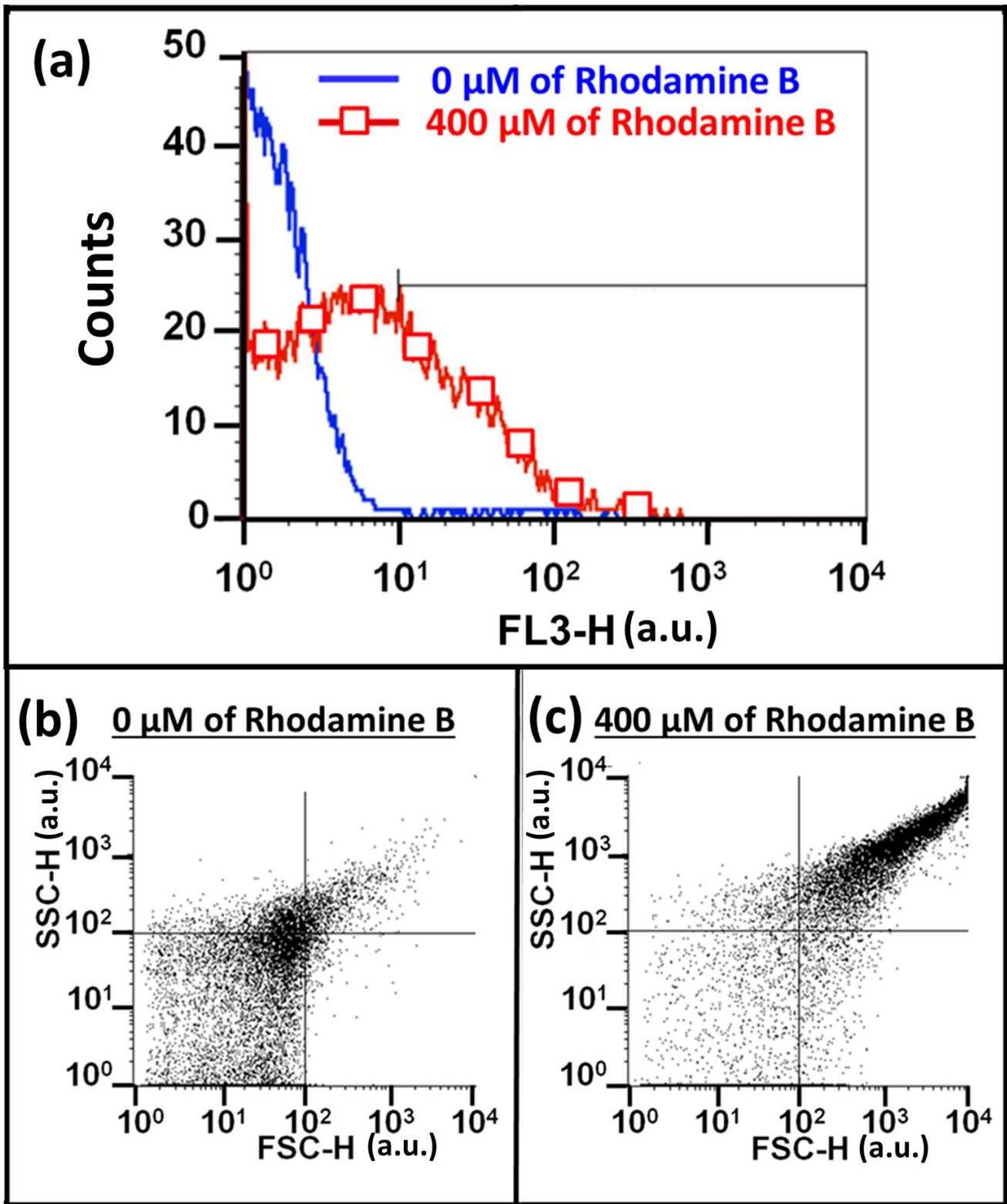


Scheme 3



**Figure 1**





**Figure 2**

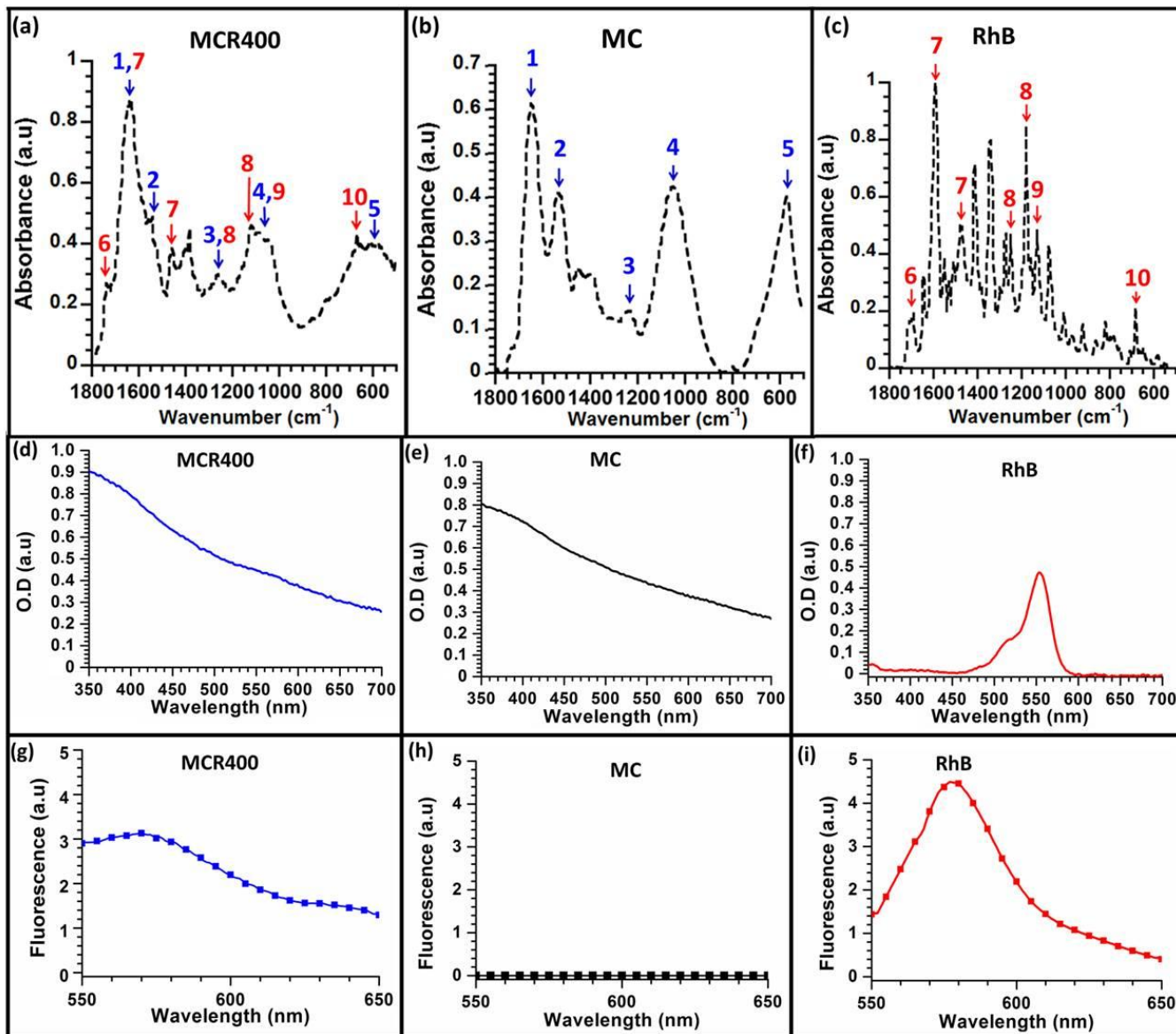
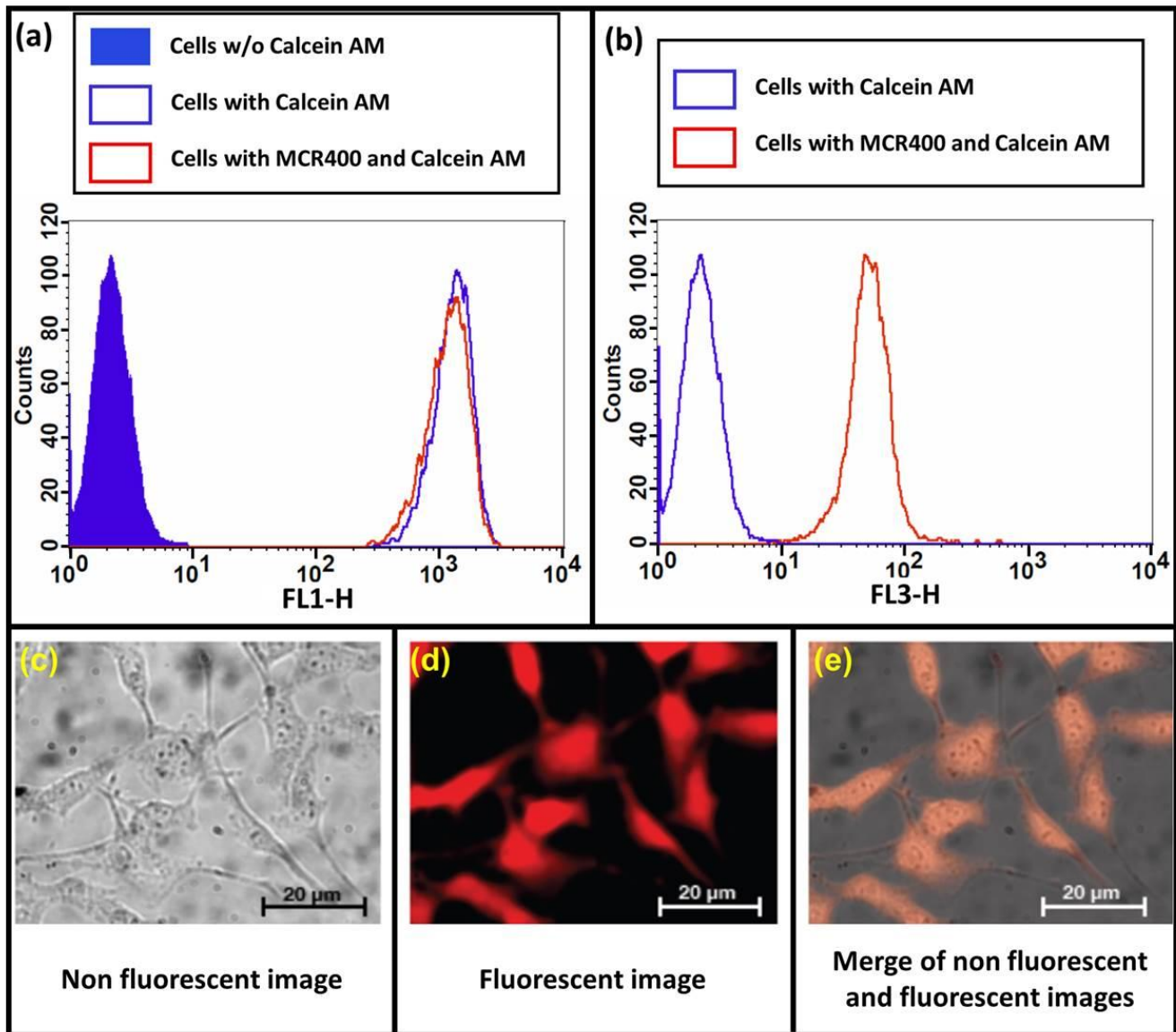


Figure 3



**Figure 4**

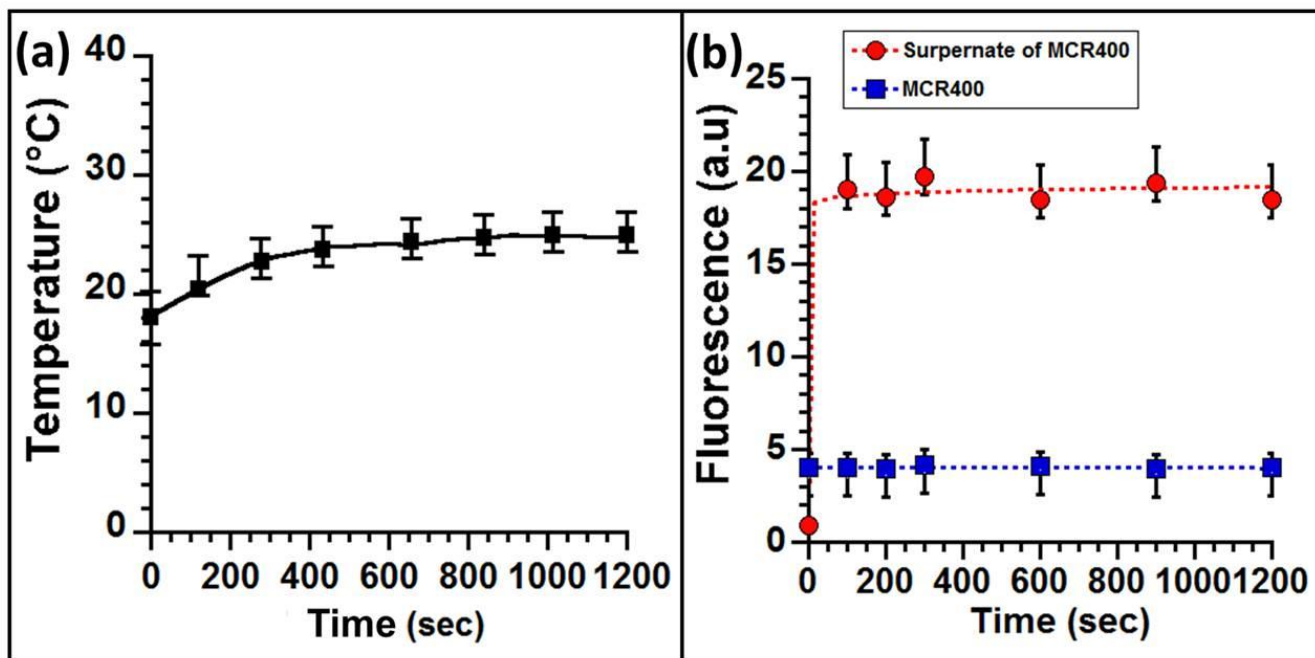


Figure 5



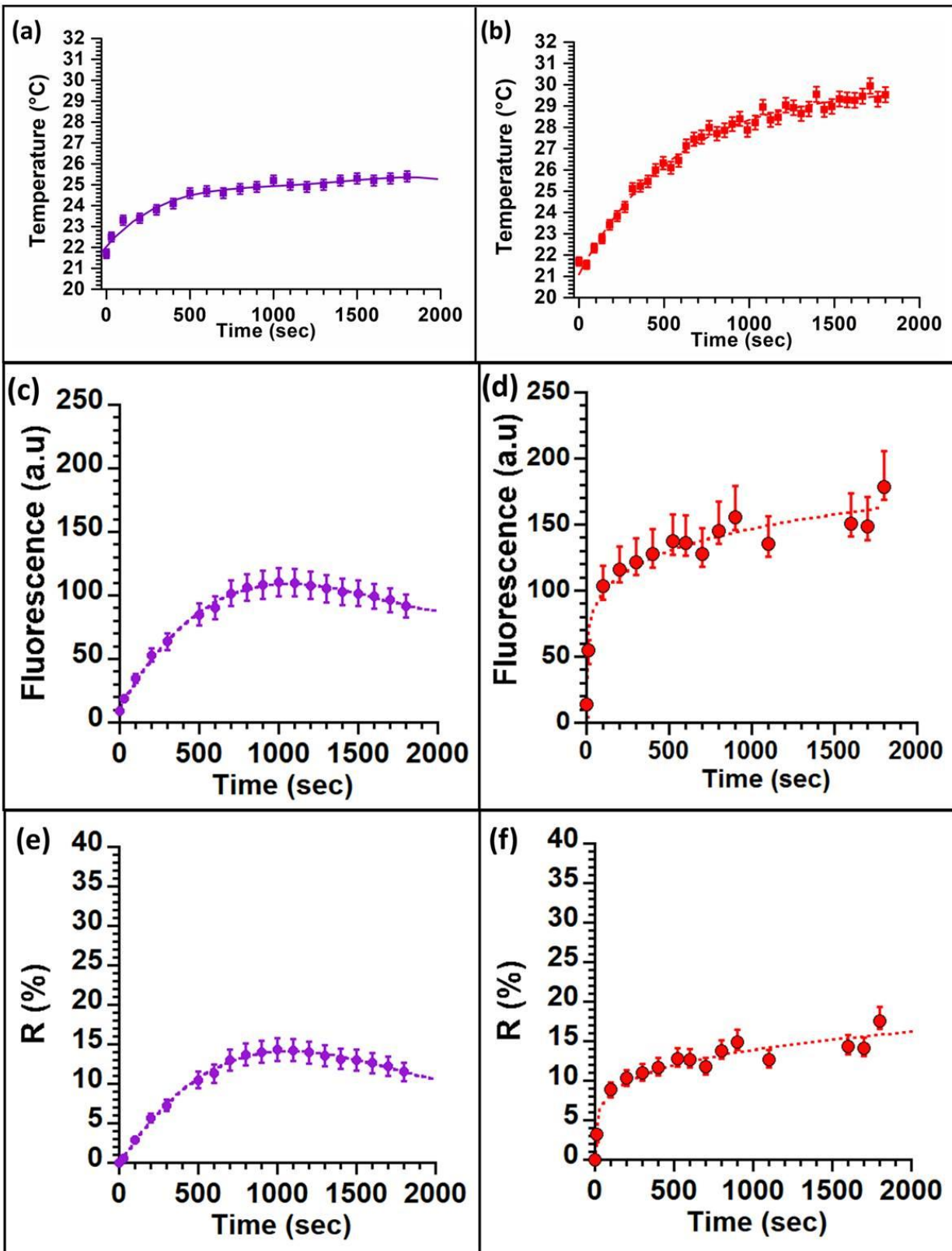


Figure 6

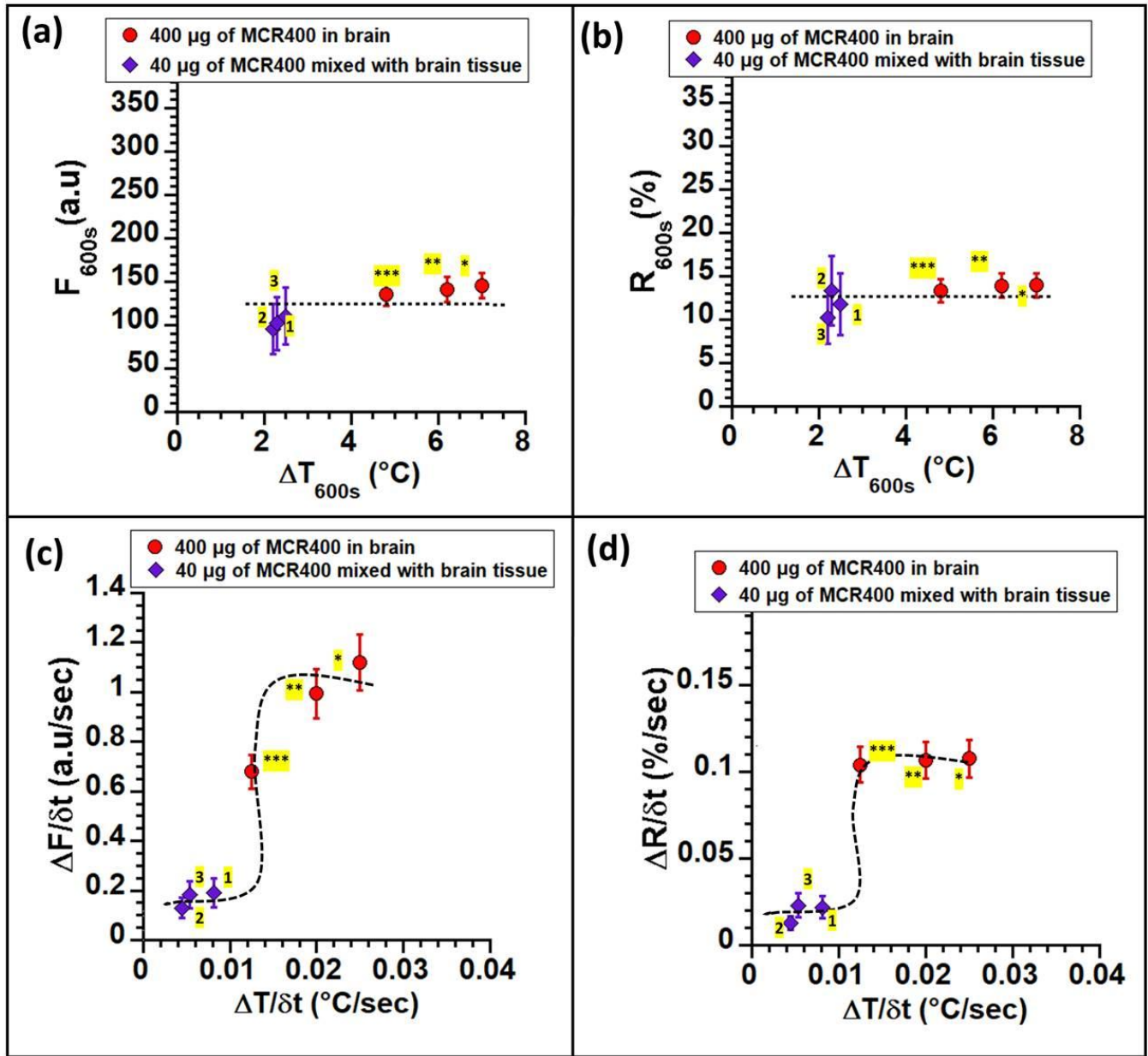


Figure 7

40  $\mu\text{g}$  of MCR400 in mouse brain, injection depth = 3 mm

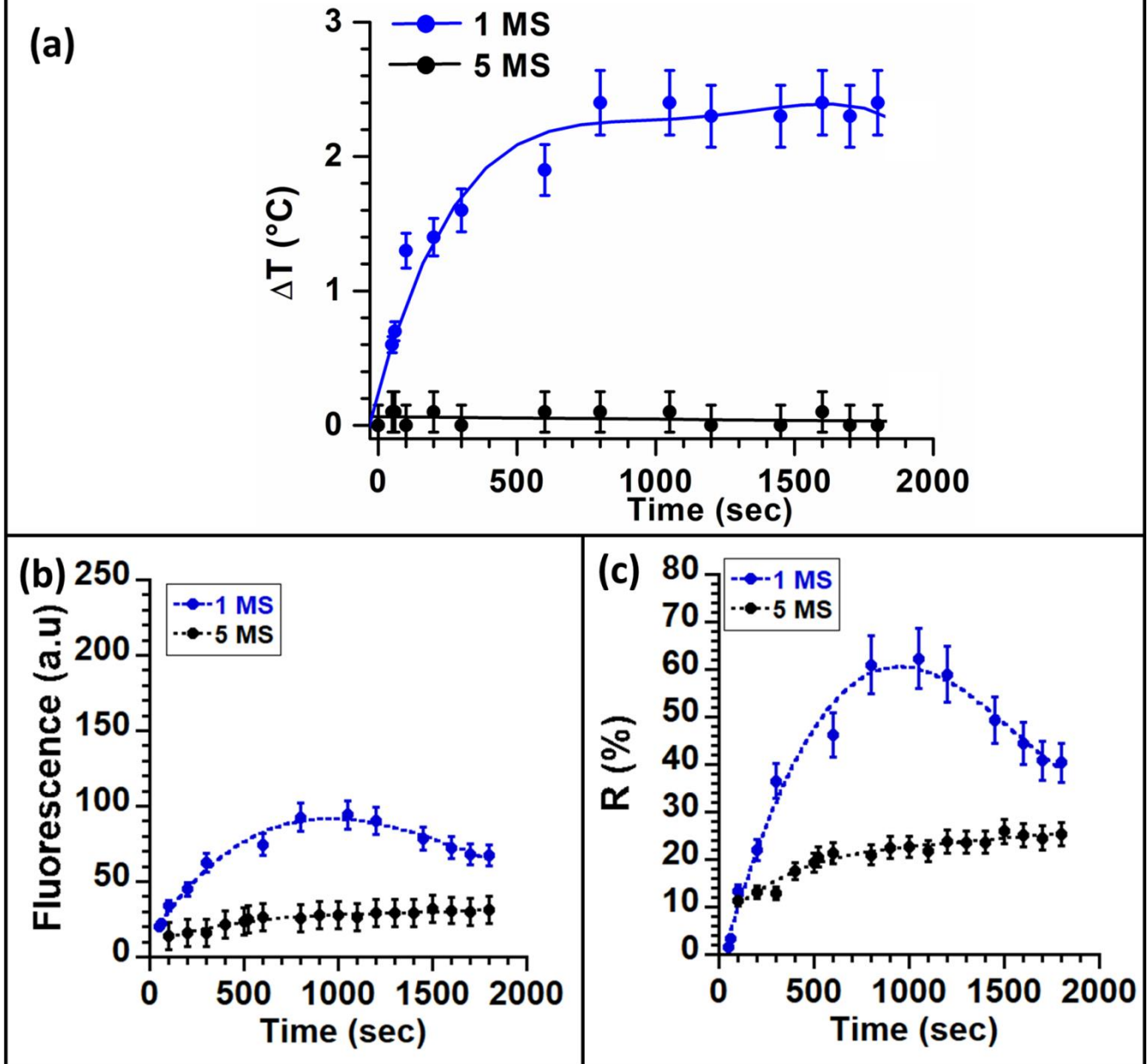


Figure 8

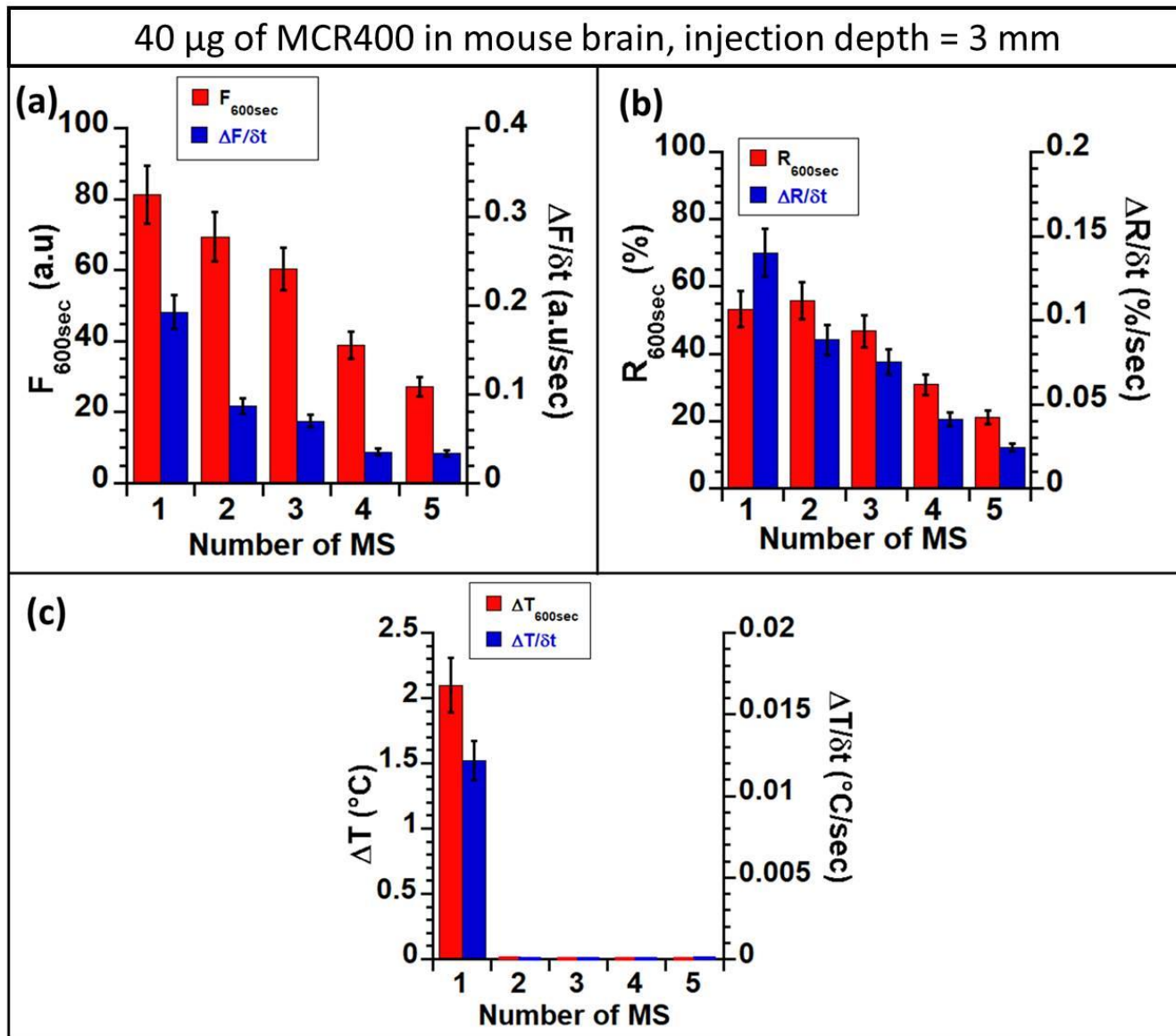


Figure 9