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1 Taylor dispersion analysis for measurement of
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22 **ABSTRACT**

23 Gadolinium-based contrast agents (GBCA) are complexes of a Gadolinium metal center and
24 a linear or macrocyclic polyamino-carboxylic acid chelating agent. These agents are
25 employed to enhance the visibility of deep abnormalities through MRI techniques. Knowing
26 the precise dimensions of various GBCA is key parameter for understanding their in-vivo and
27 pharmaco-kinetic behaviors, their diffusivity, as well as their relaxivity. However,
28 conventional size characterization techniques fall short when dealing with these tiny
29 molecules (≤ 1 nm). In this work, we propose to determine the size and diffusivity of
30 gadolinium-based contrast agents using Taylor dispersion analysis (TDA). TDA provided a
31 reliable measurement of the hydrodynamic diameter and the diffusion coefficient. The
32 obtained results were compared to DOSY NMR (Diffusion-ordered Nuclear Magnetic
33 Resonance Spectroscopy) and DFT (Density Functional Theory).

34

35

36 **KEYWORDS.** Taylor dispersion analysis, diffusion coefficient, hydrodynamic diameter,
37 gadolinium-based contrast agents, magnetic resonance imaging agents.

38 1. Introduction

39 Nuclear magnetic resonance imaging took off at the end of the 1980s with the
40 appearance of the first Gadolinium-based Contrast Agents (GBCA) dedicated to this medical
41 imaging modality (Lauffer, 1987). These agents are widely used for certain indications to
42 improve natural contrast and enhance diagnostic accuracy, especially for the diagnosis and
43 characterization of brain tumors. GBCA currently used in human clinical practice are small
44 gadolinium complexes with the metal in oxidation state +III. Their action on the signal is
45 based on the magnetic properties of Gd^{3+} , which has seven unpaired electrons distributed
46 isotopically in the seven 4f orbitals. Gadolinium belongs to the lanthanide family and is toxic
47 as a free ion, since it can compete with calcium in essential physiological processes (Spencer
48 et al., 1997). To overcome this toxicity problem, Gd^{3+} must be efficiently complexed with
49 suitable ligands while retaining its paramagnetic properties. The agent itself is not visible in
50 MRI, but its effects on protons in its immediate neighborhood are monitored. The intensity of
51 the signal depends not only on the local density of proton nuclei, but also on their
52 characteristic relaxation times (Caravan, 2006). The paramagnetic relaxation effect originated
53 by the agent makes any surrounding hydrogen atoms from water molecules to relax more
54 efficiently, and this relaxation enhancement (also called relaxivity) generates contrast on T_1 -
55 or T_2 -weighted imaging sequences. For small gadolinium complexes, the relaxivity is
56 proportional to the number of water molecules q in the first coordination sphere, and to the
57 **rotational** correlation time (τ_R) (Dumas et al., 2010). It is possible to increase τ_R by increasing
58 the molar mass of the complex (i.e. the size of the complex). Indeed, if the rotational
59 dynamics of the complex are approximated to a simple sphere, τ_R can be estimated from the
60 Debye Stokes equation:

$$61 \tau_R = \frac{4\pi\eta r^3}{3k_B T} \quad (1)$$

62 where r is the radius of the sphere, η is the viscosity of the medium, k_B is Boltzmann's
63 constant and T is the temperature. Molar mass alone does not mean that τ_R will increase in an
64 optimal way. Local segmental flexibility can allow the Gd complex moiety to rotate rapidly
65 while the overall motion is much slower.

66 The biological fate of GBCA in the body is also a crucial scientific topic. GBCAs
67 diffuse quickly after rapid intravascular injection (bolus) distributed in the different
68 anatomical territories. They are then eliminated mainly by the renal route in unchanged form
69 by glomerular filtration. Their plasma half-life is of the order of 1 to 2 hours (Le Mignon et
70 al., 1990; Staks et al., 1994; Hao et al., 2019). Nevertheless, traces of gadolinium can be
71 detected at later times in deep or elimination organs. For example, it has been shown that
72 some patients who have received repeated doses of GBCA show cerebral hypersignals in the
73 dentate nucleus 1 month after injection (Kanda et al., 2016). More recently, accumulation of
74 GBCA in the kidney, not detectable by T_1 MRI, was described (Le Fur et al., 2023). Different
75 physicochemical parameters can play a role in the fate and elimination of GBCA in the body.
76 The chemical stability of the GBCA, its diffusivity which is related to its hydrodynamic size,
77 and the viscosity of the formulation can influence the diffusion of the product within the
78 various anatomical territories (Jacquier et al., 2008)

79 From this introductive overview, it clearly appears that the ability to accurately
80 measure the size (and diffusion coefficient) of GBCA can contribute to understand their *in*
81 *vivo* behavior and paramagnetic efficiency. The usual techniques such as dynamic light
82 scattering (DLS) and electron microscopy (SEM) are suitable for polymers or nanoparticles
83 analysis (Xu, 2015) but not for molecules of low molar mass such as GBCA. In this context,
84 Taylor dispersion analysis (TDA) seems promising as an alternative sizing technique. Indeed,
85 TDA, which is based on the measurement of peak broadening of a solute plug in a laminar
86 Poiseuille flow (Taylor, 1954; Aris, 1956), enables the calculation of the molecular diffusion

87 coefficient (D) across a large range of hydrodynamic diameter (D_h , from angstrom up to 300
88 nm) (Belongia and Baygents, 1997; Chamieh et al., 2017). TDA is performed using narrow
89 bore fused silica capillaries (typically $\sim 50 \mu\text{m}$ i.d.). It presents several advantages such as low
90 sample consumption (few nL are injected for each analysis), no sample filtration (insensitive
91 to the presence of dusts), absolute method (no size calibration), rapid analysis time and
92 automation.

93 In this work, the diffusivity and the hydrodynamic radius of various commercial or
94 under-development gadolinium complexes were measured by TDA and compared to
95 diffusion-ordered NMR spectroscopy (DOSY) and Density Functional Theory (DFT)
96 calculations.

97 **2. Materials and methods**

98 **2.1 Materials**

99 Structurally, there are 2 distinct categories of GBCA: 1. Macrocyclic molecules in
100 which the Gd^{3+} ion is caged within the cavity of the ligand, and 2. Open-chain or “linear”
101 molecules (Table S1). Within each category, there are both ionic and neutral GBCA. Gd-
102 DTPA-BMA (Gadodiamide, Omniscan[®] GE-Healthcare) and Gd-BOPTA (Gadobenate
103 dimeglumine, MultiHance[®] Bracco) were diluted to 5 mM in ultrapure water coming from a
104 Milli-Q system from Millipore (Molsheim, France). Gd-DOTA (Gadoterate meglumine,
105 Dotarem[®] Guerbet), Gd-HP-DO3A (Gadoteridol, ProHance[®] Bracco), Gd-BT-DO3A
106 (Gadobutrol, Gadovist[®] Bayer Healthcare), Pyclyen based structure derivatives (PCTA) were
107 diluted to 5 mM using 10 mM Tris-HCl buffer pH 7.4 (tris(hydroxymethyl)aminomethane,
108 Sigma-Aldrich, France).

109 PCTA derivatives were prepared using previously reported protocols for D1 and D2
110 (Gadopicolenol, Elucirem[®] Guerbet) (Port et al., 2008) and for D3 (Port et al., 2000). The

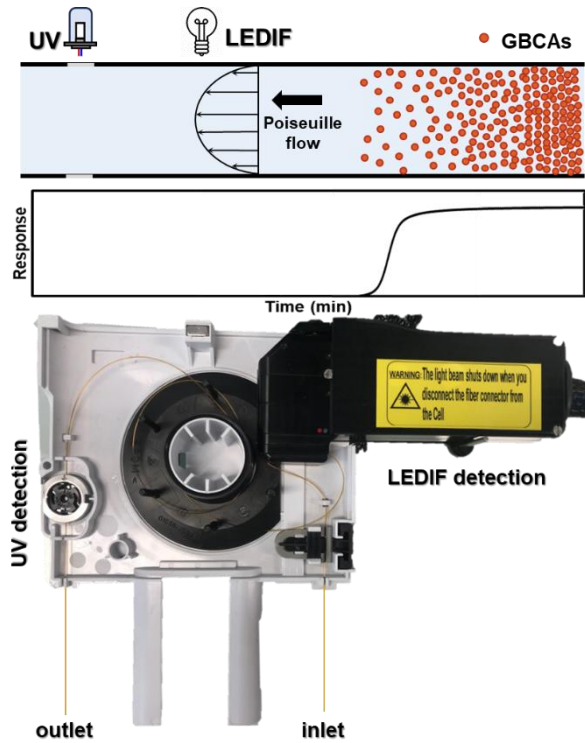
111 Europium or Yttrium analogue complexes were prepared using respectively europium or
112 yttrium chloride at the complexation step.

113 The fluorescence emission spectra were measured using a fluorescence
114 spectrofluorometer (Jasco, FP-8300, UK).

115 **2.2 Taylor dispersion analysis**

116 All experiments were performed on an Agilent 7100 CE system (Waldbronn, Germany)
117 equipped with a diode array UV-visible detector and with a Zetalif LED-induced
118 fluorescence detector (λ_{ex} 275 nm) purchased from Adelis (Toulouse, France). Analyses were
119 conducted in a bare fused-silica capillary (Polymicro technologies, USA) having a 50 μm i.d.
120 with a total length of 65 cm. The position of the LEDIF and UV detection windows were at
121 44 and 56.5 cm from the inlet side, respectively, which allows for simultaneous detection
122 from both detectors (Fig. 1).

123 Before analysis, capillaries were conditioned by flushing (at 1 bar) with water for 20
124 min followed by the background electrolyte (BGE) for 20 min. A 10 mM Tris buffer at pH
125 7.5 was used as BGE for all samples except for Gd-DTPA-BMA and Gd-BOPTA, where
126 water was used. Samples were prepared in the BGE and injected continuously at 100 mbar
127 from the inlet side of the capillary. Between each run, the capillary was rinsed by flushing
128 with the BGE for 5 min. UV detection was performed at 200 nm with a bandwidth of 4 nm.
129 The fluorescent signals were measured at an excitation wavelength of 275 nm and the signals
130 were collected from 300 to 450 nm or from 300 to 330 nm depending on the collection signal
131 filter. Analyses were performed at 25°C and the sample carousel was also kept at 25°C using
132 an external circulating water bath (Instrumat, France). Signal was acquired using
133 Chemstation software, then exported to Microsoft excel for subsequent data treatment.



134

135 **Fig. 1.** Scheme of an Agilent capillary electrophoresis cassette showing the positions of the LEDIF and UV
 136 detectors used for GBCA Taylor dispersion analysis. The upper part represents the frontal mode analysis in
 137 TDA.

138 2.3 Data treatment

139 The temporal variance σ^2 of the dispersion profile from the TDA experiment in frontal
 140 mode was obtained by fitting the elution front with a Gaussian error function (*erf*) (d'Orlyé et
 141 al., 2008; Taylor, 1953) using equation (2):

$$142 \quad y = \frac{1}{2} + \frac{1}{2} \operatorname{erf} \frac{(t - t_0)}{\sigma \sqrt{2}} \quad (2)$$

143 The sample's diffusion coefficient D and hydrodynamic radius D_h were obtained from
 144 equation (3) and equation (4) respectively.

$$145 \quad D = \frac{R_c^2 t_0}{24 \sigma^2} \quad (3)$$

$$146 \quad D_h = \frac{k_B T}{3 \pi \eta D} \quad (4)$$

147 where R_c is the capillary radius (m), t_0 is the average elution time (s), T is the temperature (K)
 148 and η is the viscosity of the carrier liquid (Pa s). The two conditions of validity of TDA

149 expressed by τ and Pe numbers and calculated using inequations (5) and (6) (Taylor, 1954;
150 Chamieh and Cottet, 2014; Cottet et al., 2014) were fulfilled:

$$151 \quad \tau = \frac{D t_0}{R_c^2} \geq 1.25 \quad (5)$$

$$152 \quad Pe = \frac{u R_c}{D} \geq 40 \quad (6)$$

153 where τ is an dimensionless characteristic time, Pe is the Peclet number and u is the linear
154 mobile phase velocity (m s^{-1}). Inequation (5) is verified when the characteristic diffusion time
155 of the solute in the capillary cross section is much lower than t_0 . Inequation (6) is valid when
156 the axial diffusion of the solute is negligible compared to Taylor dispersion.

157 In order to measure the viscosity of the carrier liquid, the capillary was filled with
158 BGE. Then, a 0.1% (v:v) DMF solution in BGE was continuously injected at 100 mbar. The
159 DMF was detected at 200 nm. The mean elution time of DMF (t_{DMF}) was measured, and the
160 relative viscosity (η) was calculated by comparing it to the elution time with pure water (t_0) at
161 the same temperature. The viscosity, being proportional to the elution time, is given by
162 equation (7) with about 3% precision (Bello et al., 1994):

$$163 \quad \eta = \frac{t_{DMF}}{t_0} \eta_0 \quad (7)$$

164 where η_0 is the viscosity of water at the same temperature. In this work, the viscosity of tris
165 buffer was found to be similar to the viscosity of water (8.92×10^{-4} Pa s).

166 **2.4 Diffusion Ordered Spectroscopy (DOSY)**

167 The method is based on a pulsed field gradient (PFG) and a stimulated spin echo (STE)
168 experiment. Analysis of the exponential decay of the signal obtained with different pulse
169 frequencies gives the diffusion coefficient D . This method can therefore be used to assess the
170 size with the Stokes-Einstein equation (equation (4)), which applies in the case of a solute

171 **assumed** to a hard sphere, with a hydrodynamic diameter D_h and evolving in a continuous
172 medium.

173 DOSY NMR spectra were recorded at 25°C on a BRUKER AVANCE III HD 500
174 spectrometer equipped with an indirect 5 mm probe head BBFO 1H/{BB}. NMR analyses
175 were performed on **samples obtained by dissolution of 10 mg of product in 700 μ L of D₂O.**
176 The spectra were performed according BRUKER's pulse programs with standard pulse
177 sequences of 1.5 s of delay, a 30° pulse and 16 scans. Viscosity of D₂O (298K): 1.1×10^{-3} Pa s
178 (Evans et al., 2013). Data processing was carried out using MestReNova software (Mestrelab
179 Research) Version 14.1.0-24037.

180 **2.5 Density Functional Theory (DFT)**

181 All calculations were performed with the Gaussian 16 program package (Frisch et al.,
182 2016), within the frame of density functional theory (DFT). We selected the hybrid, long-
183 range corrected, wB97XD density functional, which includes atom–atom dispersion
184 corrections (Chai and Head-Gordon, 2008). **We have shown recently that this functional**
185 **provides good results for Gd complexes in terms of bond distances of the metal coordination**
186 **environment and relative stability of different coordination numbers (Nucera et al., 2023).** In
187 these calculations we employed a small-core effective core potential for gadolinium (28
188 electrons in the core) (Dolg et al., 1989) and the associated ECP28MWB_GUESS basis set.
189 The Def2-TZVPP basis set was employed for all other atoms (Weigend and Ahlrichs, 2005).
190 All Gd complexes were modelled using the unrestricted method in their high-spin
191 configuration (octet). For Y complexes we used a restricted calculations with the ECP28MDF
192 (28 electrons in the core) and its associated VTZ basis set (Peterson et al., 2007). The
193 integration grid was set with the integral: superfine grid keyword. Bulk water solvent effects
194 were incorporated using a polarizable continuum model with the default options implemented
195 in Gaussian (IEFPCM) (Tomasi et al., 2005). Molecular volumes were estimated using the

196 volume = tight keyword. Input geometries for geometry optimization were obtained from
197 previous computational works (Regueiro-Figueroa and Platas-Iglesias, 2015; Esteban-Gómez
198 et al., 2012). Frequency calculations were performed to ensure that the optimized geometries
199 correspond to local energy minima.

200 **3. Results and discussion**

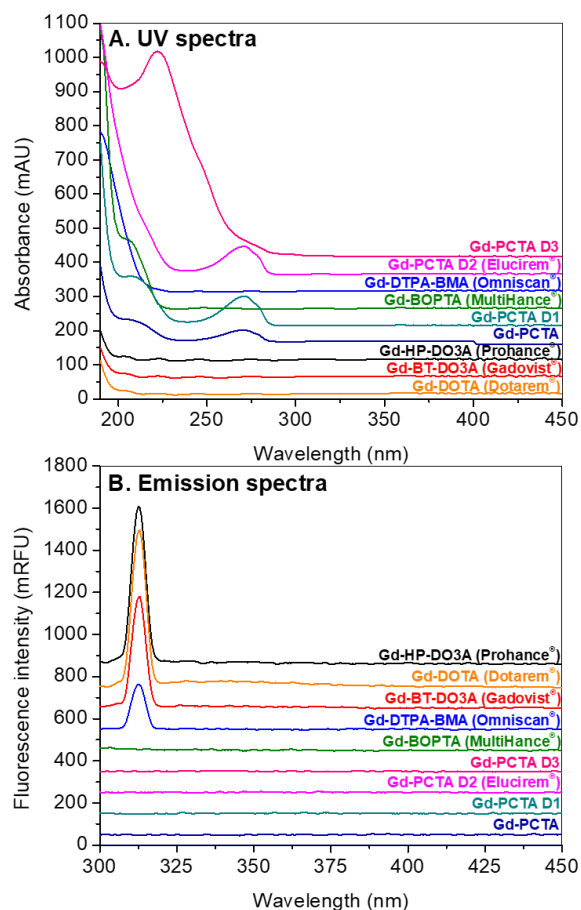
201 This work aims to determine the diffusivity and the hydrodynamic diameter of various
202 GBCA with different molar masses ranging from 500-3500 g mol⁻¹ by TDA and to compare
203 the results with DOSY-NMR and DFT calculations. For a matter of comparison with DOSY-
204 NMR, which cannot be done on GBCA directly due to their paramagnetic properties, yttrium
205 analogues were synthesized and studied by both TDA and DOSY-NMR. To evaluate the
206 fluorescence response in TDA, the europium derivatives were also synthesized and
207 investigated in comparison with GBCA. Since the UV and the fluorescence responses can be
208 very different depending on the chemical structure of GBCA, the limits of detection were
209 first determined for both detection modes, as discussed in the following section.

210 **3.1 UV and Emission spectra of Gadolinium complexes**

211 The UV and fluorescence responses of the different GBCA were investigated at the
212 same concentration of 5.0 mM. Fig. 2A shows the UV-visible spectra of the different
213 samples. As can be seen Gd-HP-DO3A, Gd-BT-DO3A and Gd-DOTA had low UV response
214 and could only be analyzed at a wavelength lower than 200 nm, where the absorption of the
215 carboxyl groups takes place (Xu et al., 2002). However, Gd-PCTA D1, Gd-PCTA D2 and
216 Gd-PCTA presented an absorption maximum at 270 nm, characteristic of the pyridyl
217 chromophore (Pellegatti et al., 2008), while Gd-PCTA D3 showed a maximum at 230 nm due
218 to aromatic cycle on each of the three pendant arms. As for Gd-DTPA-BMA and Gd-
219 BOPTA, the UV response was maximum and intense at 200 nm. For the subsequent TDA

220 analysis of the different GBCA, a common wavelength of 200 nm was used to size the
221 different samples.

222 In Fig. 2B, the emission spectra of GBCA are presented at an excitation wavelength of
223 275 nm. The results show that Gd-HP-DO3A, Gd-DOTA, Gd-BT-DO3A and Gd-DTPA-
224 BMA had a sharp emission peak at 315 nm in their decreasing order of emission intensity.
225 This emission band is characteristic of the ${}^6P_{7/2} \rightarrow {}^8S_{7/2}$ transition of Gd(III), which is
226 quenched by the presence of aromatic chromophores (Bünzli, 2010). GBCA samples
227 containing aromatic units (Gd-PCTA, Gd-PCTA D1 and Gd-PCTA D2) exhibited low
228 fluorescence emission at 300 nm, as shown in Fig. S2. Conversely, Gd-BOPTA and Gd-
229 PCTA D3 showed a non-significant fluorescence response leading to a nonlinear response in
230 the studied concentration range. Therefore, for the LEDIF responding samples, a collection
231 filter of the emitted fluorescence with a collecting range between 300 - 450 nm or between
232 300 - 330 nm were used for TDA analysis.



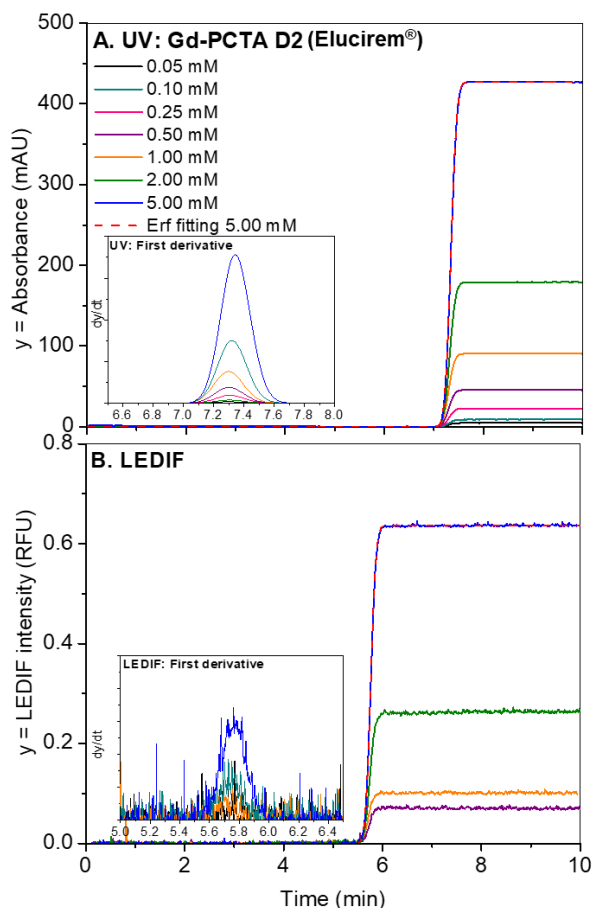
233

234 **Fig. 2.** (A) UV-Visible spectra and (B) emission spectra (excitation wavelength 275 nm) of Gadolinium
 235 complexes at a concentration of 5.0 mM. GBCA were diluted in 10 mM Tris-HCl buffer pH 7.4 except for Gd-
 236 DTPA-BMA and Gd-BOPTA diluted in ultrapure water. Sensitivity of spectrofluorometer was set at very low
 237 level. The emission spectra of Gd-PCTA, Gd-PCTA D1 and Gd-PCTA D2 at medium sensitivity level of
 238 spectrofluorometer are shown in Fig. S2.

239 3.2 Taylor dispersion analysis in frontal mode

240 Classically TDA is performed in plug mode, *i.e.* by injecting a small volume of the
 241 sample (less than 1% of the capillary volume to the detection point), and then the sample is
 242 mobilized with the BGE as a mobile phase. However, because of the dilution of the injection
 243 plug during the mobilization, and to enhance the sensitivity of detection, TDA in frontal
 244 mode was applied. Fig. 3 shows the elution profile of Gd-PCTA D2 with varying
 245 concentrations ranging from 0.05 to 5.0 mM by UV and LEDIF detections. The experimental
 246 elution profile was fitted using a Gaussian error function (*erf*) to obtain the variance and,
 247 consequently, to determine the molecular diffusion coefficient (D) and the hydrodynamic
 248 diameter (D_h). The fit is presented as a red dotted line in Fig. 3. The first derivative of the

249 elution profiles are also presented (see insert in Fig. 3), as they allow for a better visualization
 250 of the symmetry of the signal. All experimental taylorgrams of the other GBCA are presented
 251 in Fig. S3 to S10.



252 **Fig. 3.** Frontal Taylorgrams of Gd-PCTA D2 at different concentrations using (A) UV and (B) LEDIF (λ_{ex} 275
 253 nm, $\lambda_{\text{collection}}$ 300-450 nm) detections. *Erf* fitting to calculate D_h and D are plotted as a red dotted line. The insert
 254 represents the first derivative of the frontal Taylorgrams. Experimental conditions: fused silica capillary of 65 cm
 255 total length (44 cm to LEDIF detector, 56.5 cm to UV detector) x 50 μm i.d., eluent: 10 mM Tris-HCl buffer
 256 (pH 7.4), mobilization pressure 100 mbar.
 257

258 An additional advantage of the frontal mode is that the front height is directly
 259 correlated to the injected concentration. Therefore, calibration curves of GBCA were plotted
 260 and the corresponding sensitivity of detection (slope of calibration curve, a), the LOD and
 261 LOQ were determined and reported in Table 1 for both UV (at 200 and 270 nm) and LEDIF
 262 (λ_{ex} 275 nm, $\lambda_{\text{collection}}$ 300 - 450 nm and 300 - 330 nm) detections. GBCA with aromatic ring
 263 has relatively low LOD in UV (in the range of 0.01 – 0.26 mM). Comparatively, non-
 264 aromatic GBCA have higher LOD between 0.22 – 0.84 mM. LOD with LEDIF detection is in

265 interval 0.29 -1.50 mM. These results showed that the LOD of UV detection are lower than
266 the one obtained by LEDIF detection for all GBCA, except for Gd-HP-DO3A.

267 D_h , and D of GBCA were determined using eq. (3) and eq. (4) applying both detection
268 methods. Repeatabilities of TDA analysis were evaluated for each detection mode using RSD
269 ($n=6$). It was found that UV detection RSD was approximately 0.2 – 1.6%, and around 0.6 –
270 9.4% for LEDIF detection, indicating better repeatabilities when using the UV detection,
271 probably due to better sensitivity. The relative differences in the calculated D_h between the two
272 detection modes was very low (average difference of about 4%) except for Gd-DOTA (12%)
273 due to the presence of meglumine, which is a counterion of the complex. Meglumine
274 contributed to the UV response (slope at 200 nm of 1.56 AU M^{-1}) and can affect the average
275 size with this detection mode, but not with the LEDIF detector. In the case of Gd-DOTA, the
276 meglumine response at 200 nm corresponds to 59% of the overall signal (1:1
277 GBCA:meglumine molar ratio). Despite this huge contribution of the counterion to the UV
278 signal, the impact of meglumine on the Gd-DOTA size is limited since meglumine has a
279 comparable size as the GBCA. The LEDIF detector should be however preferred for TDA of
280 Gd-DOTA. As for Gd-BOPTA, the response of the meglumine is about 5% of the total signal
281 at 200 nm (1:2 GBCA : meglumine molar ratio) and therefore the impact of meglumine on
282 the size of Gd-BOPTA is minor. It is worth nothing that for all the other contrast agents, there
283 is no counter ions that can impact the measurement since they are neutral.

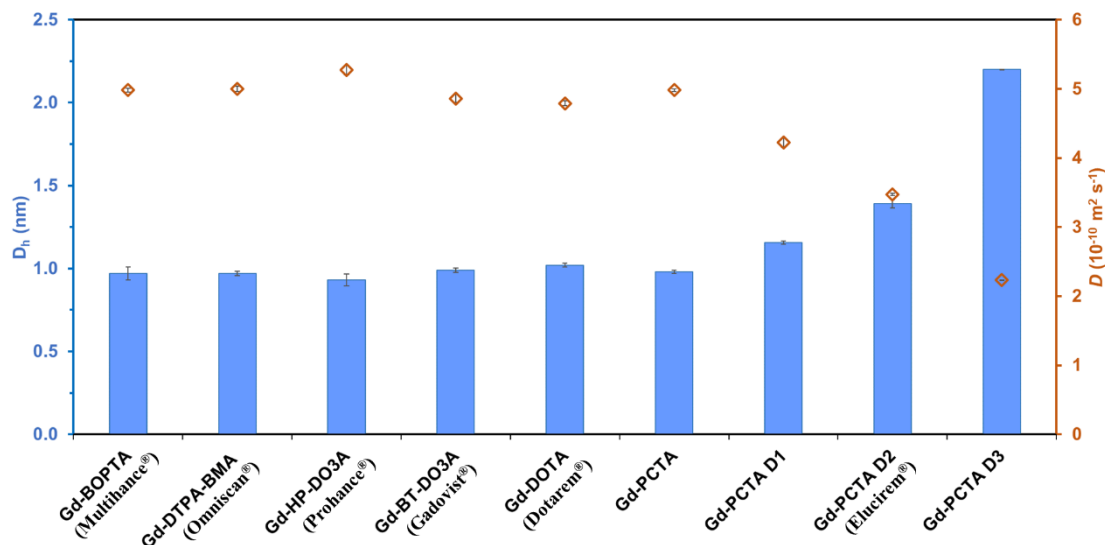
284 Fig. 4 gathers the calculated D_h and the corresponding diffusion coefficient D of all
285 GBCA based on the analysis with UV at 200 nm. The results show that the D_h were in the
286 increasing order of Gd-HP-DO3A 0.93 nm < Gd-BOPTA 0.97 nm \approx Gd-DTPA-BMA 0.97
287 nm < Gd-PCTA 0.98 nm < Gd-BT-DO3A 0.99 nm < Gd-DOTA 1.02 nm with RSD on 6
288 repetitions of about 1% or less. As for Gd-PCTA D1, Gd-PCTA D2 and Gd-PCTA D3, they
289 presented larger hydrodynamic diameters of 1.16, 1.39 and 2.20 nm, respectively. It is

290 important to remind that the diffusivity (D) of the GBCA are inversely proportional to the
291 hydrodynamic diameter (D_h) and therefore do not vary linearly one each other. Diffusion
292 coefficients of about $4.7-5.0 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ were determined for DTPA or macrocyclic cyclen
293 based GBCA, and of about $2.2-4.2 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ for PycLen based GBCA. For PCTA
294 derivatives, there is a good proportionality between the molecular weight and the
295 hydrodynamic diameter.

296 **Table 1.** Sensitivity, LOD, LOQ, D_h and D obtained by TDA using UV and LEDIF detections

Sample	M (g mol ⁻¹)	aromatic	BGE	UV detection at 200 nm and 270 nm					LEDIF λ_{ex} 275 nm $\lambda_{collection}$ 300-450 nm and $\lambda_{collection}$ 300-330 nm				
				Slope (AU M ⁻¹)	LOD ^e (mM)	LOQ ^e (mM)	D_h (RSD) (nm)	D (10 ⁻¹⁰ m ² s ⁻¹)	Slope (RFU mM ⁻¹)	LOD ^e (mM)	LOQ ^e (mM)	D_h (RSD) (nm)	D (10 ⁻¹⁰ m ² s ⁻¹)
Gd-DTPA-BMA (Omniscan [®])	573.6	no	Water	57.7 ^a	0.10 ^a	0.32 ^a	0.97 (0.8%) ^a	5.00 ^a	0.058 ^c	0.71 ^c	2.36 ^c	0.97 (9.4%) ^c	5.01 ^c
				n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	0.033 ^d	1.72 ^d	5.72 ^d	0.97 (4.9%) ^d	5.00 ^d
Gd-BOPTA (MultiHance [®])	653.7	yes	Water	51.2 ^a	0.18 ^a	0.61 ^a	0.97 (0.6%) ^{a, f}	4.98 ^{a, f}	n.d. ^c	n.d. ^c	n.d. ^c	n.d. ^c	n.d. ^c
				n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d
Gd-HP-DO3A (Prohance [®])	558.7	no	Tris-HCl	1.96 ^a	0.84 ^a	2.81 ^a	0.93 (1.2%) ^a	5.27 ^a	0.171 ^c	0.29 ^c	0.97 ^c	0.89 (1.3%) ^c	5.46 ^c
				n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	0.075 ^d	0.76 ^d	2.52 ^d	0.99 (2.1%) ^d	4.89 ^d
Gd-BT-DO3A (Gadovist [®])	604.72	no	Tris-HCl	2.65 ^a	0.70 ^a	2.35 ^a	0.99 (1.2%) ^a	4.86 ^a	0.102 ^c	1.50 ^c	5.00 ^c	0.95 (0.4%) ^c	5.03 ^c
				n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	0.053 ^d	0.39 ^d	1.28 ^d	1.01 (1.0%) ^d	4.80 ^d
Gd-DOTA (Dotarem [®])	558.7	no	Tris-HCl	2.63 ^a	0.22 ^a	0.72 ^a	1.02 (0.7%) ^{a, f}	4.79 ^{a, f}	0.202 ^c	0.56 ^c	1.87 ^c	0.90 (0.6%) ^c	5.46 ^c
				n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	0.094 ^d	1.06 ^d	3.52 ^d	0.98 (1.2%) ^d	4.98 ^d
Gd-PCTA	534.6	yes	Tris-HCl	26.2 ^a	0.45 ^a	1.50 ^a	0.98 (0.5%) ^a	4.98 ^a	0.150 ^c	0.86 ^c	2.88 ^c	1.05 (4.1%) ^c	4.67 ^c
				15.2 ^b	0.15 ^b	0.49 ^b	0.97 (1.2%) ^b	5.02 ^b	0.111 ^d	0.52 ^d	1.74 ^d	1.13 (3.7%) ^d	4.33 ^d
Gd-PCTA D1	750.6	yes	Tris-HCl	34.5 ^a	0.26 ^a	0.86 ^a	1.16 (0.9%) ^a	4.22 ^a	0.118 ^c	0.35 ^c	1.16 ^c	1.16 (0.7%) ^c	4.21 ^c
				17.7 ^b	0.22 ^b	0.75 ^b	1.11 (0.2%) ^b	4.43 ^b	0.055 ^d	0.50 ^d	1.67 ^d	1.19 (0.9%) ^d	4.08 ^d
Gd-PCTA D2 (Elucirem [®])	970.1	yes	Tris-HCl	85.6 ^a	0.12 ^a	0.40 ^a	1.39 (0.4%) ^a	3.47 ^a	0.129 ^c	0.43 ^c	1.38 ^c	1.47 (0.8%) ^c	3.32 ^c
				16.3 ^b	0.06 ^b	0.20 ^b	1.43 (0.3%) ^b	3.43 ^b	0.063 ^d	0.88 ^d	2.94 ^d	1.47 (0.8%) ^d	3.33 ^d
Gd-PCTA D3	3545	yes	Tris-HCl	1355 ^a	0.01 ^a	0.038 ^a	2.20 (0.2%) ^a	2.23 ^a	n.d. ^c	n.d. ^c	n.d. ^c	n.d. ^c	n.d. ^c
				141 ^b	0.001 ^b	0.004 ^b	2.34 (1.6%) ^b	2.09 ^b	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d

297 ^a UV at 200 nm, ^b UV at 270 nm, ^c LEDIF using $\lambda_{collection}$ 300-450 nm, ^d LEDIF using $\lambda_{collection}$ 300-330 nm. ^e LOD, LOQ were calculated by $3 \times s_{y/x}/a$ and
 298 $10 \times s_{y/x}/a$, respectively, where $s_{y/x}$ is the regression standard deviation and a is the slope of the calibration curve. **value affected by the presence of meglumine**
 299 **(counterion).**
 300 Calculated D_h from 50 μ m capillary (**d_c was optically measured**) using viscosity $\eta = 8.92 \times 10^{-4}$ Pa s at 25°C. GBCA concentration at 5.0 mM. Gd-DTPA-BMA
 301 and Gd-BOPTA diluted in ultrapure water. The other GBCA diluted in 10 mM Tris-HCl buffer pH 7.4.
 302 n.d. = not detected



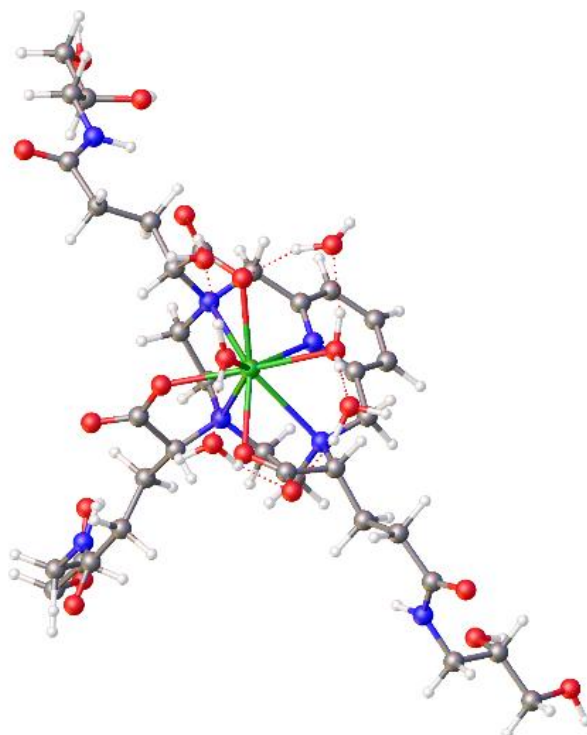
303
 304 **Fig. 4.** Hydrodynamic diameter D_h (bar graph) and diffusivity D (diamond shape) of GBCA by TDA using UV
 305 detection at 200 nm. Experimental condition as in Fig. 3 and Table 1 ($\eta = 8.92 \times 10^{-4} \text{ Pa s}$ at 25°C).

306 3.3 Diffusion Ordered Spectroscopy (DOSY)

307 Gadolinium complexes (Gd III) are highly paramagnetic due to the presence of 7
 308 electrons on the 4f level ([Xe] 4f⁷). These magnetic properties interfere with NMR
 309 spectroscopy experiments. In order to overcome this difficulty, the study of isosteric
 310 complexes was proposed (Le Fur et al., 2020). The use of diamagnetic yttrium (Y(III))
 311 complexes is one way of obtaining spectral information since these compounds have a high
 312 degree of structural similarity to Gd(III) complexes. Five yttrium complexes were prepared
 313 and DOSY experiments were carried out to evaluate their diffusion coefficients. Under our
 314 experimental conditions, the two reference complexes Y-DOTA and Y-PCTA exhibit the
 315 same D value ($3.6 \times 10^{-10} \text{ m}^2 \text{ s}$). Similarly, for the Y-PCTA-D1 complex the measured
 316 experimental value is $D = 2.9 \times 10^{-10} \text{ m}^2 \text{ s}$ whereas the Y-PCTA-D2 complex shows a value of
 317 $2.3 \times 10^{-10} \text{ m}^2 \text{ s}$. These values cannot be compared directly to D values obtained by TDA due
 318 to the difference in viscosity of the medium (D_2O vs H_2O).

319 However, by applying the Stokes-Einstein relationship (viscosity of D_2O at 298K of
 320 $1.1 \times 10^{-3} \text{ Pa s}$ (Evans et al., 2013)), the hydrodynamic diameter of the different molecules was
 321 evaluated and compared to the ones obtained by Taylor diffusion (see Table 2). Good

322 correlation between the different experimental techniques was found, confirming that yttrium
323 can be useful when the use of gadolinium is not possible.



324

325 **Fig. 5.** Molecular structure of the Gd-PCTA D2 obtained with DFT calculations.

326 **3.4 Density Functional Theory (DFT)**

327 The TDA sizes of the GBCA displayed in Fig. 4 were also obtained using DFT
328 calculations. For this purpose, the geometries of the different complexes were optimized
329 using well-established methods (details in the computational section 2.5). It is worth
330 mentioning that our models incorporated a few explicit second-sphere water molecules
331 involved in hydrogen bonding with coordinated water molecules, as we demonstrated
332 previously that this was important to describe the Gd-O_{water} distances (Esteban-Gómez et al.,
333 2012). The representative structure of the Gd-PCTA D2 complex is shown in Fig. 5. The
334 molecular volumes were subsequently estimated as the density envelope defined by an 0.001
335 e bohr⁻³ isodensity surface, as recommended by Bader (Bader et al., 1987). The diameter of
336 the complex was then estimated from the molecular volume as the diameter on a sphere with

337 that volume. These calculations give D_h values close to 1 nm for all small complexes: Gd-
 338 HP-DO3A (1.02 nm), Gd-BOPTA (1.08 nm), Gd-DTPA-BMA (1.04 nm), Gd-BT-DO3A
 339 (1.01 nm), Gd-DOTA (1.01 nm) and Gd-PCTA (0.98 nm). These values are in very good
 340 agreement with the experimental data, with deviations $< 10\%$, which confirms that these
 341 small complexes are not aggregating under the conditions of formulation (also used for TDA
 342 studies). It is clear from Table 2 that, as expected, similar D_h and D values were obtained for
 343 all lanthanides (Gd, Eu and Y) with the same ligand. The corresponding diffusion coefficients
 344 estimated from the Stokes-Einstein are also in good agreement with the experimental values,
 345 as would be expected. We note that some deviations are expected from the experimental
 346 (TDA) and theoretical data (DFT). In particular, the different charge and electrostatic
 347 properties of the complexes is expected to affect the size of the second hydration shell, and
 348 thus their hydrodynamic size provided by TDA and DOSY measurements. However, the
 349 sizes obtained with DFT are estimated from the electron density.

350 **Table 2.** Comparing D_h and D by DOSY, DFT and TDA

		PCTA		PCTA D1			PCTA D2		
		Gd	Y	Gd	Eu	Y	Gd	Eu	Y
DOSY in D ₂ O	D_h (nm) ^a	NT	1.1	NT	NT	1.4	NT	NT	1.7
	D at 298K ($10^{-10} \text{ m}^2 \text{ s}^{-1}$)	NT	3.6	NT	NT	2.9	NT	NT	2.3
DFT	D_h (nm)	1.03	1.03	1.17	NT	1.14	1.29	NT	1.27
	D at 298K ($10^{-10} \text{ m}^2 \text{ s}^{-1}$)	4.74	4.73	4.19	NT	4.28	3.78	NT	3.85
TDA in Tris- HCl	D_h (nm) ^b	0.98 ± 0.01	1.05 ± 0.05	1.16 ± 0.01	1.29 ± 0.01	1.32 ± 0.07	1.39 ± 0.03	1.39 ± 0.04	1.46 ± 0.02
	D at 298K ($10^{-10} \text{ m}^2 \text{ s}^{-1}$)	4.98 ± 0.02	4.76 ± 0.12	4.22 ± 0.07	3.80 ± 0.02	3.71 ± 0.20	3.47 ± 0.01	3.50 ± 0.12	3.36 ± 0.03

351 NT: Not tested

352 D_h : hydrodynamic diameter ; D : diffusion coefficient. All samples are 5 mM in Tris-HCl buffer for TDA.

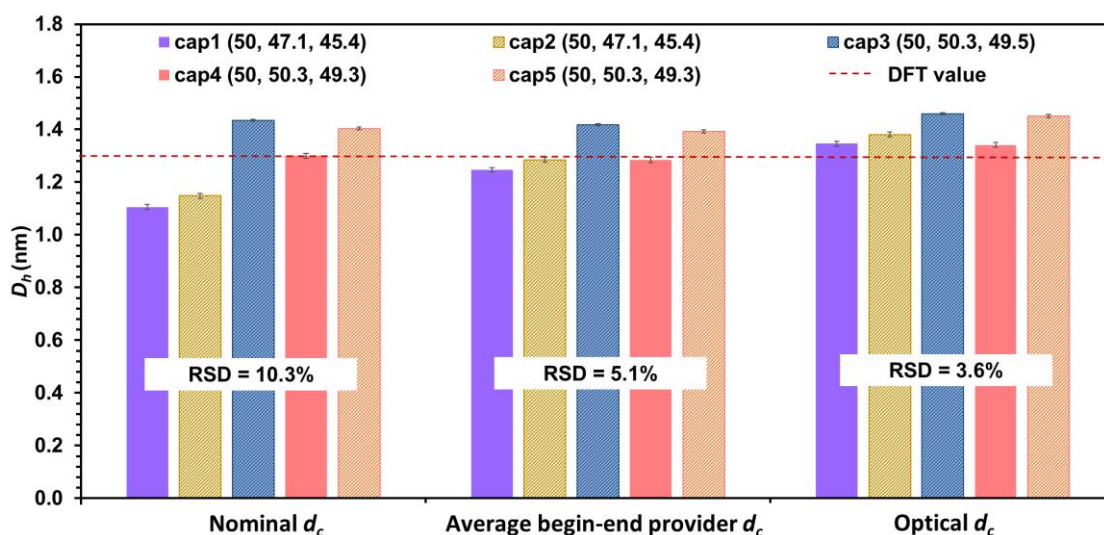
353 ^a Viscosity of D₂O (298K): $1.1232 \times 10^{-3} \text{ Pa s}$ ^b Viscosity of Tris-HCl buffer (298K): $8.92 \times 10^{-4} \text{ Pa s}$, d_c was
 354 optically measured.

355 Table 2 shows a comparison of calculated D_h and D between TDA and the other
356 methods (DOSY and DFT). The NMR DOSY method cannot measure the diffusivity directly
357 on gadolinium complexes due to their paramagnetic properties, which prevent observation of
358 ^1H signals of the ligand nuclei. Conversely, TDA works for all the complexes **whatever the**
359 **ligand and the metal (Gd, Eu and Y)**. The size obtained by TDA showed a good agreement
360 with DOSY on the yttrium diamagnetic analogues (Y-PCTA and Y-PCTA D1). The
361 deviations in diffusion coefficient resulted from the different viscosities used in the
362 calculation since NMR was conducted in deuterated water (more viscous than water). The
363 diameters of Y-PCTA, Gd-PCTA D1 and Gd-PCTA D2 estimated by DFT calculations are as
364 well comparable to the diameters determined by TDA, differing by less than 2% for Y-PCTA
365 and Gd-PCTA D1, and by 7% for Gd-PCTA D2. **We note that TDA and DOSY provide D_h**
366 **data with a significant deviation. This is likely related to the different concentrations of**
367 **GBCA used (5 mM for TDA, 15 mM for DOSY). In case some concentration-dependent**
368 **aggregation occurs, this should have a more important effect in the size measurement**
369 **performed with DOSY.**

370 **3.5 Precision of TDA measurement**

371 According to equation (3), the diffusion coefficient is directly proportional to the square
372 of the capillary diameter (d_c^2). Therefore, imprecision on the knowledge of the capillary
373 diameter might cause significant errors in D and D_h calculations and may decrease the inter-
374 capillary batch-to-batch intermediate precision of the determination. In this study, we
375 compared D_h of 5.0 mM Gd-PCTA D2 measured on 5 different capillaries for which d_c was
376 evaluated by 3 different approaches. The first approach consisted in using the nominal d_c (50
377 μm) given by the manufacturer. The second approach used the average capillary diameter
378 values given by the manufacturer for the beginning and the end of the capillary roll (namely,
379 47.1 μm for capillary 1 and capillary 2, and 50.3 μm for capillaries 3, 4 and 5). The third

380 method was based on the experimental determination of the capillary diameter using a high-
 381 resolution optical setup in our laboratory. Fig. 6 shows the GBCA D_h allowing to estimate the
 382 inter-capillary batch-to-batch intermediate precision obtained by TDA for the 5 capillaries
 383 and the 3 different approaches for d_c evaluation. The results demonstrated that using the
 384 precise diameter of capillary by optical camera provided the best inter-capillary intermediate
 385 precision for TDA sizing (RSD = 3.6%, $n=5$ capillaries). However, relatively small
 386 differences in D_h between the capillaries are remaining probably due to some temperature
 387 variations during the experiment that can affect the viscosity and thus the diffusivity.



388 **Fig. 6:** Inter-capillary intermediate precision of TDA using 5 different capillaries as indicated on the graph
 389 (nominal d_c , average d_c between the begin and end values given by the capillary provider, optical d_c determined
 390 in our laboratory, all in μm) for sizing 5.0 mM Gd-PCTA D2. D_h in nm is provided using eq.(4) and the
 391 corresponding d_c value. RSD were determined based on the 5 capillaries. Errors bars on D_h were determined for
 392 each capillary based on $n=6$ repetitions. Experimental conditions as in Fig. 3.

394 4. Conclusion

395 The aim of this work was to determine the diffusivity and the hydrodynamic diameter of
 396 GBCA by TDA and to compare these experimental values with DOSY NMR and DFT
 397 calculations. TDA was performed using both UV and LEDIF detections, using frontal mode
 398 (continuous injection of the GBCA sample into the capillary) to increase the sensitivity of
 399 detection. This study demonstrates that both detectors provide similar D and D_h values.

400 However, UV detection shows a better sensitivity and lower LOD than fluorescence
401 detection. The hydrodynamic diameters were in the order of Gd-HP-DO3A 0.93 nm < Gd-
402 BOPTA 0.97 nm \approx Gd-DTPA-BMA 0.97 nm < Gd-PCTA 0.98 nm < Gd-BT-DO3A 0.99 nm
403 < Gd-DOTA 1.02 nm < Gd-PCTA D1 1.16 nm < Gd-PCTA D2 1.39 nm < Gd-PCTA D3
404 2.20 nm. The TDA approach confirms that all the tested GBCA have high diffusivity in the
405 order of $4.7\text{-}5.0 \times 10^{-10} \text{ m}^2\text{s}^{-1}$, as confirmed by an orthogonal analytic approach such as DOSY. These
406 complexes do not aggregate in solution under the conditions used for TDA experiments, as
407 demonstrated by sizes estimated with DFT calculations. The TDA has several advantages
408 over NMR DOSY, as it can be directly applied to gadolinium complexes.

410 The pharmacokinetics and biodistribution of GBCA depends on several parameters, size
411 being among one of them. However, other properties such as stability, polarity or electrical
412 charge also play a critical role (Aime et al., 2009; He et al., 2024). Small extracellular are
413 generally eliminated via the kidney by passive glomerular filtration (He et al., 2024). The
414 largest derivative investigated here, Gd-PCTA D3, displays a different pharmacokinetic
415 profile, as it remains in the vascular compartment for a rather long time in animal models
416 (Jacquier et al., 2008). Furthermore, it is well documented that they the relaxivity response of
417 GBCA can vary very significantly with complex size, making the methodology reported here
418 very useful for the characterization of GBCA candidates.

419 **Credit authorship contribution statement**

420 Chutintorn Somnin : Investigation, Writing – original draft. Laurent Leclercq :
421 Conceptualization, Supervision, Writing – review & editing. Joseph Chamieh :
422 Conceptualization, Supervision, Writing – review & editing. Mael Le Menedeu :
423 Investigation. Christelle Medina : Conceptualization, Supervision, Writing – review &
424 editing. Olivier Rousseaux : Conceptualization, Supervision, Writing – review & editing.

425 Raphael Tripier : Supervision. Carlos Platas Iglesias : Investigation, Writing – original draft,
426 Writing – review & editing. Hervé Cottet : Conceptualization, Supervision, Writing – review
427 & editing.

428 **Declaration of competing interest**

429 The authors declare that they have no known competing financial interests or personal
430 relationships that could have appeared to influence the work reported in this paper.

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433 agreement with the University of Montpellier and the CNRS.

434 **Appendix A. Supplementary data**

435 **Tables S1** presents the structure of the GBCA and PCTA ligands.

436 **Fig. S1** displays TDA setup with UV and LEDIF detectors

437 **Fig. S2.** Emission spectra (excitation wavelength 275 nm) of Gd-PCTA, Gd-PCTA D1 and
438 Gd-PCTA D2 at a concentration of 5.0 mM.

439 **Fig. S3 to S10** present the Taylorgrams obtained for Gd-DTPA-BMA, Gd-BOPTA, Gd-HP-
440 DO3A, Gd-BT-DO3A, Gd-DOTA, Gd-PCTA, Gd-PCTA D1, Gd-PCTA D2 and Gd-PCTA
441 D3, respectively.

442 **Fig. S11** shows linear regression lines obtained for GBCA calibration curves in UV 200 nm
443 and LEDIF (λ_{ex} 275 nm, $\lambda_{\text{collection}}$ 300-450 nm).

444 **Tables S2-S12** show optimized geometries obtained with DFT.

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