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Water-Soluble Fe(II) Complexes for Theranostic Application: Synthesis, Photoacoustic Imaging, and Photothermal Conversion

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1 **Water-soluble Fe(II) complexes for theranostic application:**
2 **Synthesis, photoacoustic imaging and photothermal conversion**

3
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16
17 **Abstract:**

18 Significant effort focused on developing photoactivatable theranostics for localized image
19 guided therapy of cancer by thermal ablation. In this context iron complexes were recently
20 identified as photoactivatable theranostic agents with adequate biocompatibility and body
21 clearance. Herein, a series of Fe(II) complexes bearing polypyridine or N-heterocyclic
22 carbenes is reported that rely on rational complex engineering to red-shift their MLCT
23 based excited-state deactivation *via* a straightforward approach. The non-radiative decay
24 of their MLCT upon irradiation is exploited for theranostic purpose by combining both
25 tracking in photoacoustic imaging (PA) and photothermal therapy (PTT). The influence of
26 structural modifications introduced herein on the complexes' solubility and stability in
27 biorelevant aqueous media is discussed. The relationship between complexes' design,
28 production of contrast in photoacoustic and photothermal efficiency are explored to
29 develop tailored PA/PTT theranostic agents.

30
31 **Introduction**

32 Therapeutic agents that are trackable by medical imaging, commonly referred to as
33 theranostics, are a promising class of drug candidates for the treatment of resistant
34 cancers. It provides critical information on drug's real-time pharmacokinetics, particularly
35 *in situ* tracking of the accumulation and elimination route. This theranostic approach
36 notably highlighted the ability of some drug candidates to accumulate in tumors *in vivo*,¹
37 the *in vivo* stability of metal-ligand bond,² or the intracellular localization of the complexes.³
38 A promising avenue for theranostic imaging is photoacoustic imaging (PA), a non-invasive
39 imaging modality with hundred micrometers resolution, several centimeters imaging depth
40 and low cost.⁴ PA relies on the 'light-in sound-out principle', *i.e.* photoactivation of an agent
41 capable to convert absorbed light into heat for thermal expansion of its surroundings and
42 acoustic waves production. This ability of photothermal agents to convert light into heat is
43 also exploited in photothermal therapy (PTT) for a cost-efficient and precise treatment of
44 the diseased tissues.⁵ The selectivity toward cancer cells provided by drug photoactivation
45 is of foremost importance to limit off-target side effects and reduce drugs' therapeutic

46 dose.⁶ Additionally, therapy *via* heat production can overcome cancer resistance by
47 evading cells' repair system, although a maximum temperature threshold of 43°C was
48 fixed beyond which thermal damages increase together with temperature.⁷ Typical PA
49 and PTT setups favor near-infrared (NIR) light sources to limit thermal injuries by using
50 less energetic wavelengths, and for reduced light attenuation by the tissues and
51 endogenous molecules, particularly hemoglobin and deoxyhemoglobin.⁸ As theranostics
52 based on PA/PTT combination are receiving considerable interest, the development of
53 water soluble theranostics that are accessible in a straightforward manner and can be
54 easily finetuned to achieve NIR photoactivation is a hot topic.⁹

55 Theranostics are typically metal-based nanoparticles or organic semi-conducting particles
56 that often involve systems with a complex design.¹⁰ Therefore, finetuning their structure
57 to achieve optimized optical and physico-chemical properties can be costly and
58 demanding. On the other hand, polypyridine iron complexes are promising dual PA/PTT
59 theranostic candidates owing to their intense absorption and straightforward structural
60 modulation. The photophysics of octahedral Fe(II) complexes with a low spin 3d⁶
61 configuration are well-established,¹¹ such complexes typically displaying both highest
62 occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) that
63 are metal-centered, *i.e.* t_{2g} and e_g* respectively. Hence, upon light absorption, since d-d
64 transitions are forbidden, instead a metal-to-ligand charge transfer (MLCT) transition
65 occurs in such complexes. It rapidly deactivates through low-lying metal centered states
66 in a non-radiative manner and no competitive fluorescence or singlet oxygen production
67 occurs. Recently, exploiting the MLCT transition of iron-bis(terpyridine) complexes proved
68 of interest for PA.¹² While it highlighted the inherent benefits of this family for PA imaging,
69 expanding the scope of such complexes is required to access adequate optical and
70 physico-chemical properties for theranostic applications.

71 Herein, we propose a simple and straightforward route toward water-soluble and highly
72 stable Fe(II) complexes. The rational design of Fe(II) complexes bearing moderate to
73 strong field ligands, *i.e.* terpyridine (tpy) and 4-pyridin-2,6-bis N-heterocyclic carbene-
74 pyridine (NHC), referred to as (C[^]N[^]C), is reported herein to access photostable
75 complexes capable of light-triggered heat production due to a very short lived MLCT
76 transition.¹¹ All the complexes proved efficient for contrast production in PA and
77 photothermal conversion in PTT. Furthermore, a straightforward structural variation of the
78 complexes by quaternization into pyridinium successfully prompted a red-shift of their
79 MLCT, hence allowing enhanced PA and PTT efficiencies at irradiation wavelengths close
80 to the near-infrared region (NIR). Finally, we highlight herein the potential for PA/PTT
81 theranostics of the Fe(II)-(C[^]N[^]C) derivatives where the inert Fe-NHC interaction results
82 in high stability of the complex towards both photobleaching and biological medium.
83 Furthermore, this (C[^]N[^]C)-based complex is found to display a dramatic bathochromic
84 shift of over 80 nm of its MLCT upon methylation, hence making them promising for further
85 consideration as theranostics that might find application in the image guided therapy of
86 resistant cancers.

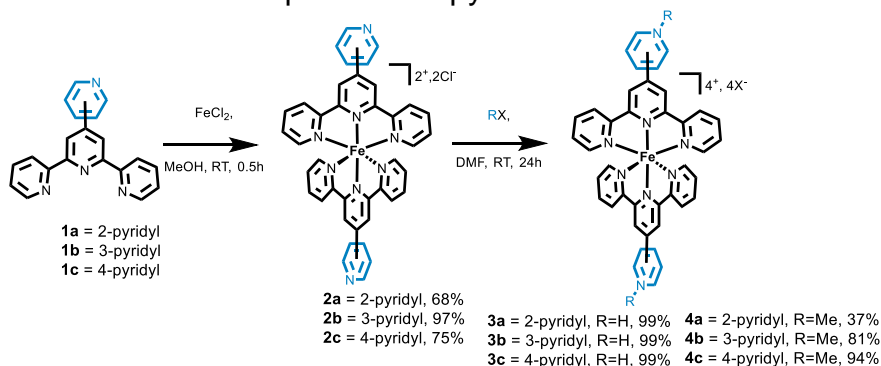
87

88 [Results and Discussion](#)

89 [Synthesis and Characterization](#)

90 Pyridine-functionalized terpyridine ligands were selected owing to their protonatable
91 nature that is anticipated to promote water-solubility, and considering their straightforward

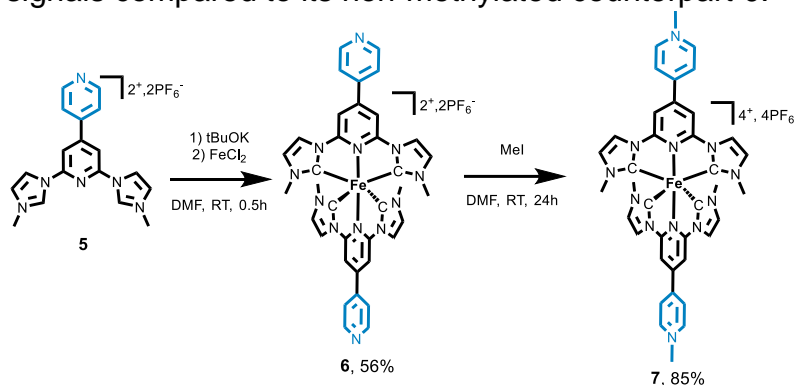
92 synthesis by Krönke reaction ensuring isolation of the ligand in sizeable quantities.
 93 Pyridines possess high electron withdrawing character that is anticipated to affect the
 94 ligands' field, and exploring the influence of their orientation, *i.e.* ortho or meta or para
 95 oriented pyridines on the optical properties of the corresponding Fe(II) complexes is
 96 proposed. The three terpyridines **1a-c** were synthesized in high yields through a modified
 97 Krönke reaction proceeding at room temperature, hence limiting the formation of side-
 98 products and making it eventually more scalable.¹³ Characterizations were in accordance
 99 with previous reports.¹⁴ Further coordination to the iron chloride precursor in a 1:2
 100 metal:ligand ratio according to reported procedure¹⁵ and isolation by precipitation in
 101 diethyl ether afforded the corresponding homoleptic complexes **2a-c** as their water soluble
 102 chloride salts, chloride ion being non-toxic (Scheme 1). Successful coordination was
 103 confirmed by ¹H NMR with a significant downfield shift up to 9.5ppm of the singlet signal
 104 corresponding to the 3' and 5' protons of the tpy. Complexes' protonation to tetra-charged
 105 complexes **3a-c**, which is expected to boost their physico- and photo-activity, was
 106 achieved quantitatively using aqueous HCl. Furthermore, bis-methylation of complexes
 107 **2a-c** to the corresponding methyl-pyridinium complexes **4a-c** is anticipated to achieve
 108 enhanced electron withdrawing effect and stability, and proceeded at room temperature
 109 at a reaction rate controlled by nitrogen's electronic density, *i.e.* a decreased reaction rate
 110 was observed from **4c**>**4b**>**4a**. This is surprising as a moderate torsion angle between the
 111 pyridine and tpy ligand in the complex **2a** was observed in solid state by X-ray
 112 diffraction.^{14a} Of note, a protocol of late-stage pyridine functionalization was preferred to
 113 the previously reported synthesis¹⁶ as it offers greater versatility in view of further adapting
 114 this approach to the functionalization with expensive moieties such as fluorophores,
 115 vectors and so forth. A general downfield shift of the protons' signals was observed in ¹H
 116 NMR upon methylation to **4a-c**, which is coherent with the pyridinium's enhanced electron
 117 withdrawing character over non-quaternized pyridine.



Scheme 1. Synthesis of the Fe(II)-terpyridine complexes studied in this work

As the metal-ligand bonds' stability in biological media is a key parameter for further development of theranostics, we propose herein an alternative to well-known tpy ligands with the use of pyridin-2,6-bis N-heterocyclic carbene-pyridine (C^N^C) ligands, that incorporate N-heterocyclic carbenes (NHCs) that are notorious for forming stable complexes (Scheme 2).¹⁷ Hence, the ligand precursor **5** was obtained in 2 steps by Suzuki coupling of pyridin-4-yl boronic acid with 4-iodo-2,6-dichloropyridine, followed by reaction with methylimidazole through a different protocol than reported elsewhere.^{11e} This imidazolium salt was further deprotonated and coordinated *in situ* to the iron precursor to yield the corresponding [Fe(C^N^C)₂]₂Cl complex **6**. The complete disappearance of the

130 singlet corresponding to the NCHN proton in the ^1H NMR spectrum of **6** attests the
 131 successful imidazolium salts' deprotonation and carbenes' coordination to the metal. The
 132 desired quaternized complex **7** is obtained *via* a similar approach to its terpyridine
 133 counterparts **4a-c**, *i.e.* in the presence of iodomethane, and shows overall downfield
 134 shifted ^1H NMR signals compared to its non-methylated counterpart **6**.



135
 136 Scheme 2. Synthesis of the Fe(II)-NHC derivative **7**

137
 138 Photophysical and physico-chemical properties in water and bio-relevant
 139 medium

140 The photophysical properties of all the complexes were investigated by absorbance
 141 spectroscopy (Table S1). As anticipated, the dicationic complexes **2a-c** showed an
 142 intense band around 575 nm, corresponding to a MLCT type transition, and the
 143 corresponding epsilon values in water are comparable to those reported in acetonitrile
 144 (Fig. 1A).^{15a} The pyridyl substitution pattern, *i.e.* ortho, meta, para, has a negligible
 145 influence on the MLCT absorption shift of complexes **2a-c**. For complex **6**, two MLCT
 146 absorptions are observed with $\lambda_{\text{N-Fe}}=510\text{nm}$ and $\lambda_{\text{C-Fe}}=398\text{nm}$ (corresponding to the Fe-
 147 pyridine or Fe-NHC transitions respectively), which are significantly blue shifted compared
 148 to its terpyridine counterpart **2c** with $\lambda_{\text{N-Fe}}=575\text{ nm}$. This can be explained by the greater
 149 ligand field and σ -donation from the NHC to the metal compared to pyridines, and the
 150 larger bite-angle of the (C^N^C) ligand than terpyridine which allows enhanced orbital
 151 overlapping.¹⁸ Protonation of the complex **2c** at pH3 is found to promote a significant
 152 bathochromic shift of the MLCT from $\lambda_{2c}=575\text{nm}$ to $\lambda_{3c}=590\text{nm}$ which is coherent with
 153 previous reports on comparable complexes,¹⁹ while the other pyridinium complexes with
 154 different pyridine orientations show a negligible MLCT shift (Table S1). A comparable
 155 effect is observed upon protonation of complex **6** at pH 3.2 with a shift of 59 nm of the
 156 pyridine-Fe MLCT transition. A more pronounced effect on the absorbance $\lambda_{\text{N-Fe}}$ is
 157 observed upon methylation of both the terpyridine and the (C^N^C) complexes. Indeed,
 158 the MLCT absorption in **4c** was red-shifted by 22 nm up to 595nm. Furthermore, while the
 159 $\lambda_{\text{N-Fe}}$ in **6** is found at 510nm which is inadequate for biological use, its quaternization to **7**
 160 promotes a significant bathochromic shift up to 595nm. Such red-shift of 85nm is markedly
 161 greater than the 22 nm shift observed in complex **2c**, hence emphasizing the potential of
 162 the (C^N^C)-based family for further optimization of their optical properties towards the
 163 NIR. Overall, a red-shift of the MLCT absorption band is observed in the trend **2c**<**3c**<**4c**
 164 and **6**<**7** coherent with the increasing electron-withdrawing properties of the quaternized
 165 pyridine.

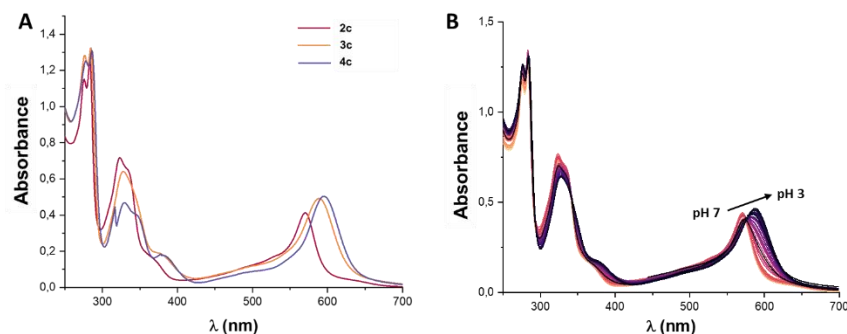


Fig. 1. A) Absorption spectra of the complexes **2c**, **3c** and **4c** in water (C=500 μ M); B) speciation of **2c** as a function of the pH.

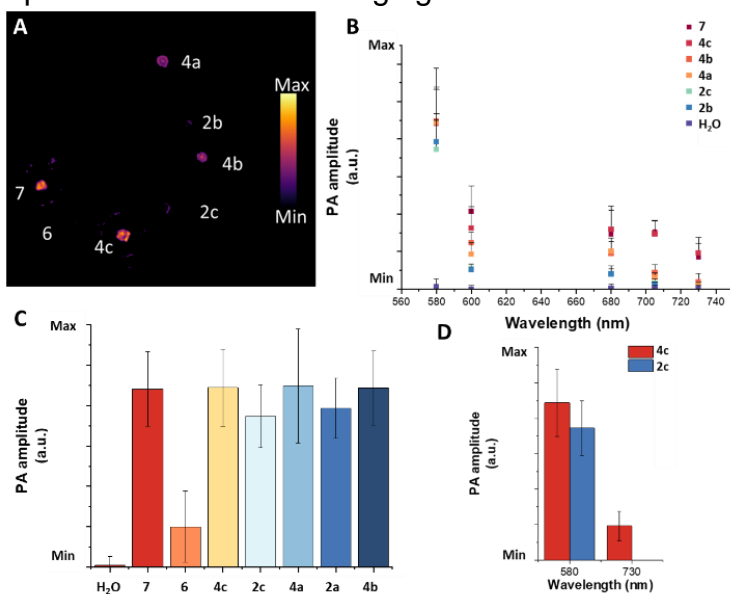
Water-solubility is a key parameter to consider in drug development as it contributes to dictating its absorption, distribution, metabolism, excretion, and toxicity (ADMET).²⁰ High water-solubility is desirable in order to limit drugs' off-target binding to the lipophilic pockets of important plasma proteins or metabolic enzymes. Log p values were measured by the 'shake flask' procedure as previously reported.²¹ All the complexes **2a-c**, **4a-c** and **6-7** proved water-soluble showing negative log p values ranging from -1.8 to -3.6 (Table S2), hence suggesting their bioavailability. However, protonation of the pyridines in complexes **3a-c** was found to modulate the complexes' optical properties, notably by redshifting their absorption maxima, and must be considered owing to the pH variations existing in the body.²² Hence, the pH dependent speciation was further explored for complexes **2c** and **3c** on the pH range 7 to 3. The spectrophotometric investigation of the species' existence as a function of pH depicted in Fig. 1B clearly showed the equilibrium between the protonated and non-protonated complexes **2c** and **3c** in the pH range of 3-5.8, beyond which no evolution is observed until pH 7. Of note, in this pH range, the MLCT absorption shows a decreased intensity, which can be ascribed to the coexistence of the protonated and non-protonated forms of the corresponding complex **2c**. This is coherent with the ligand's pKa that was predicted (by ACDLabs software) to be 4.2. A similar observation is made for complex **6** and its protonated counterpart, in the pH range 3.3-5.1, above which only complex **6** is observed (Fig. S1).

Stability in biological media is also a crucial parameter within the ADMET considerations, especially as it plays a role in maintaining the desired biological activity and hampering the formation of toxic metabolites that might result in significant deleterious effects. Gasser *et al.* emphasized the fact that rapid drug degradation in plasma is one of the exclusion criteria for drug development.²³ Therefore, the complexes **2a-c**, **4a-c** and **6-7** were incubated in serum over 72h and the complexes' stability was followed at selected time points (0, 12h, 48h, 72h) by UV-Visible absorption spectroscopy, monitoring an eventual decrease of the MLCT transition. No alteration of the MLCT transition intensity was observed irrespective of the ligands' nature or pyridine substitution, hence suggesting the stability of the metal-ligand bonds toward serum's proteins and the absence of ligand scrambling or transchelation events. Moreover, no blue shift was observed for **4a-c** and **6-7**, therefore suggesting the pyridinium stability under the tested conditions and methylation's irreversible character. Finally, the absorption spectra after 72h incubation proved unchanged from the corresponding complexes (Fig. S2), hence suggesting that

203 no non-covalent interaction occurred between the complexes and serum's proteins, which
204 could eventually alter their biodistribution profile.²⁴

205
206 Photoacoustic contrast production & photothermal efficiency

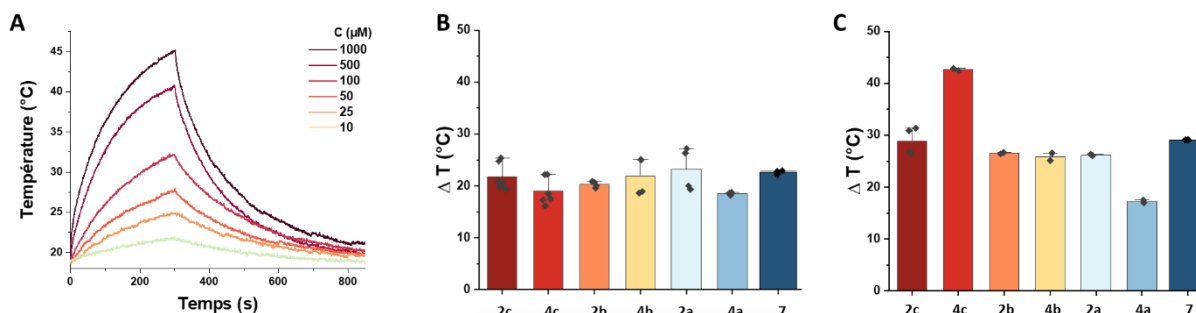
207 Thermal stability of the theranostic agent is of primary importance in view of theranostic
208 use in PA or PTT, to maintain the trackability and therapeutic efficacy under repeated
209 irradiation during a session and avoid the formation of toxic byproducts.¹⁰ All the tested
210 complexes proved stable toward repeated irradiation over 5 heating and cooling cycles,
211 as confirmed by the retained photothermal efficiency, absorbance measurements (Fig.
212 S3). Of note, the pyridiniums are found to be stable upon prolonged irradiation and do not
213 undergo demethylation. Therefore, all the complexes tested herein showed adequate
214 stability for use as photoactivated agent in PA or PTT. For the PA experiments presented
215 herein, fresh solutions of the complexes in deionized water (C=200-1000 μ M) were loaded
216 into PTFE tubes (inner diameter 0.81mm, wall thickness 0.05 mm), immersed into water
217 and irradiated at selected wavelengths with a gain of 30dB. A representative cross-section
218 view of the seven tubes mounted on a spherical holder is shown for example in Fig. 2A,
219 and highlights the enhanced PA contrast produced by the quaternized complexes **4a-c**
220 and **7** at 660nm. PA amplitude of all complexes is found to be concentration dependent
221 (Fig. S4.) and to vary significantly depending on the irradiation wavelength (Fig. 2B).
222 Indeed, PA amplitudes obtained under 580nm irradiation shown in Fig.2C suggest a
223 production of contrast in PA that is similar among all the complexes. An exception is the
224 non-quaternized (C^NC) complex **6** which PA amplitude is found by far lower to the other
225 complexes investigated herein, which was anticipated as its MLCT is blue-shifted
226 compared to others. Importantly, while the PA amplitude produced by **2c** suffers a
227 dramatic decrease when irradiated with less energetic wavelengths, *i.e.* 660nm, in the
228 case of complex **4c** that was designed to boost its photoactivity close to the NIR, high PA
229 amplitude is observed under such conditions. This behavior is also noticeable in the case
230 of **7** that shows significantly retained PA contrast in the NIR (Fig. S5). Hence, we proved
231 herein that a simple structural modification of Fe(II) complexes allows boosting their
232 photophysical properties to enable PA imaging in the NIR.



233

234 Fig. 2. Photoacoustic properties of the Fe(II) complexes (1mM in H₂O, 36°C). A) Representative PA cross-
 235 section view of the seven selected complexes ($\lambda_{\text{irrad}}=660\text{nm}$), B) variation of PA amplitude of the Fe(II)
 236 complexes depending on the irradiation wavelength, C) PA amplitude of selected complexes ($\lambda_{\text{irrad}}=580\text{nm}$),
 237 D) showing the greatest PA amplitude of quaternized complexes **7** and **4c** when irradiated with both visible
 238 and NIR wavelengths.

239
 240 PTT investigation was performed on a custom setup using non-coherent light sources
 241 (LEDs) which are preferred to laser sources as it offers a cost-effective solution that is
 242 compatible with local treatment, although it imposes reduced light power and
 243 consequently limits the possible temperature raise.²⁵ Yet, it is worth mentioning that owing
 244 to the availability and ease of use of LEDs over lasers, a growing interest for LED-
 245 activated therapies has surged,²⁶ and a LEDs-based photoactivated therapy at 635nm
 246 was approved by FDA.²⁷ PTT acquisition are expressed as a function of the LEDs'
 247 irradiance, *i.e.* the light power received by a defined area corresponding to the samples'
 248 area, and since LEDs' power vary depending on the wavelength used, the irradiance vary
 249 accordingly.²⁴ On the other hand, the ΔT is linearly correlated to complexes' concentration
 250 (Fig. 3A). As anticipated based on PA observations, all the complexes produced a
 251 significant photothermal efficiency under 565nm irradiation for 5min, with an average
 252 $\Delta T \sim 23^\circ\text{C}$ for all the complexes tested (Fig. 3B). Such temperature increase is adequate
 253 for further PTT application in cancer therapy, as cancer cells are known to undergo
 254 apoptosis upon exposure to 43°C , *i.e.* upon a temperature increase of 7°C only.⁷ Of note,
 255 overall greater ΔT are found at 660nm irradiation than 565nm due to the enhanced
 256 irradiance from the LED (0.3 W.cm^{-2} and 0.6 W.cm^{-2} respectively). Pleasantly, we
 257 observed that complexes **4c** and **7** successfully maintained a high light induced
 258 photothermal conversion upon irradiation with more redshifted LED sources, *i.e.* 660nm,
 259 while all the other complexes displayed reduced ΔT (Fig. 3C). The above-mentioned
 260 observation is in perfect agreement with the conclusions from PA imaging and confirm the
 261 rational design of complexes **4c** and **7** for boosting their potential in PA/PTT using NIR
 262 light irradiation.



263 Fig. 3. Photothermal efficiency of the Fe(II) complexes (in H₂O, 5min irradiation). A) concentration
 264 dependent variation of ΔT for complex **2c** (565nm irradiation, range 1000-10 μM), B) ΔT for complexes **2a-**
 265 **c** and **4a-c** under 565nm irradiation (1mM), C) ΔT for complexes **2a-c** and **4a-c** under 660nm irradiation
 266 (1mM).
 267
 268

269 Controlling the photoactivity of iron complexes is challenging and is a central question to
 270 multiple research fields aside biomedical applications.¹¹ Several strategies were proposed
 271 to exploit iron complexes' photosensitization of ¹O₂ for PDT therapy, notably by
 272 conjugation to fluorophores active in the visible or NIR, which efficiently enabled
 273 photosensitization and DNA photocleavage properties.²⁸ Light activation of iron-based

274 chemotherapeutics has also been shown for small molecule release such as NO or CO
275 release, in view of achieving a dual therapeutic activity.²⁹ On the other hand, non-radiative
276 deactivation processes have rarely been explored in the design of iron-based
277 chemotherapeutics.^{27c} The ability of Fe(II)-bisterpyridine complexes for light photothermal
278 conversion was highlighted by Tian *et al.* and further exploited for *in vivo* tracking of the
279 complex's biodistribution with PAI, although the stability of [Fe(terpyridine)Cl₃] complex is
280 compromised in biomimicking media.¹² However, we report herein a novel theranostic
281 complex displaying enhanced photothermal stability and inertia that could be further used
282 for PA/PTT theranostic applications.

283 Photoactivation in the NIR is highly desirable for deep light penetration and minimized
284 light diffusion by the tissues, especially as major blood components, *i.e.* hemoglobin and
285 deoxyhemoglobin, show decreased absorption beyond 600 nm and accordingly facilitated
286 PA multiplexing. While the Fe(II)-NHC complex **7** successfully displayed a significantly
287 redshifted MLCT absorption, expanding the scope of Fe(II) theranostic agents will be
288 necessary to achieve NIR absorbing Fe(II)-NHC complexes. While the strong σ -donation
289 and stability provided by the NHCs is beneficial in terms of stability, it also destabilizes the
290 HOMO level hence inducing a bathochromic shift of the MLCT.¹⁸ Gratefully, quaternization
291 of the peripheral pyridine in **7** successfully redshifted the MLCT transition corresponding
292 to N-Fe bond, which allowed photothermal activation under NIR irradiation. Yet, shifting
293 the MLCT to the therapeutic window is currently ongoing to enhance the PAI/PTT activity
294 of this family of complexes and make them more likely to clinical translation. Further
295 development could include incorporation of a more electron-withdrawing moiety than
296 pyridine in the structure³⁰ or enhancement of the π -conjugation in the ligand.³¹ Indeed, a
297 family of iron complexes that could be synthesized in a straightforward and cost-efficient
298 manner and photoactivated in the NIR would be ideal for medical imaging guided precision
299 medicine at low therapeutic dose.^{27d} Finally, the quaternization approach used herein is
300 currently being applied to complexes' functionalization with disease specific vectors to
301 achieve a dual control over therapeutics' selectivity driven by vector-target affinity for dual
302 selective photoactivatable theranostics.

303

304 [Conclusion](#)

305 We reported herein on the synthesis and photophysical characterization of a series of
306 Fe(II) complexes bearing tridentate Schiff bases, namely terpyridines and bis-NHC
307 ligands. We proposed a straightforward quaternization of the complexes to boost their
308 solubility and photophysical properties, hence making them compatible for theranostic use
309 under NIR irradiation. Exploiting their light triggered non-radiative deactivation pathway
310 enabled their use as PA imaging agent observable under NIR irradiation. Promising PTT
311 properties were also highlighted here using a LEDs-based setup, hence establishing
312 those complexes as competent water-soluble theranostic agents that could be activated
313 and tracked in the NIR. Overall, the design simplicity of the PA/PTT active complexes
314 detailed herein highlight them as promising theranostics to further explore possibilities in
315 optical imaging guided anticancer therapy.

316

317 [Supporting Information](#)

318 Additional references cited within the Supporting Information.[32]

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331
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333 Photothermal therapy.

334

335 **Conflict of interest:** The authors have no conflict of interest to declare.

336

337 **Authors contribution:** M.B. designed research. M.D., A.R.M., S.P., P.C.G. and M.B.
338 conceived, designed and performed the chemical experiments. M.B. wrote the paper and
339 M.D., A.R.M., S.P., and P.C.G. participated in manuscript writing.

340

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440 [Experimental section](#)

441 Manipulations of air and moisture sensitive compounds were carried under argon
442 atmosphere using standard Schlenk techniques and freshly distilled solvents. Reagents
443 were purchased from chemical suppliers (Sigma Aldrich Europe, BLDpharm Europe) and
444 used without further purification. ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra
445 were recorded on a Bruker Avance III 400 spectrometer at 295 K, using residual solvent
446 peak as a reference. LC-MS investigations were performed on a MicroToFq Bruker
447 coupled to a LC U3000 Thermo. IR spectra were acquired on a Shimadzu IRAffinity-1.
448 Cyclic voltammetry was performed on a Radiometer PST006 potentiostat using a
449 conventional three-electrode cell. Absorbance spectra were recorded on a Lambda 1050
450 Perkin Elmer (Shelton CT), and fluorescence spectra on a Horiba-Jobin Yvon Fluorolog 3
451 (Edison NJ), at 20°C. Photoacoustic images were acquired on a TRITOM PhotoSound
452 (Houston TX) at 36°C, using a nanosecond pulsed laser EKSPLA PhotoSonus ranging
453 from 330-1320 nm (Vilnius, Lithuania). Photothermal therapy experiments were carried out
454 using a custom setup using elements from ThorLabs (Germany): LEDs (565 nm 1000 mA,
455 660 nm 1200 mA, 730 nm 1000 mA) collimated with an adjustable collimation adapter

456 SM2F32, power was measured with a thermal sensor power meter PM160T and
457 temperature was recorded using a thermal camera optris Xi 400 « spot finder » (Optris,
458 Germany).

459
460 **General procedure for the synthesis of [Fe(tpy-Me)₂]4I complexes**
461 Iodomethane (306 μmol) is dropped under argon to a solution of [Fe(tpy)₂]2Cl (15 μmol) in
462 dried DMF (5 mL) in a sealed flask. The mixture is heated at 130°C for 1h, allowed to
463 cooldown and supplemented with 1 mL MeOH prior further precipitated by addition to 20
464 mL Et₂O. The fine powder is recovered by centrifugation (6 krpm, 5 min). Upon purification
465 on reversed phase semi-preparative HPLC using a H₂O-MeOH gradient, the desired
466 complex is obtained as dark purple-blue solid.

467 **4a. [Fe(1''-methyl-6'-(pyridin-2-yl)-[2,2':4',4''-terpyridin]-1''-ium)2]4I**

468 Purple powder (37%). ¹H NMR (d₆-DMSO, 400 MHz, 25°C): δ 4.48 (s, 6H, CH₃), 7.17 (m,
469 4H, CHar), 7.25 (m, 4H, CHar), 7.99 (m, 4H, CHar), 8.85 (m, 4H, CHar), 9.00 (m, 4H,
470 CHar), 9.64 (s, 4H, CHar); ¹³C NMR (d₆-DMSO, 101 MHz, 25°C): δ 48.8, 122.7, 125.3,
471 126.6, 128.8, 140.1, 143.5, 146.9, 152.1, 153.5, 157.9, 161.1; HRMS (ESI) calcd for
472 C₄₂H₃₄FeN₈ m/z=176.5558, found 176.5572 [M²⁺].

473 **4b. [Fe(1''-methyl-6'-(pyridin-3-yl)-[2,2':4',4''-terpyridin]-1''-ium)2]4I**

474 Purple powder (81%). ¹H NMR (d₆-DMSO, 400 MHz, 25°C): δ 4.67 (s, 6H, CH₃), 7.24 (m,
475 4H, CHar), 7.30 (d, J=5.4 Hz, 4H, CHar), 8.12 (dt, 4H, J=7.7 Hz and 1.3 Hz, CHar), 8.63
476 (m, 2H, CHar), 9.00 (d, J=8.1 Hz, 4H, CHar), 9.34 (d, J=6.1 Hz, 2H, CHar), 9.65 (d, J=8.1
477 Hz, 2H, CHar), 9.89 (s, 4H, CHar), 10.19 (s, 2H, CHar); ¹³C NMR (d₆-DMSO, 101 MHz,
478 25°C): δ 48.7, 121.5, 124.3, 127.9, 128.2, 135.7, 139.2, 142.1, 142.9, 144.7, 146.6, 152.8,
479 157.3, 160.3; HRMS (ESI) calcd for C₄₂H₃₄FeN₈ m/z=176.5558, found 176.5572 [M²⁺].

480 **4c. [Fe(1''-methyl-6'-(pyridin-4-yl)-[2,2':4',4''-terpyridin]-1''-ium)2]4I**

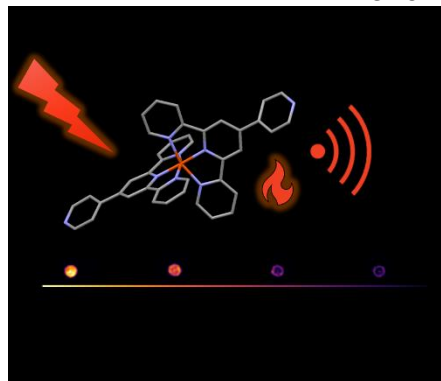
481 Dark blue powder (94%). ¹H NMR (d₆-DMSO, 400 MHz, 25°C): δ 4.80 (s, 6H, CH₃), 7.29
482 (m, 4H, CHar), 7.46 (d, J=5.5 Hz, 4H, CHar), 8.09 (m, 4H, CHar), 8.52 (t, J=7.1 and 1.4
483 Hz, 2H, CHar), 8.76 (d, J=7.9 Hz, 2H, CHar), 8.81 (d, J=7.9 Hz, 2H, CHar), 9.06 (t, J=
484 7.9Hz, 2H, CHar), 9.50 (d, J=6.2 Hz, 2H, CHar), 9.66 (s, 4H, CHar); ¹³C NMR (d₆-DMSO,
485 101 MHz, 25°C): δ 48.8, 122.7, 125.3, 126.5, 128.7, 140.0, 143.5, 146.9, 152.0, 153.4,
486 157.8, 161.1; HRMS (ESI) calcd for C₄₂H₃₄FeN₈ m/z=176.5558, found 176.5577 [M²⁺].

487
488 **6. [Fe(4-pyridyl-(2,6-bis(3-methyl-imidazol)pyridine)2]2PF₆**

489 To a solution of imidazolium salt **5** (0.077 g, 0.126 mmol) in 2 mL of anhydrous DMF was
490 added FeCl₂ (0.008 g, 0.063 mmol) and degassed the mixture with Argon for 10 min. Then
491 added t-BuOK (0.071 g, 0.632 mmol) to the above mixture and stirred at room temperature
492 for 20 min. A saturated solution of NH₄PF₆ was added (10 ml) and the precipitate was
493 collected by filtration. Then the crude was further purified on silica gel column
494 chromatography using acetone/H₂O/KNO₃(sat) = 10: 3: 0.5 mixture. The pinkish fraction
495 was collected and after the evaporation of acetone, the left solution was treated with a
496 saturated solution of NH₄PF₆. Affording the precipitation of the desired complex, it was
497 then filtered, washed with distilled water followed by ether, and dried under vacuum. The
498 isolated complex **6** was obtained as a red powder (0.035 g, 56% yield). ¹H NMR (400
499 MHz, DMSO-d₆, δ ppm): 9.03(d, J= 5.74 Hz, 2H), 8.77(s, 2H), 8.64(s, 2H), 8.36(d, J= 5.42
500 Hz, 2H), 7.41(s, 2H), 2.58(s, 6H). HRMS (ESI) calcd for C₃₆H₃₂FeN₁₂ m/z = 344.1106.
501 Found: 344.1134 [M-2PF₆]. Of note, the too low solubility of this complex in DMSO
502 prevented the acquisition of ¹³C NMR.

503
504 **7. [Fe(4-pyridinium-(2,6-bis(3-methyl-imidazol)pyridine)2]4PF6**
505 Complex **6** (0.015 g, 0.015 mmol) was charged in a 10 mL round bottomed flask and
506 dissolved in 1 mL of DMF. Then added methyl iodide (0.010 mL, 0.153 mmol) and stirred
507 at RT for 24 h. Desired complex was precipitated upon addition of saturated NH₄PF₆
508 solution and stirred until the product is completely precipitated. Complex **7** was collected
509 by vacuum filtration and washed 3 times with water and then diethyl ether and dried under
510 vacuum. The isolated complex **7** was obtained as dark blue powder (0.017 g, 85% yield).
511 ¹H NMR (400 MHz, CD₃CN, δ ppm): 8.89(d, J= 6.77 Hz, 2H), 8.59(d, J= 6.96 Hz, 2H),
512 8.25(s, 2H), 8.18(d, J= 2.20 Hz, 2H), 7.10(d, J= 2.20 Hz, 2H), 4.44(s, 3H), 2.60(s, 6H).
513 ¹³C NMR (100 MHz, CD₃CN, δ ppm): 199.28, 155.11, 152.70, 146.73, 143.20, 127.80,
514 126.26, 117.25, 104.25, 48.80, 35.36. HRMS (ESI) calcd for C₃₈H₃₆FeN₁₂ m/z =
515 358.1262. Found: 358.1262 [M-2PF₆], C₃₈H₃₇FeN₁₂ m/z = 239.0866. Found: 239.0878
516 [M-3PF₆].
517

518 **Table of contents:**



529

Iron(II) complexes bearing terpyridine or 4-pyridin-2,6-bis N-heterocyclic carbene-pyridine ligands display light-triggered photoactivation for theranostic application. Their irradiation promotes a non-radiative decay that can be exploited in photoacoustic imaging or photothermal therapy.

530

531 **Biography:**



Mathilde Bouché received her PhD in Bioinorganic Chemistry from the University of Strasbourg (France) in 2017 under the supervision of Dr. S. Bellemin-Laponnaz. After two postdoctoral stays as Fulbright scholar at Prof. Cormode's lab at the University of Pennsylvania (U.S.), and at the University of Lorraine (France), she started her independent career in 2020 as a CNRS research Associate Professor at the University of Lorraine (France). Her interdisciplinary research focusses on metal-based complexes for therapeutic and theranostic applications.