

Supplementary Information

Alkoxide Coordination of Iron(III) Protoporphyrin IX by Antimalarial Quinoline Methanols: A Key Interaction Observed in the Solid-State and Solution

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1. X-Ray Crystallography

1.1 Data Collection

Crystal data were collected on a Bruker Apex Duo diffractometer equipped with a Cu Incoatec I μ S microsource and an Oxford Cryosystems Cryostat (700 Series Cryostream Plus). Crystals were mounted on a MiTeGen mount in paratone oil, and data were collected at 100 K. Data reduction, absorption corrections and unit cell determinations were carried out using the diffractometer software.¹

1.2 Structure Solution and Refinement

The structures of MQ-Fe(III)PPIX and QD-Fe(III)PPIX were solved and refined using the SHELX-97² package implemented through XSeed.³ Diagrams were generated using POVRay,⁴ implemented through XSeed.³

Apart from some of the atoms belonging to disordered solvent molecules and disordered vinyl groups in the structure of MQ-Fe(III)PPIX, all non-hydrogen atoms were refined with anisotropic temperature factors. The hydrogen atoms located on the propionic acid groups, as well as on the piperidine (MQ) and quinuclidine (QD) nitrogen atoms, were located in the respective difference map and refined independently (using simple bond length constraints in some cases), with the exception of the hydrogen atom located on the propionic acid group of MQ-Fe(III)PPIX (**II**), which was placed, and the hydrogen atom located on the propionic acid group of QD-Fe(III)PPIX (**IV**), which was not successfully located or placed.

There is disorder displayed on the porphyrin pyrrole rings by the vinyl and methyl substituents in both structures. In MQ-Fe(III)PPIX (**I**), the vinyl groups are disordered over two positions with s.o.f's of 0.74 and 0.26. The vinyl group with occupancy of 0.74 is disordered further over two positions with s.o.f's of 0.42 and 0.32. In MQ-Fe(III)PPIX (**II**), the vinyl groups are disordered over two positions with s.o.f's of 0.52 and 0.48, with the group with occupancy 0.52 being further disordered over two positions (s.o.f's of 0.21 and 0.31). One of the trifluoromethyl groups on the MQ-Fe(III)PPIX (**II**) moiety is disordered over two positions with s.o.f's of 0.48 and 0.52 respectively. Simple bond length constraints were applied to this disordered group. In the propionate group of MQ-Fe(III)PPIX (**I**), both oxygen atoms are disordered over two positions, with s.o.f's. of 0.79 and 0.21. The

disordered acetonitrile molecules were modelled isotropically and simple bond length constraints were applied. One of the acetonitrile solvent molecules is disordered over two positions, which were modelled with s.o.f.'s of 0.65 and 0.35. The remaining disordered acetonitrile molecule was modelled with partial occupancy, and over two positions with s.o.f.'s of 0.52 and 0.28. The water molecule was also modelled with a partial occupancy of 0.20. In both QD-Fe(III)PPIX (**III**) and QD-Fe(III)PPIX (**IV**), the vinyl groups are disordered over two positions. The s.o.f.'s are 0.53 and 0.47 in (**III**) and 0.67 and 0.33 in (**IV**). The site occupancy of the included water molecule refined to 0.47.

Table S1 Crystal data, experimental and refinement parameters for MQ-Fe(III)PPIX.

Molecular formula	2(C ₅₁ H ₄₇ O ₅ FeN ₆ F ₆)·2.8CH ₃ CN·0.2H ₂ O	
Formula weight / g mol ⁻¹	2106.17	
Crystal system	Triclinic	
Space group	P1	
Unit cell dimensions (Å, °)	a = 13.655(1) b = 18.596(2) c = 20.270(2)	α = 90.523(1) β = 108.490(1) γ = 96.048(2)
Z	2	
Crystal size (mm)	0.20 × 0.10 × 0.05	
Temperature (K)	100(2)	
μ (mm ⁻¹)	0.395	
Independent reflections	24809	
R _{int}	0.0522	
Goodness of fit, S	1.029	
Final R indices [<i>I</i> > 2σ(<i>I</i>)]	R ₁ = 0.0597, wR ₂ = 0.1328	

Table S2 Crystal data, experimental and refinement parameters for QD-Fe(III)PIX.

Molecular formula	2(C ₅₄ H ₅₅ FeN ₆ O ₆)·(CH ₃ CN)·0.47(H ₂ O)	
Formula weight / g mol ⁻¹	1929.30	
Crystal system	Monoclinic	
Space group	P2 ₁	
Unit cell dimensions (Å, °)	a = 15.787 (2)	α = 90
	b = 16.517 (3)	β = 97.155 (2)
	c = 18.303 (3)	γ = 90
Z	2	
Crystal size (mm)	0.39 × 0.24 × 0.12	
Temperature (K)	100(2)	
μ (mm ⁻¹)	0.379	
Independent reflections	15591	
R _{int}	0.0596	
Goodness of fit, S	1.021	
Final R indices [<i>I</i> > 2σ(<i>I</i>)]	R ₁ = 0.0483, wR ₂ = 0.1156	

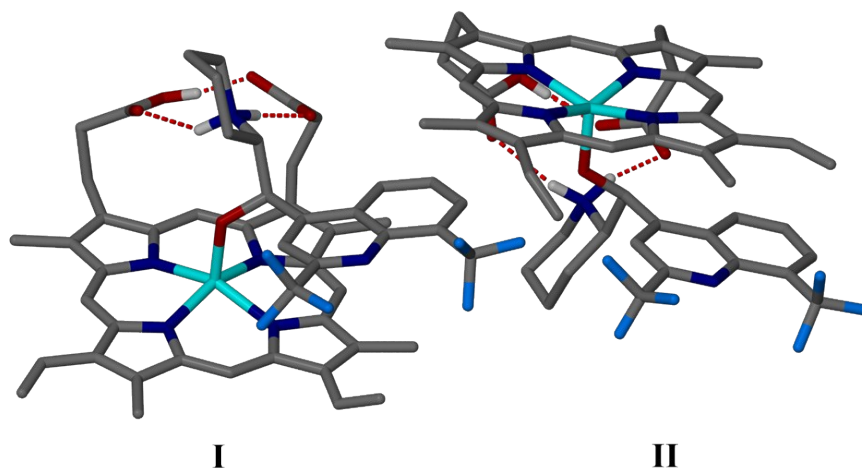


Figure S1. The asymmetric unit of the MQ-Fe(III)PPIX structure includes two unique complexes, **I** and **II**, each incorporating a different *erythro* enantiomer of MQ. Structural analysis shows that complex **I** includes the (-) isomer of MQ, while complex **II** includes the corresponding (+) isomer.

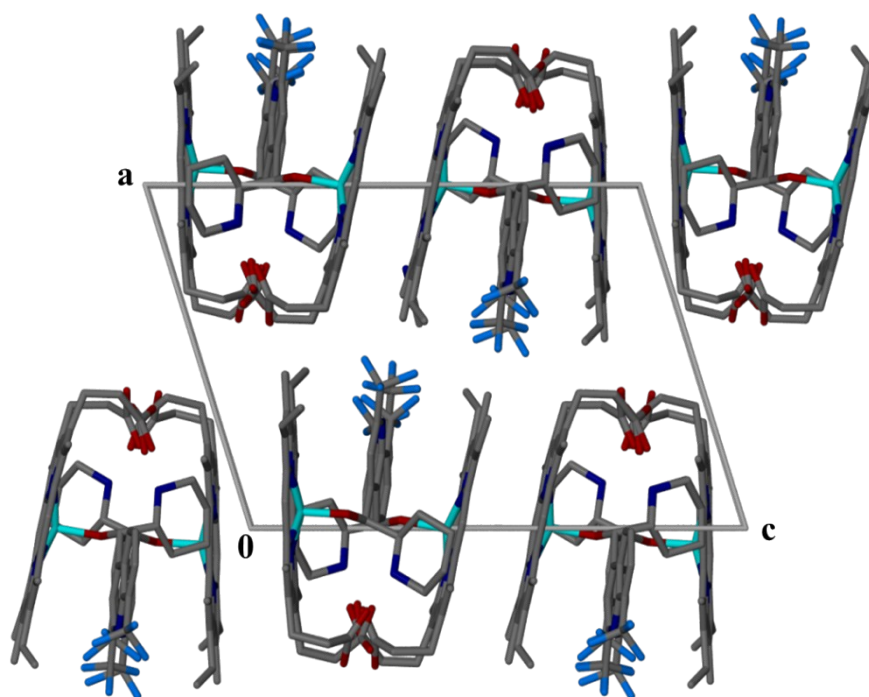


Figure S2. Crystal packing of MQ-Fe(III)PPIX viewed down the crystallographic *b*-axis as indicated by the unit cell. The offset π - π dimerization is also observed between adjacent MQ-Fe(III)PPIX moieties in the crystal packing. Disordered solvent molecules have been removed for clarity.

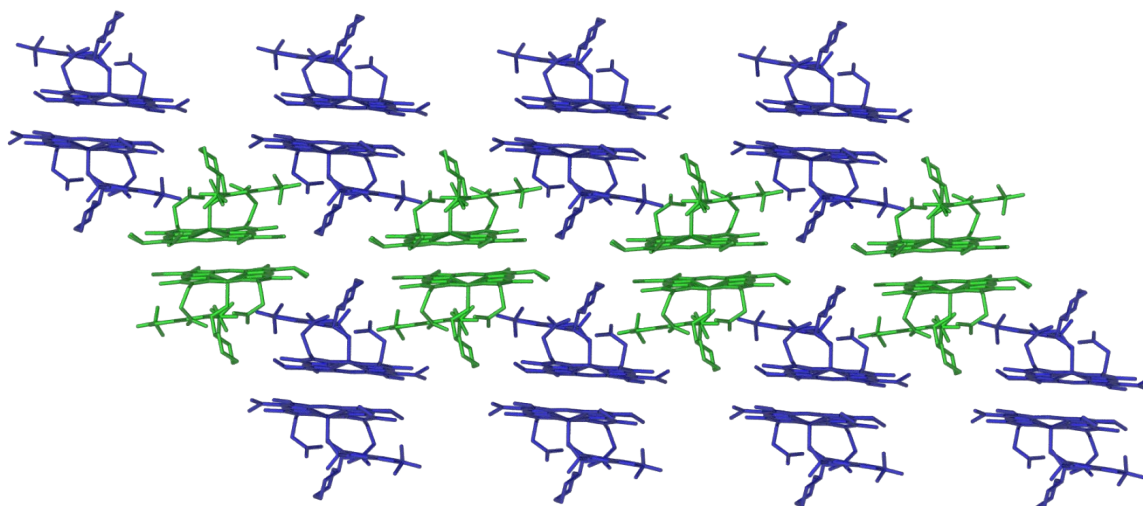


Figure S3. A sheet of molecules in the *bc* plane of the MQ-Fe(III)PPIX structure, consisting of rows of dimers of MQ-Fe(III)PPIX (**I**) (shown in green) and rows of dimers of MQ-Fe(III)PPIX (**II**) (shown in blue), both parallel to the crystallographic *b*-axis, which pack together in an alternating fashion. Solvent molecules have been removed for clarity.

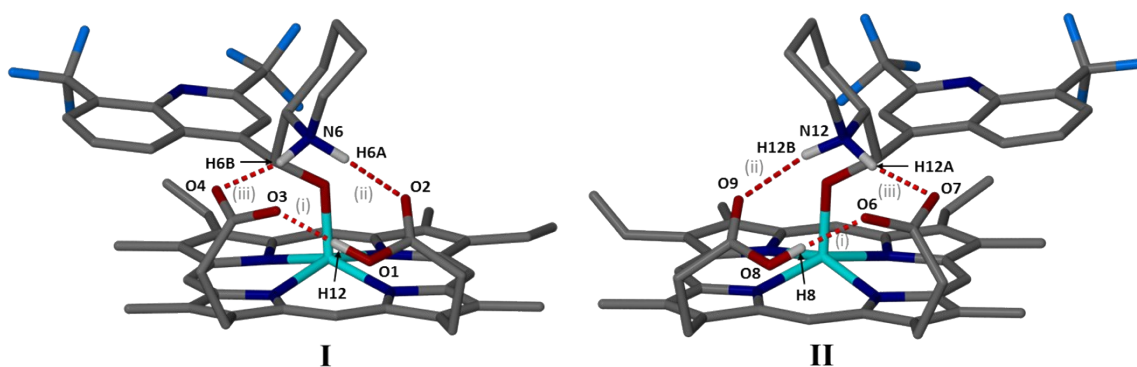


Figure S4. The intramolecular hydrogen bonding network observed in **I** and **II**, which have been reoriented relative to one another for clarity. The atom and hydrogen bond labels correspond to the nomenclature of the hydrogen bonds reported in Table S3.

Table S3 Hydrogen bond geometry in the MQ-Fe(III)PPIX crystal structure.

Complex		D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
I	(i)	O1—H12···O3	0.96(5)	1.62(5)	2.577(4)	172(5)
	(ii)	N6—H6A···O2	0.99(1)	2.21(2)	3.003(4)	137(2)
	(iii)	N6—H6B···O4	1.00(1)	1.74(2)	2.712(5)	163(4)
II	(i)	O8—H8···O6	0.84	1.70	2.536(3)	173.8
	(ii)	N12—H12B···O9	0.99(1)	2.31(2)	3.083(3)	134(2)
	(iii)	N12—H12A···O7	1.00(1)	1.74(2)	2.699(3)	159(4)

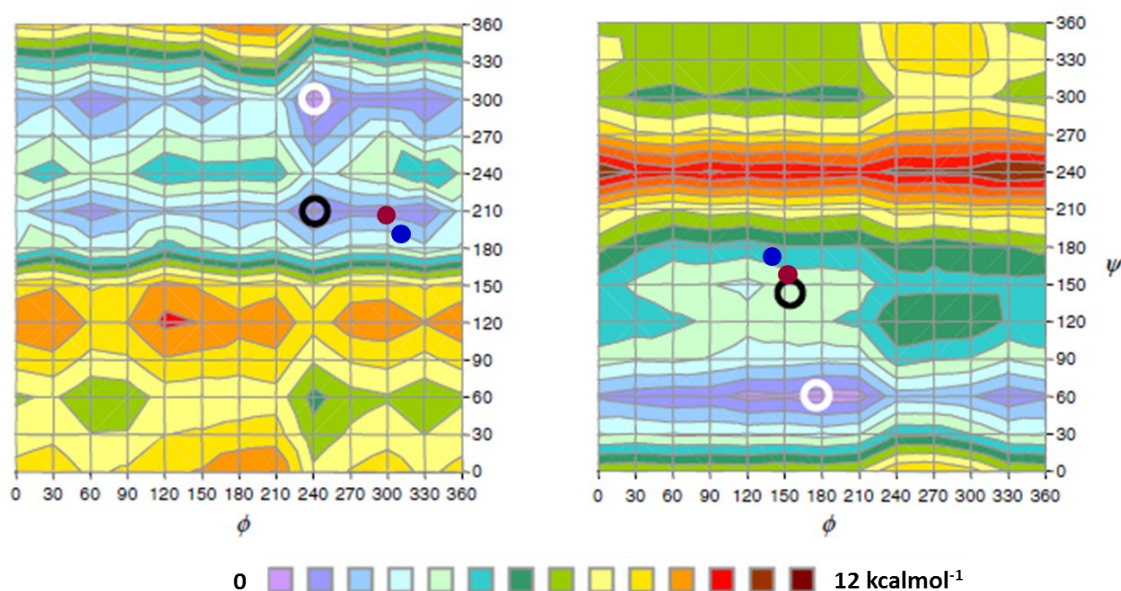


Figure S5. Strain energy surface plots for QD-Fe(III)PPIX (left) and QN-Fe(III)PPIX (right).⁵ In each, the open white circle indicates the geometry (ϕ and ψ) observed in the minimum energy (strain free) structure, while the calculated geometry and corresponding energy of the strained (intramolecular hydrogen bonded) coordination complexes is indicated by the open black circle. Experimentally-determined geometries⁶ (Table 1, main paper) and corresponding strain energies for each complex are indicated by the filled red dot. The geometries of each of the analogous MQ-Fe(III)PPIX complexes are indicated by the filled blue dot. Owing to the same relative stereochemistry in the quinoline methanol drug (axial ligand), **II** was compared to QD-Fe(III)PPIX (left), while **I** was compared to QN-Fe(III)PPIX (right).

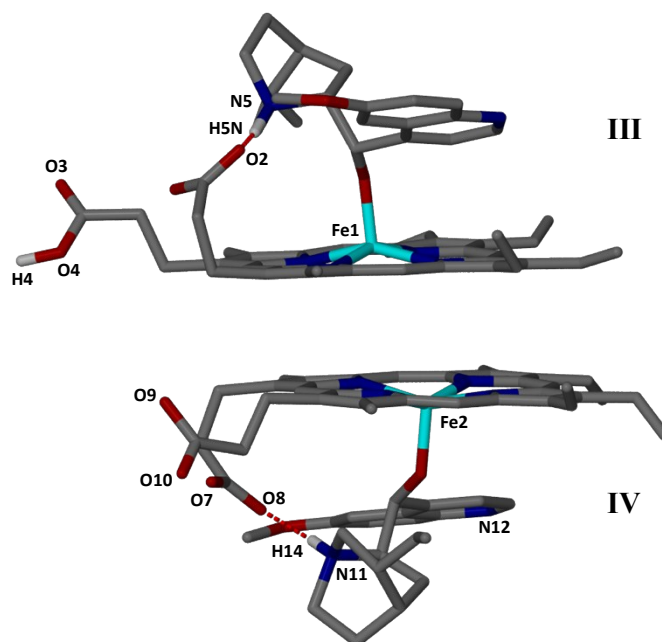


Figure S6. The asymmetric unit of the QD-Fe(III)PPIX acetonitrile solvate includes two complexes, **III** and **IV**. Hydrogen bonds are shown as broad dashed lines (red). Solvent molecules and non-relevant hydrogen atoms have been removed for clarity.

Table S4 Hydrogen bond geometry in the QD-Fe(III)PPIX complex grown from acetonitrile.

Complex	D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
III	N5—H5N···O2	1.13(5)	1.47(5)	2.553(5)	159(4)
	O4—H4···O7 ⁱ	1.05(6)	1.48(6)	2.523(5)	174(5)
IV	N11—H14···O8	0.79(5)	1.87(5)	2.661 (6)	175(5)
	O10··· N12 ^{ii*}	-	-	2.619(5)	-

Symmetry codes: (i) $-x + 1, y + \frac{1}{2}, -z + 2$; (ii) $x + 1, y, z$;

* **D···A** distance only, as no hydrogen was located or placed on O10.

Table S5 Dihedral angles in QD-Fe(III)PPIX acetonitrile solvate.

Dihedral angle	(°)
φ N2—Fe1—O5—C44 / N8—Fe2—O11—C98	296 / 295
ψ C45—C44—C40—N5 / C99—C98—C94—N11	205 / 203

Table S6 Assignment of selected peaks in the mass spectra of quinoline methanol-Fe(III)PPIX complexes.

Species		Calculated m/z	QD-Fe(III)PPIX	QN-Fe(III)PPIX	MQ-Fe(III)PPIX
[CQ+H] ⁺		320.1894	320.1900	-	320.1899
[CQ+2H] ²⁺		160.5986	160.5993	-	-
[QD+H] ⁺		325.1916	325.1922	-	-
[QN+H] ⁺		325.1916	-	325.1929	-
[MQ+H] ⁺		379.1245	-	-	379.1244
Fe(III)PPIX ⁺ (haemin – Cl ⁻)		616.1773	616.1774	616.1775	616.1772
[M+H] ⁺	QD	940.3611	940.3629	-	-
i.e. Complex of Fe(III)PPIX	QN	940.3611	-	940.3632	-
with:	MQ	994.2940	-	-	994.2946

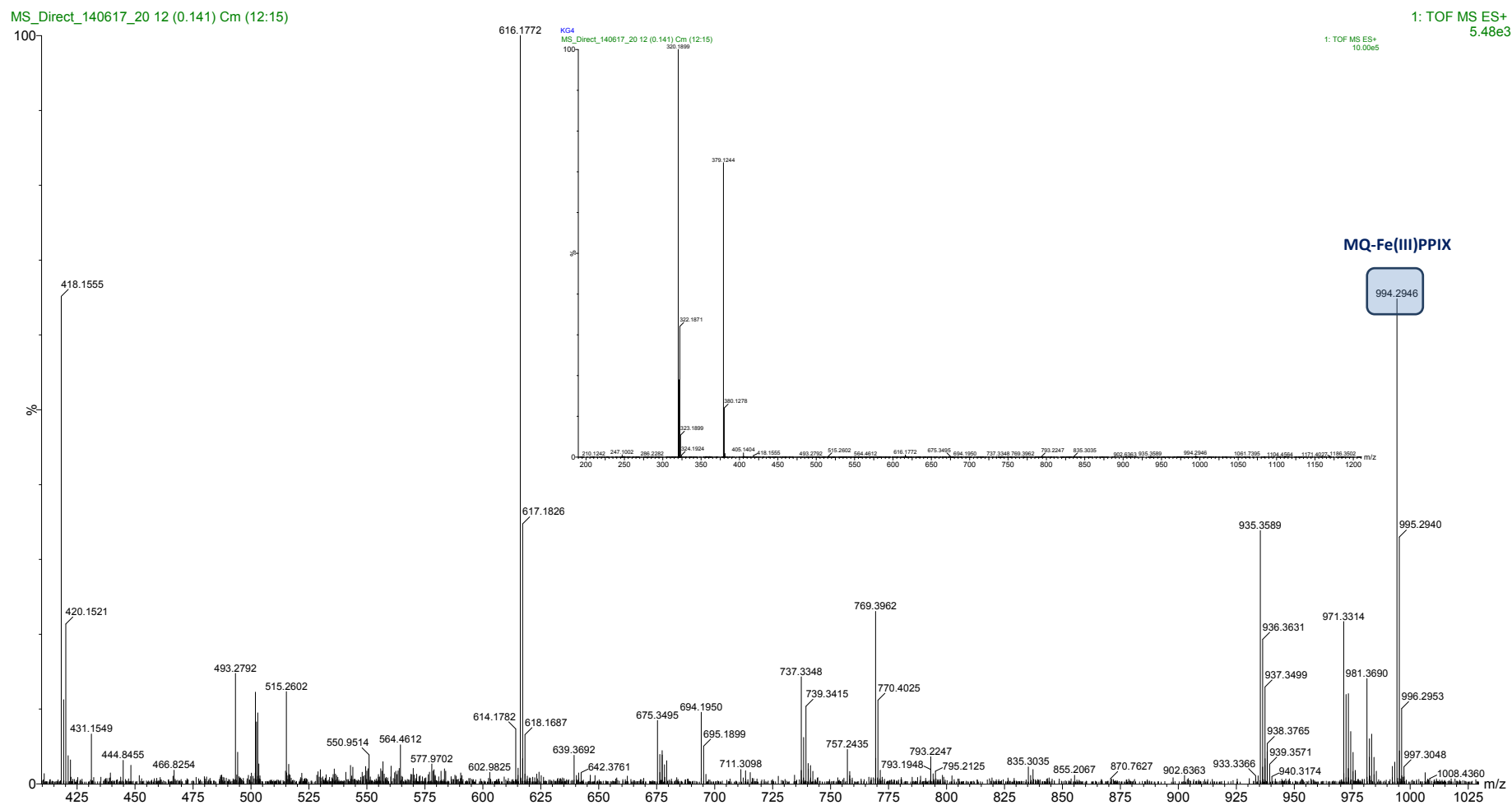


Figure S7. Positive ion mass spectrum of an acetonitrile solution containing Fe(III)PPIX, MF and CQ free base. The peak at m/z 994.2946 corresponds to $[M+H]^+$ for a 1:1 MF-Fe(III)PPIX coordination complex (calculated m/z = 994.2940). The major peak at m/z 616.1772 is assigned as the unligated porphyrin (haemin – Cl)⁺. Inset: The dominant peaks at m/z 320.1899 and 379.1244 are attributed to CQ-H⁺ and MQ-H⁺, respectively. These drugs were present in excess in solution.

MS_Direct_140617_22 9 (0.099) Cm (8:11)

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4.02e4

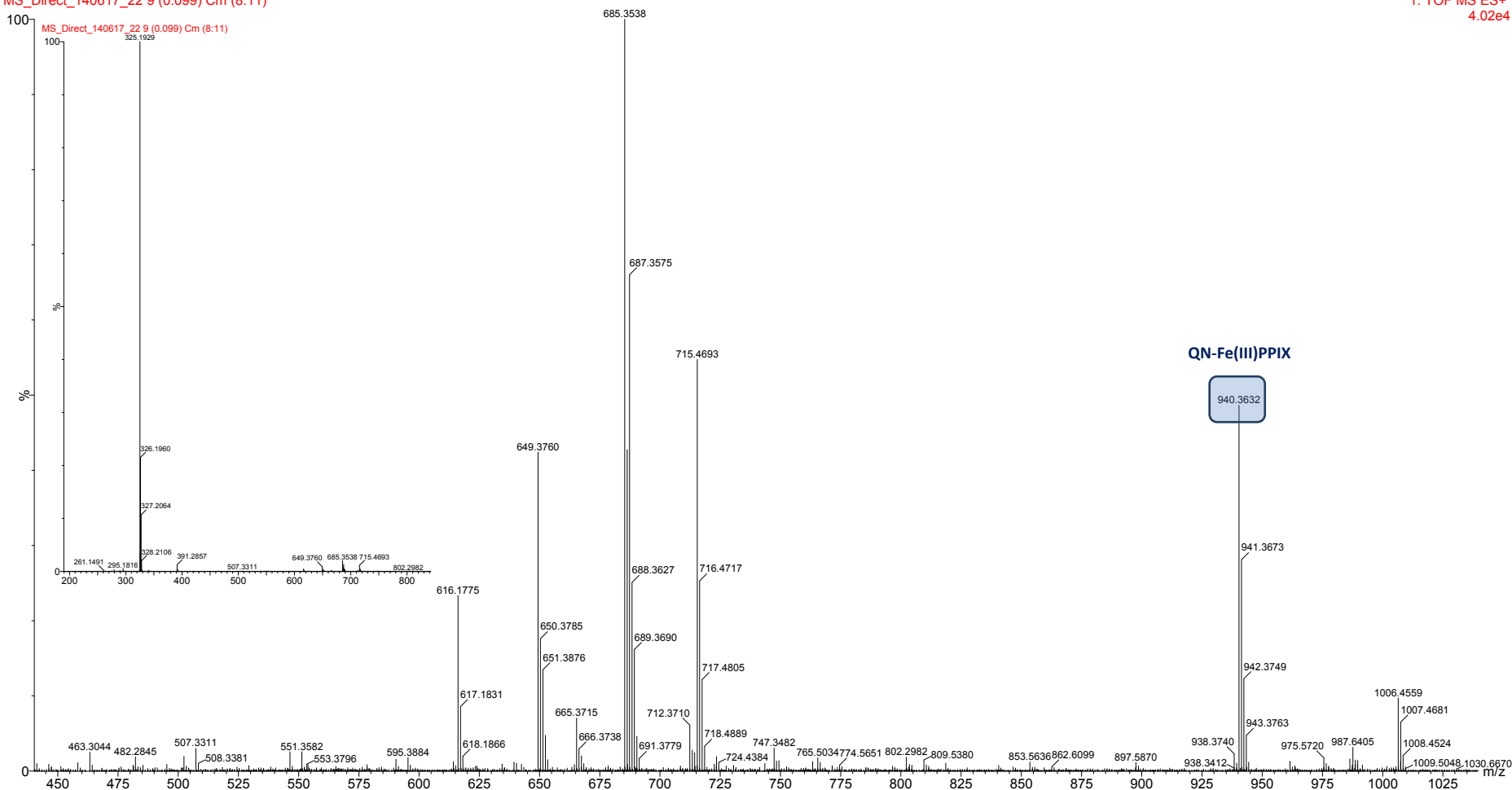


Figure S8. Positive ion mass spectrum of an acetonitrile solution containing Fe(III)PPIX and QN. The peak at m/z 940.3632 corresponds to $[M+H]^+$ for a 1:1 QN-Fe(III)PPIX coordination complex (calculated $m/z = 940.3611$). The unligated porphyrin (haemin - Cl) $^+$ is observed at m/z 616.1775. The inset shows the dominance of a peak at m/z 325.1929 which is attributed to QN-H $^+$, which was present in excess in solution.

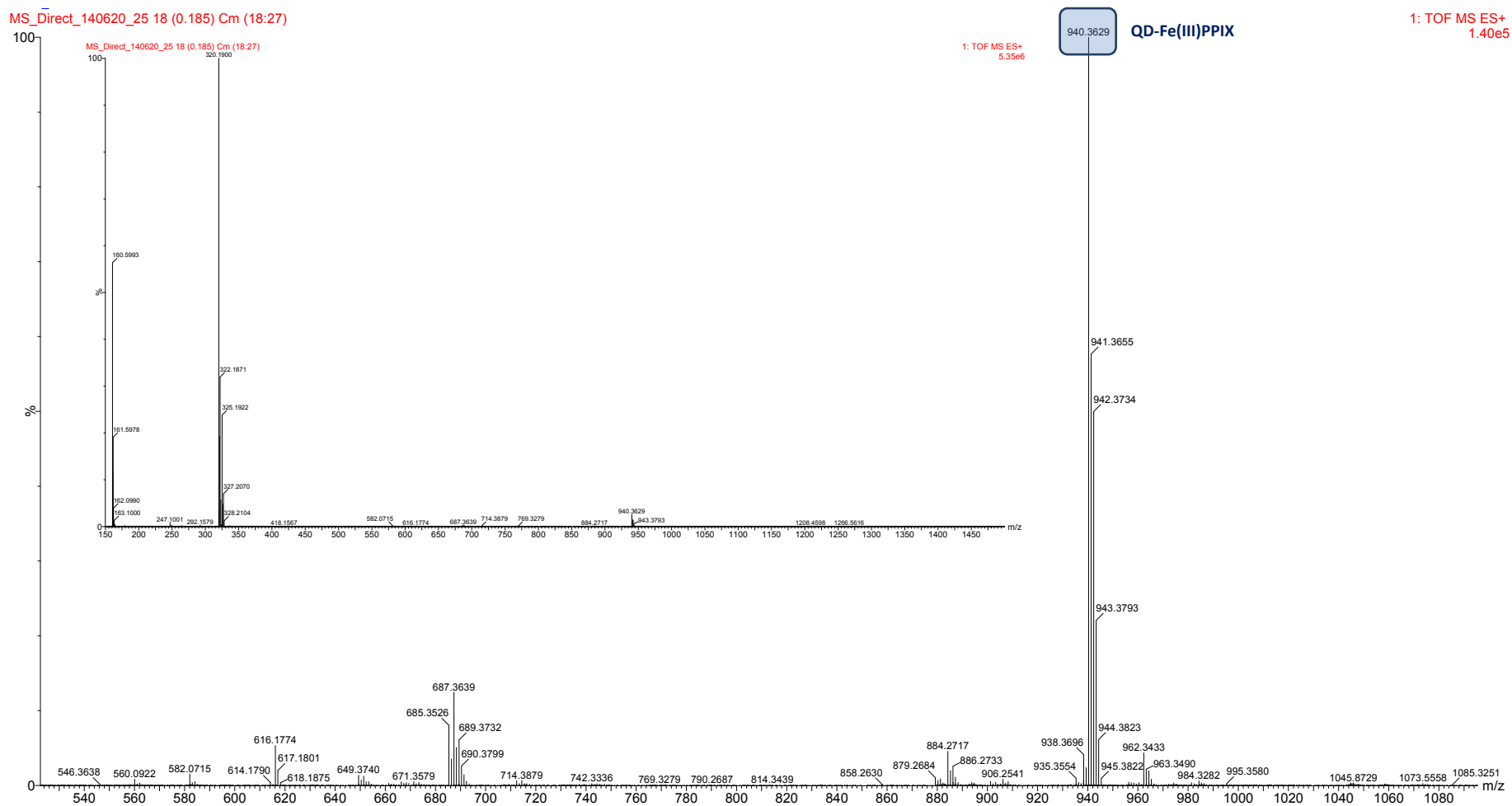


Figure S9. Positive ion mass spectrum of an acetonitrile solution containing Fe(III)PPIX, QD and CQ free base. The peak at m/z 940.3629 corresponds to $[M+H]^+$ for a 1:1 QD-Fe(III)PPIX coordination complex (calculated m/z = 940.3611). The unligated porphyrin (haemin – Cl) $^+$ is observed at m/z 616.1774. Inset: The dominant peaks at m/z 320.1900 and 325.1922 are attributed to CQ-H $^+$ and QD-H $^+$, respectively. Both drugs were present in excess in solution. The peak at 160.5993 is assigned to the CQ-2H $^{2+}$ (diprotic) species.

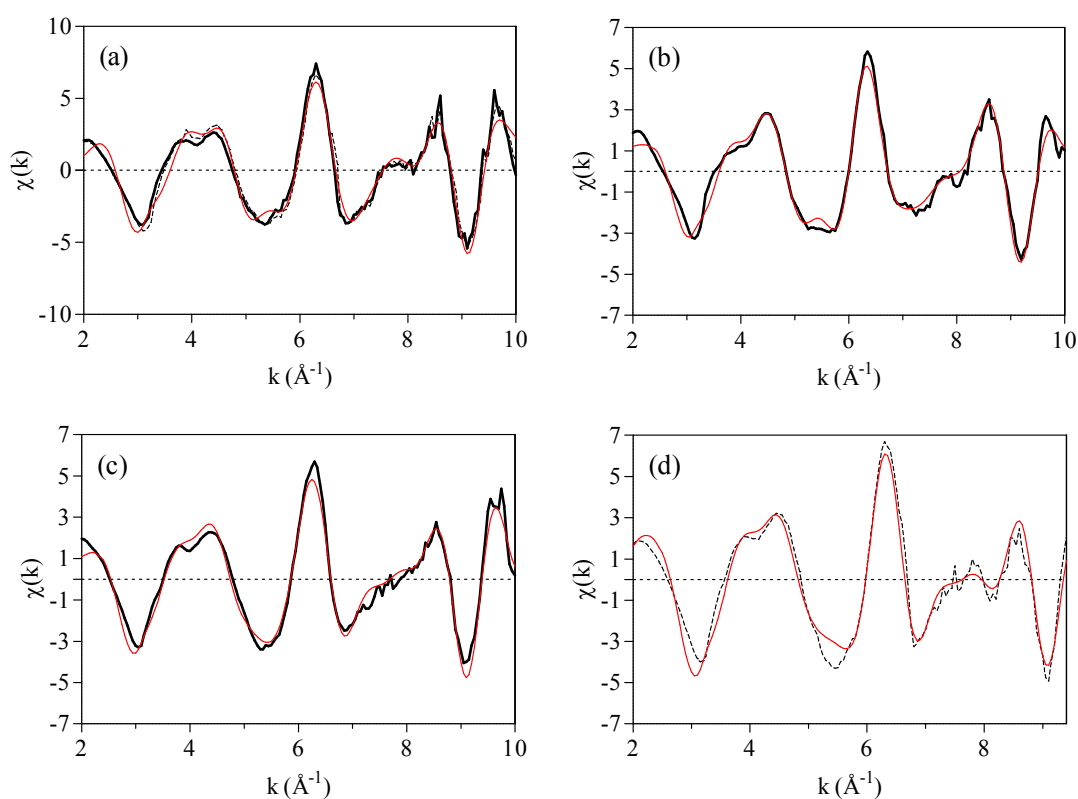


Figure S10. Experimental and fitted EXAFS spectra in k -space. (a) In the case of QD-Fe(III)PPIX, the spectra of the crystals (black solid line) and the crystallisation solution (black dashed line) were fitted to the same model (red line). Spectra were fitted over the range $2.0 \leq k \leq 10.0 \text{ \AA}^{-1}$ and $0.0 \leq R \leq 5.0 \text{ \AA}$. (b) The best fit of the model (red line) to the experimental spectrum of a crystalline sample of MQ-Fe(III)PPIX (black solid line). Spectra were fitted over the range $2.0 \leq k \leq 10.0 \text{ \AA}^{-1}$ and $0.0 \leq R \leq 5.0 \text{ \AA}$. (c) The best fit of the model (red line) to the experimental spectrum of a crystalline sample of QN-Fe(III)PPIX (black solid line). Spectra were fitted over the range $2.0 \leq k \leq 10.0 \text{ \AA}^{-1}$ and $1.0 \leq R \leq 5.0 \text{ \AA}$. (d) The best fit of the model (red line) to the experimental spectrum of the crystallisation solution of QN-Fe(III)PPIX (black dashed line). Spectra were fitted over the range $2.0 \leq k \leq 9.4 \text{ \AA}^{-1}$ and $0.0 \leq R \leq 5.0 \text{ \AA}$. All spectra (a to d) were fitted with k^3 -weighting in R -space at the final step.

Table S7 Distances (R, Å) and Debye-Waller factors (σ^2 , Å²) of all atoms relative to the absorbing iron(III) centre following EXAFS fitting.

Location	Atom	QD-Fe(III)PPIX*			QN-Fe(III)PPIX						MQ-Fe(III)PPIX#		
		N	R	σ^2	Solid-state			Solution			N	R	σ^2
		N	R	σ^2	N	R	σ^2	N	R	σ^2	N	R	σ^2
Porphyrin core	N ₁₂₃₄	4	2.05(1)	0.0003(9)	4	2.06(1)	0.001(9)	4	2.05(2)	0.002(1)	4	2.026(9)	0.0001(9)
	C _{α}	8	3.04(2)	0.0009(13)	8	3.04(2)	0.002(11)	8	3.13(2)	0.003(2)	8	3.02(1)	0.002(1)
	C _{meso}	4	3.48(4)	0.004(2)	4	3.51(1)	0.001(24)	4	3.51(1)	0.002(3)	4	3.46(3)	0.001(3)
	C _{β}	8	4.28(2)	0.0044(X)	8	4.30(2)	0.001(24)	8	4.29(2)	0.002(3)	8	4.28(1)	0.001(3)
Explicitly-refined axial (drug) atoms	O _{axial}	1	1.86(2)	0.0003(9)	1	1.86(3)	0.001(9)	1	1.85(3)	0.0004(18)	1	1.85(2)	0.0001(9)
	C ^{γ}	1	2.9(1)	0.0009(13)	1	3.1(1)	0.002(11)	1	3.0(2)	0.003(2)	1	3.13(9)	0.002(1)
	C ^{δ}	1	4.3(5)	0.004(2)	1	3.7(3)	0.001(24)	1	4.2(6)	0.002(3)	1	4.2(2)	0.001(3)
	C ^{ϵ}	1	3.7(3)	0.004(2)	1	3.5(3)	0.001(24)	1	3.5(2)	0.002(3)	1	3.9(2)	0.001(3)
Additional atoms (axial atoms of drugs and atoms from adjacent π -stacked complexes)	Fe	1	4.76(7)	0.004(5)	1	4.41(6)	0.002(61)	1	4.5(2)	0.001(6)	1	4.63(4)	0.002(5)
	Cp1	3	3.9(2)	0.004(2)	5	3.81(8)	0.004(26)	5	3.87(9)	0.003(4)	4	3.8(3)	0.007(12)
	Cp2	2	4.4(2)	0.004(2)	3	3.94(5)	0.002(42)	2	4.5(4)	0.003(11)	8	4.52(5)	0.003(12)
	Cp3	9	4.57(6)	0.004(2)	10	4.61(7)	0.008(29)	11	4.65(8)	0.008(12)	7	4.74(6)	0.005(5)
	Cp4	-	-	-	-	-	-	-	-	-	2	4.0(2)	0.005(5)
	Cn1	3	3.82(8)	0.004(2)	-	-	-	-	-	-	4	3.8(2)	0.005(6)

* EXAFS data collected from crystalline and solution samples were fitted simultaneously.

Only data obtained from the crystalline sample of MQ-Fe(III)PPIX were successfully fit.

^{γ} Corresponds to atoms C9 in QD and QN (see Figure 1, a) and C11 in MQ (see Figure 1, b). ^{δ} Corresponds to atoms C8 in QD and QN (see Figure 1, a) and C12 in MQ (see Figure 1, b). ^{ϵ} Corresponds to atoms C4' in QD and QN (see Figure 1, a) and C4 in MQ (see Figure 1, b).

Table S8 Comparative SCD distances (R, Å) relative to the iron(III) centre for atoms explicitly refined during EXAFS fitting.

Location	Atom	N	QD-Fe(III)PPIX	QN-Fe(III)PPIX	MQ-Fe(III)PPIX	
					I	II
Porphyrin core	N ₁₂₃₄	4	2.067(4)	2.06(1)	2.065(7)	2.063(7)
	C _α	8	3.08(1)	3.08(1)	3.09(1)	3.09(1)
	C _{meso}	4	3.44(2)	3.42(3)	3.44(2)	3.44(2)
	C _β	8	4.31(2)	4.30(2)	4.31(1)	4.31(1)
Explicitly-refined axial (drug) atoms	O_{axial}	1	1.862(2)	1.866(4)	1.899(2)	1.892(2)
	C ^γ	1	2.928	2.939	2.904	2.905
	C ^δ	1	4.222	4.188	4.211	4.209
	C ^ε	1	3.603	3.622	3.605	3.604

^γ Corresponds to atoms C9 in QD and QN (see Figure 1, a) and C11 in MQ (see Figure 1, b).

^δ Corresponds to atoms C8 in QD and QN (see Figure 1, a) and C12 in MQ (see Figure 1, b).

^ε Corresponds to atoms C4^γ in QD and QN (see Figure 1, a) and C4 in MQ (see Figure 1, b).

Table S9 Tentative assignment of additional atoms included in EXAFS fitting relative to atoms in the SCD structures.

Atom	QD-Fe(III)PPIX				QN-Fe(III)PPIX				MQ-Fe(III)PPIX*					
					Solid-state		Solution		I			II		
Fe	1	Fe	4.796	1	Fe	4.353	1	Fe	4.353	1	Fe1	4.566	Fe2	4.463
Cp1	3	C10	4.161	5	C39	4.148	5	C39	4.148	4	C36	3.849	C87	3.841
		C12	4.209		C46	3.751		C46	3.751		C1	3.994	N9	3.712
		C14	4.140		C11	4.182		C11	4.182		N1	3.744		
					C14	4.034		C14	4.034					
					N3	3.678		N3	3.678					
Cp2	2	C35	4.378	3	C38	4.429	2	C38	4.429	8	N6	4.448	N12	4.389
		C13	4.410		C15	4.342		C15	4.342		C2	4.533	C61	4.491
					N2	4.406					C3	4.583	C66	4.558
										N4	4.532	N8	4.526	
												N10	4.555	
Cp3	9	C41	4.629	10	C41	4.705	11	C41	4.705	7	C38	4.683	C89	4.682
		C42	4.666		C47	4.868		C47	4.868		C51	4.866	C102	4.848
		C47	4.876		C53	4.729		C53	4.729		C5	4.617	C60	4.712
		C53	4.680		N5	4.637		N5	4.637		C6	4.898	C63	4.634
		N5	4.720		C9	4.799		C9	4.799		C19	4.640	C64	4.676
		C9	4.564		C10	4.627		C10	4.627		N2	4.753	C67	4.759
		C15	4.799		C12	4.801		C12	4.801					
		C27	4.981		C13	4.696		C13	4.696					
		N2	4.659		C16	4.524		C16	4.524					
					N4	4.523		N4	4.523					
Cp4	-	-	-	-	-	-	-	-	-	2	C4	4.100		
											C20	4.385		
Cn1	3	C46	3.749	-	-	-	-	-	-	4			C62	4.047
		C11	3.792								C65	4.106		
		N3	3.756											

Atoms indicated in bold are located within the same complex as the absorbing iron(III) centre, while those shown in regular font are located in a neighbouring π -stacked complex.

* EXAFS fitting of MQ-Fe(III)PPIX required the inclusion of 25 additional atoms (of the types Cp1, Cp2, Cp3, Cp4 and Cn1). Since the recorded EXAFS spectrum is a measure of the average species present, atoms were considered from both **I** and **II**. Within the range of fitted Fe–X distances (3.8(3) – 4.74(6) Å), where X is an additional atom, a total of 30 atoms could be located relative to the absorbing iron(III) centre.

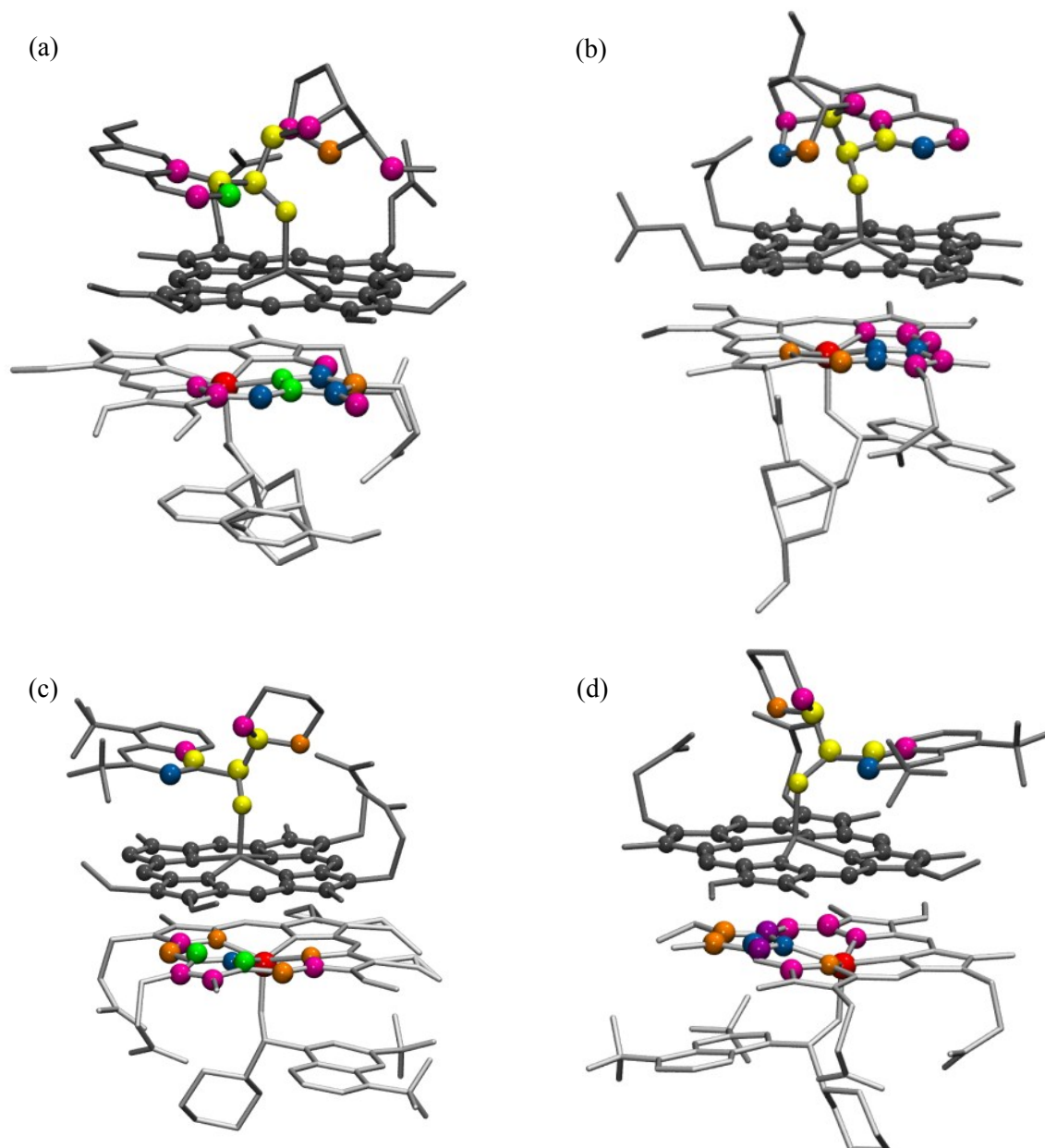


Figure S11. EXAFS models of quinoline methanol-Fe(III)PPIX coordination complexes in the solid-state. Atoms included in the EXAFS fitting of (a) QD-Fe(III)PPIX, (b) QN-Fe(III)PPIX, (c) MQ-Fe(III)PPIX (complex **I**) and (d) MQ-Fe(III)PPIX (complex **II**) are represented as spheres and overlaid with the SCD structures at the refined Fe-X (Å) distance (see Tables S7, S8 and S9). Atom colour coding: Grey – porphyrin core and yellow – explicitly-refined axial (drug) atoms (see Table S7). In the case of other axial atoms (coordinated drug) and atoms outside the complex (adjacent π -stacked porphyrin), atom colours are: blue – Cp1, orange – Cp2, pink – Cp3, purple – Cp4 and green – Cn1. These correspond to the colours used in Table S9.

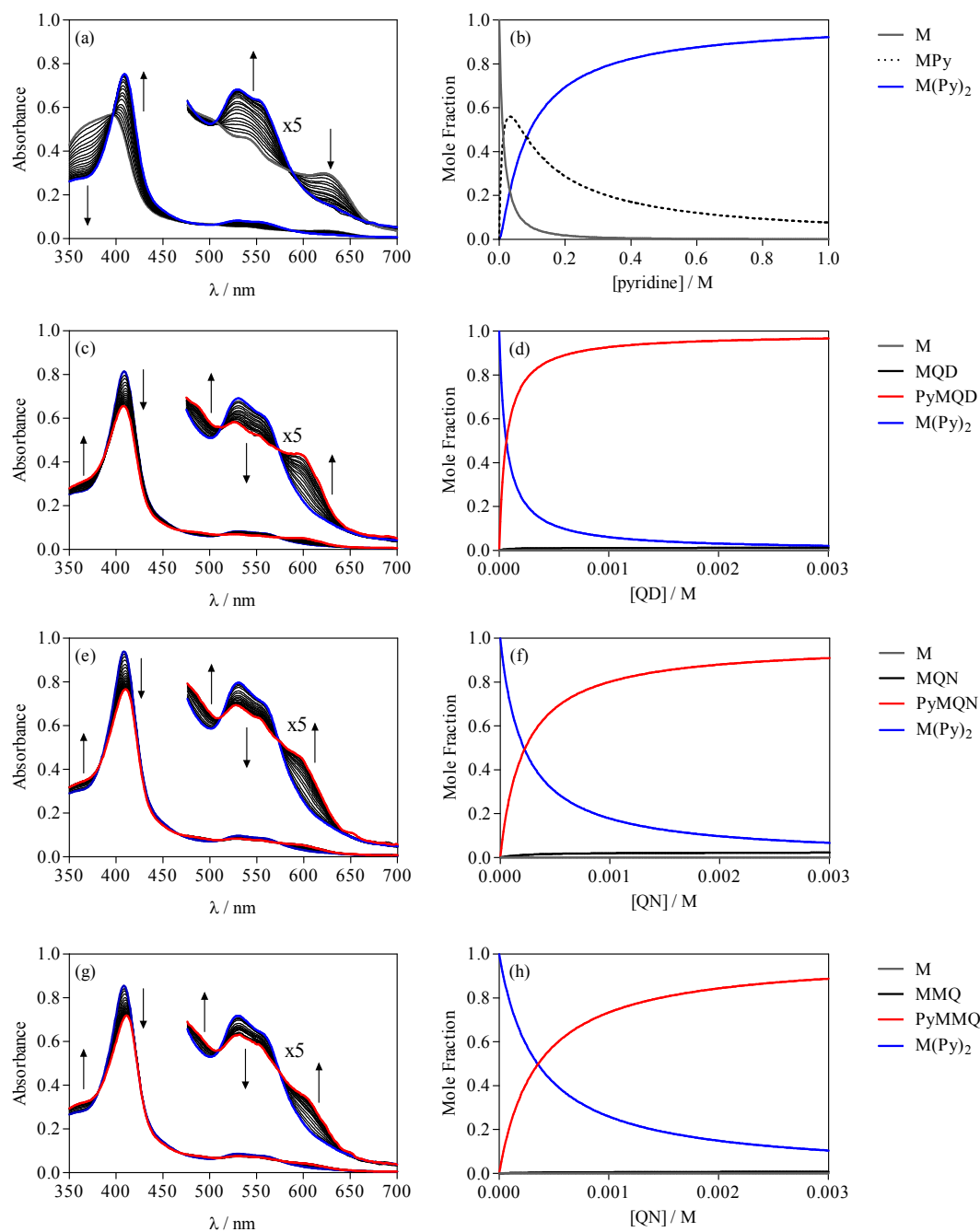


Figure S12. Spectrophotometric changes observed on titration of Fe(III)PPIX species with various ligands in acetonitrile, 25 °C. The direction of change with increasing ligand concentration is indicated with arrows. (a) The initial spectrum of Fe(III)PPIX is shown as a grey line. Increasing concentrations of pyridine result in the formation of the low-spin *bis*-pyridyl-Fe(III)PPIX complex ($M(Py)_2$, blue line.) (b) The predicted speciation plot following titration of Fe(III)PPIX with pyridine. The predicted concentration of the monopyridyl-Fe(III)PPIX species (MPy) is indicated as a dashed black line. (c, e, g) The initial spectrum of $M(Py)_2$ is shown in blue in each case. Increasing concentrations of quinoline methanol antimalarial drug (where D = quinidine (c), quinine (e), mefloquine (g)) result in the formation of six-coordinate $PyMD$ as the predominant species (red line). Experimental solutions contained 16% (v/v) pyridine. (d, f, h) The predicted speciation plots following titration of *bis*-pyridyl-Fe(III)PPIX with quinoline methanol antimalarial drug (where D = quinidine (d), quinine (f), mefloquine (h)).

Table S10. Association constants determined from spectrophotometric titration data using HypSpec analysis for Fe(III)PPIX and quinoline antimalarial drugs in acetonitrile ^a

	QD	QN	MQ
$\log \beta_4$	7.61 ± 0.07	7.32 ± 0.05	6.43 ± 0.02
$\log K_1$	4.78 ± 0.08	4.49 ± 0.06	3.60 ± 0.04
$\log K_2$	1.9 ± 0.1	1.84 ± 0.05	1.65 ± 0.08

^a Data refined using β_4 and the spectra of $PyMD$ and $M(Py)_2$ as variables.

Derivation of K_1 :

$$M(Py)_2 + D \xrightleftharpoons{K_1} PyMD + Py$$

$$K_1 = \frac{[PyMD][Py]}{[M(Py)_2][D]}$$

From eq. 4, main text:

$$[PyMD] = \beta_4[M][D][Py]$$

$$\therefore K_1 = \frac{\beta_4[M][D][Py][Py]}{[M(Py)_2][D]} = \frac{\beta_4[M][Py]^2}{[M(Py)_2]}$$

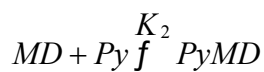
From eq. 3, main text:

$$\beta_3 = \frac{[M(Py)_2]}{[M][Py]^2}$$

$$\therefore K_1 = \frac{\beta_4}{\beta_3}$$

$$\therefore \log K_1 = \log \beta_4 - \log \beta_3$$

Derivation of K_2 :



$$K_2 = \frac{[PyMD]}{[MD][Py]}$$

From eq. 1, main text:

$$[MD] = \beta_1[M][D]$$

$$\therefore K_2 = \frac{[PyMD]}{\beta_1[M][D][Py]}$$

From eq. 4, main text:

$$\beta_4 = \frac{[PyMD]}{[M][D][Py]}$$

$$\therefore K_2 = \frac{\beta_4}{\beta_1}$$

$$\therefore \log K_2 = \log \beta_4 - \log \beta_1$$

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