EXPERIMENTAL AND THEORETICAL STUDY OF THE MECHANISM OF ACTION OF THE ANTIMALARIAL DRUG CHLOROQUINE

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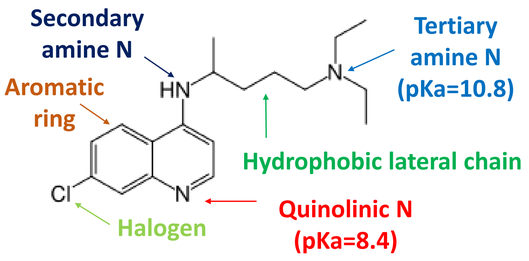
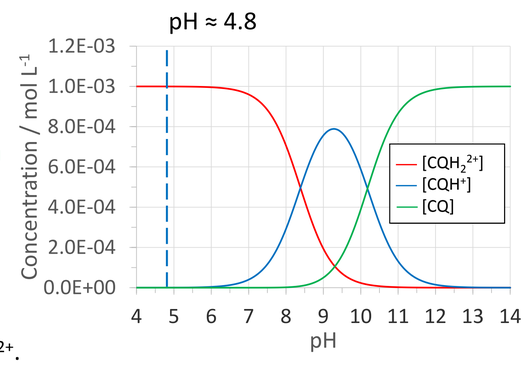
Chloroquine (CQ)





Diprotonated chloroquine (CQH_2^{2+}) is the dominant form in the parasite digestive vacuole (DV, pH ≈ 4.8). For brevity, in this work CQ=CQH_2^{2+}.

High-resolution Low-T X-ray diffraction → Solid-state Self-Recognition ← Solid and gas-phase calculations

Which are the most relevant interactions? ← **MECHANISM OF ACTION** → What is the CQ active form?

How does CQ work?

Methods

- Solid-state geometry was optimized using CRYSTAL14 (B3LYP/double-Z).
- Pair energies were evaluated on dimers extracted from the crystal through Gaussian09 (B3LYP/pob-TZVP) and PAMoC (Spackman's Experimental Charge Density Approach[1]).
- Non-covalent interactions (NCIs) were also evaluated studying the reduced density gradient (RDG [2]) through the NCImilano code[3].

Chloroquine Diphosphate Dihydrate Salt

Crystallographic data

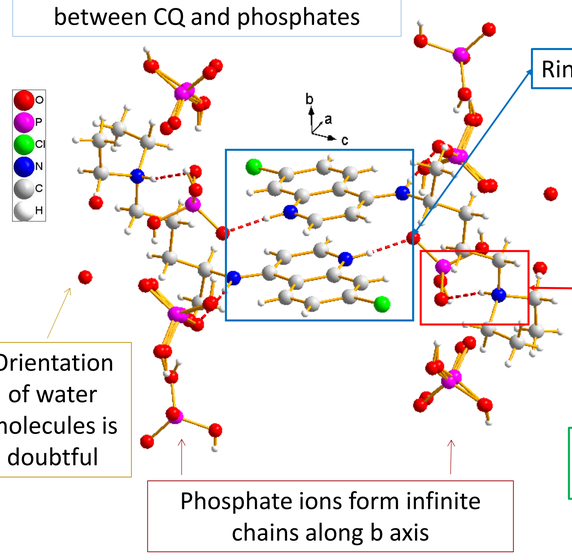
CCDC number	1471834
chemical formula	$C_{16}ClH_{38}N_3O_{10}P_2$
system	Monoclinic
space group	$P2_1/c$
Z, Z' (CQ)	4, 1
a / Å	9.7212(1)
b / Å	16.7733(2)
c / Å	15.6966(2)
β / °	105.1788(2)
V / Å³	2470.14(5)
D_x / g cm^{-1}	1.484
T / K	103(2)
λ / Å, μ / mm^{-1}	0.71073, 0.342
crystal size / mm^{-3}	0.725x0.700x0.425
reflns. collected	236057
unique reflns	20697
completeness	1.00
$\sin(\theta/\lambda)_{max}$ / A^{-1}	1.00
R_{int}	0.0277

The single crystal studied was obtained through sol-gel diffusion using water as solvent and THF as anti-solvent.

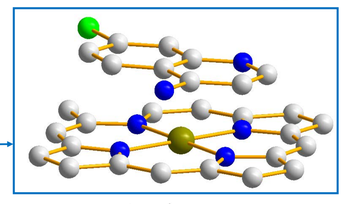
The two phosphate molecules in the asymmetric unit are independent by symmetry and one of them is disordered.

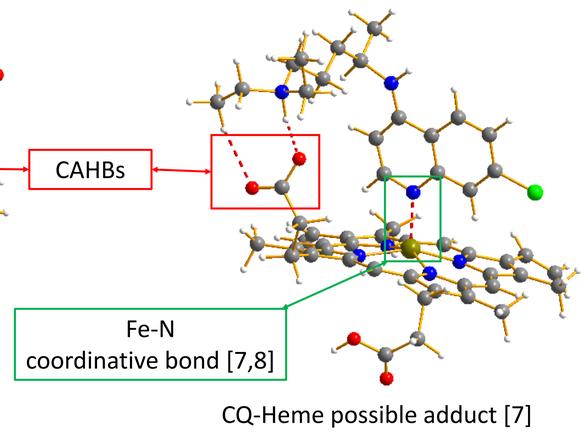
The water molecules may have a role in the stabilization of the structure, however it was not possible to determine their orientation.

Extended pattern of Charge Assisted Hydrogen Bonds (CAHBs) between CQ and phosphates



CQ-PPIX π-π interaction [5,6]





CQ-Heme possible adduct [7]

Understanding the drug:target interaction is essential to evaluate the pharmacophore and to project novel antiplasmodials.

The self-recognition of a drug is in general a good model for the drug-substrate recognition.[4]

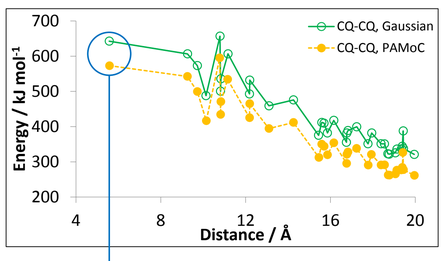
The ring-ring stacking between the CQ molecules can be considered as a model for the CQ-heme π-π interactions, proposed [5,6] as a possible mechanism of interaction.

The CAHBs between the chloroquine and the phosphate are similar to the interaction with the propionate group proposed in our recent EXAFS-DFT work.[7]

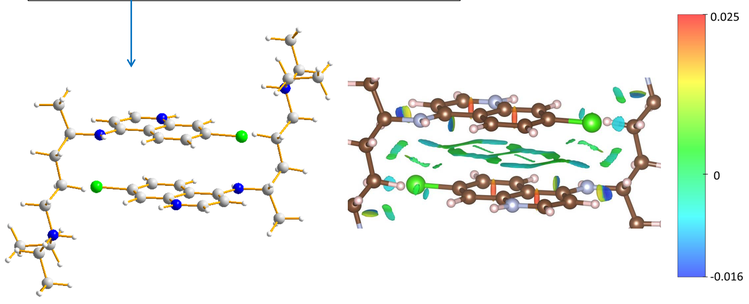
Which interactions are the most relevant?

Doubly protonated chloroquine interacts mainly with itself (CQ-CQ) and with the two symmetry independent negative phosphate ions (CQ-P1 and CQ-P2).
As expected, electrostatics dictates the crystal packing.

π-π interaction



No extra stabilization seems to come from the π-interaction



The RDG analysis shows a well shaped region between the two quinolinic rings that can be related to the interaction between the electronic π systems.
The values of $\rho \cdot \mathrm{sign}(\lambda_2)$ plotted on the RDG isosurface (0.4) are close to zero, meaning that the π-π interaction is weak and probably not structure determining.

Conclusions

1. The real dominant interaction that controls the self-recognition of the diprotonated chloroquine is electrostatic in nature.

2. The π-π bond between the quinoline rings is very weak and does not help in stabilizing the structure

⇩

The stacking with the protoporphyrin in vivo is probably not sufficiently strong to stabilize the CQ-Heme complex at acidic pH.

3. The H-bonds between the NH and the phosphate ions are quite strong and add a stabilizing contribution to the energy of the dimer

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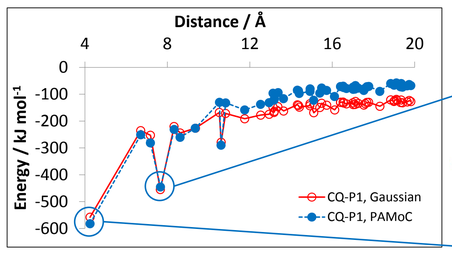
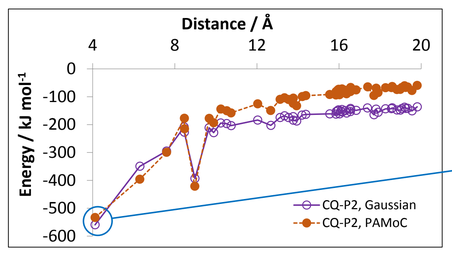
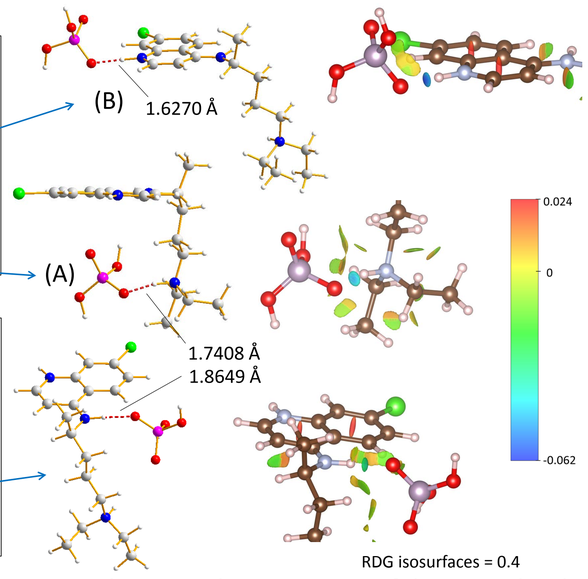
CAHBs involving the lateral chain of CQ are important features of the CQ-Heme interaction.

H-bonds







The presence of circular well-shaped RDG regions between the N-H groups of the CQ molecule and the phosphate O atoms is characteristic of the hydrogen bonds.
The negative values of $\rho \cdot \mathrm{sign}(\lambda_2)$ plotted on the RDG isosurfaces (0.4) confirm that these interactions are strongly attractive.
The study of the RDG is able to reconstruct the ranking of the H-bonds strength: the more negative is the value of $\rho \cdot \mathrm{sign}(\lambda_2)$, the stronger is the interaction. The most negative $\rho \cdot \mathrm{sign}(\lambda_2)$ value is found for the quinolinic N, while the highest one is near the non-acidic N (the secondary amine group).

Energy Decomposition

The decomposition of the energy reveals that the electrostatic term is the most important contribution; the coulombic interactions have a dominant role in determining the dimers energies and then the crystal packing.
The energy of the hydrogen bonds was estimated through the NBO analysis, using the formula: $E^2 = q_i \cdot \frac{F(i,j)^2}{\epsilon_i - \epsilon_j}$

	E_{total}	$E_{repulsion}$	$E_{dispersion}$	$E_{electrostatic}$	E_{NH-O}	$\%E_{NH-O}$
CQ-CQ	573.03	83.53	-80.95	570.46	/	/
CQ-P1(A)	-582.16	113.39	-32.90	-662.65	-123.72	21.2
CQ-P1(B)	-445.36	124.86	-20.31	-549.91	-168.31	37.8
CQ-P2	-533.32	83.65	-29.11	-587.86	-58.81	11.0

(a) All the energies are expressed in kJ mol^{-1}

The repulsion and dispersion terms in the CQ-CQ stacking interactions delete each other, confirming that possible π- π interaction are very weak.
The H-bond energies and lengths trends confirm the result obtained analyzing the reduced density gradient (RDG).

	$E_{I=0}$	E_{Ring}	E_{Chain}	% Ring	% Chain	E_{NH}	% NH
CQ-P1 (A)	-546.12	-212.87	-333.26	39.0	61.0	37.28	-6.8
CQ-P1 (B)	-456.43	-368.47	-87.96	80.7	19.3	-31.39	6.9
CQ-P2	-522.66	-214.82	-307.84	41.1	58.9	60.89	-11.7

(a) All the energies are expressed in kJ mol^{-1}

About the 82-89% of the electrostatic contributions arise from the monopole (l=0) term.
The CQ-P1 (B) has a lower energy than the other two dimers because the distance from the lateral chain is high and then its electrostatic contribution is not so relevant.

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