STUDY OF THE KEY INTERACTIONS IN THE SELF-RECOGNITION OF THE ANTIMALARIAL DRUG CHLOROQUINE

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Malaria

Malaria is one of the most virulent parasitic diseases.

Tropicalization and Globalization are expected to increase the malaria spreading, also in developed countries.

Roger, D. J. et al. Science 2000, 289, 1763-1766
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Italy, 1900
P. falciparum Resistance

P. falciparum has developed a resistance to nearly all antimalarials.

NEW ACTIVE MOLECULES ARE REQUIRED!!!
Chloroquine (CQ)

It is believed that CQ interferes with the detoxification process of the heme in digestive vacuole of the parasite (DV).

In the digestive vacuole (pH ≈ 4.8-5.0), the chloroquine is almost completely protonated (CQH$_2^{2+}$).

Detoxification mechanism

**TOXIC (ROS)**

- Quinoline-Protoporphyrin $\pi-\pi$ interactions [1,2]
- Charge Assisted Hydrogen Bond (CAHB) between CQ lateral chain and heme propionate [3]
- Fe-N coordinative bond [3,4]

**NOT TOXIC**

- Dimerization
- Crystallization

**Heme**

$$\text{Heme}$$

$$\text{DV} \quad (\text{pH} \approx 4.8-5.0)$$

$$\text{Hemozoin}$$

Self-Recognition

The study of the self-recognition features is in general a good model to understand the drug-substrate molecular interaction.

The investigation of the solid-state structure of the diprotonated chloroquine $\text{CQH}_2^{2+}$ can disclose which are the real dominant interactions.

**METHODS**

- Single crystal
- Ab-initio structure
- Experimental structure
- low-T high-resolution diffraction
- CRYSTAL14
- XD2006

**Interactions**

- Pairs energy analysis
- NCI (RDG)
- QTAIM


Chloroquine diphosphate dihydrate salt

\[ T = 103 \text{ K} \]
\[ \sin(\theta/\lambda)_{\text{max}} = 1.0 \text{ Å}^{-1} \]
\[ \lambda = 0.71073 \text{ Å} \]
\[ \text{CCDC number} = 1471834 \]

Space Group = \( P2_1/c \) (14)
\[ a = 9.7212(1) \text{ Å} \]
\[ b = 16.7733(2) \text{ Å} \]
\[ c = 15.6966(2) \text{ Å} \]
\[ \beta = 105.1788(2) \text{ Å} \]
\[ V = 2470.14(5) \text{ Å}^3 \]

Disorder and diffuse scattering at 103 K

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Infinite phosphate chains along the b axis
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Infinite phosphate chains along the b axis

Symmetry independent

Disorder
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CQ rings perpendicular to phosphate chain
Chloroquine diphosphate dihydrate salt

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Ring-Ring stacking

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Extended pattern of H-bonds between CQ and phosphates
Chloroquine diphosphate dihydrate salt

GOOD MODEL!

Extended pattern of H-bonds between CQ and phosphates

CAHBs
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Orientation and position of the water molecules is doubtful

Experimental Charge Density
Ab-initio Structure

- CRYSTAL14
- B3LYP/Double-ζ level of theory
- Grimme dispersion correction
- P2₁/c symmetry
- Cell parameters kept fixed at the experimental values; atomic coordinates fully relaxed

Bad agreement for the water molecules (shift ≈ 0.8 Å)

Good agreement for the chloroquine and phosphates molecules (RMSD = 0.014)

The water molecules are not significant in determining the intermolecular recognition pattern of CQH₂²⁺

Intermolecular interactions

Single-Point (DFT B3LYP/pob-TZVP) of pairs extracted from the crystal

- Spackman’s Experimental Charge Density Approach (ECDA)
- QTAIM analyses

3 pairs types:
• CQ-CQ
• CQ-P1
• CQ-P2

Molecular pairs with centre-of-mass distances up to 20 Å

CQ-CQ, Gaussian
CQ-CQ, PAMoC

CQ-CQ interactions have always positive energies

Expected? **YES**

We have something close to:

\[ F_{++} \rightarrow F_{++} \]

Rigid shift $63 \pm 4$ kJ/mol

Intermolecular interactions

CQ-P1 and CQ-P2 pairs have always negative energies

As expected, the crystal cohesion is dominated by the Coulombic interactions between CQ (+2) and the phosphates P1 and P2 (-1)
Intermolecular interactions

CQ-P1 and CQ-P2 pairs have always negative energies

As expected, the crystal cohesion is dominated by the Coulombic interactions between CQ (+2) and the phosphates P1 and P2 (-1)
NH···O bonds are involved in all these pairs.
The electrostatic terms (in particular the def-def contributions) give the highest contributions to the total energies

The 82-89% of the def-def values arise from the monopole terms (l=0)

Pauli repulsions overcome the dispersion contribution; the increasing trend from pair I to III is related to the NH···O bond lengths
Pairs I-II-III - NH···O bonds

NH···O bonds contributions?

<table>
<thead>
<tr>
<th>Pair</th>
<th>$E_{DFT}$</th>
<th>$E_{NH···O}$</th>
<th>$E_{NH···O} %$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (CQ-P2)</td>
<td>-559.58</td>
<td>-54.85</td>
<td>9.8</td>
</tr>
<tr>
<td>II (CQ-P1)</td>
<td>-557.95</td>
<td>-115.39</td>
<td>20.7</td>
</tr>
<tr>
<td>III (CQ-P1)</td>
<td>-454.96</td>
<td>-156.98</td>
<td>34.5</td>
</tr>
</tbody>
</table>

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

The contributions of the hydrogen bonds are particularly strong in pairs II and III.

CAHBs
## Pairs I-II-III - NH⋯O bonds

### NH⋯O bonds contributions?

#### QTAIM analysis

<table>
<thead>
<tr>
<th>Contact</th>
<th>(d_{H⋯O}/\text{Å})</th>
<th>(\rho_{\text{bcp}}/\text{e}\cdot\text{bohr}^{-3})</th>
<th>(\nabla^2\rho_{\text{bcp}}/\text{e}\cdot\text{bohr}^{-5})</th>
<th>(G_{\text{bcp}}(\text{a}))</th>
<th>(V_{\text{bcp}}(\text{a}))</th>
<th>((H_{\text{bcp}}/\rho_{\text{bcp}})/\text{au})</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (CQ-P2)</td>
<td>N9–H9⋯O5</td>
<td>1.8674</td>
<td>0.031</td>
<td>0.102</td>
<td>65.2</td>
<td>-63.6</td>
</tr>
<tr>
<td>II (CQ-P1)</td>
<td>N14–H14⋯O4</td>
<td>1.7419</td>
<td>0.045</td>
<td>0.115</td>
<td>92.5</td>
<td>-109.3</td>
</tr>
<tr>
<td>III (CQ-P1)</td>
<td>N1–H1⋯O1</td>
<td>1.6279</td>
<td>0.056</td>
<td>0.129</td>
<td>115.2</td>
<td>-145.5</td>
</tr>
</tbody>
</table>

(a) Expressed in kJ mol\(^{-1}\) Å\(^{-3}\)

The point topological analysis at bcp confirms the previous results

#### NH⋯O bonds strength

![NH⋯O bonds strength](image)

The negative values of the bond degree parameter \((H_{\text{bcp}}/\rho_{\text{bcp}})\) in the two bcp of pairs II and III, in conjunction with \(\nabla^2\rho_{\text{bcp}} > 0\), can be related to a partial covalence character of NH⋯O bonds. [1]

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Pairs I-II-III - NH···O bonds

NH···O bonds contributions?

... Again ...

The Non-Covalent Interactions (NCIs) evaluated through the study of the Reduced Density Gradient (RDG) [1] give a non local landscape of the interatomic interactions [2]

$$\text{RDG } s(r) = \frac{|\nabla \rho(r)|}{2(3\pi^2)^{1/3} \rho(r)^{4/3}}$$

Software: NCI milano [3]

Mapping the value of $\rho \cdot \text{sign}(\lambda_2)$ on RDG isosurfaces highlight the nature and the strength of the interactions:

$\lambda_2 > 0 \rightarrow$ Repulsive interaction  \hspace{1cm} |$\rho \cdot \text{sign}(\lambda_2)$| proportional to the strength of the interaction

$\lambda_2 < 0 \rightarrow$ Attractive interaction

Pairs I-II-III - NH···O bonds

Circular well-shaped RDG regions are typical for H-bonds

STRONG INTERACTIONS!

RDG isosurfaces = 0.4

ρ·sign(λ₂)
CQ-CQ Pairs

Quinoline ring-ring stacking

What about $\pi$-$\pi$ interaction?
CQ-CQ - Energy

π-π interaction?

<table>
<thead>
<tr>
<th>Pair</th>
<th>E_{DFT}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CQ-CQ</td>
<td>642.66</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pair</th>
<th>E_{Total}</th>
<th>E_{Repulsion}</th>
<th>E_{Dispersion}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CQ-CQ</td>
<td>573.03</td>
<td>83.53</td>
<td>-80.95</td>
</tr>
</tbody>
</table>

Electrostatic Energy

<table>
<thead>
<tr>
<th>E_{total}</th>
<th>E_{Pro-Pro}</th>
<th>E_{Pro-Def}</th>
<th>E_{Def-Def}</th>
</tr>
</thead>
<tbody>
<tr>
<td>570.46</td>
<td>-42.9</td>
<td>-1.02</td>
<td>614.38</td>
</tr>
</tbody>
</table>

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

As for CQ-P interactions, electrostatics energy gives the highest contribution

Two positive charges (+2) very close to each other

The dispersion and the repulsion energies delete each other

Apparently no extra contribution due to π-π interactions
Atomic interaction lines (AILs) found between the rings atoms (C,N and Cl) and between the lateral chain and the chlorines

YES, they are present!
CQ-CQ - QTAIM

π-π contribution?

<table>
<thead>
<tr>
<th>Contact</th>
<th>$d_{x\cdots y}$ / Å</th>
<th>$\rho_{bcp}$ / e·bohr$^{-3}$</th>
<th>$\nabla^2 \rho_{bcp}$ / e·bohr$^{-5}$</th>
<th>$G_{bcp}$</th>
<th>$V_{bcp}$</th>
<th>BD / au</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3···Cl</td>
<td>3.4497</td>
<td>0.007</td>
<td>0.022</td>
<td>12.4</td>
<td>-10.1</td>
<td>0.13</td>
</tr>
<tr>
<td>C6···N1</td>
<td>3.4857</td>
<td>0.005</td>
<td>0.021</td>
<td>10.1</td>
<td>-6.6</td>
<td>0.25</td>
</tr>
<tr>
<td>C4A···C8</td>
<td>3.5204</td>
<td>0.004</td>
<td>0.019</td>
<td>8.6</td>
<td>-5.0</td>
<td>0.32</td>
</tr>
<tr>
<td>C12–H12A···Cl</td>
<td>3.0079</td>
<td>0.007</td>
<td>0.021</td>
<td>11.5</td>
<td>-9.4</td>
<td>0.12</td>
</tr>
<tr>
<td>C17–H17B···Cl</td>
<td>3.2650</td>
<td>0.003</td>
<td>0.010</td>
<td>5.4</td>
<td>-4.2</td>
<td>0.13</td>
</tr>
<tr>
<td>C10–H10···Cl</td>
<td>3.3902</td>
<td>0.003</td>
<td>0.011</td>
<td>5.4</td>
<td>-3.7</td>
<td>0.23</td>
</tr>
</tbody>
</table>

(a) Expressed in kJ mol$^{-1}$Å$^{-3}$

$\rho_{bcp}$ values are one order of magnitude smaller than all the NH···O bonds

The BD is always positive

The interactions between the two rings are weak
CQ-CQ - NCI

π-π contribution?

WEAK INTERACTIONS!

ρ·sign(λ₂)
Conclusions

• The chloroquine diphosphate dihydrate salt is a good model for CQ:Heme interaction in solution:

  - Diphosphate Salt  - With Heme
    1. CQ-CQ ring stacking  \( \pi-\pi \) interactions
    2. CAHBs with the phosphates  CAHBs with the propionate group

• Electrostatics determines top ranking features in energy;
• The CAHBs give relevant contributions to the interaction energy;
• In the crystal, \( \pi-\pi \) interactions are very weak and we believe that it is improbable that in aqueous environment they are structure-driving in CQ-Heme recognition
Aknowledgement

My research group:

Dr. Leonardo Lo Presti
Prof. Silvia Rizzato
Dr. Laura Loconte
Dr. Carlo Gatti
Thank You
For Your Attention!
Chloroquine diphosphate dihydrate salt

Zig-zag arrangement of the water O atoms between different phosphate chains

Frustration of the Hydrogen Bonds

P2$_1$/c symmetry is not “completely exact”

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Intermolecular interactions

Fitting the data with $d > 10 \, \text{Å}$, $E \propto 1/d$ (Coulomb law)

Deviations at $d < 10 \, \text{Å}$ are probably due to specific intermolecular contact or aspherical electrostatic features

The top tanking pairs in energy are labeled with roman numerals in order of energy.