UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI CHIMICA

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

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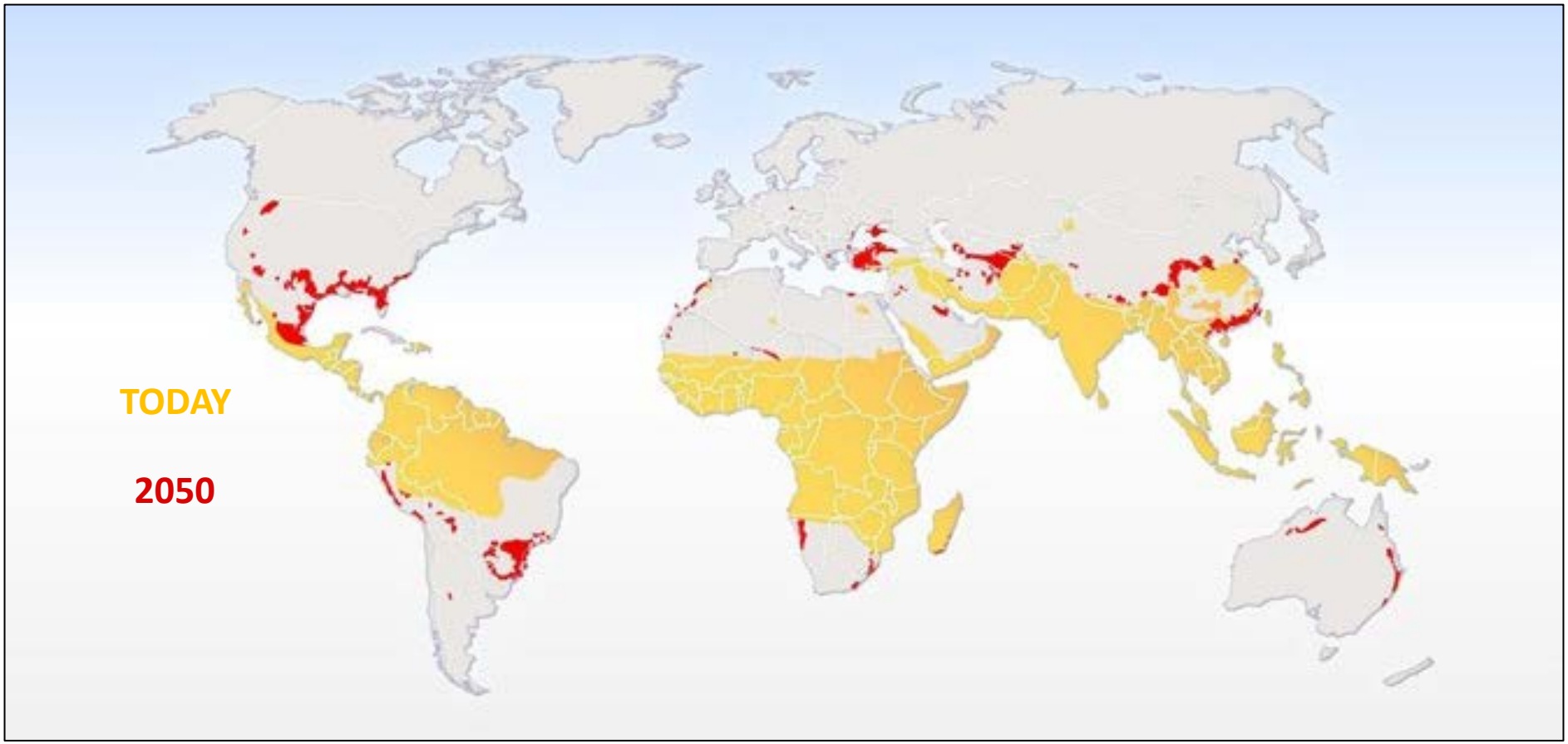
ECDM-VII, Warsaw

Malaria

Malaria is one of the most virulent parasitic disease

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



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Siraj, A.S. *et al. Science* **2014**, *343*, 1154-1158
Roger, D. J. et al. Science **2000**, *289*, 1763-1766

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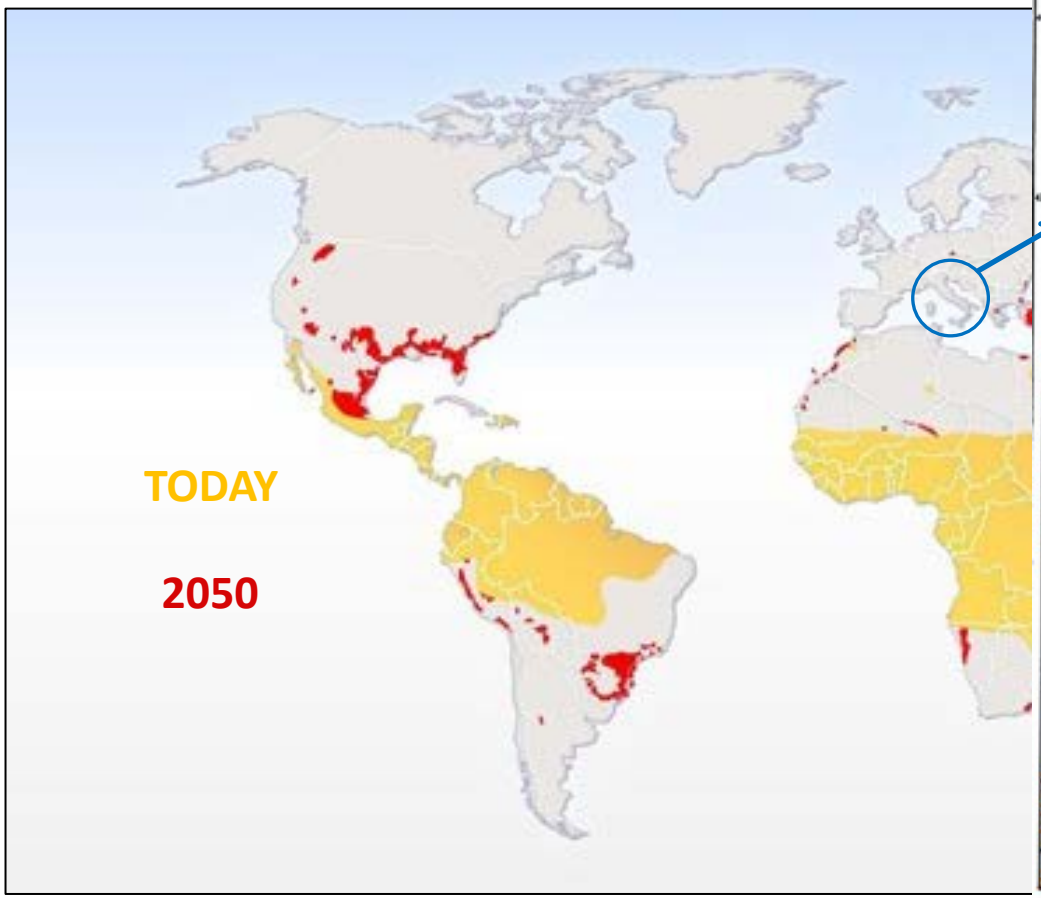

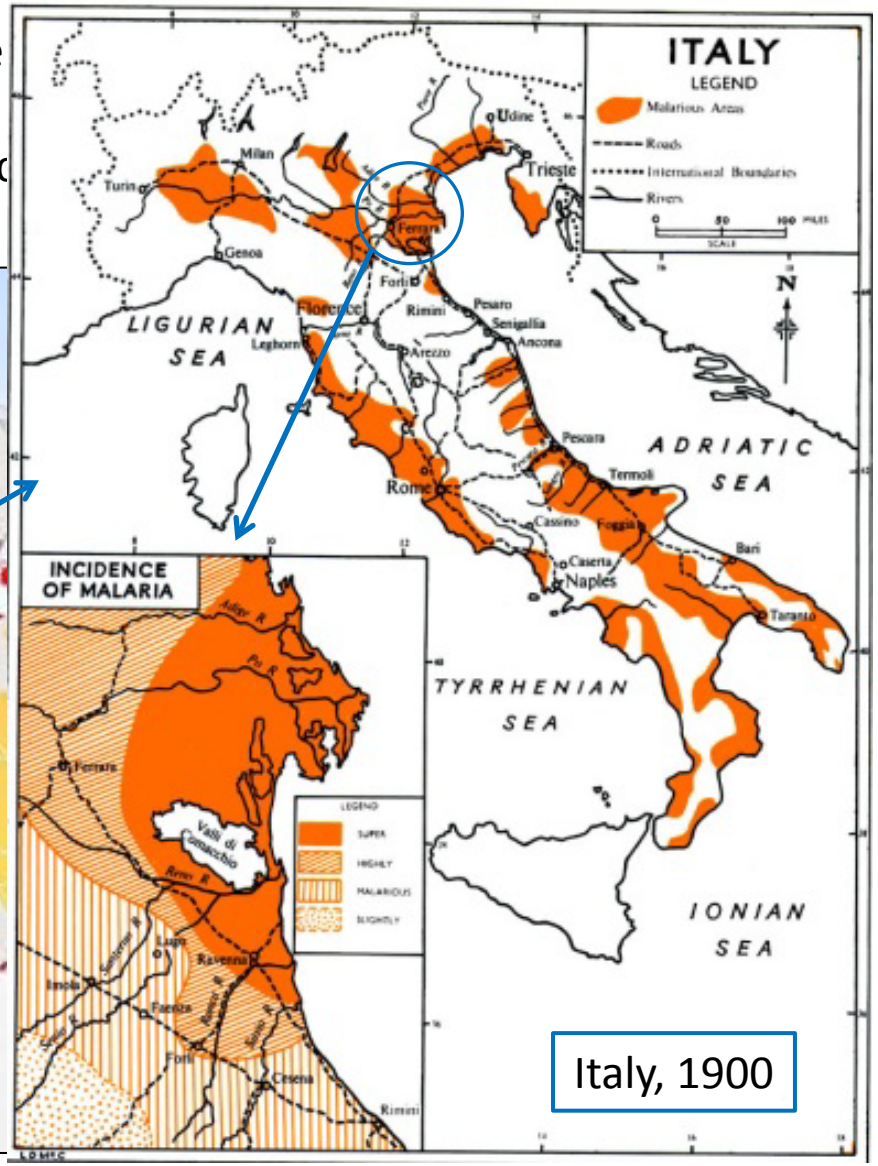

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Siraj, A.S. *et al. Science* **2014**, *343*, 1154-1158
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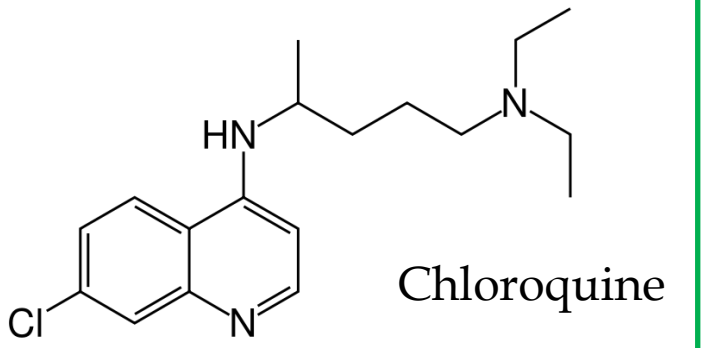
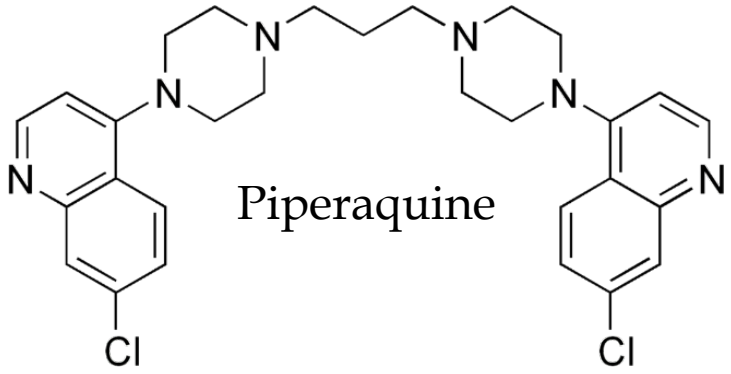
P. falciparum Resistance

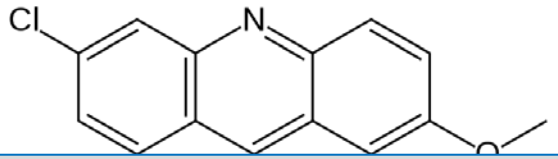


Chloroquine

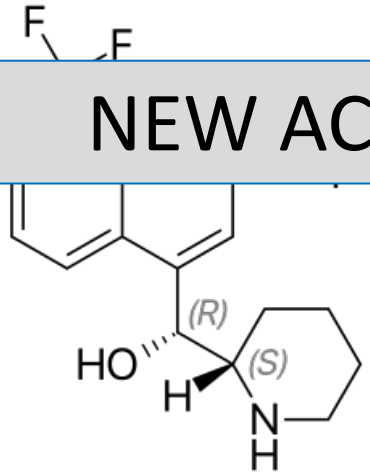


Piperaquine

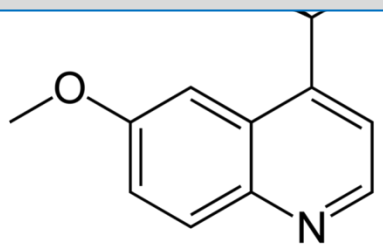
P. falciparum has developed a resistance to nearly all antimalarials



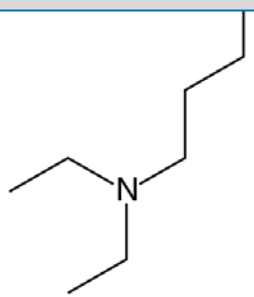
NEW ACTIVE MOLECULES ARE REQUIRED!!!



Mefloquine



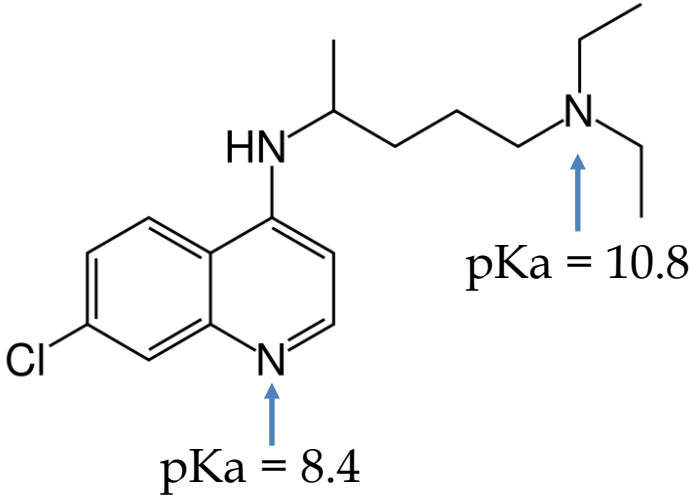
Quinine



Atabrine

White,N.J. *J.Clin.Inv.* **2004**, *113*, 1084-1092

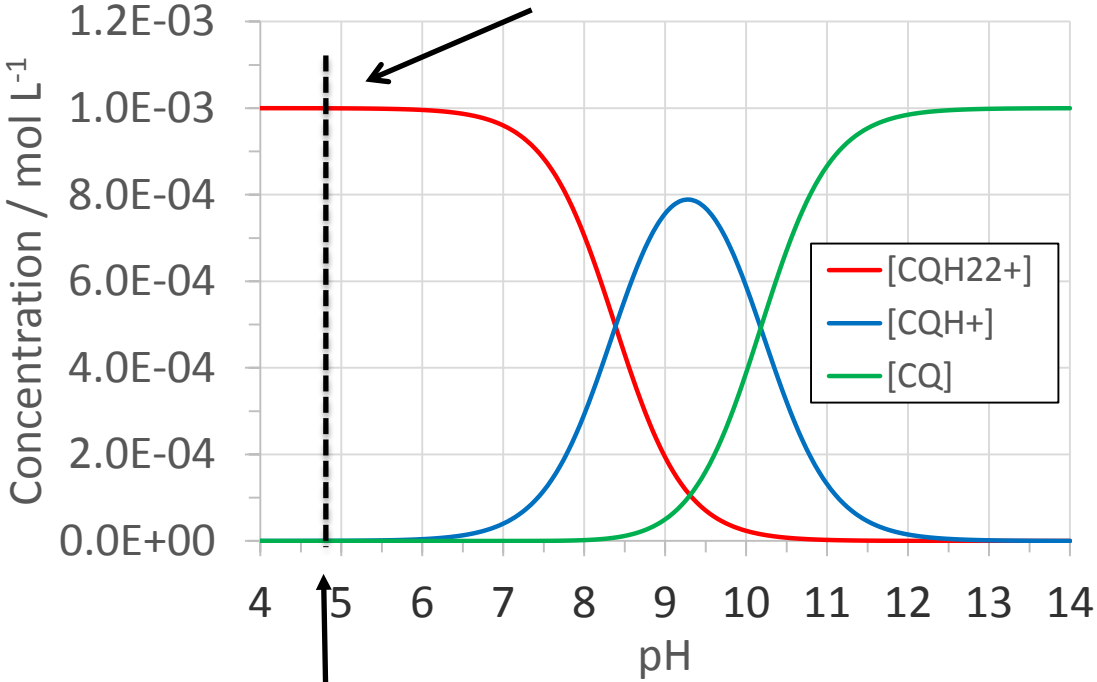
Chloroquine (CQ)



- Cheap
- Easily available
- Low side effect
- Stronger than quinine

Good starting point for novel low-cost antiplasmodial

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

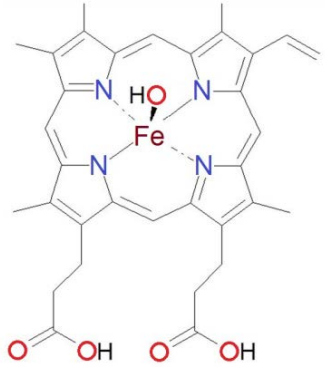


In the digestive vacuole (pH ≈ 4.8-5.0), the chloroquine is almost completelly protonated (CQH_2^{2+})

Slater *et al. Proc. Natl. Acad. Sci. USA* **1991**, *88*, 325

Detoxification mechanism

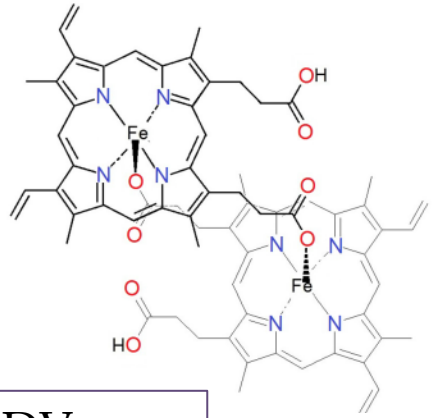
TOXIC (ROS)



Heme

Dimerization

CQ

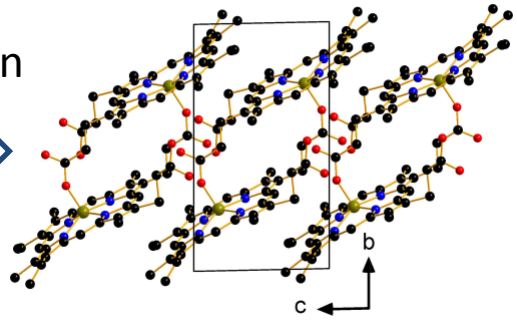


DV
(pH ≈ 4.8-5.0)

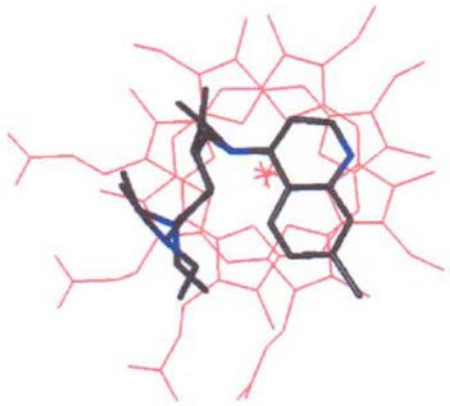
Crystallization

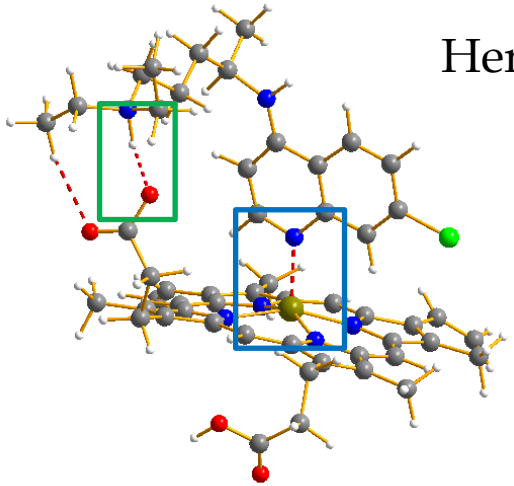
CQ

NOT TOXIC



Hemozoin





- Quinoline-Protoporphyrin π-π interactions [1,2]

- Fe-N coordinative bond [3,4]

- Charge Assisted Hydrogen Bond (CAHB) between CQ lateral chain and heme propionate [3]

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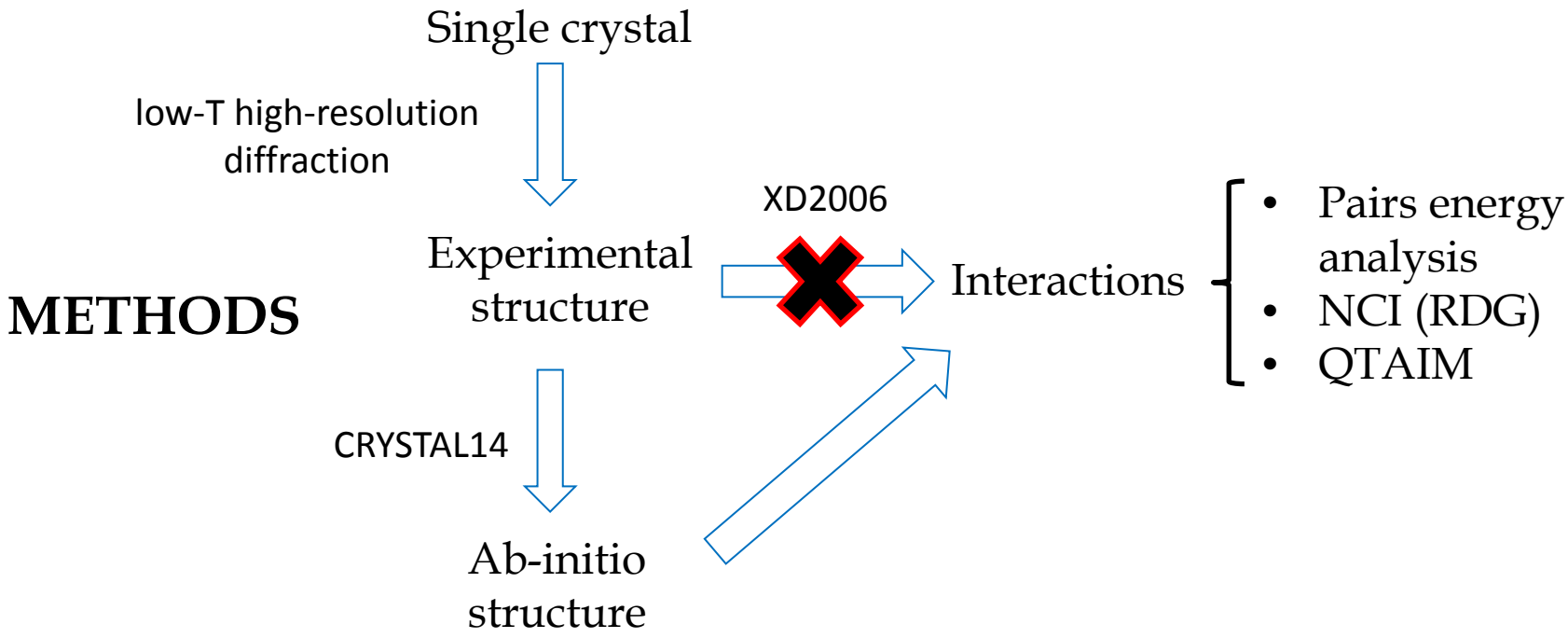
[1] Walczak *et al. J. Phys. Chem. B* **2011**, *115*, 1145; [2] Leed *et al. Biochem.* **2002**, *41*, 10245 ; [3] Macetti et al. *Phys. Scripta* **2016**, *91*,023001; [4] De Dios et al. *J. Phys. Chem. A* **2003**, *107*, 5821

Self-Recognition

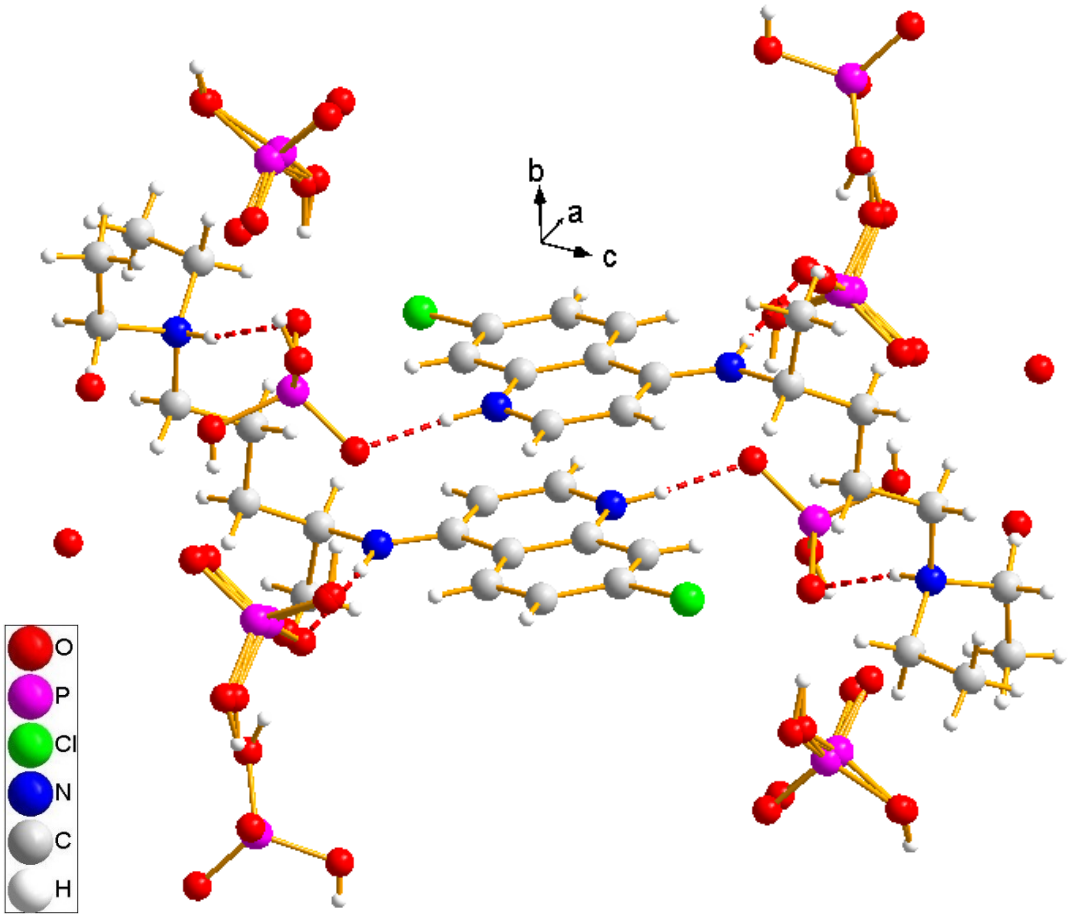
The study of the **self-recognition** features is in general a good model to **understand** the **drug-substrate** molecular interaction

The investigation of the **solid-state structure** of the diprotonated chloroquine **CQH_2^{2+}** can disclose which are the **real dominant interactions**



Destro, R. *et. al. Chem. Eur. J.* **2005**, 11, 4621– 4634.
Politzer, P. *et al. Int. J. Quantum Chem.* **2001**, 85, 676 –684.

Chloroquine diphosphate dihydrate salt



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

Space Group = $P2_1/c$ (14)
a = 9.7212(1) Å
b = 16.7733(2) Å
c = 15.6966(2) Å
β = 105.1788(2) Å
V = 2470.14(5) Å

Disorder and diffuse scattering at 103 K

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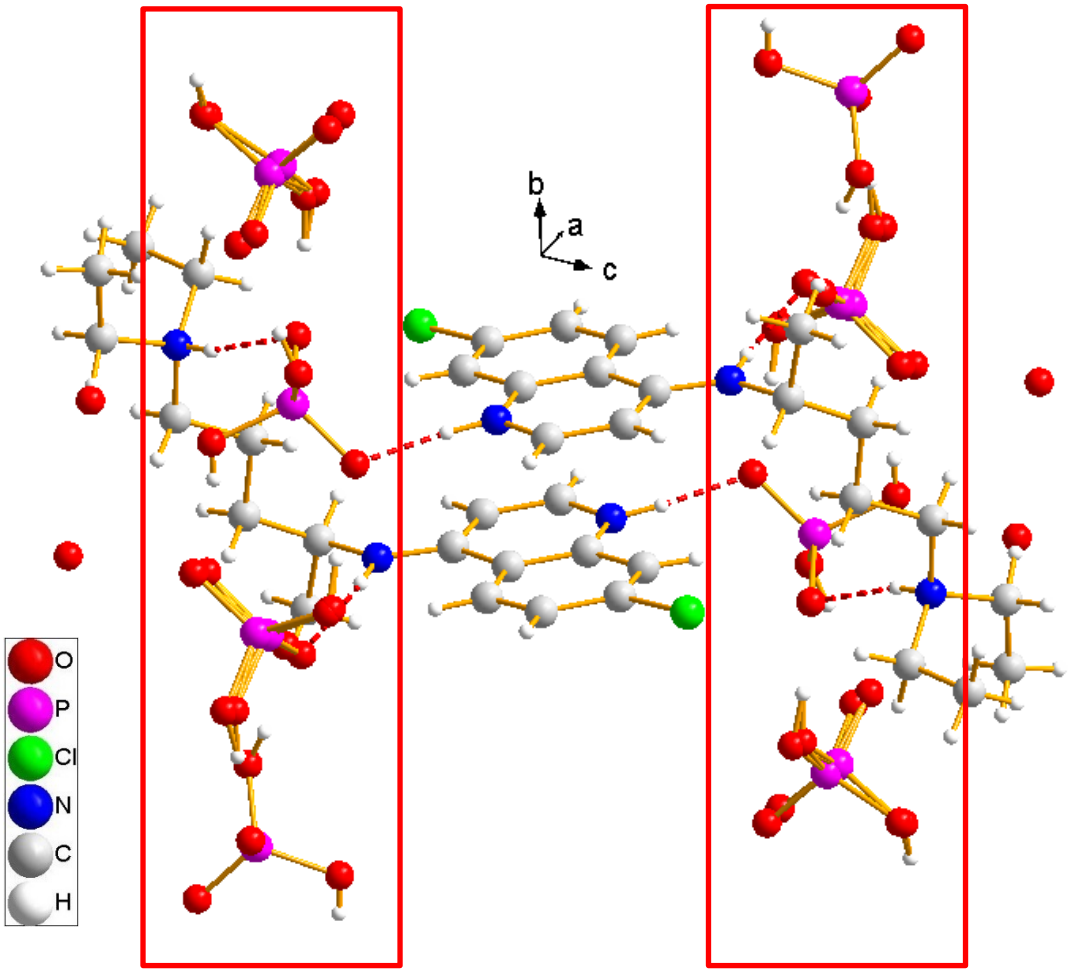
Karle, J. M. *et al Acta Cryst. C* **1988**, *44*, 1605-1608

Chloroquine diphosphate dihydrate salt



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Infinite phosphate chains along the b axis

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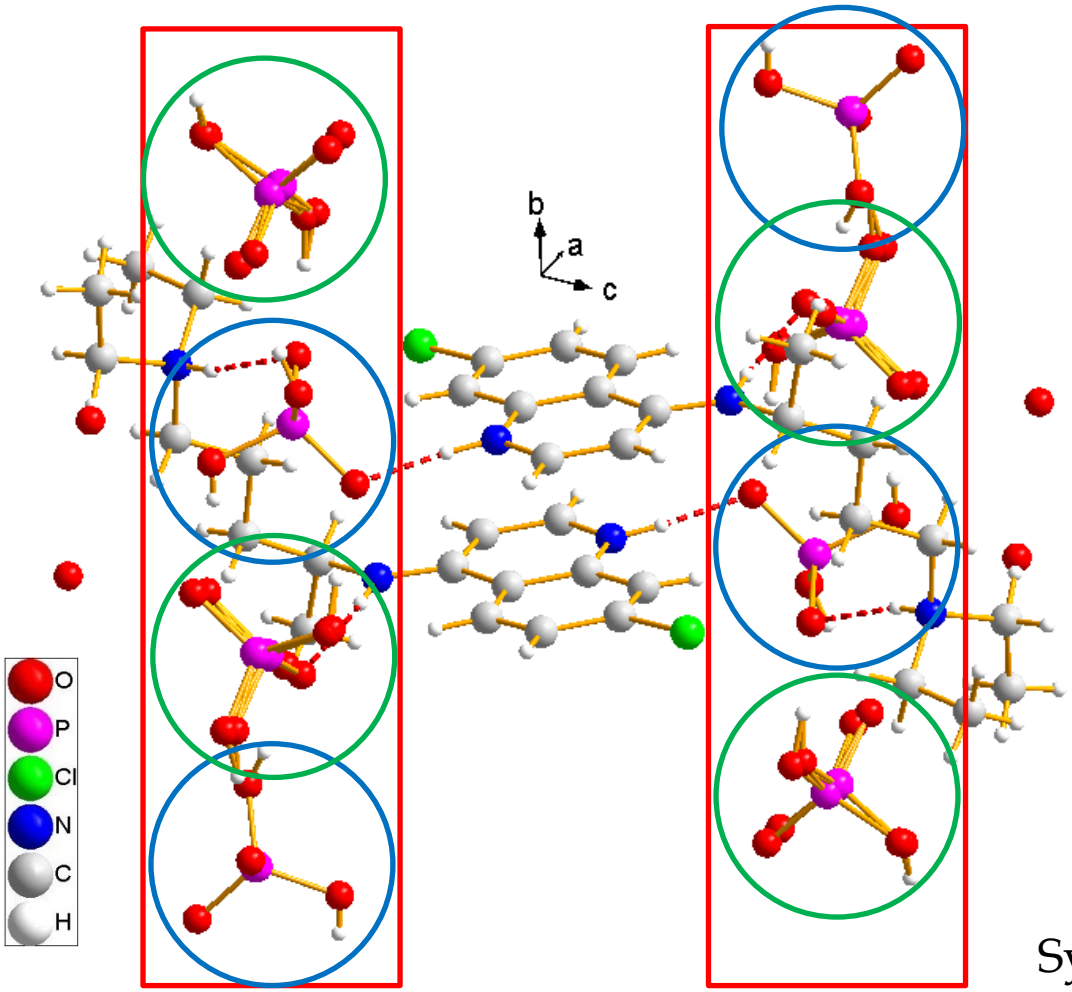

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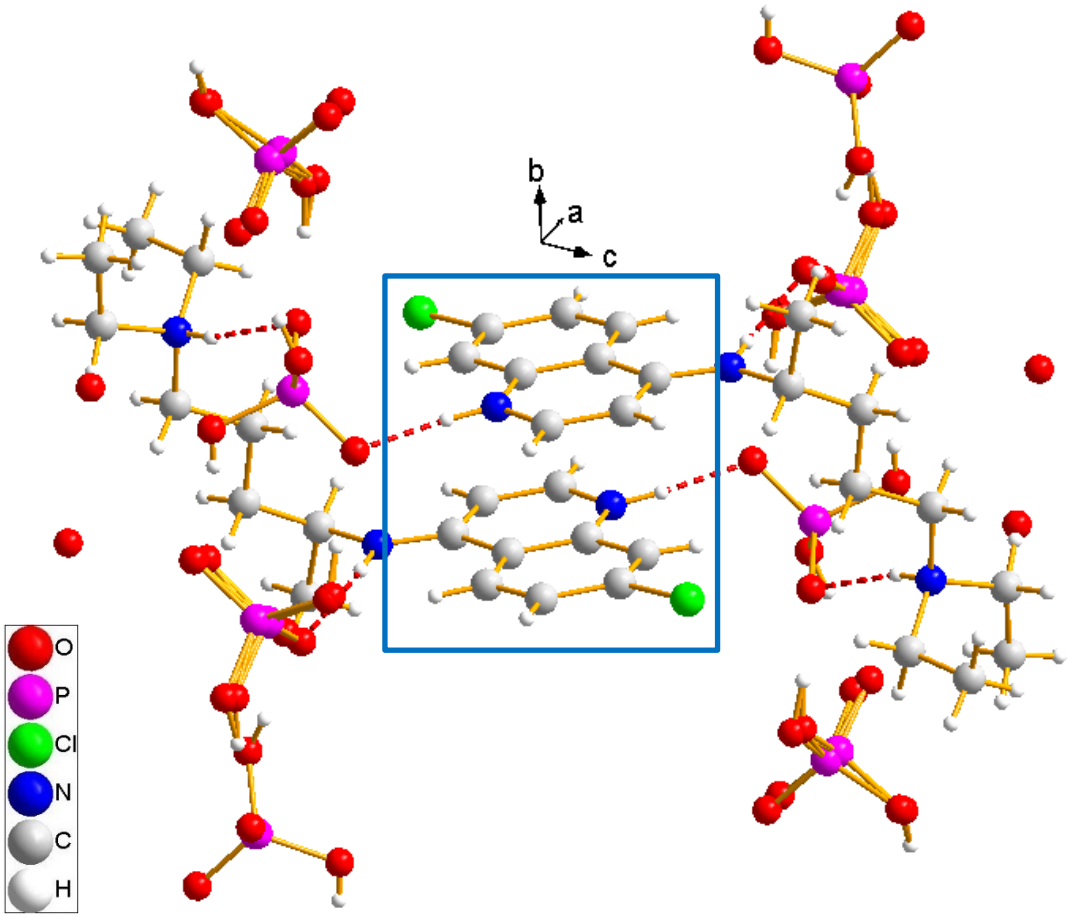
Infinite phosphate chains along the b axis

Symmetry independent: P1, P2 — Disorder

Chloroquine diphosphate dihydrate salt



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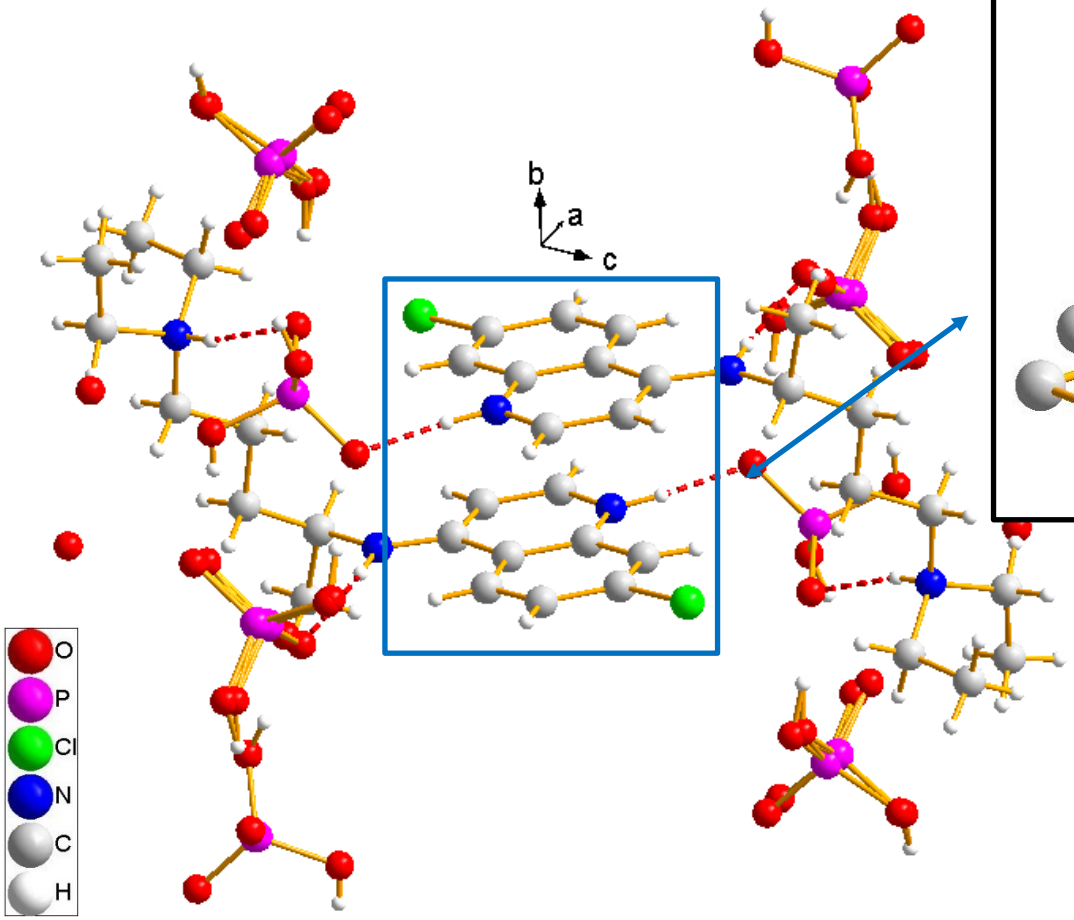
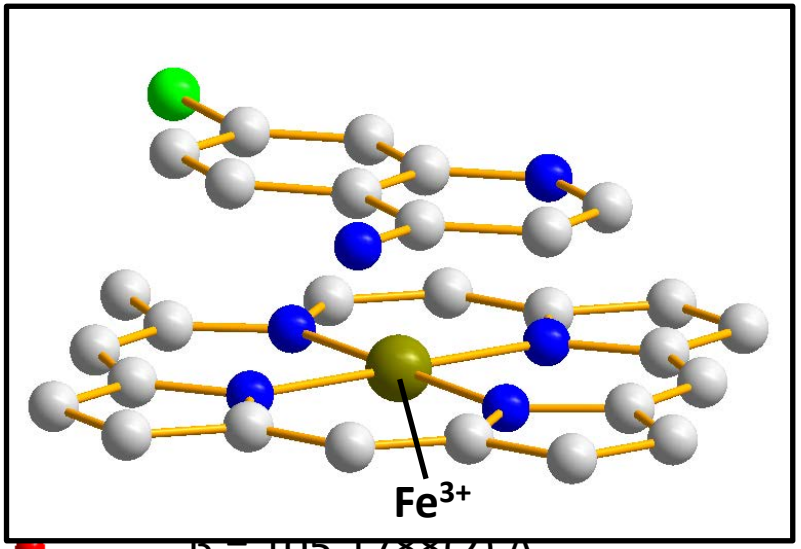
CQ rings perpendicular to phosphate chain

Chloroquine diphosphate dihydrate salt





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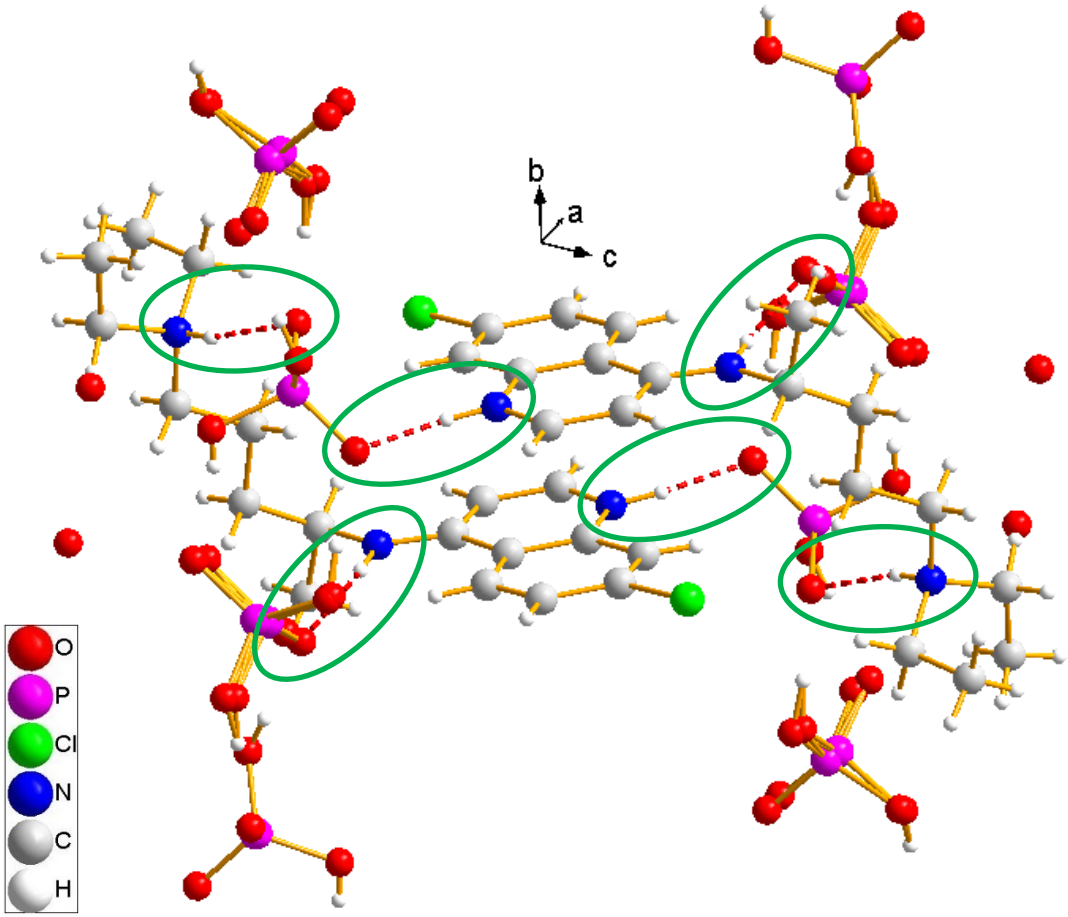
CQ rings perpendicular to phosphate chain

! Ring-Ring stacking !

Chloroquine diphosphate dihydrate salt



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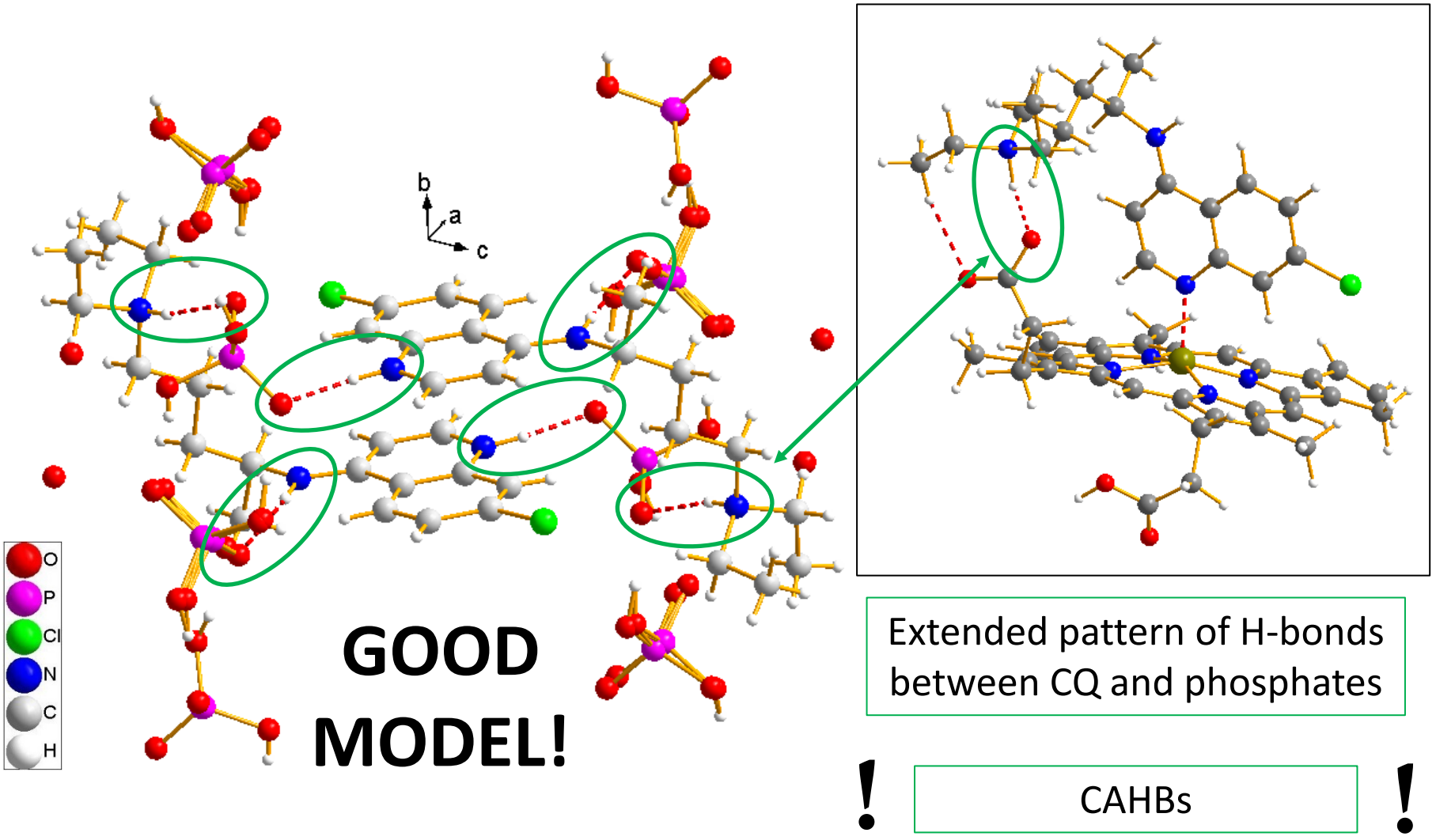
Extended pattern of H-bonds between CQ and phosphates

Chloroquine diphosphate dihydrate salt



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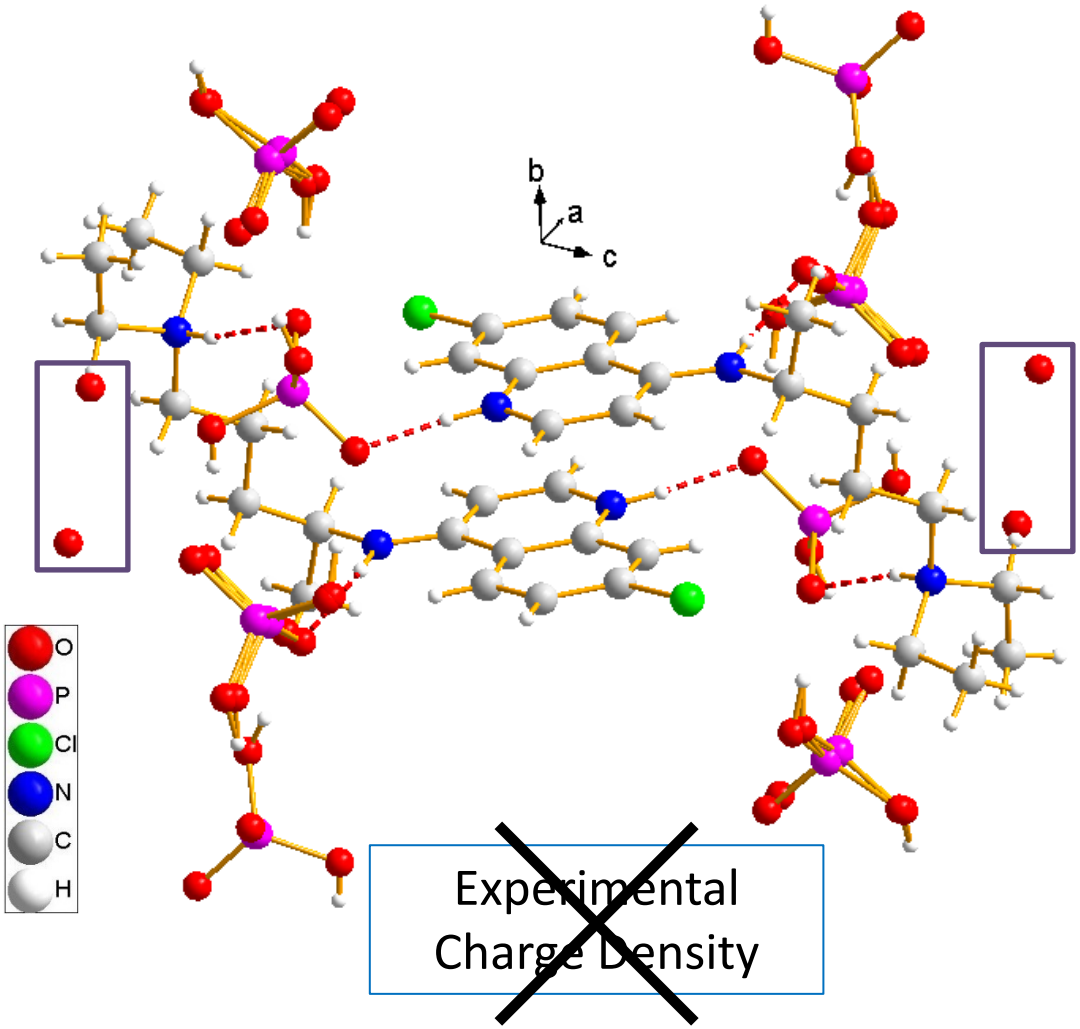

Experimental Charge Density

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Orientation and position of the water molecules is doubtful

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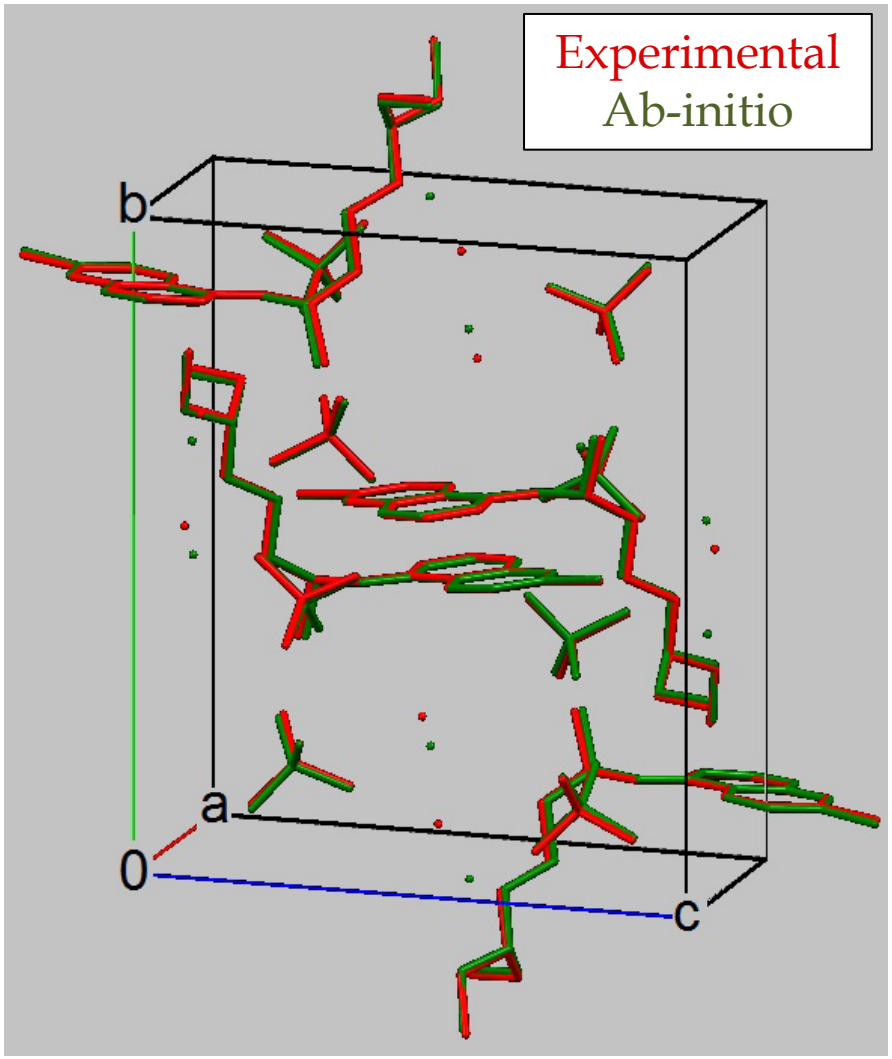
Ab-initio Structure



- CRYSTAL14
- B3LYP/Double-ζ level of theory
- Grimme dispersion correction
- $P2_1/c$ 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 CQH_2^{2+}

Grimme, S. *J. Comput. Chem.*, **2006**, 27, 1787-1799.

Intermolecular interactions

PAMoC

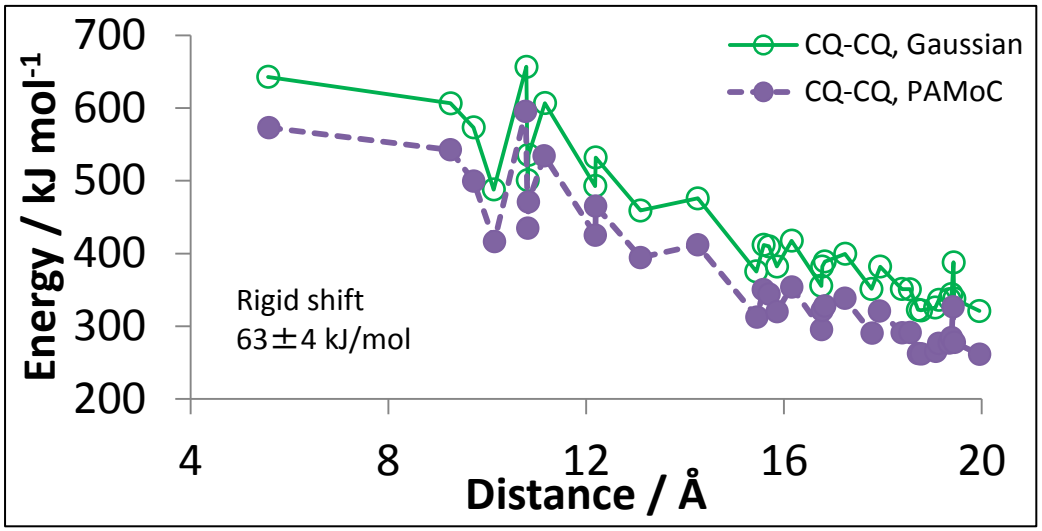
Single-Point (DFT B3LYP/pob-TZVP) of pairs extracted from the crystal

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

3 pairs types:

- CQ-CQ
- CQ-P1
- CQ-P2

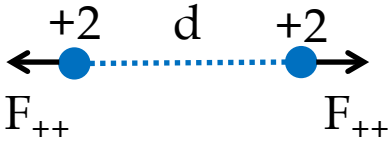
Molecular pairs with centre-of-mass distances up to 20 Å



CQ-CQ interactions have always positive energies

Expected? **YES**

We have something close to:



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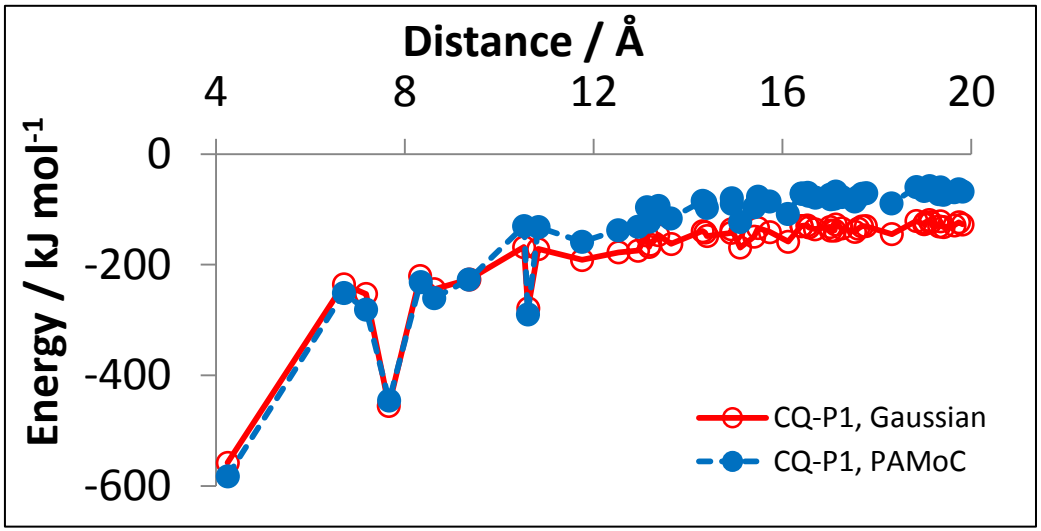
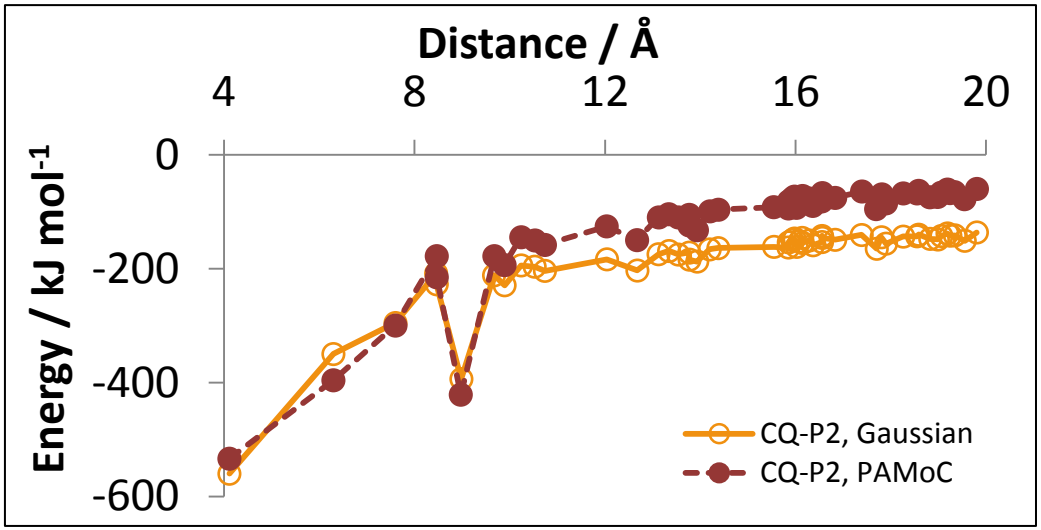
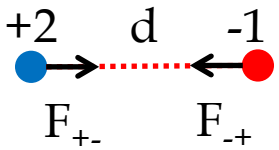
Spackman, M. A. *et al. J. Am. Chem. Soc.* **1988**, 110, 775-782.
Spackman, M. A. *J. Chem. Phys.* **1986**, 85, 6579-6586.

Intermolecular interactions



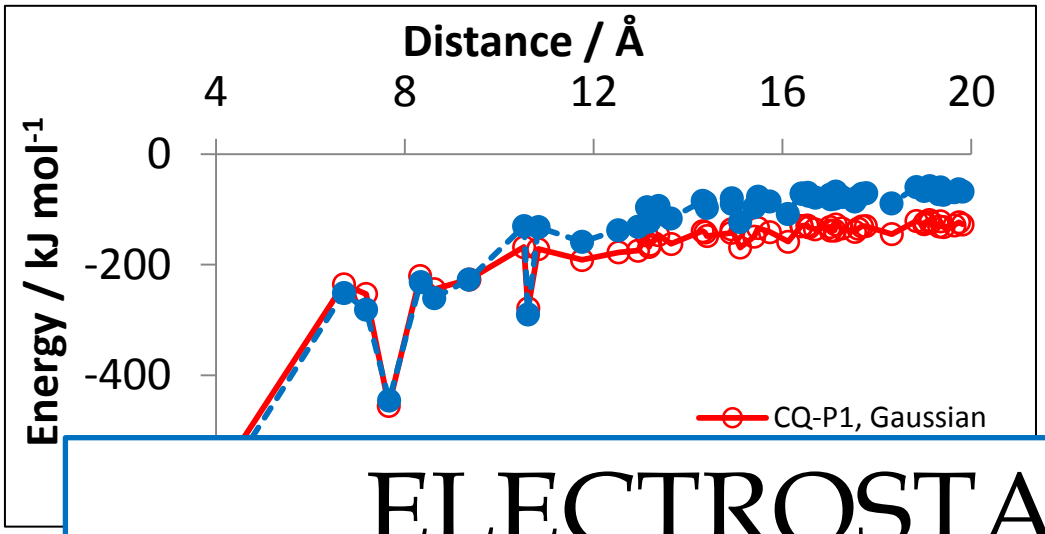


CQ-P1 and CQ-P2 pairs have always negative energies



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

Intermolecular interactions



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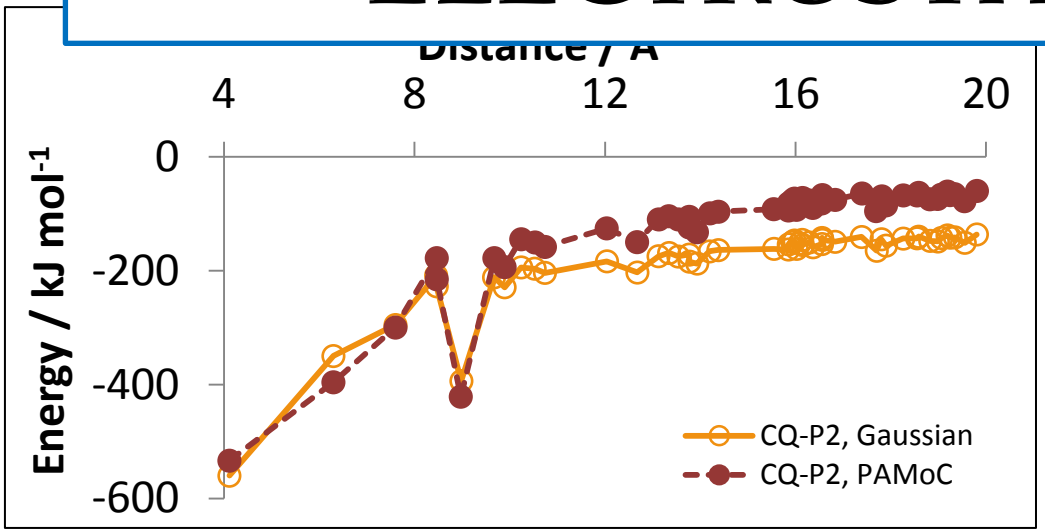
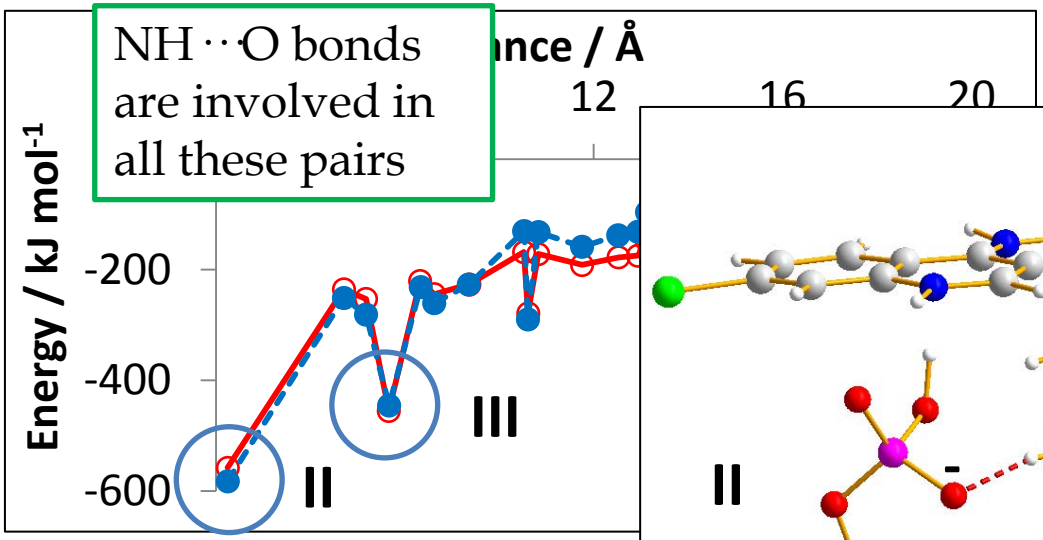
ELECTROSTATICS!

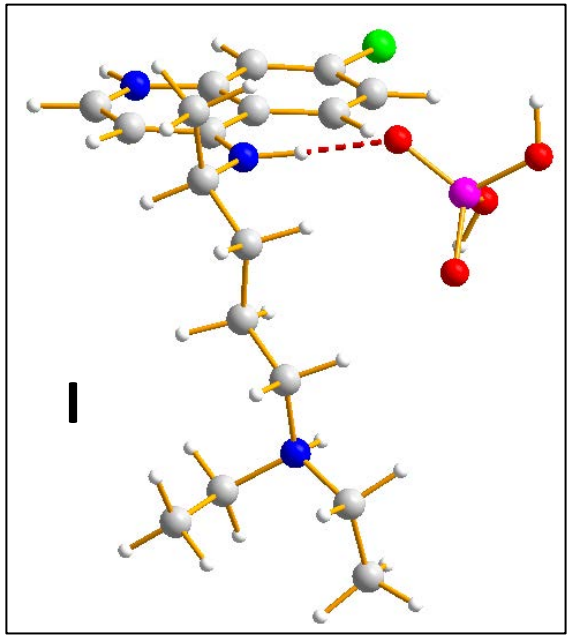


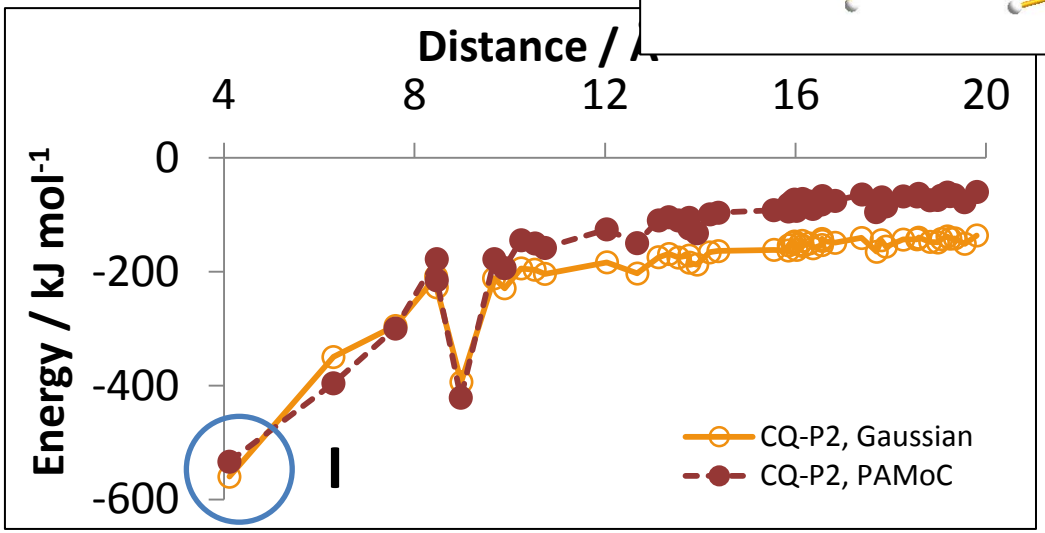
As expected, the crystal cohesion is dominated by the Coulombic interactions between CQ (+2) and the phosphates P1 and P2 (-1)

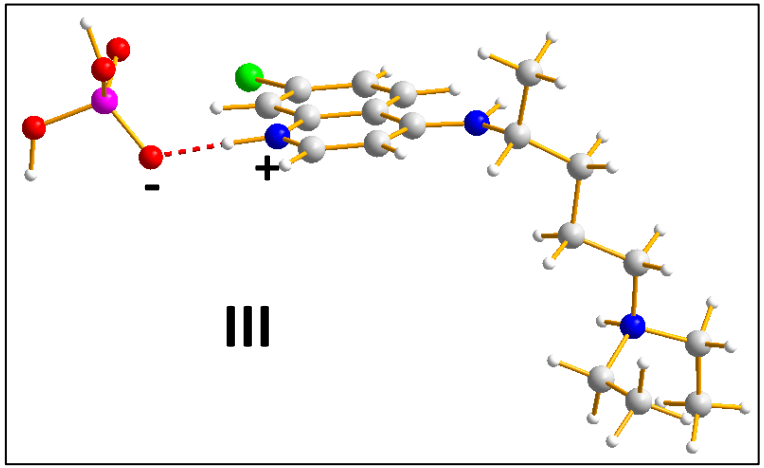
Pairs I-II-III

NH···O bonds are involved in all these pairs









Pairs I-II-III - Energy

Gaussian09

Pair	E_{DFT}
I (CQ-P2)	-559.58
II (CQ-P1)	-557.95
III (CQ-P1)	-454.96

PAMoC

				Electrostatic Energies			
Pair	E_{Total}	$E_{Repulsion}$	$E_{Dispersion}$	E_{total}	$E_{Pro-Pro}$	$E_{Pro-Def}$	$E_{Def-Def}$
I (CQ-P2)	-533.32	83.65	-29.11	-587.86	-31.60	0.92	-557.18
II (CQ-P1)	-582.16	113.39	-32.90	-662.65	-47.20	-8.27	-607.18
III (CQ-P1)	-445.36	124.86	-20.31	-549.91	-51.77	0.24	-498.38

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

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?

Pair	E_{DFT}	$E_{NH\cdots O}$	$E_{NH\cdots O}$ %
I (CQ-P2)	-559.58	-54.85	9.8
II (CQ-P1)	-557.95	-115.39	20.7
III (CQ-P1)	-454.96	-156.98	34.5

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

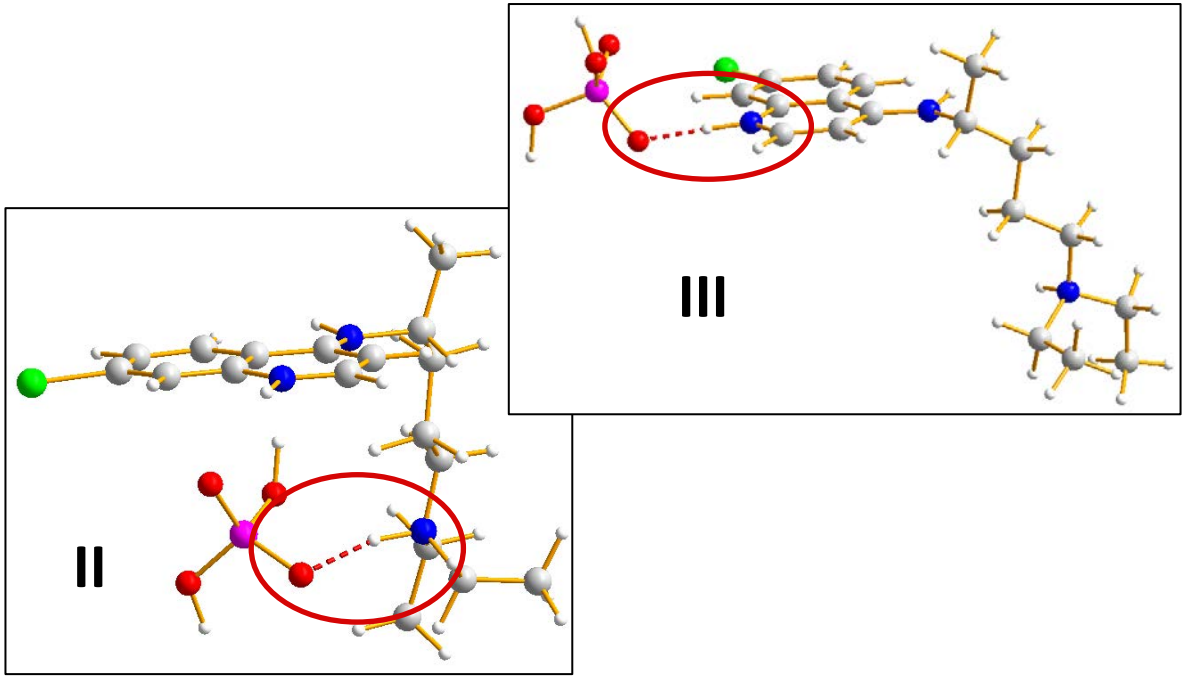
NBO analysis

$$E^2 = q_i \cdot \frac{F(i,j)^2}{\epsilon_i - \epsilon_j}$$

- q_i: donor orbital occupancy
- F(i,j): Fock matrix element
- ε_1, ε_2: orbital energies

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

	Contact	$d_{H\cdots O}$ / Å	ρ_{bcp} / e·bohr^{-3}	$\nabla^2\rho_{bcp}$ / e·bohr^{-5}	G_{bcp}(a)	V_{bcp}(a)	(H_{bcp}/ρ_{bcp}) / au
I (CQ-P2)	N9–H9···O5	1.8674	0.031	0.102	65.2	-63.6	0.02
II (CQ-P1)	N14–H14···O4	1.7419	0.045	0.115	92.5	-109.3	-0.14
III (CQ-P1)	N1–H1···O1	1.6279	0.056	0.129	115.2	-145.5	-0.21

(a) Expressed in kJ mol^{-1} Å^{-3}

The point topological analysis at bcp confirms the previous results

NH···O bonds strength

I II III

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

[1] Espinosa, E. *et al J. Chem. Phys*. **2002**, 117, 5529–5542.

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]

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

Software: NCImilano [3]

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

$\lambda_2 > 0$ → Repulsive interaction
$\lambda_2 < 0$ → Attractive interaction

$|\rho\cdot\mathrm{sign}(\lambda_2)|$ proportional to the strength of the interaction

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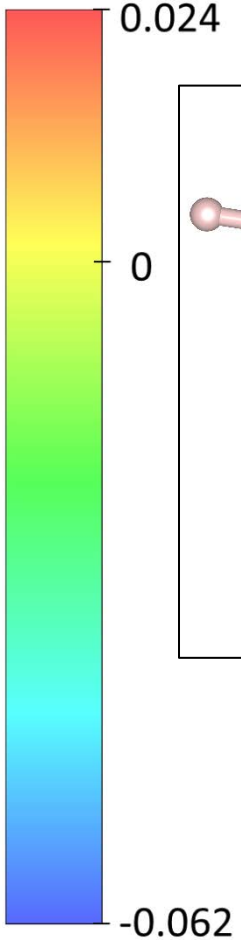
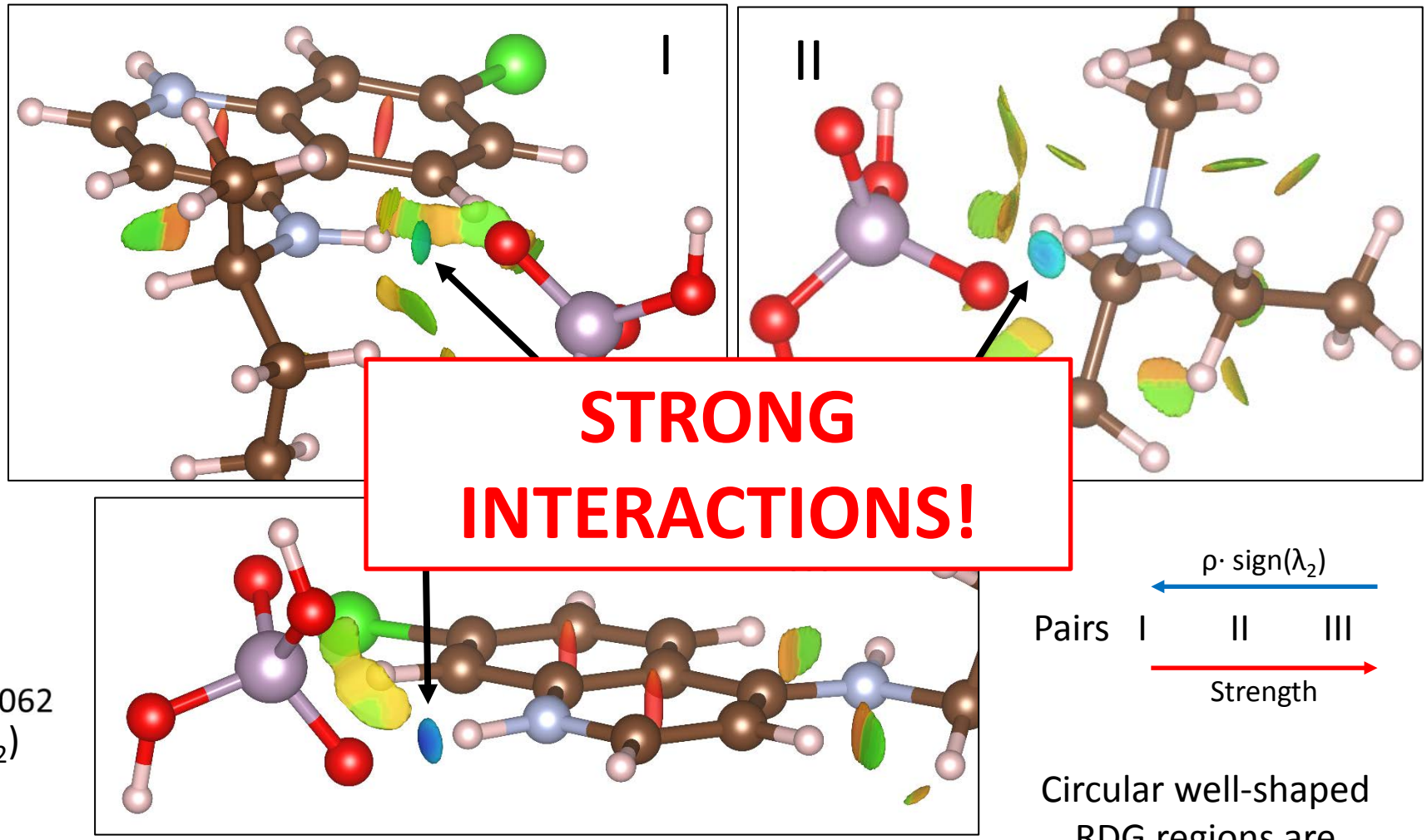
[1] Johnson, E. R. *et al. J. Am. Chem. Soc.* **2010**, 132, 6498-6506.
[2] Saleh, G. *et al. Chem. Eur. J* **2012**, *18*, 15523-15536.
[3] Saleh, G. *et al. J. Appl. Cryst.* **2013**, 46, 1513–1517.

Pairs I-II-III - NH···O bonds





STRONG INTERACTIONS!

RDG isosurfaces = 0.4

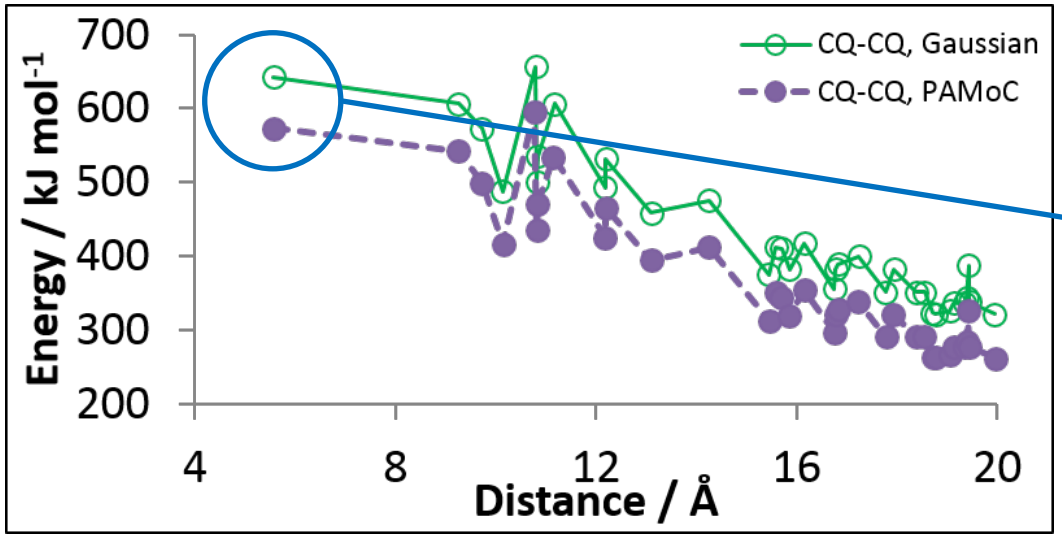
ρ· sign(λ_2)

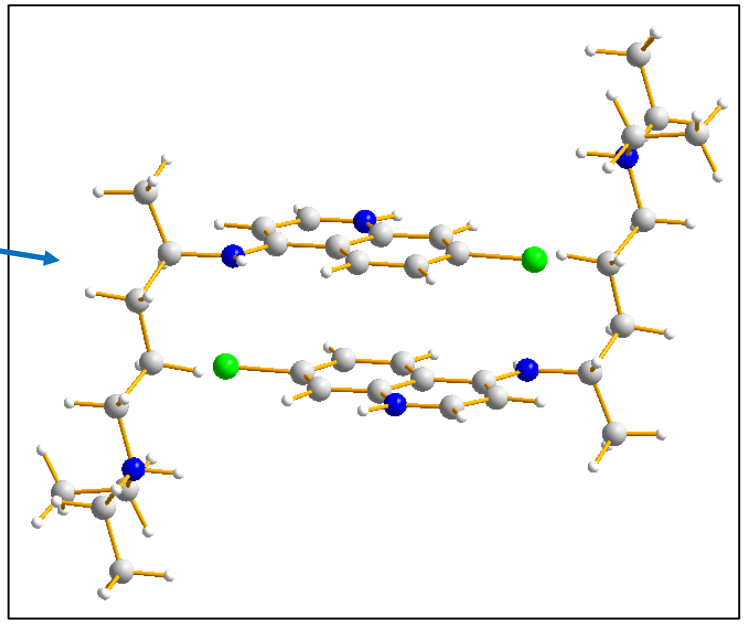
Pairs I II III

Strength

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

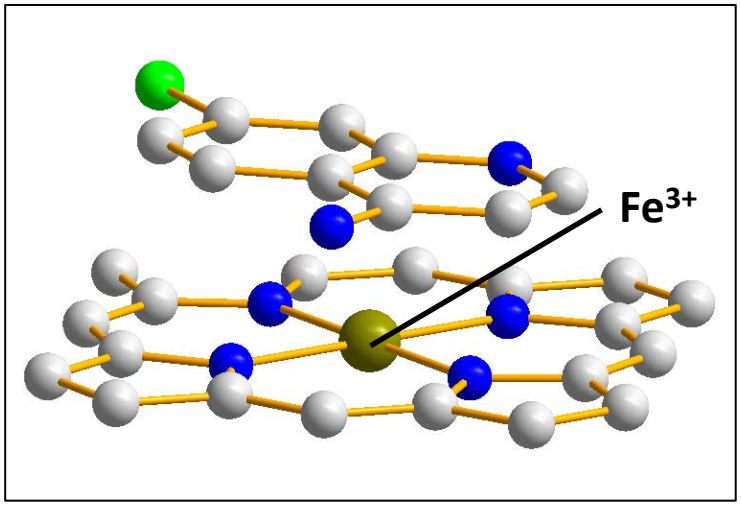
CQ-CQ Pairs





Quinoline ring-ring stacking

What about π-π interaction?



CQ-CQ - Energy

π-π interaction?

Pair	E_{DFT}
CQ-CQ	642.66

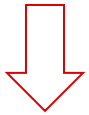
				Electrostatic Energy			
Pair	E_{Total}	$E_{Repulsion}$	$E_{Dispersion}$	E_{total}	$E_{Pro-Pro}$	$E_{Pro-Def}$	$E_{Def-Def}$
CQ-CQ	573.03	83.53	-80.95	570.46	-42.9	-1.02	614.38

(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

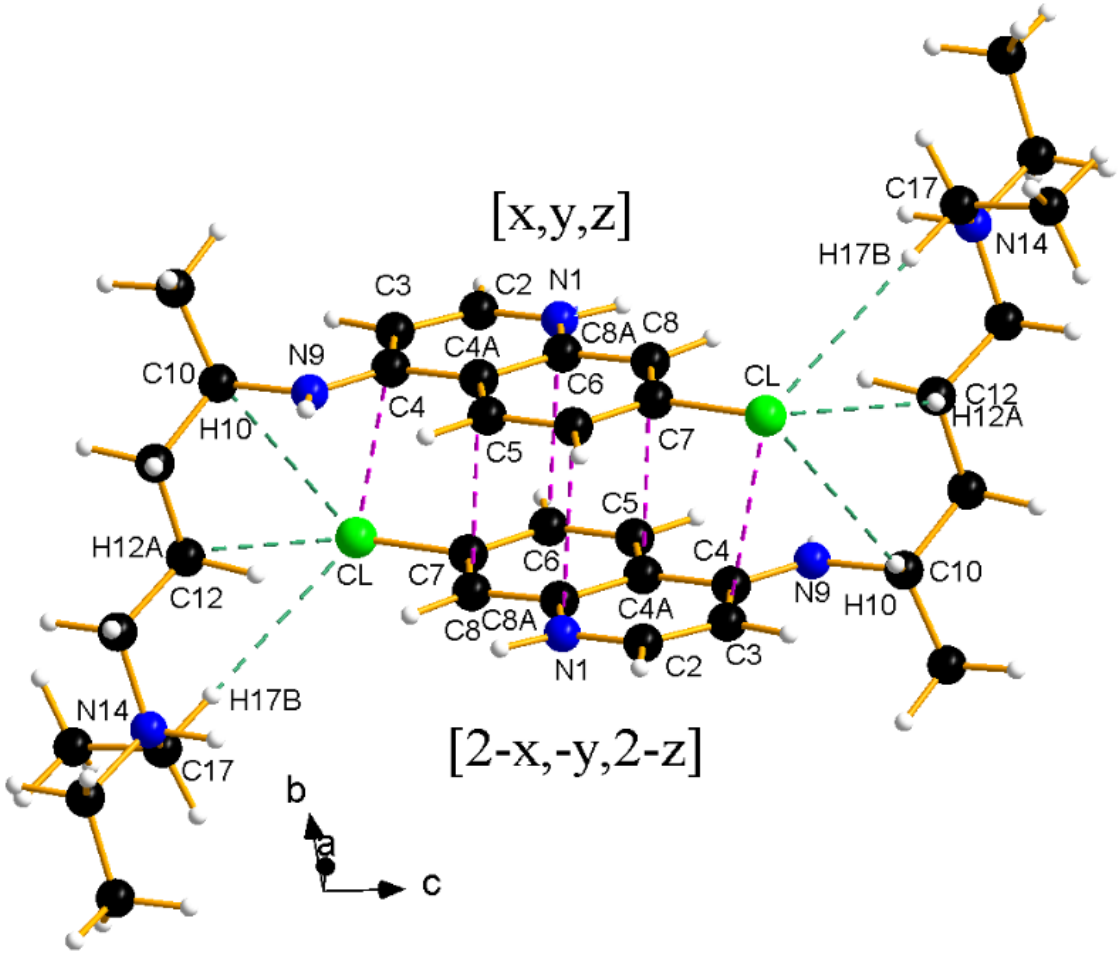
The dispersion and the repulsion energies delete each other



Apparently no extra contribution due to π-π interactions

CQ-CQ - QTAIM

π-π interaction?



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?

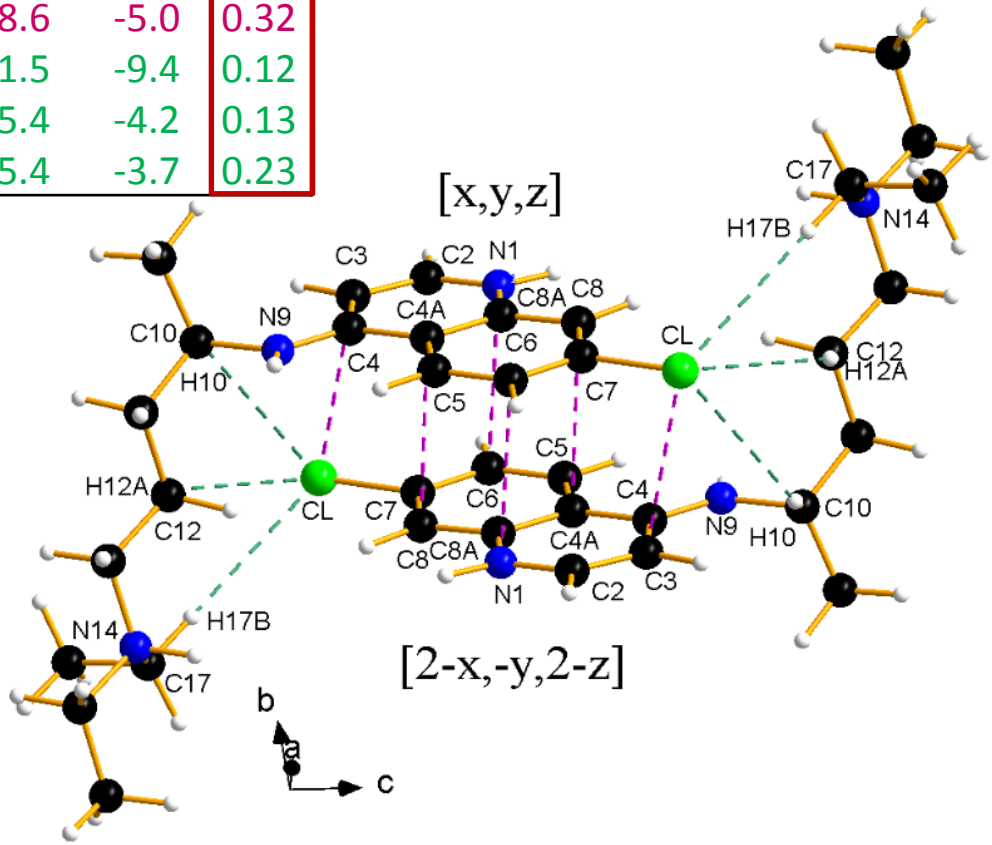
Contact	$d_{X\cdots Y}$ / Å	ρ_{bcp} / e·bohr^{-3}	$\nabla^2\rho_{bcp}$ / e·bohr^{-5}	G_{bcp}	V_{bcp}	BD / au
C3···Cl	3.4497	0.007	0.022	12.4	-10.1	0.13
C6···N1	3.4857	0.005	0.021	10.1	-6.6	0.25
C4A···C8	3.5204	0.004	0.019	8.6	-5.0	0.32
C12–H12A···Cl	3.0079	0.007	0.021	11.5	-9.4	0.12
C17–H17B···Cl	3.2650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

(a) Expressed in kJ mol^{-1}Å^{-3}

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

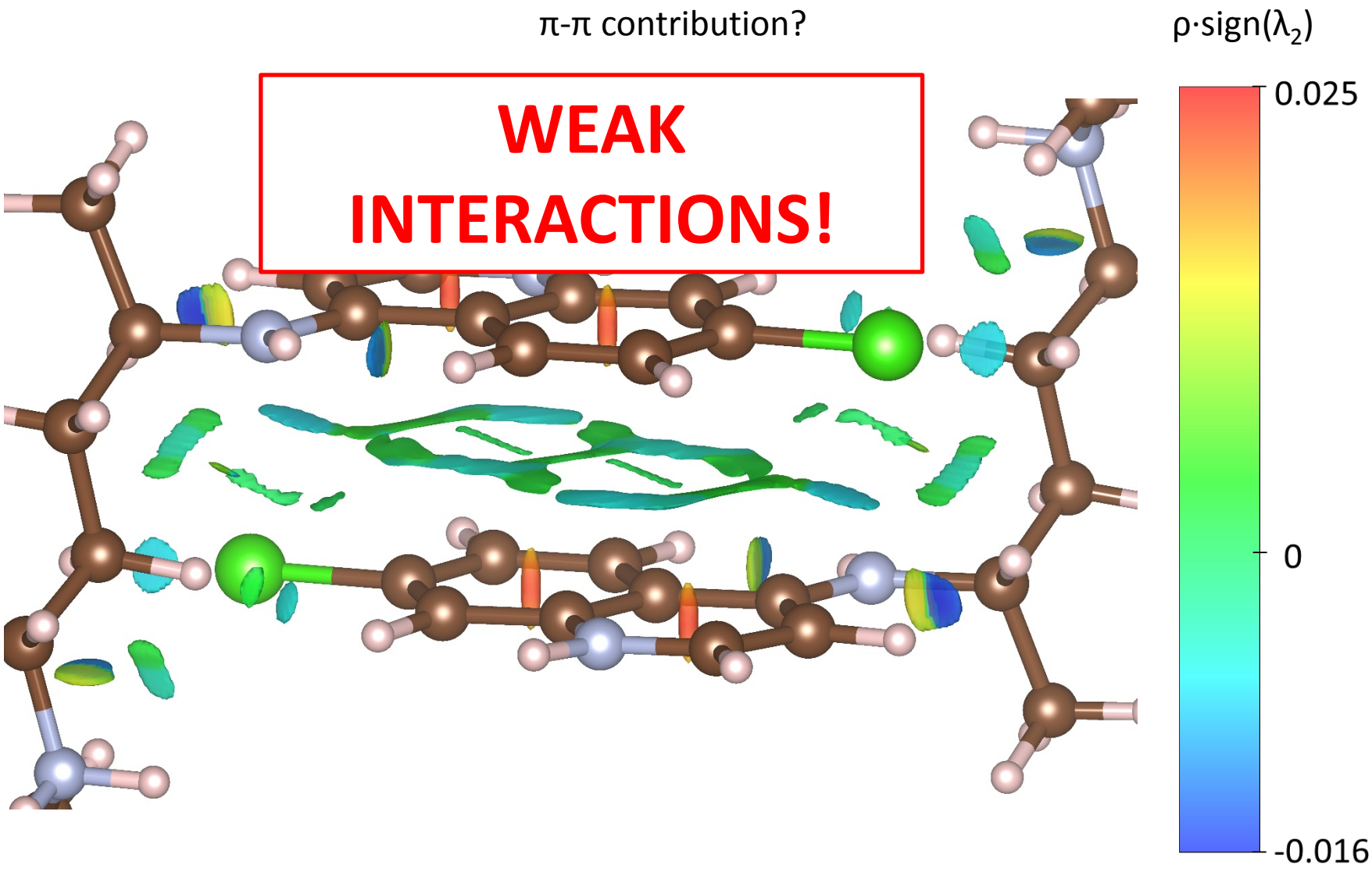
The BD is always positive

The interactions between the two rings are weak



CQ-CQ - NCI

π-π contribution?



Conclusions

- The chloroquine diphosphate dyhydrate salt is a good model for CQ:Heme interaction in solution:

- Diphosphate Salt		- With Heme
1. CQ-CQ ring stacking	⟷	CQ-PPIX π-π 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, π-π 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

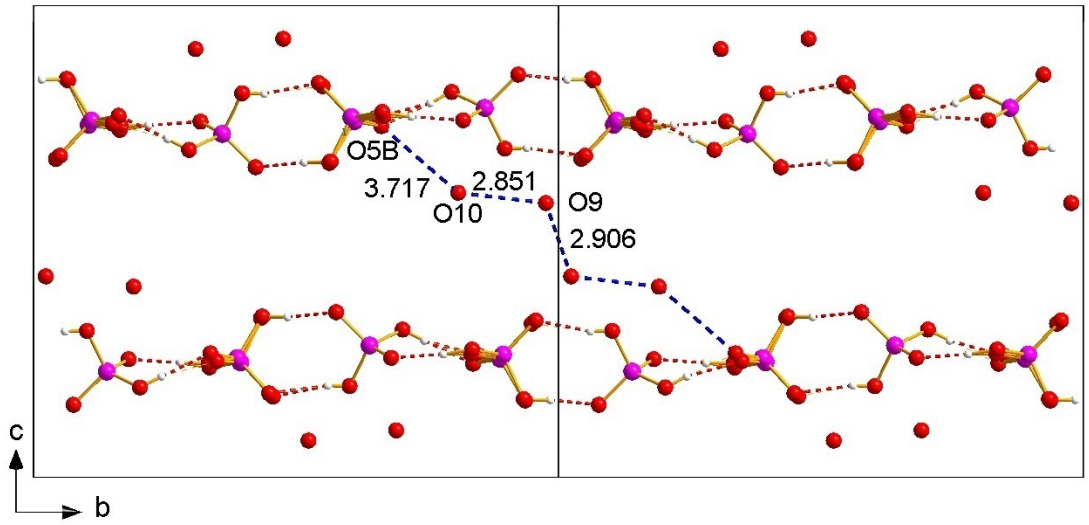
Prof. Silvia Rizzato

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”

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

Space Group = $P2_1/c$ (14)
a = 9.7212(1) Å
b = 16.7733(2) Å
c = 15.6966(2) Å
β = 105.1788(2) Å
V = 2470.14(5) Å

~~Experimental Charge Density~~

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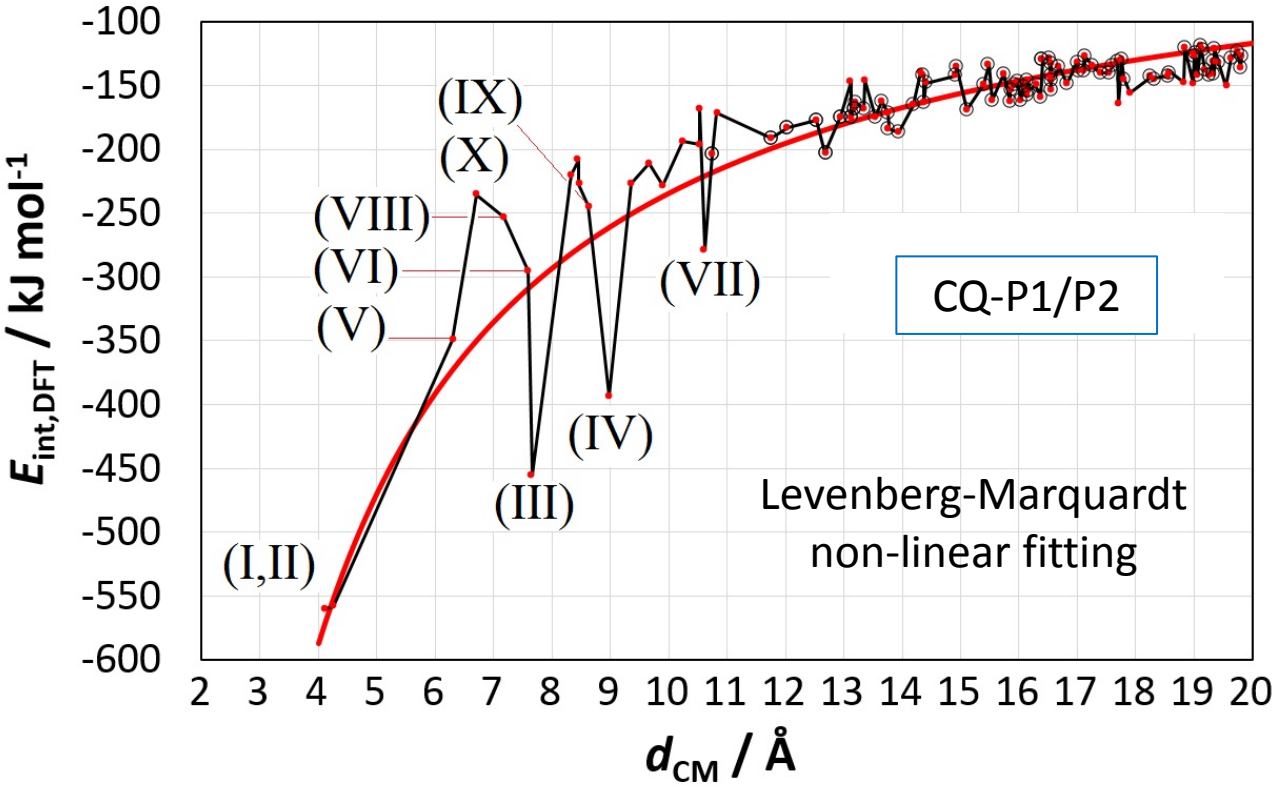
Intermolecular interactions



Fitting the data with d > 10 Å, **E ∝ 1/d** (Coulomb law)

Deviations at d < 10 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

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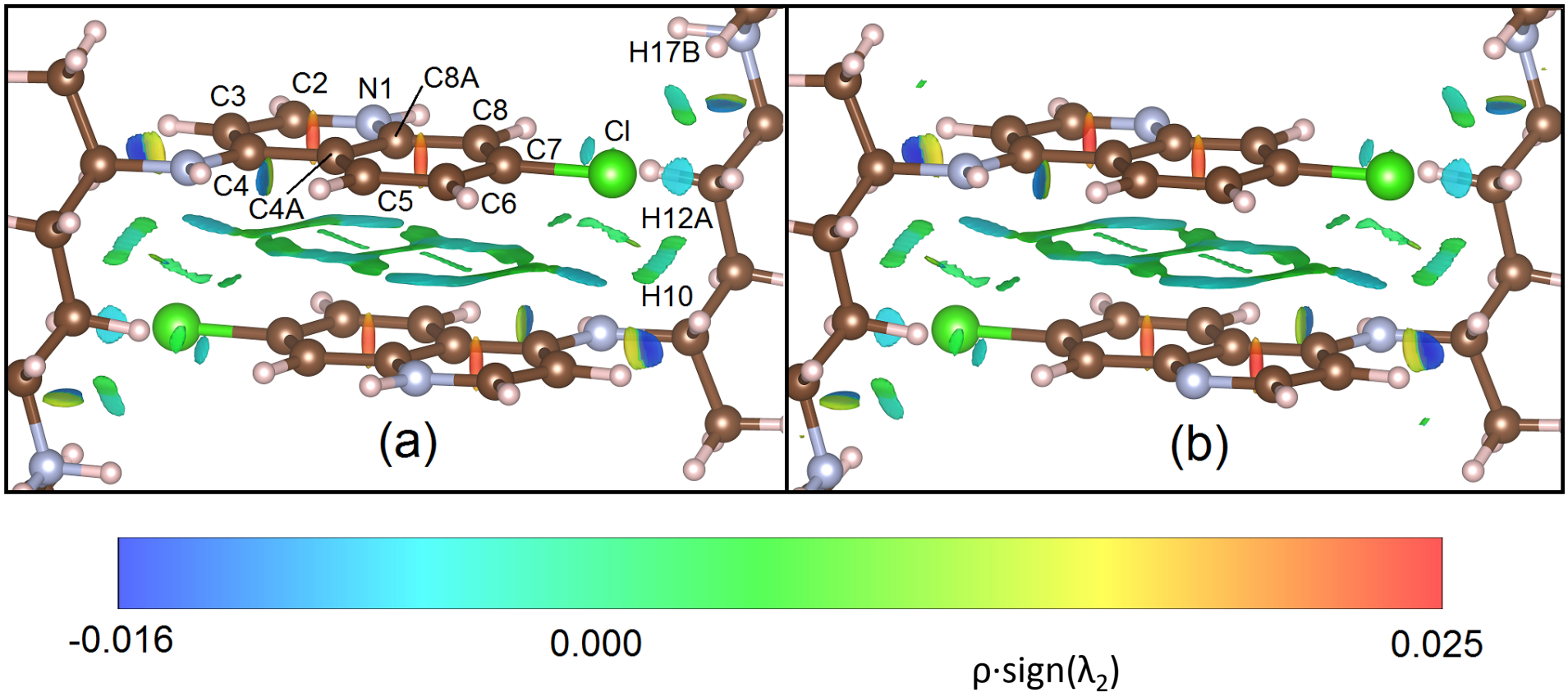
CQ-CQ - NCI



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