Proposition pour allocation de recherche, Thème (A,B,C,D,E): A

Titre de la thèse: 
**Calcium Oxalate Stones’ Morphology and Stability: Towards a Molecular Picture**

**Background**
One of the research topics of the two groups involved in the proposed project is the study of biomineralisation i.e. the formation of minerals produced by living organisms. In this frame, in vivo Calcium Oxalate (CaOx) crystallization leads to the formation of kidney and urinary stones, constituting a serious health problem to mankind. Both groups (LCMCP and Tenon Hospital) are especially interested in the study of the formation and characterization of these in vivo produced calcium oxalate minerals.

Calcium oxalate crystallization yields three main hydrates, i.e. thermodynamically favorable calcium oxalate monohydrate (COM, Whewellite), metastable calcium oxalate dihydrate (COD, Weddellite) and calcium oxalate trihydrate (COT, Caoxite).\(^1\) Monoclinic COM and tetragonal COD are the most commonly found in human stones, plants and fossils.\(^2\,^4\)

After an extensive investigation on CaOx kidney stones through FTIR, SEM and PND, we recently started to investigate the calcium oxalate polymorphs using ab initio quantum chemical methods.\(^5\) Ab initio calculations based on periodic DFT (Density Functional Theory) enable to determine the crystal structure with high precision, and compare it with experimental X-ray based crystallographic and neutron diffraction methods, but also investigate the theoretically calculated vibrational frequencies of the bulk crystal. This information gives access to simulated IR spectra obtained on perfect (defect free crystals) with high accuracy. Note that the FTIR spectra of biological whewellite is not exactly the same than the one of synthetic whewellite. The shift in the bands can be due to the size of the crystal, the presence of structural defects or other compounds. At this point it is of major importance to understand why the position of the absorption bands play a very important role in the chemical analysis of the kidney stone.

After the study of the bulk, the next step is the theoretical investigation of the different surfaces of the calcium oxalate polymorphs. Indeed, from the bulk crystal structures obtained through ab initio methods one can build for example the low index surfaces. Surface energy of the different surfaces can be calculated and the final crystal morphology predicted using the thermodynamic Wulff formalism for the construction of the crystals shape. Since the thermodynamic stability depends on the medium in which the surface is introduced, the calculation of the interaction of the calcium oxalate surface with water, urea, and other small molecules will give us the possibility to understand the change in crystal morphology of the final oxalate crystal in its natural medium. The final aim is the prediction of the shape of the kidney stone in its natural medium. On this topic it was shown recently that unusual morphological calcium oxalated dihydrate crystals were obtained in the presence of green tea extract containing catechin.\(^6\)
Indeed, the high prevalence of urolithiasis reaching up to 8-10% of the general population is mainly related to environmental factor, especially western diet\(^7\). Among calcium oxalate stones, whewellite, a calcium oxalate monohydrate crystalline form (COM) is oxalate dependent, whereas weddellite, a calcium oxalate dihydrate crystalline form (COD) is calcium dependent\(^7\). Hence, high urinary calcium and oxalate concentrations are critical factors leading to stone formation. We have recently shown that regular daily green tea intake is responsible for a significant higher prevalence of COD in female “drinkers” and COD was found in nuclei in male “drinkers” compared to “non drinkers” control hypercalciuric stone patients. Of notice, SEM analysis performed on 57 renal stones containing COD as the major component revealed a higher prevalence of square pores detected in drinkers (92% versus 40% p<0.05) with no difference for size. Moreover, COD samples (major component assessed by SPIR analysis) with regular crystal surface, sharp edges and no pore detected by SEM analysis, were selected from our stone bank (containing 80 000 samples all characterized by morpho-constitutional and SPIR analysis) and incubated with various solutes in vitro, including epigallocatechin (EGC), a polyphenol compounds, present at high concentrations in green teas (compared to black teas)\(^8\).

Incubation at room temperature with green tea extracts is responsible for the occurrence of square pores at the surface of COD crystals. Similar square holes are detected after 24 hours incubation of catechin ranging from 10 to 300 µM but also after incubation with two well-known calcium chelators: EDTA solution greater than 100 µM and sodium citrate with a concentration greater than 100 mM (Figure 2). High resolution IR spectra will first help us to determine and/or confirm the crystal structure of the polymorphs. Second, the comparison of high resolution IR spectra of the pure calcium oxalate polymorphs as well as with real kidney stone samples with the theoretically obtained spectra might help us to understand and explain quantitatively the composition of an oxalate based kidney stone. And finally ATR measurements will help us to investigate adsorption properties at the molecular level, such as adsorption geometry and energies.

Concerning the investigation of the molecular adsorption properties, the molecular catechin, but also EDTA will receive special attention because of their known effect on the kidney stone formation in particular their morphology and composition.

**Objective**
A logic continuation of the experimental work started on the characterization of calcium oxalates is the study of the adsorption properties towards small molecules in a specific medium (kidney stone). In order to obtain a molecular picture of the interaction/adsorption complex between molecules of medical interest (catechin, EDTA, citrate, etc.) and Ca oxalate crystals, IR spectra will be collected and compared with ab intio calculated vibrational spectra. This approach has shown in the past to be successful as can be seen in our former studies on the characterization of adsorption complexes\(^9,10\). Structural analysis will be performed before and under incubation of different solutions including catechin, citrate, EDTA but also solutions containing high concentrations of urea and sodium chloride in order to understand the molecular interactions leading to crystal solubilization.

**Experimental method**
Two types of samples will be analyzed: Synthesized (Ca-Oxalate Polymorphs) and Natural (kidney stones).

**Experimental: Coordinated by Tenon Hospital**
The measurements in transmission are planned for: 1) Synthesized samples; Mono, di and tri hydrate in cryostat (5 - 300 K) condition within the spectral range (6 - 2000 cm\(^{-1}\)). Very less spectroscopic information is available for this frequency range, which can easily be obtained from DFT calculations. 2) Kidney stone (samples obtained from Tenon Hospital in Paris): similarly to the synthesized samples, kidney stones will be studied using IR in transmission, but also using ATR measurements in the range 6 – 2000 cm\(^{-1}\), at room temperature.

**Theoretical: Coordinated by LCMCP**
Computational Details (calculations done in parallel with the experiments): The proposed model crystals and surfaces adsorption of selected will be geometrically optimized using periodic DFT by means of the VASP.5.4 code, CP2K and QuantumEspresso. On the optimized structures vibrational frequencies can be calculated, which together with the calculation of their intensities results in theoretical IR spectra that can be directly compared with the experiment.

**Expected Results**

We expect the following results: 1. Validation of the models used for the Ca-Oxalate bulk crystals, by comparison between calculated vibrational modes and experimental IR spectra. 2. Characterization of the clean Ca-Oxalate surfaces, hydrated or not. In particular, the kind of surface exposed and its termination (adsorption of water e.g.). 3. Adsorption geometries of the different molecules such as water, urea, catechin, EDTA, citrate, etc. 4. Theoretical prediction of crystal morphology form the ab initio quantum chemical energy calculations of the Ca-Oxalate surfaces. 5. Elucidation of the “dissolution mechanism” of kidney stone, by means of the reactivity of the Ca-Oxalate surfaces with the above mentioned molecules.

**References**