Supporting information for

Photoacoustic Measurement of Localized Optical Dichroism in Chiral Crystals

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Materials

L-Histidine (99%, HPLC) was purchased from TCI EUROPE N.V., Cobalt chloride 97% was purchased from sigma Aldrich Co 3050 Spruce Street. St Louis USA, Ethanol absolute was purchased from Bio-Lab Ltd, Hayetzira Jerusalem Israel, hydrochloric acid 37% was purchased from DAEJUNG Co, Ltd.

Crystal Characterization experimental conditions

The crystallographic structure of the Co doped L-His crystals was measured by X-ray diffraction (XRD), measurements were performed using a Bruker AXS D8 Advance diffractometer with Cu Ka ($\lambda = 1.5418$ Å) operating at 40 kV/40 mA, and collecting from 2 $\theta = 10^{\circ}$ to 80°. Images of the crystals were collected by high-resolution scanning electron microscopy (HR-SEM) using a fieldemission, FEI, Helios 600 HR-SEM. The samples were sputtered with a 3 nm layer of iridium to reduce charging effects. FTIR spectrum was collected using a Thermo Scientific Nicolet iS10 FTIR spectrometer equipped with a Smart iTR attenuated total reflectance sampler containing a single bounce diamond crystal. Data were collected and analyzed using OMNIC software. Spectra were collected in the 650-4000 cm-1 range at a spectral resolution of 4. Diffused Reflectance UV (DRUV) spectra of the powders was measured using a CARY 500 Scan UV-Vis spectrophotometer equipped with a solid sample holder. TGA measurements were performed with a Perkin Elmer model Pyris 1 TGA, Samples were heated from 30 to 850 °C at 10 °C/min under N₂ gas flow. DSC measurements were performed with a Mettler Toledo model DSC 822e equipped with a liquid-nitrogen cooling accessory and calibrated with indium, Samples were heated from 25 to 350 °C at 10 °C/min under N₂ flow. Cobalt concentrations inside of the L-His crystals were measured using Inductive Coupled Plasma (ICP). Measurements were taken using a SPECTRO ARCOS ICP-OES Multi-view FHX22 instrument, powder samples (2 mg) were dissolved in concentrated hydrochloric acid and diluted to 10 mL with distilled water to a final acid concentration of about 3% by weight. The optical activity of Co doped L-Histidine dry powder was directly measured by Polarimetry, samples were dried upon a quartz slide, optical rotation was

measured at 4 different angles with respect to the incident beam: 0, 90, 180 and 270 deg, the measurements were taken by a Jasco P-2000 polarimeter using an sodium lamp light source at 589 nm passed through an aperture of 8 mm.



Figure S1 - FTIR spectra of Co doped L-His crystals with some of the characteristic vibrations

of L-His

the graph.



Figure S2 – Diffused reflection UV (DRUV) measurements of Co doped L-His powder – Adsorption spectra (blue curve) and Reflectnce spectra (black curve) of the crystls.

Thermal decomposition of Co doped L-His crystals

The thermal decomposition of the crystals was measured using TGA and DSC as explained in the experimental section, monoclinic L-His crystals grown with ethanol in anti-solvent conditions were measured and shown to begin decomposing at 275 °C in an endothermic reaction that peaks at 281.4°C¹, in our case, the decomposition begins at 273.31 °C and the peak of the endothermic reaction occurs at 281.81 °C which is very close to the reported data. Also, the TGA measurements revealed that from 25 °C and up until 270 °C there has been very little weight loss (less than 2%) proving that the sample does not contain any impurities.



Figure S3 - TGA of Co doped L-His crystals measured under N_2 flow.



Figure S4 – DSC of Co doped L-His crystals measured under $N_{\rm 2}$ flow.

Spectral line Co Conc (ppm/mg powder)		StdDev
Co 228.616	0.3580865	0.004597
Co 237.862	0.3396945	0.001465

Measurment angle	O.R (mdeg)	STDEV
0°	+1.5	1
90°	+2.7	0.3
180°	+4	0.5
270°	+4.3	0.6
Average	+3.125	0.6

Table S1 - Cobalt concentrations measured by inductive coupled plasma (ICP), the averageconcentration from both of the spectral lines is 0.3488905 +/- 0.003031 ppm/mg powder.

Table S2 - Optical rotation of Co doped L-Histidine dry powder measured in 4 different angles $(0^0, 90^0, 180^0, 270^0)$ in correlation with the incident beam.

Computational details

The structure of L-His (80-atom unit cells) with added Co atom (for a total of 81 atoms) was allowed to relax in order to find the minimum energy structure through the optimization of the lattice parameters and the internal positions of the atoms within the unit cell. The total energy was converged with respect to both the kinetic energy cutoff and k-point sampling for both structures, the kinetic energy cutoff was (40 Ry) and gamma k-point grid was used for the calculations. All electronic structure calculations were done using density functional theory with the Perdew-Burke-Ernzerhof (PBE) exchange-correlation functional and ultrasoft pseudopotentials using the Quantum Espresso software package. The ultrasoft pseudopotentials are taken from the Rutgers university GBRV database.² In the self-consistent calculations, the energy was converged below 10–8 Ry and the force convergence criteria is set to 10–5 a.u.



Figure S5 – Crystal structures of Co doped L-His crystals - DFT calculations of Co doped L-His, the unit cell is highlighted, the red atoms represent oxygen, the light blue represent nitrogen, the brown represent carbon and the dark blue represent cobalt atoms.

Bond	Length (Å)
Co-C ₁	1.79
Co-C ₂	2.03
Co-N ₁	2.13
Co-N ₂	2.17
Co-N ₃	2.13

Table S3 - Distances between the Co ion and nearby atoms derived from DFT calulations.

Algorithm 1 Statistical Optical Dichroism Measurement

1: for $x = -N_x/2..., N_x/2$ do for $y = -N_u/2..., N_u/2$ do 2:Move excitation to position (x,y)3: for Measurement = 1..., M do 4: Switch to left circular polarization 5: $MeasureA_{left}$ 6: Switch to right circular polarization 7: 8: $MeasureA_{right}$ $D(m) = |A_{right} - A_{left}|$ 9: end for 10:end for 11: C(x, y) = percentile(D, 85) - percentile(D, 15)12:13: end for

Figure S6 – Algorithm 1: pseudocode for statistical photoacoustic measurement of unknown orientation chiral crystals.

Statistical analysis conditions

For the statistical analysis of our results, we took a 15% statistical margin (15th and 85th percentile), instead of calculating the difference between the maximal and minimal amplitude in each pixel, this was done empirically in order to enhance the stability of the pixel value and reduce the sensitivity to random noise. the lower margin would cause noisy pixel values due to statistical error, while higher-margin was found to decrease the significance of the difference between chiral and non chiral pixels.

The exact number of repetitions per pixel, M, were determined experimentally. In this experiment, the excitation laser was pointed onto a single pixel, which is known to contain chiral

crystals. We measured this pixel as described above in stages (a)-(d), for M repetitions, for different values of M (M = [5, 25,35, 50,75,100, 150]). In addition, for each value of M we repeated the experiment 10 times so that the output is a set of matrices (each of size $10X^M$) of amplitude difference values. Overall, 7 matrices, one for each value of M. from each matrix we extracted 10 pixel values (using equation 2), which corresponds to the specific value of M.

It was assumed that for M greater than some value, the pixel value will show stability over the 10 measurements. On the other hand, low values of M, meaning a low number of repetitions per pixel, will be noisy due to the unpredicted movement of the crystals and will be extremely sensitive to statistical error. The results of this experiment are shown in Figure 3. the graph presents a single pixel value as a function of M, and the error bar refers to the variety of pixel values received in the 10 experiments.

If M is high, meaning each pixel is a result of a high number of repetitions, every time we will measure this pixel it will show the same number, approximately. However, if M is low, the random nature of crystal movement will cause the pixel value to variate. The value taken as a result is M = 50 repetitions, which, as can be seen, produces a relatively stable pixel value, and yet does not require a long acquisition time per pixel.



Figure S7: Pixel value vs. the number of repetitions per pixel - When the number of repetitions is high, the calculated pixel value is stable, and its variance is low.

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- 2. K. F. Garrity, J. W. Bennett, K. M. Rabe and D. Vanderbilt, *Computational Materials Science*, 2014, **81**, 446-452.