

## Electronic Supporting Information

Table S1. Data collection and refinement statistics.

	<b>1</b> $\subset$ papain
ESRF beamline	ID23-2
Wavelength (Å)	0.8726
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit cell parameters (Å)	a = 61.03 b = 74.85 c = 86.25
Number of protein molecules per asymmetric unit	2
Resolution range (Å)	37.42 – 2.10 (2.20 – 2.10)
Completeness (%)	99.9 (100.0)
I/ $\sigma$ (I)	11.8 (5.2)
No. of measured reflections	121276 (14579)
Redundancy	5.1 (4.8)
R <sub>sym</sub> (%)	10.4 (34.3)
R <sub>work</sub> / R <sub>free</sub> factor (%)	19.20 / 24.87
N <sup>o</sup> atoms	3796
N <sup>o</sup> water molecules	332
Wilson B-factor (Å <sup>2</sup> )	17.02
Average B-factor (Å <sup>2</sup> )	21.08
RMSD	
Bonds (Å)	0.014
Angles (°)	1.018
Ramachandran (%)	
Favoured region	98.1
Allowed region	1.9
Outliers region	0.0

( ) = higher resolution shell

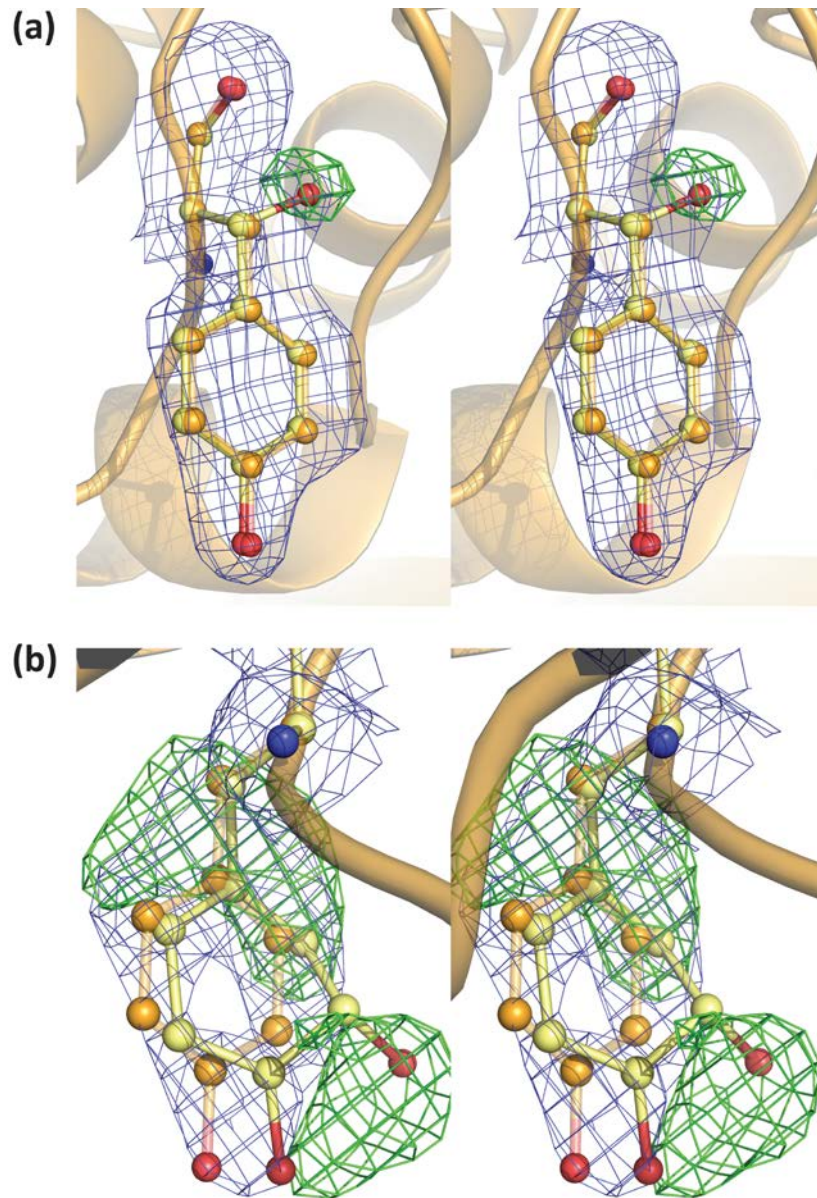


Fig. S1 Stereo image of the (Fo-Fc) omit maps showing the peaks of the hydroxylation at (a) the C $\beta$  carbon of Tyr61 and at (b) the ring of Tyr67 (see also Fig. 1).

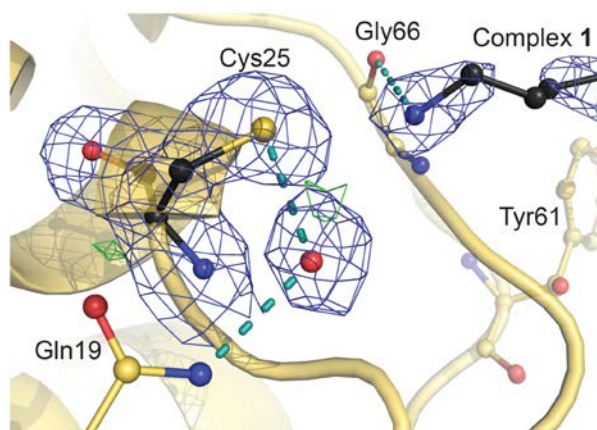


Fig. S2 Zoom of the complex **1** cleaved region in molecule A (see also Fig. 3a). The cleavage and fragmentation of the pyrrole-2,5-dione moiety as well as the H-bond between the resulting amine N atom with the carbonyl oxygen of Gly66 are clearly visible in the electron density .

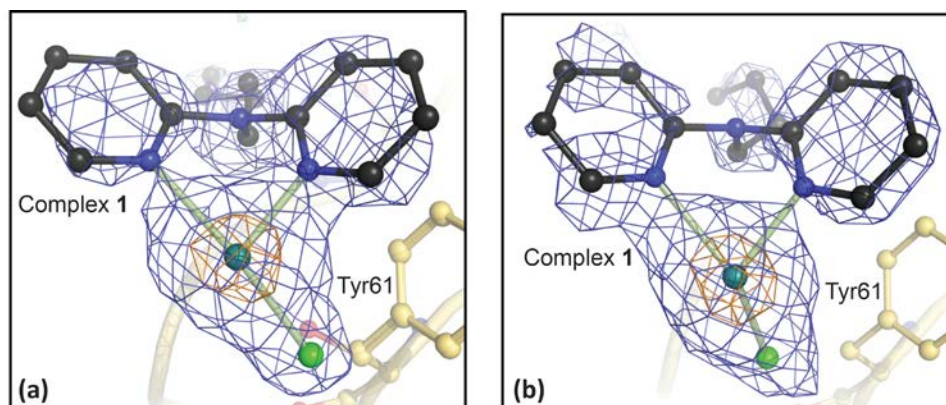


Fig. S3 Crystal structure of **1** bound to papain. Atoms are depicted as in Fig. 1; the (2Fo-Fc) electron density map is contoured at the  $1\sigma$  (blue mesh) and  $6\sigma$  (orange mesh) levels. The  $\eta^6$ -benzene ligand is not visible in the map in either **A** or **B** (see also Fig. 3).

Table S2. A comparison of relevant distance and angles at the first coordination sphere of Ru between the refined structures and ideal values (Cambridge structural database<sup>1</sup> code: TUFZEZ; nitrogen atom numbering is the one of reference 2). As indicated in the main text, there was no electron density corresponding to  $\eta^6$ -benzene, hence missing from this table.

Distance (Å)			
	Chain A	Chain B	TUFZEZ
Ru – N(1)	2.5	2.6	2.105
Ru – N(4)	2.1	2.2	2.097
Ru – Cl	2.4	2.4	2.391
Angle (°)			
N(1) – Ru – Cl	156.1	136.1	84.79
N(1) – Ru – N(4)	81.3	76.5	84.31
N(4) – Ru – Cl	103.2	114.7	85.96

## Theoretical calculations

**Tyr67: 3,4-dihydroxycyclohexadiene or 3,4-dihydroxycyclohexadienyl radical ?** Calculations were performed using programs from the Schrödinger Suite.<sup>3</sup> Density functional theory (DFT) implemented in the *Jaguar* program was used to optimize the geometries of 3,4-dihydroxycyclohexadiene and the 3,4-dihydroxycyclohexadienyl radical. The four starting models (including stereoisomers) were constructed from a tyrosine residue using *Maestro*. The B3LYP-D3 and the M06-2X-D3 functionals with the 6-31G\*\* basis set gave similar results: one of the optimized models of 3,4-dihydroxycyclohexadiene fits perfectly the crystallographic model for the hydroxylated Tyr67. Conversely, due to spin delocalization on the ring carbon atoms, optimizing the 3,4-dihydroxycyclohexadienyl radical led to models with a quasi-planar ring, a planarity that is not observed in our structure, as shown in Fig 5. The non-radical 3,4-dihydroxycyclohexadiene is thus the best alternative to explain the electron density.

**Sampling *ortho* hydroxylated-Tyr67 ring rotation.** The dihedral angle around the C $\beta$ -C $\gamma$  bond (C $\alpha$ -C $\beta$ -C $\gamma$ -C $\delta$ 1) was sampled using *Macromodel*.<sup>3</sup> It was scanned by rotation starting from the crystallographic value (95.6°) to 180° away with 4° increments. Structures with different values for C $\alpha$ -C $\beta$ -C $\gamma$ -C $\delta$ 1 angle were geometry-optimized using the OPLS2005 molecular mechanics force field<sup>4</sup> with an implicit model for water. Starting from the X-ray model of the molecule with *ortho*-hydroxylated Tyr67, hydrogen atoms were built and the hydrogen bond network was optimized using *Protein Preparation Wizard* from the Schrödinger Suite. Figure S4 depicts the energy of the model as a function of the dihedral angle value. There are two models which minima have equivalent energies. One of them is close to the crystal structure (A in Fig. S4), whereas the other one corresponds to the ring rotated by 180° where the added -OH group makes a hydrogen bond with the carbonyl group of Arg59 (B in Fig. S4). Since the latter conformation would imply an attack at the *ortho* position closest to the Ru ion, the ring could have rotated 180° around the C $\beta$ -C $\gamma$  bond after hydroxylation (see Fig. 4).

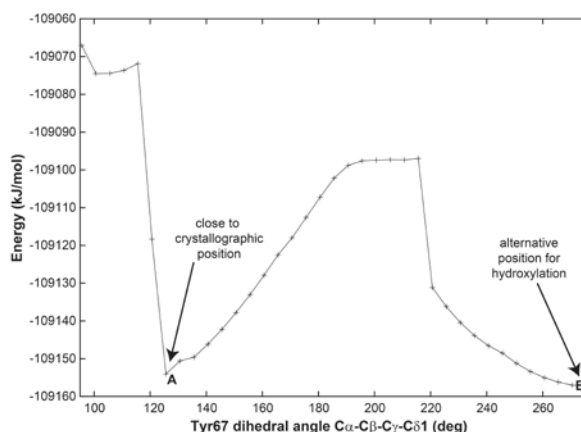


Fig. S4 The minimum energy of the system is represented as a function of the dihedral angle that allows Tyr67 ring rotation. Two minima are found, **A**, corresponding to an orientation close to that of the crystallographic model and **B**, which corresponds to a ring conformation that is rotated by 180°.

## References

- 1 C. R. Groom, I. J. Bruno, M. P. Lightfoot and S. C. Ward, *Acta Crystallogr. Sect. B Struct. Sci. Cryst. Eng. Mater.*, 2016, **72**, 171–179.
- 2 K. K. Mosny, S. R. de Gala and R. H. Crabtree, *Transit. Met. Chem.*, **20**, 595–599.
- 3 *Jaguar*, version 7.9, *Macromodel*, version 9.9, *Maestro*, version 9.4, Schrödinger, LCC, New York, NY, 2012.
- 4 W. L. Jorgensen, D. S. Maxwell and J. Tirado-Rives, *J. Am. Chem. Soc.*, **118**, 11225–11236.