



Supporting Information

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Water-based ligand screening for paramagnetic metalloproteins

Ivano Bertini^{,1,2}, Marco Fragai,^{1,3} Claudio Luchinat^{1,3} and Eleonora Talluri¹*

¹ Magnetic Resonance Center (CERM) – University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino, Italy

² Department of Chemistry – University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy

³ Department of Agricultural Biotechnology, University of Florence, Via Maragliano, 75-77, 50144 Florence, Italy.

***Prof. Ivano Bertini**

Magnetic Resonance Center (CERM)

University of Florence

Via L. Sacconi 6

50019 Sesto Fiorentino, Italy

e-mail: ivanobertini@cerm.unifi.it

Tel.: +390554574272

Fax: +390554574271

Plasmid DNA containing the gene of the human full length PP5 enzyme was kindly provided by Prof. D. Barford. The PP5 catalytic domain (PP5c, residues 169-499) was isolated from the template and then amplified by PCR. GATEWAY technology was used to clone the construct into the pENTR/TOPO/TEV vector and a LR reaction was performed to sub-clone the gene into the GATEWAY acceptor vector (pDEST30). The PP5c was expressed as His-GST-binding fusion protein in *E. Coli* BL21 (DE3) RIPL strain. PP5c was purified by Glutathione Sepharose affinity chromatography (Amersham) and then digested with TEV protease (Invitrogen). After the digestion four vector-derived amino acid residues (Gly-Ser-Phe-Thr) remain at the N-terminus of PP5c. Further purification steps were carried out by using a combination of Ni²⁺-saturated HiTrap Chelating column (Amersham) and Superdex 75 26/60 size exclusion chromatography (Amersham). The purified protein was checked by ICP mass spectrometry and found to contain one equivalent of iron and variable amounts of zinc and manganese, accounting for another equivalent. Different aliquots were dialyzed against solutions containing either MnCl₂ or ZnCl₂. Apparently, the iron ion was not displaced by dialysis, while the other ion could be fully replaced by the selected ion, either zinc(II) or manganese(II). Protein samples were concentrated to 50 μM as determined by Bradford assay and activity tested against p-nitrophenyl phosphate (pNPP, Jena Bioscience). For phosphatase activity assays pNPP was dissolved in the assay buffer solution (10mM pNPP in 20 mM Tris-HCl pH 7.4, 0.2 μg/mL BSA) and stored at 253 K until requested. The reaction was started by the addition of PP5 (50 nM) to 500 μL of the assay buffer containing 10mM pNPP. Production of pNP was followed by monitoring the increased absorbance at 405 nm using a Cary 50 Eclipse Spectrophotometer. Activity was expressed as Unit/μg enzyme where one unit is the amount of the phosphatase required to release 1 nmol of phosphate from pNPP in one minute at 295 K under the tested assay conditions. A pNPP molar extinction coefficient of 17.8 μL·nmol⁻¹·cm⁻¹ was considered. Assay was performed in triplicate and a standard error value was obtained. Endothall, exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride and cantharidin were purchased from Sigma. Compounds were dissolved in DMSO at a stock concentration of 200 mM and added to solutions of PP5. The compounds were evaluated for their ability to inhibit the hydrolysis of pNPP. The enzyme was incubated at 298 K with increasing concentration of inhibitor and the absorbance at 405 nm monitored for 3 min after the addition of the substrate. Fitting of rates as a function of inhibitor concentration provided K_i values.

The protein samples (50 μM and 370 μM) in a buffer containing Tris-HCl 50 mM, NaCl 150 mM, DTT 1 mM, at pH 7.5 were analysed by 400 MHz NMR at 295 K and by relaxometry at 288, 295, and 303 K. The NMRD profiles were collected with a Stellar fast field cycling relaxometer in

the 0.01–40 MHz proton Larmor frequency range. The instrument provides longitudinal relaxation rate measurements that are affected by an error of about $\pm 1\%$. The analysis of the temperature dependence of the longitudinal water proton relaxation rate at low field reveals that, for this paramagnetic protein, τ_M and T_{1M} are of the same order. Actually the Fe,Mn-PP5 relaxivity was not affected by the temperature in the range 288-295 K while decreases from 295 to 303 K as shown in Figure 4. T_1 experiments at high field were recorded with a simple inversion recovery scheme at 295 K on a Bruker AV400 operating at 400.1 MHz and equipped with a BBO probe.

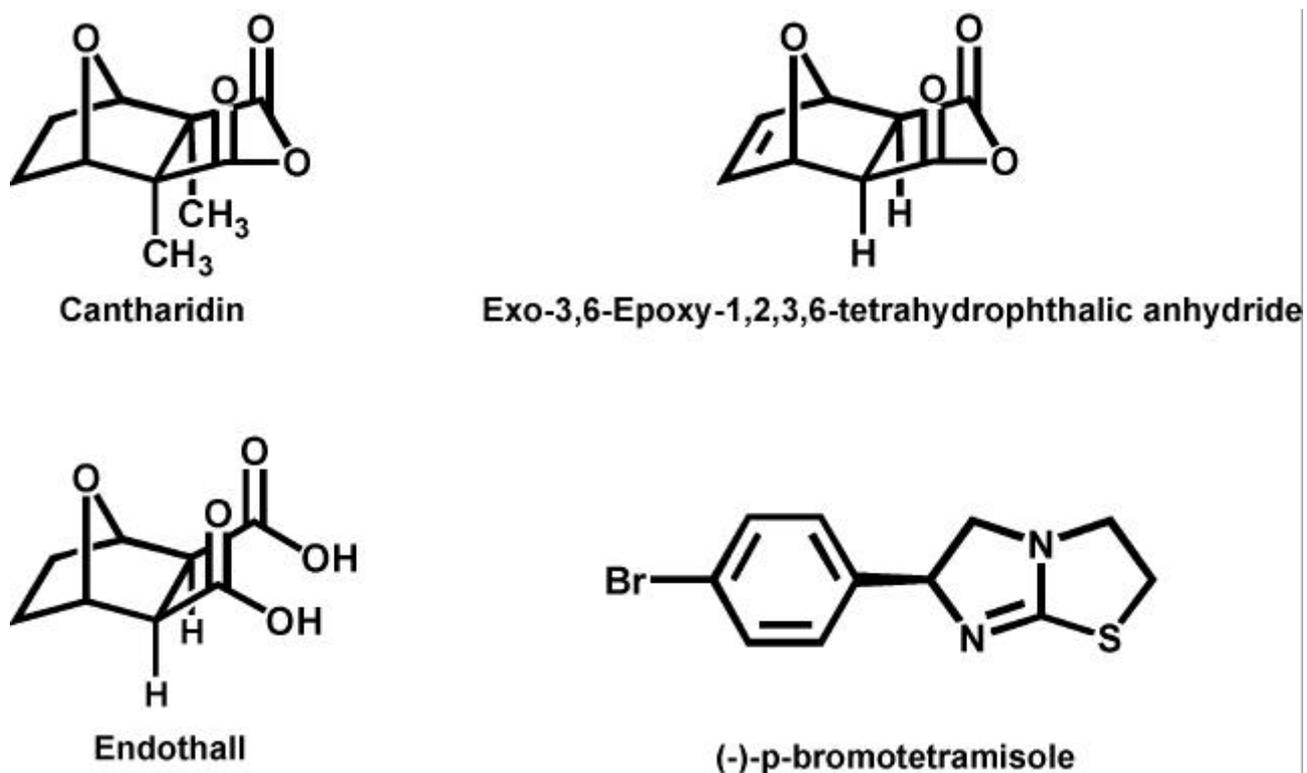


Figure S1. Chemical formula of the investigated ligands.