Electronic Supplementary Material (ESI) for Dalton Transactions.

Structure of urease inactivated by Ag(I): a new paradigm for enzyme inhibition by heavy metals

Luca Mazzei,[†] Michele Cianci,[‡] Antonio Gonzalez Vara,[†] Stefano Ciurli[,†]*

[†]Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Italy.

[‡]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Ancona, Italy.

SUPPLEMENTARY INFORMATION

Figure 1-SI. Pairwise root mean square deviation (RMSD) of Cα atoms between the α, β and γ subunits (red, purple and blues, respectively) between the native (PDB code 4CEU) and SPU inhibited in the presence of silver nitrate (PDB code 6G48).

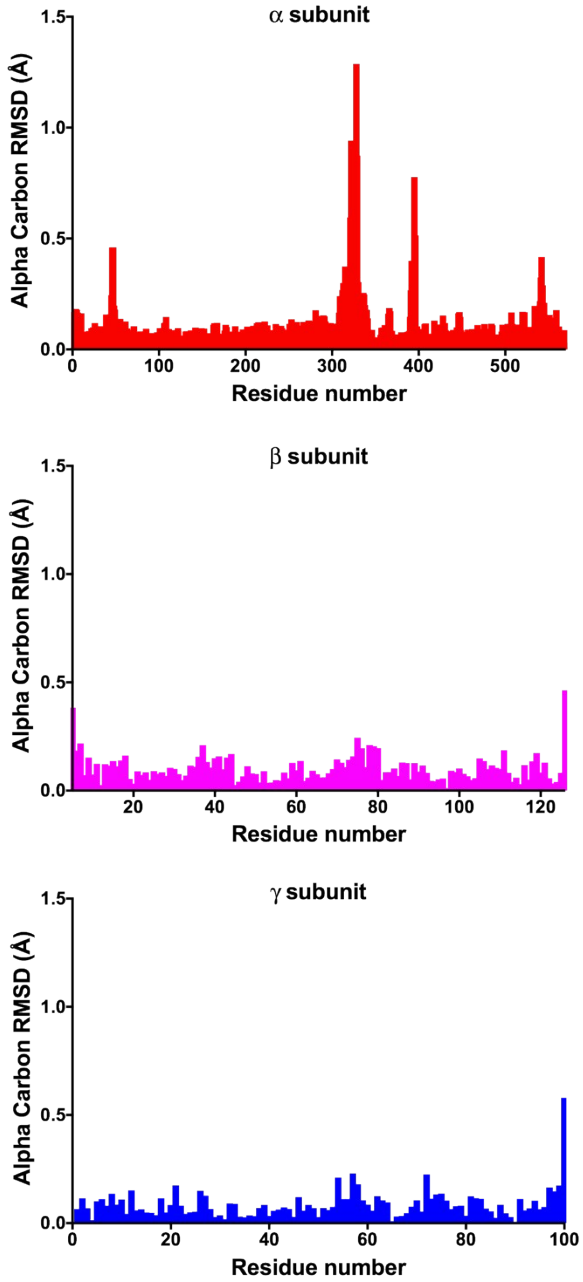

Figure 2-SI. Multiple sequence alignment of ureases from different organisms, chosen within the UNIPROT database as those reviewed and for which their existence has been evidenced at the protein level. Each sequence is designated by the corresponding UNIPROT code and the source organism. The aligned amino acid sequences correspond to the region involved in the Ni(II)-binding in the active site and to the region affected by the binding of Ag(I) in urease from *Sporosarcina pasteurii* (SPU, *). Amino acid residues involved in Ni(II) binding for all ureases and those binding Ag(I) in SPU are shown in blue and red, respectively.

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|P41020|Sporosarcina_pasteurii*        135 DTHVHFINPDQVDVALANGITTLFGGGTGPAEGSKATTVTPGPWNIEKMLKSTEGLPINVGILGKGHGSSIAPIMEQIDAGAAGLKIHEDWGATPASIDRSLTVADEADVQVAIHSDTLN 254
|P0CB00|Ureaplasma_urealyticum         139 DTHVHWLEPEIVPVALDGGITTVIAGGTGMNDGTKATTVSPGKFWVKSALQAADGLPINAGFLAKGQGM-EDPIFEQIVAGACGLKIHEDWGATGNAIDLALTVAEKTDVAVAIHTDTLN 257
|P50047|Streptococcus_salivarius     136 DLHVHYISADLPEFGLDNGITTLFGGGTGPADGSNATTCTPGKFHITRMLQAVDDMPANFGFLAKGVGSETEVVEEQIKAGAAGIKTHEDWGATYAGIDNSLKVADKYDVSFAVHTDSLN 255
|P9WFF1|Mycobacterium_tuberculosis    139 DCHVHLICPQIIVEALAAGTTTIIGGGTGPAEGTKATTVTPGEWHLARMLESLDGWPVNFALLGKGNTVNPDALWEQLRGGASGFKLHEDWGSTPAAIDTCLAVADVAGVQVALHSDTLN 258
|P77837|Bacillus_subtilis            135 DTHIHFICPQQMEVALSSGVTTLLGGGTGPATGSKATTCTSGAWYMARMLEAAEEFPINVGFLGKGNASDKAPLIEQVEAGAIGLKLHEDWGTTPSAIKTCMEVVDEADIQVAIHTDTIN 254
|P69996|Helicobacter_pylori           134 DTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAAEEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVAIHTDTLN 253
|Q08716|Helicobacter_felis           134 DTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHTDTLN 253
|Q2YPD5|Brucella_abortus             134 DTHVHFISPQQVDEALNAGITCMVGGGTGPAHGTLATTCTPGPWHIARLIQSFDGLPMNIGVFGKGNASLPGALEEMVRAGACGLKLHEDWGCTPAAIDNCLSVADHFDVQVAIHTDTLN 253
|Q8G2P8|Brucella_suis               134 DTHVHFISPQQVDEALNAGITCMVGGGTGPAHGTLATTCTPGPWHIARLIQSFDGLPMNIGVFGKGNASLPGALEEMVRAGACGLKLHEDWGCTPAAIDNCLSVADHFDVQVAIHTDTLN 253
|Q9L644|Prochlorococcus_marinus       134 DTHIHFICPQQIETALASGVTTMLGGGTGPATGTNATTCTPGAFHISRMIQSAEAFPVNLGFFGKGNSSNETNLFEQVNAGACGLKLHEDWGTTPSTINSCLNVADTLDVQVCIHTDTLN 253
|O87402|Synechococcus_sp.            134 DTHIHFICPQQIETALASGMTTLMGGGTGPATGTNATTCTPGAFHIGRMLQAAEGLPVNLGFFGKGNASTPEALEEQVRAGACGLKLHEDWGTTPATIDACLSVADRMDVQVCIHTDTLN 253
|Q07397|Bacillus_sp.                 134 DAHIHFICPQQIETALASGVTTMIGGGTGPATGTNATTCTPGPWNIHRMLQAAEEFPINLGFLGKGNCSDEAPLKEQIEAGAVGLKLHEDWGSTAAAIDTCLKVADRYDVQVAIHTDTLN 253
|P18314|Klebsiella_aerogenes          132 DTHIHWICPQQAEEALVSGVTTMVGGGTGPAAGTHATTCTPGPWYISRMLQAADSLPVNIGLLGKGNVSQPDALREQVAAGVIGLKIHEDWGATPAAIDCALTVADEMDIQVALHSDTLN 251
|P17086|Proteus_mirabilis            132 DTHIHFICPQQAQEGLVSGVTTFIGGGTGPVAGTNATTVTPGIWNMYRMLEAVDELPINVGLFGKGCVSQPEAIREQITAGAIGLKIHEDWGATPMAIHNCLNVADEMDVQVAIHSDTLN 251
|O00084|Schizosaccharomyces_pombe      402 DSHVHFICPQQIEEALASGITTMYGGGTGPSTGTNATTCTPNKDLIRSMLRSTDSYPMNIGLTGKGNDSGSSSLKEQIEAGCSGLKLHEDWGSTPAAIDSCLSVCDEYDVQCLIHTDTLN 521
|P07374|Canavalia_ensiformis         405 DCHVHYICPQLVYEAISSGITTLVGGGTGPAAGTRATTCTPSPTQMRLMLQSTDDLPLNFGFTGKGSSSKPDELHEIIKAGAMGLKLHEDWGSTPAAIDNCLTIAEHHDIQINIHTDTLN 524
|E0ZS48|Oryza_sativa               403 DCHVHFICPQLAEEAIASGITTLVGGGTGPAHGTCATTCTPSPSHMKLMLQSTDELPINMGFTGKGNTTKPDGLAEIIKAGAMGLKLHEDWGSTPAAIDNCLSVAEAFDIQVNIHTDTLN 522
|P67404|Staphylococcus_aureus          136 DTHVHFINPEQAEVALESGITTHIGGGTGASEGSKATTVTPGPWHIHRMLEAAEGLPINVGFTGKGQATNPTALIEQINAGAIGLKVHEDWGATPSALSHALDVADEFDVQIALHADTLN 255
|Q4A0J5|Staphylococcus_saprophyticus  136 DTHVHFINPEQSQVALESGITTHIGGGTGASEGTKATTVTPGPWHLHRMLLAAESLPLNIGFTGKGQAVNHTALVEQIHAGAIGLKVHEDWGATPSALDHALQVADDYDVQIALHADTLN 255
|P42873|Staphylococcus_xylosus        136 DTHVHFVNPEQSQVALESGITTHIGGGTGASEGAKATTVTPGPWHLHRMLLAAESLPLNIGFTGKGQAVNHTALVEQIHAGAIGLKVHEDWGATPSALDHALQVADDYDVQIALHADTLN 255
|Q79VJ3|Corynebacterium_glutamicum    135 DTHVHFLGTDQVNTALASGITTMIGGGTGPSQASMATTVTPGQWNTYNMLSAFEGMPMNFGILGKGHGSSKSPLAEQVRAGAIGLKIHEDWGATPSSINTALEVADDMDIQVALHSDTLN 254
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|P41020|Sporosarcina_pasteurii*        255 EAGFLEDTVRAINGRVIHSFHVEGAGGGHAPDIMAMAGHPNVLPSSTNPTRPFTVNTIDEHLDMLMVCHHLKQNIPEDVAFADSRIRPETIAAEDILHDLGIISMMSTDALAMGRAGEMV 374
|P0CB00|Ureaplasma_urealyticum         258 EAGFVEHTIAAMKGRTIHAYHTEGAGGGHAPDILESVKYAHILPASTNPTIPYTVNTIAEHLDMLMVCHHLNPKVPEDVAFADSRIRSQTIAAEDLLHDMGAISIMSSDTLAMGRIGEVV 377
|P50047|Streptococcus_salivarius     256 EGGFMENTLESFQGRTVHTFHTEGSGGGHAPDIMVFAGKENILPSSTNPTNPYTTNAIGELLDMVMVCHHLDPKIPEDVSFAESRVRKQTVAAEDVLHDMGALSIMTSDAMAMGRVGEVA 375
|P9WFF1|Mycobacterium_tuberculosis    259 ETGFVEDTIGAIAGRSIHAYHTEGAGGGHAPDIITVAAQPNVLPSSTNPTRPHTVNTLDEHLDMLMVCHHLNPRIPEDLAFAESRIRPSTIAAEDVLHDMGAISMIGSDSQAMGRVGEVV 378
|P77837|Bacillus_subtilis            255 EAGFLENTLDAIGDRVIHTYHIEGAGGGHAPDIMKLASYANILPSSTTPTIPYTVNTMDEHLDMMMVCHHLDAKVPEDVAFSHSRIRAATIAAEDILHDIGAISMTSSDSQAMGRVGEVI 374
|P69996|Helicobacter_pylori           254 EAGCVEDTMAAIAGRTMHTFHTEGAGGGHAPDIIKVAGEHNILPASTNPTIPFTVNTEAEHMDMLMVCHHLDKSIKEDVQFADSRIRPQTIAAEDTLHDMGIFSITSSDSQAMGRVGEVI 373
|Q08716|Helicobacter_felis           254 EAGCVEDTLEAIAGRTIHTFHTEGAGGGHAPDVIKMAGEFNILPASTNPTIPFTKNTEAEHMDMLMVCHHLDKSIKEDVQFADSRIRPQTIAAEDQLHDMGIFSITSSDSQAMGRVGEVI 373
|Q2YPD5|Brucella_abortus             254 EGGFVEDTLNAFKGRTIHSFHTEGAGGGHAPDIIRVCQYPNVLPASTNPTRPYTVNTIAEHLDMLMVCHHLSPAIPEDIAFAESRIRKETIAAEDILHDMGAFSIISSDSQAMGRVGEMI 373
|Q8G2P8|Brucella_suis               254 EGGFVEDTLNAFKGRTIHSFHTEGAGGGHAPDIIRVCQYPNVLPASTNPTRPYTVNTIAEHLDMLMVCHHLSPAIPEDIAFAESRIRKETIAAEDILHDMGAFSIISSDSQAMGRVGEMI 373
|Q9L644|Prochlorococcus_marinus       254 EAGFVEDTIAAIAGRTIHTFHTEGAGGGHAPDIIKICGENNVLPSSTNPTRPYTKNTLEEHLDMLMVCHHLDSKIPEDIAFAESRIRRETIAAEDILHDIGAFSIIASDSQAMGRVGEVI 373
|O87402|Synechococcus_sp.            254 EAGFVEDTIAAIKGRTIHTFHTEGAGGGHAPDIIKICGEANVLPSSTNPTRPYTRNTLEEHLDMLMVCHHLDPRIPEDVAFAESRIRRETIAAEDILHDLGAFSIIASDSQAMGRVGEVI 373
|Q07397|Bacillus_sp.                 254 EGGFVEDTLKAIDGRVIHTYHTEGAGGGHAPDIIKAAGFPNILPSSTNPTRPYTINTLEEHLDMLMVCHHLDANIPEDIAFADSRIRKETIAAEDVLHDLGVFSMISSDSQAMGRVGEVI 373
|P18314|Klebsiella_aerogenes          252 ESGFVEDTLAAIGGRTIHTFHTEGAGGGHAPDIITACAHPNILPSSTNPTLPYTLNTIDEHLDMLMVCHHLDPDIAEDVAFAESRIRRETIAAEDVLHDLGAFSLTSSDSQAMGRVGEVI 371
|P17086|Proteus_mirabilis            252 EGGFYEETVKAIAGRVIHVFHTEGAGGGHAPDVIKSVGEPNILPASTNPTMPYTINTVDEHLDMLMVCHHLDPSIPEDVAFAESRIRRETIAAEDILHDMGAISVMSSDSQAMGRVGEVI 371
|O00084|Schizosaccharomyces_pombe      522 ESSFVEGTFKAFKNRTIHTYHVEGAGGGHAPDIISLVQNPNILPSSTNPTRPFTTNTLDEELDMLMVCHHLSRNVPEDVAFAESRIRAETIAAEDILQDLGAISMISSDSQAMGRCGEVI 641
|P07374|Canavalia_ensiformis         525 EAGFVEHSIAAFKGRTIHTYHSEGAGGGHAPDIIKVCGIKNVLPSSTNPTRPLTSNTIDEHLDMLMVCHHLDREIPEDLAFAHSRIRKKTIAAEDVLNDIGAISIISSDSQAMGRVGEVI 644
|E0ZS48|Oryza_sativa               523 ESGCVEHTIAAFKDRTIHTYHSEGAGGGHAPDIIKVCGVKNVLPSSTNPTRPFTLNTVDEHLDMLMVCHHLDRNIPEDVAFAESRIRAETIAAEDILHDMGAISIISSDSQAMGRIGEVI 642
|P67404|Staphylococcus_aureus          256 EAGFMEDTMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAFSNILPSSTNPTLPYTHNTVDEHLDMVMITHHLNAAIPEDIAFADSRIRKETIAAEDVLQDMGVFSMISSDSQAMGRVGEVI 375
|Q4A0J5|Staphylococcus_saprophyticus  256 EAGFMEETMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAYANILPSSTNPTLPYTVNTIDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDMGVFSMVSSDSQAMGRVGEVI 375
|P42873|Staphylococcus_xylosus        256 EAGFMEETMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAYSNILPSSTNPTLPYTVNTIDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDIGVFSMVSSDSQAMGRVGEVI 375
|Q79VJ3|Corynebacterium_glutamicum    255 EAGFVEDTIEAIAGRVIHTFHTEGAGGGHAPDLIRVAALPNVLPASTNPTLPYTRNTVEEHLDMVMVAHHLNPDIPEDVAFADSRIRAETIAAEDVLHDMGIFSITSSDSQAMGRVGETI 374
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Table 1-SI. Data collection, processing and refinement statistics for Ag(I)-inhibited SPU.

Data collection	
Wavelength (Å)	0.9677
Detector	DECTRIS EIGER X 4M
Crystal-to-Detector distance (mm)	101.1
Oscillation angle (degrees)	0.100
Number of images	800
Space group	$P6_322$
Unit cell (*a, b, c,* Å)	131.1, 131.1, 189.1
Resolution range (Å)[a]	45.43 – 1.91
Total number of reflections[a]	674571 (42240)
Unique reflections[a]	74481 (4553)
Multiplicity[a]	9.1 (9.3)
Completeness[a] (%)	99.7 (100.0)
R_{sym}[a,b] (%)	13.7 (162.2)
R_{pim}[a,c] (%)	5.1 (59.1)
Mean I half-set correlation CC(1/2)[a]	0.998 (0.755)
Mean I/σ(I)[a]	13.0 (1.5)
Refinement statistics	
Monomers in the asymmetric unit	3
R_{factor}[d] (%)	16.69
R_{free}[d] (%)	20.89
Cruickshank's DPI for coordinate error[e] based on R_{factor} (Å)	0.127
Wilson plot B-factor (Å^2)	24.2
Average all atom B-factor[f] (Å^2)	32.337
RMS (bonds)[d]	0.14
RMS (angles)[d]	1.62
Total number of atoms	6751
Total number of water molecules	472
Solvent content (%)	55.11
Matthews Coefficient (Å^3/Da)	2.74
Ramachandran plot[g]	
Most favored regions (%)	89.8
Additionally allowed regions (%)	9.3
Generously allowed regions (%)	0.7
Disallowed regions (%)	0.2

[a] Highest resolution bin in parentheses;

[b] $R_{sym} = \sum_{hkl}\sum_{j}|I_j - \langle I\rangle| / \sum_{hkl}\sum_{j} I_j$, where I is the intensity of a reflection, and $\langle I\rangle$ is the mean intensity of all symmetry related reflections j;

[c] $R_{p.i.m.} = \sum_{hkl}\left\{[1/(N-1)]\sum_{j}|I_j - \langle I\rangle|\right\}^{½} / \sum_{hkl}\sum_{j} I_j$, where I is the intensity of a reflection, and $\langle I\rangle$ is the mean intensity of all symmetry related reflections j, and N is the multiplicity;[1]

[d] Taken from REFMAC;[2] R_{free} is calculated using 5% of the total reflections that were randomly selected and excluded from refinement;

[e] $DPI = R_{factor} \cdot D_{max} \cdot compl^{-⅓} \sqrt{\frac{N_{atoms}}{(N_{refl} - N_{params})}}$, where N_{atoms} is the number of the atoms included in the refinement, N_{refl} is the number of the reflections included in the refinement, D_{max} is the maximum resolution of reflections included in the refinement, *compl* is the completeness of the observed data, and for isotropic refinement, $N_{params} \approx 4N_{atoms}$;[3]

[f]Taken from BAVERAGE;[4]
[g]Taken from PROCHECK.[4]

Table 2-SI. Selected distances and angles around the Ni(II) ions in the crystal structure of native SPU (PDB code 4CEU) and Ag(I)-inhibited SPU (PDB code 6G48).

PDB code	**4CEU**	**6G48**
Ni(1) - αLys220* Oθ1	1.94	1.98
Ni(1) - O_B[a]	2.08	2.08
Ni(1) - O_1	2.24	2.16
Ni(1) - αHis249 Nδ	2.03	2.03
Ni(1) - αHis275 Nε	2.02	2.06
Ni(2) - αLys220* Oθ2	2.08	2.16
Ni(2) - O_B	2.12	2.16
Ni(2) - O_2	2.07	2.15
Ni(2) - αHis137 Nε	2.11	2.02
Ni(2) - αHis139 Nε	2.08	2.13
Ni(2) - αAsp363 Oδ1	2.10	2.19
Ni(1) ••• Ni(2)	3.67	3.67
O_1 ••• O_2	2.37	2.20
L - Ni - L Angles (°)		
αLys220* Oθ1 - Ni(1) - αHis249 Nδ	100.4	105.3
αLys220* Oθ1 - Ni(1) - αHis275 Nε	107.2	105.2
αLys220* Oθ1 - Ni(1) - O_B	96.6	95.1
αLys220* Oθ1 - Ni(1) - O_1	108.2	105.2
αHis249 Nδ - Ni(1) - αHis275 Nε	98.6	97.8
αHis275 Nε - Ni(1) - O_B	94.6	94.7
O_B - Ni(1) - O_1	67.0	63.6
O_1 - Ni(1) - αHis249 Nδ	89.3	92.5
αHis249 Nδ - Ni(1) - O_B	154.2	152.2
αHis275 Nε - Ni(1) - O_1	141.6	143.9
αLys220* Oθ2 - Ni(2) - αHis137 Nε	90.8	89.8
αLys220* Oθ2 - Ni(2) - αHis139 Nε	91.7	88.2
αLys220* Oθ2 - Ni(2) - O_2	92.9	91.6
αLys220* Oθ2 - Ni(2) - O_B	95.6	95.7
αAsp363 Oδ1 - Ni(2) - αHis137 Nε	82.8	87.6
αAsp363 Oδ1 - Ni(2) - αHis139 Nε	86.4	89.2
αAsp363 Oδ1 - Ni(2) - O_2	94.5	92.0
αAsp363 Oδ1 - Ni(2) - O_B	89.1	88.4
O_2 - Ni(2) – O_B	67.7	64.3
O_B - Ni(2) - αHis137 Nε	95.0	96.0
αHis137 Nε- Ni(2) - αHis139 Nε	108.5	111.3
αHis139 Nε- Ni(2) - O_2	88.4	88.4
αLys220* Oθ2 - Ni(2) - αAsp363 Oδ1	172.4	175.4
O_B - Ni(2) - αHis139 Nε	155.3	152.5
O_2- Ni(2) - αHis137 Nε	162.6	160.3
Ni(1) - O_B - Ni(2)	122.1	120.0

[a] O_B, O_1 and O_2 indicate the water/hydroxide molecules in the bridging position and bound to Ni(1) and Ni(2), respectively.

1. M. Weiss, *J. Appl. Crystal.*, 2001, **34**, 130-135.
2. G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr. D*, 1997, **53**, 240-255.
3. D. W. Cruickshank, *Acta Crystallogr. D*, 1999, **55**, 583-601.
4. M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin and K. S. Wilson, *Acta Crystallogr. D*, 2011, **67**, 235-242.