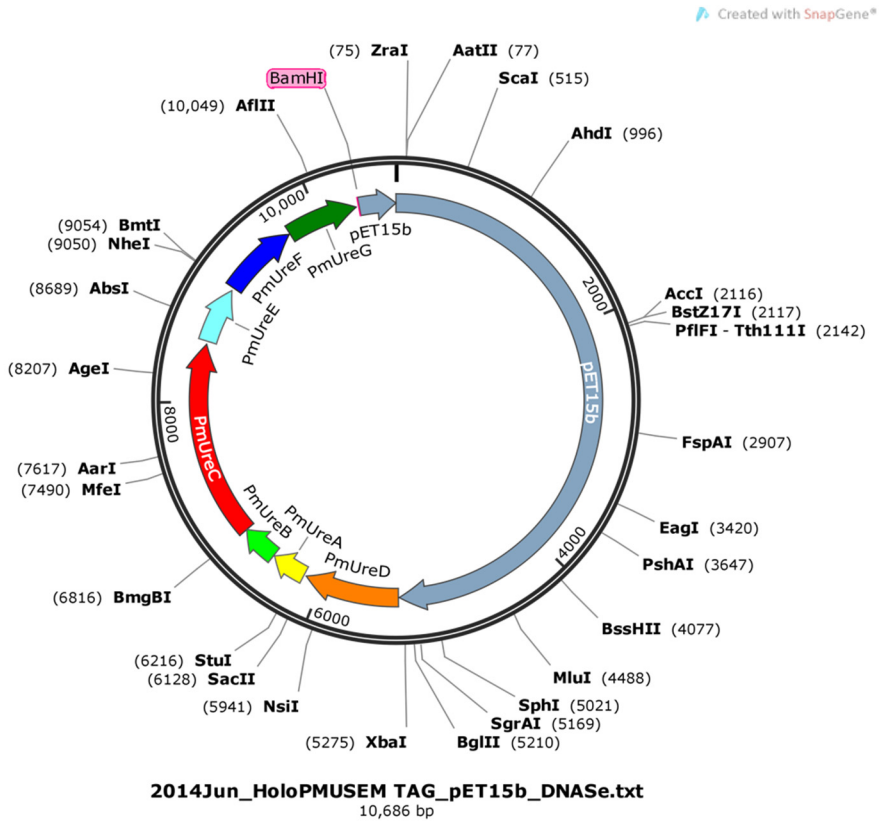
International Journal of
Molecular Sciences

MDPI

Supplementary Material

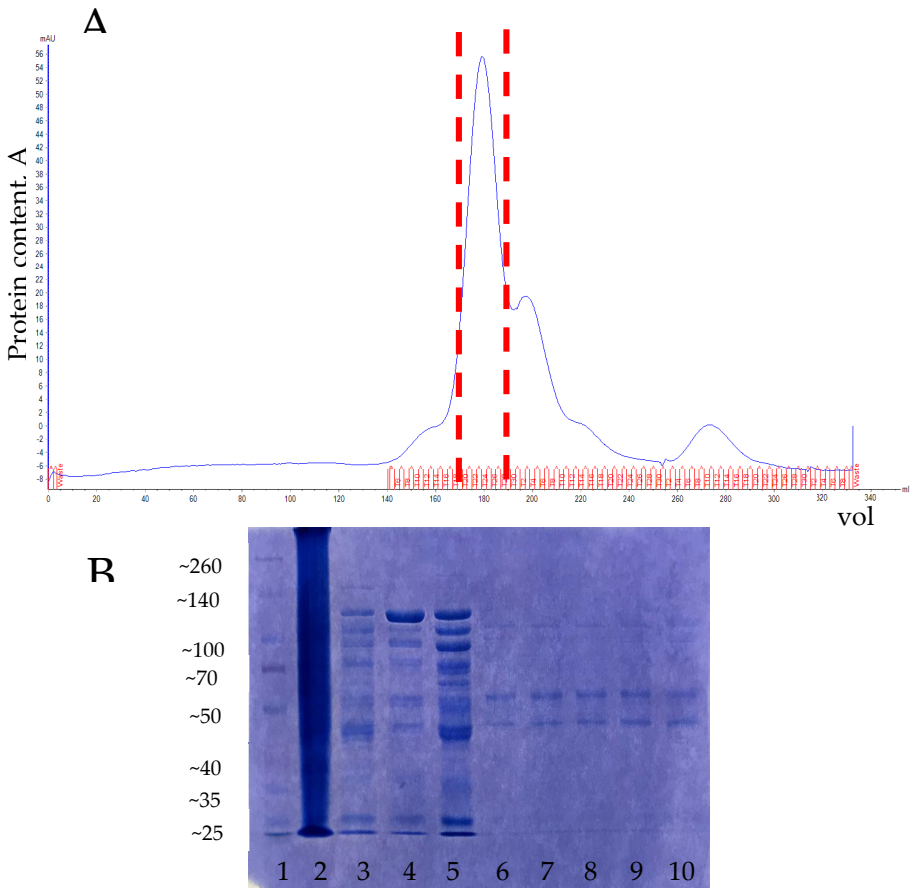
Proteus mirabilis urease: Unsuspected non-enzymatic properties relevant to pathogenicity



Supplementary Figure S1. Restriction Map of the D*ureR* holoPMU::pET15b plasmid

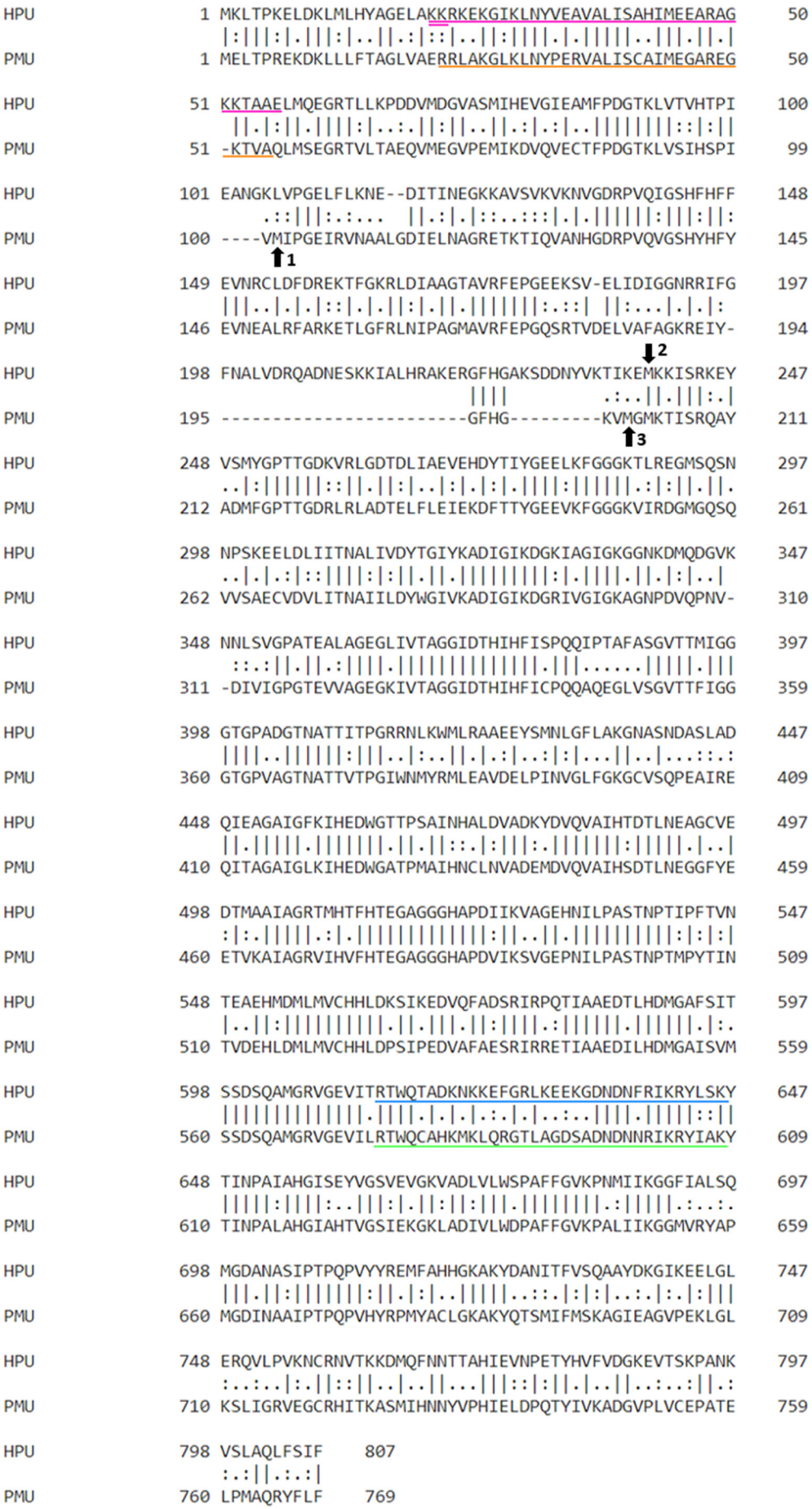
The modified operon f D*ureR* holoPMU contains seven genes in tandem *ure*D-*ure*ABC-*ure*EFG: three structural genes (*ure*A, *ure*B and *ure*C) and four genes encoding accessory and regulatory proteins (*ure*D, *ure*E, *ure*F, and *ure*G), thus encoding a fully active *Proteus mirabilis* urease (PMU) urease. The modified operon was inserted into a pET15b plasmid

between XbaI and BamHI restriction sites. The figure was prepared with the Snapgene software (from Insight Science; available at www.snapgene.com).



Supplementary Figure S2. Purification of recombinant *P. mirabilis*

Purification PMU consisted of a sequence of ion exchange and molecular size exclusion chromatographies. Panel A shows the chromatographic patterns obtained in the Superdex 200™ 26/60 column. The dashed lines indicate fractions with ureolytic activity. Panel B shows a SDS-PAGE 12 % of samples during the purification protocol (1- Molecular mass markers, 2- crude extract, 3- active pool after Q-Sepharose column, 4 and 5- active pool from the first Source 15Q column, 6,7,8,9 and 10 are fractions from the Superdex S200 column). The protein band with Mr ~60kDa in lanes 6-10 corresponds to subunit C of *P. mirabilis* urease.


HPU 1 MKLTPKELDKLMLHYAGELAKKRKEKGIKLNYVEAVALISAHIMEEARAG 50
PMU 1 MELTPREKDKLLLFTAGLVAERRLAKGLKLNYPERVALISCAIMEGAREG 50
HPU 51 KKTAAELMQEGRTLLKPDDVMDGVASMIHEVGIEAMFPDGTKLVTVHTPI 100
PMU 51 -KTVAQLMSEGRTVLTAEQVMEGVPEMIKDVQVECTFPDGTKLVSIHSPI 99
HPU 101 EANGKLVPGELFLKNE--DITINEGKKAVSVKVKNVGDRPVQIGSHFHFF 148
PMU 100 ----VMIPGEIRVNAALGDIELNAGRETKTIQVANHGDRPVQVGSHYHFY 145
1
HPU 149 EVNRCLDFDREKTFGKRLDIAAGTAVRFEPGEEKSV-ELIDIGGNRRIFG 197
PMU 146 EVNEALRFARKETLGFRLNIPAGMAVRFEPGQSRTVDELVAFAGKREIY- 194
2
HPU 198 FNALVDRQADNESKKIALHRAKERGFHGAKSDDNYVKTIKEMKKISRKEY 247
PMU 195 ----------GFHG---------KVMGMKTISRQAY 211
3
HPU 248 VSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSN 297
PMU 212 ADMFGPTTGDRLRLADTELFLEIEKDFTTYGEEVKFGGGKVIRDGMGQSQ 261
HPU 298 NPSKEELDLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVK 347
PMU 262 VVSAECVDVLITNAIILDYWGIVKADIGIKDGRIVGIGKAGNPDVQPNV- 310
HPU 348 NNLSVGPATEALAGEGLIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGG 397
PMU 311 -DIVIGPGTEVVAGEGKIVTAGGIDTHIHFICPQQAQEGLVSGVTTFIGG 359
HPU 398 GTGPADGTNATTITPGRRNLKWMLRAAEEYSMNLGFLAKGNASNDASLAD 447
PMU 360 GTGPVAGTNATTVTPGIWNMYRMLEAVDELPINVGLFGKGCVSQPEAIRE 409
HPU 448 QIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVAIHTDTLNEAGCVE 497
PMU 410 QITAGAIGLKIHEDWGATPMAIHNCLNVADEMDVQVAIHSDTLNEGGFYE 459
HPU 498 DTMAAIAGRTMHTFHTEGAGGGHAPDIIKVAGEHNILPASTNPTIPFTVN 547
PMU 460 ETVKAIAGRVIHVFHTEGAGGGHAPDVIKSVGEPNILPASTNPTMPYTIN 509
HPU 548 TEAEHMDMLMVCHHLDKSIKEDVQFADSRIRPQTIAAEDTLHDMGAFSIT 597
PMU 510 TVDEHLDMLMVCHHLDPSIPEDVAFAESRIRRETIAAEDILHDMGAISVM 559
HPU 598 SSDSQAMGRVGEVITRTWQTADKNKKEFGRLKEEKGDNDNFRIKRYLSKY 647
PMU 560 SSDSQAMGRVGEVILRTWQCAHKMKLQRGTLAGDSADNDNNRIKRYIAKY 609
HPU 648 TINPAIAHGISEYVGSVEVGKVADLVLWSPAFFGVKPNMIIKGGFIALSQ 697
PMU 610 TINPALAHGIAHTVGSIEKGKLADIVLWDPAFFGVKPALIIKGGMVRYAP 659
HPU 698 MGDANASIPTPQPVYYREMFAHHGKAKYDANITFVSQAAYDKGIKEELGL 747
PMU 660 MGDINAAIPTPQPVHYRPMYACLGKAKYQTSMIFMSKAGIEAGVPEKLGL 709
HPU 748 ERQVLPVKNCRNVTKKDMQFNNTTAHIEVNPETYHVFVDGKEVTSKPANK 797
PMU 710 KSLIGRVEGCRHITKASMIHNNYVPHIELDPQTYIVKADGVPLVCEPATE 759
HPU 798 VSLAQLFSIF 807
PMU 760 LPMAQRYFLF 769

Supplementary Figure S3. Alignment of amino acid sequences of *Proteus mirabilis* and *Helicobacter pylori* ureases

Protein sequences for bacterial urease subunits were collected from NCBI - Protein database (https://www.ncbi.nlm.nih.gov/protein/) and aligned using the EMBOSS Needle algorithm [1]. *Proteus mirabilis* urease (GenBank: M31834.1), *Helicobacter pylori* urease (PDB 19EZ). Conserved amino acid positions are marked (|) for identity,(:) for strong similarity and (.) for similarity. Sequences targeting intracellular compartments are shown in bold underlined letters. The NLS in the N-terminal domain of HPU (positions 21-56) and PMU (positions 22-54) were predicted by cNPS mapper with scores of 4.9 and 4.7, respectively. The sequence 21KKRKEK26 in HPU was previously identified as a NSL [2]. The NLS in the C-terminal domain of HPU (positions 613-646) and PMU (positions 575-608) were predicted by cNPS mapper with scores of 4.4 and 5.6, respectively. Arrows 1 and 2 indicate the N-terminal Met residues of PMU's subunit B and C, respectively, and arrow 3 indicates the N-terminal Met residue of HPU's subunit B.

Reference

1. Needleman, S.B.; Wunsch, C.D. A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J. Mol. Biol.* **1970**, *48*, 443–453, doi:10.1016/0022-2836(70)90057-4.
2. Suarez, I.; Bodega, G.; Fernandez, B. Glutamine synthetase in brain: Effect of ammonia. *Neurochem. Int.* **2002**, *41*, 123–142, doi:10.1016/s0197-0186(02)00033-5.