

Figure S1. Sequence and structural prediction of UreA. The primary sequence corresponding to Gene Bank GI: 67516273 is shown along with transmembrane helix predictions (orange shading). Secondary structure elements resulting from homology modelling based on the X-ray structures of vSGLT (Protein Data Bank ID 3DH4) or Mhp1 (Protein Data Bank ID 2X79) are indicated by blue and green lines below and above the sequence, respectively. The red rectangle shows the position of an intracellular helix (ICH3/4), where T133 and R141 are located. The red brackets indicate the sequence included in the structural models. Amino acids mutated in this work are shown in red and substitutions are signalled below each residue.

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UreA 62 FSTAGRSVKSLVASAVVSSWTWAATLLQSSAVAYQYGTSGPFFYASGATVQIILFATLAIELKRRAPN---AHTFLEAIRA
ScDUR3 48 FTTAGRSVKTGLVAAAVVSSWIWCSTLLTSSSTKEYADGIFGGYAYAAGACFQIIAFAILAIKTKQMAPN---AHTYLELVRT
AtDUR3 73 FNTAGRNVKTGLIASVIVSQWTWAATLLQSSNVAWQYGVSGPFWYASGATIQVLLFGVMAIEIKRKAPN---AHTVCEIVKA
PiDUR3 47 FTSASRSVKPGLIASGIVSAWTWAATLLQSSAVAYRYGIGSPWWYAGATVQVLLFAQLAAKLNAPY---AHTWLEIVAT
CaDUR3 48 FATAGRSVKTGLIAACIVSSWTWSATLNGSTSQVYNNVCGSIFYAMGATVQITLFACLAIKARERAPA---AHTYLEIVKVG
OsDUR3 78 FNTAGRSVKTGLIASVIVSQWTWAATLLQSSNVAWQYGVSGPFWYASGATIQVLLFGVMAIEIKRKAPN---AHTVCEIVRA
PutP 35 YILGGRSLGPFVTALSAGASDMGWLMLGLPGAVFLSGISESWIAIGLTLGAWINWKLAVAGRLRVHTEYNNALTLDPDYFTG
hSGLT 58 FFLAGRSMVWWPIGASLFSANIGSGHFVGLAGTGAASGLAIGGFENNALVLVVLGWLFPVIYIKAGVV-----TMPEYLRK
NIS 47 FFTGGRRLAALPVGLSLSASFMSAVQVLGVPSEAYRYGLKFLWMLCGLLNSVLTALLFMPVFYRLGLT-----STYEYLEM
vSGLT 44 YFLAGKSLPWWAVGASLIAANISAEQFIGMSGSGYSILGLAIASYEWMSAITLIIVGKYFLPIFIEKGIY-----TIPEFVEK

UreA 141 RYG-TVVHLVFI VFCMLTNI LVTAMLLT---GSAVLNSMTGVPVVAACFLLP IGVVLYTLFGG IKAFTITDMYHTVIV
ScDUR3 127 RYG-KIGHGCYLFYATATN I LVTSMLLT---SGSAVFSDLTGMNTIASCFLLPVGVVVYTLFGG IKAFTFLTDYMHMTCVII
AtDUR3 152 RWG-TATHIVFLVFCLATN VVVTAMLLL---GSAVNVNALTGVNLYAASFLIPLGVVVYTLAGGLKATFLASVYHSHVIVH
PiDUR3 126 RWG-TVAHLIFMFFGLATN I VSSMLVL---GSAFVTSLTGMSTIAACFLIPLGVVLYVVGGRMSTLLCDYTHTTVLF
CaDUR3 127 RYG-TTTHFVYIFWGLVTN I LVTSMLLS---GSAFVNDLTGMNIVAAI LLLPLGVVYTLFGG IKAFTFLTDYAHTVVLI
OsDUR3 157 RWG-TPAHLVFLTFCLLTN VIVTAMLLL---GSAVNVNALTGVNLYAASFLIPLGVVVYTLAGGLKATFLASVYHSHVIVH
PutP 117 RFE-DKSRILRIISALV I LFFFTIYCASGIVAGARLFESTFCMSYETALWAGAAATILYTFITGGFLAVSWTDTVQASLMI
hSGLT 135 RFGGQRIQVYLSLLSLLY I FTKISADIFS---GAIFINLAI GLNLYLAI FLLLAITALYTTTGG LAAVIYTDLTQTVIML
NIS 124 RFS-RAVRLCGTLQYI VATMLYTGIVY---APALILNQVTCID I WASLLSTGIC ICFEYTA VGGMKAVVWTDVQVQVVML
vSGLT 121 RFN-KKLKTI LAVFWI SLYIFVNLTSVLY---LGGLALETII C I PLMYSILGLALFALVYSIY GGLS AVVWTDVIVQVFFLV

UreA 423 ATGLHYAGIGMG-YLYLLMGV I ISSAVFPGAMTLVWKQONWIAAAA S PVLGLAMSLVAWL-VTTKTEYGVFTVETTGAN
ScDUR3 416 SVGLYGGISMG-YIYEMMG I ISSAVLPVVLTLCKDMNLVAAVV S PILGTGLAIMSWL-VCTKSLYKELTVDTTFMDY
AtDUR3 447 AVVLNKAQVSLG-WMYLAMG V I GSAVPIPIAEFMLLWSKANAFGAILGATSGCVFGIITWL-TTAKTQYGRVLDSTGKNG
PiDUR3 426 GVIFFYIGVSMG-WLYTFMG V I LGSVPIALCITWSKASRAGCVGGAVIGFVVGIAAWLGVTAAYNAGVINVTSGGNY
CaDur3 415 AIGLYYAKVSMG-YLYEMMG V I IGGAVLSSALTILSKRQNWHAATFTPII STALAIMAWL-VCTKKKFSVTYTKTFEDD
OsDur3 452 AVVLNVAGVSLG-WMYLAMG V I GSAVPIPIALLLWSKANAVGAMGGAVSGCALGVAVWL-TVAKVQYGRVNLDTTGRNA
PutP 390 AANPENRVLGLVSYAWAGFGA AFGPVVL---FSVMWSRMRTRNGALAGMIIG-ALTIVVWK-----QFGWLG L-----Y
hSGLT 442 PIVQSAQSQQLFDYIQS I TSYLGPPIAAVFLLAIFWKRVRNEP GAFWGLILGLLIGISRMI---TEFAYGTGSCMEP SNCP
NIS 402 AALSSLLGGGV LQGSFTVMG V I SGPLLGAFILGMFLPACNTPGVLAGLGAGLALS LWWALGATLYPPEQOTMRVLPSSAA
vSGLT 414 APMLGGIGQAFQ-YIQEY TGLVSPGILAVFLLGLFWKKTTSKGA I IGVVASIPFALFLKF---MPLSMFPMDQMLYTLFF

UreA 565 -----SPSQQ QEE-----EEIRKLNKAAFISRCLTVGMVICFL I LWPIMPYGSYVFSKFKFFT
ScDUR3 573 DVRVNNEKLI EPNLGVV I SNAIFQEDDTQLQNELDEEQRELARG LK IAYFLCVFFALAFVLPVMPMYGSKYI FSKFKFFT
AtDUR3 566 -----PAEELREEK-----LRRAKAWIVKWLGVFT I LIVVIVPVLSPAR-VFSRGYFW
PiDUR3 576 -----QSELD PVG-----LKKAFKFAAWSSVIMTV I FMILPLPLFGASTVFSTTGFT
CaDur3 563 -----VIASQLVEG EREQYQ-----KEKEILNKSFKIC C I VCTLAICLVVLPMPPLYGTYI FSKFKFFT
OsDur3 578 -----DELKEER-----LVHAKRWIVRWGLVFTAV I VVAWPALS LPAR-RYSLGYFT
PutP 459 -----
hSGLT 546 -----LLTKPIPDVHLYRLCWSLRNSKEERIDLDAEEN I QEGPKE
NIS 518 -----ALDSFYAISYLYYGALGTLTTVLCGAL I SCLTGPTKRSTLA
vSGLT 503 -----INDDDPK

UreA 619 GWVVVGIIWLFGTAFGV I LFLWEGRSSIKRVGKMLLLD-----AMGRQWKSSALV GQDDEESEESGSAVTPSEKIVA
ScDUR3 653 GWVVVMI IWLFFSAFAVCIY P LWEGRHGIIYTLRGLYWD-----LSGQTYKLREWQNSNPQDLHVVT S QISARAHQSS
AtDUR3 614 FWAIVAIAGWTIGSIV I IGLPLWESWDTIKSVCMGMFTNDRVMK KLDLNLHRLRALTMAVPEAEKIY--LLELEKTKK--
PiDUR3 625 VVVVIGILWTFCSAFAV VLYPLWESREALIMISKGM I QG-YLCQRVSGKFPADAKEEVPALKGGLMTMIMMVNKLDPHL
CaDur3 623 GWVVVF I IWL FYSAFQV I I FPIWESRTDLYHTFRGIYWD-----LTGQTYKLKAWQNEHPEEMHAVRSQ LQAQLSR TAV
OsDur3 624 LWAAVAIAAGTVGSSV I ILLVPAESWTTITKVCAGMFTND AVYDRLLD VNLRLRAIMGAMPEAEKRYQLHETEMHPAGT
PutP 459 -----FIFGSGIVVFS L LGKAPSAM-----
hSGLT 587 TIEIETQVPEKKKGI FFRAYD LFCGLGQHGA PKMTEEEKAMKMTDTSEKPLWRTV LNVNGI I LVTAVFCHAYFA--
NIS 560 PGLLWWDLARQ T ASVAPKEE VAILDDNLVKGPEELPTGNKK PPGFLPTNEDRLFFLGQKELEGAGSWTPCVGHGGRDQO
vSGLT 510 GISVTS SSMFVTRDSFNIAAY G I MIVLAVLYTLFW-----

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Figure S2. Multiple sequence alignment of UreA and homologues of known function vs. representative members of the Sodium/solute Symporter Superfamily (SSS). Fully conserved amino acids are shaded in black, structurally conserved amino acids are shaded in grey. Mutated amino acids in UreA by classical or spontaneous mutagenesis are highlighted in blue and red-lined boxes respectively. Dotted vertical lines represent omitted portions of the alignment, which do not bear conserved residues mutated in this work. The proteins included in the alignment are UreA of *Aspergillus nidulans* (GI: 67516273) and its homologues ScDUR3 of *Saccharomyces cerevisiae* (GI: 51013791), AtDUR3 of *Arabidopsis thaliana* (GI: 9758728), PiDUR3 of *Paxillus involutus* (sequence kindly provided by Morel *et al.*, 2008), CaDUR3 of *Candida albicans* (GI: 68484979), OsDUR3 of *Oryza sativa* (GI: 115483686). SSS members include the sodium/proline symporter, PutP, of *Escherichia coli* (GI: 91210108), the sodium/glucose symporter, hSGLT, of *Homo sapiens* (GI: 4507031), the sodium/iodide symporter, NIS, of *H. sapiens* (GI: 4507035) and the sodium/galactose symporter, vSGLT, of *Vibrio parahaemolyticus* (GI: 8134671).

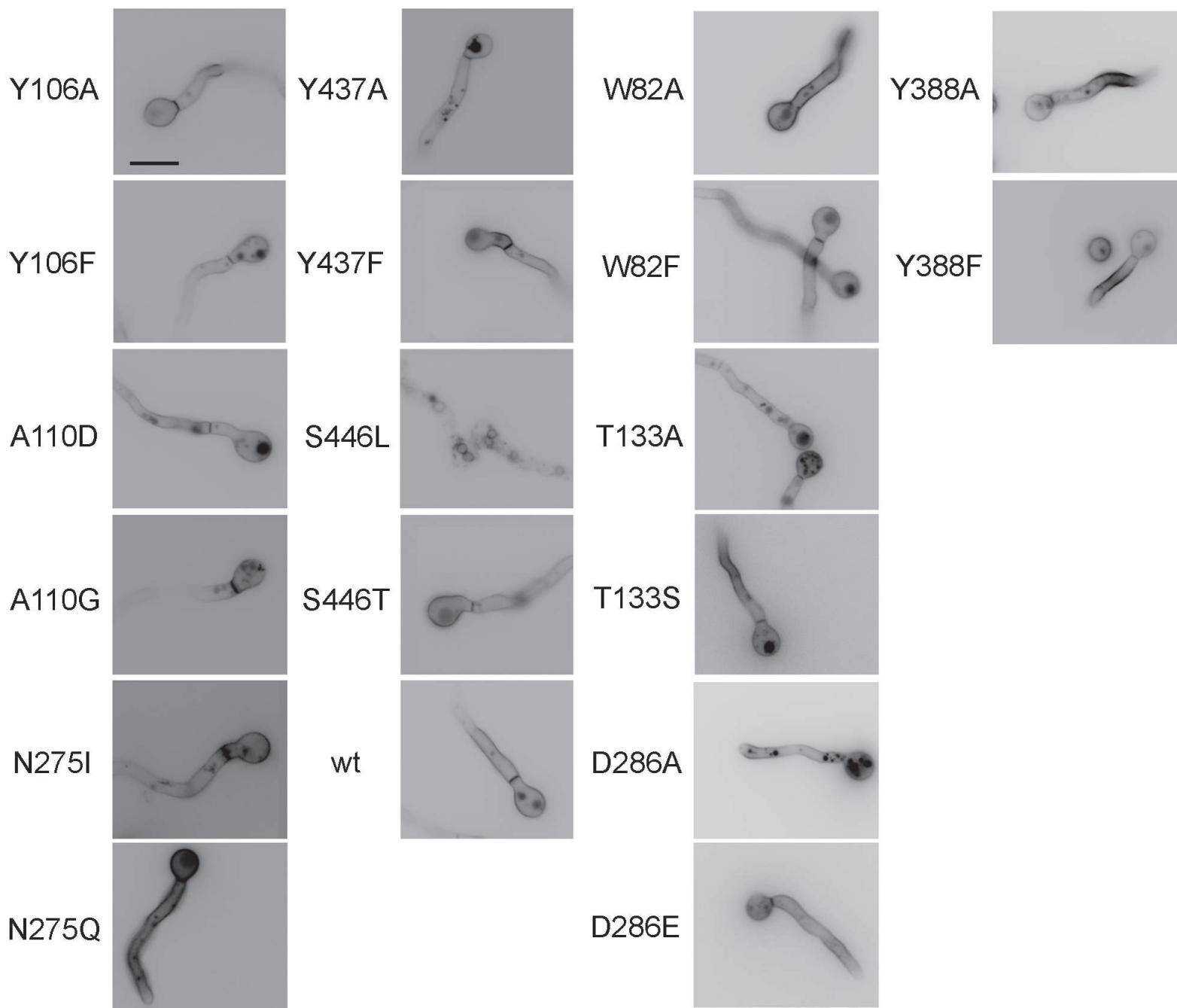


Figure S3. Epifluorescence microscopy of UreA mutants grown in de-repressing conditions. Wild type UreA-GFP localization is shown as control. The bar represents 10 μ m.

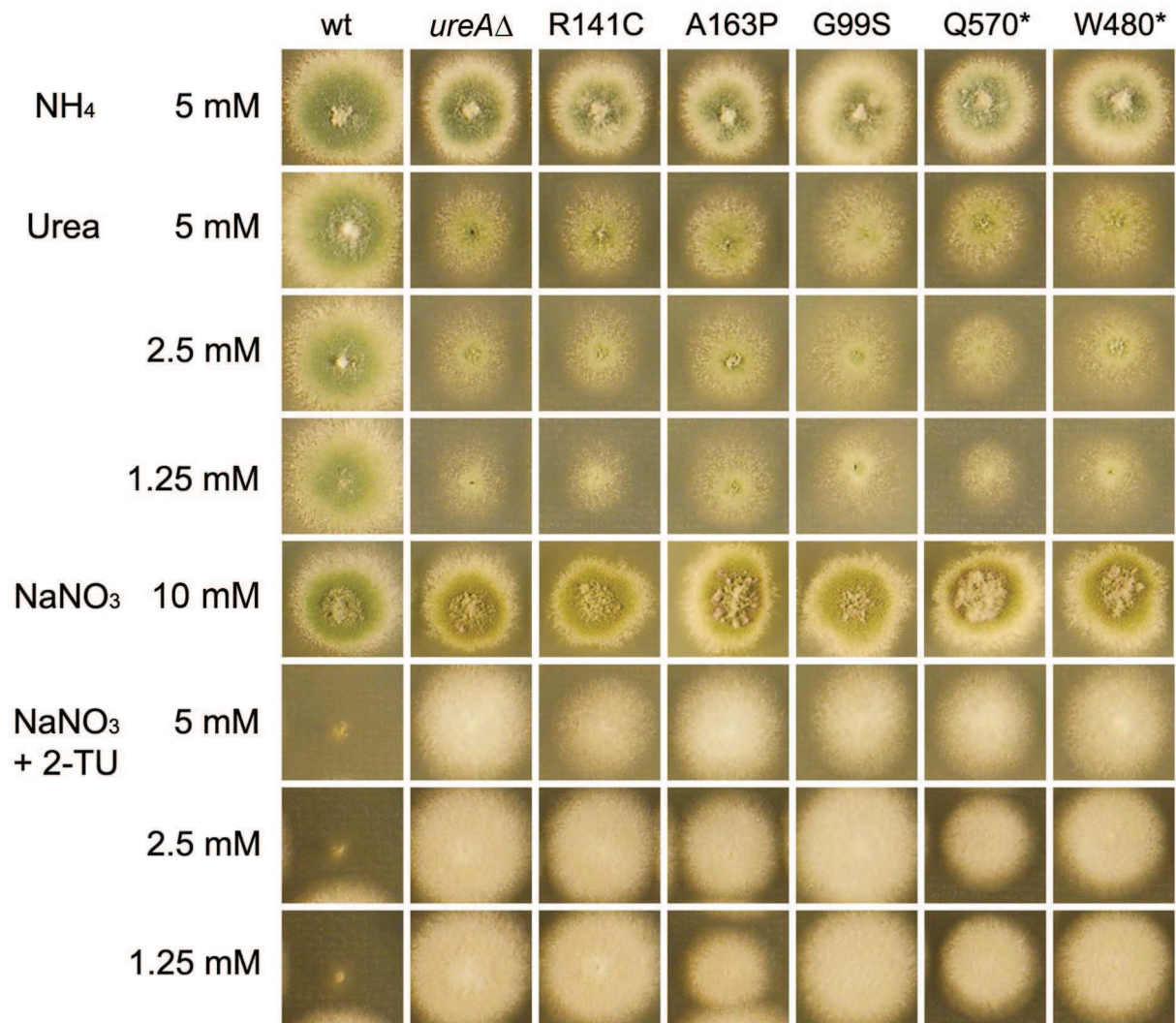


Figure S4. Growth test at 37 °C of mutant *UreA* strains isolated by random mutagenesis. Strains were grown on urea as sole nitrogen source or on 2-thiourea to test resistance. In this latter case 10 mM NaNO₃ was used as nitrogen source. Growth on ammonium 5 mM or 10 mM NaNO₃ was used as a control. A *wt* and a *ureA*Δ strain are shown as positive and negative control, respectively. Similar results were obtained at 25 °C (data not shown).

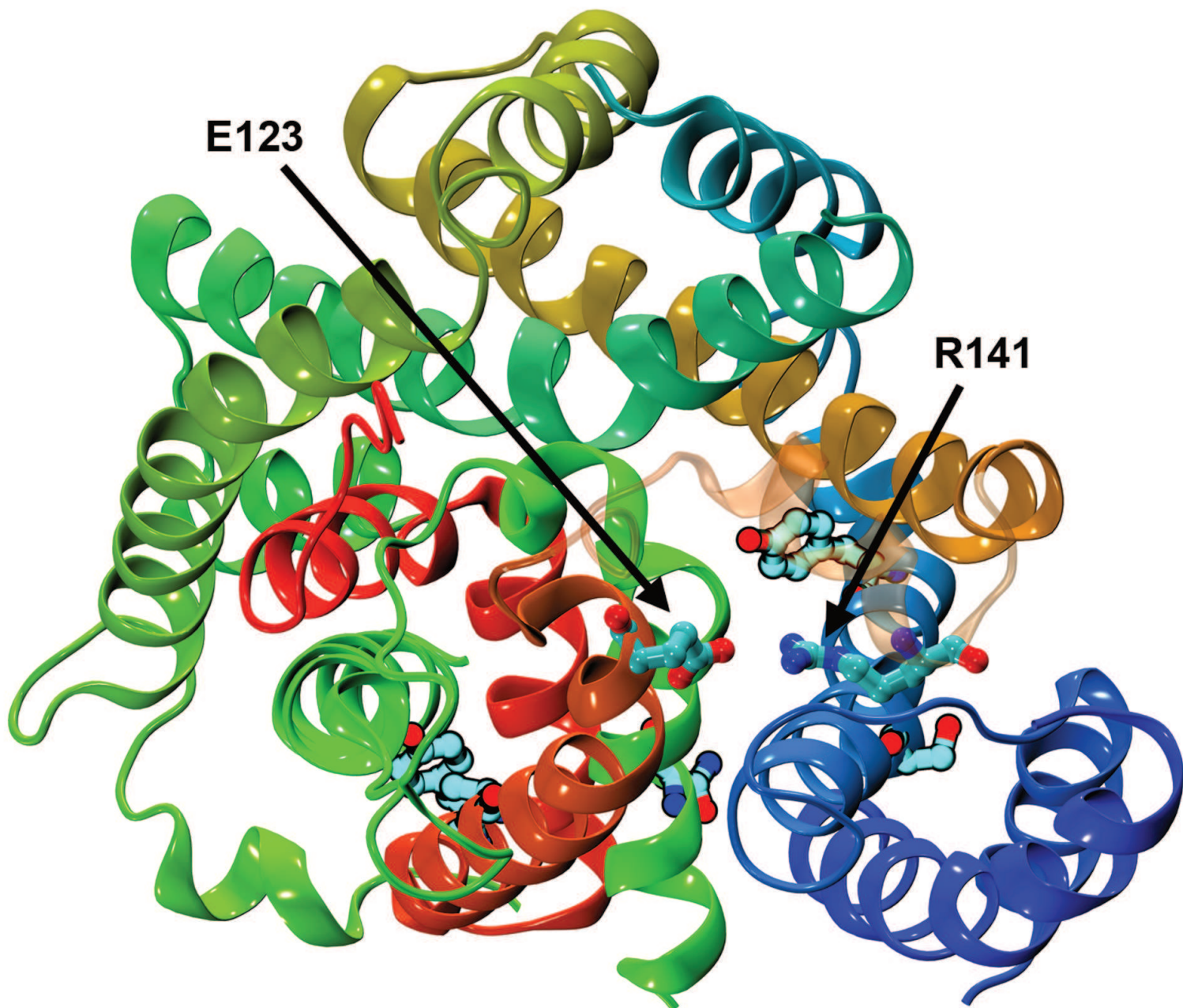


Figure S5. Molecular representation of the UreA model in the conformation presenting an outward facing cavity seen from the extracellular side. R141 and E123, which can form a salt bridge are indicated and shown in glossy color. ICH3/4 is shown as semitransparent in orange.

Table S1. *Aspergillus nidulans* strains used and constructed during this study.

Strain	Genotype
MVD 001	<i>pabaA1 veA1</i>
P002	<i>yA2 pabaA1</i>
MVD 10A	<i>ureA::gfp::AFpyrG riboB2 pyrG89 pyroA4 ΔnkuA::argB veA1</i>
MVD 13A	<i>ureAΔ::riboB riboB2 pyrG89 pyroA4 ΔnkuA::argB veA1</i>
MVD 14A	<i>yA2 ureAΔ::riboB riboB2 pyrG89 pyroA4 ΔnkuA::argB veA1</i>
MVD 100	<i>ureA1 biA1 veA1</i>
MVD 103	<i>ureA905 pabaA1 veA1</i>
MVD 205	<i>wA3 yA2 pabaA1 hhoA::mrfp::pyrGAf</i>
MVD 105*	<i>yA2 ureA1::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
MVD 106*	<i>yA2 ureA905::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
W82A*	<i>yA2 ureAW82A::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
W82F*	<i>yA2 ureAW82F::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
Y106A*	<i>ureAY106A::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
Y106F*	<i>ureAY106F::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
A110D*	<i>ureAA110D::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
A110G*	<i>ureAA110G::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
T133A*	<i>yA2 ureAT133A::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
T133S*	<i>ureAT133S::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
N275I*	<i>ureAN275I::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
N275Q*	<i>ureAN275I::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
D286A*	<i>yA2 ureAD286A::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
D286E*	<i>yA2 ureAD286E::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
Y388A*	<i>ureAY388A::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
Y388F*	<i>ureAY388F::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
Y437A*	<i>ureAY437A::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
Y437F*	<i>ureAY437F::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
S446L*	<i>ureAS446L::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
S446T*	<i>ureAS446T::gfp::AFpyrG pyrG89 pyroA4 riboB2 nkuA::argB veA1</i>
MVD 313*	<i>ureAS446L::gfp::AFpyrG hhoA::mrfp::AFpyrG pabaA1 (pyrG89) veA1</i>

* Obtained in this study.

Table S2. Oligonucleotides used in this study

Primer name	5' to 3' Sequence
Ure5-F	GAAACCTGGAGCAGTCGAAG
Ure3-R	CCCGATTTCTGAGACAAGGA
Ure5-N	GCACCGATGACAAGGGAGAT
Ure3-N	ACCAATGGATCTGGCACTAAAC
W82A-F	GCTGTCGTGAGCAGT <u>GCT</u> ACCTGGGCAGCTACTCTGCTG
W82A-R	GAGTAGCTGCCCAGGT <u>AGC</u> ACTGCTCACGACAGCAGAGG
W82F-F	GCTGTCGTGAGCAGT <u>TTT</u> ACCTGGGCAGCTACTCTGCTG
W82F-R	AGAGTAGCTGCCCAGGT <u>GAA</u> ACTGCTCACGACAGCAGAG
Y106A-F	CCTCGGGGCCGTTCTT <u>GC</u> AGCATCGGGTTGGTCTTC
Y106A-R	GAAGACCAACCCGATGCT <u>TGC</u> GAAGAACGGCCCCGAGG
Y106F-F	CCTCGGGGCCGTTCTT <u>TTT</u> GCATCGGGTTGGTCTTC
Y106F-R	GAAGACCAACCCGATGCA <u>AA</u> GAAGAACGGCCCCGAGG
A110D-F	GACAGTTTGCAGGCG <u>AC</u> ACCGTTCAGATCATC
A110D-R	GATGATCTGAACGGT <u>GTC</u> GCCTGCAAACCTGTC
A110G-F	ACTGACAGTTTGCAGGCG <u>GGC</u> ACCGTTCAGATCATCTTG
A110G-R	CAAGATGATCTGAACGGT <u>GCC</u> GCCTGCAAACCTGTCAGT
T133A-F	CGCCTAACGCACAC <u>GC</u> ATTCTGGAAGCCATCCGTG
T133A-R	TGGCTTCCAGGAAT <u>TGC</u> GTGTGCGTTAGGCGCGCGTC
T133S-F	CGCCTAACGCACACT <u>TC</u> ATTCTGGAAGCCATCCGTG
T133S-R	TGGCTTCCAGGAAT <u>GAG</u> TGTGCGTTAGGCGCGCGTC
N275I-F	ATCTTCTGGGTGCATC <u>ATC</u> CTCGTCGGTAACTTC
N275I-R	GAAGTTACCGACGAGG <u>AT</u> GATGACCCAGAAGAT
N275Q-F	GTATCTTCTGGGTGCATC <u>CAG</u> CTCGTCGGTAACTTCG
N275Q-R	CGAAGTTACCGACGAGC <u>TG</u> GATGACCCAGAAGATAC
D286A-F	GCACTGTCTTCTG <u>GCC</u> AACGGCTACTACAACAAGG
D286A-R	TTGTTGTAGTAGCCGTT <u>GGC</u> CAGGAAGACAGTGCCGAAG
D286E-F	GCACTGTCTTCTG <u>GAG</u> AACGGCTACTACAACAAGG
D286E-R	TTGTTGTAGTAGCCGTT <u>CTC</u> CAGGAAGACAGTGCCGAAG
Y388A-F	GTGTCCTCGATCCTCACCC <u>GCC</u> GATATCTACCAAGCCTAC
Y388A-R	TAGGCTTGGTAGATATC <u>GGC</u> GGTGAGGATCGAGGACAC
Y388F-F	TGTCCTCGATCCTCACCT <u>TT</u> CGATATCTACCAAGCCTA
Y388F-R	TAGGCTTGGTAGATATC <u>GAA</u> GGTGAGGATCGAGGACA
Y437A-F	CGGTATGGGCTATCTC <u>GCT</u> CTTCTCATGGGCGTC
Y437A-R	GACGCCCATGAGAAG <u>AGC</u> GAGATAGCCCATACCG
Y437F-F	GTATGGGCTATCTC <u>TT</u> CCTTCTCATGGGCG
Y437F-R	CGCCCATGAGAAG <u>GAA</u> GAGATAGCCCATAC
S446L-F	GCGTCATCATCTCC <u>TT</u> AGCCGTGTTCCCGGGC
S446L-R	GCCCGGGAACACGGCT <u>AA</u> GGAGATGATGACGCC
S446T-F	GCGTCATCATCTCC <u>AC</u> AGCCGTGTTCCCGG
S446T-R	CCGGGAACACGGCT <u>G</u> TGGAGATGATGACGCC

Mutated codons are underlined. Modified bases are denoted in black.

Table S3. Summary of modelling data

	Template (PDB id)	Identity, E and p values	Ramachandran plot statistics	Averaged G-Factors*
Inward Facing	3DH4	18 % 3,77E ⁻⁶⁵ 1.2E ⁻⁶⁹	a.a. most favored regions: 91.7 % a.a. in allowed regions:7.8 % a.a. in disallowed regions:0.5 %	Psi-psi: 0.28 Chi1-Chi2: 0.09 Chi1 only: 0.12 Omega: -0.38
Outward Facing	2JLN	14% 5.6 E ⁻⁰⁹ 1.8E ⁻¹³	a.a. most favored regions: 92.1 % a.a. in allowed regions:7.7 % a.a. in disallowed regions:0.2 %	Psi-psi: 0.26 Chi1-Chi2: -0.6 Chi1 only: 0.25 Omega: -0.34

* G-factors are a measure of unusual values for stereo chemical properties. Very negative values are indicative of significant deviations from standard conformations.