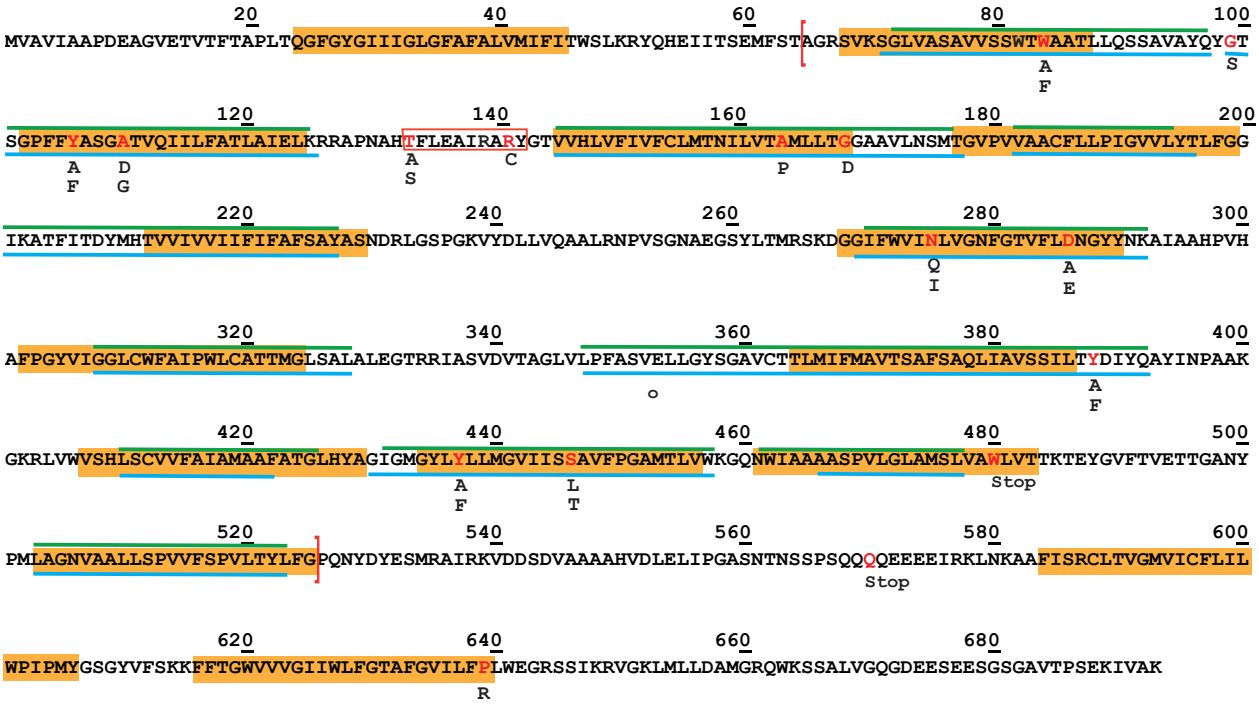

Figure S1. Sequence and structural prediction of UreA. The primary sequence corresponding to Gene Bank GI: 67516273 is shown along with transmembrane helix predictions (orange shading). Secondary structure elements resulting from homology modelling based on the X-ray structures of vSGLT (Protein Data 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.

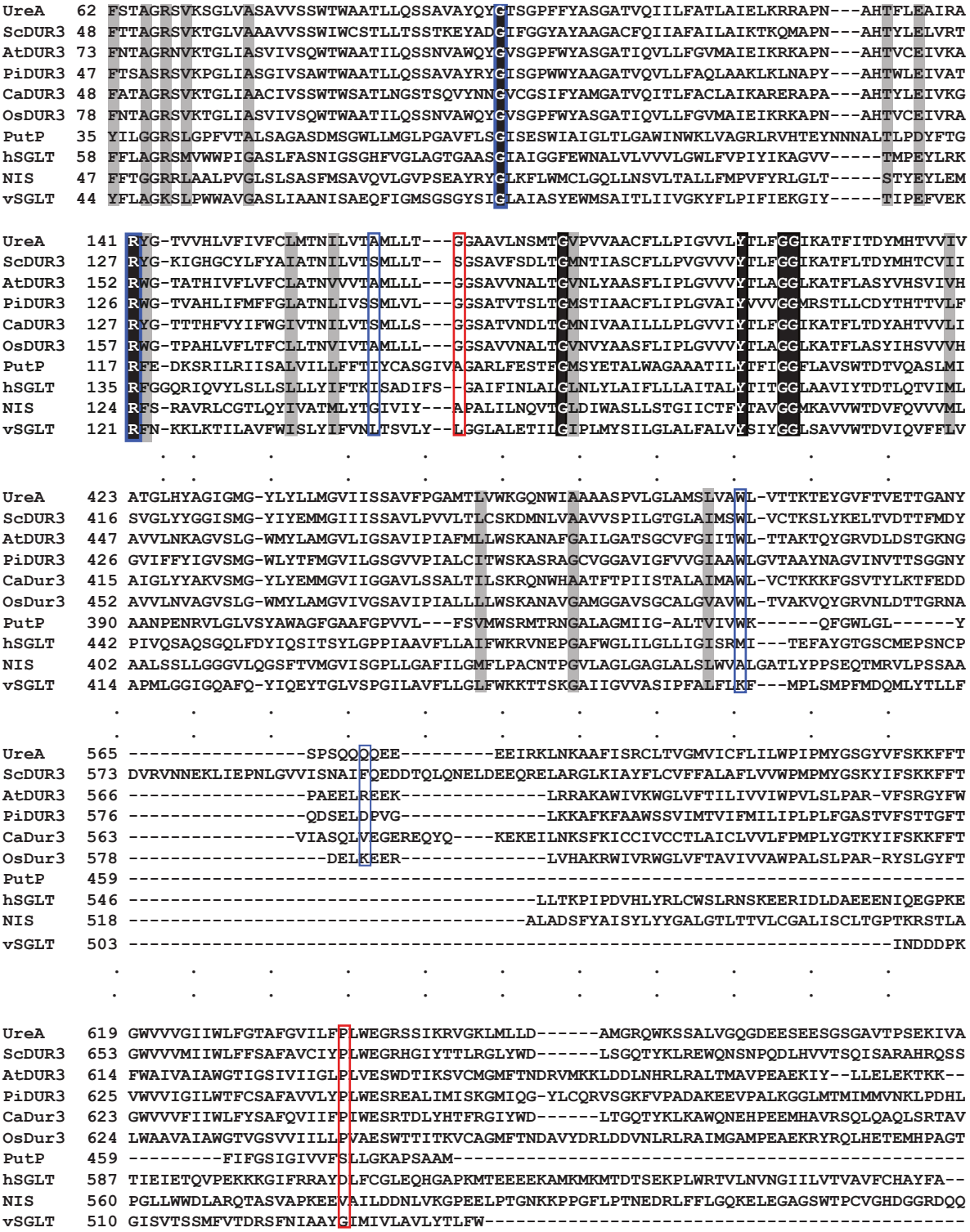

Figure S2. Multiple sequence alignment of UreA and homologues of known function *vs.* representative members of the Sodium/solute Symporter Superfamily (SSS). Fully conserved amino acids are shaded in black, structurally conserved amino acids are shaded in grey. Mutated amino acids in UreA by classical 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 a l.*, 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).

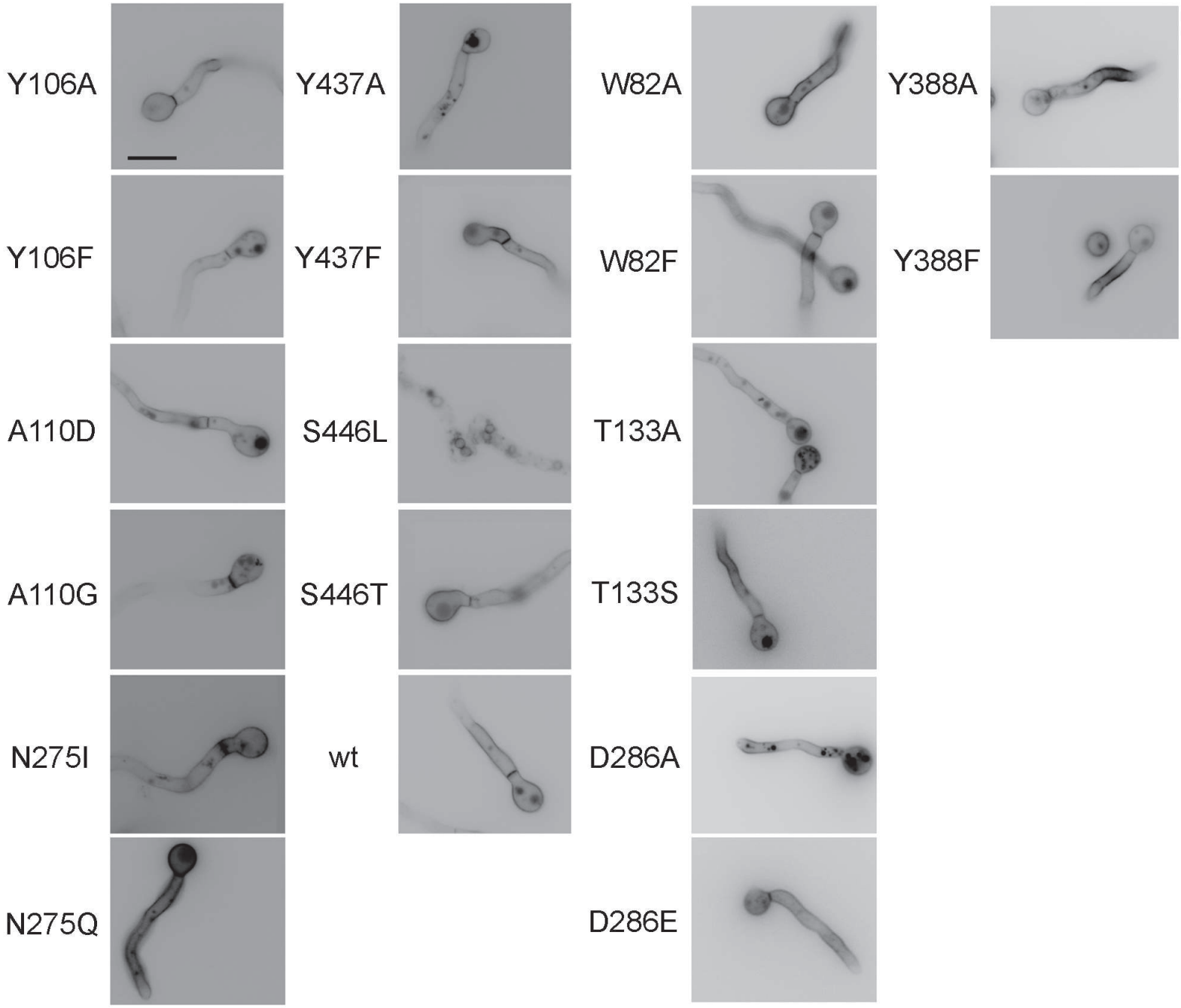

Figure S3. Epifluorescence microscopy of UreA mutants grown in de-repressing conditions. Wild type UreA-GFP localization is shown as control. The bar represents 10 µm.

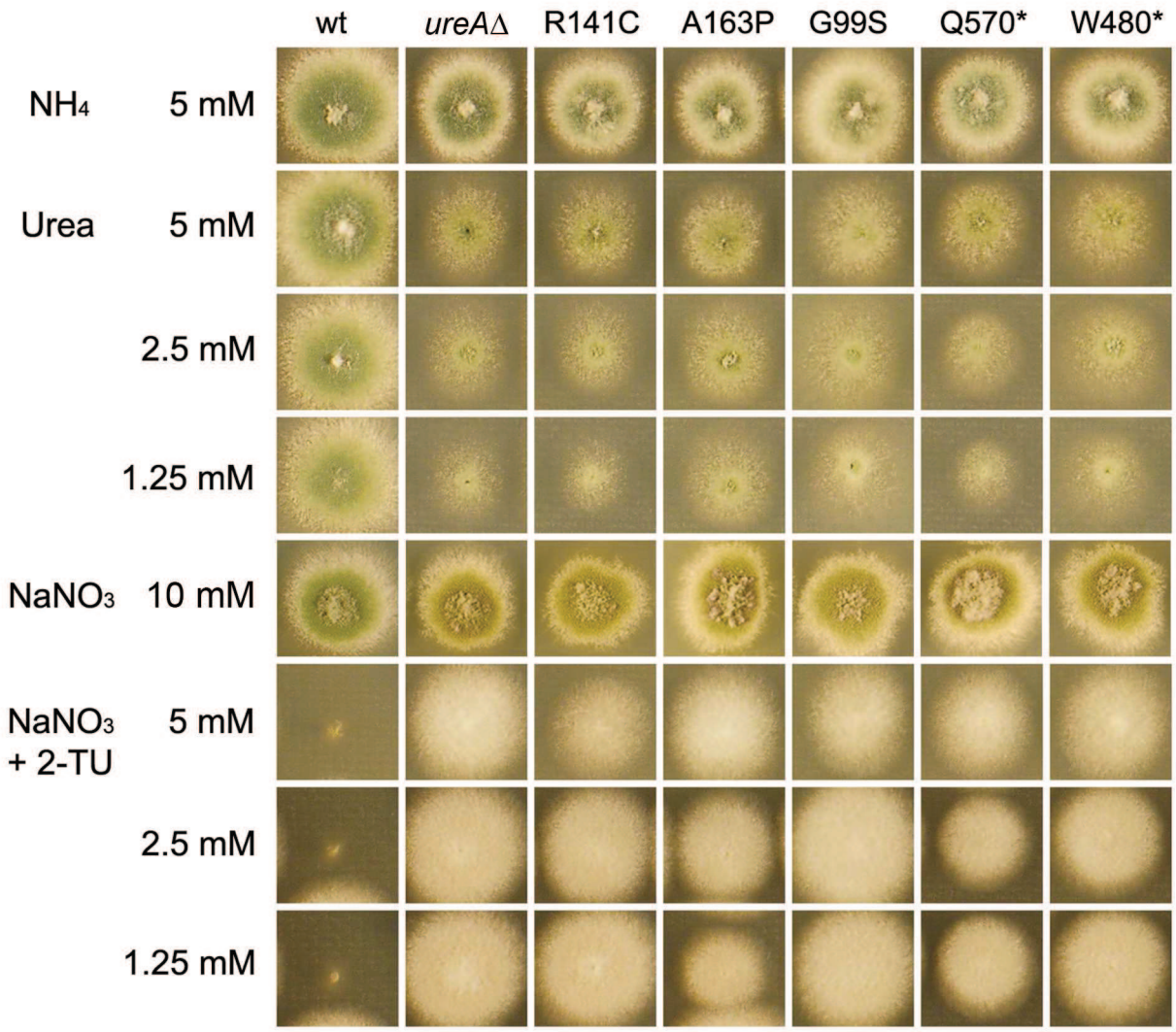

Figure S4. Growth test at 37 ºC of mutant UreA strains isolated by random mutagenesis. Strains were grown on urea as sole nitrogen source or on 2-thiourea to test resistance. In this latter case 10 mM $NaNO_3$ was used as nitrogen source. Growth on ammonium 5 mM or 10 mM $NaNO_3$ was used as a control. A *wt* and a *ureA*Δ strain are shown as positive and negative control, respectively. Similar results were obtained at 25 ºC (data not shown).

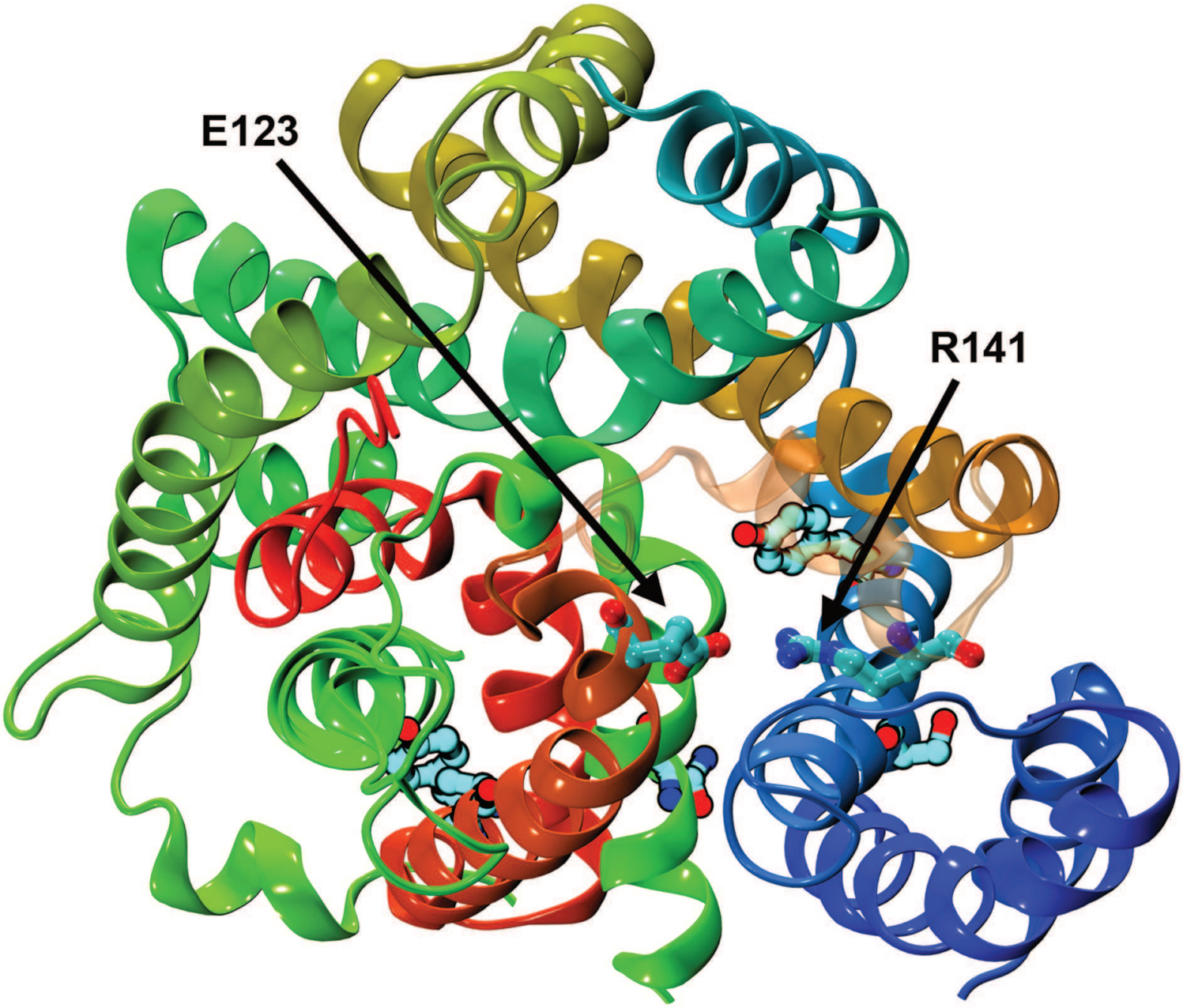

Figure S5. Molecular representation of the UreA model in the conformation presenting an outward facing cavity seen from the extracellular side. R141 and E123, which can form a salt bridge are indicated and shown in glossy color. ICH3/4 is shown as semitransparent in orange.

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

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

* 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**GCT**ACCTGGGCAGCTACTCTGCTG
W82A-R	GAGTAGCTGCCCAGGT**AGC**ACTGCTCACGACAGCAGAGG
W82F-F	GCTGTCGTGAGCAGTT**TC**ACCTGGGCAGCTACTCTGCTG
W82F-R	AGAGTAGCTGCCCAGGT**GA**AACTGCTCACGACAGCAGAG
Y106A-F	CCTCGGGGCCGTTCTTC**GCA**GCATCGGGTTGGTCTTC
Y106A-R	GAAGACCAACCCGATGC**TGC**GAAGAACGGCCCCGAGG
Y106F-F	CCTCGGGGCCGTTCTTCT**T**TGCATCGGGTTGGTCTTC
Y106F-R	GAAGACCAACCCGATGCA**A**AGAAGAACGGCCCCGAGG
A110D-F	GACAGTTTGCAGGCG**A**CACCGTTCAGATCATC
A110D-R	GATGATCTGAACGGTG**T**CGCCTGCAAACTGTC
A110G-F	ACTGACAGTTTGCAGGCG**G**CACCGTTCAGATCATCTTG
A110G-R	CAAGATGATCTGAACGGTG**C**CGCCTGCAAACTGTCAGT
T133A-F	CGCCTAACGCACAC**G**CATTCCTGGAAGCCATCCGTG
T133A-R	TGGCTTCCAGGAATG**C**GTGTGCGTTAGGCGCGCGTC
T133S-F	CGCCTAACGCACAC**T**CATTCCTGGAAGCCATCCGTG
T133S-R	TGGCTTCCAGGAATG**A**GTGTGCGTTAGGCGCGCGTC
N275I-F	ATCTTCTGGGTCATCA**T**CCTCGTCGGTAACTTC
N275I-R	GAAGTTACCGACGAGG**A**TGATGACCCAGAAGAT
N275Q-F	GTATCTTCTGGGTCATC**C**A**G**CTCGTCGGTAACTTCG
N275Q-R	CGAAGTTACCGACGAG**C**T**G**GATGACCCAGAAGATAC
D286A-F	GCACTGTCTTCCTGG**C**CAACGGCTACTACAACAAGG
D286A-R	TTGTTGTAGTAGCCGTTG**G**CCAGGAAGACAGTGCCGAAG
D286E-F	GCACTGTCTTCCTGGA**G**AACGGCTACTACAACAAGG
D286E-R	TTGTTGTAGTAGCCGTT**C**TCCAGGAAGACAGTGCCGAAG
Y388A-F	GTGTCCTCGATCCTCACC**GC**CGATATCTACCAAGCCTAC
Y388A-R	TAGGCTTGGTAGATATCG**GC**GGTGAGGATCGAGGACAC
Y388F-F	TGTCCTCGATCCTCACC**TT**CGATATCTACCAAGCCTA
Y388F-R	TAGGCTTGGTAGATATCG**A**AGGTGAGGATCGAGGACA
Y437A-F	CGGTATGGGCTATCTC**GCT**CTTCTCATGGGCGTC
Y437A-R	GACGCCCATGAGAAG**AGC**GAGATAGCCCATACCG
Y437F-F	GTATGGGCTATCTCT**T**CCTTCTCATGGGCG
Y437F-R	CGCCCATGAGAAGG**A**AGAGATAGCCCATAC
S446L-F	GGCGTCATCATCTCC**TT**AGCCGTGTTCCCGGGC
S446L-R	GCCCGGGAACACGGCT**A**AGGAGATGATGACGCC
S446T-F	GGCGTCATCATCTCC**A**CAGCCGTGTTCCCGG
S446T-R	CCGGGAACACGGCTG**T**GGAGATGATGACGCC

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.