# Analysis of the thermal stability of a mercuric reductase from the Red Sea Atlantis II hot brine environment by site-directed mutagenesis

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## Supplementary Figures

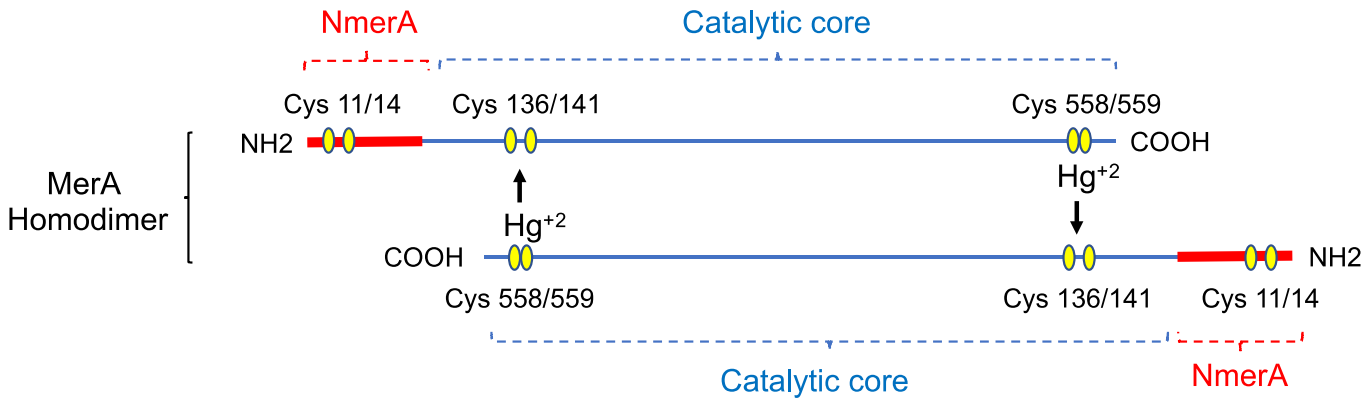



**Fig. S1. Cartoon showing the structure of mercuric reductase dimer and the binding and catalytic sites.**

This simplified diagram represents the structure of the mercuric reductase homodimer. The six pairs of cysteines in the merA homodimer are highlighted in yellow, and the N-terminal domain is highlighted in red. Cys 11/14 pair serves to bind $Hg^{2+}$ and transfer it from ligands in the cytoplasm to the redox-active cysteines for reduction under physiological conditions in which intercellular thiols are depleted. Cys 136/141 pair is the redox-active cysteine involved in catalysis. Cys 558/559 pair binds $Hg^{2+}$ and transfers it to the active site of the other subunit for reduction.

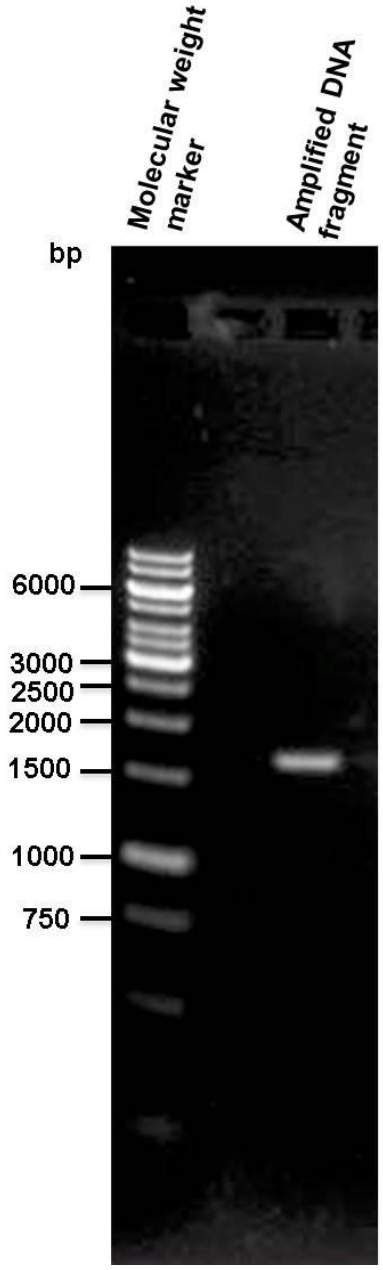



**Fig. S2. PCR amplification of metagenomic Atlantis-II LCL mercuric reductase**.

Products of PCR amplification of ATII-LCL metagenomic DNA analyzed on 1% agarose gel electrophoresis. Lane 1, molecular weight marker; Lane 2, amplified DNA fragment.

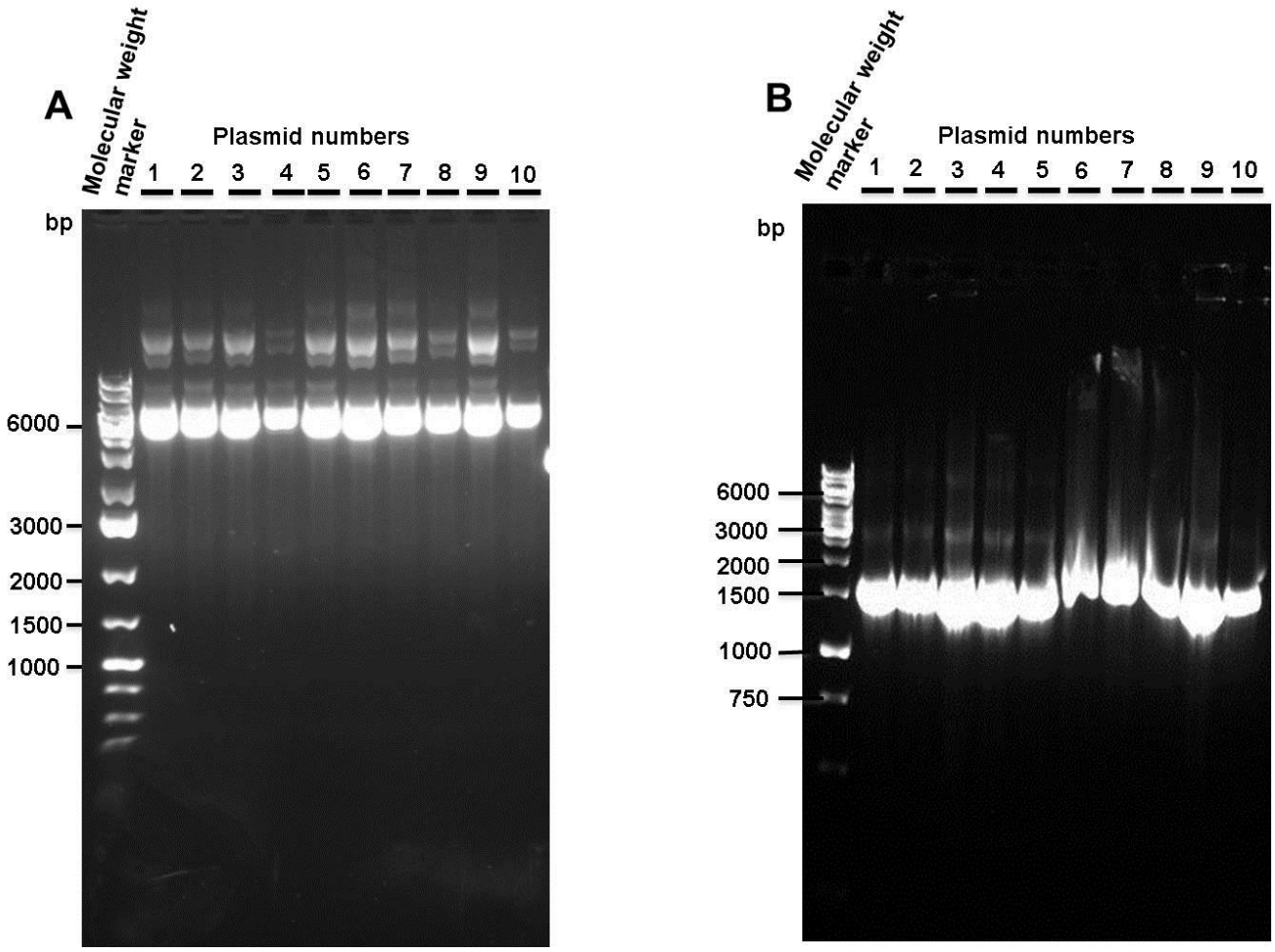
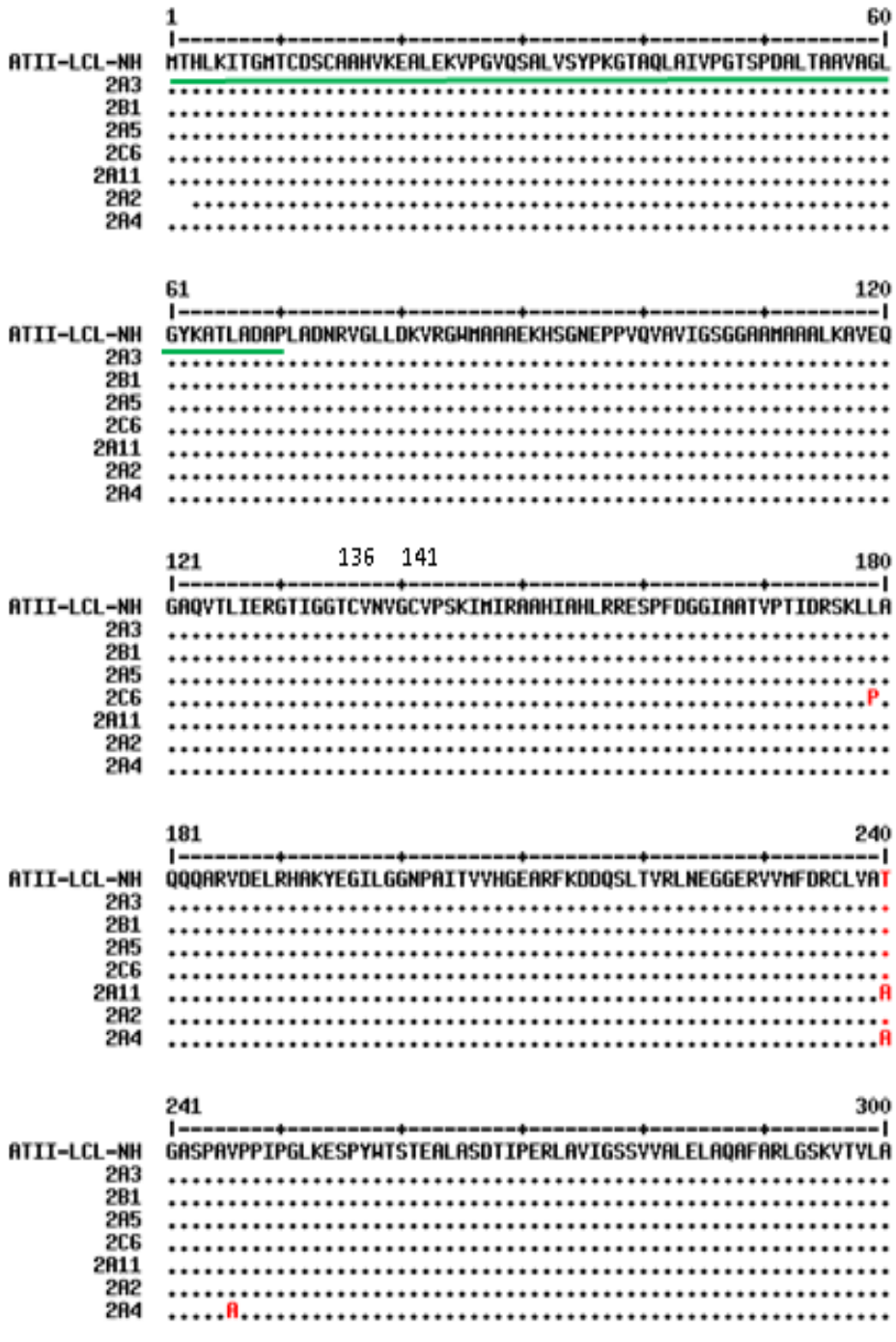



**Fig. S3. Analysis of randomly selected clones from the ATII-LCL *merA* library**.

A) Analysis of recombinant plasmids from ten randomly selected clones on 1% agarose gel electrophoresis. B) Analysis of the size of inserted DNA fragments by PCR. PCR parameter and primers used in the amplification process are described in the experimental procedures section. MWM refer to molecular weight marker.


1 60
ATII-LCL-NH MTHLKITGMTCDSCAAHVKEALEKVPGVQSALVSYPKGTAQLAIVPGTSPDALTAAVAGL
2A3 ....................................................
2B1 ....................................................
2A5 ....................................................
2C6 ....................................................
2A11 ....................................................
2A2 ....................................................
2A4 ....................................................
61 120
ATII-LCL-NH GYKATLADAPLADNRVGLLDKVRGWMAAAEKHSGNEPPVQVAVIGSGGAAMAAALKAVEQ
2A3 ....................................................
2B1 ....................................................
2A5 ....................................................
2C6 ....................................................
2A11 ....................................................
2A2 ....................................................
2A4 ....................................................
121 136 141 180
ATII-LCL-NH GAQVTLIERGTIGGTCVNVGCVPSKIMIRAAHIAHLRRESPFDGGIAATVPTIDRSKLLA
2A3 ....................................................
2B1 ....................................................
2A5 ....................................................
2C6 ....................................................P.
2A11 ....................................................
2A2 ....................................................
2A4 ....................................................
181 240
ATII-LCL-NH QQQARVDELRHAKYEGILGGNPAITVVHGEARFKDDQSLTVRLNEGGERVVMFDRCLVAT
2A3 ....................................................
2B1 ....................................................
2A5 ....................................................
2C6 ....................................................
2A11 ....................................................A
2A2 ....................................................
2A4 ....................................................A
241 300
ATII-LCL-NH GASPAVPPIPGLKESPYWTSTEALASDTIPERLAVIGSSVVALELAQAFARLGSKVTVLA
2A3 ....................................................
2B1 ....................................................
2A5 ....................................................
2C6 ....................................................
2A11 ....................................................
2A2 ....................................................
2A4 .....A.....................................................

43 .

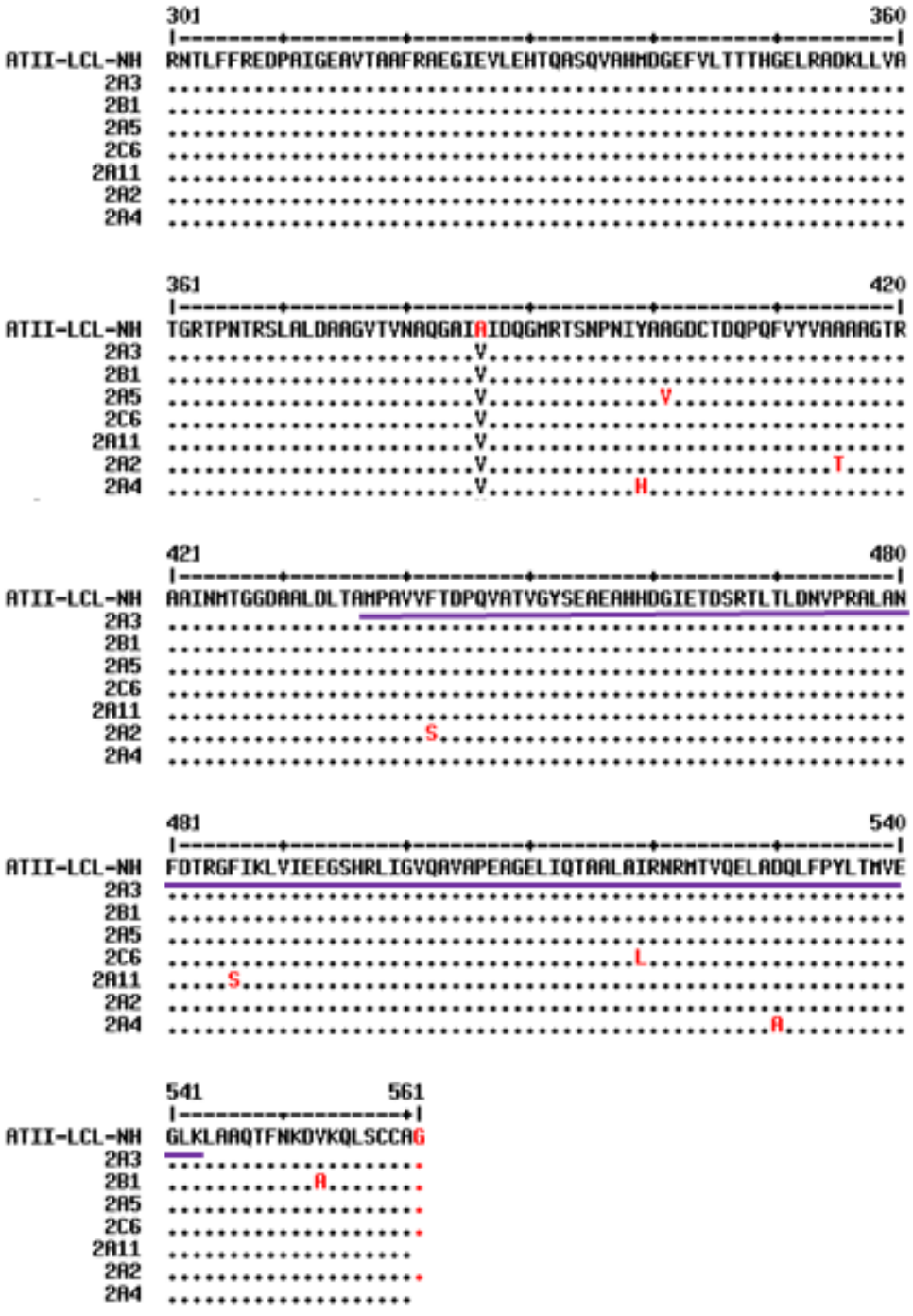
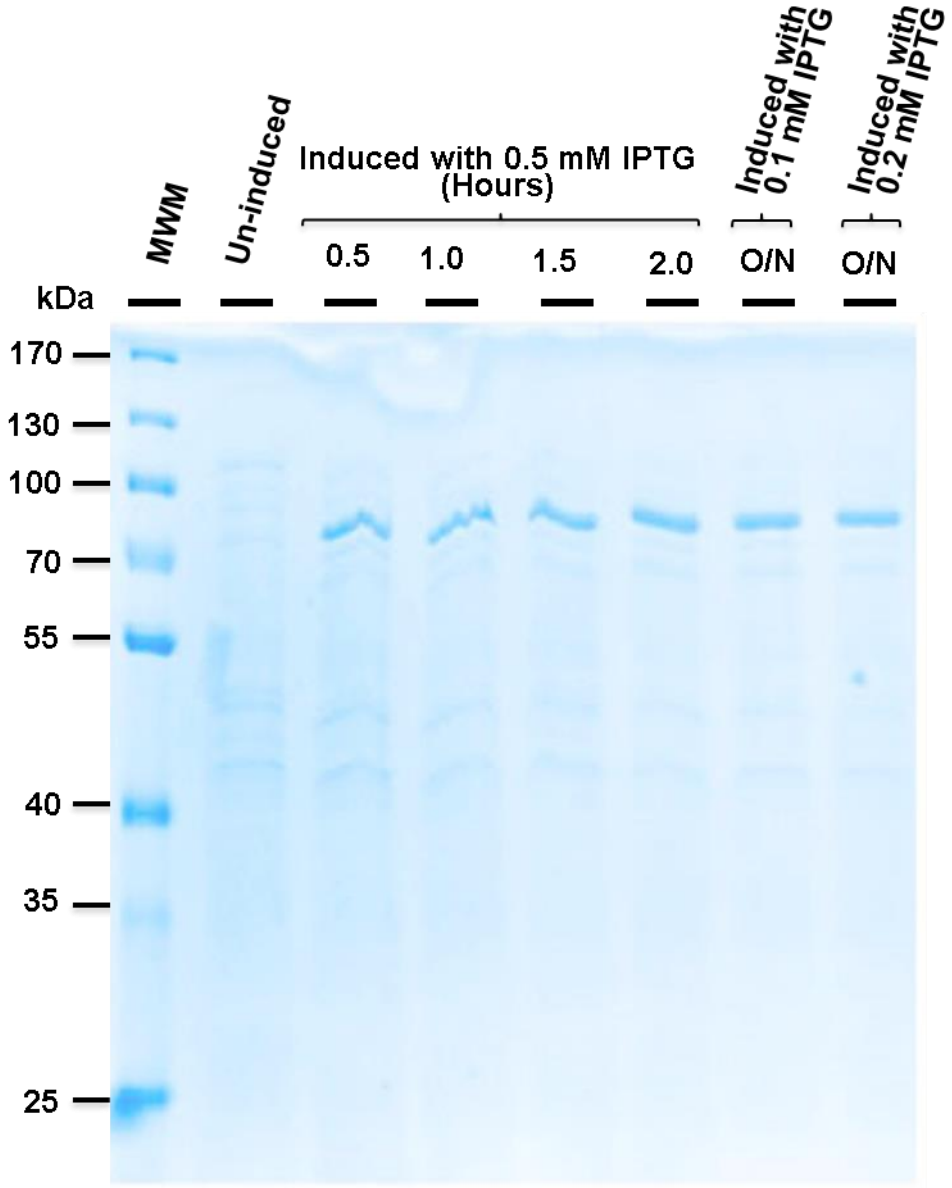

301 360
ATII-LCL-NH RNTLFFREDPAIGEAVTAAFRAEGIEVLEHTQASQVAHMDGEFVLTTTHGELRADKLLVA
2A3 .....
2B1 .....
2A5 .....
2C6 .....
2A11 .....
2A2 .....
2A4 .....
361 420
ATII-LCL-NH TGRTPNTRSLALDAAGVTVNAQGAIAIDQGMRTSNPNIYAAGDCTDQPQFVYVAAAAGTR
2A3 .....V.....
2B1 .....V.....
2A5 .....V.....V.....
2C6 .....V.....
2A11 .....V.....
2A2 .....V.....T.....
2A4 .....V.....H.....
421 480
ATII-LCL-NH AAINMTGGDAALDLTAMPAVVFTDPQVATVGYSEAEAHHDGIETDSRTLTLDNVPRALAN
2A3 .....
2B1 .....
2A5 .....
2C6 .....
2A11 .....
2A2 .....S.....
2A4 .....
481 540
ATII-LCL-NH FDTRGFIKLVIEEGSHRLIGVQAVAPEAGELIQTAALAIRNRMTVQELADQLFPYLTMVE
2A3 .....
2B1 .....
2A5 .....
2C6 .....L.....
2A11 .....S.....
2A2 .....
2A4 .....A.....
541 561
ATII-LCL-NH GLKLAAQTFNKDVKQLSCCAG
2A3 .....
2B1 .....A.....
2A5 .....
2C6 .....
2A11 .....
2A2 .....
2A4 .....

**Fig. S4. Multiple sequence alignment of non-redundant and full-length MerA sequences.**

Multiple sequence alignment of eight non-redundant full-length MerA sequences obtained from the ATII-LCL MerA library were aligned by Multalin (1). The first sequence of the figure, ATII-LCL-NH, is a MerA sequence identified also in a separate library. The amino acid variants/substitutions are in red letters. The NmerA domain (2) is underlined in green. The Dimerization domain (3) is underlined in purple.




58

59 **Fig. S5. Induction of ATII-LCL-NH MerA at different conditions.**

60 SDS-PAGE 10% of the induced ATII-LCL-NH-merA gene was performed as described in the

61 figure. First lane is the molecular weight marker (MWM).

62 .

63

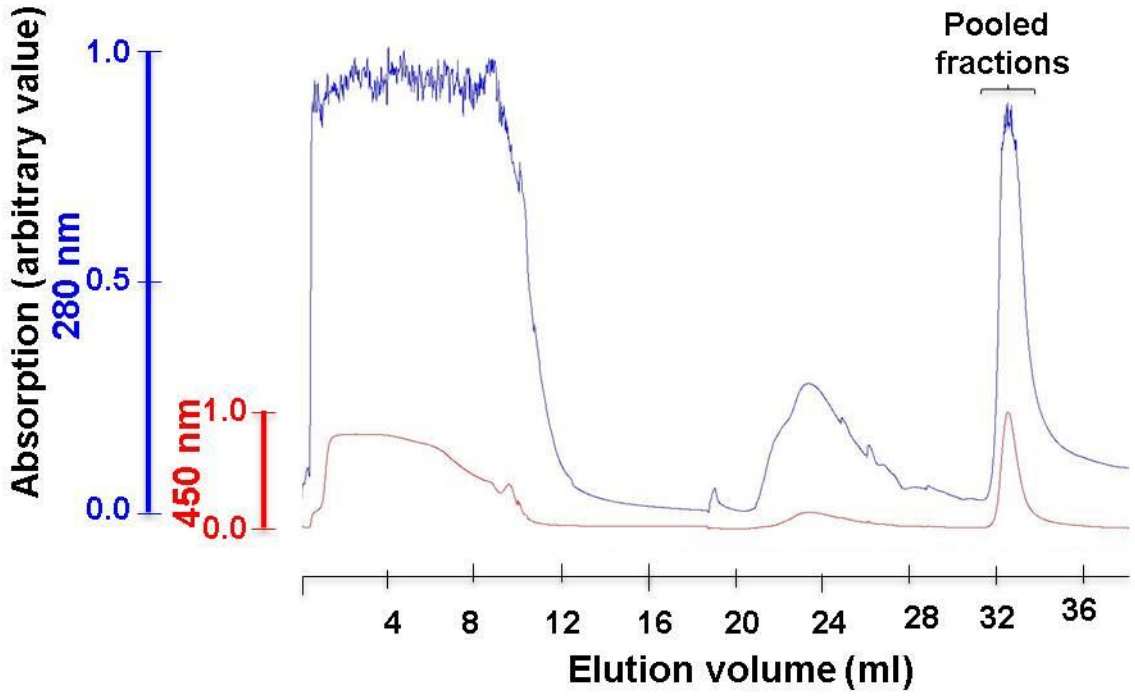



**Fig. S6. Purification profile of MerA ATII-LCL-NH recombinant enzyme using HisTrap affinity column**.

The ATII-LCL-NH *E. coli* cell lysate was uploaded into 1 ml HisTrap column equilibrated with 40 mM imidazole using the AKTA purifier machine. On-line AKTA monitor adjusted at 280 nm and 450 nm was used to follow the elution profile of proteins and FAD respectively. 250 µl aliquots were collected in each fraction. Elution of the protein was achieved by changing the buffer to 500 mM imidazole at 28 ml of the elution volume. Pooled fractions containing ATII-LCL-NH MerA are indicated in the figure.

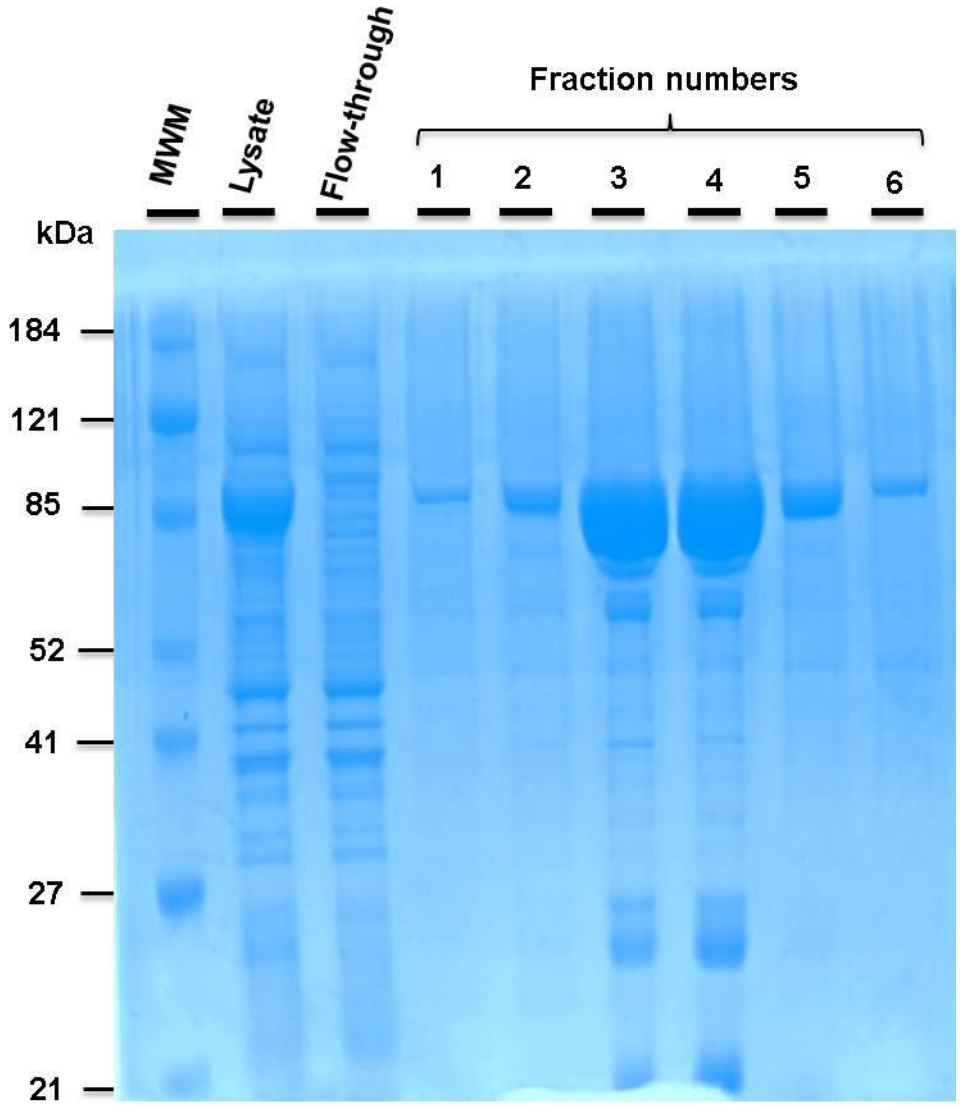
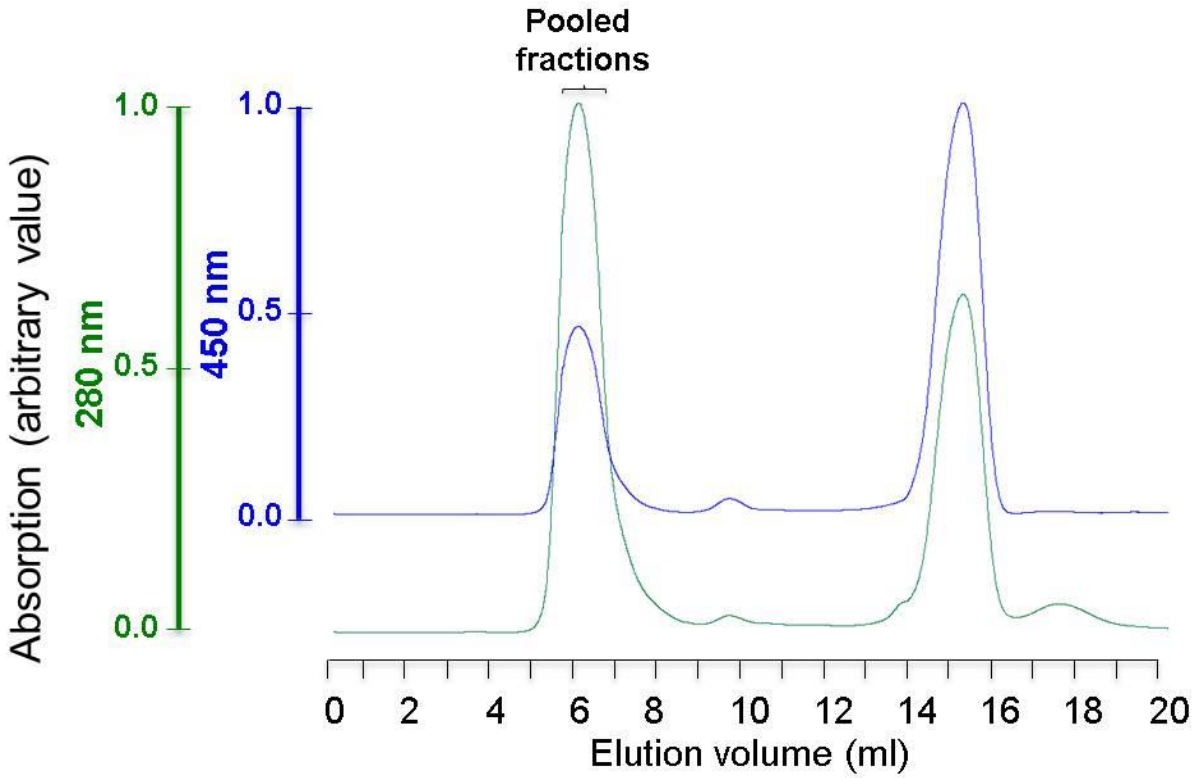



**Fig. S7. Visualization of the HisTrap purification process by SDS-PAGE.**

Aliquots from the HisTrap purification process were analyzed by SDS-PAGE as described in the experimental procedures. Fractions 1 to 6 refer to the pooled fractions indicated in Fig. S6.




80 .

81 **Fig. S8. Purification profile of MerA ATII-LCL-NH recombinant enzyme on Superdex 75**

82 **column**.

83 Pooled fractions from the HisTrap column were uploaded into 24-ml Superdex column

84 equilibrated with PBS buffer using the AKTA purifier machine. On-line AKTA monitor adjusted

85 at 280 nm and 450 nm was used to follow the profile of elution of proteins and FAD

86 respectively. Aliquots, 250 µl, were collected. Pooled fractions are indicated in the figure.

87

88

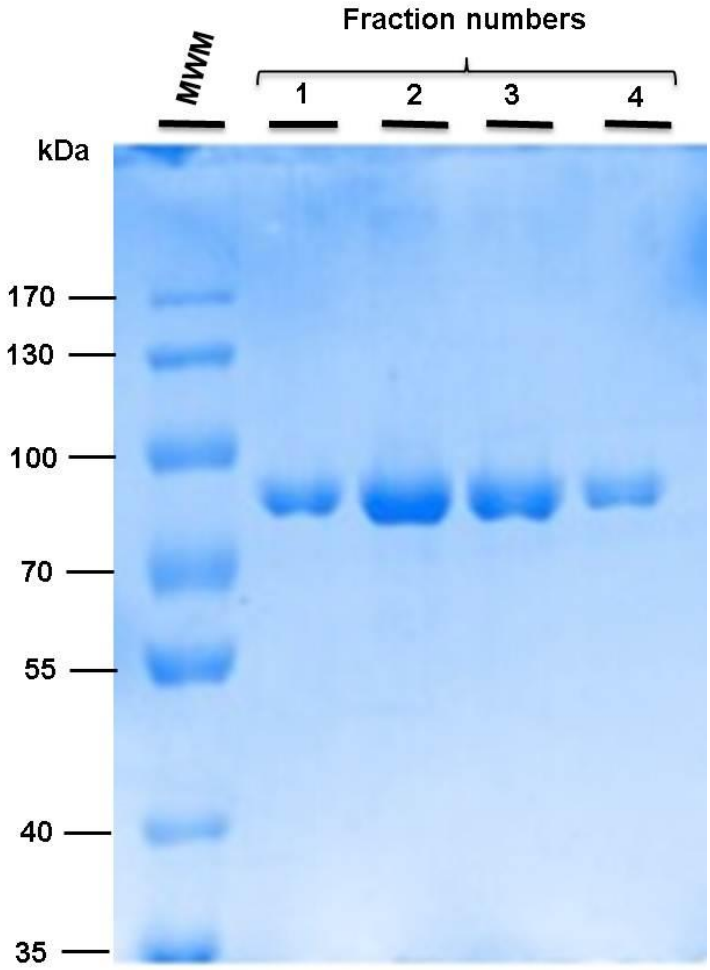



**Fig. S9. Assessment of Superdex 75 purification process by SDS-PAGE**.

Aliquots from the Superdex 75 purification process were analyzed on 10% SDS-PAGE as indicated in the experimental procedures. Fractions 1 to 4 refer to the pooled fractions indicated in Fig. S8.

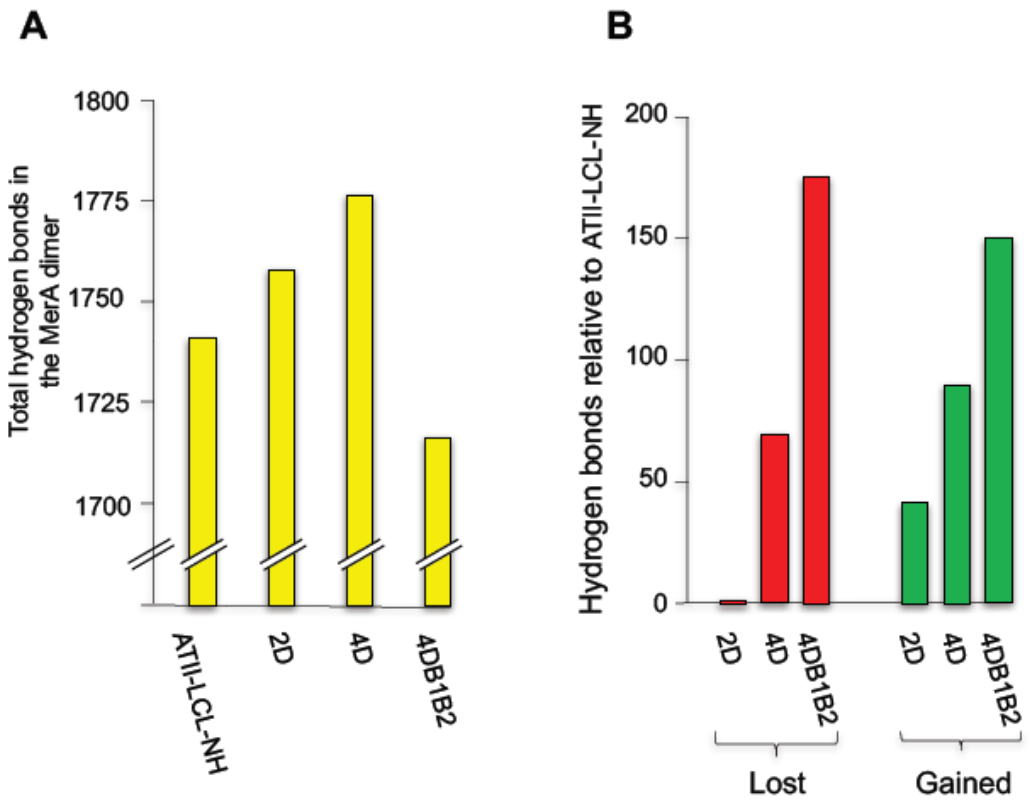
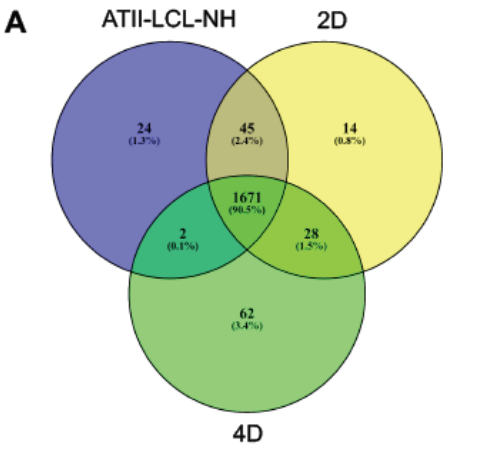
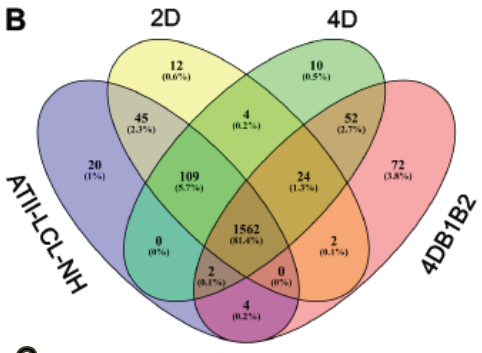


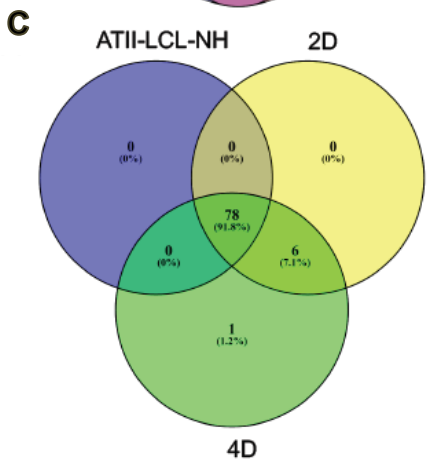

**Fig. S10. Total hydrogen bonds of ATII-LCL-NH and its mutants.**

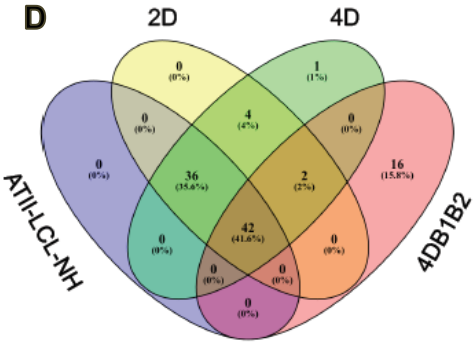
A) Total hydrogen bonds in the indicated MerAs were calculated by Hydrogen Bond Calculator version 1.1 (4). B) The loss and gain of hydrogen bonds in each MerA indicated in the figure were computationally calculated.


A
ATII-LCL-NH
2D
4D
24
(1.3%)
45
(2.4%)
14
(0.8%)
1671
(90.5%)
2
(0.1%)
28
(1.5%)
62
(3.4%)


B
2D
4D
ATII-LCL-NH
4DB1B2
12
(0.6%)
10
(0.5%)
45
(2.3%)
4
(0.2%)
52
(2.7%)
20
(1%)
109
(5.7%)
24
(1.3%)
72
(3.8%)
1562
(81.4%)
0
(0%)
2
(0.1%)
2
(0.1%)
0
(0%)
4
(0.2%)

104


C
ATII-LCL-NH
2D
4D
0
(0%)
0
(0%)
0
(0%)
78
(91.8%)
0
(0%)
6
(7.1%)
1
(1.2%)


D
2D
4D
ATII-LCL-NH
4DB1B2
0
(0%)
1
(1%)
0
(0%)
4
(4%)
0
(0%)
0
(0%)
36
(35.6%)
2
(2%)
16
(15.8%)
42
(41.6%)
0
(0%)
0
(0%)
0
(0%)
0
(0%)
0
(0%)

105

**Fig. S11. Venn diagram of hydrogen bonding and salt bridges in ATII-LCL-NH and its mutants.**

Venn diagrams of total hydrogen bonds and salt bridges in ATII-LCL-NH, 2D, and 4D mutants were created and drawn using Venny software version 2.1 (5). A) Diagram of total potential hydrogen bonds in ATII-LCL-NH and 2D mutant. B) Diagram of ATII-LCL-NH together with 2D, 4D, and 4DB1B2 mutants. C) A Venn diagram of total potential salt bridges in ATII-LCL-NH and 2D mutant. D) Diagram of ATII-LCL-NH together with 2D, 4D, and 4DB1B2 mutants.

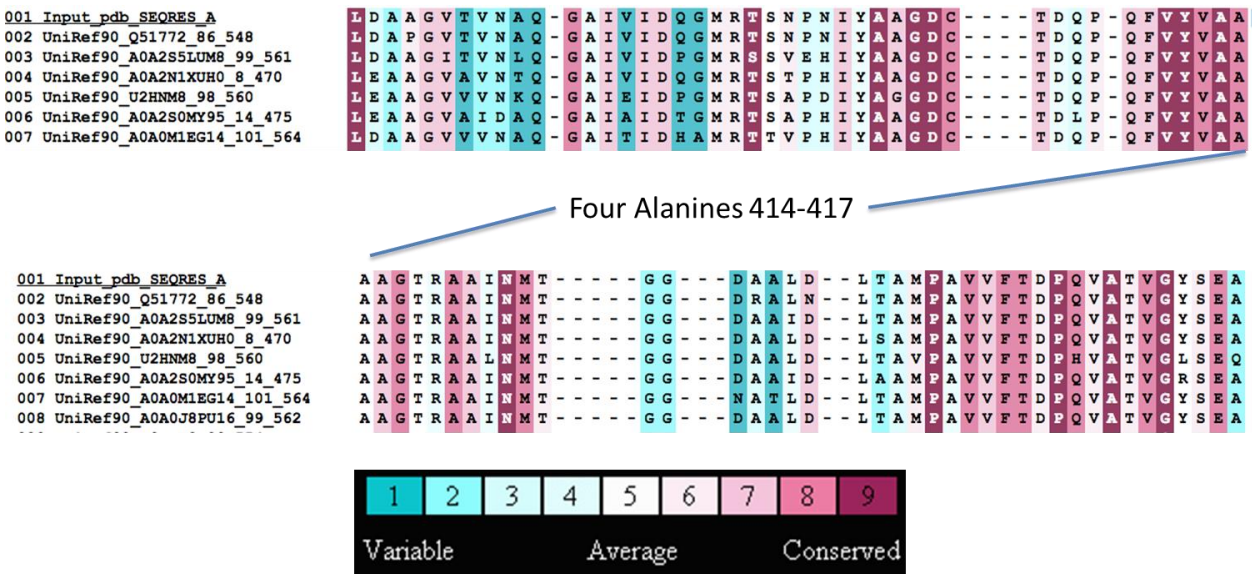



**Fig. S12. ConSurf Alignment of ATII-LCL-NH MerA model and homologous mercuric reductases**.

ConSurf software (6) was used to calculate and analyse the evolutionary conservation of every amino acid. Ala 414 is highlighted in dark purple indicating its highest conservation among homologous mercuric reductase enzymes, while Ala 416 is highlighted in white denoting its average variability. A color-coded key is provided.

## Supplementary figures references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