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Supporting information for article:

A disulfide-bond cascade mechanism for arsenic(III) *S*-adenosylmethionine methyltransferases

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Supporting information

Figure S1. Multiple sequence alignment of ArsM and As3MT orthologs. The CmArsM sequence from *Cyanidioschyzon* sp. 5508 (accession number ACN39191) with the C-terminal 28 residues replaced with a histidine tag (underlined) was aligned with human AS3MT (accession number AAV68045), rat AS3MT (accession number NP_543166) and CrArsM from *Chlamydomonas reinhardtii* (accession number XP_001703619). The C-terminal sequences of each of the latter three were deleted at the same position as CmArsM. The four conserved cysteine residues are highlighted in yellow background. Identical residues are shaded in black, and conservative replacements in grey. The four conserved cysteine residues are numbered according to the CmArsM sequences.

Figure S2. (A) **Structure of CmArsM with bound Rox(III)**. Cartoon diagram (colored in light orange) representation of Rox(III) (PDB ID: 4RSR). The conserved cysteine residues are represented by ball-and-stick and colored green (carbon), blue (nitrogen) or yellow (sulfur). The dark blue sphere is the arsenic atom, and the light blue sphere is a Ca^{++} ion in the SAM binding site. PhAs(III) is bound between conserved residues Cys174 and Cys224. (B) **A disulfide bond between Cys44-Cys72 in the Rox(III)-bound structure.** Coloring is as in Fig. S2A. The four conserved cysteine residues are shown as ball-and-stick. The arsenic atoms are coordinated with thiolates of Cys174 and Cys224 and oxygen (red) of Cys174. The length of the disulfide bond is approximately 2.1 Å in both structures.

Figure S3. Stereo view and electron density map of the N-termini of (A) PhAs(III)-bound and (B) Rox(III)-bound CmArsM. The 2Fo-Fc electron density map is shown for

residues 44 to 50 contoured at 1.0 σ colored in light blue. Residues 44-50 and 71-74 are represented in sticks and colored in cyan (carbon), blue (nitrogen), red (oxygen) and yellow (sulfur).

Figure S4. (A) **Superposition of the N-terminal domain of Rox(III)-bound CmArsM with the SAM-bound structure**. Superimposition of Rox(III)-bound CmArsM (light orange) with the SAM-bound (light grey) structure has an RMSD of 1.32 Å. The loop shift (6.5 Å) in N-terminal loop is proposed to lead to disulfide bond formation between Cys72 and Cys174. Rox(III) cysteine residues are represented as ball-and-stick, with coloring of atoms the as in Fig. 1. SAM is shown in ball-and-stick and colored in purple (carbon), blue (nitrogen) or red (oxygen). (B) **Modeling the complex of CmArsM with SAM and aromatic arsenicals**. The ternary complex of CmArsM with bound SAM and Rox(III) was modeled by superposition of their individual structures. The distances from the S-methyl group of SAM and the sulfur atom of conserved cysteine residues and from the arsenic atom of Rox(III) are shown.

Figure S5. Superimposition of CmArsM with ligands. (A) The structure of CmArsM with bound PhAs(III) (light orange) was superimposed on the Rox(III)-bound (marine) structure. (B) The two structures were superimposed on the As(III)-bound structure (red). (C) Superposition of the SAM-bound structure (light grey) with PhAs(III)-bound and Rox(III)-bound CmArsM. SAM, PhAs(III) and Rox(III) are shown in ball-and-stick.

Figure S6. Methylation of PhAs(III) by CmArsM. Methylation of 10 μM PhAs(III) was assayed for 2 h at 60 °C, as described in Materials and Methods, and the products analyzed by reverse phase HPLC−ICP-MS.

Fig. S1

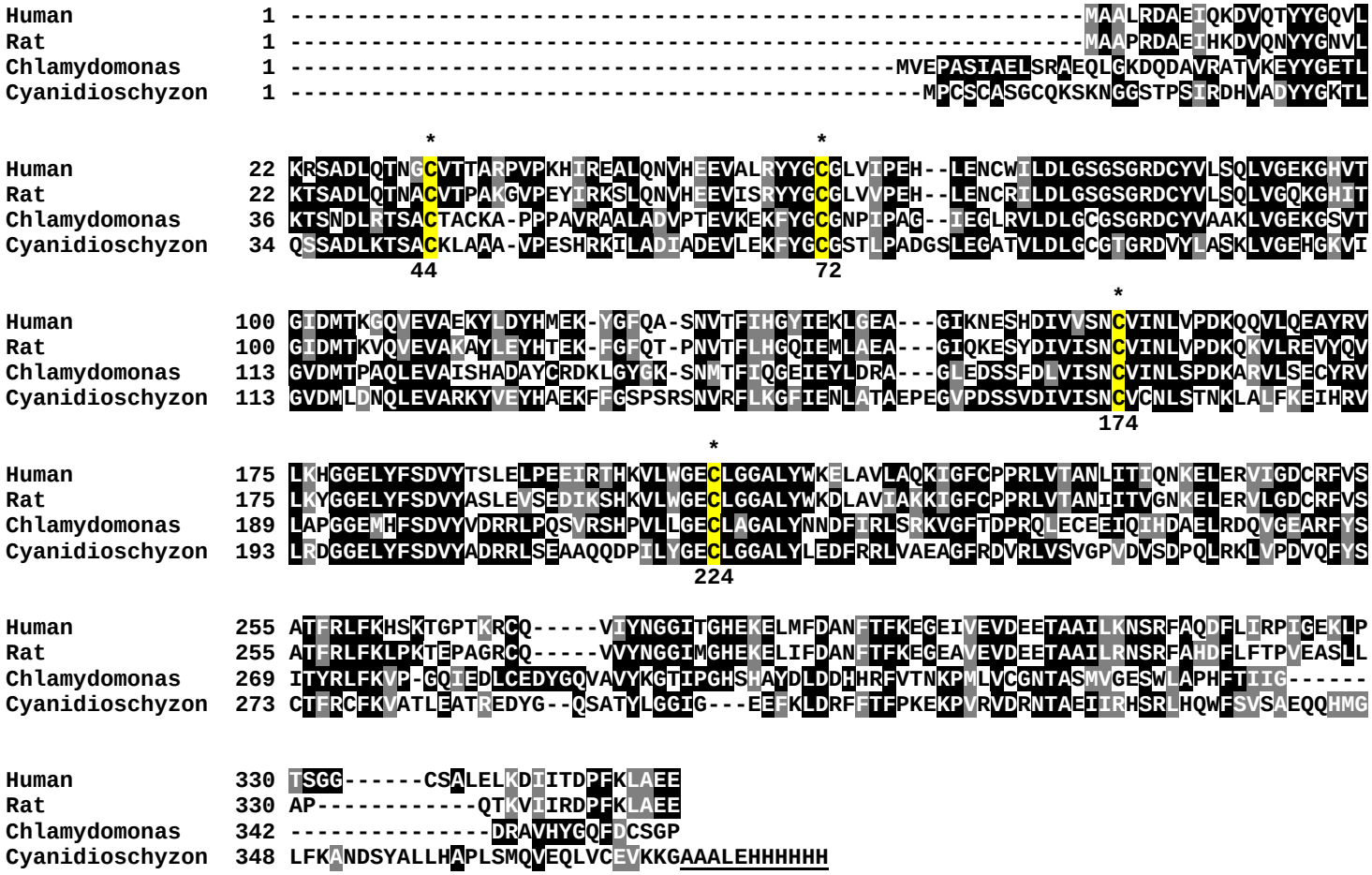

Human
Rat
Chlamydomonas
Cyanidioschyzon
44
72
174
224
AAALEHHHHHH

Fig. S2

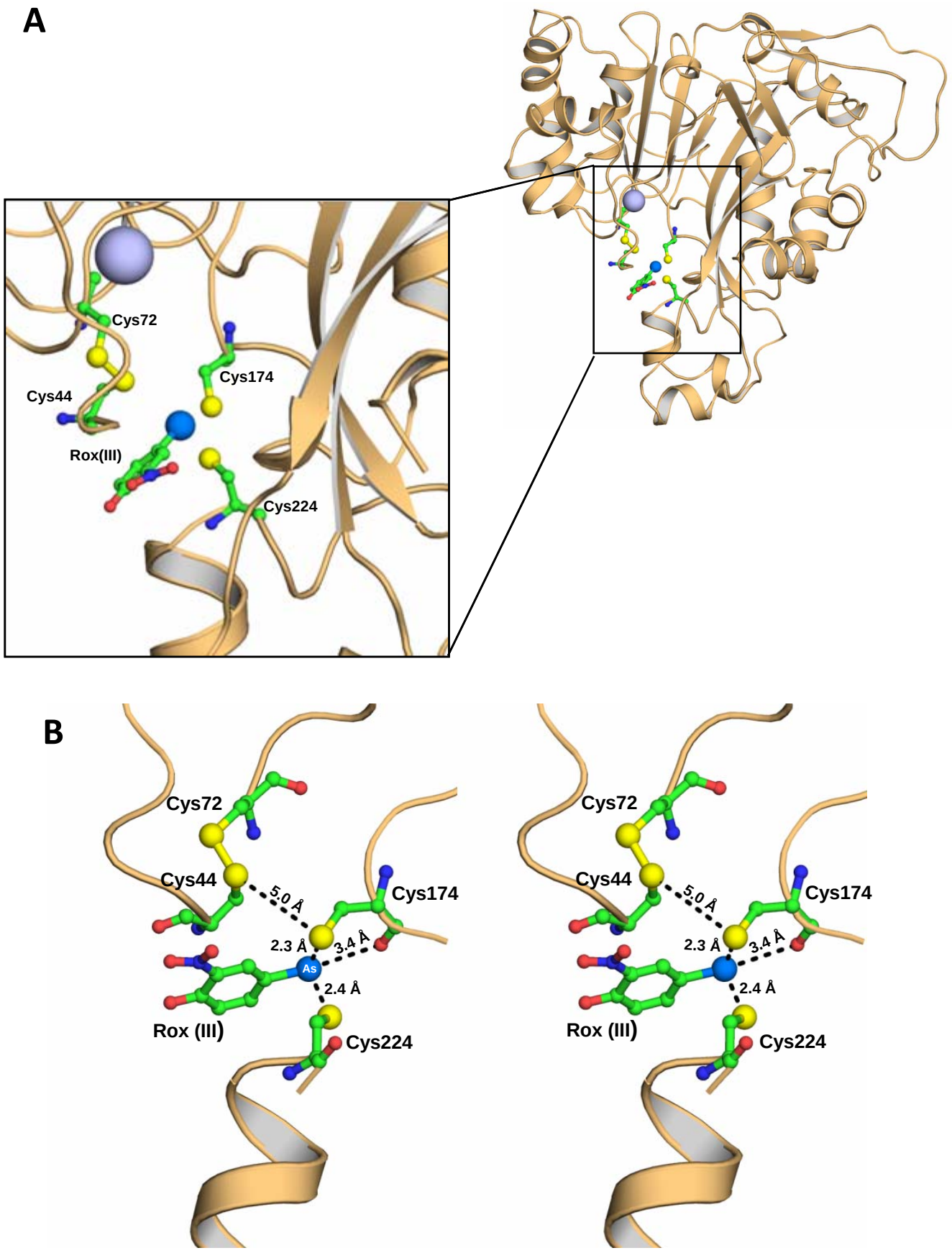

A
Cys72
Cys174
Cys44
Rox(III)
Cys224
B
Cys72
Cys44
5.0 Å
Cys174
2.3 Å
3.4 Å
As
2.4 Å
Rox (III)
Cys224
Cys72
Cys44
5.0 Å
Cys174
2.3 Å
3.4 Å
2.4 Å
Rox (III)
Cys224

Fig. S3

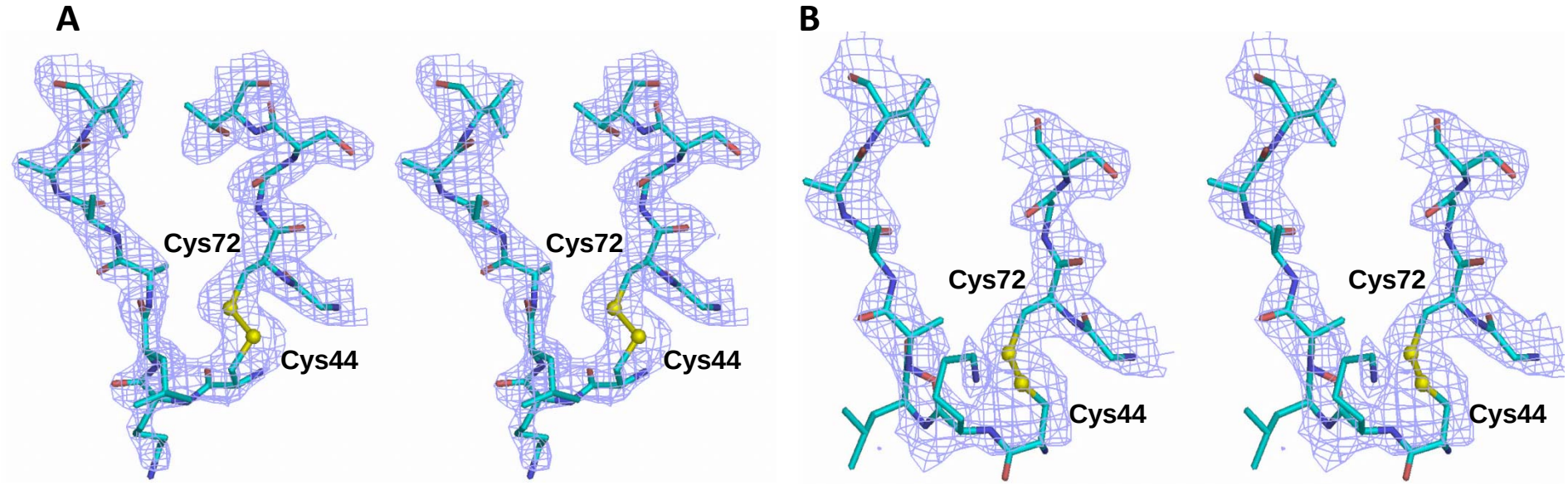


Fig. S4

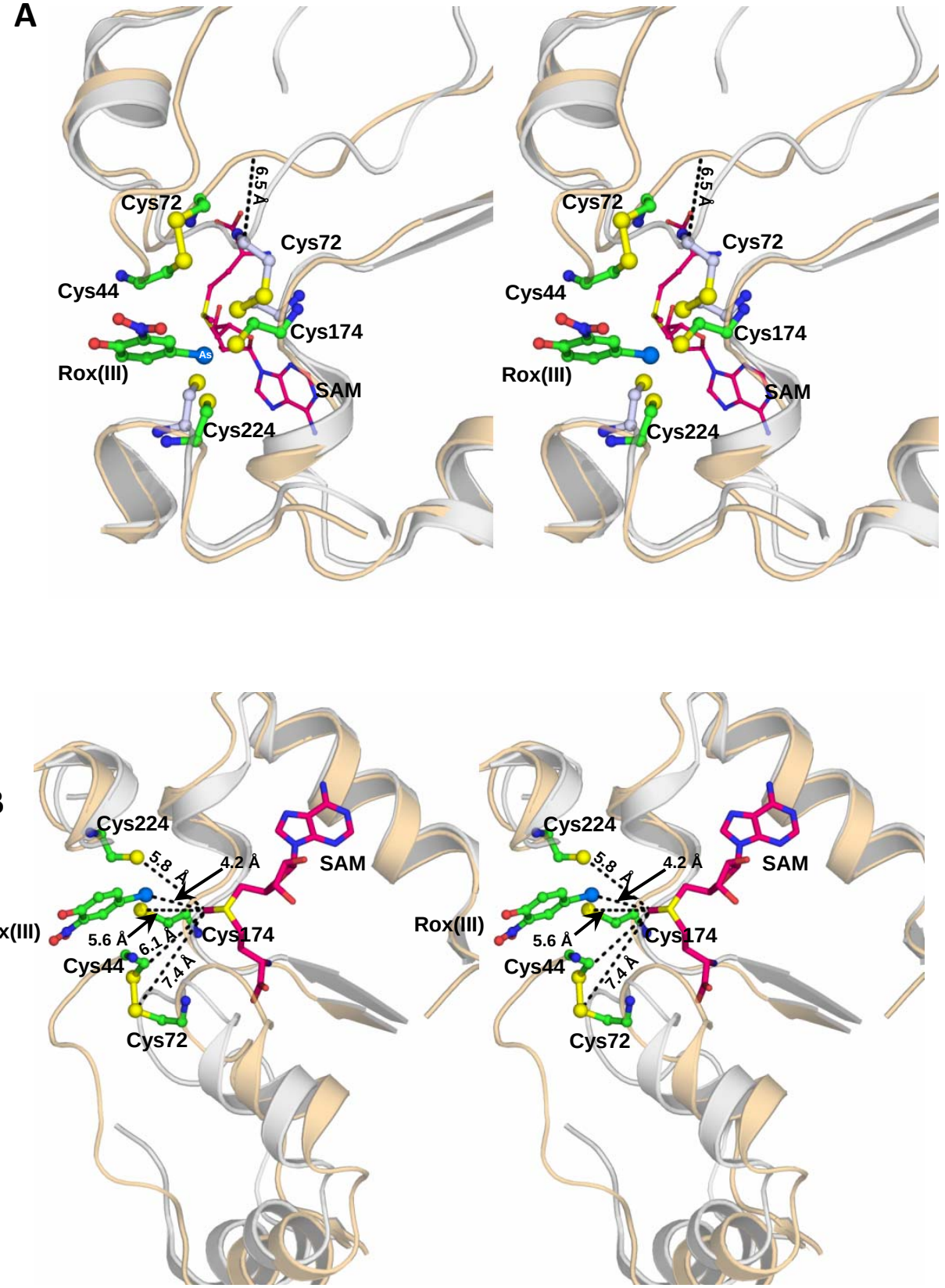

A
6.5 Å
Cys72
Cys72
Cys44
Cys174
As
Rox(III)
SAM
Cys224
6.5 Å
Cys72
Cys72
Cys44
Cys174
Rox(III)
SAM
Cys224
B
Cys224
5.8 Å
4.2 Å
SAM
Rox(III)
5.6 Å
6.1 Å
Cys174
Cys44
7.4 Å
Cys72
Rox(III)
Cys224
5.8 Å
4.2 Å
SAM
5.6 Å
Cys174
Cys44
7.4 Å
Cys72

Fig. S5

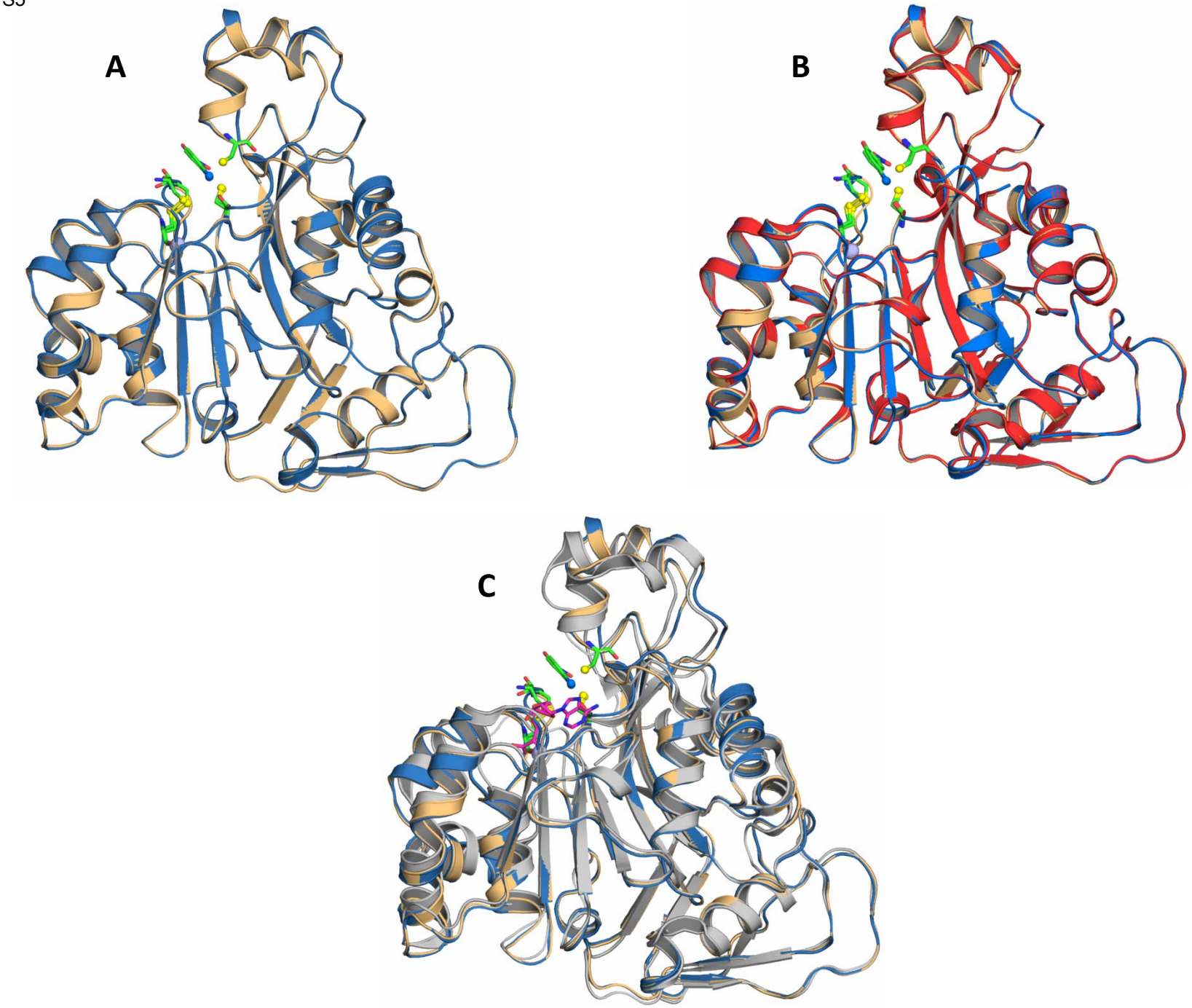
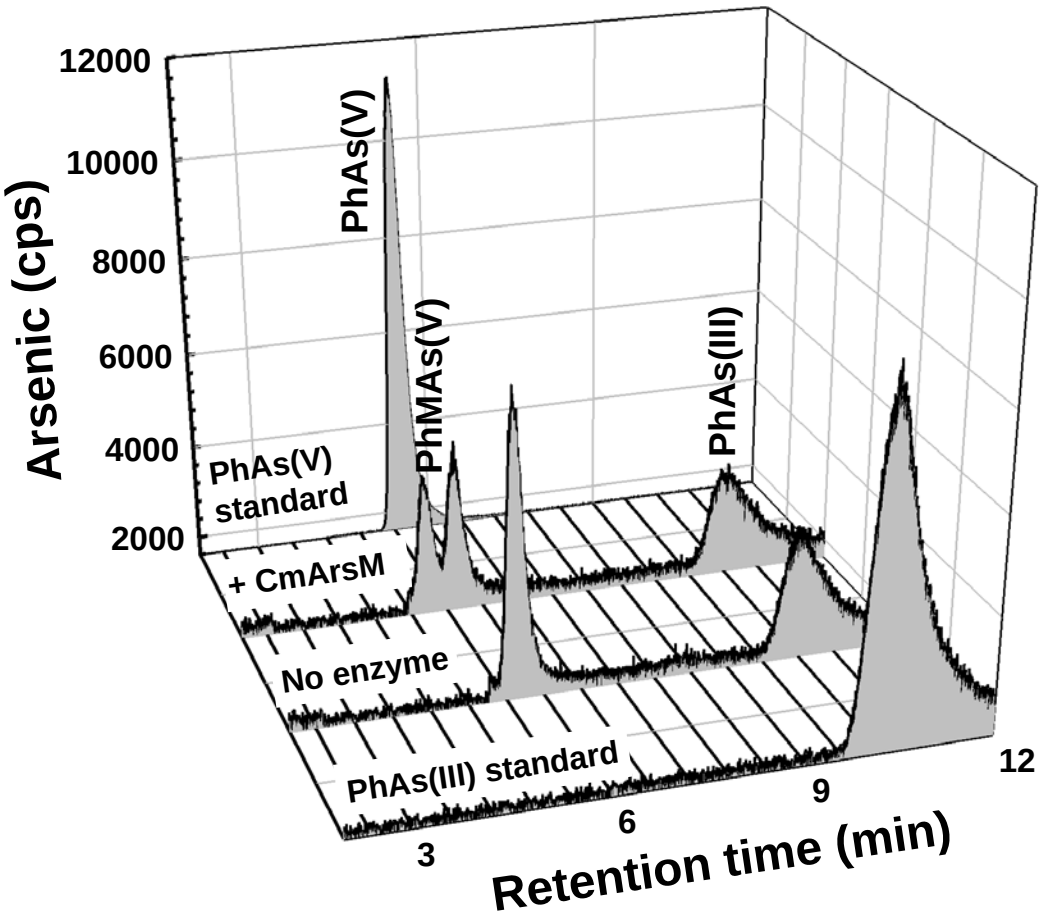


Fig. S6


Arsenic (cps)
12000
10000
8000
6000
4000
2000
PhAs(V)
PhMAs(V)
PhAs(III)
PhAs(V) standard
+ CmArsM
No enzyme
PhAs(III) standard
3
6
9
12
Retention time (min)