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Supporting information for article:

Structural and biochemical characterization of mitochondrial citrate synthase 4 from *Arabidopsis thaliana*

Kazuya Nishio and Tsunehiro Mizushima

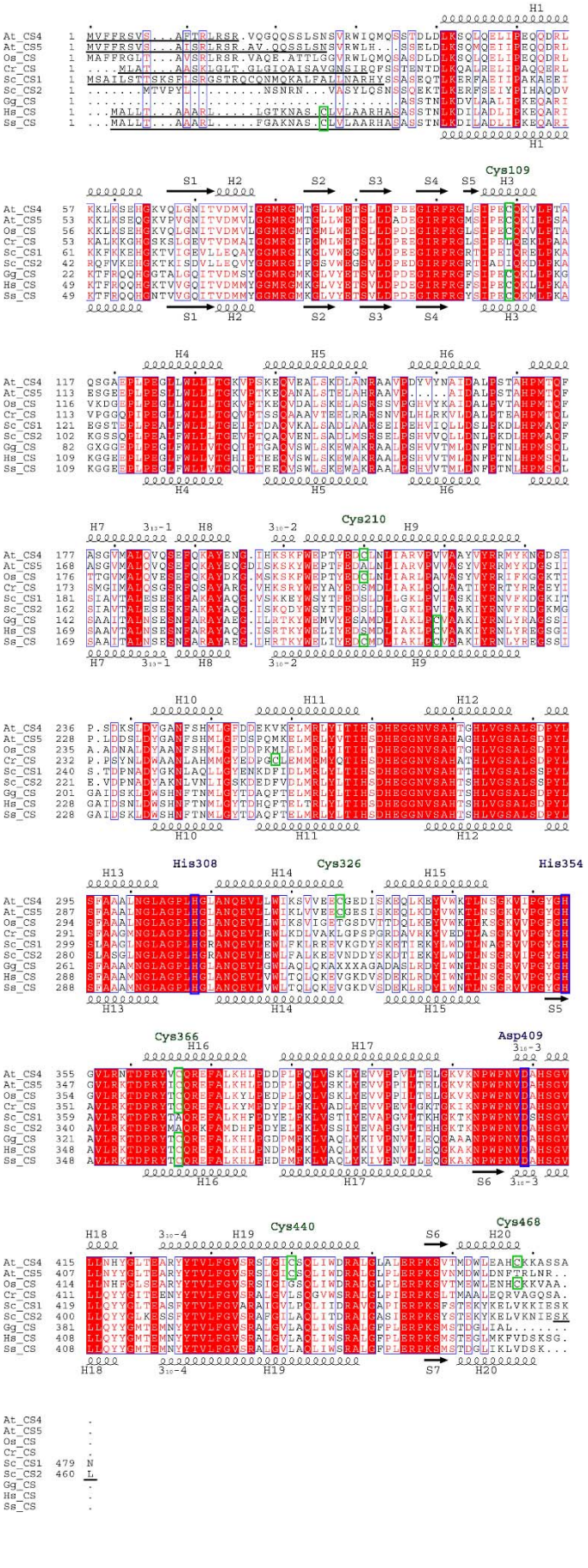

At_CS4
At_CS5
Os_CS
Cr_CS
Sc_CS1
Sc_CS2
Gg_CS
Hs_CS
Ss_CS
Cys109
Cys210
His308
Cys326
His354
Cys366
Asp409
Cys440
Cys468

Figure S1 Amino acid sequence alignment of Type I CSs. Cs sequences [*Arabidopsis thaliana* (At_CS4/CSY4, At_CS5/CSY5), *Oryza sativa* (Os_CS), *Chlamydomonas reinhardtii* (Cr_CS), *Saccharomyces cerevisiae* (Sc_CS1/Cit1, Sc_CS2/Cit2), *Gallus gallus* (Gg_CS), *Homo sapiens* (Hs_CS), and *Sus scrofa* (Ss_CS)] were obtained from the Uniprot database [https://www.uniprot.org/; (UniProt, 2019)]. The alignment and secondary structure depiction were performed using *Clustal O* (Sievers *et al.*, 2011) and *ESPript* (Robert & Gouet, 2014), respectively. Blue and green highlighted residues are three functionally important and cysteine residues on CSs, respectively. The residues underlined in black indicate putative targeting signals as referred from the Uniprot database, except Os_CS and Cr_CS. Putative targeting signal of Os_CS and Cr_CS are from a previous report (Schmidtmann *et al.*, 2014).

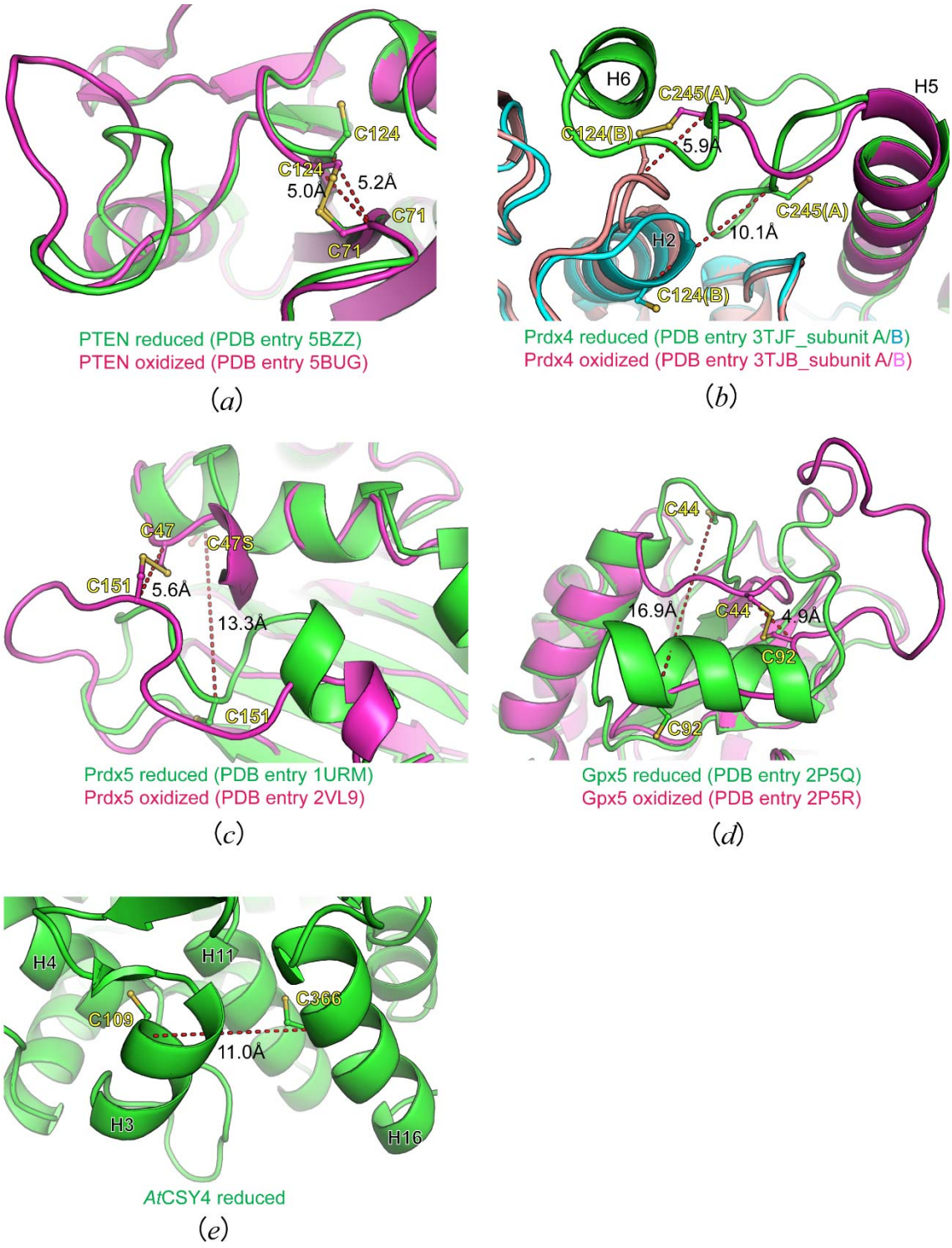
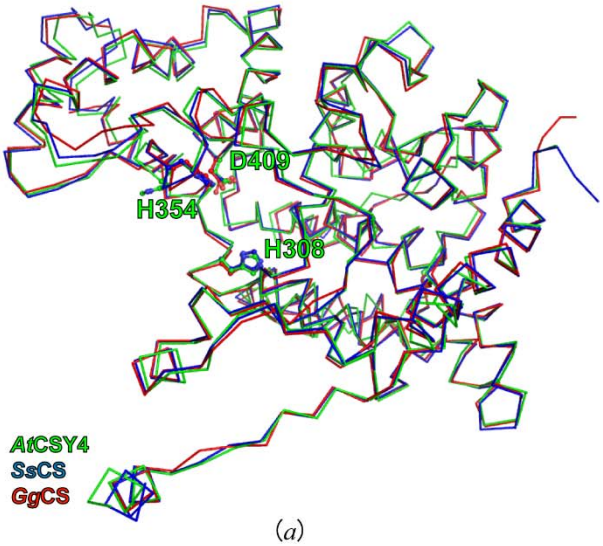
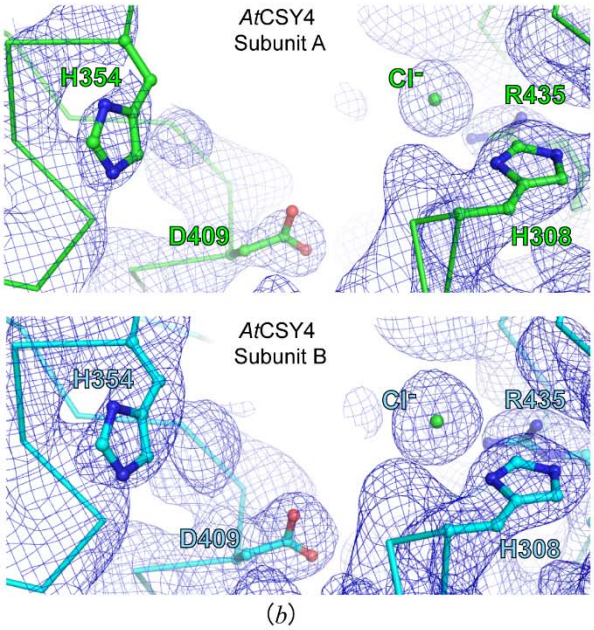


Figure S2 A close-up view of the redox-sensitive cysteine residues in PTEN, Prdx4, Prdx5, Gpx5, and *At*CSY4. (*a*) Comparison of the Cα–Cα distance and structural differences between the reduced (green; PDB entry 5BZZ) and oxidized (magenta; PDB entry 5BUG) forms of

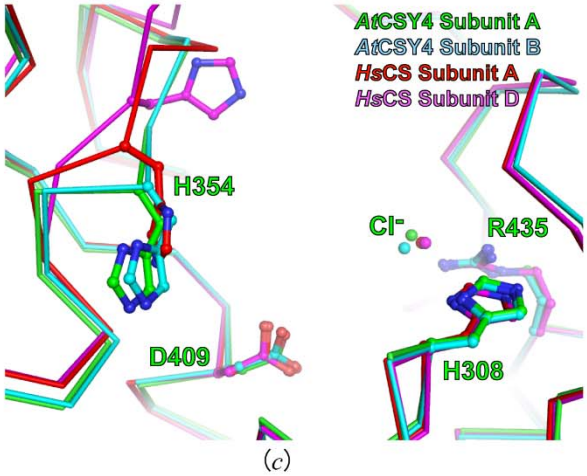
PTEN. (*b*) Comparison of the Cα–Cα distance and structural differences between the reduced (green, subunit A and cyan, subunit B; PDB entry 3TJF) and oxidized (magenta, subunit A and pink, subunit B; PDB entry 3TJB) forms of Prdx4. (*c*) Comparison of the Cα–Cα distance and structural differences between the reduced (green; PDB entry 1URM, C47S mutant) and oxidized (magenta; PDB entry 2VL9) forms of Prdx5. (*d*) Comparison of the Cα–Cα distance and structural differences between the reduced (green; PDB entry 2P5Q) and oxidized (magenta; PDB entry 2P5R) forms of Gpx5. (*e*) The Cα–Cα distance between Cys109 and Cys366 in the reduced form of *At*CSY4.


D409
H354
H308
AtCSY4
SsCS
GgCS

(*a*)


AtCSY4
Subunit A
H354
Cl-
R435
D409
H308
AtCSY4
Subunit B
H354
Cl-
R435
D409
H308

(*b*)


AtCSY4 Subunit A
AtCSY4 Subunit B
HsCS Subunit A
HsCS Subunit D
H354
Cl-
R435
D409
H308

(*c*)

Figure S3 Conformation of the active site in CSs. (*a*) Comparison of *At*CSY4 (green) with porcine (blue; PDB entry 3ENJ) and chicken (red; PDB entry 5CSC) CSs. (*b*) A close-up view of the active site in *At*CSY4. The $2F_o - F_c$ electron density map showed by a blue mesh was contoured at the 1σ level. The three functionally important residues and Cl^- ion binding residues (Arg435) are represented by a stick model. (*c*) Close-up view of the conserved active site residues in *At*CSY4 (green, subunit A and cyan, subunit B) and *Hs*CS (red, subunit A and magenta, subunit D; PDB entry 5UZR). The three functionally important residues and Cl^- ion binding residues (Arg435) are represented by a stick model. Residue numbering is based on the active-site residues of *At*CSY4.