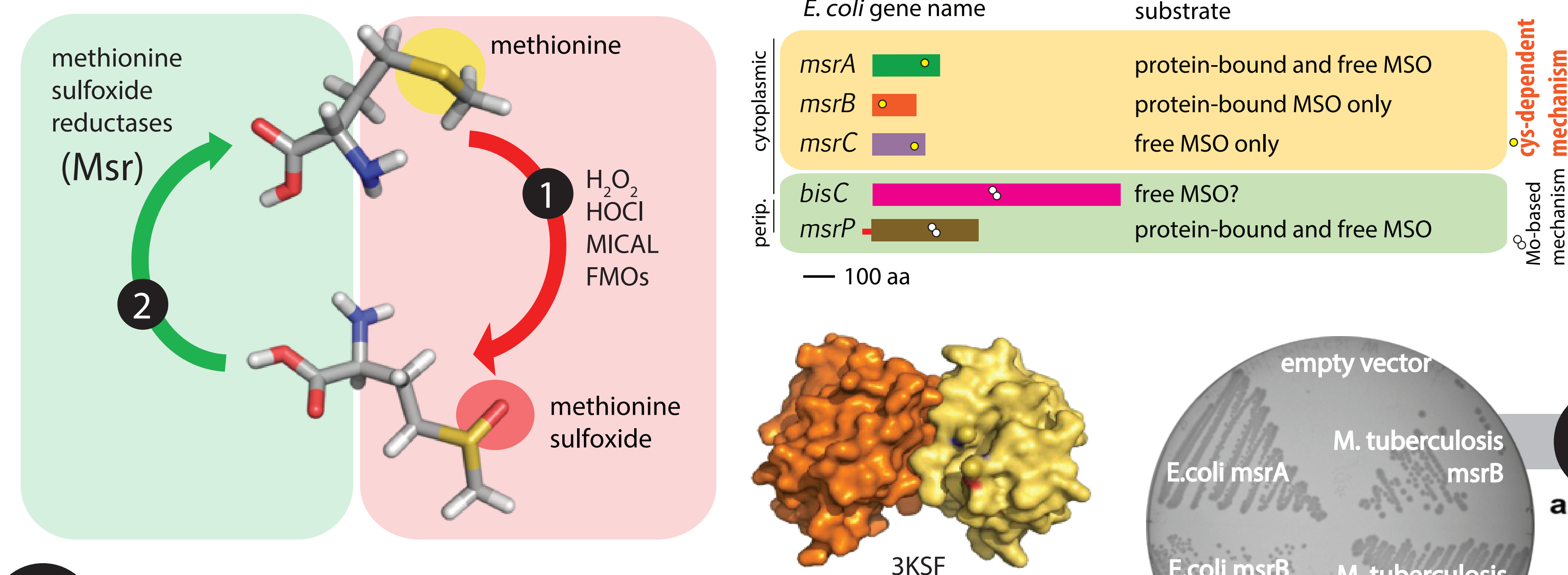


Conservation and mechanism of the methionine reductase C family

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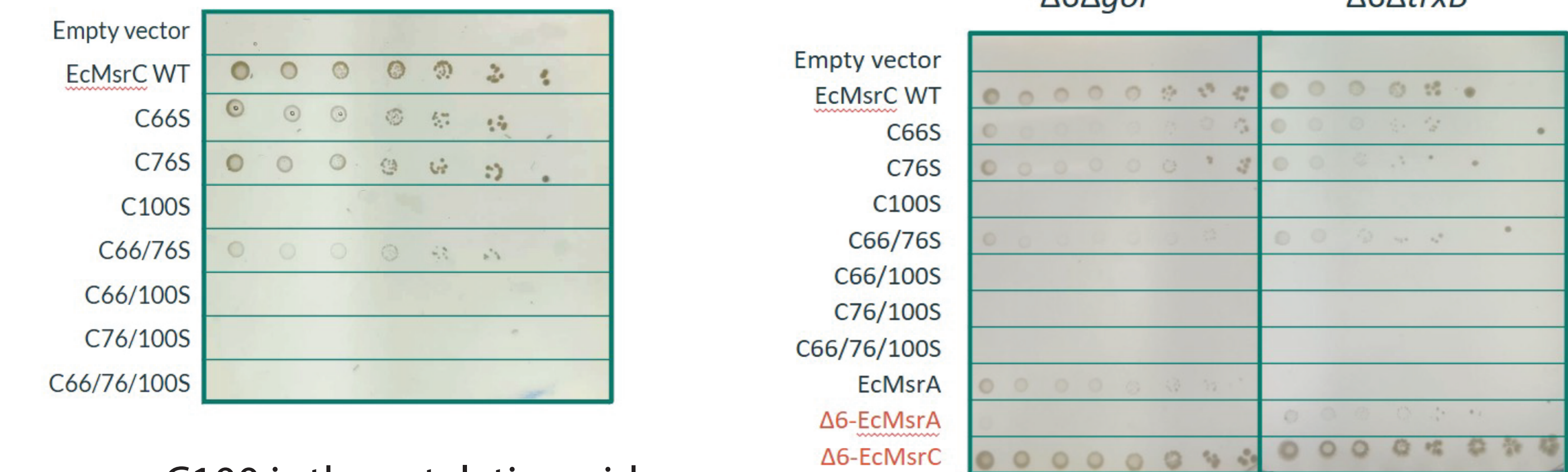
Oxidation of methionine to methionine sulfoxide (MSO) is reversed by methionine sulfoxide reductases (Msr), a family of six evolutionarily-unrelated proteins that converge on this activity. Here we explore the mechanism of MsrC, an unusual Msr active on free-MSO with evolutionary origins on ligand-binding GAF-domain containing proteins involved in signaling.

A Methionine sulfoxide reductases



C in-cell activity assay

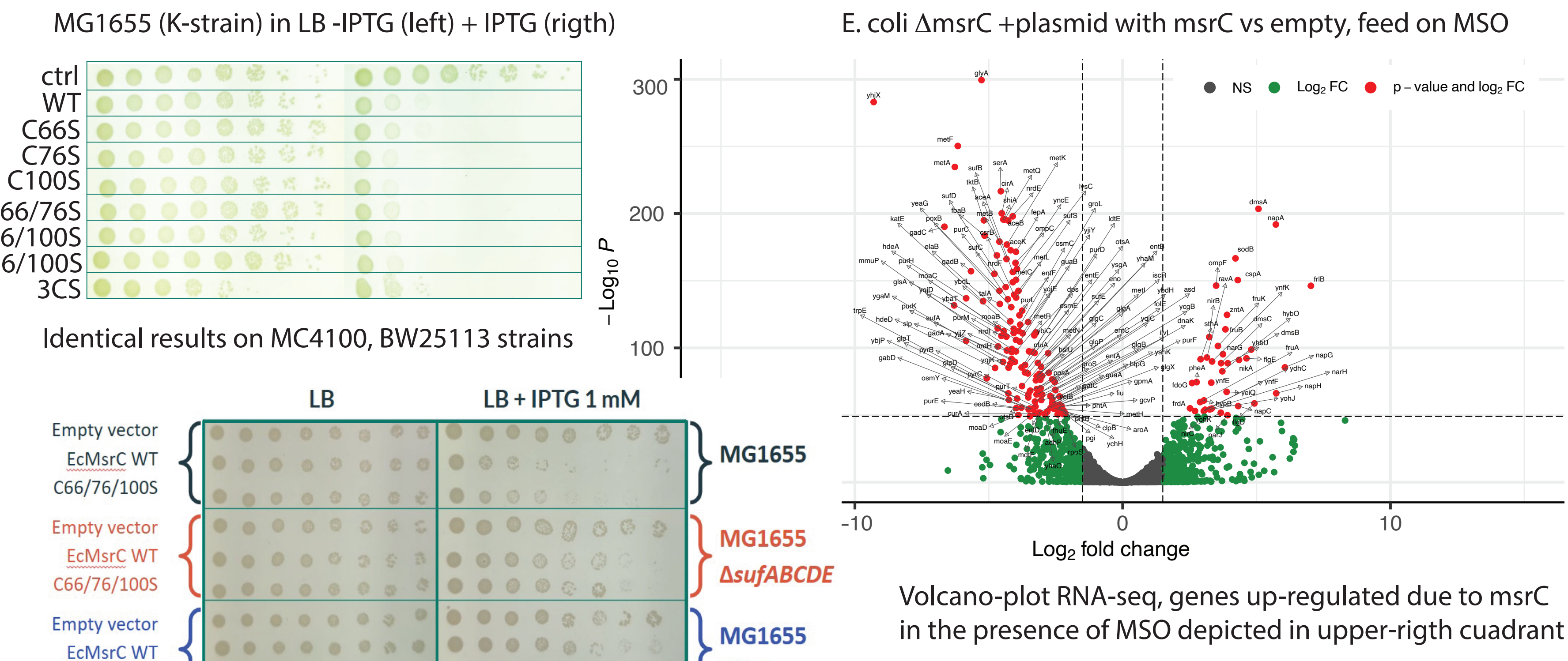
$\Delta 6$ is a Met-auxotroph *E. coli* strain with no MSRs. NO growth with MSO as the only source of Met. Growth recovered when an ACTIVE Msr is expressed from plasmid



C100 is the catalytic residue.
Both **Trx** and/or **GSH/Grx** system can support redox activity.

E Expression of *msrC* changes gene expression pattern

Recombinant MsrC is produced with high-yield on B-type strains. However, when expressed on K-type *E. coli* cells, compromises cell growth. This phenomena is independent of its redox mechanism. Expression of *msrC* on a *msrC*/KO strain alters gene expression (RNAseq). The mechanism is related to SUF and/or FNR.

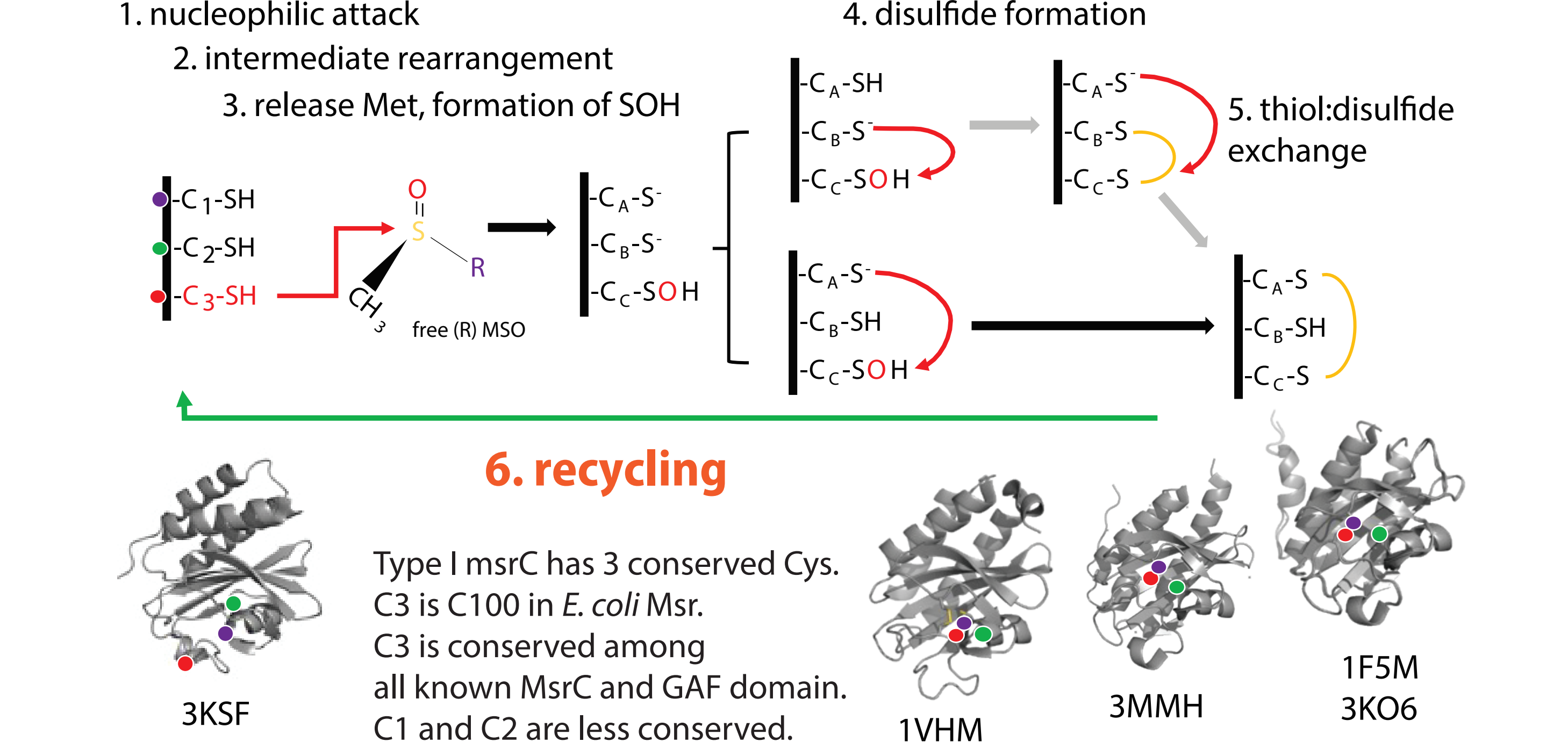


H MsrC "protein" is the effector

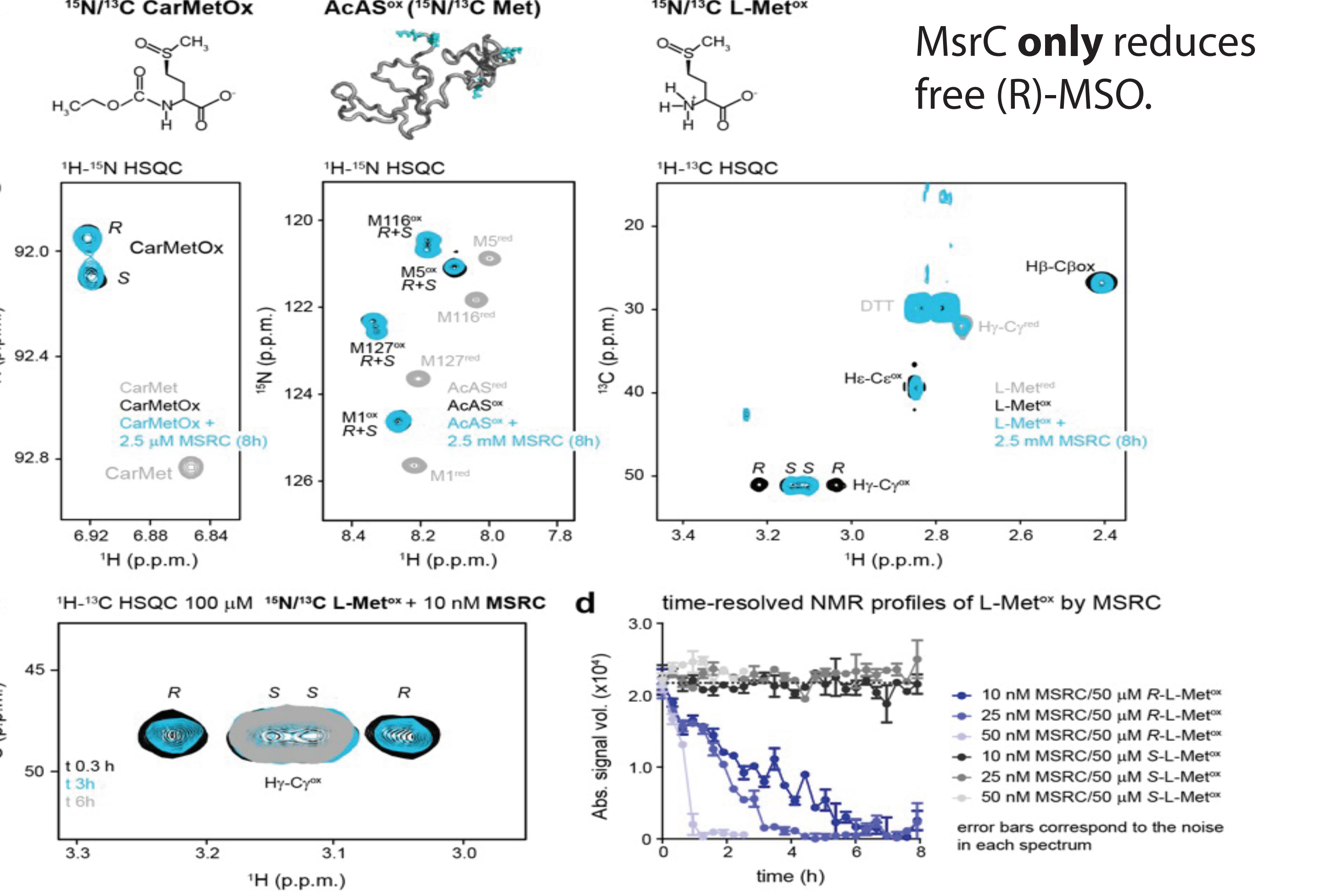
The "toxic" phenotype is mediate by MsrC PROTEIN and it's not hidden in the RNA squence (cod.sin=same protein seq from a different RNA seq).

In-vivo mechanism and effect of MsrC on gene expression may represent two sides of the same mechanism. MsrC relies on C100 to be active, and both Trx/Grx systems can reduce it. MsrC impacts gene expression in response to MSO. This phenotype is linked to SUF and FNR systems, FeS cluster proteins involved in stress response..

B Mechanism of MsrC

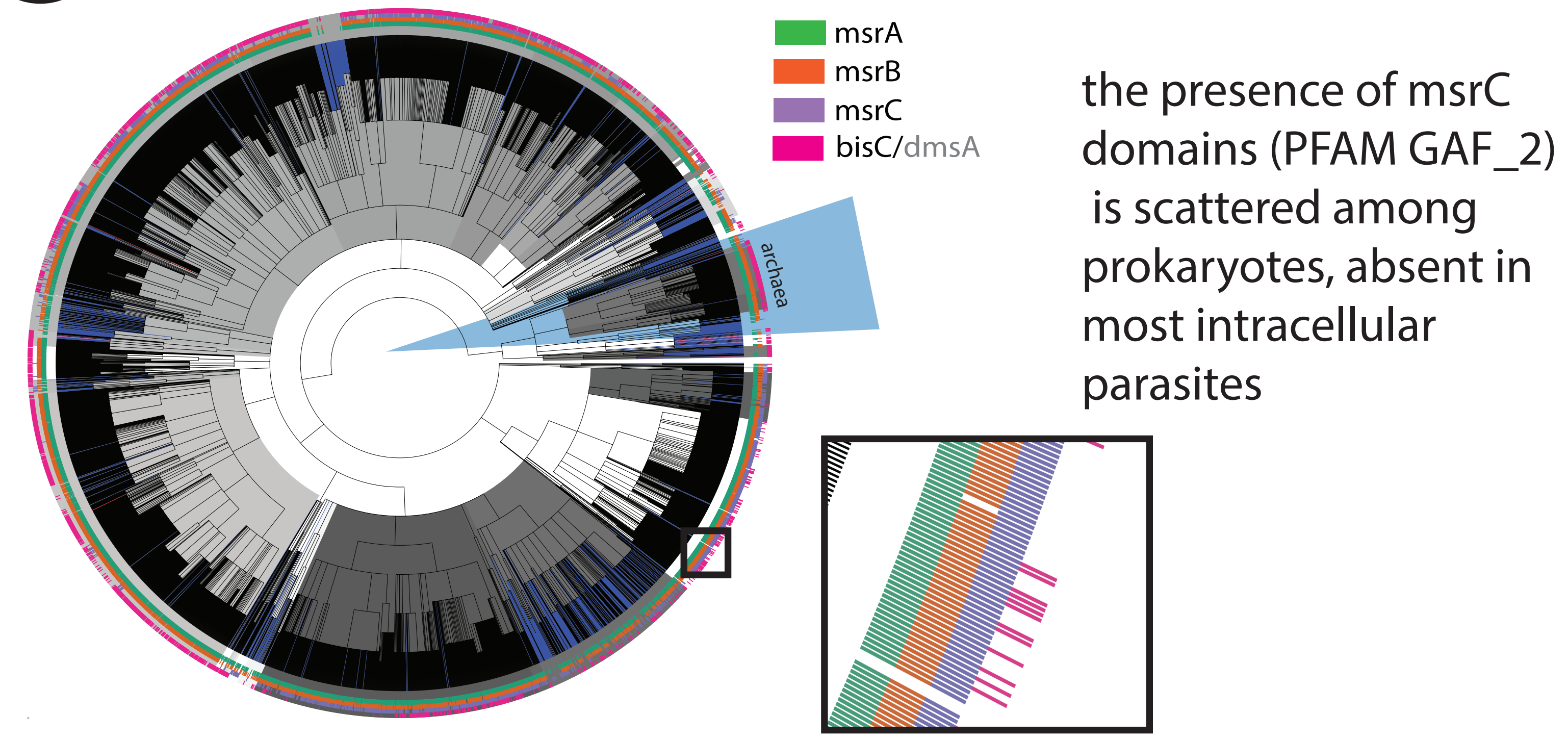


D in vitro activity assay



MsrC is a dimeric enzyme with 3 titrable thiols per unit, all of the with pKa around 8 (not shown). k_2 MSO similar to *msrA* (not shown).

F Conservation of *msrC* (GAF_2) in prokaryotes



G *msrC* is a subtype of redox-active GAF domain

