A cysteinyl-tRNA synthetase variant confers resistance against selenite toxicity and decreases selenocysteine misincorporation

Selenocysteine (Sec) is the 21st genetically encoded amino acid in organisms across all domains of life. Although structurally similar to cysteine (Cys), the Sec selenoglutamate group has unique properties that are attractive for protein engineering and biotechnology applications. Production of designer proteins with properties that are attractive for protein engineering and biotechnology applications. Production of designer proteins with properties that are attractive for protein engineering and biotechnology applications. Production of designer proteins with properties that are attractive for protein engineering and biotechnology applications.

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This article contains Figs. S1–S4 and Tables S1–S4.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) MK579384.1

Both authors contributed equally to this work.

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A rare cysteinylation-tRNA synthetase for selenite resistance

**Figure 1. Selenium metabolic pathways in E. coli and S. cerevisiae that lead to free Sec.** Pathways specific for Sec are shown in red and blue, respectively. 1, in S. cerevisiae, SeMet is imported through amino acid permeases (30), whereas selenite is taken up through phosphate transporters and a monocarboxylate transporter (57, 58). 2, SeMet enters the Met metabolic cycle and is converted to Se-adenosylmethionine (SeAM) by SAM synthase (33). 3, The Met salvage pathway converts Se-adenosylmethionine back to SeMet. Se-adenosylmethionine is also a substrate of methyltransferases, which release Se-adenosylhomocysteine (SeAH) as a product of methylation reactions. Se-adenosylhomocysteine is hydrolyzed to selenohomocysteine (SeHCys) by S-adenosylhomocysteine hydrolase. Selenohomocysteines can be methylated back to SeMet by synthase (59). 4, enzymes of the trans-sulfuration pathway convert selenohomocysteine to Sec (30). Cystathionine synthases and cystathionine lyases interconvert selenohomocysteine and selenocystathionine (SeCyst). Selenium from selenite can also replace sulfur in the trans-sulfuration pathway. 5, reduction of selenite to selenide occurs nonenzymatically and involves GSH and other organic selenium intermediates (60). 6, selenide reacts with O-acetylcysteine in yeast to form selenohomocysteine. In E. coli, selenide can react with SAM to produce methylselenol (MSe) (61), or it can be a substrate of O-acetylserine sulffhydrilases AB (cysM and cysR) for the production of free Sec (60, 62) (8, 9), because methionyl-tRNA synthetase and CysRS cannot discriminate between sulfur- and selenium-containing amino acids. SeMet and Sec are ligated onto tRNA and misincorporated at Met and Cys codons, respectively, during protein synthesis (figure was adapted from Ref. 59).

growth (19), which may be in part due to the misincorporation of Sec. Interestingly, certain plant species are resistant to high levels of selenium and accumulate selenium compounds in their tissues (selenium accumulator plants). With the identification of a Sec-rejecting CysRS from the selenium accumulator Astragalus bisulcatus (15, 20), it has been suggested that selenium tolerance is gained by reducing Sec misincorporation.

As our work on site-directed Sec insertion into proteins (21) would benefit from a CysRS with reduced affinity for Sec, we decided to search for such an enzyme. We hypothesized that expression of a Sec-rejecting CysRS variant in yeast or _E. coli_ would prevent nonspecific incorporation of Sec into cellular proteins _in vivo_. Moreover, this work would help inform our mechanistic understanding of selenium tolerance in selenium accumulator plant species.

**Results**

**A. bisulcatus encodes a CysRS with a rare H240N variation next to the active site**

Early studies that compared the substrate specificity of CysRS enzymes revealed that _A. bisulcatus_ CysRS has a 3-fold higher apparent _K_m for Cys and fails to use Sec as substrate in the ATP–PP, exchange reaction (15, 22), suggesting that it evolved a mechanism to discriminate Cys from Sec. However, the mechanism by which _A. bisulcatus_ CysRS achieves a higher substrate specificity is unknown. To investigate the molecular basis of _A. bisulcatus_ CysRS activity and specificity, we first determined the primary sequence of _A. bisulcatus_ CysRS. We grew _A. bisulcatus_ from seeds, extracted RNA from leaf tissue, synthesized cDNA, and amplified cysS using primers designed for a cysS sequence from the closely related species _Astragalus chrysophlorus_ (23). The resulting _A. bisulcatus_ CysRS cDNA encoded a sequence of 538 amino acids (Fig. S1). Using multiple sequence alignments, we compared _A. bisulcatus_ CysRS with all the available CysRS sequences in the UniProt database. This analysis revealed that _A. bisulcatus_ CysRS has a rare H240N variation in the direct vicinity of the active site of the enzyme (Fig. 2A). Our analysis of CysRS sequences showed that the His → Asn change is rare, as it was only found in 59 of the 28,576 analyzed sequences (a representative alignment is shown in Fig. 2B; all His variants are shown in Fig. S2).

Asn-240 of _A. bisulcatus_ CysRS corresponds to His-235 of _E. coli_ CysRS, which is responsible for coordinating a catalytic zinc ion that is thought to be critical for CysRS amino acid specificity (Fig. 2A) (24, 25). Based on this finding, we hypothesized that Asn-240 plays a role in rejecting Sec from the _A. bisulcatus_ CysRS active site.

Among species that encode CysRS with the His → Asn variation, we were unable to establish a strict correlation to selenium-rich lifestyles due to the lack of information for each organism. However, we identified the His → Asn variation in CysRS of _Triticum urartu_. _T. urartu_ is the diploid progenitor of the A genome of the polyploid wheat species _Triticum aestivum_ (26), one of the most widely consumed crops in the world. _T. aestivum_ represents one of the largest sources of dietary selenium for the human population (27) and is more tolerant to selenium than tobacco, soybean, and rice (28). It is possible that _T. urartu_ thrives in soils high in selenium relative to other wheat species and contributes to selenium tolerance in _T. aestivum_.

**Transplanting the His → Asn variation to _E. coli_ CysRS increases selenium tolerance of _E. coli_**

To test whether the His → Asn variation found in _A. bisulcatus_ CysRS defines higher CysRS specificity toward Cys over Sec, we used _E. coli_ CysRS as a model system. We introduced a low-copy plasmid encoding either _E. coli_ wildtype (WT) CysRS or CysRS-H235N into _E. coli_ UQ818 cells (29), which encode a temperature-sensitive cysS (ts-cysS) allele that prevents growth at 42 °C. As expected, _E. coli_ UQ818 was unable to grow at 42 °C in the absence of plasmid-borne _E. coli_ cysS (Fig. 3A), whereas expression of cysS and cysS-H235N from a low-copy plasmid complemented _E. coli_ ts-cysS at the same temperature (Fig. 3A). We then grew cells in the presence of sodium selenite. We used sodium selenite as a Sec source because selenite is reduced to HSe⁻ and then infiltrates Cys biosynthesis (Fig. 1), resulting in the formation of free Sec (10, 19). The inability of CysRS to effectively distinguish Cys from Sec leads to the formation of Sec-tRNA^Cys^*. As a result, Sec is misincorporated in place of Cys residues in proteins. This ultimately jeopardizes the sustainability of _E. coli_ and other bacteria (10). Interestingly, at the lowest selenite concentration tested (1 mm), cells expressing WT CysRS showed a significant growth defect, whereas CysRS-H235N-containing cells were only marginally affected (Fig. 3A). Higher concentrations of selenite amplified the phenotype of the WT cysS strain, which was unable to grow at a concentration of 5 mm. In contrast, although the growth rate was
slower, the cysS-H235N strain grew at a selenite concentration of 10 mM. Unexpectedly, we found that substitution of His-235 with alanine (CysRS-H235A) can also increase the selenite tolerance of the cells, albeit less effectively than CysRS-H235N (Fig. 3A), indicating that other amino acids at this position may also alter the specificity of the enzyme. Furthermore, even though at the permissive temperature (30 °C) cells express chromosomally encoded CysRS, only cells expressing CysRS-H235N or CysRS-H235A were able to grow at high selenite concentration (Fig. 3B). Together, these results suggest that the single His → Asn change found in A. bisulcatus CysRS may be responsible for improving the specificity of CysRS toward Cys, thereby lessening mistranslation with Sec and increasing selenium tolerance.

**The His → Asn variation in S. cerevisiae CysRS increases selenomethionine tolerance**

In yeast, free Sec is synthesized by the conversion of SeMet by the trans-sulfuration pathway (Fig. 1 and Ref. 30). Production of free Sec causes misincorporation of Sec at Cys codons, leading to toxicity and protein aggregation (31). In contrast to Sec, SeMet incorporation at Met codons is tolerated at over 90% of Met codons when trans-sulfuration of SeMet to Sec is disrupted (32, 33). Based on our results with *E. coli*, we reasoned that an *S. cerevisiae* CysRS variant containing a His → Asn mutation in the homologous position of *E. coli* and *A. bisulcatus* CysRS should increase its discrimination against Sec and alleviate growth inhibition caused by SeMet conversion to Sec. We constructed an *S. cerevisiae* CysRS-H395N variant and expressed the allele in a YNL247W (yeast cysS)-knockout strain. On rich medium, growth of the YNL247W-knockout strain containing a centromeric plasmid expressing either *S. cerevisiae* CysRS or CysRS-H395N resembled that of WT strain (Fig. 4). However, in the presence of 15 μM SeMet, the CysRS-H395N strain had a clear growth advantage. When reducing the amount of Met in the media to limit Cys biosynthesis and increase the ratio of Sec to Cys in cells, resistance to SeMet gained by CysRS-H395N became more pronounced (Fig. 4). These results suggest that...
S. cerevisiae CysRS-H395N discriminates against Sec, reducing the frequency of Sec misincorporation at Cys codons.

Substrate characterization of the E. coli CysRS-H235N variant

To directly test whether replacing His-235 with Asn affects the specificity and efficiency of E. coli CysRS, we performed biochemical assays to obtain the steady-state kinetic parameters using recombinant E. coli CysRS. ATP–PPi exchange assays showed that WT CysRS and CysRS-H235N catalyzed Cys-AMP formation with almost identical efficiency ($k_{\text{cat}}/K_m$) (Table 1). Similarly, although CysRS-H235N showed a lower $K_m$, both WT and CysRS-H235N formed Sec-AMP with comparable efficiency. Surprisingly, both enzymes catalyzed Sec-AMP synthesis slightly more efficiently than Cys-AMP, indi-
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Table 1
Steady-state kinetic parameters for amino acid activation by E. coli CysRS

| CysRS     | $K_a$ (µM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$·µM$^{-1}$) | Relative $k_{cat}/K_m$ | Specificity factor* |
|-----------|------------|----------------------|----------------------------------|------------------------|---------------------|
| Cys       |            |                      |                                  |                        |                     |
| WT        | 16.6 ± 1  | 29.8 ± 2  | 1.81                           | 1.0                    |                     |
| H235N     | 15.4 ± 4  | 34.0 ± 3  | 2.34                           | 0.79                   |                     |
| Sec       |            |                      |                                  |                        |                     |
| WT        | 10.9 ± 2  | 37.4 ± 1  | 3.29                           | 1.0                    | 0.55                |
| H235N     | 6.81 ± 2  | 23.7 ± 1  | 3.87                           | 0.85                   | 0.60                |

* Specificity factor (Cys/Sec) = $k_{cat}/K_m$(Cys)/$k_{cat}/K_m$(Sec).

ciating that CysRS cannot differentiate between these two amino acids during the first step of aminoacylation.

We next determined the kinetic parameters for Cys and Sec in the aminoacylation reaction using in vitro transcribed E. coli tRNA$^\text{Cys}$. Aminoacylation experiments revealed that CysRS-H235N esterifies tRNA$^\text{Cys}$ with Cys with the same efficiency as WT CysRS. Moreover, whereas both enzymes attach Sec to tRNA$^\text{Cys}$ with similar catalytic efficiencies (Table 2), Sec esterification is catalyzed ~8 times less efficiently than Cys. Thus, although both Cys and Sec are “activated” with equal efficiency, CysRS is better suited for aminoacylation with Cys than Sec. Together, our enzyme-kinetic results (with pure CysRS and tRNA$^\text{Cys}$) indicate that the H235N mutation in E. coli CysRS does not alter the substrate specificity at either of the two steps of aminoacylation.

The CysRS-H235N variant reduces mistranslation of Cys codons as Sec

We hypothesized that E. coli CysRS-H235N increases the tolerance of selenite by decreasing the frequency of Sec misincorporation. To measure the extent of Sec misincorporation in cells encoding E. coli CysRS-H235N, we grew E. coli UQ818 cells containing plasmid-borne copies of either cysS or cysS-H235N to mid-log phase at 42 °C in the presence of 1 mM selenite and detected Sec at Cys codons by MS. First, we analyzed peptide species containing Sec at Cys-48 of purified sfGFP from each strain (Fig. S3) and observed an overall decrease in the peak intensities of Sec-containing peptides as a result of CysRS-H235N expression (Table S2 and Fig. S4). Moreover, sfGFP purified from the WT CysRS strain grown in the absence of selenite did not yield any detectable peptides containing Sec.

We then investigated Sec misincorporation frequency across hundreds of endogenous E. coli proteins. As reported in a recent isoTOP-ABPP method (34), Sec-containing proteins were selectively enriched from cell lysates of WT and mutant strains by labeling with an isotopically light (cysS) or heavy (cysS-H235N) IA-alkyne probe (35) at pH 5.75. This low-pH isoTOP-ABPP platform results in significant deactivation of Cys reactivity through protonation, thereby allowing for improved selenopeptide identification. Following IA-alkyne labeling, peptides were conjugated to a chemically cleavable biotin azide tag and then enriched on streptavidin beads (Fig. 5A). After an on-bead trypsin digestion, IA-labeled peptides were selectively eluted and analyzed by LC/MS-MS/MS. IA-conjugated Sec and Cys peptides were identified and quantified between the cysS and cysS-H235N samples. Thereby, the degree of misincorporation of Sec at Cys codons can be globally characterized across hundreds of E. coli proteins.

We observed an ~3-fold decrease in Sec-peptide identification (spectral counts) in the cysS-H235N strain relative to the cysS strain when both were grown in 1 mM selenite (Fig. 5B and Table S3), with no significant difference in Cys-peptide identification. Importantly, in the absence of selenite supplementation in the growth media, no significant difference in the number of Cys-containing peptides between these two samples was observed, and there were virtually no selenopeptides identified. Over 400 Sec-containing peptides were quantified by our low-pH isoTOP-ABPP platform, all with light to heavy (L/H) ratios of >1, indicating significantly increased Sec misincorporation (Fig. 5C and Table S4). The median L/H ratio for these Sec-containing peptides was 4.3, which is ~3-fold greater than the median L/H ratio (1.5) for the ~1600 Cys-containing peptides quantified (Fig. 5D, Table S4). For ~190 of the peptides with Cys-to-Sec substitutions, both the Sec and Cys versions of the peptide could be independently identified and quantified by our low-pH isoTOP-ABPP platform. In all cases, the Cys-containing peptide had a lower L/H ratio (<2) than the corresponding selenopeptide (Fig. 5E and Table S4). The Sec variants could be easily distinguished from the equivalent Cys variant by both the increased mass of their parent ion and the unique isotopic envelope conferred by the presence of the selenium atom (Fig. 5E, cmoA, ldhA, and clpB inserts). Taken together, these results demonstrate that the H235N mutation in E. coli CysRS reduces nonspecific Sec incorporation at Cys codons.

**Table 2**
Steady-state kinetic parameters for aminoacylation by E. coli CysRS

| CysRS | $K_m$ (µM) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$·µM$^{-1}$) | Relative $k_{cat}/K_m$ | Specificity factor* |
|-------|------------|----------------------|----------------------------------|------------------------|---------------------|
| Cys   |            |                      |                                  |                        |                     |
| WT    | 5.33 ± 2.5 | 140 ± 77             | 25.8                             | 1.0                    |                     |
| H235N | 7.47 ± 2.9 | 156 ± 25             | 23.7                             | 1.1                    |                     |
| Sec   |            |                      |                                  |                        |                     |
| WT    | 24.2 ± 12  | 50.3 ± 6.6           | 3.01                             | 1.0                    | 8.57                |
| H235N | 15.9 ± 1.9 | 45.9 ± 3.3           | 2.34                             | 1.0                    | 8.14                |

* Specificity factor (Cys/Sec) = $k_{cat}/K_m$(Cys)/$k_{cat}/K_m$(Sec).
3-fold and indicate that selenite toxicity is mediated in part by Sec misincorporation.

**Discussion**

In this study, we found that CysRS from the selenium-tolerant plant *A. bisulcatus* has a rare H240N variation in the vicinity of the catalytic site. By introducing this variation into CysRS from *E. coli* and *S. cerevisiae*, we engineered *E. coli* and *S. cerevisiae* cells with increased tolerance to high selenium levels in growth medium. Our quantitative mass-spectral analysis revealed that the selenium-tolerant *E. coli* strain contained significantly less Sec incorporation at Cys positions across hundreds of *E. coli* proteins, indicating that higher selenium tolerance stems from the ability of the engineered cells to reject nonspecific incorporation of Sec into cellular proteins. This finding suggests that the H240N variation found in *A. bisulcatus* CysRS can be used to enhance an organism’s tolerance to selenium.

Although *E. coli* cells carrying the *cysS-H235N* allele are more tolerant to selenite, they are not completely resistant. This may be due to the residual misincorporation of Sec by CysRS-H235N and other toxic side effects associated with selenium, which generates reactive oxygen species that cause oxidative stress, induction of antioxidant enzymes, and depletion of GSH in plants (for a review, see Ref. 17). To combat selenium toxicity, organisms use distinct defense mechanisms. For example, selenium accumulator plant species can compartmentalize or store organic selenium compounds in specialized cells along the periphery of the plant (36). Alternatively, some plants generate volatile selenium forms from organic selenium compounds (e.g. SeMet and Sec) that are first methylated and later metabolized to dimethyl selenide or dimethyl diselenide, which escape from leaf tissue (37–39). Although this pathway is not exclusive to selenium accumulators, selenium hyperaccumulators (species that accumulate > 1 mg/g of dry weight) have an increased flux of selenium through the sulfur assimilation pathway.
pathway (40). Sec methylation has also been suggested to prevent tRNA acylation with Sec by plant CysRS (15). Interestingly, overexpression of A. bisulcatus selenocysteine methyltransferase in Arabidopsis and Indian mustard increases tolerance and accumulation of selenium (40–42), which has potential for phytoremediation of selenium-contaminated soils (43).

Notably, our study illustrates the discrepancy that occasionally arises between in vitro data (collected by using pure enzyme and substrate molecules) and in vivo experiments where the enzyme and substrate molecules are subjected to global competition with other cellular components. Although our in vitro assays did not reveal any difference in substrate specificity between the WT CysRS and CysRS-H235N, the in vivo experiments show that the CysRS variation remarkably improves E. coli and S. cerevisiae growth in the presence of toxic selenium concentrations and decreases Sec misincorporation 3-fold in vivo compared with other cellular components. Although our enzyme and substrate molecules are subjected to global competition, our work suggests that E. coli strains carrying CysRS-H235N should be better for accurate selenoprotein production (21). The CysRS variant would reduce the amount of Sec-tRNA Sec formed in E. coli strains engineered for selenoprotein production that accumulate free Sec from the hydrolysis of excess Sec-tRNA Sec.

Experimental procedures

Isolation of A. bisulcatus RNA and cDNA synthesis

A. bisulcatus seeds were purchased from Prairie Moon Nursery (Winona, MN) and germinated in moist growth nutrients provided with the seeds. Once germinated the seedlings were planted in standard gardening topsoil (Miracle Grow) containing 24% nitrogen (N), 8% phosphate (P2O5), and 16% soluble potash (K2O). Plants were grown in a growth chamber at 22 °C for 2 months, receiving light for 16 h/day and water once every 2 days. RNA was extracted and purified from 100 mg of leaf tissue using a Qiagen RNeasy Plant Mini kit according to the manufacturer’s protocol. The SuperScript® III First-Strand Synthesis System (Invitrogen) was used to prepare cDNA from 1 μg of total A. bisulcatus RNA following the manufacturer’s protocol. After synthesis, RNA was degraded by addition of RNase H (40 units) for 30 min at 37 °C.

Sequencing of A. bisulcatus CysRS

An aliquot (2 μL) of the first-strand cDNA synthesis reaction was used as template for PCR. Primers 5UTR-F and 3UTR-R (Table S1) were designed to anneal to a CysRS cDNA sequence from the closely related Astragalus species A. chrysoscholorus (mRNA sequence listed in Fig. S1a). PCR amplification resulted in a product with the expected size of A. chrysoscholorus CysRS. This DNA product was sequenced (Fig. S1b), and a BLASTP analysis confirmed its homology with other known CysRS sequences.

Cloning of E. coli cysS

E. coli cysS was PCR-amplified from −150 bp of the cysS translational start codon to +100 bp of the stop codon using genomic DNA (E. coli MG1655) and primers EcCysRS-F and EcCysRS-R (Table S1). pGEX-2T was linearized by PCR using primers pGEXlin-1 and pGEXlin-2, and cysS PCR products were cloned in by Gibson assembly in replacement of the GST tag. The H235N and H235A mutations were introduced by inverse PCR of the plasmid using primer H235N-1 with H235N-2 and primer H235A-1 with H235A-2, respectively. pGEX-2T without the GST tag was used as the empty vector control.

S. cerevisiae strains and cloning of CysRS

To analyze the effects of S. cerevisiae CysRS-H395N, we purchased (Dharmacon) the YNL247W heterozygous knockout strain (record number 21197) (44). This strain was transformed with pRS316-CysRS and sporulated, and tetrads were dissected on a YPD plate. Spore colonies were patched onto a YPD plate containing 200 μg/ml G418 and a plate lacking uracil to select for a haploid strain having both the YNL247W deletion and pRS316-CysRS. The Ura+ G418-resistant haploid strain was transformed with pRS313-CysRS-H395N and then plated onto 5-fluoroorotic acid medium to remove pRS316-CysRS.

YNL247W, from −500 bp of the translational start to +300 bp of the stop codon, was PCR-amplified from genomic DNA using primer yCysS-1 with yCysS-2 and cloned by Gibson assembly into BamHI/EcoRI-cut pRS316 and pRS313. H395N was introduced by two-step PCR. Primer yCRSp-F with yCRSqc-R and primer yCRSqc-F with yCRSt-R were used to amplify the front and back half of YNL247W while introducing H395N. The two fragments were annealed and extended for 5 min at 72 °C followed by PCR amplification with primer yCRSp-F with yCRSt-R. The PCR product was ligated into pRS313 by restriction enzyme cloning using Xmal/Xbal.

Multiple sequence alignment of CysRS

All CysRS sequences were retrieved from UniProt and aligned using Clustal Omega (45) with the default parameters. The alignment file was imported into Jalview version 2.10.4b1 (46), and residues are illustrated using ClustalW coloring.

Growth rate experiments

E. coli strain UQ818 (29) expressing either E. coli CysRS, CysRS-H235N, or CysRS-H235A from pGEX-2TΔGST was grown to stationary phase in LB medium containing 100 μg/ml ampicillin. E. coli UQ818 containing pGEX-2TΔGST was used as the empty vector control strain. Each strain was diluted to an optical density of 0.01 in the same medium with increasing concentrations of sodium selenite (indicated in Fig. 2) and grown for 12 h at both 30 and 42 °C. Growth rates were measured using a BioTek Synergy HT plate reader and Gen5 software version 2.00.18.

Yeast strains BY4741, KSH1, and KSH2 were grown in synthetic complete (SC) medium containing 32.5 μM Met to stationary phase. Cell densities were normalized and spotted in 10-fold serial dilutions onto plates containing either YPD, SC
with 65 μM Met and 15 μM SeMet, or SC with 32.5 μM Met and 15 μM SeMet. All plates were grown at 30 °C, the YPD plate was incubated for 2 days, and SC plates were incubated for 4 days.

**Protein purification**

*E. coli* WT CysRS was purified as described previously (47). Site-directed mutagenesis was used to replace His with Asn at position 235 of *E. coli* CysRS. pET28a.EcCysRS-H235N, the resulting plasmid, was transformed into *E. coli* BL21(DE3) cells. *E. coli* H235N CysRS overexpression was induced with 0.15 mM isopropyl β-d-1-thiogalactopyranoside at an optical density of 0.6 overnight at 23 °C. Cells were sonicated in buffer containing 50 mM Tris-HCl, pH 7.4, 0.3 M NaCl, and protease inhibitor mixture tablets (cOmplete, Roche Applied Science). Lysed cells were centrifuged, and the supernatant was run through TALON metal affinity resin (Clontech). The His-tagged protein was eluted with a linear gradient of imidazole. The concentrations of WT CysRS and CysRS-H235N were calculated using active-site titration (48).

**ATP–PPi exchange assays**

Steady-state kinetic parameters for CysRS were obtained in buffer containing 150 mM HEPES (pH 7.5), 10 mM KF, 10 mM MgCl₂, 10 mM DTT, 0.2 mg/ml BSA, 1 mM PPi, 1 mM ATP, and [γ-32P]ATP, and varying concentrations of Cys and Sec (ranging from 2.5 to 4000 μM). Reactions were initiated by addition of 0.2 μM CysRS, and the reaction course was monitored by spotting 1 μl of reaction mixture on a PEl-cellulose TLC plate (prewashed with water). Plates were run in buffer containing 0.75 KHzPO₄ and 1 M urea for ~45 min. Sec (50 mM) was freshly prepared as described previously (49, 50). Briefly, 2.1 mg of Sec (Acros Organics) was dissolved with 5 μl of 0.2 n HCl and 5 μl of 0.2 M tris(2-carboxyethyl)phosphine (Sigma-Aldrich) in 0.1 M Tris-HCl, pH 8. Dilutions of Sec were made with 0.1 M tris(2-carboxyethyl)phosphine in 0.1 M Tris-HCl, pH 8. Cys solutions were prepared using the same protocol.

**tRNA aminoacylation assays**

*E. coli* tRNA^{Cys} was transcribed in vitro using T7 RNA polymerase and labeled at the 3'-end adenosine with [32P]phosphate as described previously (47, 51). Aminoacylation experiments were carried out in buffer containing 50 mM HEPES (pH 7.3), 10 mM ATP, 2 mM DTT, 10 mM MgCl₂, 50 mM KCl, 20 μM *E. coli* tRNA^{Cys} (containing trace amounts of 32P-labeled tRNA), and varying concentrations of Sec and Cys (3–600 μM). 50–100 nM CysRS was used to start the reactions. Reaction time points were quenched in a solution of 0.1 unit/μl P1 nuclease (Sigma-Aldrich) in 200 mM sodium acetate (pH 4.5) and incubated at room temperature for 1 h. 1 μl of the quenched solution was spotted on a PEI-cellulose TLC plate (prewashed with water). Plates were run in 5% acetic acid and 0.1 M ammonium acetate.

**GFP purification**

*E. coli* UQ818 containing pCDF-sfGFP and expressing either *E. coli* CysRS or CysRS-H235N from pGEX-2TAGST were grown to stationary phase in LB medium containing 50 μg/ml spectinomycin and 100 μg/ml ampicillin at 30 °C. Each strain was then diluted 1:500 and grown at 42 °C in the same medium to an optical density of 2.0. Cells were then harvested; resuspended in buffer containing 50 mM NaH₂PO₄, 300 mM NaCl, 8 mg of lysozyme, 5 μg/ml DNase I, and protease inhibitor mixture tablets (cOmplete); and incubated for 30 min at room temperature. Cells were further lysed by sonication in the same buffer. Cell lysates were centrifuged, and the supernatant was incubated with nickel-nitrilotriacetic acid resin (Qiagen) for 1 h at 4 °C. The resin was washed four times with 8 ml of buffer containing 50 mM NaH₂PO₄, 300 mM NaCl, and 20 mM imidazole. The His-tagged protein was eluted by gravity flow in the same buffer except with 250 mM imidazole. Purified protein elutions were resolved by SDS-PAGE on a 12% polyacrylamide gel, and bands corresponding to the molecular weight of sfGFP were excised for MS analysis.

**Low-pH isoTOP-ABPP**

Lysates from *E. coli* UQ818 cells containing plasmid-borne copies of cysS or cysS-H235N grown at 42 °C to mid-log phase in the presence or absence of 1 mM selenite were generated by sonication in Sec enrichment buffer (50 mM MES, 50 mM NaH₂PO₄, and 100 mM NaCl (pH 5.75)). Lysate concentration was determined by Bradford assay, and the concentration was adjusted to 2 mg/ml with Sec enrichment buffer. For nonquantitative experiments, 1 ml of lysate was labeled with 100 μM isotopically light iodoacetamide-alkyne probe for 1 h at 25 °C. Samples were then conjugated to a diazo biotin azide tag (Click Chemistry Tools) by CuAAC (100 μM azo tag, 1 mM tris(2-carboxyethyl)phosphine, 100 μM tris[(1-benzyl-1H,1,2,3-triazol-4-yl) methyl] amine, and 1 mM CuSO₄) for 1 h at 25 °C (34, 35, 52). For quantitative experiments, 1 ml of lysate was labeled with either isotopically light iodoacetamide-alkyne (cysS, +Se) or isotopically heavy iodoacetamide-alkyne (cysS-H235N, +Se) probes for 1 h at 25 °C. Next, both samples were conjugated to a diazo biotin azide tag by CuAAC (100 μM azo tag, 1 mM tris(2-carboxyethyl)phosphine, 100 μM tris[(1-benzyl-1H,1,2,3-triazol-4-yl) methyl] amine, and 1 mM CuSO₄) for 1 h at 25 °C and then combined.

For both quantitative and nonquantitative experiments, proteins were precipitated by centrifugation (at 6,500 × g for 10 min at room temperature), resuspended in ice-cold MeOH, and repelleted (at 6,500 × g for 10 min at 4 °C). The protein pellet was washed two more times in ice-cold MeOH and then resuspended with 1 ml of 1.2% SDS in PBS by sonication and heating (5 min at 95 °C). Samples were diluted into 5 ml of PBS and 100 μl of streptavidin-agarose beads and rotated at 4 °C overnight. The beads were then incubated with rotation at 25 °C for 3 h followed by washes with 0.2% SDS in PBS (5 ml), PBS (3 × 5 ml), and water (3 × 5 ml). Between washes, the beads were pelleted by centrifugation (at 1,400 × g for 3 min). The beads were transferred to screw-cap Eppendorf tubes and resuspended in 6 ml urea in PBS (500 μl) and 10 mM DTT (diluted from 20× stock in water) and heated for 20 min at 65 °C. Iodoacetamide (20 mM; diluted from 20× stock in water) was added, and the samples were incubated at 37 °C for 30 min. The beads were pelleted by centrifugation and resuspended in 200 μl of 2 M urea in PBS, 1 mM CaCl₂ (diluted from 100× stock in water), and 2 μg of sequence-grade modified trypsin (Promega). The tryptic
digestion was incubated overnight at 37 °C. The beads were washed in PBS (3 × 500 μl) and water (3 × 500 μl).

Labeled peptides were eluted from the beads by sodium dithionite–mediated cleavage of the diazo biotin azide tag. Beads were incubated with 50 μl of 50 mM sodium dithionite in PBS, rotating at 25 °C for 1 h. After centrifugation, the supernatant was collected and saved. The beads were washed twice more with 75 μl of 50 mM sodium dithionite, and all the collected supernatant fractions were combined. The beads were washed twice more with 75 μl water, and the supernatant fractions were combined with the previous fractions. To the total sample volume (350 μl), 17.5 μl of formic acid (Sigma) was added, and the samples were stored at −20 °C.

**LC/LC-MS/MS and data processing**

Mass spectrometry was performed using a Thermo LTQ Orbitrap Discovery mass spectrometer coupled to an Agilent 1200 series HPLC. Labeled peptide samples were pressure-loaded onto a 250-mm fused-silica desalting column packed with 4 cm of Aqua C18 reverse-phase resin (Phenomenex). Peptides were eluted onto a 100-mm fused-silica biphasic column packed with 10 cm of C18 resin and 4 cm of Partisphere strong cation-exchange resin (Whatman) using a five-step multidimensional LC-MS protocol (MudPIT) (53). Each of the five steps used a salt push (0, 50, 80, 100, and 100%) followed by a gradient of 5–100% buffer B in buffer A (buffer A: 95% water, 5% acetonitrile, and 0.1% formic acid; buffer B: 20% water, 80% acetonitrile, and 0.1% formic acid). The flow rate through the column was ∼0.25 μl/min with a spray voltage of 2.75 kV. One full MS1 scan (400–1800 molecular weight) was followed by eight data-dependent scans with dynamic exclusion enabled (30 s; repeat of 1).

The tandem MS data, generated from the five MudPIT runs, was analyzed by the SEQUEST algorithm (54). The precursor ion mass tolerance was set at 50 ppm, and the fragment-ion mass tolerance was set to 0 (default setting). Static modification of Cys residues (+57.0215 m/z; iodoacetamide alkylation) was assumed with no enzyme specificity. Modified residues were identified by searching against the *E. coli* reverse-concatenated nonredundant UniProt database. Data sets were independently searched for differential Cys modification by either the light (+306.1481) or heavy (+312.1682) IA-alkyne probe. Modified Sec residues were identified through searching for differential Cys modifications by the light (+354.0925) or heavy (+360.1126) IA-alkyne with an additional mass of +47.9444 (the difference in mass between selenium and sulfur).

MS2 spectra matches were assembled into protein identifications and filtered using DTASelect2.0 (55) with the -trypstat and -modstat options applied. Peptides were restricted to fully tryptic (-y 2) with a found modification (-m 0) and a ΔCN score greater than 0.06 (-d 0.06). Single peptides per locus were also allowed (-p 1) as were redundant peptides identified from multiple proteins, but the database contained only a single consensus splice variant for each protein. For quantitative experiments, peptide L/H ratios were calculated using the cimage quantification package using either the assembled DTASelect files for Cys or Sec identification (56).

**Mass spectrometry analysis**

Gel bands were cut into small pieces, washed with 0.8 ml of water for 10 min with rocking, and then washed with 0.8 ml of 50% acetonitrile and 100 mM NH4HCO3 for 30 min on a tilt table. After a final 30-min wash with 50% acetonitrile and 25 mM NH4HCO3, the gel bands were soaked in 4.5 mM DTT at 37 °C for 30 min. DTT was then removed, and 10 mM iodoacet- nitrite was added and incubated in the dark at room temperature for 30 min. Gel bands were then washed with 800 μl of 50% acetonitrile and 100 mM NH4HCO3 for 15 min on a tilt table with a final 15-min wash with 50% acetonitrile and 25 mM NH4HCO3. Supernatants were removed, and samples were dried by speed vacuum. Each band was resuspended in 100 μl of 25 mM NH4HCO3 containing 0.5 μg of digestion-grade trypsin (Promega, V5111) and incubated at 37 °C for 16 h. 7.5 μl of digest was diluted into 7.5 μl of MS loading buffer (0.2% TFA and 2% acetonitrile in water) and placed in a vial for LC-MS/MS analysis (5 μl was injected).

LC-MS/MS analysis was performed on a Thermo Scientific Q Exactive Plus equipped with a Waters nanoAcquity UPLC system utilizing a binary solvent system (buffer A: 100% water and 0.1% formic acid; buffer B: 100% acetonitrile and 0.1% formic acid). Trapping was performed at 5 μl/min 99.5% buffer A for 3 min using a Waters M-Class Symmetry® C18 180-μm × 20-mm trap column. Peptides were separated using an Acquity UPLC M-Class Peptide BEH C18 column (130 Å; 1.7 μm; 75 μm × 250 mm) at 37 °C and eluted at 300 nl/min with the following gradient: 3% buffer B at initial conditions, 5% B at 1 min, 25% B at 45 min, 50% B at 65 min, 90% B at 70 min, 90% B at 75 min, and return to initial conditions at 77 min. MS was acquired in profile mode over the 300–1,700 m/z range using one microscan, 70,000 resolution, automatic gain control target of 3E6, and a maximum injection time of 45 ms. Data-dependent MS/MS was acquired in centroid mode on the top 20 precursors per MS scan using one microscan, 17,500 resolution, automatic gain control target of 1E5, maximum injection time of 100 ms, and an isolation window of 1.7 m/z. Precursors were fragmented by higher-energy collisional dissociation activation with a collision energy of 28%. MS/MS was collected on species with an intensity threshold of 1E4, charge states of 2–6, and peptide match preferred. Dynamic exclusion was set to 20 s.

Peptide identification data were analyzed using Proteome Discoverer software for peak picking (version 2.2, Thermo Scientific) and searched in-house using the Mascot algorithm (version 2.6.1) (Matrix Science). The data were searched against a custom database containing sfGFP sequences with Sec, Cys, or any other of the 19 canonical amino acids at position 48 as well as the Swiss-Prot database with taxonomy restricted to *E. coli*. Search parameters included trypsin digestion with up to two missed cleavages, peptide mass tolerance of ±0.10 ppm, MS/MS fragment tolerance of ±0.02 Da, variable modifications of Met oxidation, Cys and Sec modified with propionamide or carbamidomethyl, Cys to dehydroalanine substitution, and Asn and Gln deamidation. Normal and decoy database searches were run with the confidence level set to 95% (*p* < 0.05).

Progenesis QI (Nonlinear Dynamics) was used to analyze the raw data and compare peptides between the CysRS and CysRS-
H235N samples. Uncharacterized peptide features common to both samples were aligned by plotting m/z versus retention time for each peak. The MS/MS data for each aligned feature were extracted, and peptides were identified using Mascot. The normalization factor by which the peptide ions vary as a whole between samples was calculated using the mean of the log abundance ratios of peptide ions that fall within robust estimated limits (Progenesis QI default parameters). Peptide ion intensities were normalized using this calculated factor. Peptides with a score >15 and within a mass tolerance of 10 ppm were used in the analysis.

**References**

1. Reich, H. J., and Hondal, R. J. (2016) Why nature chose selenium. *ACS Chem. Biol.* 11, 821–841 CrossRef Medline

2. Mousa, R., Notis Dardashti, R., and Metanis, N. (2017) Selenium and selenocysteine in protein chemistry. *Angew. Chem. Int. Ed. Engl.* 56, 15818–15827 CrossRef Medline

3. Nauser, T., Dockheer, S., Kissner, R., and Koppenol, W. H. (2006) Catalysis of electron transfer by selenocysteine. *Biochemistry* 45, 6038–6043 CrossRef Medline

4. Singh, R., and Whitesides, G. M. (1991) Selenols catalyze the interchange reactions of dithiols and disulfides in water. *Biochemistry* 30, 6038–6043 CrossRef Medline

5. Huber, R. E., and Criddle, R. S. (1967) Comparison of chemical properties of selenocysteine and selenocystine with their sulfur analogs. *Arch. Biochem. Biophys.* 122, 164–173 CrossRef Medline

6. Forchhammer, K., Leinfelder, W., Boesmiller, K., Veprek, B., and Böck, A. (1991) Selenocysteine synthase from *Escherichia coli*. Nucleotide sequence of the gene (sela) and purification of the protein. *J. Biol. Chem.* 266, 6318–6323 Medline

7. Yuan, J., Palourea, S., Salazar, J. C., Su, D., O’Donoghue, P., Hohn, M. J., Cardoso, A. M., Whitman, W. B., and Soll, D. (2006) RNA-dependent conversion of phosphoserine forms selenocysteine in eukaryotes and archaea. *Proc. Natl. Acad. Sci. U.S.A.* 103, 18923–18927 CrossRef Medline

8. Xu, X. M., Carlson, B. A., Mix, H., Zhang, Y., Saira, K., Glass, R. S., Berry, M. J., Gladyshev, V. N., and Hatfield, D. L. (2007) Biosynthesis of selenocysteine on its tRNA in eukaryotes. *PLoS Biol.* 5, e4 CrossRef Medline

9. Yoshizawa, S., and Böck, A. (2009) The many levels of control on bacterial selenoprotein synthesis. *Biochim. Biophys. Acta* 1790, 1404–1414 CrossRef Medline

10. Müller, S., Heider, J., and Böck, A. (1997) The path of unspecific incorporation of sulfur in *Escherichia coli*. *Arch. Microbiol.* 168, 421–427 CrossRef Medline

11. Young, P. A., and Kaiser, I. I. (1975) Aminoacylation of *Escherichia coli* cysteine tRNA by selenocysteine. *Arch. Biochem. Biophys.* 171, 483–489 CrossRef Medline

12. Burnell, J. N. (1977) Cysteinyl-tRNA synthetase from *Phaseolus aureus*: purification and properties. *Plant Physiol.* 60, 670–674 CrossRef Medline

13. Shrift, A., Bechard, D., and Harcup, C. (1976) Utilization of selenocysteine by a cysteinyl-tRNA synthetase from *Phaseolus aureus*. *Plant Physiol.* 58, 248–252 CrossRef Medline

14. Burnell, J. N., Karle, J. A., and Shrift, A. (1980) Reduction of dL-selenocysteine and isolation of l-selenocysteine. *J. Inorg. Biochem.* 12, 343–351 CrossRef Medline

15. Burnell, J. N. (1979) Cysteinyl-tRNA synthetase from *Astragalus* species. *Plant Physiol.* 63, 1095–1097 CrossRef Medline

16. Combs, G. F. (2001) Selenium in global food systems. *Br. J. Nutr.* 85, 517–547 CrossRef Medline

17. Gupta, M., and Gupta, S. (2016) An overview of selenium uptake, metabolism, and toxicity in plants. *Front. Plant. Sci.* 7, 2074 CrossRef Medline

18. Girling, C. A. (1984) Selenium in agriculture and the environment. *Agric. Ecosyst. Environ.* 11, 37–65 CrossRef

19. Weiss, K. F., Ayres, J. C., and Kraft, A. A. (1965) Inhibitory action of selenite on *Escherichia coli*, *Proteus vulgaris*, and *Salmonella thompson*. *J. Bacteriol.* 90, 857–862 Medline

20. Brown, T. A., and Shrift, A. (1981) Exclusion of selenium from proteins of selenium-tolerant *Astragalus* species. *Plant Physiol.* 67, 1051–1053 CrossRef Medline

21. Mukai, T., Sevostyanova, A., Suzuki, T., Fu, X., and Söll, D. (2018) A facile method for producing selenocysteine-containing proteins. *Angew. Chem. Int. Ed. Engl.* 57, 7215–7219 CrossRef Medline

22. Burnell, J. N., and Shrift, A. (1981) Correction: Cysteinyl-tRNA synthetase from *Astragalus* species. *Plant Physiol.* 68, 788 CrossRef

23. Çakır, Ö, Turgut-Kara, N., Ari, Ş., and Zhang, B. (2015) *De novo* transcrip-tome assembly and comparative analysis elucidate complicated mechanism regulating *Astragalus chrysoclorus* response to selenium stimuli. *PLoS One* 10, e0153677 CrossRef Medline

24. Newberry, K. J., Hou, Y. M., and Perona, J. J. (2002) Structural origins of amino acid selection without editing by cysteinyl-tRNA synthetase. *EMBO J.* 21, 2778–2787 CrossRef Medline

25. Zhang, C. M., Christian, T., Newberry, K. J., Perona, J. J., and Hou, Y. M. (2003) Zinc-mediated amino acid discrimination in cysteinyl-tRNA synthetase. *J. Mol. Biol.* 327, 911–917 CrossRef Medline

26. Ling, H. Q., Zhao, S., Liu, D., Wang, J., Sun, H., Zhang, C., Fan, H., Li, D., Dong, L., Tao, Y., Gao, C., Wu, H., Li, Y., Cui, Y., Guo, X., et al. (2013) Draft genome of the wheat A-genome progenitor *Triticum urartu*. *Nature* 496, 87–90 CrossRef Medline

27. Litov, R. E., and Combs, G. F., Jr. (1991) Selenium in pediatric nutrition. *Pediatrics* 87, 339–351 Medline

28. Lyons, G. H., Stangoulis, J. C., and Graham, R. D. (2005) Tolerance of wheat (*Triticum aestivum* L.) to high soil and solution selenium levels. *Plant Soil* 270, 179–188 CrossRef

29. Bohman, K., and Isaksson, L. A. (1979) Temperature-sensitive mutants in cysteinyl-tRNA ligase of *E. coli* K-12. *Mol. Gen. Genet.* 176, 53–55 CrossRef Medline

30. Lazard, M., Dauplais, M., Blanquet, S., and Plateau, P. (2015) Trans-sulfuration pathway seleno-amino acids are mediators of selenomethionine toxicity in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 290, 10741–10750 CrossRef Medline

31. Plateau, P., Saveanu, C., Lestini, R., Dauplais, M., Decourty, L., Jacquier, A., Blanquet, S., and Lazard, M. (2017) Exposure to selenomethionine causes selenocysteine misincorporation and protein aggregation in *Saccharomyes cerevisiae*. *Sci. Rep.* 7, 44761 CrossRef Medline

32. Bockhorn, J., Balar, B., He, D., Seittomer, E., Copeland, R. P., and Kinzy, T. G. (2008) Genome-wide screen of *Saccharomyces cerevisiae* null allele strains identifies genes involved in selenomethionine resistance. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17682–17687 CrossRef Medline

33. Malkowsky, M. G., Quartley, E., Friedman, A. E., Babulski, J., Kon, Y., Woffley, J., Said, M., Luft, J. R., Phizicky, E. M., DeTitta, G. T., and Grayhack, E. J. (2007) Blocking S-adenosylmethionine synthesis in yeast allows selenomethionine incorporation and multiwavelength anomalous disper-

A rare cysteinyl-tRNA synthetase for selenite resistance
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471–479

45. Tabb, D. L., McDonald, W. H., and Yates, J. R., 3rd (2002) DTASelect and contrast: tools for assembling and comparing protein identifications from shotgun proteomics. J. Proteome Res. 1, 21–26 CrossRef Medline

46. McDermott, J. R., Rosen, B. P., and Liu, Z. (2010) Jen1p: a high affinity orthophosphate transporter. J. Biol. Chem. 285, 32029–32037 CrossRef Medline

47. McDermott, J. R., Rosen, B. P., and Liu, Z. (2010) Jen1p: a high affinity selenite transporter in yeast. Mol. Biol. Cell 21, 3934–3941 CrossRef Medline

48. Lazard, M., Dauplais, M., Blanquet, S., and Plateau, P. (2010) Uptake of selenite by Saccharomyces cerevisiae involves the high and low affinity orthophosphate transporters. J. Biol. Chem. 285, 32029–32037 CrossRef Medline

49. El-Bakkoury, M., Bangham, R., Benito, R., El-Bakkoury, M., Bangham, R., Benito, R., et al. (2002) Functional profiling of the Saccharomyces cerevisiae genome. Nature 418, 387–391 CrossRef Medline

50. Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J., Thompson, J. D., and Higgins, D. G. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539 CrossRef Medline

51. Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., and Barton, G. J. (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25, 1189–1191 CrossRef Medline

52. Vargas-Rodriguez, O., Englert, M., Merkuryev, A., Mukai, T., and Söll, D. (2018) Recoding of the selenocysteine UGA codon by cysteine in the presence of a non-canonical RNA 5′′′ and elongation factor SelB. RNA Biol. 15, 471–479 CrossRef Medline

53. Franklyn, C. S., First, E. A., Perona, J. J., and Hou, Y. M. (2008) Methods for kinetic and thermodynamic analysis of aminoacyl-tRNA synthetases. Methods 44, 100–118 CrossRef Medline

54. So, B. R., An, S., Kumar, S., Das, M., Turner, D. A., Hadad, C. M., and Musier-Forsyth, K. (2011) Substrate-mediated fidelity mechanism ensures accurate decoding of proline codons. J. Biol. Chem. 286, 31810–31820 CrossRef Medline

55. Hondal, R. J., Nilsson, B. L., and Raines, R. T. (2001) Selenocysteine in native chemical ligation and expressed protein ligation. J. Am. Chem. Soc. 123, 5140–5141 CrossRef Medline

56. Ledoux, S., and Uhlenbeck, O. C. (2008) [3′-32P]-labeling tRNA with nucleotideyltransferase for assaying aminoacylation and peptide bond formation. Methods 44, 74–80 CrossRef Medline

57. Qian, Y., Martel, J. P., Pace, N. J., Ballard, T. E., Johnson, D. S., and Weerapana, E. (2013) An isotopically tagged azobenzene-based cleavable linker for quantitative proteomics. ChemBioChem 14, 1410–1414 CrossRef Medline

58. Weerapana, E., Speers, A. E., and Cravatt, B. F. (2007) Tandem orthogonal proteolysis-activity-based protein profiling (TOP-ABPP)—a general method for mapping sites of probe modification in proteomes. Nat. Protoc. 2, 1414–1425 CrossRef Medline

59. Eng, J. K., McCormack, A. L., and Yates, J. R. (1994) An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. J. Am. Soc. Mass Spectrom. 5, 976–989 CrossRef Medline

60. Tabb, D. L., McDonald, W. H., and Yates, J. R., 3rd (2002) DTASelect and contrast: tools for assembling and comparing protein identifications from shotgun proteomics. J. Proteome Res. 1, 21–26 CrossRef Medline

61. Zhang, X., Zhai, T., Cai, C., Huang, J., Wang, T., and Li, X. (2020) CrossMed: A cross-organism Metlin database. J. Proteome Res. 19, 1609–1614 CrossRef Medline

62. Fimm, A. L., and Loughlin, R. E. (1977) Isolation and characterization of cyst mutants of Escherichia coli K12. J. Gen. Microbiol. 103, 37–43 CrossRef Medline