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# Chemoproteomic Approach for the Quantitative Identification of Arsenic-Binding Proteins

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## Abstract

Arsenic is a widespread environmental contaminant, and long-term exposure to arsenic in drinking water is known to be associated with the development of many human diseases. Identification of arsenic-binding proteins is important for understanding the mechanisms underlying the toxic effects of arsenic species. Here, we developed a chemoproteomic strategy, relying on the use of a biotin-As(III) probe, stable isotope labeling by amino acids in cell culture, and liquid chromatography–tandem mass spectrometry analysis, to identify quantitatively As(III)-binding proteins. Over 400 proteins were enriched from the lysate of HEK293T cells with streptavidin beads immobilized with the biotin-As(III) probe. Competitive labeling experiments in the presence or absence of *p*-aminophenylarsenoxide (PAPAO) revealed 51 candidate As(III)-binding proteins, including several molecular chaperones and cochaperones, that is, HSPA4, HSPA4L, HSPH1, HOP1, FKBP51, and FKBP52. We also validated, by employing western blot analysis, the ability of HSPA4, a member of heat shock protein 70 (HSP70) family, in binding with PAPAO and sodium arsenite in vitro. Together, our work led to the identification of a number of new As(III)-interaction proteins, and our results suggest that As(III) may perturb proteostasis partly through binding directly with molecular chaperones.

## Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.2c00244.

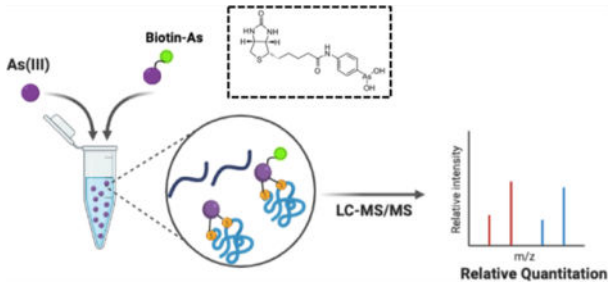
MS and MS/MS results for HSPH1 obtained from competitive labeling experiments, and structure analysis showing the potential As(III)-binding Cys residues in HSPA4 (PDF)

Excel file containing the processed LC–MS/MS data (XLSX)

Excel file containing the GO analysis results (XLSX)

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemrestox.2c00244

The authors declare no competing financial interest.




## INTRODUCTION

Arsenic is ubiquitously mobilized into the environment, and long-term human exposure to arsenic species can result in a myriad of health disorders, including cancer, diabetes mellitus, and cardiovascular diseases.[1–3] Arsenic and its derivatives have also been used as chemotherapeutic agents, where arsenic trioxide (ATO) was found to be very effective in treating acute promyelocytic leukemia patients carrying the PML-RAR$\alpha$ fusion oncoprotein.[4]

Many studies have been conducted to examine the biological consequences of arsenic exposure; however, the mechanisms through which arsenic species elicit their toxic effects are not fully understood. Identification of arsenic-binding proteins is important for understanding the mechanisms underlying the biological effects of arsenic exposure.[5] Trivalent arsenicals have a tendency to bind to sulfhydryl groups in vicinal cysteine (Cys) residues in proteins.[5,6] Moreover, the binding of arsenic with Cys residues in proteins can alter protein conformation, which may result in their aggregations and/or aberrant interactions with other biomolecules.[7,8] In particular, As(III) was shown to interact with many zinc finger proteins, especially those carrying a $Cys_3His$ or $Cys_4$-type of zinc fingers, for example, CLIP170, FANCL, PARP1, PML, RNF20, RNF40, and so on.[7–14] Many other mammalian proteins were also found to interact with arsenic, including hemoglobin,[15] tubulin,[16] actin,[17] arsR,[5] nicotinic receptor,[18] galectin-1,[19,20] and hexokinase 1.[21] Nevertheless, some of the observed toxic effects of arsenicals cannot be attributed to their interactions with these proteins.[5] Thus, there are likely other yet identified arsenic-binding proteins.

Several proteomic approaches were employed to identify arsenic-binding proteins. Along this line, a global profiling using a human proteome microarray with 16,368 proteins led to the identification of 360 arsenic-binding proteins, including hexokinase 1 (HK1), the rate-limiting enzyme of the glycolysis pathway, where the activity of its homologous enzyme HK2 can be inhibited by As(III).[21] In addition, Zhang et al.[22] employed a *p*-aminophenylarsenoxide (PAPAO)–biotin conjugate to treat MCF-7 human breast cancer cells and identified 50 arsenic-binding proteins using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. Moreover, Yan et al.[23] employed click chemistry with an azide-labeled arsenical to selectively capture As(III)-binding proteins in A549 cells for liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis, which led to the identification of 48 arsenic-binding proteins.

Here, we employed a biotin-conjugated As(III) probe,[24] together with stable isotope labeling by amino acids in cell culture (SILAC)[25] and LC–MS/MS, to label, enrich, and identify candidate As(III)-binding proteins from the entire human proteome in the absence or presence of an excess amount of As(III) (i.e., PAPAO). We were able to uncover a number of candidate arsenic-binding proteins, including several molecular chaperones. We also confirmed the binding of one of these proteins, HSPA4, with PAPAO and $NaAsO_2$.

## EXPERIMENTAL SECTION

### Cell Culture.

HEK293T human embryonic kidney epithelial cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Corning) and 1% penicillin–streptomycin solution (GE Healthcare). The cells were maintained in a humidified incubator with 5% $CO_2$ at 37 °C, and the culture medium was changed in every 2–3 days as needed.

For SILAC experiments, heavy medium was freshly prepared by adding [$^{13}C_6$, $^{15}N_2$]-L-lysine (Lys-8) and [$^{13}C_6$]-L-arginine (Arg-6) to lysine, arginine-depleted DMEM medium (Thermo Scientific) until their final concentrations reached 0.798 and 0.398 mM, respectively. The light medium was prepared in a similar way with the use of unlabeled lysine (Lys-0) and arginine (Arg-0). The SILAC media were further supplemented with 10% dialyzed FBS and 1% penicillin/streptomycin. HEK293T cells were cultured in the heavy-DMEM medium for at least six cell doublings to ensure complete heavy isotope incorporation.

### Biotin-As Probe for the Competitive Labeling of As(III)-Binding Proteins.

HEK293T cells were harvested at approximately 80% confluency. The resulting cell pellet was incubated on ice for 30 min with 1% protease inhibitor cocktail (Sigma) containing prechilled CelLytic M cell lysis reagent (Sigma-Aldrich). The mixture was centrifuged at 16,000 *g* for 30 min at 4 °C, and the supernatant, which contained cellular proteins, was loaded onto an NAP-10 column (Amersham Biosciences) to remove endogenous small molecules, and the ensuing proteins were reconstituted in a buffer containing 50 mM HEPES (pH 7.4), 75 mM NaCl, and 5% glycerol. Protein concentrations were subsequently quantified by using Quick Start Bradford Protein Assay (Bio-Rad).

The biotin-As probe was synthesized following published procedures by coupling PAPAO with the activated pentafluorophenyl ester of biotin.[18,24] In the forward SILAC experiment, heavy protein lysate (1.0 mg each) was first incubated with 80 $\mu$M PAPAO at 4 °C for 30 min. The biotin-As probe was then added to the mixture, or to the same amount of light lysate without preincubation with PAPAO, until the final concentration of the probe reached 20 $\mu$M. After incubation at room temperature with gentle shaking for 2 h, the biotin-As-conjugated proteins were enriched from the two mixtures separately by incubating with high-capacity streptavidin beads (Sigma-Aldrich) at room temperature for 2 h. The reverse SILAC experiment was conducted in a similar fashion except that the light-labeled lysate was preincubated with 80 $\mu$M PAPAO. After removal of unbound proteins by washing

sequentially with 1× PBS buffer (6 mL), 10× PBS (6 mL), and water (6 mL), the enriched heavy- and light-biotin-conjugated proteins were mixed and subjected to filter-aided sample preparation,[26] denatured in 8 M urea, reduced with dithiothreitol (20 mM), and alkylated with iodoacetamide (55 mM). The protein mixture was then subjected to buffer exchange with 50 mM ammonium bicarbonate and digested with trypsin at an enzyme/protein ratio of 1:100. Prior to LC–MS and MS/MS analyses, the tryptic peptides were desalted with C18 ZipTip (Agilent), concentrated by Speed-Vac, and reconstituted in 20 μL of 0.1% formic acid.

### LC-MS/MS Analysis and Data Processing.

LC–MS/MS experiments were conducted on a *Q* Exactive Plus quadrupole–Orbitrap mass spectrometer equipped with a Flex nanoelectrospray ion source (Thermo Fisher Scientific) and coupled with an UltiMate 3000 RSLCnano system (Dionex, Thermo Fisher). Peptide mixtures were loaded onto a home-made 4 cm capillary trapping column (150 μm i.d.) packed with the ReproSil-Pur 120 C18-AQ stationary phase material (5 μm and 120 Å in particle and pore sizes, respectively, Dr. Maisch GmbH HPLC) with buffer A (0.1% formic acid in water) at a flow rate of 3 μL/min. The peptides were eluted from the trapping column and resolved on a 20 cm long fused silica analytical column (75 μm i.d.) packed in-house with the ReproSil-Pur 120 C18-AQ stationary phase material (3 μm in particle size and 120 Å in pore size, Dr. Maisch GmbH HPLC). A 200 min linear gradient of 3–45% buffer B (0.1% formic acid in 80% $CH_3CN$) in buffer A (0.1% formic acid in water) was used, and the flow rate was 300 nL/min.

The mass spectrometer was operated in a data-dependent acquisition mode. Full-scan mass spectra were acquired using an Orbitrap mass analyzer at a resolution of 35,000 with lock mass option being enabled for the ion of $m/z$ 445.120025. Up to 25 most abundant ions detected in full-scan MS with charge state of ≥2 were sequentially isolated and fragmented at a normalized collisional energy of 28, an activation *Q* value of 0.25, and an activation time of 10 ms.

The raw LC–MS/MS data were processed using the Maxquant search engine (version 1.5.2.8) against a human fasta database (Uniprot Human Proteome Fasta: UP000005640_9606), which contained 78,120 entries. The mass tolerances for precursor and fragment ions were set at 10 and 20 ppm, respectively. The maximum number of tryptic miss-cleavages was two per peptide. For SILAC-based quantification experiments, lysine (+8 Da) and arginine (+6 Da) mass shifts introduced by heavy stable isotope labeling and cysteine carbamidomethylation were considered as fixed modifications. The reverse database search option was enabled to filter the search results with a maximum false discovery rate of 1%.

Gene enrichment and functional annotation analyses of newly identified As-binding proteins represented in Table 1 were performed using the DAVID tool (https://david.ncifcrf.gov/tools.jsp). The Gene Ontology (GO) analysis classified the list of genes and selected the annotation categories according to their associated biological processes, cellular components, molecular functions, and cancer pathways in the Kyoto Encyclopedia of Genes and Genomes pathway database.

### Western Blot.

HEK293T cells were lysed following the above-described procedures, and 1.0 mg of whole-cell protein lysate was used for each pull-down and western blot experiment. High-capacity streptavidin beads (40 μL) were washed with PBS and incubated with 1 mL of 20 μM biotin-As at room temperature for 2 h. Excess free biotin-As was removed by washing sequentially with 10× PBS, 1× PBS, and water. The resulting biotin-As-saturated streptavidin beads were then added to 1.0 mg of whole-cell protein lysates preincubated with 0, 0.5, 1.0, and 2.0 μM PAPAO or $NaAsO_2$. The mixtures were agitated at room temperature for 2 h. The resin was subsequently washed with 1× PBS (6 mL), 10× PBS (6 mL), and water (6 mL), and the bound proteins were eluted from the streptavidin beads by heating in 25 μL of 2× SDS loading buffer at 95 °C for 10 min. The resulting mixtures were centrifuged, and the supernatants were subjected to western blot analysis. HSPA4 antibody (Abclonal, #A19705, 1:1000 dilution) and antirabbit IgG (whole molecule)–peroxidase antibody produced in goat (Sigma, #A0545, 1: 5000 dilution) were used as the primary and secondary antibodies, respectively.

### Statistical Analysis.

Mean and standard deviation of protein ratios were determined from the normalized ratios for the abundances of the heavy over light-labeled proteins obtained from three forward SILAC and three reverse SILAC labeling experiments.

## RESULTS AND DISCUSSION

### Proteome-Wide Profiling of As(III)-Binding Proteins.

In this study, we aim to identify candidate As(III)-binding proteins from the lysate of HEK293T cells using LC–MS/MS. Toward this end, we devised a chemoproteomic method by using a biotin-conjugated As(III) probe, which can react with cysteine residues and install biotin tag(s) on the resulting proteins. To minimize false-positive identification of As(III)-binding proteins arising from nonspecific adsorption of proteins to streptavidin beads, we employed a SILAC-based workflow by incorporating competitive binding in the presence of an excess amount of PAPAO. In this vein, PAPAO can also bind to proximal cysteine residues in As(III)-binding proteins, thereby diminishing their labeling with the biotin-As probe; thus, those proteins exhibiting attenuated labeling efficiencies in lysates preincubated with PAPAO are considered candidate arsenic-binding proteins.

We first incubated separately the biotin-As probe (20 μM) with the same amount of light and heavy lysates of HEK293T cells, where the heavy cell lysate was preincubated with an excess amount (80 μM) of PAPAO (forward labeling, Figure 1). To minimize bias introduced from incomplete SILAC labeling, we also performed reverse labeling experiments, where an excess amount of PAPAO was preincubated with the light cell lysate instead.

It is of note that treating cells directly with biotin-As should also allow for identification of As(III)-binding proteins from living cells. In the present study, we employed relatively high concentrations of biotin-As probe (20 μM) and its competitor (PAPAO, 80 μM) to achieve facile identification of As(III)-binding proteins. Incubating cells with these concentrations

of As(III) species may promote cell death and alter the expression of cellular proteins, the latter of which may complicate data interpretation. Hence, we chose to conduct the aforementioned cell-free experiments with the use of lysates.

Biotin-labeled proteins from the reaction mixtures were enriched by using high-capacity streptavidin agarose beads, digested with trypsin, and the ensuing peptides were analyzed on a *Q* Exactive Plus quadrupole–Orbitrap mass spectrometer (Figure 1). We used peak intensity ratios of the light- and heavy-labeled peptides from the two experimental states to derive the biotin-As labeling ratio. To classify a protein as a candidate As(III)-binding protein, we employed a cut-off ratio of 0.5 for the peptide intensities derived from the labeling experiments with the presence of the competitor (i.e., PAPAO) over those without.

The LC-MS/MS results led to the quantification of a total of 409 proteins, and 51 of them are considered candidate arsenic-binding proteins using the aforementioned criterion. The list contained several previously reported arsenic-binding proteins. For example, thioredoxin (TXN), which exhibits a high binding specificity toward PAPAO with a ratio of 0.18 (Figure 2), was shown to possess a highly conserved CysGlyProCys sequence motif that can bind to As(III).[27] We also identified PMSB6, transketolase (TKT), and phosphoglycerate kinase 1 (PGK1) as candidate As(III)-binding proteins, with the SILAC protein ratios being 0.31, 0.51, and 0.27, respectively (Tables 1 and S1). These proteins were previously shown to bind to As(III).[21,22] GO analysis of the candidate As(III)-binding proteins revealed protein folding, extracellular exosome, RNA binding, and pyruvate metabolism as the most enriched biological process, cellular component, molecular function, and KEGG pathway, respectively (Table S2). Together, our results revealed a number of candidate As(III)-binding proteins, several of which were previously shown to bind to As(III).

### HSPA4 Can Bind to PAPAO and $NaAsO_2$.

Among the candidate As(III)-binding proteins, 70 kDa heat shock protein 4 (HSPA4) and 110 kDa heat shock protein 1 (HSPH1) exhibit relatively high specificities toward binding PAPAO, with the quantified ratios being 0.37 and 0.23, respectively (Figures 3a, S1). In addition, the quantification results are highly reproducible among the different replicate experiments (Table 1). The representative MS and MS/MS results for the light- and heavy-labeled forms of a tryptic peptide derived from HSPA4 are shown in Figure 3.

Heat shock proteins (HSPs) constitute the major components of molecular chaperones that facilitate the folding and maturation of newly synthesized proteins and the selection of unfolded proteins for degradation in different subcellular compartments such as cytosol, endoplasmic reticulum, and mitochondria.[28,29] HSPA4 is a member of the HSP70 family, and this family of HSPs and their cochaperones participate in many important cellular processes, including protein folding, ubiquitination regulation, and other aspects of the proteostasis network.[30]

We next examined further the interaction between HSPA4 and PAPAO and $NaAsO_2$ by employing biotin-As pull-down followed by western blot analysis. To this end, we incubated the lysate of HEK293T cells with biotin-As-immobilized streptavidin beads in the presence of increasing concentrations (0, 0.5, 1, to 2 $\mu$M) of PAPAO or $NaAsO_2$. Our western

blot results showed that the amount of HSPA4 retained on the biotin-As-conjugated beads decreases with increasing concentrations of the added free PAPAO or $NaAsO_2$. These results substantiated the specific interaction between HSPA4 and As(III) (Figure 4).

Arsenic is known to affect protein functions through the formation of adducts with sulfhydryl groups in closely spaced cysteines.[5] Thus, we examined the possibility of As(III) in accessing specific Cys residues in HSPA4. While there is currently no high-resolution structure of human HSPA4, sequence alignment showed a 66% amino acid identity between HSPA4 and HSPH1, which is also a candidate As(III)-binding protein (Figure S2a and Table 1). Thus, we analyzed the X-ray crystal structure of HSPH1 (PDB code: 6GFA) and found that Cys139, Cys166, Cys375, and Cys379 are in close proximity (Figure S2b). In particular, Cys375 and Cys379 are located on the protein surface and within an $\alpha$-helix that may allow for the conjugation with As(III). Moreover, two Cys residues (i.e., Cys12 and Cys33) are situated near the nucleotide-binding pocket of HSPA4 and are in close proximity (Figure S2). Thus, these two Cys residues may also bind with arsenic, which may elicit conformational change(s) to the protein, perturb its binding with ATP, and compromise its chaperone activity (Figure S2).

## CONCLUSIONS

Arsenic species are widely present in the environment, and ATO has been successfully employed in the treatment of acute promyelocytic leukemia. A number of studies have been conducted to examine their toxic mechanisms of action. Binding to cellular proteins constitutes a key molecular event leading to the toxic effects of arsenic exposure.[5] By employing SILAC-based metabolic labeling, together with competitive labeling with a biotin-As probe in the presence or absence of PAPAO, we were able to identify 51 candidate As(III)-binding proteins. It will be important to extend the method for assessing how binding of the biotin-As probe to cellular proteins is affected by inorganic As(III), for example, sodium arsenite.

It is worth discussing the advantages and limitations of our method. Compared to the previously reported approach with the use of proteome microarray,[21] our method obviates the need in generating recombinant proteins, which is time-consuming and costly. In addition, recombinant proteins may not behave the same way as those expressed endogenously in human cells. Relative to previously published MS-based methods,[22,23] our strategy incorporates competitive labeling using PAPAO and a SILAC-based quantitative proteomic workflow, which minimizes false-positive identifications of As(III)-binding proteins emanating from nonspecific adsorption to streptavidin beads. Owing to the inherent limitations of the data-dependent acquisition method, some low-abundance As(III)-binding proteins may escape LC–MS/MS detection. Therefore, prefractionation at the protein (e.g., SDS-PAGE separation of the bound proteins prior to tryptic digestion) or peptide (e.g., fractionation of tryptic digestion mixtures with strong-cation exchange chromatography prior to online LC–MS/MS analysis) level may enable identification of those As(III)-binding proteins that are present at low levels in cell lysates.

We confirmed the proteomic results by showing that the binding of HSPA4 to biotin-As could be competed off by PAPAO or $NaAsO_2$ (Figure 4). By analyzing the structure of HSPH1, which exhibits a very high level of sequence identity as HSPA4, we found that the Cys residues located near the nucleotide-binding pocket of the protein (i.e., Cys12 and Cys33) may constitute a binding site for As(III). This raises the possibility that As(III) binding may disrupt the ATPase activity of HSPA4 and perturb its chaperone function.

Aside from HSPA4, our quantitative proteomic analysis also led to the discovery of HSPA4L and HSPH1 as well as several cochaperones of HSP90, including HOP, FKBP51, and FKBP52, which are encoded by *STIP1*, *FKBP5* and *FKBP4* genes, respectively,[29,31] as candidate As(III)-binding proteins (Table 1). Moreover, two of the proteasomal subunits, that is, PSMB6 and PSMA6, are also potential As(III)-binding proteins (Table 1). Organisms are equipped with multiple mechanisms to maintain proteome integrity, including ribosomal quality control, molecular chaperones for promoting the folding/maturation and for minimizing the aggregation of proteins, and proteasomal degradation of aggregated proteins that are difficult to refold.[32,33] Thus, our current study, along with previously published work, suggests that arsenic exposure elicits proteotoxic stress through multiple pathways.

Together, we developed a chemoproteomic method for the quantitative identification of candidate arsenic-binding proteins, which provides insights into mechanisms underlying the toxic effects of arsenic species.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

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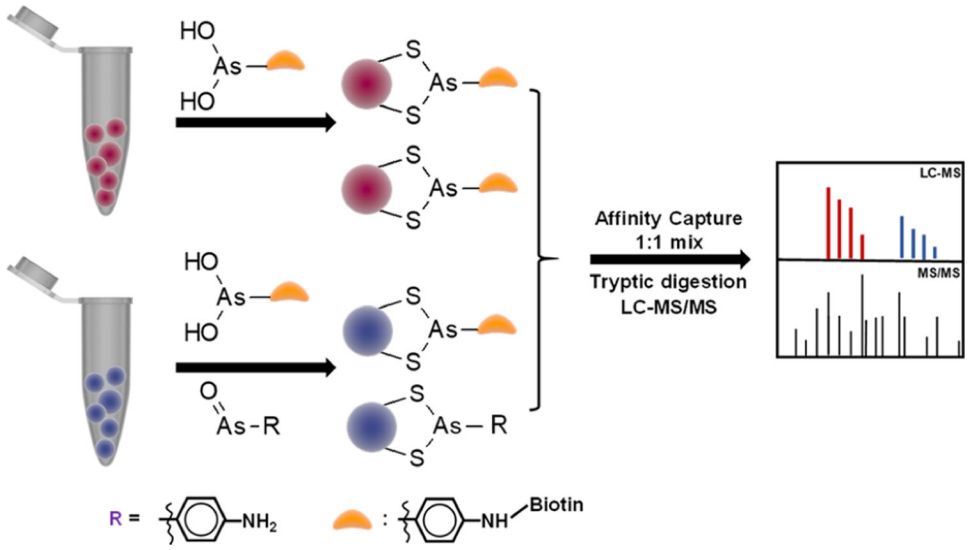


**Figure 1.**

SILAC-based competition strategy for proteome-wide identification of As(III)-binding proteins. Equal amount of biotin-As probe was incubated individually with the same amount of light and heavy lysates of HEK293T cells, where the heavy cell lysate was preincubated with an excess amount of PAPAO (in forward experiment). Biotin-labeled proteins from the reaction mixtures were enriched by using high-capacity streptavidin agarose beads, mixed at 1:1 ratio, digested with trypsin, and subjected to LC–MS/MS analysis.

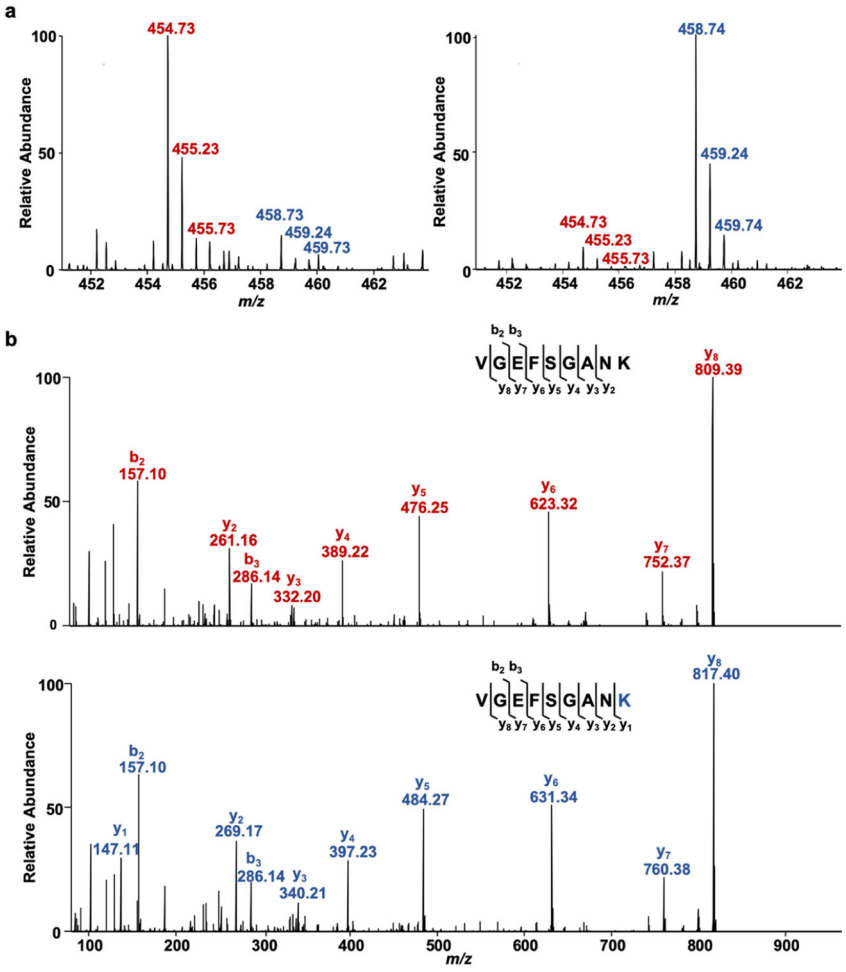


**Figure 2.**

LC-MS/MS revealed TXN as a candidate As(III)-binding protein. (a) Comparison of quantification results of a tryptic peptide from TXN, VGEFSGANK, obtained from forward and reverse competitive labeling experiments with 20 $\mu$M biotin-As in the presence or absence of 80 $\mu$M PAPAO; (b) MS/MS of light- and heavy-arginine-containing peptide, VGEFSGANK.

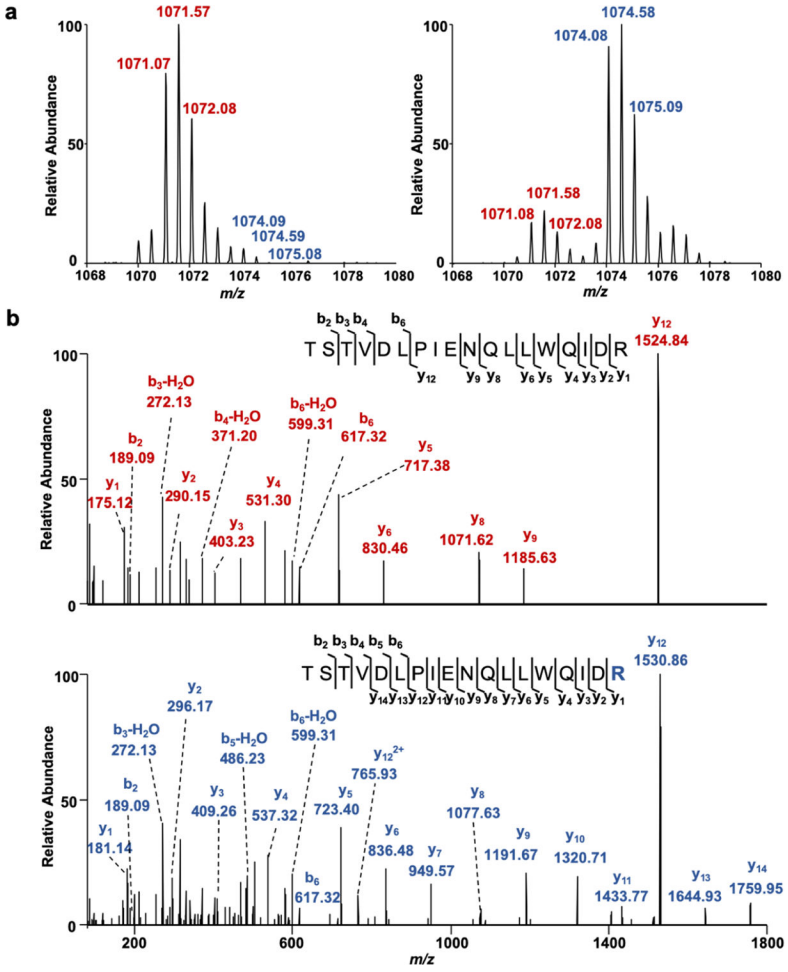


**Figure 3.**

LC–MS/MS revealed HSPA4 as a candidate As(III)-binding protein. (a) Comparison of quantification results for a tryptic peptide, TSTVDLPIENQLLWQIDR, of HSPA4 obtained from forward and reverse competitive labeling experiments with 20 $\mu$M biotin-As in the presence or absence of 80 $\mu$M PAPAO. (b) Representative MS/MS of the light- and heavy arginine-labeled peptide, TSTVDLPIENQLLWQIDR.

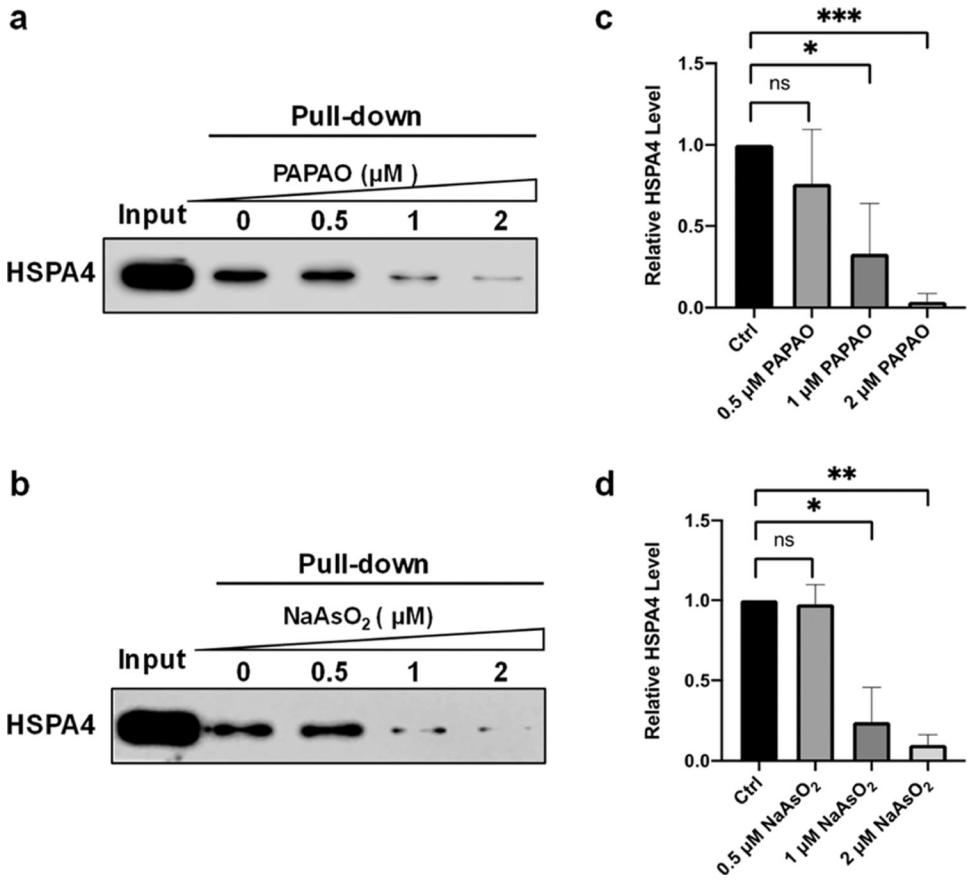


**Figure 4.**

Competitive binding of As(III) to HSPA4. Streptavidin affinity pull-down followed by western blot analysis revealed the competitive interactions of HSPA4 with As(III) and biotin-As probe. The interaction between biotin-As and HSPA4 was diminished upon a 2 h preincubation of the biotin-As-bound beads with 0.5, 1, and 2 $\mu$M PAPAO (a) or $NaAsO_2$ (b). Quantification results obtained from three separate experiments. The data represent mean ± S.D. The $p$ values were calculated using the two-tailed, paired $t$-test, *** $p < 0.001$.

**Table 1.**

Candidate PAPAO-Binding Proteins[a]

| gene ID | gene names | ave | S. D. |
|---|---|---|---|
| P10599 | TXN [20] | 0.176 | 0.041 |
| Q92598 | HSPH1 | 0.233 | 0.126 |
| Q02790 | FKBP4 | 0.254 | 0.183 |
| P34932 | HSPA4 [22] | 0.255 | 0.098 |
| P06733 | ENO1 [23] | 0.262 | 0.131 |
| P00558 | PGK1 [23] | 0.273 | 0.138 |
| Q9UBE0 | SAE1 | 0.281 | 0.139 |
| P31948 | STIP1 [23] | 0.284 | 0.129 |
| Q96JB1 | DNAH8 | 0.293 | 0.081 |
| Q9NR45 | NANS | 0.293 | 0.162 |
| P40926 | MDH2 | 0.309 | 0.250 |
| P28072 | PSMB6 [21] | 0.313 | 0.217 |
| O95757 | HSPA4L | 0.313 | N.A. |
| Q8IX12 | CCAR1 | 0.318 | 0.084 |
| Q96G61 | NUDT11 | 0.328 | N.A. |
| P49189 | ALDH9A1 | 0.328 | 0.099 |
| P13804 | ETFA | 0.340 | N.A. |
| P14174 | MIF | 0.340 | 0.198 |
| Q9Y490 | TLN1 | 0.355 | N.A. |
| Q96ER3 | SAAL1 | 0.356 | 0.148 |
| P24666 | ACP1 | 0.360 | 0.219 |
| P60900 | PSMA6 | 0.363 | 0.234 |
| Q9NXG2 | THUMPD1 | 0.368 | 0.070 |
| P49419 | ALDH7A1 | 0.370 | N.A. |
| Q6P2E9 | EDC4 | 0.376 | N.A. |
| O43175 | PHGDH | 0.389 | 0.192 |
| P62917 | RPL8 | 0.394 | 0.153 |
| P62241 | RPS8 | 0.397 | 0.157 |
| P27694 | RPA1 | 0.399 | N.A. |
| P14618 | PKM | 0.410 | 0.168 |
| Q13126 | MTAP | 0.411 | 0.115 |
| P84103 | SRSF3 | 0.419 | 0.188 |
| Q9BWD1 | ACAT2 | 0.434 | N.A. |
| P41227 | NAA10 | 0.437 | N.A. |
| P25398 | RPS12 | 0.446 | 0.305 |
| Q15185 | PTGES3 | 0.447 | 0.092 |
| Q13451 | FKBP5 [29] | 0.450 | 0.186 |
| Q9BY32 | ITPA | 0.451 | 0.235 |
| P11310 | ACADM | 0.454 | 0.225 |

| gene ID | gene names | ave | S. D. |
|---|---|---|---|
| Q9UQ80 | PA2G4 | 0.454 | 0.189 |
| Q96GK7 | FAHD2A | 0.460 | 0.249 |
| P07900 | HSP90AA1 [23] | 0.467 | 0.151 |
| P61978 | HNRNPK | 0.467 | 0.157 |
| P26599 | PTBP1 | 0.473 | 0.147 |
| Q08211 | DHX9 | 0.484 | 0.218 |
| Q04760 | GLO1 | 0.486 | 0.237 |
| Q86VP6 | CAND1 | 0.491 | N.A. |
| P13667 | PDIA4 | 0.491 | 0.238 |
| Q9NQW7 | XPNPEP1 | 0.493 | 0.252 |
| Q8NBS9 | TXNDC5 | 0.493 | 0.270 |
| Q00610 | CLTC | 0.499 | 0.185 |

[a]Known As(III)-binding proteins are underlined. “N.A.”, not available. The data representative quantification results obtained from three forward and three reverse labeling experiments (see Table S1 for results obtained from individual experiments).