

Arsenic Binding to Proteins

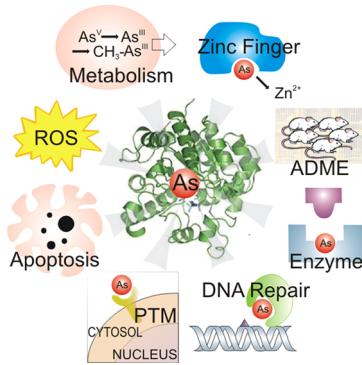
Shengwen Shen,[†] Xing-Fang Li,[†] William R. Cullen,[‡] Michael Weinfeld,[§] and X. Chris Le*,[†]

[†]Department of Laboratory Medicine and Pathology, 10-102 Clinical Sciences Building, University of Alberta, Edmonton, Alberta, Canada, T6G 2G3

[‡]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada, V6T 1Z1

[§]Department of Oncology, Cross Cancer Institute, University of Alberta, 11560 University Avenue, Edmonton, Alberta, Canada, T6G 1Z2

Supporting Information



CONTENTS

1. Introduction	7769	4.4. Biotinylated Arsenical Pull-Down	7779
2. Chemical Basis and Biological Implications of Arsenic Binding to Proteins	7770	4.5. Metalloproteomic Approaches	7780
2.1. Arsenic Biomethylation	7771	5. Characterization of Protein Binding to Arsenic Species	7780
2.2. Enzyme Inhibition	7771	5.1. Binding Sites	7780
2.2.1. Pyruvate Dehydrogenase (PDH)	7771	5.1.1. X-ray Crystallography and Nuclear Magnetic Resonance (NMR) Spectroscopy	7781
2.2.2. Thioredoxin	7771	5.1.2. Molecular Modeling and Site-Directed Mutagenesis	7781
2.2.3. DNA Repair Enzymes	7772	5.1.3. Electrospray Ionization Mass Spectrometry (ESI-MS)	7781
2.3. Resistance to Arsenic	7772	5.2. Binding Stoichiometry	7782
2.3.1. Metallothionein	7772	5.3. Binding Affinity	7782
2.3.2. Ars Operon	7773	5.4. Binding Kinetics	7783
2.4. Arsenic Binding to Hemoglobin and Accumulation of Arsenic in Rat Blood	7774	6. Summary and Perspectives	7784
2.5. Arsenic-Induced Apoptosis	7775	Associated Content	7785
2.6. Arsenic Binding to Other Proteins	7776	Supporting Information	7785
3. Determination of Arsenic Species Bound to Proteins	7776	Author Information	7785
3.1. Fractionation of Protein-Bound Arsenic	7776	Corresponding Author	7785
3.2. Release of Arsenic Species from Proteins	7777	Notes	7785
3.3. Determination of Arsenic Species Released from Proteins	7777	Biographies	7785
3.4. Direct Analysis of Arsenic Species by X-ray Spectroscopy	7777	Acknowledgments	7786
4. Identification of Arsenic-Binding Proteins	7777	References	7786
4.1. Autoradiography	7777		
4.2. Fluorescent Arsenical Probes	7778		
4.3. Affinity Chromatography	7778		
4.3.1. Protein-Specific Immunological Affinity Chromatography	7778		
4.3.2. Arsenical Affinity Chromatography	7778		

1. INTRODUCTION

Arsenic is a trace element found in the earth's crust at an average concentration of $\sim 5 \mu\text{g/g}$ (ppm). Although its relative abundance in the earth's crust is about 54th, arsenic can become concentrated in some parts of the world because of natural mineralization. Arsenic is a component of 245 minerals, associated most frequently with other metals such as copper, gold, lead, and zinc in sulfidic ores.^{1–3} When disturbed by natural processes, such as weathering, biological activity, and volcanic eruption, arsenic may be released into the environment. Anthropogenic activities, such as combustion of fossil fuels, mining, ore smelting, and well drilling, also mobilize and introduce arsenic into the environment.

Chronic exposure to arsenic from groundwater has been recognized to cause the largest environmental health disaster in the world, putting more than 100 million people at risk of cancer and other arsenic-related diseases.^{4,5} Because of its

Received: January 18, 2012

Published: June 28, 2013

prevalence in the environment, potential for human exposure, and the magnitude and severity of health problems it causes, the United States Agency for Toxic Substances and Disease Registry (ATSDR) has ranked arsenic as No. 1 on its Priority List of Hazardous Substances for many years. The recent priority list, posted in 2011 (<http://www.atsdr.cdc.gov/SPL/index.html>), shows arsenic as No. 1, ahead of lead, mercury, and polychlorinated biphenyls (PCBs).

Epidemiological studies of populations exposed to high levels of arsenic due to ingestion from water, including those from Taiwan,^{6–8} Argentina,^{9,10} Chile,^{11,12} West Bengal, India,^{13,14} Bangladesh,^{15–17} and Inner Mongolia, China,^{18,19} have repeatedly shown strong associations between the exposure to high concentrations of arsenic and the prevalence of several cancers,^{20–23} most severely bladder, lung, and skin cancers. Arsenic is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (EPA). Chronic exposure to elevated concentrations of arsenic has also been associated with the increased risk of a number of noncancerous effects.^{24–27} Although the adverse health effects arising from exposure to arsenic have been well-recognized, the mechanism(s) of action responsible for the diverse range of health effects are complicated and poorly understood.^{26–30}

It is believed that inorganic arsenite (HAsO_4^{2-}), which is a molecular analogue of phosphate (HPO_4^{2-}), can compete for phosphate anion transporters and replace phosphate in some biochemical reactions.²⁸ For example, generation of adenosine-5'-triphosphate (ATP) during oxidative phosphorylation can be inhibited by the replacement of phosphate with arsenate. Depletion of ATP by arsenate has been observed in cellular systems.²⁸ However, the replacement of phosphate in DNA by arsenic is not firmly established.^{31–35}

The toxicity of trivalent arsenicals likely occurs through the interaction of trivalent arsenic species with sulfhydryl groups in proteins. Arsenic binding to a specific protein could alter the conformation and function of the protein as well as its recruitment of and interaction with other functional proteins. Therefore, there has been much emphasis on studies of arsenic binding to proteins, for the purpose of understanding arsenic toxicity and developing arsenic-based therapeutics.

This review summarizes various aspects of arsenic binding to proteins. It discusses the chemical basis and biological implications and consequences of arsenic binding to proteins. It also describes analytical techniques and the characterization of arsenic binding, including the binding affinity, kinetics, and speciation.

2. CHEMICAL BASIS AND BIOLOGICAL IMPLICATIONS OF ARSENIC BINDING TO PROTEINS

Trivalent arsenicals have high affinity for sulfhydryl groups and can bind to reduced cysteines in peptides and proteins.^{36–60} Figure 1 illustrates schematically the binding of inorganic arsenite (iAs^{III}), monomethylarsinous acid (MMA^{III}), and dimethylarsinous acid (DMA^{III}) to cysteine residues in proteins. This chemical basis has been well-known. For example, the toxicity of many chemical warfare agents in use during and after World War I, such as arsine halides, was based on their binding to protein dithiols.^{61–63} Lewisite (β -chlorovinyldichloroarsine) was known to react with some enzymes by forming a ring involving two thiol groups in the enzymes.⁶⁴ To counteract the toxicity of these poison gases during World War I, the British developed dithioglycerol

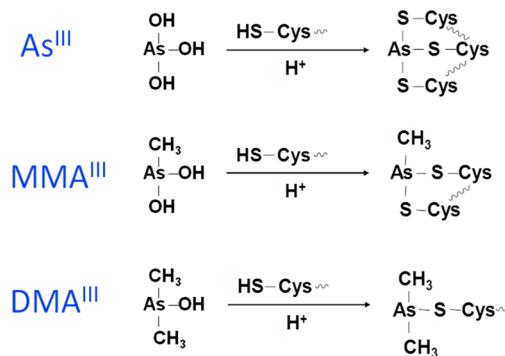


Figure 1. Binding of inorganic arsenite (iAs^{III}), monomethylarsinous acid (MMA^{III}), and dimethylarsinous acid (DMA^{III}) to cysteines in a protein.

(British anti-lewisite, BAL), which could bind arsenic to form very stable complexes, preventing arsenic from binding to proteins.

Arsenic binding to a specific protein could alter the conformation of the protein, resulting in loss of its function, and affect its recruitment of and interaction with other proteins and DNA. Figure 2 shows a model of iAs^{III} binding to the

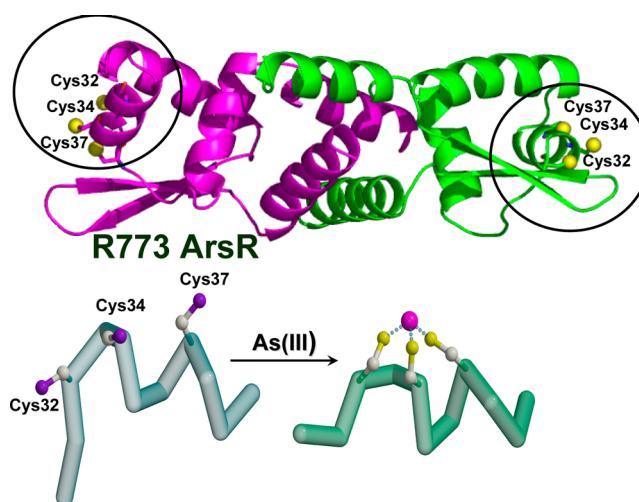


Figure 2. Binding of iAs^{III} to the *ArsR* repressor from *E. coli* plasmid R773 (P15905) results in conformational change of the repressor. iAs^{III} binds to Cys32, Cys34, and Cys37 of the *ArsR* repressor. Unwinding the helix disrupts DNA binding, resulting in dissociation of the repressor from the operator site. Dissociation of the repressor induces gene expression. (Adapted from ref 65.)

Escherichia coli repressor protein *ArsR*. In each subunit, two cysteine residues are within an α -helix, which must unravel from one end in order to bind iAs^{III} . Melting of the helix produces the conformational change that dissociates the repressor from the DNA, inducing gene expression. This *ArsR* binds iAs^{III} at three cysteine residues, Cys32, Cys34, and Cys37. Cys32 and Cys34 are within the $\alpha 1$ helix in the DNA binding site. Their thiolates are oriented such that iAs^{III} cannot bind until the helix unwinds, bringing all three thiolates into position to form the three-coordinate iAs^{III} binding site. Unwinding the helix disrupts DNA binding, resulting in dissociation of the repressor from the operator site and hence gene expression.⁶⁵

The importance of direct interaction of arsenic compounds with proteins has also been highlighted by the observation that the successful treatment of patients suffering from acute promyelocytic leukemia (APL) with As_2O_3 is likely due to the binding of arsenic to cysteine residues in zinc fingers of the aberrant promyelocytic leukemia-retinoic acid receptor (PML-RAR) fusion protein expressed by these patients.^{66,67}

2.1. Arsenic Biomethylation

Biomethylation is the major metabolic pathway for inorganic arsenic (iAs) in nearly all organisms, including humans and most animal species.⁶⁸ Arsenic biomethylation involves specific reductases^{27,30,69–73} and methyltransferases.^{50,68,74–78} The major methyltransferase responsible for arsenic biomethylation is arsenic (+3 oxidation state) methyltransferase (AS3MT, previously named Cty19). This enzyme catalyzes transfer of the methyl group of S-adenosylmethionine (SAM) to trivalent arsenic.^{50,68,74,75} The crystallographic structure of AS3MT from the thermophilic eukaryotic red alga *Cyanidioschyzon merolae* has recently been obtained.^{75,79} It reveals binding domains for arsenic and SAM (Figure 3). Although a crystal structure of human AS3MT is not available, Cys156 and Cys206, among the 14 cysteine residues, were demonstrated to be the active sites of human AS3MT.^{80–82}

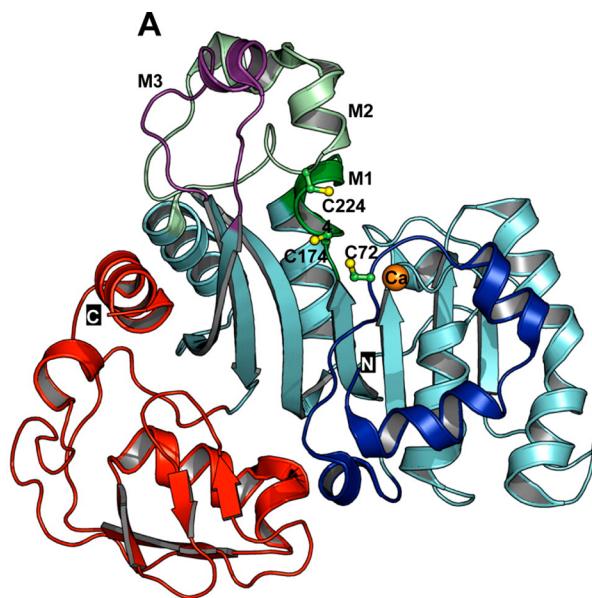


Figure 3. Ribbon representation of ligand-free AS3MT (CmArsM) from the thermophilic eukaryotic alga *C. merolae*. N and C indicate the N- and C-terminal domains and are colored blue and red, respectively. Cysteine residues are shown as balls and sticks and colored green (carbon) and yellow (sulfur). Three cysteine residues, C72, C174, and C224, are believed to be involved in arsenic binding. (Reprinted with permission from ref 75. Copyright 2012 American Chemical Society.)

2.2. Enzyme Inhibition

Arsenic may inactivate up to 200 enzymes.^{83,84} Most of the enzyme inhibition studies used iAs^{III} and some used organic arsenicals, particularly phenylarsine oxide (PAO). Enzymes that were shown to be inhibited by arsenic include glutathione reductase,^{49,85–87} glutathione S-transferase,⁸⁸ glutathione peroxidase,⁸⁸ thioredoxin reductase,^{89–91} thioredoxin peroxidase,⁹² DNA ligases,⁹³ Arg-tRNA protein transferase,^{94,95} trypanothione reductase,⁸⁶ IκB kinase β (IKK β),⁹⁶ pyruvate kinase

glectin 1,⁹⁷ protein tyrosine phosphatase,^{98–101} JNK phosphatase,¹⁰² Wip1 phosphatase,¹⁰³ and E3 ligases c-CBL and SIAH1.¹⁰⁴

2.2.1. Pyruvate Dehydrogenase (PDH). The enzyme complex pyruvate dehydrogenase (PDH) is one of the most studied enzyme systems. A search for natural dithiol compounds to mimic BAL led to the discovery of its cofactor, lipoic acid.¹⁰⁵ PDH complexes from prokaryotes and eukaryotes, which have molecular weights in excess of 10^6 Da, are formed by noncovalent interactions between multiple enzymes. Arsenite inhibits the PDH complex by binding to the lipoic acid moiety. MMA^{III} was shown to be a more potent inhibitor of the PDH complex than iAs^{III}.^{105,106} The PDH complex oxidizes pyruvate to acetyl-CoA, a precursor to intermediates of the citric acid cycle. The citric acid cycle degrades the intermediates, providing the mitochondria with reducing equivalents needed for electron transport and the subsequent production of ATP. Inhibition of the PDH complex can block the citric acid cycle and ultimately decrease the production of ATP, resulting in cell damage and death. On the basis of the fact that As_2O_3 was far more inhibitory to the intracellular PDH complex than to the purified PDH complex (actually similar phenomena were observed for other enzymes), the PDH complex (and perhaps other dithiol-containing enzymes) was proposed by Samikkannu et al.¹⁰⁷ to be inhibited by arsenite via reactive oxygen species (ROS) production rather than direct thiol binding as in the case of PAO. However, a recent study¹⁰⁵ conducted under conditions free of ROS generation suggested that all three trivalent arsenic species (iAs^{III}, MMA^{III}, and DMA^{III}) at biologically relevant concentrations inhibited the PDH complex and the 2-oxoglutarate dehydrogenase (KGDH) complex by direct binding. The reduced form of the lipoic acid moiety appeared to be essential for the binding. No pentavalent arsenicals (iAs^V, MMA^V, and DMA^V) were found to inhibit the enzymes. All enzymes post-translationally modified by the addition of the lipoic acid cofactor are mitochondrial and are important for respiration.¹⁰⁵ It is conceivable that trivalent arsenic binds to PDH or KGDH complexes and inhibits their activity, which depletes the mitochondrial NADH pool and leads to oxidative stress, producing ROS, which is otherwise suppressed or balanced. Thus, the binding of arsenic to those enzymes may be the initial point of action to inhibit the activity of the enzymes. However, subsequently generated ROS may have further effects on the enzymatic inhibition.

2.2.2. Thioredoxin. The direct binding between thioredoxin (Trx) and arsenic has been characterized using mass spectrometry.¹⁰⁸ All of the four trivalent arsenic species under study (iAs^{III}, MMA^{III}, DMA^{III}, and PAO) bound to human Trx to form complexes at room temperature while only MMA^{III}, DMA^{III}, and PAO formed complexes with *E. coli* Trx, consistent with arsenic coordination chemistry and the availability of cysteine residues in these two proteins for binding. Trx proteins from both human and *E. coli* have the highly conserved –CysGlyProCys– region, but human Trx has three more cysteine residues (Cys62, Cys69, and Cys73). Human Trx showed slightly higher binding constants than *E. coli* Trx to the same arsenic species.¹⁰⁸ Pentavalent arsenic species (iAs^V, MMA^V, and DMA^V) could be reduced by excess Trx at 37 °C to their trivalent counterparts, which in turn could bind to Trx.

The thioredoxin system consists of two oxidoreductase enzymes, thioredoxin and thioredoxin reductase.¹⁰⁹ The thioredoxin system complements the function of the

glutathione system in maintaining reductive intracellular redox potential and protecting against toxicity. Thioredoxin is a small protein (~12 kDa) containing a highly conserved sequence, –CysGlyProCys–. It was first identified in *E. coli* and subsequently found to be present in most eukaryotic and prokaryotic species.¹⁰⁹ Thioredoxin is the major ubiquitous disulfide reductase responsible for maintaining proteins in their reduced state, in which process thioredoxin itself is oxidized and subsequently regenerated at the expense of NADPH via thioredoxin reductase. Thioredoxin has been implicated in a multitude of biological activities.¹¹⁰ As an electron carrier, thioredoxin activates the catalytic cycles of biosynthetic enzymes, such as ribonucleotide reductases, methionine sulfoxide reductases, and sulfate reductases. It protects cytosolic proteins from aggregation or inactivation via formation of intra- or intermolecular disulfides. Thioredoxin can regulate the activity of transcription factors such as NF-κB, AP-1, and p53. Thioredoxin plays a central role in thiol redox control of cell functions by modulation of the transcription events of target genes. It can interact with other proteins to form functional protein complexes. For example, thioredoxin is an essential processivity factor for bacteriophage T7 DNA polymerase. Its activity has been found outside of the cell, in the cytoplasm, in the nucleus, and in the mitochondria.¹¹¹

As an important cellular dithiol redox enzyme, thioredoxin is required for the methylation of arsenic catalyzed by AS3MT.^{112,113} Trivalent arsenic compounds, especially the methylated metabolite MMA^{III}, were found to inhibit thioredoxin reductase in cultured rat hepatocytes.⁹⁰ Arsenite was also shown to induce NF-κB activation and to affect AP-1 and NF-κB DNA binding activity by upregulating the expression of the *Trx* gene.¹¹⁴ Thioredoxin peroxidase II was identified as an arsenic-binding protein in mammalian cells.⁹² Thioredoxin stimulates cell growth and has antiapoptotic properties. Modulation of thioredoxin was implicated in the apoptosis of APL cancer cells treated with arsenic trioxide (As_2O_3).⁸⁹

2.2.3. DNA Repair Enzymes. All three trivalent arsenicals (iAs^{III}, MMA^{III}, and DMA^{III}) can inhibit DNA repair.^{115–117} These trivalent arsenicals were found to inhibit the activities of several DNA repair proteins, including poly(ADP-ribose) polymerase-1 (PARP-1), formamidopyrimidine-DNA glycosylase (Fpg), and xeroderma pigmentosum group A protein (XPA), each containing a zinc finger DNA binding domain.^{118,119} The interactions between these repair proteins and arsenic compounds resulted in the release of zinc. As the first identified member of the PARP superfamily, PARP-1 is believed to mediate the major fraction of cellular poly(ADP-ribosylation). Although its role is not fully understood, there is strong evidence that PARP-1 contributes to base excision repair (BER), perhaps also to nucleotide excision repair (NER)^{120,121} and to the nonhomologous end joining pathway of DNA double-strand break (DSB) repair.¹²² PARP-1 contains two zinc fingers, where each zinc is complexed through three cysteines and one histidine residue (C3H1). Zinc fingers are essential for the recognition of damaged DNA. Recently, the direct binding between arsenic and peptides derived from both zinc fingers of human PARP-1 (apo-PARPzf) was observed using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS).^{45,123} A 72-Da *m/z* shift corresponded to the covalent binding of iAs^{III} to the PARPzf peptides after the release of three hydrogen atoms from the three cysteine residues of the C3H1 zinc finger peptides. Displacement of

Zn²⁺ from the PARPzf peptides by iAs^{III} was also demonstrated to be dependent on the iAs^{III} concentration.

XPA binds specifically to damaged DNA and plays a central role in the first step of NER. XPA contains a single C4 zinc finger domain and zinc is complexed by Cys105, Cys108, Cys126, and Cys129. Trivalent arsenicals (iAs^{III}, MMA^{III}, and DMA^{III}) were shown to release zinc from the zinc finger domain of the XPA protein (XPAzf). Methylated trivalent arsenicals were more efficient than inorganic arsenite in releasing zinc.¹¹⁵ It was further demonstrated that equimolar MMA^{III} could react with XPAzf and yield a mixture of mono- and diarsenical adducted XPAzf.¹²⁴ The unprotected thiols in the MMA^{III}-XPAzf were further oxidized to form an intramolecular disulfide. There was no complex formation between iAs^{III} and XPAzf.

2.3. Resistance to Arsenic

All cells have regulatory mechanisms for controlling the intracellular concentrations of metals and metalloids. Metallothioneins, found in both eukaryotes and prokaryotes, are small proteins (2–7 kDa) high in thiolate sulfurs that bind metal ions for storage and/or for detoxification. Phytochelatins are present in plants and some fungi.¹²⁵ They are cysteine-rich peptides that bind heavy metals and metalloids for detoxification, such as in the case of hyperaccumulation of arsenic.^{126–128} By contrast, metallochaperones constitute a different type of metal-binding protein, serving as an intracellular shuttles for metal ions. Metallochaperones sequester metals in the cytoplasm and deliver them to protein targets.¹²⁹ The targets can be metalloenzymes, where metals are utilized as enzyme cofactors, or membrane transporters, which predominantly transport ATPases. These metallochaperones are involved in metal ion homeostasis and detoxification.¹²⁹

2.3.1. Metallothionein. Metallothioneins (MT I and II) are highly inducible by metals (such as Zn²⁺, Cd²⁺, Co²⁺, Ni²⁺, Ag⁺, Hg²⁺, and Bi³⁺), alkylating agents, oxidizing agents, glucocorticoids, and inflammatory signals.^{130–138} Arsenic was also reported to induce metallothioneins.^{139–144} The mechanism by which arsenic induces metallothioneins remained unclear until recently when metal-activated transcription factor 1 (MTF1), a member of the zinc finger family, was found to be involved in *Mt1* induction.¹³⁰ iAs^{III} and PAO covalently bound the C-terminal cysteine cluster (Cys632, Cys634, Cys636, Cys638, and Cys653) of MTF1, which in turn induced the binding of MTF1 to the metal response elements (MRE) of endogenous *Mt1*.

Metallothioneins have also been found to directly bind to many metals such as Zn²⁺, Cd²⁺, Co²⁺, Ni²⁺, Fe²⁺, Cu⁺, Ag⁺, Au⁺, Pt²⁺, Bi³⁺, Hg²⁺, and Tc^{IV}O, either *in vivo* or *in vitro*.^{145–148} The direct binding between metallothioneins and arsenic was studied by several research groups.^{149–159} In a large excess of arsenic over metal-free human metallothionein 2 (apo-hMT-2), up to six iAs^{III} could bind to hMT-2 (Figure 4), on the basis of results obtained from inductively coupled plasma atomic emission spectrometry (ICP-AES), UV absorption spectrometry, MALDI-TOF-MS, and electrospray ionization (ESI) TOF-MS.^{149,152,155} Evidence from MALDI-TOF-MS¹⁴⁹ and ESI-TOF-MS^{151,152,155} also suggested that the binding of one iAs^{III} resulted in the loss of three protons from the protein. The binding stoichiometry was consistent with the fact that 20 cysteine residues are contained in mammalian metallothioneins and maximally six iAs^{III} can bind to the protein.

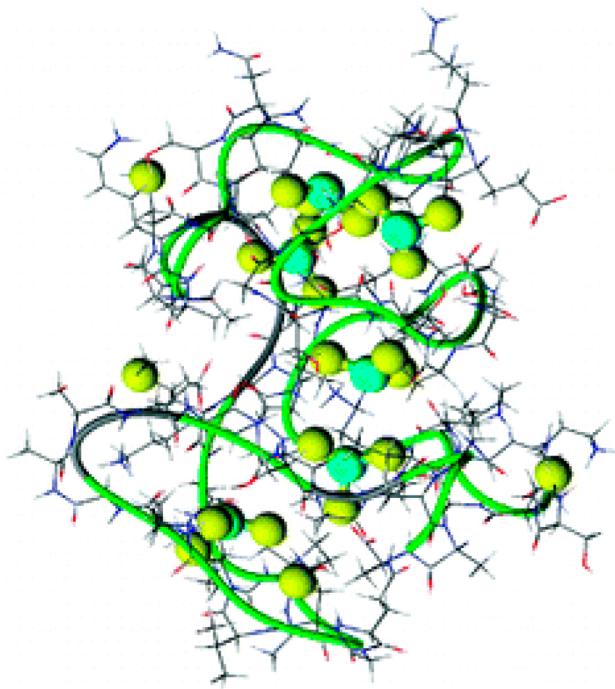


Figure 4. A model depicting six arsenic atoms bound to 18 cysteines in metallothionein. (Reprinted with permission from ref 155. Copyright 2008 American Chemical Society.)

A detailed binding study between six arsenic species (iAs^{III} , MMA^{III} , DMA^{III} , iAs^{V} , MMA^{V} , DMA^{V} , and TMAO^{V}) and

rabbit metallothionein II (rMT II) was conducted by use of HPLC–ICP-MS and ESI-quadrupole-TOF-MS.¹⁵¹ The binding stoichiometry revealed by the complementary mass spectrometry data demonstrated that each apo-rMT II molecule could bind up to six iAs^{III} , 10 MMA^{III} , and 20 DMA^{III} , consistent with the coordinate chemistry of the individual trivalent arsenicals (Figure 5). Because the reduced product, trimethylarsine, from TMAO^{V} , has no binding sites for thiol groups, no binding was observed from the reaction between MT and TMAO^{V} . As expected, no binding occurred between apo-rMT II and pentavalent arsenic species. A downward mass shift of MT was observed in the presence of pentavalent arsenicals, however, implying the oxidation of $-\text{SH}$ to disulfide in MT, associated with reduction of pentavalent arsenicals to their trivalent counterparts.

2.3.2. Ars Operon. Resistance to arsenite and antimonite in many prokaryotes is conferred by an ars operon, which often contains three genes, *arsR*, *arsB*, and *arsC*. Some operons have two extra genes, *arsD* and *arsA*, first identified in the *E. coli* plasmid R773 ars operon.^{160–162} Cells expressing the arsRDABC operon are more resistant to iAs^{V} and iAs^{III} than those expressing the arsRBC operon. The first two genes, *arsR* and *arsD*, encode regulatory proteins. The *arsA* gene encodes the transport ATPase and the *arsB* gene encodes the membrane translocase subunit of an arsenic pump. The last gene of the operon, *arsC*, encodes an arsenate reductase that is required to catalyze the reduction of iAs^{V} to iAs^{III} prior to extrusion. Figure 6 shows arsenic detoxification in *E. coli* by the plasmid R773 arsRDABC operon.

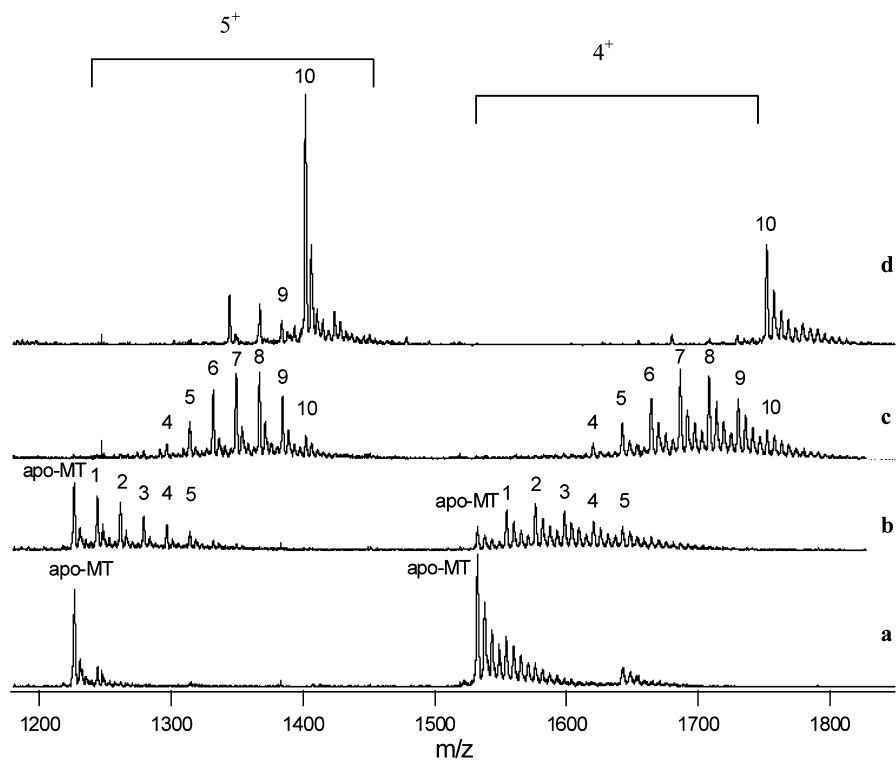


Figure 5. Mass spectra showing the number of monomethylarsonous acid (MMA^{III}) molecules bound to metallothionein (MT). The solutions contained 7 μM metallothionein (rMT-IIa) and increasing concentrations of MMA^{III} . The concentration ratios of MMA^{III} to MT in the solutions were (a) 1:5, (b) 1:1, (c) 5:1, and (d) 50:1. The ions carrying 5^+ and 4^+ charges are shown. The numbers on the peaks represent the number of MMA^{III} bound to the MT molecule. For example, peak 6 represents MT-(CH_3As)₆. A maximum of 10 MMA^{III} molecules were bound to a single MT that contained 20 cysteines. (Adapted from ref 151.)

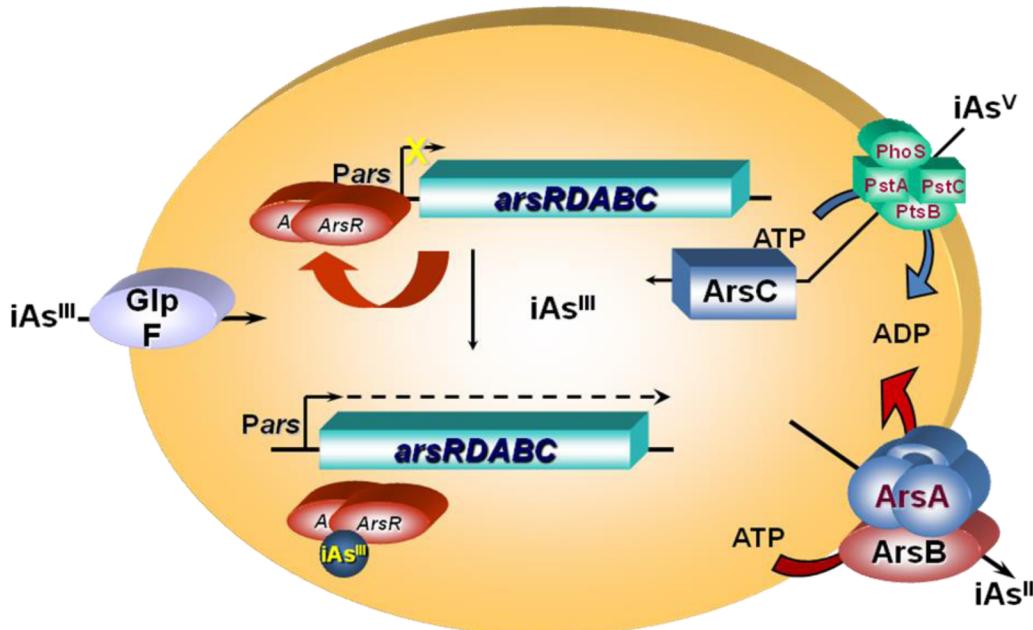


Figure 6. Schematic representation of arsenic interaction with the *arsRDABC* operon and related enzymes in *E. coli*. ArsR is an *iAs*^{III}-responsive transcriptional repressor that binds to the *ars* promoter, repressing transcription. Binding of *iAs*^{III} to ArsR results in dissociation of the repressor from the DNA and hence gene expression. *iAs*^V is taken up by the phosphate transporter, while *iAs*^{III} is taken up by the aquaglyceroporin GlpF. *iAs*^V is reduced to *iAs*^{III} by ArsC. Intracellular *iAs*^{III} is extruded from the cells by ArsB alone or by the ArsAB ATPase. (Modified from ref 163.)

ArsR is a homodimer of two 117-residue monomers with high affinity for the *arsRDABC* promoter. ArsR binds to the DNA and represses expression of the operon to a basal level in the absence of *iAs*^{III}. But in the presence of arsenic, the binding of *iAs*^{III} to ArsR changes its conformation and thus ArsR dissociates from the DNA, enabling transcription. ArsD also functions as a homodimer of two 120-residue subunits. ArsD and ArsR bind to the same operator site but the affinity of ArsD is 2 orders of magnitude lower than that of ArsR. Hence, ArsD has a weaker *ars* operon repressor activity and it binds to the *ars* operon when produced in high concentrations, such as after prolonged stimulation of transcription of the *arsD* gene following induction by the toxic *iAs*^{III}. It has been proposed that ArsD controls the upper level of expression of the operon, preventing the buildup of ArsB, which at high levels appears to be toxic to the cell. Although the affinity of ArsD for *iAs*^{III} is an order of magnitude lower than that of ArsR, ArsD binds to high levels of *iAs*^{III} and then dissociates from the DNA, allowing the operon to transcribe. Thus, ArsR and ArsD together form a regulatory circuit that controls the expression of *arsRDABC* operon at both high and low levels of *iAs*^{III}.^{162,163} Recently another role of *arsD* was identified.¹⁶⁴ It serves as an arsenic metallochaperone that delivers *iAs*^{III} to the ArsA ATPase. Interaction with ArsD increases the affinity of ArsA for *iAs*^{III}, resulting in increased efflux and resistance at environmental concentrations of arsenic.

In almost all the encoded proteins of the *arsRDABC*, arsenic–thiol interaction plays an essential role for their induction or function.¹⁶¹ ArsR, ArsD, and ArsA each contain critical cysteine residues that interact with *As*^{III}. Coordination of *As*^{III} with some critical cysteine thiolates in ArsR (Cys32, Cys34, and Cys37) and ArsD (Cys12, Cys13, Cys112, and Cys 113) results in a conformational change in the repressors that disrupts their interaction with the operator DNA. Three critical cysteine residues (Cys12, Cys13, and Cys18) were also reported to be required of ArsD to exert its metallochaperone

activity.¹⁶⁴ Cys113, Cys172, and Cys422 are involved in the metallostimulation of the ArsA ATPase. The strong arsenic–thiol interactions provide an excellent way to sense arsenic and to turn on the genes transcriptionally or the ArsA ATPase allosterically. In the ArsC protein, Cys12 is involved in forming a covalent thioester intermediate with arsenate. The thioester is further reduced by glutaredoxin and glutathione, producing a Cys12–S–*As*^{III} intermediate, from which arsenite is released upon hydrolysis.¹⁶⁵ ArsB contains a single cysteine residue that was shown not to be essential for its function. Low-affinity oxyanion binding instead of a very strong arsenic–thiol interaction seems to be appropriate for this membrane transporter protein to be effective, binding the substrate on one side of the membrane and releasing it on the other.¹⁶²

2.4. Arsenic Binding to Hemoglobin and Accumulation of Arsenic in Rat Blood

There are noticeable differences in the half-life of arsenic in blood between animal species. Most arsenic can be rapidly eliminated from blood in humans, with a half-life of about 1 h. Studies on hemodialysis patients indicate that part of the arsenic is bound to transferrin.¹⁶⁶ However, arsenic is retained in rat blood considerably longer than in blood of other species. The accumulation of arsenic in rat red blood cells was reported more than 50 years ago.¹⁶⁷ The cat can also accumulate arsenic in blood, albeit to a lesser extent than does the rat.¹⁶⁸ Binding of arsenic to hemoglobin in red blood cells was proposed as the site of accumulation in rat blood.¹⁶⁹

Hemoglobin makes up to about 97% of the red blood cell's dry content in mammals and, therefore, is a reasonable target protein of arsenic in red blood cells. Preferential accumulation of arsenic in rat blood was attributed to arsenic binding to hemoglobin.¹⁶⁹ Rat hemoglobin showed higher affinity for trivalent arsenic species than human hemoglobin (Figure 7). Both hemoglobins are tetramers, each consisting of two α -chains and two β -chains. Rat hemoglobin has three cysteines in

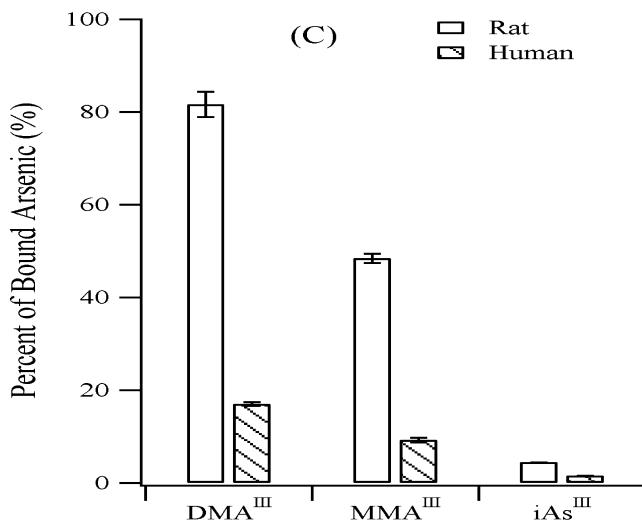


Figure 7. Comparison of rat hemoglobin (rHb) and human hemoglobin (hHb) binding to three trivalent arsenicals (iAs^{III}, MMA^{III}, and DMA^{III}). Higher percentages of the trivalent arsenicals are bound to rHb than to hHb. (Reprinted with permission from ref 169. Copyright 2004 American Chemical Society.)

each α -chain (Cys13, Cys104, and Cys111) and two cysteines in each β -chain (Cys93 and Cys125). Human hemoglobin has only one cysteine in each α -chain (Cys104) and two cysteines in each β -chain (Cys93 and Cys112). Although previous *in vivo* studies¹⁷⁰ showed that DMA^V was detected in rat hemoglobin, further investigations revealed that DMA^{III} is the actual binding form of arsenic to rat hemoglobin and Cys13 is the preferential binding site for arsenic retention.^{60,171,172} It is interesting that cat hemoglobin also contains Cys13.⁶⁰ Whether this coincidence is responsible for arsenic accumulation in the cat has not been investigated.

2.5. Arsenic-Induced Apoptosis

Arsenic (in the form of As₂O₃) has been successfully used as a chemotherapeutic agent in treating certain types of cancers, especially relapsed or *all-trans*-retinoic acid (ATRA)-resistant acute promyelocytic leukemia (APL). As₂O₃-induced apoptosis was suggested to be the mechanism of the therapeutic effect of

As₂O₃.¹⁷³ Although apoptosis can be triggered by many different pathways, direct binding of iAs^{III} to cysteine residues in zinc fingers (ZFs) located in the promyelocytic leukemia protein (PML) was demonstrated to be one of the plausible modes of action leading to APL cell apoptosis.^{66,67} The conserved cysteines in both zinc fingers contained within PML-R and PML-B (Cys60, Cys77, Cys80 in ZF1 and Cys72, Cys88, Cys91 in ZF2) were indicated to be coordinated with iAs^{III}. Apo-PML-R (PML-R without zinc) could bind one or two arsenic atoms at a clinically relevant dose of As₂O₃, determined by MALDI-TOF-MS. Arsenic binding induces PML oligomerization, which facilitates the SUMOylation (a reversible post-translational modification involving conjugation to small ubiquitin-related modifier proteins) and subsequent degradation of PML-RAR α (a fusion protein of PML and retinoic acid receptor alpha) and/or PML proteins essential for the growth of APL cells (Figure 8).

Mitochondria play a vital role in the cell, providing most of the cell's energy and participating in Ca²⁺, redox, and pH homeostasis. A major mitochondrial dysfunction is likely to cause cell death.^{174,175} With few exceptions, mitochondria represent an essential component of many apoptotic pathways.^{176,177} Proteins present in the mitochondrial intermembrane space have been shown to induce apoptosis in response to a variety of apoptotic stimuli.¹⁷⁴ Mitochondrial membrane permeabilization is critical for the release of proapoptotic factors from mitochondria. The mitochondrial permeability transition pore (MPTP), a multiprotein complex that is located at the contact sites of the inner and outer membranes, is responsible for mitochondrial permeabilization. It is comprised of the voltage-dependent anion channel (VDAC) and the adenine nucleotide translocator (ANT), the two most abundant MPTP components of the inner and outer mitochondrial membranes, respectively, in association with other proteins. ANT is a 30 kDa protein and has three cysteines (Cys57, Cys160, and Cys257). PAO and some trivalent arsenic–glutathione derivatives have been shown to cross-link the critical thiols (Cys160 and Cys257) in ANT and therefore lock ANT into an open configuration, leading to apoptosis.^{178–181} Since there are numerous redox-sensitive mitochondrial proteins that are arsenic binding targets,^{182–186} including

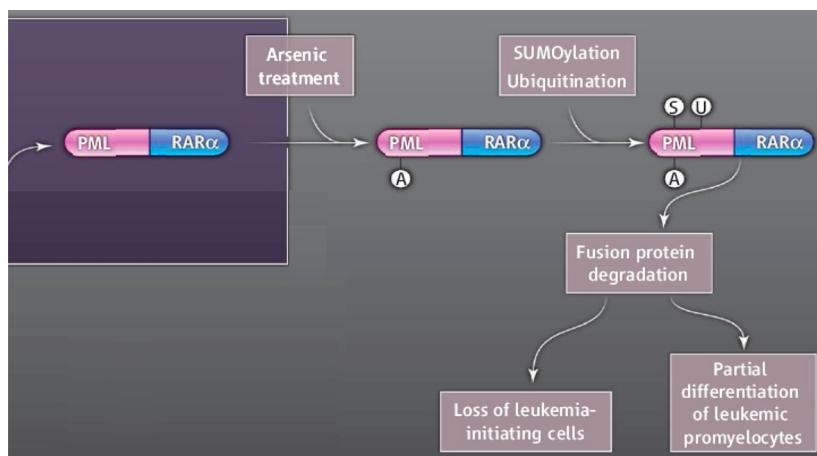


Figure 8. A proposed mechanism for the treatment of acute promyelocytic leukemia (APL) using inorganic arsenic. Binding of arsenic to the promyelocytic leukemia (PML) retinoic acid receptor alpha (RAR α) fusion protein triggers SUMOylation and ubiquitination and ultimately leads to the degradation of the oncoprotein and cell death. (Reprinted with permission from ref 66 and Kogan, S.C. 10.1126/science.1189198. Copyright 2010 American Association for the Advancement of Science.)

PDH, VDAC, tubulin, glucocorticoid receptor, thioredoxin, thioredoxin reductase, thioredoxin peroxidase, and glutathione S-transferase, their contribution to arsenic-induced apoptosis cannot be precluded. Thiol modification/redox regulation in mitochondria was acknowledged to be relevant in inducing apoptosis.^{187,188} For instance, inhibition of thioredoxin reductase has been suggested to be responsible for APL cell apoptosis induced by As_2O_3 .⁸⁹ Both the N-terminal dithiols and the C-terminal selenothiol may participate in the reaction with the arsenic compound. Arsenic may also mimic glucocorticoids to induce apoptosis by binding to mitochondrial glucocorticoid receptor.¹⁸⁹

2.6. Arsenic Binding to Other Proteins

Trivalent arsenicals also bind many other proteins, including tubulin,^{190,191} actin,¹⁹² glucocorticoid receptor,^{193,194} and estrogen receptor α .^{42,195} It is notable that iAs^{III} binds Kelch-like ECH-associated protein 1 (Keap1), an antioxidant sensing protein, at low K_d values.¹³⁴ Actin-tethered Keap1 that binds to nuclear factor erythroid 2-related factor 2 (Nrf2) constitutes a protein triad that is indispensable for induction of the phase II response, a major cellular reaction to oxidative/electrophile stress. Nrf2 is involved in inducing phase II and antioxidative proteins and regulates both the basal and inducible expressions of many cytoprotective genes through a common antioxidant response element (ARE). Reaction of oxidative/electrophile inducers, such as iAs^{III}, with Keap1 releases Nrf2 for nuclear translocation and activation of ARE.¹⁹⁶ The primary sensors for these inducers are the reactive cysteine thiols of Keap1.

Two cysteine residues are frequently found separated by two other amino acid residues (CXXC), a motif known to be present in a variety of metal-binding proteins and oxidoreductases.¹⁹⁷ There is also a positive correlation between the occurrence of cysteine in proteins and the complexity of organisms,¹⁹⁷ indicating that evolution takes advantage of increased use of cysteine residues. In humans, several hundred good binding sites for trivalent arsenicals were proposed to be present in each organ.^{42,198}

3. DETERMINATION OF ARSENIC SPECIES BOUND TO PROTEINS

Arsenic can exist in different oxidation states (commonly +V, +III, +I, 0, and -III), depending on the redox status and pH of the environment and biological activities. More than 50 arsenic species have been identified in the natural environment and biological systems.¹⁹⁹ It has been recognized that environmental behavior, physical and chemical properties, toxicity, mobility, and biotransformation differ enormously between individual arsenic species.⁷⁴ For example, Figure 9 shows cell viability as a function of the concentration of arsenic in the form of six arsenic species.²⁰⁰ The relative cytotoxicity of these six arsenic species varies by up to 5 orders of magnitude. Therefore, it is essential to identify and quantify individual arsenic species.

3.1. Fractionation of Protein-Bound Arsenic

Dialysis, ultrafiltration, gel filtration chromatography, and protein precipitation have been employed to differentiate the protein-bound arsenic from the free arsenic species. Dialysis and ultrafiltration are both membrane-based techniques, aimed at separating high molecular weight molecules from small molecules. The molecular weight cutoff of the membranes is typically 10 or 25 kDa.^{201–206} After ultrafiltration or dialysis, the protein fraction is retained for analysis. Compared to

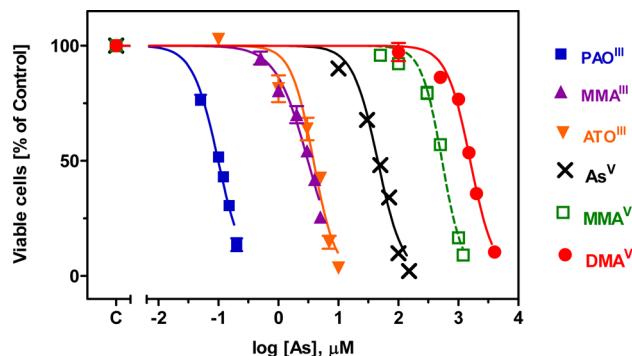


Figure 9. Toxicity of six arsenic compounds to HL-60 cells after a 48-h incubation. (Adapted from ref 200.)

ultrafiltration, which can be accelerated by centrifugation or pressurization, dialysis usually takes longer to complete and requires frequent change of the buffer outside of the dialysis sac. Moreover, dialysis usually results in sample dilution, and thus, subsequent sample concentration or redissolution after freeze-drying is sometimes needed to enable detection of trace amounts of arsenic.

Ultrafiltration is a convenient technique to evaluate the binding percentage when radiolabeled arsenic is used in an assay system, because the radioactivity retained in the ultrafiltration apparatus after washing can be taken as a measure of the extent of radiolabeled arsenic binding to proteins and expressed as a percentage of the total radionuclide in the assay system.²⁰⁷ It should be pointed out that washing residues and repeated ultrafiltration can remove free arsenic more efficiently, but this could result in a loss of arsenic-protein binding.¹⁶⁶ Conceivably, centrifugation and filtration could be applied to studying arsenic binding to nonsoluble proteins, which would not be amenable to chromatographic methods of analysis.

Gel filtration chromatography enables separation of the unbound arsenic species from the protein–arsenic complexes. Absorbance at 280 nm is typically used for protein detection, while arsenic species are detected by either atomic spectrometry or radiochemical methods. Peaks appearing at the same elution times on both protein and arsenic detectors are assigned to the arsenic–protein complexes, although orthogonal separation techniques, each relying on a different separating mechanism, such as molecular size or charge, may be needed to avoid coelution and erroneous peak assignment.^{208,209} Gel filtration chromatography can be used to fractionate arsenic-binding proteins, giving additional information on the molecular weight range of the proteins. Both denaturing and nondenaturing polyacrylamide gel electrophoresis (PAGE) methods have been used as alternatives to determine the molecular weights of arsenic-binding proteins.^{201,210,211}

Although generally regarded as a crude fractionation method, ammonium sulfate fractionation can also be used to maximize fractionation efficiency and partially purify arsenic-binding proteins prior to gel electrophoresis or gel filtration chromatography.²¹¹ The binding between radiolabeled arsenite and rabbit liver cytosolic proteins was examined, both *in vitro* and *in vivo*, by combining ammonium sulfate fractionation and gel electrophoresis. Protein precipitation is not only an effective way of separating proteins from contaminating small molecules but also serves as a preconcentration step for proteins from dilute solutions. Trichloroacetic acid (TCA) and ace-

tone^{210,212–218} are commonly used to precipitate arsenic-bound proteins. Similar to nonspecific retention of arsenic on filter membranes during ultrafiltration,^{202,207,219,220} nonspecific adsorption of arsenic to sample tubes was also acknowledged in an *in vitro* binding study with radiolabeled arsenic²¹⁹ and needs to be accounted for when determining the arsenic content in the protein pellet.²¹¹ Furthermore, the precipitating agents, such as 10% TFA, could affect the stability of arsenic–protein complexes.^{210,221}

3.2. Release of Arsenic Species from Proteins

Treatment with thiol-reactive agents or chelators, such as *N*-ethylmaleimide, 5,5'-dithio-bis(2-nitrobenzoic acid), β -mercaptoethanol, dithiothreitol, and 2,3-dimercaptopropanol, failed to quantitatively release most protein-bound arsenicals.²²⁰ Because arsenic–protein complexes are sensitive to NaOH treatment,²¹³ alkaline conditions have been explored to release arsenic from proteins. However, interconversion of iAs^{III} to iAs^V was observed, during the treatment with a strong base, tetramethylammonium hydroxide (TMAH).²²² Likewise, oxidation with concentrated H₂O₂ at room temperature was able to release all the protein-bound arsenic species as free pentavalent arsenicals.^{223–225}

Acidic conditions have also been explored to release arsenic from proteins,^{213,226} although the use of TCA or HCl alone could not quantitatively release protein-bound arsenicals.^{213,226} By including CuCl in HCl (pH 1), Styblo et al.²²⁰ have developed a method of liberating protein-bound arsenicals. CuCl was originally used by Reinke et al.²²⁷ to reduce iAs^V to iAs^{III}, which was subsequently extracted and analyzed polarographically for the determination of iAs^{III} and iAs^V in fish and shellfish. This method was later modified²⁰² by increasing the treatment temperature from 37 to 100 °C to shorten the treatment time. The reaction mechanism of how CuCl releases arsenic from proteins has not been investigated. It is possible that complexation of the cuprous ion with proteins is responsible for displacing arsenic from the cysteine residues in the proteins. Complexation of the cuprous ion with proteins is the basis for protein determination by the bicinchoninic acid (BCA) and Lowry methods.

A systematic study²²⁶ was conducted to examine release of arsenic species from liver proteins by four different acids (2 M HCl, 20% TCA, 2 M H₂SO₄, and 2 M HNO₃), alone or in combination with heat treatment. The releasing capability of the four acids was in the descending order of HNO₃ > H₂SO₄ > TCA > HCl. HNO₃ (2 M) and 1 min heating at 110 °C proved to quantitatively (>99%) release all arsenic from the liver protein fraction. Similar to alkaline treatments, acidic treatments can change the oxidation status of arsenic species. For example, MMA^{III} and DMA^{III} were oxidized to their pentavalent counterparts upon TCA treatment, and iAs^V was partially reduced to iAs^{III} upon TFA treatment.²²⁶ Although TFA at 4 °C did not influence the chemical form of DMA^V and TMAO, a hot 2 M HNO₃ treatment resulted in detectable loss of methyl groups from MMA^V and DMA^V. In the presence of proteins (rabbit liver cytosol), partial methylation of MMA^V to DMA^V was also observed after the 2 M HNO₃ treatment.²²⁶ These observations suggest the importance of recognizing the integrity and artifacts of arsenic species during sample treatment.

Lu et al.²²⁸ have recently developed a method based on proteolytic digestion to release protein-bound arsenicals, aimed at preserving both the original methylation and oxidation status

of the bound arsenic. Under mild alkaline conditions (pH 8.0), no oxidation of iAs^{III} and MMA^{III} was observed and up to 61% DMA^{III} (the least stable arsenic species) remained unoxidized.²²⁸ The recovery ranged from 93% to 106% with a digestion time as short as 30 min. Thus, the method not only quantitatively releases protein-bound arsenicals but also minimizes species conversion (at least in the As^{III} state). The recent identification of a pentavalent arsenic–peptide complex (the glutathione conjugated form of dimethylmonothioarsinic acid) in cabbage emphasizes the importance of preserving the oxidation state of protein-bound arsenicals.²²⁹ The protease digestion method can be expected to achieve this goal because of its mildness toward arsenicals. However, it remains to be seen if this method can be extended from model proteins to complex protein samples from diverse biological sources, where protease inhibitors can be present. Removal of protease inhibitors will be necessary in those cases prior to protease digestion.

3.3. Determination of Arsenic Species Released from Proteins

After release of arsenicals from proteins, the arsenicals can be determined using a range of speciation techniques, as discussed in several reviews.^{230–233} The most commonly used techniques for speciation of arsenic in solution involve a combination of high-performance liquid chromatography (HPLC) separation with inductively coupled plasma mass spectrometry (ICP-MS) and/or electrospray ionization mass spectrometry (ESI-MS) detection.^{234–238}

3.4. Direct Analysis of Arsenic Species by X-ray Spectroscopy

Direct speciation of arsenic protein fractions without releasing arsenic can be achieved by using X-ray absorption spectroscopy,²³⁹ especially EXAFS (extended X-ray absorption fine structure) and XANES (X-ray absorption near edge spectroscopy). These techniques can reveal the electronic environment around the arsenic atom and therefore provide information as to whether arsenic is trivalent or pentavalent and whether arsenic is bound to sulfur, oxygen, or carbon.^{240–247} Therefore, XAS is invaluable for probing the kinetically labile arsenic–protein complexes that are prone to dissociation or ligand exchange during sample handling.²³⁵ Complete identification of arsenic species is best achieved by complementing XAS information with structural information obtained by using other techniques.

4. IDENTIFICATION OF ARSENIC-BINDING PROTEINS

4.1. Autoradiography

Radioactive isotopes of ⁷³As and ⁷⁴As (⁷³As has a longer half-life and lower γ -emission) have been used, although organic arsenic compounds have labeling options with radioisotopic carbon.^{202,248} Whole-body autoradiography of mice after intravenous injection of ⁷³As compounds provided information on the tissue distribution of arsenic.^{170,249} The studies on arsenic distribution and binding involving the use of radiolabeled arsenic compounds in animals and humans have been thoroughly reviewed.²⁵⁰ In general, keratin-rich tissues (such as the hair, nails, and skin) with high cysteine content exhibit the highest concentrations and longest retention. At the cellular level, arsenic–protein binding occurs mainly in the cytosol, most notably in liver and kidney. Greater retention of arsenic was observed with arsenite compared to arsenate, and differences in

arsenic accumulation were noted in the liver, kidney, and skin. In the bloodstream, arsenic is distributed between erythrocytes and plasma and binds to proteins in both compartments.

Although the radiotracer can be monitored and its activity measured typically with a scintillation detector equipped with a thallium-activated sodium iodide crystal [NaI(Tl)],²⁵¹ identification of arsenic-binding proteins requires more complementary techniques for separation and characterization. Since the heme moiety strongly absorbs light at 420 nm, simultaneous detection of both ⁷⁴As and the heme moiety after size exclusion chromatography (SEC) revealed that arsenic was bound to hemoglobin in packed blood cells of rabbits intraperitoneally injected with ⁷⁴As-labeled arsenate.²⁰⁴ This binding was also observed in the red blood cells of rats, as SEC showed that the ⁷⁴As was predominantly associated with a protein that had a molecular mass of ~60 kDa and a strong absorption band at 420 nm.²⁰⁹ To study potential arsenic binding partners in serum, cation and anion exchange separation schemes with fast protein liquid chromatography were applied to serum that had been incubated *in vitro* with ⁷⁴As-labeled arsenate. The protein fractions so isolated were further separated by isoelectric focusing and identified as asialotransferrin, sialotransferrin, and albumin.²⁰⁹

Other useful radiolabeled arsenic species include phenylarsine oxide derivatives. *N*-[³H]acetyl-4-aminophenylarsine oxide and 4-[¹²⁵I]iodophenylarsine oxide were synthesized and employed as covalent affinity reagents to study arsenic–protein interaction.¹⁹⁰

4.2. Fluorescent Arsenical Probes

Wang et al.²⁵² identified two naturally occurring tetracysteine-containing sequences that bind to FlAsH [4,5-bis(1,3,2-dithioarsolan-2-yl)fluorescein] by selecting against the *Shewanella oneidensis* and *E. coli* proteomes. SlyD, a metallochaperone in the assembly of [Ni–Fe] clusters in the hydrogenase biosynthetic pathway, was identified as a high-affinity biarsenical binding protein in these two prokaryotic organisms. Fluorescent biarsenical affinity probes FlAsH and its many analogues, such as ReAsH (see structures in Table S1 of Supporting Information), were initially developed by Tsien and co-workers^{253,254} to selectively label a range of proteins in both eukaryotic and bacterial organisms fused to an engineered tetracysteine binding sequence, CysCysXXCysCys, where the central XX is preferentially ProGly. The biarsenical dyes undergo a dramatic enhancement of fluorescence upon binding to the proteins. Because endogenous proteins that would bind FlAsH are rare, fluorescent biarsenical probes permit the easy detection of the peptide-tagged proteins over the background. These fluorescent biarsenicals could also be used to detect endogenous cysteine-rich proteins.^{66,255,256}

More recently, a naphthalimide-based fluorescent monoarsenical probe, NPE (see the structure in Table S1 of Supporting Information), has been developed by Huang et al.²⁵⁷ to detect and image vicinal dithiol proteins both *in vitro* and in living cells.

4.3. Affinity Chromatography

4.3.1. Protein-Specific Immunological Affinity Chromatography. Plasma from rabbits intraperitoneally injected with ⁷⁴As-labeled arsenate was analyzed by transferrin-specific affinity chromatography by using an *N*-hydroxysuccinimide antirabbit transferrin HiTrap column.²⁰⁴ Up to 18% of total plasma ⁷⁴As was found to bind to transferrin. Similarly, a transferrin-specific affinity column was used to further confirm

the identity of transferrin in serum of patients on continuous ambulatory peritoneal dialysis.¹⁶⁶ Transferrin is capable of binding to a wide range of synergistic anions at its iron binding site, and formation of an iron–arsenate–transferrin complex is presumably responsible for arsenate–transferrin binding.²⁰⁴

4.3.2. Arsenical Affinity Chromatography. Chromatography using trivalent arsenicals as affinity ligands was first devised by Hannestad et al.²⁵⁸ to retain and separate mono- and dithiols, making use of interactions between the thiols and trivalent arsenicals. The affinity adsorbent was prepared by directly binding *p*-aminophenylarsine oxide to CNBr-activated Sepharose 6B. The arsenic affinity chromatography was later modified by several research groups to study covalent arsenical–protein interaction or to purify proteins. In their investigation into the complex effects of the trivalent arsenical PAO on insulin-dependent hexose uptake in 3T3-L1 adipocytes, Hoffman and Lane¹⁹⁰ prepared affinity resins by covalently coupling agarose, derivatized with a 10-atom spacer arm having a terminal hydroxysuccinimide active ester, to 4-aminophenylidithioarsine of β -mercaptoethanol (β -ME). The captured proteins were eluted off from this arsenic affinity column using β -mercaptoethanol in 1% Triton X-100 or SDS, further separated on SDS-PAGE, and detected by selective staining with 4-[¹²⁵I]iodophenylarsine oxide. Several proteins from 3T3-L1 adipocytes were identified to be able to bind arsenic, including the insulin-responsive glucose transporter GLUT4 and tubulin. Zhou et al.²⁵⁹ covalently linked 4-aminophenylarsine oxide to carboxymethyl (CM)-Bio-Gel A activated through an *N*-hydroxysuccinimide ester. They used this column to purify human plasma lecithin:cholesterol acyltransferase (LCAT). LCAT is a dithiol enzyme that has two reduced cysteine residues (Cys31 and Cys184) within its catalytic site. Binding of these cysteines to the arsenical affinity column enabled the capture and subsequent elution (with a buffer containing 2,3-dimercaptopropanesulfonic acid), affording a resultant 13-fold increase in specific activity of the enzyme and an overall yield of 11%. Kaleb et al.²⁶⁰ prepared affinity resins based on Sepharose 4B linked with aminohexanoyl-4-aminophenylarsine oxide to purify proteins containing vicinal dithiols from L1210 murine leukemia lymphoblasts. A one-step purification of the L-triiodothyronine recombinant rat c-erb A β 1 T3 receptor synthesized in yeast was achieved with this affinity chromatography, resulting in a 62-fold increase in specific activity and a yield of 11%. CNBr-activated Sepharose 4B was derivatized with 6-aminohexanoic acid to constitute a spacer arm prior to coupling with freshly prepared 4-aminophenylarsine oxide (high solubility in DMSO) via carbodiimide. Elution was performed with β -mercaptoethanol or dithiothreitol (DTT) in buffers. The dithiol proteins were identified by specific labeling with *N*-iodoacetyl-3-[¹²⁵I]-iodotyrosine ([¹²⁵I]IAIT). A different method of preparing PAO-Sepharose 4B was reported by Berleth et al.²⁶¹ to partially purify ubiquitin-protein ligase (E3). This method was subsequently adopted by Boerman and Napoli²⁶² to partially purify a microsomal retinol dehydrogenase (RoDH).

Arsenic affinity chromatography was also used by Winski and Carter²⁶³ to characterize arsenic binding proteins in rat red blood cells. Proteins retained on the column were displaced by sequential washes with cysteine, glutathione, and dimercaptopropanesulfonate (DMPS) after removing nonspecific binding materials with buffer and ethylene glycol. Hemoglobin seemed to be the main arsenic-binding protein in the rat red blood cell, which was captured on the arsenic affinity column and eluted

with 100 mM glutathione. Using the same arsenic affinity chromatographic strategy, Menzel et al.¹⁹² identified two arsenic-binding proteins to be tubulin and actin on the basis of their molecular weights. A highly arsenite-inducible protein, heme oxygenase 1 (HO1), failed to bind to the arsenic affinity column because it is devoid of cysteines. Chang et al.⁹² used commercial PAO-agarose resins to isolate As^{III}-modulated proteins from Chinese hamster ovary (CHO) and SA7 (arsenic-resistant CHO) cells. Following washes with 2 M NaCl in Tris buffer to remove the nonspecific binding proteins, the specific binding proteins were sequentially eluted with 20 mM β-ME and 20 mM DTT. Galectin 1 (Gal-1; in the β-ME-eluted fraction from CHO cells), glutathione S-transferase P-form (GST-P), and thioredoxin peroxidase II (TPX-II) were identified by partial amino acid sequence analysis. TPX-II was preferentially expressed in SA7 cells (which were routinely cultured and maintained in As^{III}-containing medium), but not in CHO or SA7N (a revertant of SA7 cells cultured in regular medium). In contrast, Gal-1 was specifically identified in CHO and SA7N cells, but not in SA7 cells. The specific binding of Gal-1 and TPX-II to As^{III} was verified by both coimmunoprecipitation from mammalian cells treated with As^{III} and coelution of recombinant Gal-1 and TPX-II with As^{III} from arsenic-treated transfected *E. coli*.

Mizumura et al.²⁶⁴ prepared three different arsenic-bound Sepharose resins (As^V-immobilized Sepharose, type A; As^{III}-diglutathione-immobilized Sepharose, type B; and As^{III}-immobilized Sepharose, type C) to investigate the binding of hepatic cytosolic proteins to pentavalent, glutathione-conjugated, and trivalent arsenicals. They synthesized As^V-immobilized Sepharose by conjugating NHS-activated Sepharose 4 Fast Flow gel with p-arsanilic acid. This gel was further converted to As^{III}-diglutathione-immobilized Sepharose gel after reduction with GSH, which could be stabilized in the presence of GSH. The As^{III}-diglutathione-immobilized Sepharose gel was hydrolyzed to produce As^{III}-immobilized Sepharose gel in the absence of GSH. Hepatic cytosolic proteins bound to each Sepharose were eluted with GSH, DTT, or LDS (lithium dodecyl sulfate) sample buffer. SDS-PAGE followed by EZBlue staining showed no proteins bound to pentavalent arsenic. MALDI-MS/MS was able to identify the binding of protein disulfide isomerase-related protein 5 (PDSIRPS) and peroxiredoxin 1/enhancer protein (PRX1/EP) to trivalent arsenic. Four proteins, peroxiredoxin 2, cytosolic pyrophosphatase, phosphoglycerate kinase 1, and KM-102-derived reductase-like factor (equivalent to thioredoxin reductase), were identified to specifically bind to glutathione-conjugated trivalent arsenic, possibly involving substitution of a cysteine residue of the proteins for glutathione at the As-SG site. He and Ma¹³⁰ conjugated 4-amino-phenylarsine oxide to Affigel to make PAO affinity beads, which were used to probe binding of arsenic to Kelch-like ECH-associated protein 1 (Keap1), nuclear factor erythroid 2-related factor 2 (Nrf2), and the carboxyl half of metal-activated transcription factor 1 (MTF1). The PAO affinity materials have also been used to isolate some other enzymes, such as adenine nucleotide translocase (ANT) and protein tyrosine phosphatases (PTP), from cell lysates.^{188,265} In all these identified proteins, multiple cysteine residues are present for arsenic binding.

Reversible oxidation on proteins of vicinal thiols to form disulfides is believed to be an important means of redox-based regulation of cell signaling, metabolism, and gene expression. Gitler et al.²⁶⁶ introduced a general method to identify and

enrich vicinal thiol proteins (VTPs) present in intact cells in the oxidized, disulfide state. They first blocked unoxidized thiols *in situ* by incubation of murine leukemia L1210 cells with cell permeable *N*-ethylmaleimide (NEM) and then reduced oxidized thiols with DTT. These proteins containing reduced thiols could be enriched by using PAO affinity chromatography. It was a challenge to the use of PAO affinity chromatography for the isolation of readily oxidizable VTPs because it required a delicate balance between maintaining the thiols in the reduced state necessary for PAO binding and the possible competition of DTT for the binding. Conceivably, the use of DTT could have led to an underestimation of the proteins able to bind PAO in the previous studies. By substituting tris(2-carboxyethyl)phosphine (TCEP), a nonthiol reducing agent, for DTT, Foley et al.²⁶⁷ improved the capture of VTPs from a Triton X-100-soluble rat brain extract by using PAO-affinity chromatography. After unoxidized thiols were alkylated with iodoacetamide (IAM), the oxidized thiols in proteins were reduced with TCEP prior to loading the protein sample onto a PAO affinity column. The eluted fractions were analyzed by SDS-PAGE, and protein bands were subjected to in-gel tryptic digestion and MALDI-MS/MS analysis for protein identification. The two most abundant proteins were identified as albumin and triose phosphate isomerase (TPI). The catalytic subunit of protein phosphatase 2A (PP2Ac) from rat brain was also revealed, by the improved method, to contain reversibly oxidized thiols.²⁶⁸ Similarly, protein phosphatase 2B (Calcineurin, CaN) and thioredoxin were isolated and identified from a cytosolic fraction of polymorphonuclear leukocytes (PMN) as involved in redox regulation, along with four other proteins (glutathione S-transferase P1-1, calgranulin, L-plastin, and cofilin).²⁶⁹

Our group²⁷⁰ has developed a targeted proteomic approach to identify arsenic-binding proteins in human cells involving arsenical affinity chromatography. The affinity resins were synthesized by reaction of Eupergit C beads with 4-amino-phenylarsine oxide (NAPO^{III}). After being eluted with increasing levels of DTT in buffer with or without 1% SDS, the bound proteins were subjected to in-gel or in-solution digestion prior to LC-ESI-MS/MS analysis. This approach demonstrated that arsenic-binding proteins could be identified in the presence of a large excess of nonspecific proteins. Fifty proteins in the nuclear fraction and 24 proteins in the membrane/organelle fraction were identified in the A549 human lung carcinoma cell line.

4.4. Biotinylated Arsenical Pull-Down

Zhang et al.²⁷¹ designed an in-solution pull-down approach to identify arsenic-binding proteins in human breast cancer MCF-7 cells. An arsenic–biotin conjugate was synthesized by coupling the pentafluorophenol ester of biotin with *p*-aminophenylarsine oxide. Improved synthesis of arsenic–biotin conjugates in a one-pot procedure was reported.²⁷² Arsenic-binding proteins were pulled down with streptavidin resins and subsequently released, separated by SDS-PAGE, and identified by MALDI-MS/MS. Analyses of MCF-7 cells resulted in tentative identification of 50 proteins. Two proteins, β-tubulin and pyruvate kinase M2, were confirmed further by Western blotting and molecular modeling. The same approach was applied to demonstrate that arsenic bound to PML and PML-RARα fusion protein, but not to RARα alone.⁶⁶ This direct binding was shown to be important in the anticancer activity of As₂O₃ in patients with acute promyelocytic leukemia (APL).

Donoghue et al.²⁷³ devised a membrane-impermeable trivalent arsenical to identify closely spaced thiols in mammalian cell-surface proteins undergoing redox reactions. Because GSH is constitutively secreted by mammalian cells but is not taken up by these cells, an arsenic probe for the cell-surface proteins could be developed by conjugating GSH to arsenic. Aminophenylarsine oxide was therefore coupled to the thiol of GSH to produce 4-(*N*-(*S*-glutathionylacetyl)amino)-*p*-phenylarsine oxide (*p*-GSAO), which was shown to bind tightly to dithiols but not to monothiols. To identify cell-surface proteins that contain closely spaced thiols, they attached a biotin moiety through a spacer arm to the primary amino group of the γ -glutamyl residue of GSAO to afford GSAO-B. The biotin moiety was expected to prevent cleavage of the γ -glutamyl residue by membrane-bound γ -glutamyl transpeptidases.²⁷⁴ Cells were labeled with GSAO-B in the absence or presence of 2,3-dimercaptopropanol (DMP), lysed, and incubated with streptavidin-agarose beads to collect biotin-labeled proteins. The bound proteins were eluted with 50 mM DTT in buffer, resolved on SDS-PAGE, transferred to PVDF membranes, and blotted with specific antibodies against candidate proteins. Alternatively, after labeling with GSAO-B, proteins were resolved on SDS-PAGE, transferred to PVDF membranes, and blotted with streptavidin-peroxidase to detect the GSAO-B label. There were 10 distinct proteins on the surface of bovine aortic endothelial (BAE) cells and 12 on the surface of human fibrosarcoma (HT1080) cells that incorporated GSAO-B. Protein disulfide isomerase (PDI) was labeled with GSAO-B on the cell surfaces of both cell types. The pattern of labeling of the cell lysate with GSAO-B was very different from the pattern of labeling of the cell surface proteins, reflecting the membrane-impermeable nature of GSAO-B. The interaction of GSAO-B with PDI and thioredoxin was also demonstrated in vitro with purified proteins. The adenine nucleotide translocase (ANT) was captured by GSAO-B, from isolated rat liver mitochondria, when the arsenical moiety of GSAO was either at the ortho (*o*-GSAO-B) or para (*p*-GSAO-B) position on the phenyl ring.²⁷⁵ The GSAO-B-bound proteins were then pulled down by streptavidin- or avidin-coated beads and eluted with DTT. By a similar strategy, GSAO-B has been demonstrated to be retained in the cytosol predominantly by covalent reaction with a dithiol in the 90 kDa heat shock protein (Hsp90), followed by eukaryotic translation elongation factor 2 and filamin A.²⁷⁶ Apart from GASO-B, three novel biotinylated PAO compounds with spacers of varying length were also synthesized as bifunctional reagents to examine spatially close thiols in *Torpedo* nicotinic receptors.²⁷⁷

Aside from identifying arsenic binding proteins, arsenical probes and PAO affinity chromatography have been employed to confirm the interaction of arsenic and the proteins in question.^{66,134,196,278,279} They can further be used to ascertain the critical thiols for binding in proteins after site-directed mutagenesis.^{134,161,280}

4.5. Metalloproteomic Approaches

General metalloproteomic approaches combining efficient separation of metalloproteins by liquid chromatography or electrophoresis with highly sensitive elemental detection can be applied to identification of arsenic-binding proteins in biological samples.^{201,281} Several excellent review papers have been dedicated to the analytical chemistry approaches in metalloproteomic or metallomic research.^{232,282–288} Proteomics

approaches using mass spectrometry (with MALDI and ESI) remain the state-of-the-art for high-throughput protein identification.^{289,290}

5. CHARACTERIZATION OF PROTEIN BINDING TO ARSENIC SPECIES

Although numerous cysteine-containing proteins that can bind arsenic have been identified, not many arsenic-binding proteins have been thoroughly characterized in terms of their binding sites, binding stoichiometry, and binding affinity. Part of the reason for this paucity is that purified proteins are not readily available in sufficient amounts for structural determination and binding characterization. One early study that employed a fractional occupancy assay with a ⁷⁵As tracer indicated that the average dissociation constant (K_d) for all proteins capable of binding arsenite in rabbit liver was 18.4 μM .²¹¹

5.1. Binding Sites

Cysteines and histidines are known to be most frequently involved in binding metals such as iron, zinc, and copper.^{291,292} The binding between arsenic and histidines, however, has never been unequivocally established. In an NMR spectroscopic study of complexation of arsenicals to rabbit hemoglobin,²⁹³ a direct interaction of As^{III} with histidine was discounted because there was no NMR spectral modification observed to a buffered solution of histidine when As^{III} was added. As^{III} seemed to bind to Cys93 but not to other neighboring amino acids of Hb. Similarly, histidine tags in recombinant Trx and ArsD were shown not to bind iAs^{III}.^{108,164} Rosen and co-workers^{162,294–296} frequently substituted serine residues for cysteine residues in their site-directed mutagenesis studies to define the role of the cysteine residues in arsenic binding, on the premise that the interaction of serine with arsenite is weak. So far, the only binding ligand of arsenic identified in the ArsRDABC operon proteins is cysteine thiolates. In a recent examination of amino acid constituents that can potentially bind arsenic, Kitchin and Wallace⁴² showed that amino acids other than cysteine in their synthetic peptides did not bind arsenite at all. Adequate cysteine content and vicinal cysteine residues were shown to be required for high-affinity arsenite binding with the peptides.^{42,43} Arsenite was shown to preferentially bind zinc finger peptides containing three or four cysteine residues and interact selectively with zinc finger proteins containing a C3H1 or C4 but not with a C2H2 motif in releasing zinc and reducing DNA-binding capacity of the proteins.⁴⁵ In sum, cysteine (and selenocysteine as in selenoproteins, considering its lower pK_a and therefore increased nucleophilicity²⁹⁷) is the only naturally occurring amino acid that has been demonstrated to be responsible for trivalent arsenic–protein binding. Other amino acid residues in close proximity to cysteine may serve as proton acceptors and enhance thiol reactivity toward arsenic.²⁹⁸

Li and Pickart⁹⁴ demonstrated that vicinal thiols were not involved in the binding of phenylarsine oxide to Arg-tRNA protein transferase. They proposed that structural features other than cysteines and/or serines were responsible for the binding. Arg-tRNA protein transferase is an enzyme containing 15 cysteine residues, and multiple cysteine residues are essential for its activity. Despite using site-directed mutagenesis to mutate specific cysteine residues, it was challenging to rule out the role in the arsenic binding of any remaining cysteine residues that could be brought into spatial proximity, even in the presence of urea because “unfolded proteins are conformationally mobile and structurally accommodating”.²⁹⁹ The final

possibility of “redundancy in the abilities of cysteine residues of the transferase to bind arsine oxide” could not be excluded but was dismissed as unlikely by the authors.⁹⁴ However As^{III} has lately been demonstrated to be able to distort a polypeptide structure so that As^{III} can capture thiols in the reorganized structure in order to satisfy its desire for thiolate coordination.^{299–301} Therefore, it is possible that the inhibition of Arg-tRNA protein transferase occurs due to conformational distortion of the enzyme induced by arsenic cross-linking of thiol groups.

Besides sulfhydryl groups, hydroxyl groups in small molecules were demonstrated to have some potential to form a ring structure with phenylarsine oxide in the gas phase.²⁵⁸ In a crystal structure of the arsenite–dithiothreitol complex, As^{III} bound with two sulfur thiolates and one hydroxyl oxygen.³⁰² Therefore, in proteins, hydroxyl-containing serine appears to be another possible arsenic binding ligand. In support of this possibility, several studies^{303–305} have implicated the role of the active site serine bound by arsenic in the inhibition of enzymes, including those containing no cysteine. In these studies the enzymes were all serine hydrolases and the arsenicals interacting with the active site serine were all pentavalent. The only exception is cholinesterases, of which arsenite but not arsenate is a potent inhibitor.^{306,307} A tripartite oxyanion hole in proximity to the active site is usually required to stabilize the transition state complex between pentavalent arsenic and the catalytic serine.³⁰⁵

5.1.1. X-ray Crystallography and Nuclear Magnetic Resonance (NMR) Spectroscopy. X-ray crystallography and NMR spectroscopy are the two primary techniques employed to determine three-dimensional structures of the metal binding sites of proteins. The first example of covalent modification of serine by pentavalent arsenic was observed with X-ray crystallography.³⁰⁴ In the study, the crystal structures of the acetyl esterase HerE and its complexes with an inhibitor, dimethylarsinic acid, were determined and a covalent bond via the O_γ of Ser160 seemed to be formed between the enzyme and arsenic. For this experiment, HerE was crystallized in the presence of 100 mM cacodylate (dimethylarsinic acid) without DTT. This was different from the DTT-mediated reaction of cysteine with the arsenic center of the cacodylate present in the crystallization buffer (usually containing 100 mM cacodylate and 5 mM DTT) observed with several proteins. In the latter case, the presence of DTT results in the reduction of cacodylate by DTT and subsequent reaction with exposed cysteine residues.^{308–312} X-ray crystallography was also employed by Rosen and co-workers to study the mechanism of arsenic binding in arsenate reductase ArsC.¹⁶⁵ The crystal structures of ArsC and its complexes with arsenate (substrate) and arsenite (product) were determined. Arsenate forms a thioester with the catalytic residue Cys12. Arsenite also binds the Cys12 thiol to form an unusual bicoordinate thiarsahydroxyl intermediate ArsC-Cys12-As⁺-OH, which is essential to prevent the suicide inhibition of the enzyme by its reaction product (arsenite). Rosen and co-workers have also recently obtained the X-ray crystallographic and NMR information^{313,314} about the ArsD arsenic metallochaperone in attempts to examine the structural changes in the protein in response to metalloid binding and upon interaction with ArsA. Molecular modeling was additionally performed to probe the metal binding sites of ArsD to incorporate induced changes upon arsenic binding since the apoprotein (without bound arsenic) was used in their structural determinations. Conserved cysteine residues Cys12, Cys13, and

Cys18 form a tricoordinate binding site for As^{III}, which corroborated their previous determination of As^{III} binding sites on this protein by using site-directed mutagenesis combined with arsenic analysis.³¹⁵ The coordination of As^{III} with three sulfur atoms in ArsD was further demonstrated by X-ray absorption spectroscopy.¹⁶⁴

Compared with X-ray crystallography, NMR generally offers a lower-resolution structure, but it does not require crystallization. After transmetalation to Cd^{II}, which served as an NMR spectroscopic probe, the Zn^{II} enzyme alkaline phosphatase was studied on its binding with arsenate.³¹⁶ Arsenate, an enzyme inhibitor, bridged Ser102 and zinc-binding sites. This binding geometry was confirmed by a subsequent X-ray crystallographic study of alkaline phosphatase.³¹⁷

5.1.2. Molecular Modeling and Site-Directed Mutagenesis. Molecular modeling, a term for computational protein structure prediction, can contribute to the protein determination process and deal with large proteins that are impossible with either X-ray crystallography or NMR.^{318,319} Many review articles and monographs^{318,320,321} have been dedicated to the topic of molecular modeling, and it has been used to determine the arsenic binding site in the example of ArsD mentioned above.³¹³

The site-directed mutagenesis approach is generally applicable to identify the roles of specific residues in ligand binding, specificity, and catalysis, which in turn is guided by knowledge of the 3-D structures of proteins. The importance of site-directed mutagenesis and subsequent quantitative kinetic analysis of mutant enzymes has been highlighted in structural biology in the case of adenylate kinase, where a discrepancy between the X-ray model and the NMR model existed.³²² Site-directed mutagenesis has been intensively used by Rosen and co-workers to identify arsenic binding sites of Ars operon proteins. For instance, a combination of site-directed mutagenesis and gel permeation chromatography analysis revealed that ArsA had a high-affinity metalloid binding site composed of Cys113 and Cys422.³²³ The 2.3 Å crystal structure of ArsA crystallized in the presence of Sb^{III} and MgADP showed that ArsA had two other bound metalloid atoms, one bound to Cys172 and His453 and the other bound to His148 and Ser420. Further site-directed mutagenesis analysis revealed that His418 and Ser420 had little effect on metalloid binding while the C172A single mutant and C172A/H453A double mutant exhibited significantly decreased affinity for Sb^{III}, suggesting that C172 is the third residue that participates in high-affinity binding.²⁹⁶ A site-directed mutagenic approach was also employed in search for a third arsenic ligand in ArsR, Cys37, which forms a tricoordinate complex with As^{III} together with highly conserved Cys32 and Cys34.¹⁶¹ The As-S coordination environment in ArsR was confirmed by X-ray absorption spectroscopy of ArsR stoichiometrically treated with arsenite. Cys95, Cys96, and Cys102, the only three cysteine residues in an ArsR family protein in *Acidithiobacillus ferrooxidans*, were also identified to form a tricoordinate As^{III} binding site.²⁹⁵

5.1.3. Electrospray Ionization Mass Spectrometry (ESI-MS). The development of soft ionization methods (MALDI and ESI) for mass spectrometry has facilitated the study of metal ion binding to macromolecules, including proteins. With ESI-MS, noncovalent protein–metal interactions in solution can be maintained during desolvation and transfer to the gas phase. Therefore, solution-phase complex formation can be detected and monitored by mass spectrometry in simple “mix-and-measure” experiments, which makes ESI-MS a more

natural choice than MALDI-MS.³²⁴ Binding stoichiometries can be determined directly from the measured mass. In favorable cases binding affinities and binding kinetics may be obtained from the measured intensity ratios of the free and bound protein ions.³²⁴ Structural information such as binding sites may also be obtained using tandem MS (MS/MS) or top-down strategies. In combination with chemical modifications, the capability of binding site determination by ESI-MS can be extended.³²⁵ As further evidence that trivalent arsenicals have a strong preference to bind to cysteine residues in proteins, ESI-MS studies revealed that trivalent arsenicals failed to bind purified hemoglobin and thioredoxin after reactive cysteine sulphydryl groups were blocked with *N*-ethylmaleimide (NEM) or monobromobimane.^{108,169}

ESI-MS has enabled identification of a preferential binding cysteine in rat hemoglobin (rHb).⁶⁰ In vitro binding of trivalent arsenicals to hemoglobin generated the corresponding arsenic–hemoglobin complexes with all possible stoichiometries depending on the relative concentrations of arsenicals and hemoglobin.¹⁶⁹ In vivo, however, a DMA^{III}–rHb complex was exclusively observed on the α -chain with a stoichiometry of 1:1 in rats exposed to iAs^V, MMA^V, or DMA^V. A mass shift of 104 Da was indicative of the binding of one DMA^{III} [$(CH_3)_2AsOH$, FW 122] to the α -unit with the loss of one H₂O molecule. No complexes of iAs^{III} or MMA^{III} with rHb were detected from any of the treated rats. It has been known that in the rat iAs^{III} and MMA^{III} can be excreted into the bile in the form of As(GS)₃ and MeAs(GS)₂, respectively, while DMA^{III} is released into the bloodstream and efficiently taken up by red blood cells.^{326,327} The identity of DMA^{III} bound to rHb was confirmed by HPLC analysis of arsenic species released from the protein: only DMA^{III} and its oxidation product DMA^V were observed.⁶⁰ Collision-induced dissociation (CID) tandem mass spectrometry¹⁷² revealed two characteristic fragment ions associated with a DMA^{III}-tagged cysteine residue that were equally observable from DMA^{III} complexes with cysteine and glutathione. From measuring the accurate masses of the DMA^{III}-tagged internal ions (di-, tri-, and tetrapeptides) and comparing with the theoretical masses for peptides encompassing the three cysteine residues (Cys13, Cys104, and Cys111) in the rHb α -unit, Cys13 was determined to be the only binding site of DMA^{III}. The observation of exclusively DMA^{III}-tagged fragment ions at Cys13 was not due to differential production by CID fragmentation, because the internal ions from all three cysteine residues could be detected from rHb α –(DMA^{III})₃ that was formed by incubating rHb with a 100-fold molar excess of DMA^{III}. The unbiased fragmentation was further confirmed by CID MS/MS analysis of DMA^{III} complexes with three synthetic nonapeptides embedding each of the cysteine residues in the α -chain.¹⁷² The relative reactivity of cysteine residues in rHb α -chain was determined to be in the order of Cys13 \gg Cys111 > Cys104. The preferential binding of Cys13 to DMA^{III} is probably because Cys13 in rat Hb is located in an open hydrophobic pocket that provides easy access and a favorable binding microenvironment for moderately hydrophobic DMA^{III}.⁶⁰ In the β -chain, Cys125 is more reactive than Cys93, and glutathione preferentially binds Cys125.

5.2. Binding Stoichiometry

In order to obtain information on metal-binding sites from X-ray spectroscopy, it is necessary to have access to well-defined structures of stoichiometric preparations of metal–protein complexes. For X-ray crystallography, sufficient amounts of the

ligand have to be present to ensure the formation of a fully occupied stoichiometric complex obtained either by cocrystallization or by soaking the ligand into a fully grown crystal.³²⁸ Mass spectrometry offers a rapid and precise determination of metal–protein binding stoichiometry, providing integer numbers of bound metal ions per protein molecule, even when not all protein molecules are fully metal-loaded. Titration-based methods, isothermal titration calorimetry included, may yield noninteger numbers of metal ions (the average metal content) per protein molecule under similar circumstances.³²⁹ The binding stoichiometries of As^{III} were shown by using either ESI-MS or MALDI-MS to be consistent with the number of available cysteines in proteins such as metallothionein (shown in Figures 4 and 5) and hemoglobin examined at saturating arsenic concentrations.^{149,152–155,169}

5.3. Binding Affinity

The binding affinity (association constant, K_a ; dissociation constant, K_d) of arsenic–protein/peptide binding can be determined by measuring changes in either the properties of the protein/peptide or in the partition of arsenic. Commonly adopted procedures to determine K_d include absorbance shift of protein/peptide or arsenical,^{254,330,331} intrinsic tryptophan fluorescence quenching,^{164,299,332} the reduction of thiol content due to arsenic binding,²⁷³ and the changes in the protein electrophoretic mobility measured by capillary electrophoresis.⁹⁷ Alternatively, partition of arsenic in free and protein/peptide-bound forms after reaching binding equilibrium can be quantified to determine the binding affinity after separating the two forms by using size exclusion liquid chromatography,^{108,169} dialysis,^{248,333–335} or filtration.^{43,198} Use of radioactive or fluorescently labeled arsenic compounds offers a fast and convenient determination of arsenic–protein binding affinity.^{254,336} As a technique that directly measures the heat released or absorbed during a biomolecular binding event, isothermal titration calorimetry (ITC) can simultaneously determine several binding parameters (binding stoichiometry, binding constant, binding enthalpy, and entropy) in one experiment and has been used to study arsenic–protein binding.³³⁷ Information readily obtained from ITC, such as formation of multiple titration species fitting a sequential-binding model, was proven to be useful for data modeling, distinguishing this technique from other titration methods. For example, a stability constant ($K_a = 1/K_d$) of 2×10^6 was determined with ITC for the 1:3 arsenite complex with GSH,³³⁸ for which unusually high values of 2×10^{33} and 1×10^{32} were reported by Rey et al.³³⁹ on the basis of their near-UV absorption and potentiometric data, respectively. The binding affinities between arsenic and several thiol-containing biomolecules were also determined with ESI-MS by Schmidt and co-workers,^{212,340–342} but their values were inconsistent. One issue with quantifying both the unbound and bound species using ESI-MS is that these species may have different ESI-MS responses and their intensities may not be calibrated against a single species. Thus, ESI-MS should be used to determine relative binding affinities rather than absolute values.^{341,343,344} K_d values obtained by MS should be used with caution in the absence of validation from other solution-phase techniques.³⁴³

The arsenic–protein binding affinity can also be determined indirectly in inhibition studies by measuring the inhibitory effect of arsenic on ligand binding or enzyme activity. When measured this way, the dissociation constant of binding of the

inhibitor is termed the inhibition constant, K_i (equivalent to K_d). The inhibition of enzyme activity by arsenic compounds was shown to be either competitive or noncompetitive (mixed),^{345–356} demonstrating the ability of arsenic to bind to free enzymes.³⁵⁷ A majority of the binding constants for arsenic–protein binding were obtained indirectly. IC_{50} , the half-maximal inhibitory concentration, which can often be related to K_i by the Cheng–Prusoff equation [$K_i = IC_{50}/(1 + [S]/K_m)$],³⁵⁸ is not a direct indicator of affinity and may vary with experimental conditions such as substrate concentrations. Therefore, IC_{50} values reported in the literature are not included in this review. When arsenic acts as the substrate for an enzyme, K_m , the Michaelis constant, can be determined using Michaelis–Menten kinetics. K_m is the concentration of substrate at which enzyme activity is half-maximal. Its values include not only the affinity of the substrate for the enzyme but also the rate at which the enzyme-bound substrate is converted to the product in the catalytic reaction. Thus, K_m can be interpreted as a crude measurement of the affinity of the substrate for the enzyme.³⁵⁹ A large K_m indicates weak affinity. K_m values reported in the literature for arsenic-containing compounds are included in Table S3 of the Supporting Information. A schematic comparison with statistical analysis is shown in Figure 10 on the K_d (or K_i) and K_m values for

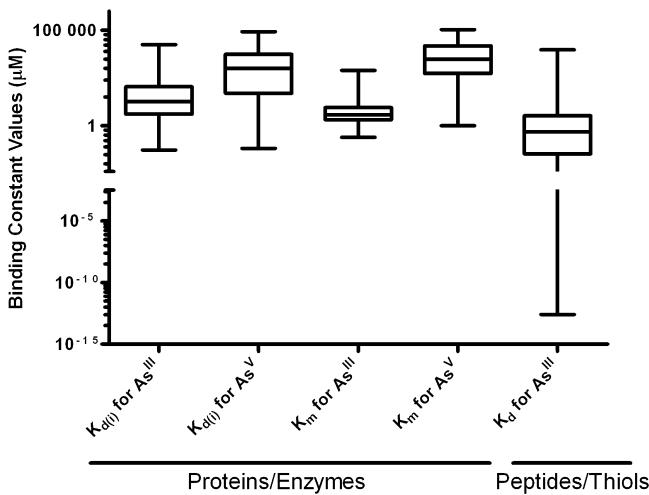


Figure 10. A comparison of binding affinity of trivalent and pentavalent arsenicals to proteins.

trivalent and pentavalent arsenicals separately. K_d values of the binding between trivalent arsenicals and small peptides/thiols are included as well (see Supporting Information, Table S4). Strict comparison of the listed binding constants cannot always be made since different arsenic compounds, methods, and assay conditions have been used. For example, in the absence of tetrahedral oxyanions, such as sulfate, oxidative inactivation of ArsC (arsenate reductase) encoded by *Staphylococcus aureus*, a highly oxygen sensitive redox enzyme, was observed during the course of the enzymatic assay, leading to significantly lower V_{max} and K_m values.^{355,360}

Several interesting inferences can be made from the enzymatic studies showing K_m or K_i values: (A) Reflective of product inhibition in enzymatic reactions, arsenite is a potent inhibitor of arsenate reductase, for which arsenate is the substrate. Similarly, MMA^{III} is an inhibitor of iAs^{III} methylation by arsenic methyltransferase from rabbit liver, suggesting a possibility that MMA^{III} is the enzymatic reaction product of

arsenic methyltransferase from iAs^{III}.^{361,362} (B) Arsenic reductases (arsenate reductase and GSTO1-1) generally have higher K_m values than arsenic methyltransferases (including AS3MT), indicating that the reduction step is rate-limiting for the overall process of arsenic metabolism. (C) Pentavalent arsenicals are substrates (showing K_m) or inhibitors (showing K_i) of many enzymes, as shown in Table S3 (Supporting Information), probably as a result of their mimicking a tetrahedral phosphorus atom in substrate or product analogues. These enzymes include phosphorylases,^{363–368} phosphatases,^{335,348,369–375} phosphoenolpyruvate mutase,³⁷⁶ glycerol-3-phosphate dehydrogenase,³⁷⁷ adenylate kinase,³⁷⁸ enolase,³⁷⁹ ethanolamine-phosphate cytidylytransferase,³⁸⁰ triosephosphate isomerase,^{381–383} ornithine carbamoyltransferase,^{384,385} acetyl esterase,³⁰⁴ chymotrypsin,^{305,386} subtilisin,^{305,386} and angiotensin-converting enzyme.³⁸⁷ Ubiquitin, which has esterase, carbonic anhydrase, and phosphatase activities, was also inhibited by arsenate.³⁸⁸ The majority of the enzymes mentioned above fall into the category of serine hydrolases. Serine hydrolases form covalent intermediates with their substrate through the hydroxyl group of the active site serine, and the catalytic action can be nearly irreversibly inhibited by organophosphorus compounds, accounting for the notoriously toxic effects of organophosphorus compounds.³⁸⁹ The anomaly manifested by cholinesterases (acetylcholinesterase and butyrylcholinesterase) for which arsenite but not arsenate is an inhibitor, may be related to their unique structures compared to other serine hydrolases. They contain a deep and narrow active site groove with two separate ligand binding sites: an acylation site or A-site at the base of the groove and a peripheral anionic site or P-site at the groove mouth, with a strong electrostatic dipole aligned with the groove leading to its active site.^{390,391} Ligand binding to the P-site can result in steric blockade of the A-site and inhibit substrate hydrolysis.³⁹² The anionic nature of the P-site³⁹³ allows entry of cationic but not anionic (e.g., arsenate) ligands to the active site groove. Inhibition of acetylcholinesterase by arsenite was shown to be pH-dependent,³⁹⁴ possibly reflecting the change with pH of the dissociated state of arsenious acid (neutral). Arsenobetaine (AsB) and arsenocholine (AsC) were shown to inhibit butyrylcholinesterase.³⁹⁵ (D) Arsenate is a potent inhibitor of alkaline or acid phosphatase ($K_i < 1$ mM) while arsenite inhibits the same enzyme with $K_i \geq 17$ mM, indicating that reasonably low K_i (or K_d) values can differentiate specific from nonspecific binding between arsenic and proteins. However, it is difficult to establish a universal benchmark K_i (or K_d) below which a specific binding is defined because of different methods and assay conditions used across the studies. (E) With regard to possible arsenic–DNA interactions, the dissociation constant (K_d) of arsenite–DNA binding was determined spectroscopically to be around 8–90 μ M^{396,397} and can be even lower in the case of arsenic binding to DNA aptamers.³⁹⁸ However, no specific binding of either arsenate or arsenite to DNA was observed by using vacuum filtration binding assays,¹⁹⁸ and the nature of arsenic–DNA binding is noncovalent.³⁹⁹

5.4. Binding Kinetics

A few studies have reported rate constants (association rate constant, k_{on} ; dissociation rate constant, k_{off}) for the binding between arsenicals and proteins/peptides (Table S5 in Supporting Information). Various kinetic methods can be employed for the determination of rate constants. Radioactive arsenic offers an extremely sensitive yet discontinuous assay,⁴⁴

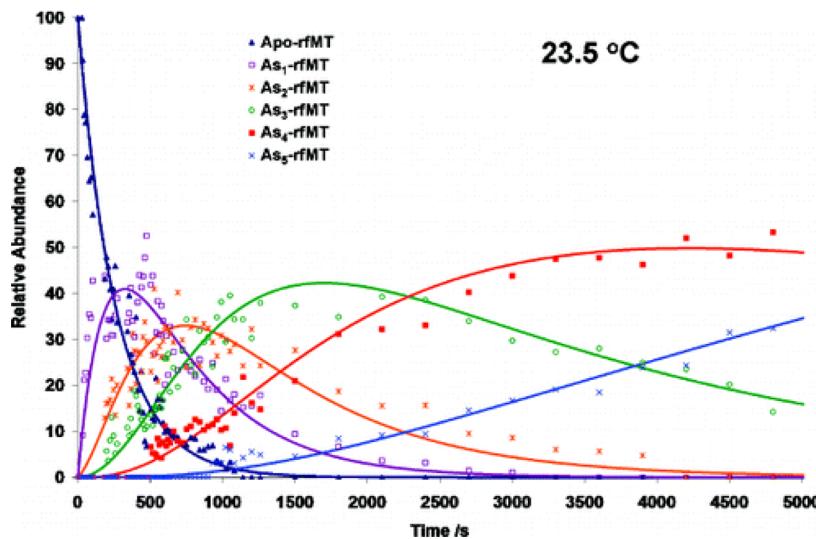


Figure 11. Kinetic data showing the relative abundance of the various arsenic–metallothionein species detected using electrospray mass spectrometry. The solution was analyzed following reaction of 9 μM apo-rfMT with 108 μM arsenite at 23.5 $^{\circ}\text{C}$. The experimental data have a relative standard error of 7%. (Reprinted with permission from ref 153.)

requiring off-line separation of bound from free arsenic, which needs large sample size, excessive analysis time, and time profile reconstruction. Assays based on UV–vis absorption or fluorescence spectroscopy are most convenient since the reaction can be monitored continuously and thus remain the major methods for studying the reaction kinetics between arsenic and proteins/peptides. The application of these assays requires optical changes accompanying the reaction. These changes can result from ligand to metal charge-transfer,^{330,400,401} use of chromophoric substrates,^{87,254,301,331,336,394} or intrinsic tryptophan quenching.²⁹⁹ Obviating the need for chromophoric probes, time-resolved ESI-MS has been shown to be a powerful tool for kinetic studies in online experiments.^{402,403} Capable of simultaneous monitoring of multiple intermediate species, ESI-MS affords accurate rate parameters of biochemical reactions and provides insights into reaction mechanism.⁴⁰³ Stillman and co-workers have acquired k_{on} values and depicted mechanisms for the binding between arsenite and metallothioneins using time-resolved ESI-MS.^{152–155} Figure 11 shows an example of reaction kinetics between iAs^{III} and metallothionein. Current restriction(s) with this technique lie(s) in the incompatibility of the electrospray process with nonvolatile components, such as high concentrations of salts, that may be required in some biochemical reactions. Under such circumstances online microdialysis for rapid desalting of the reaction mixture immediately prior to ESI-MS analysis is necessary and can be achieved with an automated microfluidic system, without significantly compromising the time resolution.⁴⁰⁴

The kinetics of the binding between arsenic and protein thiols have also been investigated at the single molecule level. By constructing transmembrane protein pores formed by mutated α -hemolysin in a planar bilayer, Shin et al.^{405,406} studied the interaction of 4-sulfophenylarsonous acid with a strategically positioned cysteine in the protein. The k_{on} , k_{off} , and K_d values were derived from recorded fluctuations in the ionic current flowing through a single transmembrane protein pore after the arsenical was added to the trans side of the bilayer.

Generally speaking, arsenic binds both proteins and peptides with k_{off} values of similar orders of magnitude (10^{-3} – 10^{-6} s^{-1}).

Arsenic binds to proteins with much lower k_{on} values than to small peptides, possibly reflecting that an additional energy barrier has to be overcome in restructuring proteins for arsenic binding. This energetic expense to disrupt preorganized conformations has been evidenced in the case of arsenite binding to human metallothionein α -domain with a slower than expected k_{on} for the first arsenite binding event when k_{off} was assumed to be constant and insignificant.¹⁵⁵

6. SUMMARY AND PERSPECTIVES

The biological effects of arsenic are diverse and the mechanisms of arsenic toxicity and carcinogenicity are complicated. Arsenic–sulfur interactions are the chemical basis of most of these effects. Trivalent arsenicals bind to thiols that are contained in numerous intracellular and cell-surface proteins, and this arsenic–protein binding often triggers cellular responses. For example, arsenic binding to PML or some mitochondrial proteins is a key step leading to cell differentiation or apoptosis induced by As₂O₃. A low baseline cellular glutathione content and a high expression level of the primary membrane transporter aquaglyceroporin 9 that mediates uptake of As₂O₃ into cells predetermine the selectivity of APL toward As₂O₃.⁴⁰⁷ Generation of cellular ROS is often implicated in the inhibition of various enzymes by arsenic; however, it cannot supplant the role of direct protein binding by trivalent arsenicals. ROS generation itself may result from thiol chelation or complexation in dithiol-containing enzymes, such as pyruvate dehydrogenase (PDH), by trivalent arsenic metabolites.

Direct protein binding by pentavalent arsenicals is mainly manifested in phosphate utilizing enzymes that normally alkylate, acylate, or phosphorylate the phosphate,⁴⁰⁸ with conceivable biological implications. Serine hydrolases are often the proteins that bind pentavalent arsenicals at the active site serine residue: sulphydryl groups do not seem to play an important role in their enzymatic activities. Whether pentavalent arsenicals bind to these proteins in a covalent way or as a coordination complex of certain thermodynamic and kinetic properties needs to be examined. Future arsenic–protein binding studies should include a variety of arsenicals on

selected proteins in order to fully understand the nature and diversity of arsenic binding to proteins.

The issue of nonspecific association needs to be addressed in protein binding to arsenicals. Stable and specific interaction between arsenic and biomolecules may be necessary to sustain the biological impact of arsenic. For example, arsenite binds peptides containing three (C3) or four (C4) cysteine residues more stably than those containing two cysteine (C2) residues,^{45,46} which may explain the finding that cellular arsenic exposure resulted in loss of zinc from C3 or C4 (PARP-1 and XPA), but not C2 (SP-1, APTX, and PARP-1 zinc finger mutants) zinc finger proteins.⁴⁵ Similarly, nonspecific association between arsenite and DNA¹⁹⁸ demonstrates that arsenic does not directly react with DNA and may account for the fact that inorganic arsenic is inactive or extremely weak in its ability to induce gene mutations.^{409,410}

With the development of integrated metallomic approaches, more arsenic-binding proteins are expected to be identified under physiologically and pathologically relevant conditions. However, binding specificity should be enshrined as the key concept and scrutinized when identifying novel arsenic-binding proteins with putative arsenic-binding domains demonstrated.

ASSOCIATED CONTENT

Supporting Information

Tables S1–S5 that list the structures of arsenic species, structures of common thiols, K_d values, and K_{on} and K_{off} values. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

AUTHOR INFORMATION

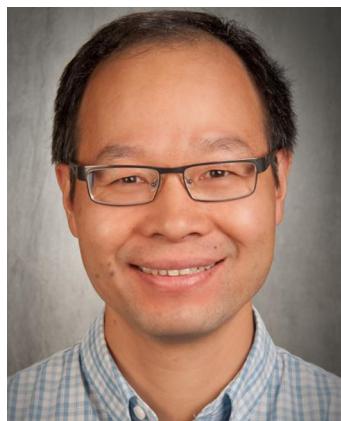
Corresponding Author

*Telephone: (780) 492-6416. Fax: (780) 492-7800. E-mail: xc.le@ualberta.ca.

Notes

The authors declare no competing financial interest.

Biographies



Shengwen Shen obtained his Ph.D. from the Department of Public Health Sciences at the University of Alberta, Edmonton, Canada, in 2006. He performed his postdoctoral research in the Department of Laboratory Medicine and Pathology at the University of Alberta from 2006 to 2008. He then worked in the Department of Agricultural, Food and Nutritional Science (2008–2010), and is currently a research associate in the Department of Laboratory Medicine and Pathology at the University of Alberta (2010–present). His current research focuses

on the cocarcinogenicity of arsenic, with an emphasis on the roles of arsenic in protein binding and inhibition of DNA repair.



Xing-Fang Li is Professor of Analytical and Environmental Toxicology in the Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada. She received her B.Sc. (1983) from Hangzhou University (Hangzhou, China), M.Sc. (1986) from the Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences (Beijing, China), M.Sc. (1990) from Brock University (St. Catharines, Canada), and Ph.D. (1995) from the University of British Columbia (Vancouver, Canada). She joined the Faculty of Medicine and Dentistry at the University of Alberta in 2001 and was promoted through the ranks to Professor in 2010. She received the W.A.E. McBryde Medal from the Canadian Society for Chemistry in 2010 and Killam Annual Professorship from the University of Alberta in 2012. Her research interests include studies of protein interaction with small molecules, selection of DNA aptamers, detection of microbial pathogens, characterization of new drinking water disinfection byproducts, and studies of toxicity of water contaminants and human health effects.



William R. Cullen received a Ph.D. from Cambridge University (Cambridge, U.K.) in 1959. He joined the Faculty of the Chemistry Department, University of British Columbia (Vancouver, Canada) in 1958. He was promoted to Professor in 1969. In 1998 he became Professor Emeritus at UBC and Adjunct Professor at the Royal Military College of Canada (Kingston, Canada) in 2000. He is a Fellow of the Royal Society of Canada. His research has focused on the chemistry of arsenic with increasing emphasis on biological and environmental problems. His book *Is Arsenic an Aphrodisiac: The Sociochemistry of an Element* was published by the Royal Society of Chemistry in 2008.



Michael Weinfeld received his B.A. degree in Chemistry from Oxford University (Oxford, U.K.) in 1978 and his Ph.D. from the University of London in 1982. After postdoctoral research at the Chalk River Nuclear Laboratories (Ontario, Canada) and the Cross Cancer Institute (Edmonton, Canada), he joined the Faculty of Medicine and Dentistry at the University of Alberta and Cross Cancer Institute in 1989. His current research interests include DNA damage and repair, with a special focus on the repair of DNA strand breaks and arsenic carcinogenesis.



X. Chris Le is Distinguished University Professor and Director of the Analytical and Environmental Toxicology Division in the Faculty of Medicine and Dentistry, University of Alberta (Edmonton, Canada). He holds a Canada Research Chair (Tier 1) in Bioanalytical Technology and Environmental Health. He is an elected Fellow of the Royal Society of Canada (Academy of Science). He received a B.Sc. in Chemistry from Wuhan University (Wuhan, China) in 1983, an M.Sc. in Environmental Chemistry from the Chinese Academy of Sciences (Beijing, China) in 1986, a second M.Sc. in Analytical Chemistry from Brock University (St. Catharines, Canada) in 1988, and a Ph.D. in Analytical Environmental Chemistry from the University of British Columbia (Vancouver, Canada) in 1993. With the support of a Killam Postdoctoral Fellowship, he pursued postdoctoral research in Bioanalytical Chemistry at the University of Alberta (Canada) in 1994. His research on arsenic speciation in biological and environmental systems focuses on arsenic binding to proteins, human exposure to arsenic species, and mechanisms of arsenic metabolism and health effects. Another area of his research deals with bioanalytical chemistry, focusing on the development of DNA and protein binding assays for measuring proteins, DNA damage, and molecular interactions. Dr. Le is an Associate Editor for *Environmental Health Perspectives*.

ACKNOWLEDGMENTS

The authors thank the Canadian Institutes of Health Research, the National Sciences and Engineering Research Council of Canada, the Canada Research Chairs Program, Alberta Cancer Foundation, Alberta Health, and Alberta Innovates for funding this work. The authors also thank Dr. Barry P. Rosen (Florida International University) for helpful discussion and sharing unpublished data.

REFERENCES

- (1) Cullen, W. R. *Is Arsenic an Aphrodisiac? The Sociochemistry of an Element*; RSC Publishing: Cambridge, U.K., 2008; 412 pp.
- (2) Oremland, R. S.; Stolz, J. F. *Science* **2003**, *300*, 939.
- (3) Nordstrom, D. K. *Science* **2002**, *296*, 2143.
- (4) Jain, C. K.; Ali, I. *Water Res.* **2000**, *34*, 4304.
- (5) Bhattacharya, P.; Welch, A. H.; Stollenwerk, K. G.; McLaughlin, M. J.; Bundschuh, J.; Panaullah, G. *Sci. Total Environ.* **2007**, *379*, 109.
- (6) Chen, C.; Chuang, Y.; You, S.; Lin, T.; Wu, H. *Br. J. Cancer* **1986**, *53*, 399.
- (7) Chen, C.; Chen, C.; Wu, M.; Kuo, T. *Br. J. Cancer* **1992**, *66*, 888.
- (8) Chiou, H. Y.; Hsueh, Y. M.; Liaw, K. F.; Horng, S. F.; Chiang, M. H.; Pu, Y. S.; Shinn-Nan Lin, J.; Huang, C. H.; Chen, C. J. *Cancer Res.* **1995**, *55*, 1296.
- (9) Hopenhayn-Rich, C.; Biggs, M. L.; Fuchs, A.; Bergoglio, R.; Tello, E. E.; Nicoll, H.; Smith, A. H. *Epidemiology* **1996**, *117*.
- (10) Steinmaus, C.; Yuan, Y.; Kalman, D.; Rey, O. A.; Skibola, C. F.; Dauphine, D.; Basu, A.; Porter, K. E.; Hubbard, A.; Bates, M. N. *Toxicol. Appl. Pharmacol.* **2010**, *247*, 138.
- (11) Smith, A. H.; Marshall, G.; Yuan, Y.; Liaw, J.; Ferreccio, C.; Steinmaus, C. *Am. J. Epidemiol.* **2011**, *173*, 414.
- (12) Smith, A. H.; Goycolea, M.; Haque, R.; Biggs, M. L. *Am. J. Epidemiol.* **1998**, *147*, 660.
- (13) Chowdhury, T. R.; Mandal, B. K.; Samanta, G.; Basu, G. K.; Chowdhury, P.; Chanda, C.; Karan, N.; Lodh, D.; Dhar, R.; Das, D. In *Arsenic Exposure and Health Effects*; Abernathy, C. O., Calderon, R. L., Chappell, W. R., Eds.; Chapman & Hall: London, 1997; p 93.
- (14) Chatterjee, A.; Das, D.; Mandal, B. K.; Chowdhury, T. R.; Samanta, G.; Chakraborti, D. *Analyst* **1995**, *120*, 643.
- (15) Argos, M.; Kalra, T.; Rathouz, P. J.; Chen, Y.; Pierce, B.; Parvez, F.; Islam, T.; Ahmed, A.; Rakibuz-Zaman, M.; Hasan, R. *Lancet* **2010**, *376*, 252.
- (16) Sohel, N.; Persson, L. Å.; Rahman, M.; Streatfield, P. K.; Yunus, M.; Ekström, E. C.; Vahter, M. *Epidemiology* **2009**, *20*, 824.
- (17) Lindberg, A. L.; Sohel, N.; Rahman, M.; Persson, L. Å.; Vahter, M. *Environ. Health Perspect.* **2010**, *118*, 533.
- (18) Luo, Z. D.; Zhang, Y. M.; Ma, L.; Zhang, G. Y.; He, X.; Wilson, R.; Byrd, R. W.; Griffiths, J. G.; Lai, S.; He, L.; Grumski, K.; Lamm, S. H. In *Arsenic Exposure and Health Effects*; Abernathy, C. O., Calderon, R. L., Chappell, W. R., Eds.; Chapman & Hall: London, 1997; p 55.
- (19) Mo, J.; Xia, Y.; Wade, T. J.; Schmitt, M.; Le, X. C.; Dang, R.; Mumford, J. L. *Environ. Health Perspect.* **2006**, *114*, 835.
- (20) Cantor, K. P.; Lubin, J. H. *Toxicol. Appl. Pharmacol.* **2007**, *222*, 252.
- (21) Celik, I.; Gallicchio, L.; Boyd, K.; Lam, T. K.; Matanoski, G.; Tao, X.; Shiels, M.; Hammond, E.; Chen, L.; Robinson, K. A. *Environ. Res.* **2008**, *108*, 48.
- (22) Heck, J. E.; Andrew, A. S.; Onega, T.; Rigas, J. R.; Jackson, B. P.; Karagas, M. R.; Duell, E. J. *Environ. Health Perspect.* **2009**, *117*, 1718.
- (23) Smith, A. H.; Ercumen, A.; Yuan, Y.; Steinmaus, C. M. *J. Expo. Sci. Environ. Epidemiol.* **2009**, *19*, 343.
- (24) Chen, C. J.; Wang, S. L.; Chiou, J. M.; Tseng, C. H.; Chiou, H. Y.; Hsueh, Y. M.; Chen, S. Y.; Wu, M. M.; Lai, M. S. *Toxicol. Appl. Pharmacol.* **2007**, *222*, 298.
- (25) Chen, Y.; Graziano, J. H.; Parvez, F.; Liu, M.; Slavkovich, V.; Kalra, T.; Argos, M.; Islam, T.; Ahmed, A.; Rakibuz-Zaman, M. *BMJ* **2011**, *342*, d2431 DOI: 10.1136/bmj.d2431.
- (26) National Research Council. *Arsenic in Drinking Water*; National Academy Press: Washington, DC, 1999.

- (27) National Research Council. *Arsenic in Drinking Water: Update*; National Academy Press: Washington, DC, 2001.
- (28) Hughes, M. F. *Toxicol. Lett.* **2002**, *133*, 1.
- (29) Hughes, M. F.; Beck, B. D.; Chen, Y.; Lewis, A. S.; Thomas, D. J. *Toxicol. Sci.* **2011**, *123*, 305.
- (30) Aposhian, H. V.; Aposhian, M. M. *Chem. Res. Toxicol.* **2006**, *19*, 1.
- (31) Reaves, M. L.; Sinha, S.; Rabinowitz, J. D.; Kruglyak, L.; Redfield, R. J. *Science* **2012**, *337*, 470.
- (32) Erb, T. J.; Kiefer, P.; Hattendorf, B.; Günther, D.; Vorholt, J. A. *Science* **2012**, *337*, 467.
- (33) Fekry, M. I.; Tipton, P. A.; Gates, K. S. *ACS Chem. Biol.* **2011**, *6*, 127.
- (34) Denning, E. J.; MacKerell, A. D., Jr. *J. Am. Chem. Soc.* **2011**, *133*, 5770.
- (35) Wang, J.; Gu, J.; Leszczynski, J. *Chem. Commun.* **2012**, *48*, 3626.
- (36) Summers, A. O. *Curr. Opin. Microbiol.* **2009**, *12*, 138.
- (37) Thompson, D. J. *Chem. Biol. Interact.* **1993**, *88*, 89.
- (38) Wiberg, N.; Holleman, A. *Holleman-Wiberg's Inorganic Chemistry*; Academic Press: New York, 2001.
- (39) Kitchin, K. T.; Wallace, K.; Andrewes, P. In *Arsenic Exposure and Health Effects V*; Chapell, W. R., Abernathy, C. O., Calderon, R. L., Thomas, D. J., Eds.; Elsevier B.V.: Amsterdam, 2003; p 345.
- (40) Cullen, W.; McBride, B.; Reglinski, J. *Inorg. Biochem.* **1984**, *21*, 179.
- (41) Thayer, J. S. *Appl. Organomet. Chem.* **2002**, *16*, 677.
- (42) Kitchin, K. T.; Wallace, K. *Toxicol. Appl. Pharmacol.* **2005**, *206*, 66.
- (43) Kitchin, K. T.; Wallace, K. *J. Biochem. Mol. Toxicol.* **2006**, *20*, 35.
- (44) Kitchin, K. T.; Wallace, K. *J. Biochem. Mol. Toxicol.* **2006**, *20*, 48.
- (45) Zhou, X.; Sun, X.; Cooper, K. L.; Wang, F.; Liu, K. J.; Hudson, L. G. *J. Biol. Chem.* **2011**, *286*, 22855.
- (46) Zhao, L.; Chen, S.; Jia, L.; Shu, S.; Zhu, P.; Liu, Y. *Metalomics* **2012**, *4*, 988.
- (47) Lopez, S.; Miyashita, Y.; Simons, S., Jr. *J. Biol. Chem.* **1990**, *265*, 16039.
- (48) Bellacchio, E.; Paggi, M. G. *J. Cell. Physiol.* **2008**, *214*, 681.
- (49) Styblo, M.; Serves, S. V.; Cullen, W. R.; Thomas, D. J. *Chem. Res. Toxicol.* **1997**, *10*, 27.
- (50) Marapakala, K.; Qin, J.; Rosen, B. P. *Biochemistry* **2012**, *51*, 944.
- (51) Fluharty, A.; Sanadi, D. *Proc. Natl. Acad. Sci. U. S. A.* **1960**, *46*, 608.
- (52) Fluharty, A. L.; Sanadi, D. *J. Biol. Chem.* **1961**, *236*, 2772.
- (53) Fletcher, M. J.; Sanadi, D. *Arch. Biochem. Biophys.* **1962**, *96*, 139.
- (54) Craven, P. A.; DeRupertis, F. R. *Biochim. Biophys. Acta Enzymol.* **1978**, *524*, 231.
- (55) Bagui, T. K.; Ghosh, M.; Datta, A. K. *Biochem. J.* **1996**, *316*, 439.
- (56) Joshi, S.; Hughes, J. B. *J. Biol. Chem.* **1981**, *256*, 11112.
- (57) Spiegel, A. M.; Brown, E. M.; Aurbach, G. D. *J. Cyclic Nucleotide Res.* **1976**, *2*, 393.
- (58) González-Segura, L.; Mújica-Jiménez, C.; Muñoz-Clares, R. A. *Chem. Biol. Interact.* **2009**, *178*, 64.
- (59) Chang, Y. Y.; Kuo, T. C.; Hsu, C. H.; Hou, D. R.; Kao, Y. H.; Huang, R. N. *Arch. Toxicol.* **2012**, *86*, 911.
- (60) Lu, M.; Wang, H.; Li, X.; Arnold, L. L.; Cohen, S. M.; Le, X. C. *Chem. Res. Toxicol.* **2007**, *20*, 27.
- (61) Stocken, L. A.; Thompson, R. *Physiol. Rev.* **1949**, *29*, 168.
- (62) Stocken, L.; Thompson, R. *Biochem. J.* **1946**, *40*, 529.
- (63) Stocken, L.; Thompson, R. *Biochem. J.* **1946**, *40*, 535.
- (64) Stevenson, K. J.; Hale, G.; Perham, R. N. *Biochemistry* **1978**, *17*, 2189.
- (65) Ordóñez, E.; Thiagarajan, S.; Cook, J. D.; Stemmler, T. L.; Gil, J. A.; Mateos, L. M.; Rosen, B. P. *J. Biol. Chem.* **2008**, *283*, 25706.
- (66) Zhang, X. W.; Yan, X. J.; Zhou, Z. R.; Yang, F. F.; Wu, Z. Y.; Sun, H. B.; Liang, W. X.; Song, A. X.; Lallemand-Breitenbach, V.; Jeanne, M. *Science* **2010**, *328*, 240.
- (67) Jeanne, M.; Lallemand-Breitenbach, V.; Ferhi, O.; Koken, M.; Le Bras, M.; Duffort, S.; Peres, L.; Berthier, C.; Soilihi, H.; Raught, B. *Cancer Cell* **2010**, *18*, 88.
- (68) Styblo, M.; Drobná, Z.; Jaspers, I.; Lin, S.; Thomas, D. J. *Environ. Health Perspect.* **2002**, *110*, 7667.
- (69) Aposhian, H. V. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 397.
- (70) Board, P. G.; Coggan, M.; Chelvanayagam, G.; Easteal, S.; Jermini, L. S.; Schulte, G. K.; Danley, D. E.; Hoth, L. R.; Griffor, M. C.; Kamath, A. V. *J. Biol. Chem.* **2000**, *275*, 24798.
- (71) Zakharyan, R. A.; Tsaprailis, G.; Chowdhury, U. K.; Hernandez, A.; Aposhian, H. V. *Chem. Res. Toxicol.* **2005**, *18*, 1287.
- (72) Messens, J.; Martins, J. C.; Van Belle, K.; Brosens, E.; Desmyter, A.; De Gieter, M.; Wierszeski, J. M.; Willem, R.; Wyns, L.; Zegers, I. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 8506.
- (73) Messens, J.; Silver, S. *J. Mol. Biol.* **2006**, *362*, 1.
- (74) Cullen, W. R.; Reimer, K. J. *Chem. Rev.* **1989**, *89*, 713.
- (75) Ajees, A. A.; Marapakala, K.; Packianathan, C.; Sankaran, B.; Rosen, B. P. *Biochemistry* **2012**, *51*, 5476.
- (76) Li, J.; Waters, S. B.; Drobna, Z.; Devesa, V.; Styblo, M.; Thomas, D. J. *Toxicol. Appl. Pharmacol.* **2005**, *204*, 164.
- (77) Fomenko, D. E.; Xing, W.; Adair, B. M.; Thomas, D. J.; Gladyshev, V. N. *Science* **2007**, *315*, 387.
- (78) Hamdi, M.; Yoshinaga, M.; Packianathan, C.; Qin, J.; Hallauer, J.; McDermott, J. R.; Yang, H.; Tsai, K.; Liu, Z. *Toxicol. Appl. Pharmacol.* **2012**, *162*, 185.
- (79) Qin, J.; Lehr, C. R.; Yuan, C.; Le, X. C.; McDermott, T. R.; Rosen, B. P. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 5213.
- (80) Song, X.; Geng, Z.; Li, X.; Zhao, Q.; Hu, X.; Zhang, X.; Wang, Z. *Biochimie* **2010**, *93*, 369.
- (81) Song, X.; Geng, Z.; Zhu, J.; Li, C.; Hu, X.; Bian, N.; Zhang, X.; Wang, Z. *Chem. Biol. Interact.* **2009**, *179*, 321.
- (82) Song, X.; Geng, Z.; Li, X.; Hu, X.; Bian, N.; Zhang, X.; Wang, Z. *Biochimie* **2010**, *92*, 1397.
- (83) Ratnaike, R. N. *Postgrad. Med. J.* **2003**, *79*, 391.
- (84) Webb, J. L. In *Enzyme and Metabolic Inhibitors* Academic Press: New York, 1966; Vol. III, p 595.
- (85) Muller, S.; Walter, R. D.; Fairlamb, A. H. *Mol. Biochem. Parasitol.* **1995**, *71*, 211.
- (86) Cunningham, M. L.; Zvelebil, M. J. J. M.; Fairlamb, A. H. *Eur. J. Biochem.* **1994**, *221*, 285.
- (87) Knowles, F. C. *Arch. Biochem. Biophys.* **1985**, *242*, 1.
- (88) Chouchane, S.; Snow, E. T. *Chem. Res. Toxicol.* **2001**, *14*, 517.
- (89) Lu, J.; Chew, E. H.; Holmgren, A. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 12288.
- (90) Lin, S.; Del Razo, L. M.; Styblo, M.; Wang, C.; Cullen, W. R.; Thomas, D. J. *Chem. Res. Toxicol.* **2001**, *14*, 305.
- (91) Lin, S.; Cullen, W. R.; Thomas, D. J. *Chem. Res. Toxicol.* **1999**, *12*, 924.
- (92) Chang, K. N.; Te Chang Lee, M. F. T.; Chen, Y. C.; Lee, L. W.; Lee, S. Y.; Lin, P. J.; Huang, R. N. *Biochem. J.* **2003**, *371*, 495.
- (93) Snow, E. T.; Hu, Y.; Yan, C. C.; Chouchane, S. Modulation of DNA Repair and Glutathione Levels in Human Keratinocytes by Micromolar Arsenite. In *Arsenic Exposure and Health Effects*; Chappell, W. R., Abernathy, C. O., Calderon, R. L., Eds.; Elsevier: Oxford, UK, 1999; p 243.
- (94) Li, J.; Pickart, C. M. *Biochemistry* **1995**, *34*, 15829.
- (95) Li, J.; Pickart, C. M. *Biochemistry* **1995**, *34*, 139.
- (96) Kapahi, P.; Takahashi, T.; Natoli, G.; Adams, S. R.; Chen, Y.; Tsien, R. Y.; Karin, M. *J. Biol. Chem.* **2000**, *275*, 36062.
- (97) Lin, C. H.; Huang, C. F.; Chen, W. Y.; Chang, Y. Y.; Ding, W. H.; Lin, M. S.; Wu, S. H.; Huang, R. N. *Chem. Res. Toxicol.* **2006**, *19*, 469.
- (98) Zhang, X. Y.; Bishop, A. C. *Biochemistry* **2008**, *47*, 4491.
- (99) Walton, Z. E.; Bishop, A. C. *Bioorg. Med. Chem.* **2010**, *18*, 4884.
- (100) Davis, O.; Bishop, A. C. *Bioconjugate Chem.* **2012**, *23*, 272.
- (101) Rehman, K.; Chen, Z.; Wang, W. W.; Wang, Y. W.; Sakamoto, A.; Zhang, Y. F.; Suzuki, N.; Naranmandura, H. *Toxicol. Appl. Pharmacol.* **2012**, *263*, 273.
- (102) Cavigelli, M.; Li, W. W.; Lin, A.; Su, B.; Yoshioka, K.; Karin, M. *EMBO J.* **1996**, *15*, 6269.

- (103) Yoda, A.; Toyoshima, K.; Watanabe, Y.; Onishi, N.; Hazaka, Y.; Tsukuda, Y.; Tsukada, J.; Kondo, T.; Tanaka, Y.; Minami, Y. *J. Biol. Chem.* **2008**, *283*, 18969.
- (104) Mao, J. H.; Sun, X. Y.; Liu, J. X.; Zhang, Q. Y.; Liu, P.; Huang, Q. H.; Li, K. K.; Chen, Q.; Chen, Z.; Chen, S. J. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 21683.
- (105) Bergquist, E. R.; Fischer, R. J.; Sugden, K. D.; Martin, B. D. *J. Organomet. Chem.* **2009**, *694*, 973.
- (106) Petrick, J. S.; Jagadish, B.; Mash, E. A.; Aposhian, H. V. M. *Chem. Res. Toxicol.* **2001**, *14*, 651.
- (107) Samikkannu, T.; Chen, C. H.; Yih, L. H.; Wang, A. S. S.; Lin, S. Y.; Chen, T. C.; Jan, K. Y. *Chem. Res. Toxicol.* **2003**, *16*, 409.
- (108) Wang, Z.; Zhang, H.; Li, X. F.; Le, X. C. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3658.
- (109) Powis, G.; Montfort, W. R. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 421.
- (110) Arnér, E. S. J.; Holmgren, A. *Eur. J. Biochem.* **2000**, *267*, 6102.
- (111) Gromer, S.; Urić, S.; Becker, K. *Med. Res. Rev.* **2004**, *24*, 40.
- (112) Waters, S. B.; Devesa, V.; Del Razo, L. M.; Styblo, M.; Thomas, D. *J. Chem. Res. Toxicol.* **2004**, *17*, 404.
- (113) Thomas, D. J. *Toxicol. Sci.* **2009**, *107*, 309.
- (114) Hu, Y.; Jin, X.; Snow, E. T. *Toxicol. Lett.* **2002**, *133*, 33.
- (115) Schwerdtle, T.; Walter, I.; Hartwig, A. *DNA Repair* **2003**, *2*, 1449.
- (116) Shen, S.; Lee, J.; Weinfeld, M.; Le, X. C. *Mol. Carcinog.* **2008**, *47*, 508.
- (117) Shen, S.; Wang, C.; Weinfeld, M.; Le, X. C. *Chin. Sci. Bull.* **2013**, *58*, 214.
- (118) Walter, I.; Schwerdtle, T.; Thuy, C.; Parsons, J. L.; Dianov, G. L.; Hartwig, A. *DNA Repair* **2007**, *6*, 61.
- (119) Hartwig, A.; Blessing, H.; Schwerdtle, T.; Walter, I. *Toxicology* **2003**, *193*, 161.
- (120) Flohr, C.; Bürkle, A.; Radicella, J. P.; Epe, B. *Nucleic Acids Res.* **2003**, *31*, 5332.
- (121) Luijsterburg, M. S.; Lindh, M.; Acs, K.; Vrouwe, M. G.; Pines, A.; van Attikum, H.; Mullenders, L. H.; Dantuma, N. P. *J. Cell Biol.* **2012**, *197*, 267.
- (122) Audebert, M.; Salles, B.; Calsou, P. *J. Biol. Chem.* **2004**, *279*, S5117.
- (123) Ding, W.; Liu, W.; Cooper, K. L.; Qin, X. J.; de Souza Bergo, P. L.; Hudson, L. G.; Liu, K. J. *J. Biol. Chem.* **2009**, *284*, 6809.
- (124) Piątek, K.; Schwerdtle, T.; Hartwig, A.; Bal, W. *Chem. Res. Toxicol.* **2008**, *21*, 600.
- (125) Cobbett, C.; Goldsbrough, P. *Plant Biol.* **2002**, *53*, 159.
- (126) Raab, A.; Feldmann, J.; Meharg, A. A. *Plant Physiol.* **2004**, *134*, 1113.
- (127) Zhang, W.; Cai, Y. *Anal. Chem.* **2003**, *75*, 7030.
- (128) Ma, L. Q.; Komar, K. M.; Tu, C.; Zhang, W.; Cai, Y.; Kellenley, E. D. *Nature* **2001**, *409*, 579.
- (129) Lin, Y. F.; Walmsley, A. R.; Rosen, B. P. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 15617.
- (130) He, X.; Ma, Q. *J. Biol. Chem.* **2009**, *284*, 12609.
- (131) Andrews, G. K. *Biochem. Pharmacol.* **2000**, *59*, 95.
- (132) Klaassen, C. D.; Liu, J.; Choudhuri, S. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 267.
- (133) Margoshes, M.; Vallee, B. L. *J. Am. Chem. Soc.* **1957**, *79*, 4813.
- (134) He, X.; Ma, Q. *J. Pharmacol. Exp. Ther.* **2010**, *332*, 66.
- (135) Stillman, M. J.; Shaw, C. F.; Suzuki, K. T. (Eds.) *Metallothioneins: Synthesis, Structure, and Properties of Metallothioneins, Phytochelatins, and Metal–Thiolate Complexes*; Wiley-VCH: New York, 1992.
- (136) Suzuki, K. T.; Imura, N.; Kimura, M. (Eds.) *Metallothionein III: Biological Roles and Medical Implications*; Birkhäuser: Boston, 1993.
- (137) Henkel, G.; Krebs, B. *Chem. Rev.* **2004**, *104*, 801.
- (138) Sutherland, D. E.; Willans, M. J.; Stillman, M. J. *Biochemistry* **2010**, *49*, 3593.
- (139) Albores, A.; Koropatnick, J.; Cherian, M. G.; Zelazowski, A. J. *Chem. Biol. Interact.* **1992**, *85*, 127.
- (140) Kreppel, H.; Bauman, J. W.; Liu, J. I. E.; McKim, J. M.; Klaassen, C. D. *Toxicol. Sci.* **1993**, *20*, 184.
- (141) Falnoga, I.; Zeleník Pevec, A.; Šlejkovec, Z.; Žnidarič, M. T.; Zajc, I.; Mlakar, S. J.; Marc, J. *Biol. Trace Elem. Res.* **2012**, *149*, 331.
- (142) Macías-Barragán, J.; Huerta-Olvera, S.; Ramos-Márquez, M. E.; Armendáriz-Borunda, J.; Siller-López, F. *Toxicol. Lett.* **2006**, *164*, S137.
- (143) Zhou, X. D.; Sens, D. A.; Sens, M. A.; Namburi, V. B.; Singh, R. K.; Garrett, S. H.; Somji, S. *Toxicol. Sci.* **2006**, *91*, 467.
- (144) Ng, J.; Seawright, A.; Moore, M. *Toxicology* **2001**, *164*, 59.
- (145) Kaegi, J. H. R.; Schaeffer, A. *Biochemistry* **1988**, *27*, 8509.
- (146) Singh, S.; Mulchandani, A.; Chen, W. *Appl. Environ. Microbiol.* **2008**, *74*, 2924.
- (147) Suzuki, T.; Ohata, S.; Togawa, T.; Himeno, S.; Tanabe, S. *J. Toxicol. Sci.* **2007**, *32*, 321.
- (148) Langdon, C.; Winters, C.; Stürzenbaum, S.; Morgan, A.; Charnock, J.; Meharg, A.; Pearce, T. G.; Lee, P.; Semple, K. T. *Environ. Sci. Technol.* **2005**, *39*, 2042.
- (149) Toyama, M.; Yamashita, M.; Hirayama, N.; Murooka, Y. *J. Biochem.* **2002**, *132*, 217.
- (150) Chen, C. L.; Whanger, P. D. *J. Inorg. Biochem.* **1994**, *54*, 267.
- (151) Jiang, G.; Gong, Z.; Li, X. F.; Cullen, W. R.; Le, X. C. *Chem. Res. Toxicol.* **2003**, *16*, 873.
- (152) Ngu, T. T.; Stillman, M. J. *J. Am. Chem. Soc.* **2006**, *128*, 12473.
- (153) Ngu, T. T.; Lee, J. A.; Rushton, M. K.; Stillman, M. J. *Biochemistry* **2009**, *48*, 8806.
- (154) Ngu, T. T.; Lee, J. A.; Pinter, T. B. J.; Stillman, M. J. *J. Inorg. Biochem.* **2010**, *104*, 232.
- (155) Ngu, T. T.; Easton, A.; Stillman, M. J. *J. Am. Chem. Soc.* **2008**, *130*, 17016.
- (156) Merrifield, M.; Ngu, T.; Stillman, M. *Biochem. Biophys. Res. Commun.* **2004**, *324*, 127.
- (157) Ngu, T. T.; Dryden, M. D.; Stillman, M. J. *Biochem. Biophys. Res. Commun.* **2010**, *401*, 69.
- (158) Ngu, T. T.; Stillman, M. J. *IUBMB Life* **2009**, *61*, 438.
- (159) Guo, Y.; Ling, Y.; Thomson, B. A.; Siu, K. W. M. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1787.
- (160) Rosen, B. P. *FEBS Lett.* **2002**, *529*, 86.
- (161) Shi, W.; Dong, J.; Scott, R. A.; Ksenzenko, M. Y.; Rosen, B. P. *J. Biol. Chem.* **1996**, *271*, 9291.
- (162) Xu, C.; Zhou, T.; Kuroda, M.; Rosen, B. P. *J. Biochem.* **1998**, *123*, 16.
- (163) Fu, H.; Jiang, X.; Rosen, B. P. In *Biological Chemistry of Arsenic, Antimony and Bismuth*; Sun, H., Ed.; John Wiley & Sons: Hoboken, NJ, 2009; p 181.
- (164) Yang, J.; Rawat, S.; Stemmler, T. L.; Rosen, B. P. *Biochemistry* **2010**, *49*, 3658.
- (165) Martin, P.; DeMel, S.; Shi, J.; Gladysheva, T.; Gatti, D. L.; Rosen, B. P.; Edwards, B. F. P. *Structure* **2001**, *9*, 1071.
- (166) Zhang, X.; Cornelis, R.; De Kimpe, J.; Mees, L.; Lameire, N. *Clin. Chem. (Washington, D. C.)* **1998**, *44*, 141.
- (167) Hunter, F.; Kip, A.; Irvine, J., Jr. *J. Pharmacol. Exp. Ther.* **1942**, *76*, 207.
- (168) Lanz, H., Jr.; Wallace, P.; Hamilton, J. *Univ. Calif. (Berkeley) Pub. Pharmacol.* **1949**, *2*, 263.
- (169) Lu, M.; Wang, H.; Li, X. F.; Lu, X.; Cullen, W. R.; Arnold, L. L.; Cohen, S. M.; Le, X. C. *Chem. Res. Toxicol.* **2004**, *17*, 1733.
- (170) Vahter, M.; Marafante, E.; Dencker, L. *Arch. Environ. Contam. Toxicol.* **1984**, *13*, 259.
- (171) Lu, M.; Li, X. F.; Le, X. C.; Weinfeld, M.; Wang, H. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1523.
- (172) Lu, M.; Wang, H.; Wang, Z.; Li, X. F.; Le, X. C. *J. Proteome Res.* **2008**, *7*, 3080.
- (173) Waxman, S.; Anderson, K. C. *Oncologist* **2001**, *6*, 3.
- (174) Wang, X. *Genes Dev.* **2001**, *15*, 2922.
- (175) Wang, C.; Youle, R. J. *Annu. Rev. Genet.* **2009**, *43*, 95.
- (176) Gupta, S. *Int. J. Oncol.* **2003**, *22*, 15.
- (177) Desagher, S.; Martinou, J. C. *Trends Cell Biol.* **2000**, *10*, 369.

- (178) Don, A. S.; Kisker, O.; Dilda, P.; Donoghue, N.; Zhao, X.; Decollonge, S.; Creighton, B.; Flynn, E.; Folkman, J.; Hogg, P. J. *Cancer Cell* **2003**, *3*, 497.
- (179) Belzacq, A. S.; El Hamel, C.; Vieira, H. L.; Cohen, I.; Haouzi, D.; Metivier, D.; Marchetti, P.; Brenner, C.; Kroemer, G. *Oncogene* **2001**, *20*, 7579.
- (180) Larochette, N.; Decaudin, D.; Jacotot, E.; Brenner, C.; Marzo, I.; Susin, S. A.; Zamzami, N.; Xie, Z.; Reed, J.; Kroemer, G. *Exp. Cell Res.* **1999**, *249*, 413.
- (181) Zheng, Y.; Shi, Y.; Tian, C.; Jiang, C.; Jin, H.; Chen, J.; Almasan, A.; Tang, H.; Chen, Q. *Oncogene* **2003**, *23*, 1239.
- (182) Wong, H. L.; Liebler, D. C. *Chem. Res. Toxicol.* **2008**, *21*, 796.
- (183) Carré, M.; André, N.; Carles, G.; Borghi, H.; Brichese, L.; Briand, C.; Braguer, D. *J. Biol. Chem.* **2002**, *277*, 33664.
- (184) Psarra, A.; Hermann, S.; Panayotou, G.; Spyrou, G. *Biochem. J.* **2009**, *422*, 521.
- (185) Miranda-Vizcute, A.; Damdimopoulos, A. E.; Spyrou, G. *Antioxid. Redox Signaling* **2000**, *2*, 801.
- (186) Requejo, R.; Chouchani, E. T.; James, A. M.; Prime, T. A.; Lilley, K. S.; Fearnley, I. M.; Murphy, M. P. *Arch. Biochem. Biophys.* **2010**, *504*, 228.
- (187) Marchetti, P.; Decaudin, D.; Macho, A.; Zamzami, N.; Hirsch, T.; Susin, S. A.; Kroemer, G. *Eur. J. Immunol.* **1997**, *27*, 289.
- (188) McStay, G. *Biochem. J.* **2002**, *367*, 541.
- (189) Sionov, R. V.; Cohen, O.; Kfir, S.; Zilberman, Y.; Yefenof, E. *J. Exp. Med.* **2006**, *203*, 189.
- (190) Hoffman, R. D.; Lane, M. D. *J. Biol. Chem.* **1992**, *267*, 14005.
- (191) Li, Y. M.; Broome, J. D. *Cancer Res.* **1999**, *59*, 776.
- (192) Menzel, D. B.; Hamadeh, H. K.; Lee, E.; Meacher, D. M.; Said, V.; Rasmussen, R. E.; Greene, H.; Roth, R. N. *Toxicol. Lett.* **1999**, *105*, 89.
- (193) Spuches, A. M.; Wilcox, D. E. *J. Am. Chem. Soc.* **2008**, *130*, 8148.
- (194) Simons, S. S.; Chakraborti, P. K.; Cavanaugh, A. H. *J. Biol. Chem.* **1990**, *265*, 1938.
- (195) Stoica, A.; Pentecost, E.; Martin, M. B. *Endocrinology* **2000**, *141*, 3595.
- (196) He, X.; Ma, Q. *Mol. Pharmacol.* **2009**, *76*, 1265.
- (197) Miseta, A.; Csutora, P. *Mol. Biol. Evol.* **2000**, *17*, 1232.
- (198) Kitchin, K. T.; Wallace, K. J. *Inorg. Biochem.* **2008**, *102*, 532.
- (199) Reimer, K. J.; Koch, I.; Cullen, W. R. *Met. Ions Life Sci.* **2010**, *7*, 165.
- (200) Charoensuk, V.; Gati, W. P.; Weinfeld, M.; Le, X. C. *Toxicol. Appl. Pharmacol.* **2009**, *239*, 64.
- (201) Naranmandura, H.; Suzuki, K. T. *Chem. Res. Toxicol.* **2008**, *21*, 678.
- (202) Styblo, M.; Thomas, D. J. *Toxicol. Appl. Pharmacol.* **1997**, *147*, 1.
- (203) Delnomdedieu, M.; Styblo, M.; Thomas, D. J. *Chem. Biol. Interact.* **1995**, *98*, 69.
- (204) De Kimpe, J.; Cornelis, R.; Mees, L.; Vanholder, R. *Toxicol. Sci.* **1996**, *34*, 240.
- (205) Marafante, E.; Vahter, M.; Envall, J. *Chem. Biol. Interact.* **1985**, *56*, 225.
- (206) Vahter, M.; Marafante, E. *Chem. Biol. Interact.* **1983**, *47*, 29.
- (207) Styblo, M.; Delnomdedieu, M.; Thomas, D. J. *Chem. Biol. Interact.* **1996**, *99*, 147.
- (208) Cornelis, R.; Borguet, F.; De Kimpe, J. *Anal. Chim. Acta* **1993**, *283*, 183.
- (209) Cornelis, R.; Kimpe, J. *J. Anal. At. Spectrom.* **1994**, *9*, 945.
- (210) Schmidt, A.; Steier, S.; Otto, M. *Talanta* **2009**, *77*, 1830.
- (211) Bogdan, G. M.; Sampayo-Reyes, A.; Aposhian, H. V. *Toxicology* **1994**, *93*, 175.
- (212) Schmidt, A.; Steier, S.; Otto, M. *Talanta* **2009**, *77*, 1830.
- (213) Vahter, M.; Marafante, E. *Biol. Trace Elem. Res.* **1989**, *21*, 233.
- (214) Buchet, J. P.; Lauwers, R. *Arch. Toxicol.* **1985**, *57*, 125.
- (215) Oladimeji, A. A. *Ecotoxicol. Environ. Saf.* **1985**, *9*, 1.
- (216) Piotrowski, J. K.; Szymańska, J. A. *J. Toxicol. Environ. Health, Part A* **1976**, *1*, 991.
- (217) Arpadjan, S.; Eelik, G.; Taskesen, S.; Güeer, S. *Food Chem. Toxicol.* **2008**, *46*, 2871.
- (218) Hanlon, D. P.; Ferm, V. H. *Environ. Res.* **1986**, *40*, 380.
- (219) Styblo, M.; Yamauchi, H.; Thomas, D. J. *Toxicol. Appl. Pharmacol.* **1995**, *135*, 172.
- (220) Styblo, M.; Hughes, M. F.; Thomas, D. J. *J. Chromatogr. B: Biomed. Appl.* **1996**, *677*, 161.
- (221) Schmidt, A. C.; Mattusch, J.; Wennrich, R. *Microchim. Acta* **2005**, *151*, 167.
- (222) Reyes, L. H.; Mar, J. L. G.; Rahman, G. M.; Seybert, B.; Fahrenholz, T.; Kingston, H. M. *Talanta* **2009**, *78*, 983.
- (223) Naranmandura, H.; Suzuki, N.; Suzuki, K. T. *Chem. Res. Toxicol.* **2006**, *19*, 1010.
- (224) Chen, B.; Cao, F.; Yuan, C.; Lu, X.; Shen, S.; Zhou, J.; Le, X. C. *Anal. Bioanal. Chem.* **2013**, *405*, 1903.
- (225) Chen, B.; Arnold, L. L.; Cohen, S. M.; Thomas, D. J.; Le, X. C. *Toxicol. Sci.* **2011**, *124*, 320.
- (226) De Kimpe, J.; Cornelis, R. *Toxicol. Environ. Chem.* **1999**, *71*, 279.
- (227) Reinke, J.; Utthe, J. F.; Freeman, H. C.; Johnston, J. R. *Environ. Lett.* **1975**, *8*, 371.
- (228) Lu, M.; Wang, H.; Li, X.; Lu, X.; Le, X. C. *J. Chromatogr. A* **2009**, *1216*, 3985.
- (229) Raab, A.; Wright, S. H.; Jaspars, M.; Meharg, A. A.; Feldmann, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 2594.
- (230) Gong, Z.; Lu, X.; Ma, M.; Watt, C.; Le, X. C. *Talanta* **2002**, *58*, 77.
- (231) Francesconi, K. A.; Kuehnelt, D. *Analyst* **2004**, *129*, 373.
- (232) Dietz, C.; Sanz, J.; Sanz, E.; Muñoz-Olivas, R.; Cámarra, C. *J. Chromatogr. A* **2007**, *1153*, 114.
- (233) Ma, R.; McLeod, C. W.; Tomlinson, K.; Poole, R. K. *Electrophoresis* **2004**, *25*, 2469.
- (234) Feldmann, J. *TrAC Trends Anal. Chem.* **2005**, *24*, 228.
- (235) Mounicou, S.; Szpunar, J.; Lobinski, R. *Chem. Soc. Rev.* **2009**, *38*, 1119.
- (236) Szpunar, J.; Lobinski, R.; Prange, A. *Appl. Spectrosc.* **2003**, *57*, 102.
- (237) Harrington, C. F.; Clough, R.; Hansen, H. R.; Hill, S. J.; Pergantis, S. A.; Tyson, J. F. *J. Anal. At. Spectrom.* **2009**, *24*, 999.
- (238) B'hymer, C.; Caruso, J. *J. Chromatogr. A* **2004**, *1045*, 1.
- (239) Patel, P. C.; Goulhen, F.; Boothman, C.; Gault, A. G.; Charnock, J. M.; Kalia, K.; Lloyd, J. R. *Arch. Microbiol.* **2007**, *187*, 171.
- (240) Feldmann, J.; Devalla, S.; Raab, A.; Hansen, H. R. In *Organic Metal and Metalloid Species in the Environment: Analysis, Distribution, Processes and Toxicological Evaluation*; Hirner, A. V., Emons, H., Eds.; Springer Verlag: Berlin, 2004; p 41.
- (241) Whaley-Martin, K.; Koch, I.; Moriarty, M.; Reimer, K. *Environ. Sci. Technol.* **2012**, *46*, 3110.
- (242) Caumette, G.; Koch, I.; Moriarty, M.; Reimer, K. *J. Sci. Total Environ.* **2012**, *432*, 243.
- (243) Sun, X.; Tsang, C.; Sun, H. *Metallomics* **2009**, *1*, 25.
- (244) Munro, K. L.; Mariana, A.; Klavins, A. I.; Foster, A. J.; Lai, B.; Vogt, S.; Cai, Z.; Harris, H. H.; Dillon, C. T. *Chem. Res. Toxicol.* **2008**, *21*, 1760.
- (245) Miot, J.; Morin, G.; Skouri-Panet, F.; Férand, C.; Aubry, E.; Briand, J.; Wang, Y.; Ona-Nguema, G.; Guyot, F.; Brown, G. E. *Environ. Sci. Technol.* **2008**, *42*, 5342.
- (246) Mustra, D. J.; Warren, A. J.; Wilcox, D. E.; Hamilton, J. W. *Chem. Biol. Interact.* **2007**, *168*, 159.
- (247) Matzapetakis, M.; Farrer, B. T.; Weng, T.; Hemmingsen, L.; Penner-Hahn, J. E.; Pecoraro, V. L. *J. Am. Chem. Soc.* **2002**, *124*, 8042.
- (248) Chong, S.; Dill, K.; McGown, E. *J. Biochem. Toxicol.* **1989**, *4*, 39.
- (249) Vahter, M.; Marafante, E.; Lindgren, A.; Dencker, L. *Arch. Toxicol.* **1982**, *51*, 65.
- (250) Pott, W. A.; Benjamin, S. A.; Yang, R. S. H. *Rev. Environ. Contam. Toxicol.* **2001**, *169*, 165.
- (251) Das, A. K.; Chakraborty, R.; Luisa Cervera, M.; de la Guardia, M. *Microchim. Acta* **1996**, *122*, 209.

- (252) Wang, T.; Yan, P.; Squier, T. C.; Mayer, M. U. *ChemBioChem* **2007**, *8*, 1937.
- (253) Griffin, B. A.; Adams, S. R.; Tsien, R. Y. *Science* **1998**, *281*, 269.
- (254) Adams, S. R.; Campbell, R. E.; Gross, L. A.; Martin, B. R.; Walkup, G. K.; Yao, Y.; Llopis, J.; Tsien, R. Y. *J. Am. Chem. Soc.* **2002**, *124*, 6063.
- (255) Stroffekova, K.; Proenza, C.; Beam, K. G. *Pflügers Arch.-Eur. J. Physiol.* **2001**, *442*, 859.
- (256) Hearps, A. C.; Pryor, M. J.; Kuusisto, H. V.; Rawlinson, S. M.; Piller, S. C.; Jans, D. A. *J. Fluoresc.* **2007**, *17*, 593.
- (257) Huang, C.; Yin, Q.; Zhu, W.; Yang, Y.; Wang, X.; Qian, X.; Xu, Y. *Angew. Chem., Int. Ed.* **2011**, *50*, 7551.
- (258) Hannestad, U.; Lundqvist, P.; Sörbo, B. *Anal. Biochem.* **1982**, *126*, 200.
- (259) Zhou, G.; Jauhainen, M.; Stevenson, K.; Dolphin, P. J. *J. Chromatogr. B: Biomed. Sci. Appl.* **1991**, *568*, 69.
- (260) Kalef, E.; Walfish, P. G.; Gitler, C. *Anal. Biochem.* **1993**, *212*, 325.
- (261) Berleth, E. S.; Kasperek, E. M.; Grill, S. P.; Braunscheidel, J. A.; Graziani, L. A.; Pickart, C. M. *J. Biol. Chem.* **1992**, *267*, 16403.
- (262) Boerman, M. H. E. M.; Napoli, J. L. *Arch. Biochem. Biophys.* **1995**, *321*, 434.
- (263) Winski, S. L.; Carter, D. E. *J. Toxicol. Environ. Health, Part A* **1995**, *46*, 379.
- (264) Mizumura, A.; Watanabe, T.; Kobayashi, Y.; Hirano, S. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 119.
- (265) Jin, Y. J.; Yu, C. L.; Burakoff, S. J. *J. Biol. Chem.* **1999**, *274*, 28301.
- (266) Gitler, C.; Zarmi, B.; Kalef, E. *Anal. Biochem.* **1997**, *252*, 48.
- (267) Foley, T. D.; Stredny, C. M.; Coppa, T. M.; Gubbiotti, M. A. *Neurochem. Res.* **2010**, *35*, 306.
- (268) Foley, T. D.; Melideo, S. L.; Healey, A. E.; Lucas, E. J.; Koval, J. A. *Neurochem. Res.* **2011**, *36*, 232.
- (269) Bognumil, R.; Ullrich, V. *Methods Enzymol.* **2002**, *348*, 271.
- (270) Yan, H.; Wang, N.; Weinfeld, M.; Cullen, W. R.; Le, X. C. *Anal. Chem.* **2009**, *81*, 4144.
- (271) Zhang, X.; Yang, F.; Shim, J. Y.; Kirk, K. L.; Anderson, D. E.; Chen, X. *Cancer Lett.* **2007**, *255*, 95.
- (272) Heredia-Moya, J.; Kirk, K. L. *Bioorg. Med. Chem.* **2008**, *16*, 5743–5746.
- (273) Donoghue, N.; Yam, P. T. W.; Jiang, X. M.; Hogg, P. J. *Protein Sci.* **2000**, *9*, 2436.
- (274) Donoghue, N.; Hogg, P. J. *Meth. Enzymol.* **2002**, *348*, 76.
- (275) Dilda, P. J.; Decollogne, S.; Rossiter-Thornton, M.; Hogg, P. J. *Cancer Res.* **2005**, *65*, 11729.
- (276) Park, D.; Don, A. S.; Massamiri, T.; Karwa, A.; Warner, B.; MacDonald, J.; Hemenway, C.; Naik, A.; Kuan, K. T.; Dilda, P. J. *J. Am. Chem. Soc.* **2011**, *133*, 2832.
- (277) Moaddel, R.; Sharma, A.; Huseni, T.; Jones, G. S., Jr.; Hanson, R. N.; Loring, R. H. *Bioconjugate Chem.* **1999**, *10*, 629.
- (278) Bennett, T. A.; Edwards, B. S.; Sklar, L. A.; Rogelj, S. J. *Immunol.* **2000**, *164*, 4120.
- (279) Foley, T. D.; Clark, A. R.; Stredny, E. S.; Wierbowski, B. M. *Cell. Mol. Neurobiol.* **2011**, *1*.
- (280) Li, S.; Chen, Y.; Rosen, B. P. *Mol. Microbiol.* **2001**, *41*, 687.
- (281) Gong, Z.; Jiang, G.; Cullen, W. R.; Aposhian, H. V.; Le, X. C. *Chem. Res. Toxicol.* **2002**, *15*, 1318.
- (282) Mounicou, S.; Lobinski, R. *Pure Appl. Chem.* **2008**, *80*, 2565.
- (283) Lobinski, R.; Becker, J. S.; Haraguchi, H.; Sarkar, B. *Pure Appl. Chem.* **2010**, *82*, 493.
- (284) Garcia, J. S.; Magalhães, C. S.; Arruda, M. A. Z. *Talanta* **2006**, *69*, 1.
- (285) Ge, R.; Sun, H. *Sci. China Ser. B: Chem.* **2009**, *52*, 2055.
- (286) Sekhon, B. *Proc. Natl. Acad. Sci. India Section B-Biol. Sci.* **2008**, *78*, 299.
- (287) Haraguchi, H. *J. Anal. At. Spectrom.* **2003**, *19*, 5.
- (288) Jakubowski, N.; Lobinski, R.; Moens, L. *J. Anal. At. Spectrom.* **2003**, *19*, 1.
- (289) Aebersold, R.; Mann, M. *Nature* **2003**, *422*, 198.
- (290) Lin, D.; Tabb, D. L.; Yates, J. R. *Biochim. Biophys. Acta, Proteins Proteomics* **2003**, *1646*, 1.
- (291) Thilakaraj, R.; Raghunathan, K.; Anishetty, S.; Pennathur, G. *Bioinformatics* **2007**, *23*, 267.
- (292) Fomenko, D. E.; Marino, S. M.; Gladyshev, V. N. *Mol. Cell* **2008**, *26*, 228.
- (293) Delnomdedieu, M.; Basti, M. M.; Styblo, M.; Ottos, J. D.; Thomas, D. J. *Chem. Res. Toxicol.* **1994**, *7*, 621.
- (294) Bhattacharjee, H.; Rosen, B. P. *J. Biol. Chem.* **1996**, *271*, 24465.
- (295) Qin, J.; Fu, H. L.; Ye, J.; Bencze, K. Z.; Stemmler, T. L.; Rawlings, D. E.; Rosen, B. P. *J. Biol. Chem.* **2007**, *282*, 34346.
- (296) Zhou, T.; Radaev, S.; Rosen, B. P.; Gatti, D. L. *EMBO J.* **2000**, *19*, 4838.
- (297) Jacob, C.; Giles, G. I.; Giles, N. M.; Sies, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4742.
- (298) Torrance, J. W. *The Geometry and Evolution of Catalytic Sites and Metal Binding Sites*. Ph.D. Thesis, European Bioinformatics Institute, Robinson College, Cambridge, U.K., 2008.
- (299) Ramadan, D.; Rancy, P. C.; Nagarkar, R. P.; Schneider, J. P.; Thorpe, C. *Biochemistry* **2008**, *48*, 424.
- (300) Touw, D. S.; Nordman, C. E.; Stuckey, J. A.; Pecoraro, V. L. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 11969.
- (301) Ramadan, D.; Cline, D. J.; Bai, S.; Thorpe, C.; Schneider, J. P. *J. Am. Chem. Soc.* **2007**, *129*, 2981.
- (302) Cruse, W. B. T.; James, M. N. G. *Acta Crystallogr. Sect. B: Struct. Crystallogr. Cryst. Chem.* **1972**, *28*, 1325.
- (303) Brenna, O.; Perrella, M.; Pace, M.; Pietta, P. G. *Biochem. J.* **1975**, *151*, 291.
- (304) Zhu, X.; Larsen, N. A.; Basran, A.; Bruce, N. C.; Wilson, I. A. *J. Biol. Chem.* **2003**, *278*, 2008.
- (305) Glazer, A. N. *J. Biol. Chem.* **1968**, *243*, 3693.
- (306) Jain, M. K. In *Handbook of Enzyme Inhibitors*; Wiley: New York, 1982.
- (307) Zollner, H. *Handbook of Enzyme Inhibitors*; VCH: New York, 1993; p 1065.
- (308) Tsao, D. H. H.; Maki, A. H. *Biochemistry* **1991**, *30*, 4565.
- (309) Thompson, A. A.; Peersen, O. B. *EMBO J.* **2004**, *23*, 3462.
- (310) Maignan, S.; Guilloteau, J. P.; Zhou-Liu, Q.; Clément-Mella, C.; Mikol, V. *J. Mol. Biol.* **1998**, *282*, 359.
- (311) Tête-Favier, F.; Cobessi, D.; Boschi-Muller, S.; Azza, S.; Branlant, G.; Aubry, A. *Structure* **2000**, *8*, 1167.
- (312) Raman, C. S.; Li, H.; Martásek, P.; Babu, B. R.; Griffith, O. W.; Masters, B. S. S.; Poulos, T. L. *J. Biol. Chem.* **2001**, *276*, 26486.
- (313) Ye, J.; Ajees, A. A.; Yang, J.; Rosen, B. P. *Biochemistry* **2010**, *49*, S206.
- (314) Ye, J.; He, Y.; Skalicky, J.; Rosen, B. P.; Stemmler, T. L. *Biomol. NMR Assign.* **2011**, *5*, 109.
- (315) Lin, Y. F.; Yang, J.; Rosen, B. P. *J. Biol. Chem.* **2007**, *282*, 16783.
- (316) Gettins, P.; Coleman, J. E. *J. Biol. Chem.* **1984**, *259*, 4987.
- (317) Sowadski, J. M.; Handschumacher, M. D.; Krishna Murthy, H. M.; Foster, B. A.; Wyckoff, H. W. *J. Mol. Biol.* **1985**, *186*, 417.
- (318) Coutinho, E. *Pharma Times* **2006**, *38*, 10.
- (319) Forster, M. J. *Micron* **2002**, *33*, 365.
- (320) Schwede, T.; Sali, A.; Eswar, N.; Peitsch, M. C. Protein Structure Modeling. In *Computational Structural Biology*; World Scientific Publishing: Singapore, 2008.
- (321) Fiser, A. *Expert Rev. Proteom.* **2004**, *1*, 97.
- (322) Tsai, M. D.; Yan, H. *Biochemistry* **1991**, *30*, 6806.
- (323) Ruan, X.; Bhattacharjee, H.; Rosen, B. P. *J. Biol. Chem.* **2006**, *281*, 9925.
- (324) Konermann, L.; Stocks, B. B.; Pan, Y.; Tong, X. *Mass Spectrom. Rev.* **2010**, *29*, 651.
- (325) Lim, J.; Vachet, R. W. *Anal. Chem.* **2003**, *75*, 1164.
- (326) Suzuki, K. T.; Tomita, T.; Ogra, Y.; Ohmichi, M. *Chem. Res. Toxicol.* **2001**, *14*, 1604.
- (327) Shiobara, Y.; Ogra, Y.; Suzuki, K. T. *Chem. Res. Toxicol.* **2001**, *14*, 1446.

- (328) Schlichting, I. X-ray Crystallography of Protein–Ligand Interactions. In *Protein–Ligand Interactions: Methods and Applications*; Nienhaus, G. U., Ed.; Humana Press: Totowa, NJ, 2005; p 155.
- (329) Hu, P.; Ye, Q. Z.; Loo, J. A. *Anal. Chem.* **1994**, *66*, 4190.
- (330) Hille, R.; Stewart, R. C.; Fee, J. A.; Massey, V. *J. Biol. Chem.* **1983**, *258*, 4849.
- (331) Cline, D. J.; Thorpe, C.; Schneider, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 2923.
- (332) Chen, Y.; Rosen, B. P. *J. Biol. Chem.* **1997**, *272*, 14257.
- (333) Jiang, H.; Ding, J.; Chang, P.; Chen, Z.; Sun, G. *Chromatographia* **2010**, *71*, 1075.
- (334) Lazdunski, C.; Petitclerc, C.; Chappelet, D.; Lazdunski, M. *Biochem. Biophys. Res. Commun.* **1969**, *37*, 744.
- (335) Petitclerc, C.; Lazdunski, C.; Chappelet, D.; Moulin, A.; Lazdunski, M. *Eur. J. Biochem.* **1970**, *14*, 301.
- (336) Chen, B.; Cao, H.; Yan, P.; Mayer, M. U.; Squier, T. C. *Bioconjugate Chem.* **2007**, *18*, 1259.
- (337) Wilcox, D. E. *Inorg. Chim. Acta* **2008**, *361*, 857.
- (338) Spuches, A. M.; Kruszyna, H. G.; Rich, A. M.; Wilcox, D. E. *Inorg. Chem.* **2005**, *44*, 2964.
- (339) Rey, N. A.; Howarth, O. W.; Pereira-Maia, E. C. *J. Inorg. Biochem.* **2004**, *98*, 1151.
- (340) Schmidt, A. C.; Neustadt, M.; Otto, M. *J. Mass Spectrom.* **2007**, *42*, 771.
- (341) Schmidt, A. C.; Steier, S. *J. Mass Spectrom.* **2010**, *45*, 870.
- (342) Schmidt, A. C.; Mickein, K. *J. Mass Spectrom.* **2012**, *47*, 949.
- (343) Raji, M.; Frycak, P.; Beall, M.; Sakrout, M.; Ahn, J. M.; Bao, Y.; Armstrong, D.; Schug, K. *Int. J. Mass Spectrom.* **2007**, *262*, 232.
- (344) Gabelica, V.; Galic, N.; Rosu, F.; Houssier, C.; De Pauw, E. *J. Mass Spectrom.* **2003**, *38*, 491.
- (345) Pizauro, J. M.; Ciancaglini, P.; Leone, F. A. *Mol. Cell. Biochem.* **1995**, *152*, 121.
- (346) Crans, D. C.; Simone, C. M.; Holz, R. C.; Que, L., Jr. *Biochemistry (N. Y.)* **1992**, *31*, 11731.
- (347) Hirano, K.; Iizumi, Y.; Mori, Y.; Toyoshi, K.; Sugiura, M.; Iino, S. *J. Biochem.* **1985**, *97*, 1461.
- (348) Saha, A. K.; Crans, D. C.; Pope, M. T.; Simone, C. M.; Glew, R. H. *J. Biol. Chem.* **1991**, *266*, 3511.
- (349) Pappas, P. W.; Leiby, D. A. *J. Cell. Biochem.* **1989**, *40*, 239.
- (350) Cyboron, G. W.; Wuthier, R. E. *J. Biol. Chem.* **1981**, *256*, 7262.
- (351) Fernandes, S. S.; Furriel, R. P. M.; Petenisci, S. O.; Leone, F. A. *Biochem. Pharmacol.* **1999**, *58*, 841.
- (352) Sugiura, Y.; Kawabe, H.; Tanaka, H.; Fujimoto, S.; Ohara, A. *J. Biol. Chem.* **1981**, *256*, 10664.
- (353) Rezende, A. A.; Pizauro, J. M.; Ciancaglini, P.; Leone, F. A. *Biochem. J.* **1994**, *301*, 517.
- (354) Eaton, R. H.; Moss, D. W. *Biochem. J.* **1967**, *102*, 917.
- (355) Messens, J.; Martins, J. C.; Brosens, E.; Van Belle, K.; Jacobs, D. M.; Willem, R.; Wyns, L. *J. Biol. Inorg. Chem.* **2002**, *7*, 146.
- (356) Messens, J.; Hayburn, G.; Desmyter, A.; Laus, G.; Wyns, L. *Biochemistry* **1999**, *38*, 16857.
- (357) Zakharyan, R. A.; Ayala-Fierro, F.; Cullen, W. R.; Carter, D. M.; Aposhian, H. V. *Toxicol. Appl. Pharmacol.* **1999**, *158*, 9.
- (358) Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
- (359) Wildfang, E.; Zakharyan, R. A.; Aposhian, H. V. *Toxicol. Appl. Pharmacol.* **1998**, *152*, 366.
- (360) Ji, G.; Garber, E. A. E.; Armes, L. G.; Chen, C. M.; Fuchs, J. A.; Silver, S. *Biochemistry* **1994**, *33*, 7294.
- (361) Kedderis, G. L.; Elmore, A. R.; Crecelius, E. A.; Yager, J. W.; Goldsworthy, T. L. *Chem. Biol. Interact.* **2006**, *161*, 139.
- (362) Hayakawa, T.; Kobayashi, Y.; Cui, X.; Hirano, S. *Arch. Toxicol.* **2005**, *79*, 183.
- (363) Helmreich, E.; Cori, C. F. *Proc. Natl. Acad. Sci. U. S. A.* **1964**, *51*, 131.
- (364) Helmreich, E.; Cori, C. F. T. *Proc. Natl. Acad. Sci. U. S. A.* **1964**, *52*, 647.
- (365) De Verdier, C. H.; Gould, B. J. *Biochim. Biophys. Acta* **1963**, *68*, 333.
- (366) Eis, C.; Nidetzky, B. *Biochem. J.* **2002**, *363*, 335.
- (367) Nidetzky, B.; Eis, C.; Albert, M. *Biochem. J.* **2000**, *351*, 649.
- (368) Schinzel, R.; Drueckes, P. *FEBS Lett.* **1991**, *286*, 125.
- (369) Bhattacharjee, H.; Sheng, J.; Ajees, A. A.; Mukhopadhyay, R.; Rosen, B. P. *Biochemistry* **2010**, *49*, 802.
- (370) Keng, Y. F.; Wu, L.; Zhang, Z. Y. *Eur. J. Biochem.* **1999**, *259*, 809.
- (371) Zhang, Y. L.; Zhang, Z. Y. *Anal. Biochem.* **1998**, *261*, 139.
- (372) Szajn, H.; Csopak, H.; Folsch, G. *Biochim. Biophys. Acta, Enzymol.* **1977**, *480*, 154.
- (373) Whisnant, A. R.; Gilman, S. D. *Anal. Biochem.* **2002**, *307*, 226.
- (374) VanEtten, R. L.; Waymack, P. P.; Rehkopf, D. M. *J. Am. Chem. Soc.* **1974**, *96*, 6782.
- (375) Zhang, Z. Y.; Van Etten, R. L. *Arch. Biochem. Biophys.* **1990**, *282*, 39.
- (376) Chawla, S.; Mutenda, E. K.; Dixon, H. B.; Freeman, S.; Smith, A. W. *Biochem. J.* **1995**, *308*, 931.
- (377) Mutenda, E. K.; Sparkes, M. J.; Dixon, H. B. *Biochem. J.* **1995**, *310*, 983.
- (378) Adams, S. R.; Sparkes, M. J.; Dixon, H. B. *Biochem. J.* **1984**, *221*, 829.
- (379) Chawla, S.; Dixon, H. B. *J. Enzyme Inhib. Med. Chem.* **1995**, *8*, 255.
- (380) Visedo-Gonzalez, E.; Dixon, H. B. *Biochem. J.* **1989**, *260*, 299.
- (381) Lambeir, A. M.; Opperdoes, F. R.; Wierenga, R. K. *Eur. J. Biochem.* **1987**, *168*, 69.
- (382) Krietsch, W. K. G.; Pentchev, P. G.; Klingenberg, H.; Hofstätter, T.; Bücher, T. *Eur. J. Biochem.* **1970**, *14*, 289.
- (383) Nickbarg, E. B.; Knowles, J. R. *Biochemistry* **1988**, *27*, 5939.
- (384) Legrain, C.; Stalon, V. *Eur. J. Biochem.* **1976**, *63*, 289.
- (385) Wargnies, B.; Legrain, C.; Stalon, V. *Eur. J. Biochem.* **1978**, *89*, 203.
- (386) Glazer, A. N. *Proc. Natl. Acad. Sci. U. S. A.* **1968**, *59*, 996.
- (387) Galardy, R. E.; Kortylewicz, Z. P. *Biochem. J.* **1985**, *226*, 447.
- (388) Taniguchi, N.; Matsumoto, H. *Comp. Biochem. Physiol., Part B: Comp. Biochem.* **1985**, *81*, 587.
- (389) Kovach, I. M. *J. Enzyme Inhib. Med. Chem.* **1988**, *2*, 199.
- (390) Johnson, J. L.; Cusack, B.; Hughes, T. F.; McCullough, E. H.; Fauq, A.; Romanovskis, P.; Spatola, A. F.; Rosenberry, T. L. *J. Biol. Chem.* **2003**, *278*, 38948.
- (391) Auletta, J. T.; Johnson, J. L.; Rosenberry, T. L. *Chem. Biol. Interact.* **2010**, *187*, 135.
- (392) Rosenberry, T. L. *J. Mol. Neurosci.* **2010**, *40*, 32.
- (393) Ripoll, D. R.; Faerman, C. H.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 5128.
- (394) Wilson, I. B.; Silman, I. *Biochemistry* **1977**, *16*, 2701.
- (395) Fulladosa, E.; Debord, J.; Villaescusa, I.; Bollinger, J. C.; Murat, J. C. *Environ. Chem. Lett.* **2007**, *5*, 115.
- (396) Nafisi, S.; Sobhanmanesh, A.; Alimoghaddam, K.; Ghavamzadeh, A.; Tajmir-Riahi, H. A. *DNA Cell Biol.* **2005**, *24*, 634.
- (397) Mandal, S.; Ghosh, A.; Pati, B.; Das, A. *Toxicol. Environ. Chem.* **2009**, *91*, 219.
- (398) Kim, M.; Um, H. J.; Bang, S. B.; Lee, S. H.; Oh, S. J.; Han, J. H.; Kim, K. W.; Min, J.; Kim, Y. H. *Environ. Sci. Technol.* **2009**, *43*, 9335.
- (399) Phatak, P.; Dai, F.; Butler, M.; Nandakumar, M.; Gutierrez, P. L.; Edelman, M. J.; Hendriks, H.; Burger, A. M. *Clin. Cancer Res.* **2008**, *14*, 4593.
- (400) Stewart, R. C.; Hille, R.; Massey, V. *J. Biol. Chem.* **1984**, *259*, 14426.
- (401) George, G. N.; Bray, R. C. *Biochemistry* **1983**, *22*, 1013.
- (402) Zechel, D. L.; Konermann, L.; Withers, S. G.; Douglas, D. *Biochemistry* **1998**, *37*, 7664.
- (403) Konermann, L.; Pan, J.; Wilson, D. J. *Compr. Anal. Chem.* **2008**, *52*, 103.
- (404) McMullen, J. P.; Jensen, K. F. *Org. Process Res. Dev.* **2011**, *15*, 398.
- (405) Shin, S. H.; Luchian, T.; Cheley, S.; Braha, O.; Bayley, H. *Angew. Chem.* **2002**, *114*, 3859.

- (406) Shin, S. H.; Steffensen, M. B.; Claridge, T. D. W.; Bayley, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 7412.
- (407) Dilda, P. J.; Hogg, P. J. *Cancer Treat. Rev.* **2007**, *33*, 542.
- (408) Dixon, H. B. F. *Adv. Inorg. Chem.* **1996**, *44*, 191.
- (409) Rossman, T.; Stone, D.; Molina, M.; Troll, W. *Environ. Mutagen.* **1980**, *2*, 371.
- (410) Jacobson-Kram, D.; Montalbano, D. *Environ. Mutagen.* **1985**, *7*, 787.