The Mechanisms of Arsenic bioremediation from water by the Green Microalgae *Chlorella vulgaris*

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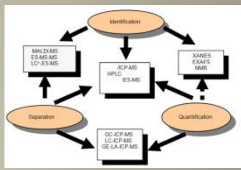
In collaboration with



TESLA
Trace element Speciation Laboratory Aberdeen

Leonardo Pantoja, Diane Purchase, Huw Jones, Jörg Feldmann and Hemda Garelick

OUTLINE

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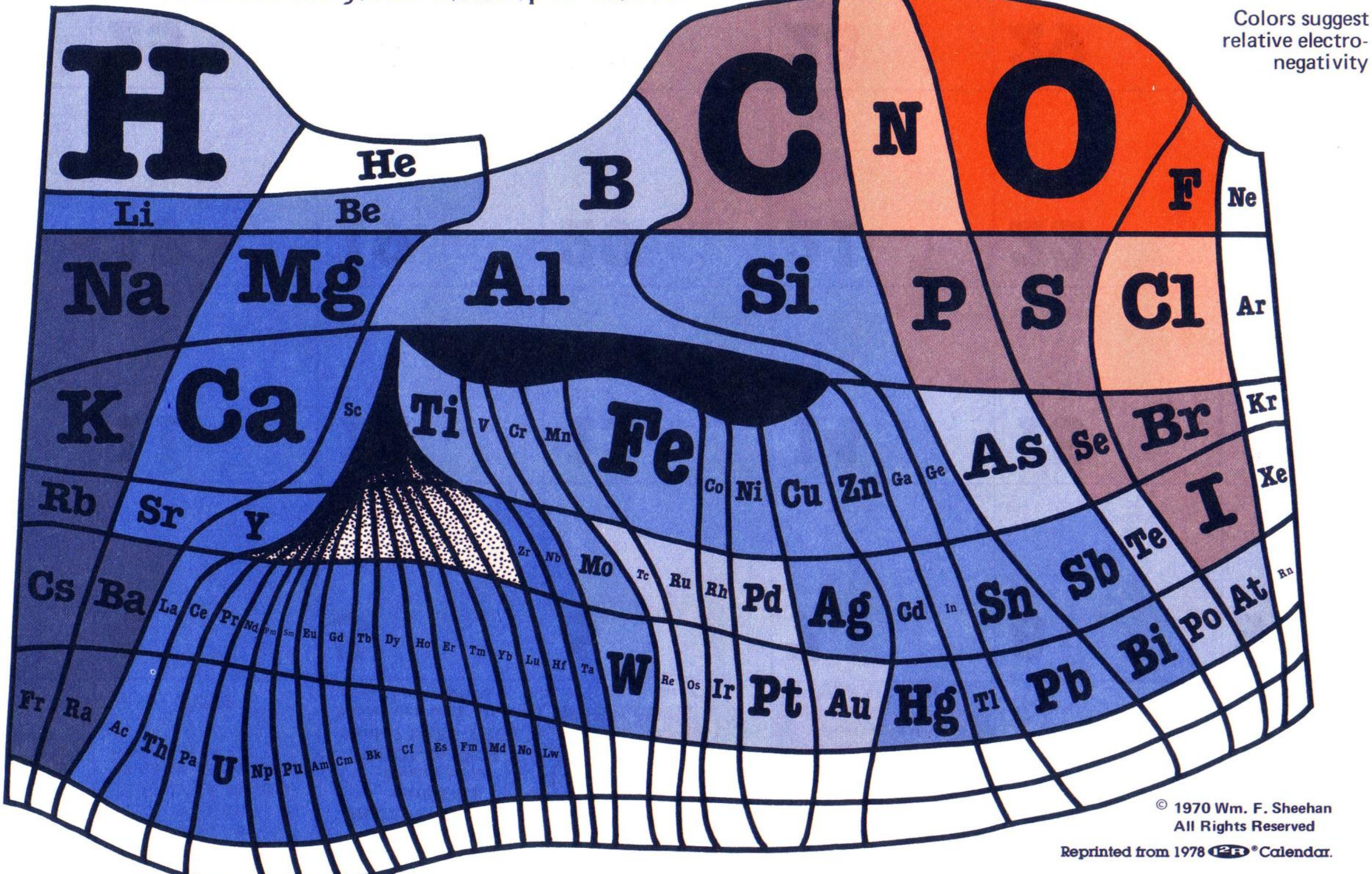
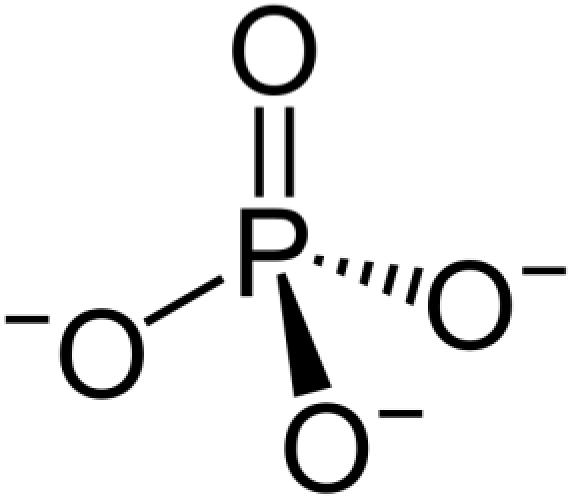
- Toxicity of arsenic to *C. vulgaris*
  - ASTM E1218 and flow cytometry
- Focused sonication for extraction for As-phytochelatin complexes
- Formation of As-GS/PC complexes as detoxification mechanism
  - Exposure to As(III) (Sodium arsenite)
  - Exposure to DMA(V) (Dimethylarsinic acid)
  - Exposure to As(V) (Sodium arsenate)
- Total As-GS/PC formation
- Transport of As-GS/PC to vacuoles
- Conclusions

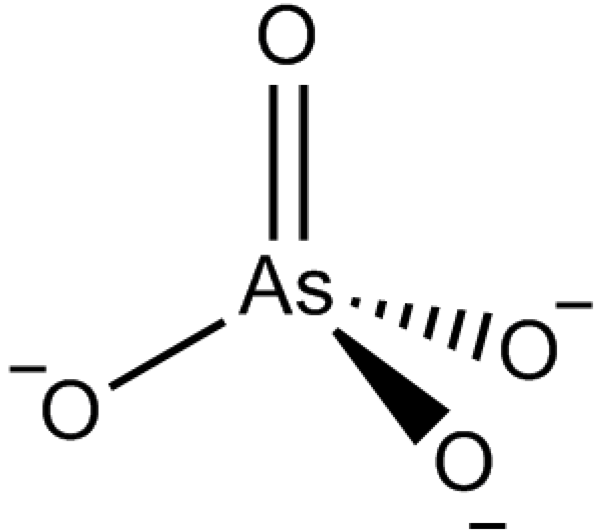
The Elements According to Relative Abundance

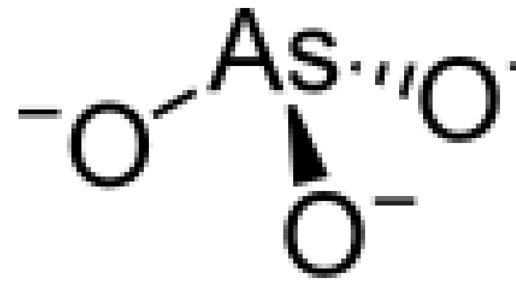
A Periodic Chart by Prof. Wm. F. Sheehan, University of Santa Clara, CA 95053
Ref. Chemistry, Vol. 49, No. 3, p 17–18, 1976

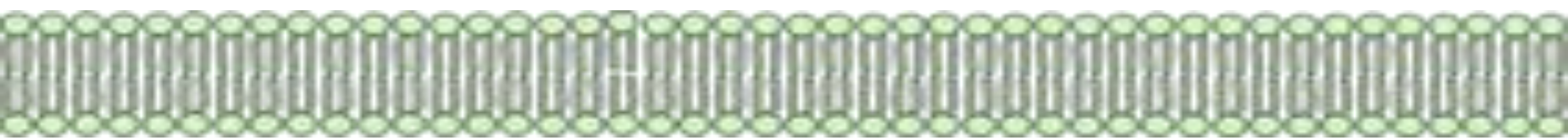


Arsenic toxicity (similarities)

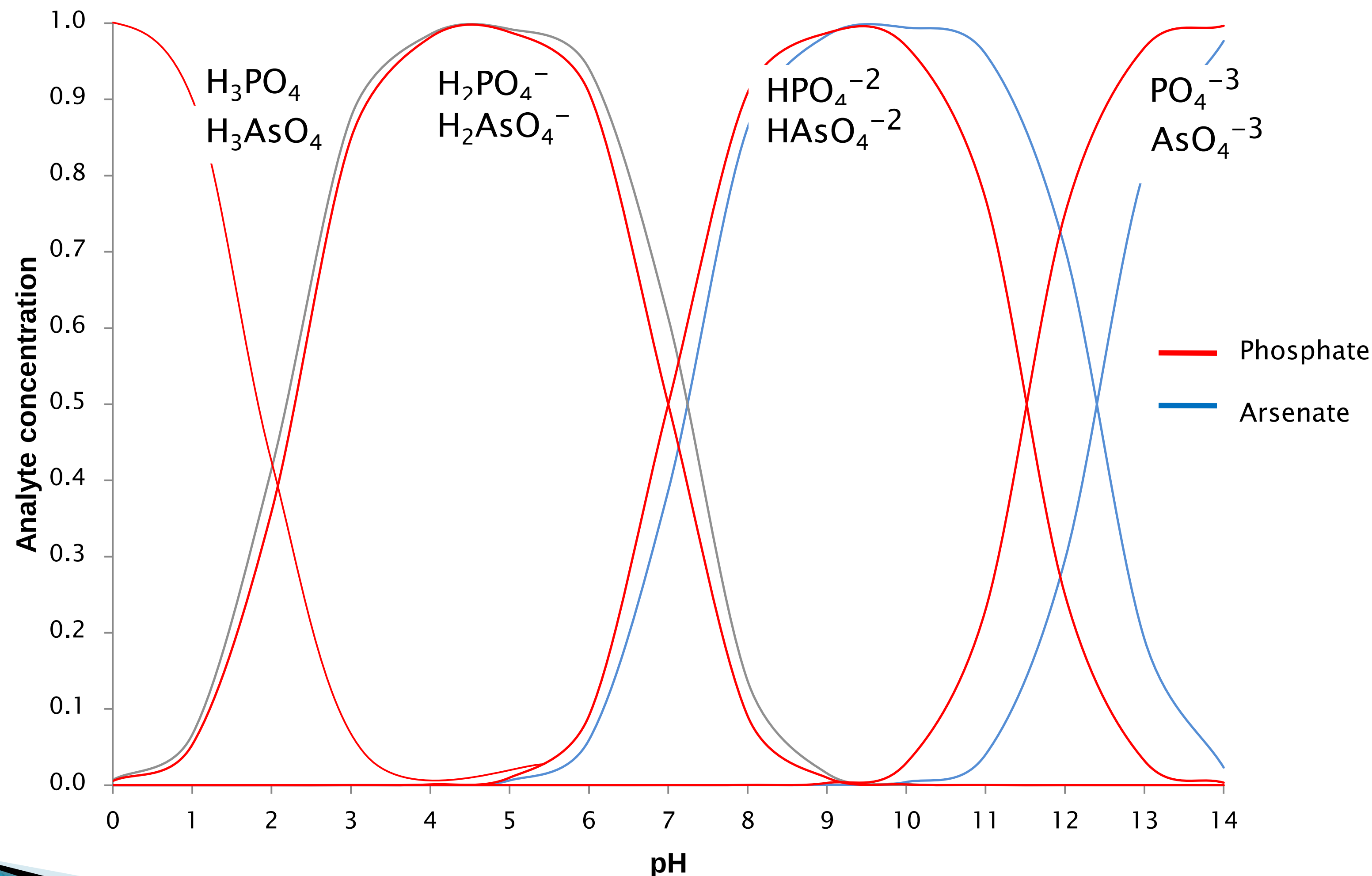








Arsenic toxicity (similarities)



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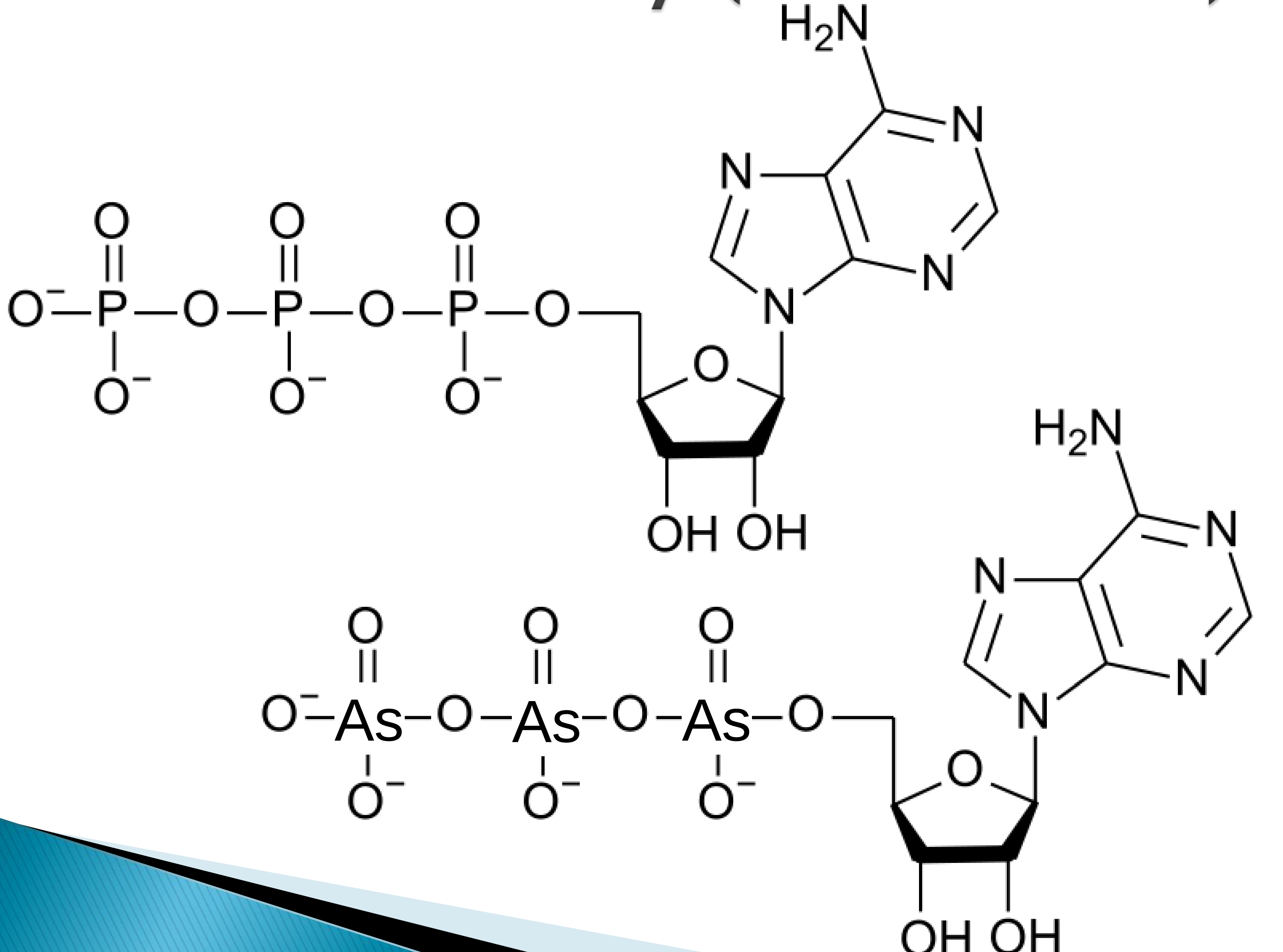

TOXICITY OF ARSENIC TO *C. VULGARIS*

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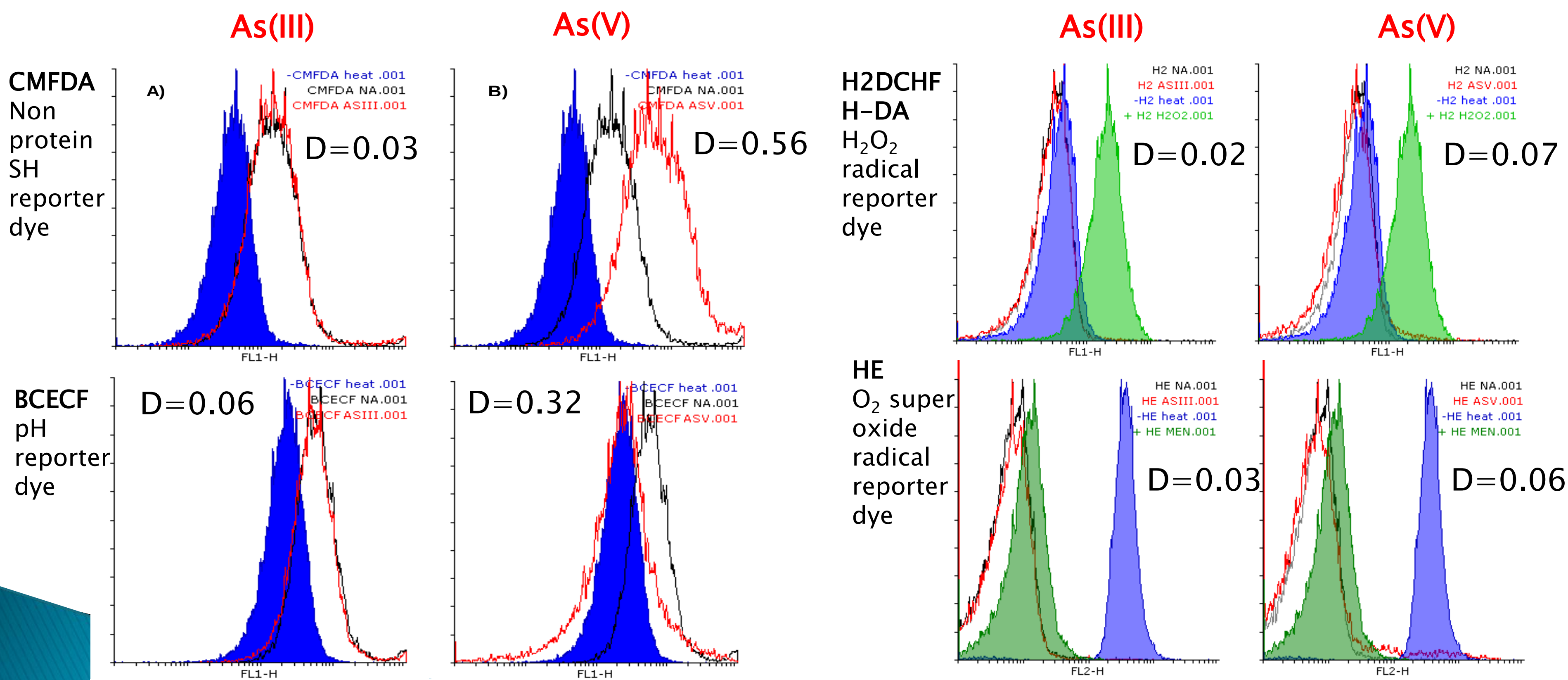
- Toxicity of As(III) (72 h @ 0.3mg L^{-1} phosphate)
  IC_{50}=64.23 mg L^{-1}
- Toxicity of As(V) (72 h @ 0.3mg L^{-1} phosphate)
  IC_{50}=1.07 mg L^{-1}

→ Chlorophyll as surrogate for cell health



n=20,000 cells, Strong D>0.3, Moderate 0.2< D <= 0.3, Weak 0.15< D <=0.20, Negligible D <= 0.15

Kolmogorov-Smirnov statistics

ENHANCED DETERMINATION OF ARSENIC-PHYTOCHELATIN COMPLEXES IN *C. VULGARIS* USING FOCUSED SONICATION EXTRACTION*

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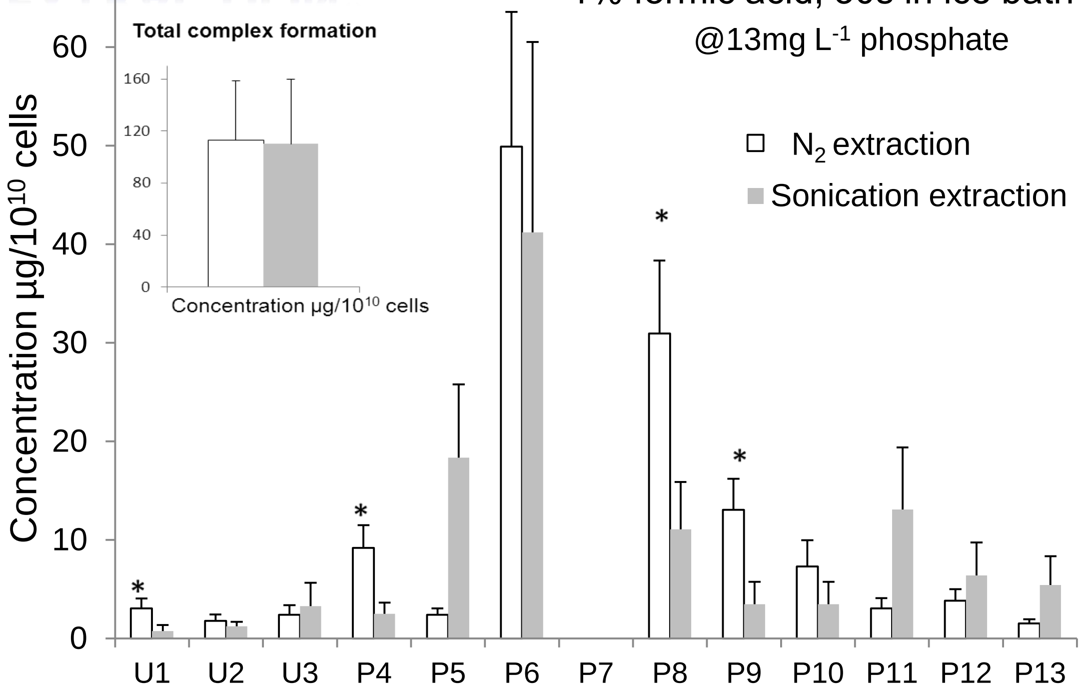
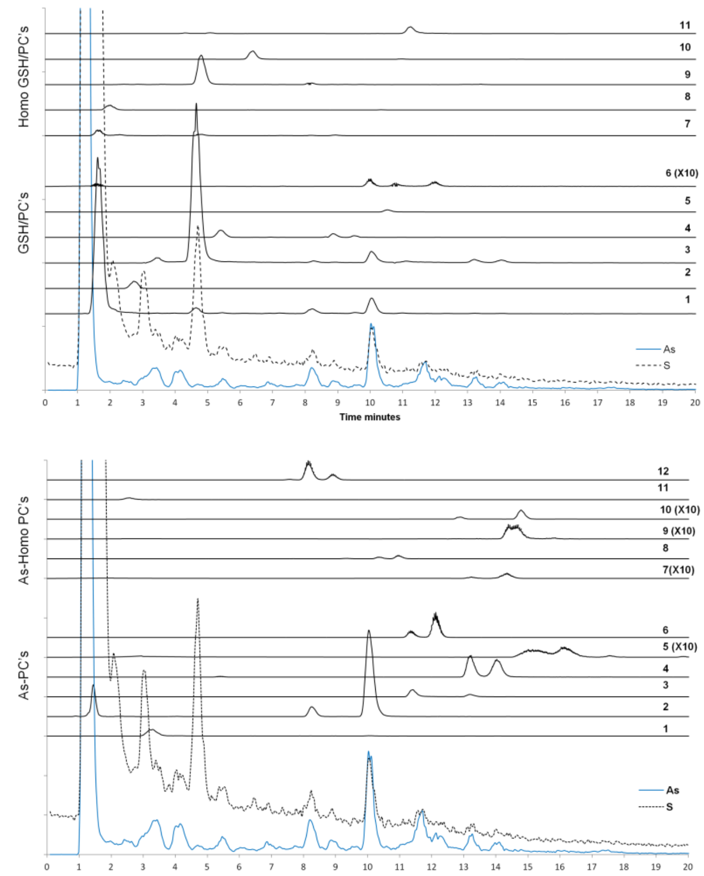
1% formic acid, 30s in ice bath (4˚C)

@13mg L^{-1} phosphate



Online HPLC-ICPMS/ESMS



21 peptides

Sample	µg As g^{-1}		% Recovery
Total extraction C. vulgaris	83.2	SE = 2.04, n= 6	
Sonication C. vulgaris	59.2	SE = 1.14, n = 6	71.10%
Quality control	µg As L^{-1}		
Total extraction Kelp	23.5	SE = 0.60, n = 9	
Sonication Kelp	22.2	SE = 0.28, n = 11	94.70%
SRM 2669	48.1	%RSD = 2.16	
Certifed value	50.7	± 6.3 (95% CI)	

SE= Standard error , n = Number of samples, % RSD = Relative standard deviation

*Leonardo Pantoja, Diane Purchase, Huw Jones, Jörg Feldmann and Hemda Garelick. Anal. Methods, 2014,6, 791-797

EXPOSURE TO As(III)*

Cells exposed to 50mg L^{-1} for 48h

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Unbound peptides

Molecule	Formula	Monoisotopic mass ($M+H^+$ or $M+2H^+$)	Experimental mass	Difference ppm
GSH/PC				
GSH	$C_{10}H_{17}N_3O_6S$	308.0916	308.0912	-1.34
GSSG	$C_{20}H_{32}N_6O_{12}S_2$	613.1598	613.1598	0
Reduced PC_2	$C_{18}H_{29}N_5O10S_2$	540.1434	540.1433	-0.16
Oxidised PC_2	$C_{18}H_{27}N_5O_{10}S_2$	538.1278	538.1289	2.04
Reduced PC_3	$C_{26}H_{41}N_7O_{14}S_3$	772.1952	772.1952	-0.01
Oxidised PC_3	$C_{26}H_{39}N_7O_{14}S_3$	770.1795	770.1795	0
Reduced PC_4	$C_{34}H_{53}N_9O_{18}S_4$	1004.247	Not found	
Ala GSH/PC				
γ-(Glu-Cys)-Ala	$C_{11}H_{19}N_3O_6S$	322.1073	322.1072	-0.21
γ-$(Glu\text{-}Cys)_2$-Ala	$C_{19}H_{31}N_5O_{10}S_2$	554.1591	554.1578	-2.2
desGly GSH/PC				
γ-(Glu-Cys)	$C_8H_{14}N_2O_5S$	251.0702	251.0706	1.55
γ-$(Glu\text{-}Cys)_2$	$C_{16}H_{26}N_4O_9S_2$	483.1219	483.1217	-0.58
γ-$(Glu\text{-}Cys)_3$	$C_{24}H_{38}N_6O_{13}S_3$	715.1737	715.1747	1.41

Glutathione/PC homologues (terminal amino acid Gly substituted by Ala, Ser, Gln, Glu or is absent)

Arsenic bound peptides

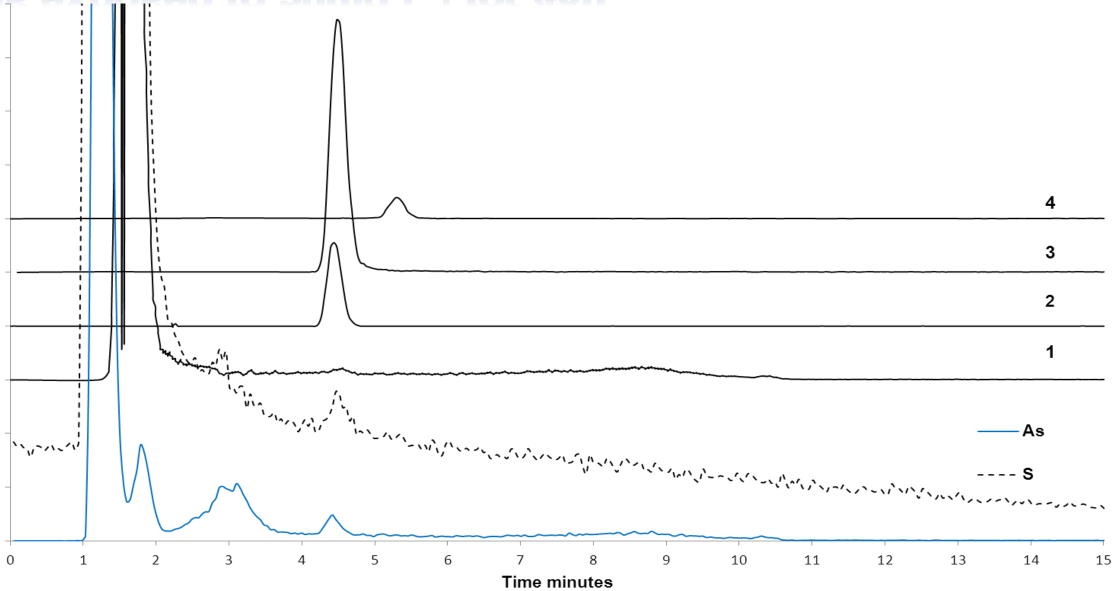
Molecule	Formula	Monoisotopic mass ($M+H^+$ or $M+2H^+$)	Experimental mass	Difference ppm
GSH/PC				
As(III)-PC_2	$C_{18}H_{28}N_5O_{11}S_2As$	630.0443	630.0437	-0.88
As(III)-PC_3	$C_{26}H_{38}N_7O_{14}S_3As$	844.0933	844.0931	-0.19
GS-As(III)-PC_2	$C_{28}H_{43}N_8O_{16}S_3As$	460.0666	460.0663	-0.06
		919.1253	919.1247	-0.69
As(III)-$(PC_2)_2$	$C_{36}H_{54}N_{10}O_{20}S_4As$	576.0925	576.0923	-0.37
As(III)-PC_4	$C_{34}H_{50}N_9O_{18}S_4As$	1076.1451	1076.1455	0.37
MMA(III)-PC_2	$C_{19}H_{30}N_5O_{10}S_2As$	628.0728	628.0729	0.12
Ala GSH/PC				
As(III)-γ-$(Glu\text{-}Cys)_3$-Ala	$C_{27}H_{40}N_7O_{14}S_3As$	858.109	858.1082	-0.87
GS-As(III)-γ-$(Glu\text{-}Cys)_2$-Ala	$C_{29}H_{45}N_8O_{16}S_3As$	467.0744	467.0744	0.09
As(III)-γ-$((Glu\text{-}Cys)_2)_2$-Ala	$C_{37}H_{57}N_{10}O_{20}S_4As$	583.1003	583.101	1.19
MMA(III)-γ-$(Glu\text{-}Cys)_2$-Ala	$C_{20}H_{32}N_5O_{10}S_2As$	642.0885	642.0889	0.66
desGly GSH/PC				
As(III)-γ-$(Glu\text{-}Cys)_2$	$C_{16}H_{25}N_4O_{10}S_2As$	573.0306	573.0318	2.11
GS-As(III)-γ-$(Glu\text{-}Cys)_2$	$C_{26}H_{40}N_7O_{15}S_3As$	431.5558	431.5558	-0.17

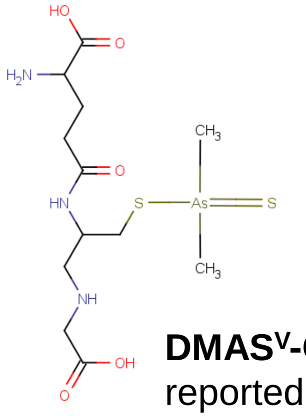
Newly reported peptides

*Leonardo Pantoja, Diane Purchase, Huw Jones, Jörg Feldmann and Hemda Garelick. Anal. Methods, 2014,6, 791-797

EXPOSURE TO DMA*

Cells exposed to 50mg L^{-1} for 48h





$DMAS^{V}$-GS has only been reported once in *Brassica Oleracea* plants:

Cabbage, broccoli, cauliflower, kale, Brussels sprouts, collard greens, savoy, among other

1 GSH (308), **2 $DMAS^{V}$-GS (444)**, **3** Reduced PC_2 (540) and **4** Oxidised PC_2 (538), As and S signals from HR-ICP-MS

@13mg L^{-1} phosphate

Molecule	Formula (M)	Monoisotopic mass ($M+H^+$)	Experimental mass	Difference ppm
GSH	$C_{10}H_{17}N_3O_6S$	308.0916	308.0918	0.65
Red PC_2	$C_{18}H_{29}N_5O_{10}S_2$	540.1434	540.1433	-0.16
Ox PC_2	$C_{18}H_{28}N_5O_{10}S_2$	538.1278	538.1288	1.86
$DMAS^{V}$-GS	$C_{12}H_{23}N_3O_6S_2As$	444.0244	444.0247	0.56

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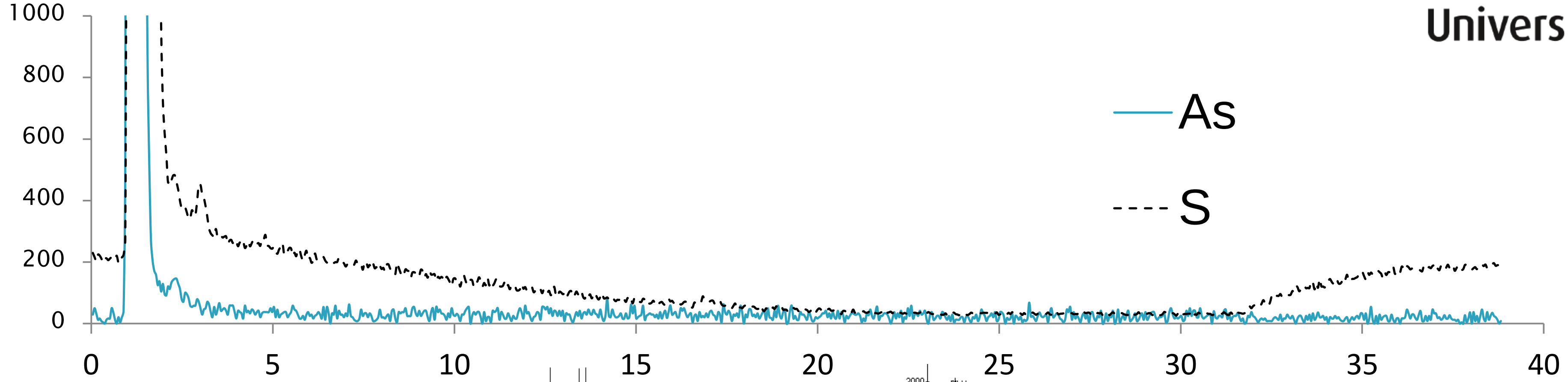
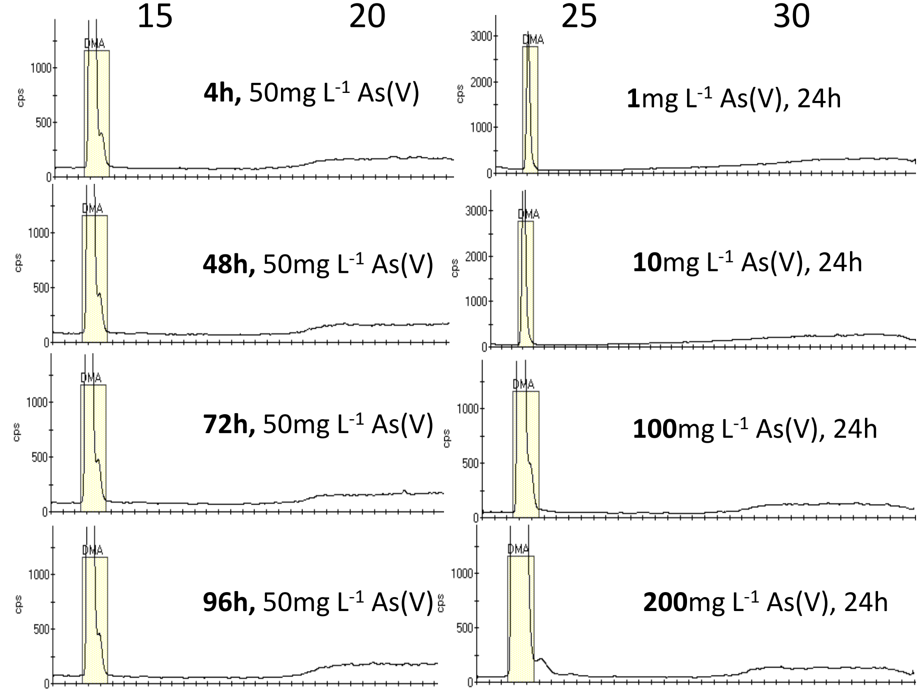
EXPOSURE TO As(V)*

Cells exposed to 50mg L^{-1} for 48h



@13mg L^{-1} phosphate

At this level of phosphate cells are not under stress



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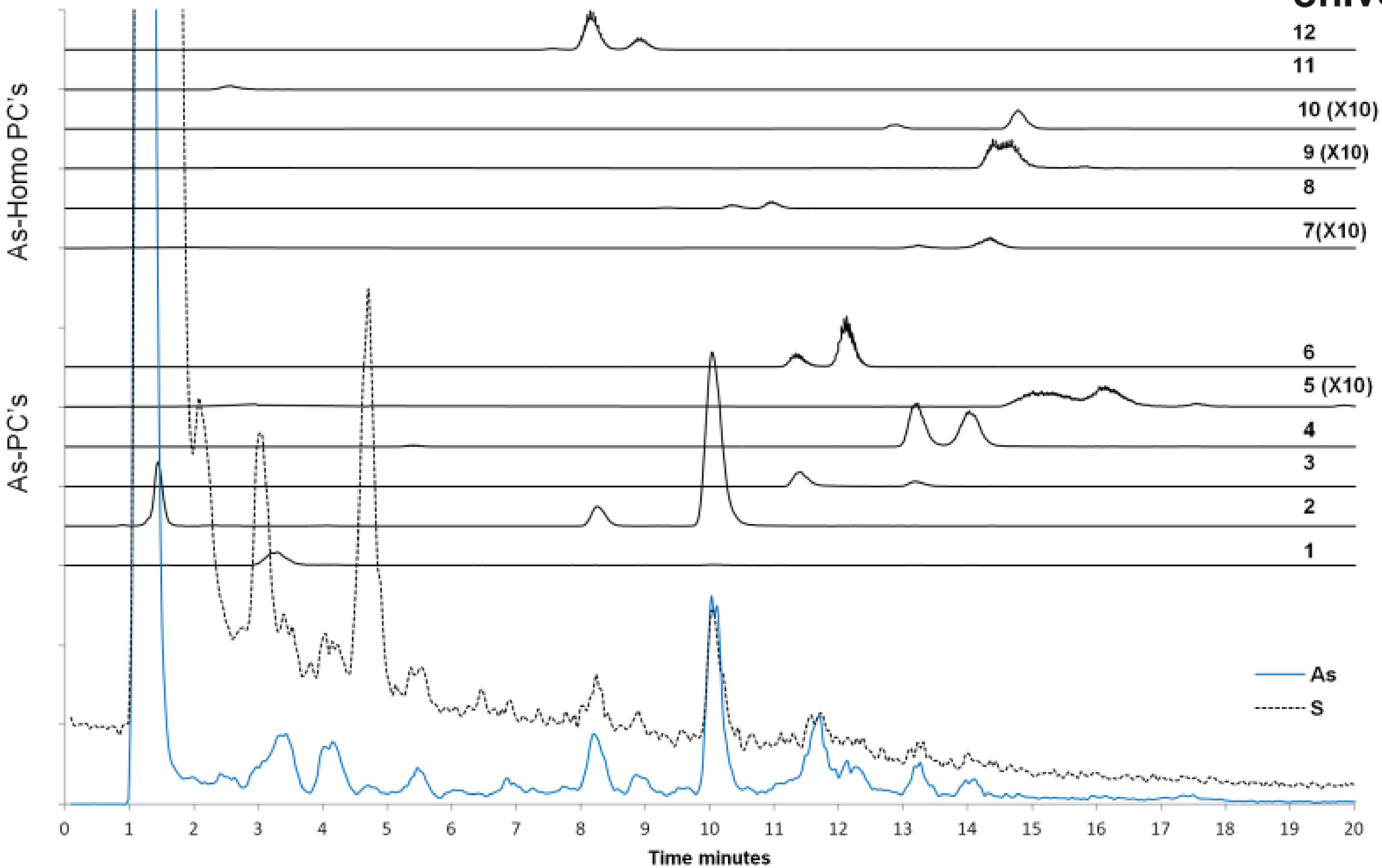
EXPOSURE TO As(III)*

@13mg L^{-1} phosphate



TOTAL As-GS/PC FORMATION

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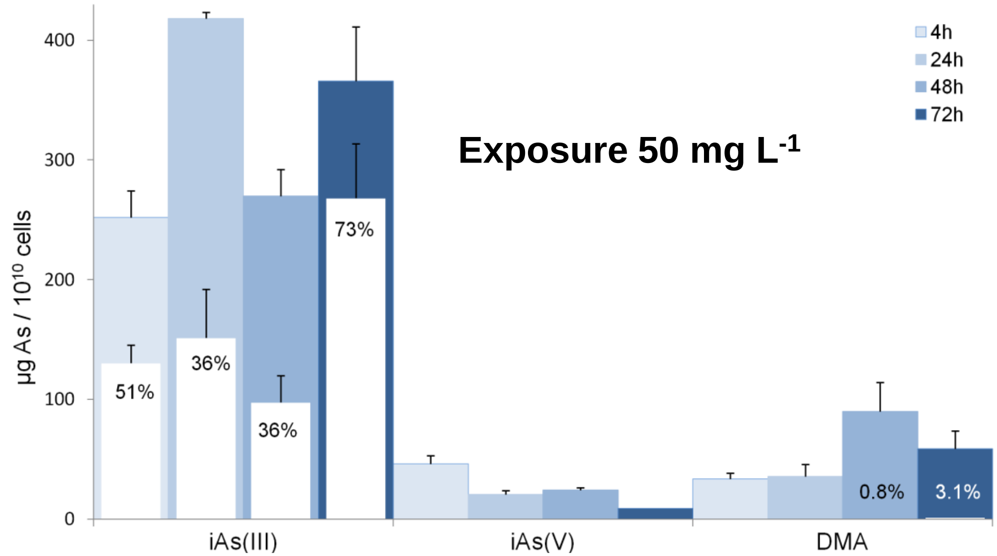
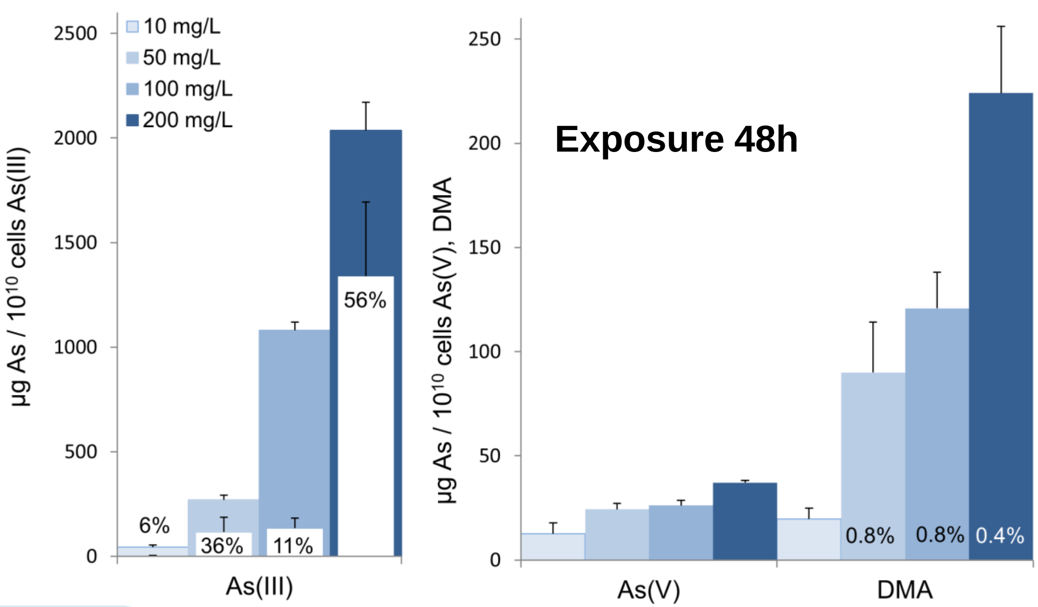
@13mg L^{-1} phosphate

- Coloured bars - total amount of arsenic
- White bars- amount of As-GS/PC (percentage of total arsenic)





Vertical bars denote + 1 standard error, n = 3

TRANSPORT OF As-GS/PC TO VACUOLES

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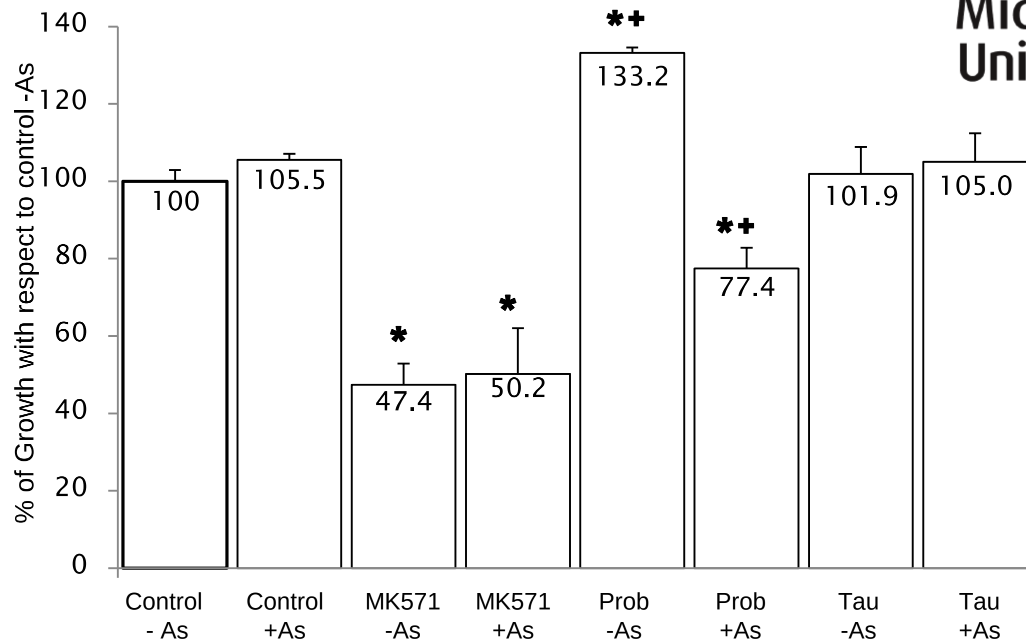
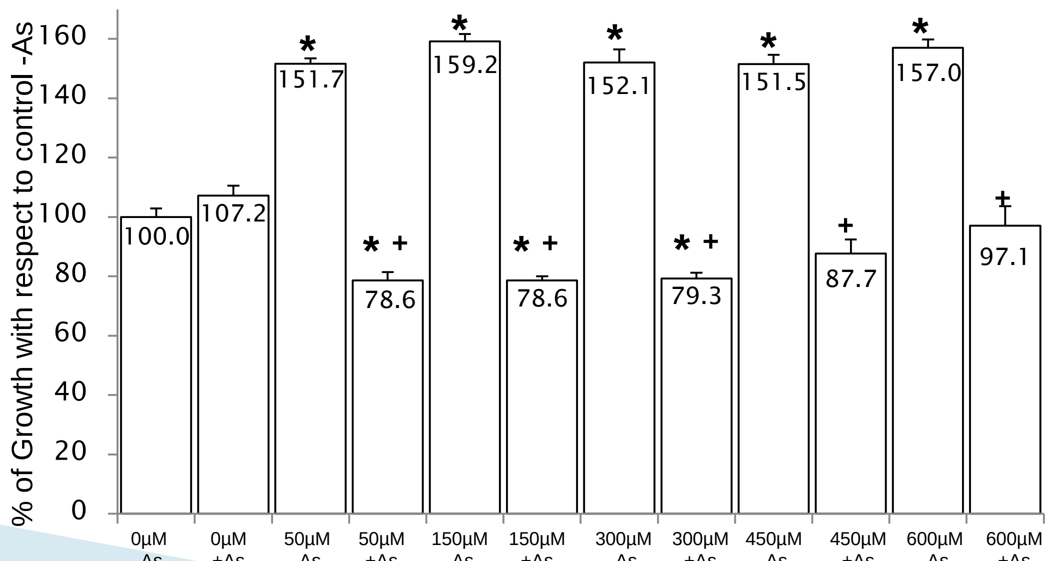
Toxicity (72 h) to As(III) (50 mg L^{-1})
@13mg L^{-1} phosphate



Test the presence of ABCC1 and ABCC2 inhibitors:

- MK571(25 μM)
- Probenecid (Prob, 500 μM)
- Sodium taurocholate (Tau, 50 μM).

ABC = ATP-binding cassette transporter
MRP = Multidrug resistance-associated protein



* (P < 0.05) with respect to control
\+ (P < 0.05) with respect to absence/presence of arsenic
Vertical bars + 1 standard error
Control n=6, experiments n = 3

TRANSPORT OF As-GS/PC TO VACUOLES

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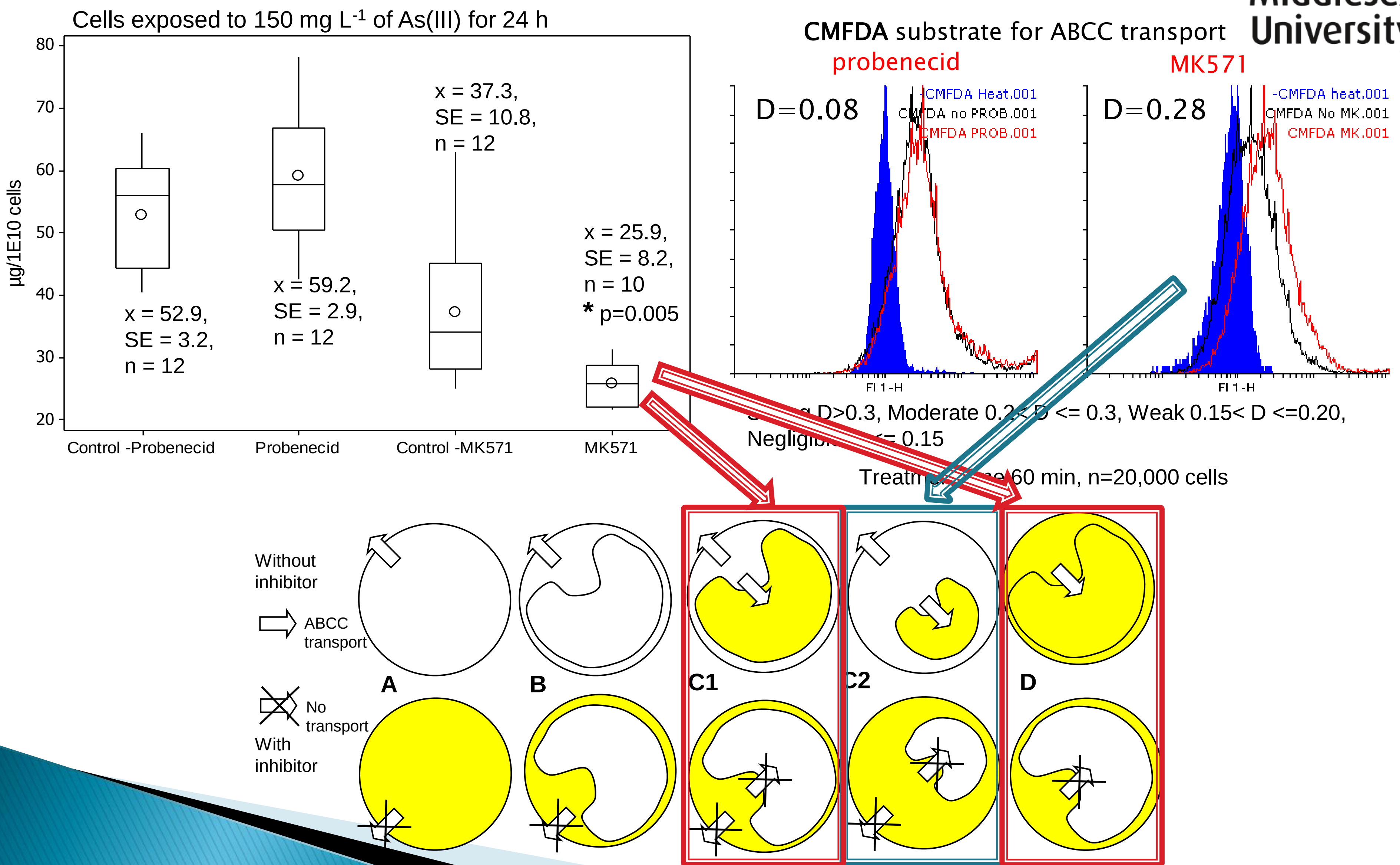

CONCLUSIONS

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- As(V) is more toxic than As(III) to *C. vulgaris* cells at the same concentration of phosphate
- As(III) triggers the formation of **As-GS/PC** molecules
- As(V) does not trigger the formation of **As-GS/PC** molecules when cells are not under stress
- DMA triggers the formation of **DMAS^{V}-GS** but it is unlikely that this is part of a detoxification mechanism
- ABCC1 and ABCC2 are involved in **As-GS/P**C transport to acidic vacuoles in *C. vulgaris*

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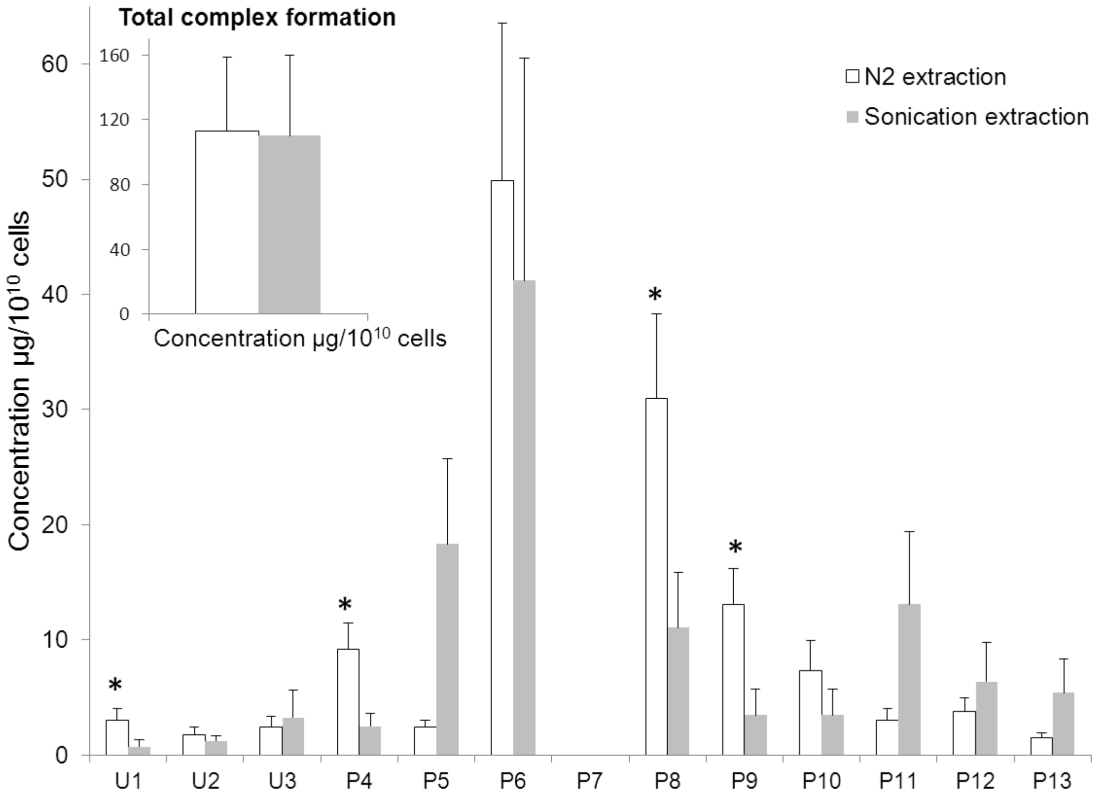
Thank you for listening.

Any questions?



U1-3	Unknowns
P4	GS-As(III)-PC_2/GS-As(III)- γ-$(Glu\text{-}Cys)_2$,
P5	As(III)- γ-$(Glu\text{-}Cys)_2$
P6	GS-As(III)-PC_2
P7	GS-As(III)- γ-$(Glu\text{-}Cys)_2$-Ala
P8	As(III)-PC_3/ MMA(III)-PC_2
P9	MMA(III)-PC_2
P10	As(III)-PC_3/ As(III)-$(PC_2)_2$
P11	As(III)-$(PC_2)_2$/ As(III)-γ-$(Glu\text{-}Cys)_3$-Ala/ As(III)- γ-$((Glu\text{-}Cys)_2)_2$-Ala/ MMA(III)- γ-$(Glu\text{-}Cys)_2$-Ala,
P12	As(III)-PC_4
P13	As(III)-PC_4