Immobilized Organo-Mercurial Lyase on Zeolite Using a Solid Binding Peptide

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Abstract Methylmercury (MeHg) compounds can form naturally, are highly toxic, and of concern because of their tendency to bio-accumulate. Certain bacteria have evolved mechanisms that can tolerate MeHg by first demethylating MeHg compounds, before further processing. Drawing inspiration from this demethylation mechanism controlled by a single organo-mercurial lyase in a protonolysis reaction, this research uses a recombinant gene that produces this lyase plus an additional polypeptide that selectively binds to zeolite particles, effectively tethering the enzyme to the solid substrate. This work is part of a broader attempt to create a fixed bed reactor for de-methylation of MeHg. Enzyme immobilization was achieved using a solid binding peptide (SBP) with high affinity for faujasite zeolite (FZ), the choice of binding substrate in the present work. The lyase is coded for by the *merB* gene, and a sequence with highly conserved active site homology was obtained from *E.coli* plasmid R8361b. The SBP plus *merB* sequence was designed such that the SBP was positioned either on the N or C-terminal of the construct. The DNA was synthesized commercially, and expressed in *E.coli* (BL21DE3 Star) using pET100® vector. Sanger sequencing was used to confirm construct in transformed cells using standard T7 oligos. Expression was lactose induced, and SDS-PAGE electrophoresis was used to confirm protein production and size. LC-MS/MS and sequence bio-analytics confirmed peptide sequence. Silica binding assays using SDS-PAGE confirmed binding of the enzyme to the silica substrate. Enzyme functionality results using a non-methylated mercuric compound were inconclusive, however the enzyme has not been assessed using MeHg compounds at this stage.

Keywords: *merB*, methylmercury, enzyme immobilization, demethylation

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1. Introduction

Methylated mercury species (MeHg) are highly toxic compounds that form mostly via bio-genic mechanisms under certain conditions, particularly anoxic environments with high anionic ligand content [1]. Methylmercury formation is often mediated by microbial activity, in particular by sulfur and iron reducing bacteria (SRB and FeRB respectively), methanogens, and firmicutes [2,3]. The proclivity for such toxic compounds to bio-accumulate in the food web [4], and therefore impact human health, is one of the main factors MeHg compounds have received much research attention over the past decade. Apart from several transgenic plant studies aimed at bioremediation [5,6], few studies have focused on bio-remediation of MeHg in aqueous bodies.

One strategy that may prove beneficial in this regard is inspired directly from evolved microbial metabolic mechanisms that deal with MeHg via *de-methylation*. Such de-methylating bacteria are ubiquitous and represented by a wide range of taxa [7]. The overall microbial strategy often involves the intracellular reduction of $\text{Hg}^{2+} \rightarrow \text{Hg}^0$ by a mercuric reductase enzymatic reaction, whereby Hg^0 then naturally volatilizes and passively diffuses from the

cell and out of the immediate surrounds into the atmosphere [8]. Where it is necessary for microbes to deal with MeHg, the reduction reaction is preceded by MeHg intracellular interaction with an organo-mercurial lyase, which cleaves the C-Hg bond through a protonolysis reaction [9], producing CH_4 and Hg^{2+} , where mercury is then passed to the mercuric reductase enzyme. It appears that the reductase enzyme activates release of the lyase bound Hg^{2+} [10]. The C-Hg enzymatic cleavage confers broad spectrum Hg resistance. HgR microbes with broad spectrum capabilities are characterized by the presence of the *merB* gene, which codes for the organo-mercurial lyase.

The mechanistic function of the *merB* product has not been fully elucidated, however it is highly likely to function as proposed by Parks *et.al.*, [9]. The enzyme itself is relatively small at around 24kDa, having been structurally characterized through crystallography, including various mutants, which have helped deduce the functional domain, key amino residues, and mechanistic architecture [11,12]. The may give this enzyme the ability to remain functional while immobilized on an extracellular solid substrate.

Immobilizing technologies greatly reduce the complexity of deploying this bio-catalytic strategy for remediation purposes, as living cells pose problems in terms of delivery to heterogeneous sites, geographical containment

within the contaminated plume (particularly so in a water column), nutrient supply, and competition from indigenous microbes etc. [13]. The concept explored in this work thus involves the extraction and immobilization of the *merB* gene product on a solid substrate, that being a faujasite type aluminosilicate zeolite (FZ) in particle form. This work represents the first stage of a broader project such that a fixed bed bio-catalytic reactor may be constructed to de-methylate aqueous MeHg compounds *in situ*.

Enzyme immobilization techniques often suffer from orientation and target accessibility issues [14]. An approach that may overcome these problems is the use of solid binding peptides (SBP's) that selectively recognize and bind to solid surfaces [15]. The SBP-enzyme conjugate can be either C or N terminal, depending on the active site location and orientation required, with the SBP used as a linker to tether the enzyme to the substrate. One such class of SBPs has a high affinity for certain zeolites and can thus be used to immobilize enzymes on silica-based substrates [16]. Zeolite is an ideal substrate for this work due to its inert and robust nature, and because it is readily synthesized and inexpensive. This particular SBP is highly amorphous, overcoming issues with accessibility and steric hindrance, and has been used to immobilize over 25 enzymes on silica based substrates which retained functionality [17]. The immobilization of the enzyme on a silica substrate can be achieved via the expression of a fusion protein that contains this SBP linker and the organo-mercurial lyase, which is then extracted and purified from *E. coli* cells and bound to solid particles. These particles may then be packed in a column, and it is envisioned that MeHg concentrations may be reduced or eliminated in aqueous media which interacts with these particles.

2. Materials and Methods

2.1. Recombinant Protein Construct

Two constructs were designed with the SBP flanking either N or C terminal of the *merB* sequence. The SBP used is a truncated version of one previously described [17,21]. *merB* sequence was derived from GenBank: U77087.1, *E. coli* plasmid R831b, and codon optimized for expression in *E. coli* BL21 DE3 Star. Constructs synthesized commercially by GeneArt®. Oligos designed as per requirements and synthesized commercially by Life Technologies Corporation. Constructs synthesized in a T7 mediated expression ready pET100® vector with existing N terminal polyhistidine-tag. These are designated N[FZ]SBP+*merB* and C[FZ]SBP+*merB* for N and C terminal zeolite binding SBP position respectively. *E. coli* DH5α (Life Technologies Corporation) cells as stock held in 20% glycerol at -80°C. Expression was carried out using *E. coli* BL21 DE3 Star cells (Life Technologies Corporation). The SBP linker amino acid sequence is (VKQTATSREEPPRLPSKHRPG)₃VKTQTAS.

2.2. Transformation and DNA Confirmation

Chemically competent *E. coli* DH5α and BL21 DE3 Star cells transformed using heat shock method. S.O.C.

media by Thermo Fischer (Cat No. 15544034). Incubation at 37°C (shaking at 120 rpm) with 100µg/mL Ampicillin (Amp) (Thermo Fischer Cat No. 11593027) doped media. Sanger sequencing was performed by University of Nevada, Reno, at the Nevada Genomics Centre (NGC), using approximately 500ng plasmid template to 10 Pmol primer. Plasmid extraction using Qiagen QIAprep® Spin Miniprep Kit (Cat No. 27104). Oligos supplied by NGC (Fwd): T7 primer 5' TAATACGACTCACTATAGGG. Pairwise sequence alignment was performed using Emboss Needle ©.

2.3. Expression and Purification

Lactose based auto-induction was performed using two-stage media. Cells were first grown to stationary phase using RM media plus glucose (per L: 20g casamino acids, 100mL 10x M9 salts, 1mL 1M MgCl₂, 10mL glucose, Amp to 1mM in dH₂O) at 37°C with shaking at 120rpm. Glucose ensures leaky T7 promoter induction can be tightly controlled, and was monitored regularly to ensure supply using glucose assays (Genzyme Diagnostics Glucose [Trinder] Assay Cat No. 22032). **ZYM-5052 Media** ((per L: 2 mL 1M MgSO₄, 40mL 25x *M. Sola* (per L: 88.75g NaHPO₄, 85g KH₂PO₄, 67g NH₄Cl, 17.75g Na₂SO₄), 2g lactose, 5mL 100% glycerol, 0.5g glucose)) was inoculated to OD₆₀₀ ≈ 0.5 with RM media stationary phase cells. Protein expression quantified using Thermo Fischer EZQ™ Protein Quantification Kit Cat No. R33200. Non induced cells, and cells containing neither N[FZ]SBP+*merB* or C[FZ]SBP+*merB* as controls. 500µL aliquots were removed at various time-points, pelleted (7 x 10³rpm^{-min} x 2mins), boiled in 80µL 2x SDS at 97°C for 7mins, centrifuged (7 x 10³rpm^{-min} for 3mins), and protein expression checked (for size) using 10µL soluble product on (NuPAGE™ 12% Bis-Tris) protein gels (1X MOPS/SDS) at 120V, followed by Coomassie Blue™ staining. Pelleted samples were stored at -20°C where necessary.

Protein purified using Ni-NTA agarose (Invitrogen, Cat. No. R901-01) by gravity fed column (Bio-Rad Poly-Prep® chromatography column (Cat. 731-1550). Binding buffer (20mM Tris, 500mM NaCl, 10mM Imidazole, 10% glycerol, pH 8.0). Wash buffer (20mM Tris, 100mM NaCl, 30mM Imidazole, pH 8.0). Elution buffer (20mM Tris, 100mM NaCl, 300mM Imidazole, pH 8.0) Synthesis of the desired amino sequence(s) was 100% confirmed by tandem mass spectrometry after trypsin digestion of excised polyacrylamide gel bands. LC-MS/MS was performed by the Nevada Proteomics Centre at the University of Nevada, Reno, using an LC gradient of 60-90 min with data analysis performed using Scaffold Version 4.8.4 with peptide threshold of 80% and protein (min 2) threshold of 99%.

2.4. Zeolite Binding Assay

Post purification, the enzyme was mixed with silica particles (SiliaFlash® Irregular Silica Gels, 60Å (Cat No. R12030B); Zeolite Y CBV100, Zeolyst Int; Sigma-Aldrich Silica Gel, 60Å (Cat 9385); and Faujasite Type Zeolite, Sigma-Aldrich (BCR704-10G)). The binding assay is described by Sunna *et. al.* (2013). Briefly, approximately 100mg particles are first vortexed in 1mL

wash buffer (10 mM Tris-HCl, pH 7.5, 100mM NaCl and 1% Triton-X100), repeated twice, and dried under N₂. Soluble protein was mixed with particles and incubated by slow rotation at room temperature for 1h. Any unbound fraction was removed after centrifugation at 10×10^3 rpm for 30s. The residual silica pellet was washed by vortexing with 100 μ L of 100 mM Tris-HCl buffer, pH 8.0. Washing was repeated twice and wash fraction samples retained. Bound protein was eluted using 100 μ L of 2x SDS PAGE-loading buffer followed by incubation at 99°C for 10 m. Pellet was vortexed briefly every 2 m during this elution step. The various fractions were analyzed using SDS-PAGE with Coomassie Blue™ staining.

2.5. Functional Assay

Functionality was assessed using an indirect assay on 250mL culture. Cells were pelleted (5krpm 15mins at 4°C), resuspended and briefly washed in lysis buffer (50mM Tris-HCl (pH 7.5), 1mM β -mercaptoethanol, 1mM PMSF, 0.5mM EDTA, 5%^{v/v} glycerol) before centrifugation (5krpm 15mins at 4°C). Cells were resuspended in 2mL lysis buffer and sonicated on ice for 15s. The mixture was centrifuged (15krpm 10mins at 4°C) and 20 μ L soluble fraction added to assay solution (50mM Tris-HCl (pH 7.5), 1mM L-Cys, 1 μ M 4-Chloromercuribenzoic acid (PCMB)) and absorbance at λ_{250} monitored every minute for 20 mins. Crude extract and FZ bound enzyme were tested.

3. Results and Discussion

3.1. Protein Expression

Figure 1. Average total protein expression ($\text{mg} \cdot \text{mL}^{-1}$) over time where T_0 is $\text{OD}_{600} = 0.5$ for C[FZ]SBP+merB and N[FZ]SBP+merB transformants (control was IDOLDH+)

Protein expression was first unsuccessfully attempted using IPTG, where it was noted that growth rates and viability were so variable after induction at $\text{OD}_{600} = 0.4 - 0.6$ at varying concentrations between 0.1 and 2.0 μ M final concentration IPTG that data sets could not be replicated. There is evidence that elevated levels of merB product can be toxic, somewhat modulated by inclusion body formation [18]. Although the expected product is soluble, the insoluble fraction was also analyzed but no evidence was obtained for the expected product in this fraction. The T7 promoter used in this plasmid (pET100®) is known to be somewhat leaky [19], and to rule out toxicity problems that may be occurring, we used a glucose and lactose based induction

method to tightly control expression, which proved much more successful.

Using total protein concentration as a proxy for growth rates, post induction batches were monitored every 2h for 8hrs, then sampled again at 24h. Figure 1., shows total soluble protein content after expression was induced using the glucose-lactose induction method. This result is much more in line with expectations, although of note is that the C[FZ]SBP+merB variant consistently does not seem to do as well, and in particular struggles consistently between 3 and 4h mark post induction. No attempt was made to solve that puzzle, although there is some evidence that C terminal additions to constructs are more difficult to express successfully [20]. Conversely, between 3 and 4h post induction appears to be the time with the highest growth rate for N[FZ]SBP+merB. Induction time was always at or adjusted to the same cell density so this cannot explain this variable. In any case, cells with N terminal construct performed consistently better using lactose based induction, and although C[FZ]SBP+merB cells did eventually grow at a comparable rate, they took additional time to recover post induction and appear to have suffered some considerable viability issues as well.

SDS-PAGE was used to help detect soluble protein fraction. Figure 2, is an image of the Coomassie Blue™ stained SDS-PAGE gel, indicating products of the expected MW of approximately 34.8kDa. Lane 14 is overspill and can be ignored. The induced bands are clearly not visible 2h post induction and we surmise this is due to residual glucose availability, thus repressing expression. Controls were both IPTG induced at $\text{OD}_{600}=0.5$ and these gel fractions represent 8h post induction.

Figure 2. SDS-PAGE gel indicating product at the expected MW of 34.8 kDa for both C and N(SBP)+merB constructs. Lane 1 is Bio-Rad protein ladder, Lane 2 is pUC19 control, Lanes 3-7 are C(SBP)+merB 2,4,6,8 and 24h post induction (respectively), Lane 8 is IDOLDH(+) control, and Lanes 9-13 are N(SBP)+merB 2,4,6,8 and 24h post induction (respectively). Lane 14 is overspill and should be ignored

In order to confirm N[FZ]SBP+merB growth, cells were again grown until 8h post induction, this time using auto induction method only. As is clearly visible in Figure 3, the N terminal variant successfully grew, and the time series clearly indicates any toxicity issues seem to have been mitigated as progressively more protein is found in the soluble fraction as growing time is extended.

The wells were each loaded with 10 μ L sample ruling out inconsistent volumes, indicating the sample was progressively more concentrated with the desired product.

Figure 3. SDS-PAGE gel image of N[FZ]SBP+merB using auto induction media Lane 1. Bio-Rad Broad Spectrum protein ladder, Lane 2. Non-induced, Lane 3. 0.5 OD600, Lane 4. 2h post induction, Lane 5. 4h post induction, Lane 6. 6h post induction, Lane 7. 8h post induction

3.2. Sequencing

Cloning confirmation of the desired construct was obtained using Sanger Sequencing, and expression was confirmed using LC-MS/MS after trypsin digestion (data in supplementary). The results for C terminal variant were not in line with predicted results for either. In particular, C[FZ]SBP+merB variant mass spectrometry results were poor. There was no match for a peptide adjacent to the C term although one was predicted. MS results for N[FZ]SBP+merB were more conclusive, showing 8 peptide matches representing 80aa's with over 25% coverage, including coverage of the SBP conjugate. Due to the many problems associated with the C terminal variant it is likely the N terminal variant would be easier to scale up production.

The SBP has an amorphous structure which can assist with enzyme orientation and steric hindrance issues. This structure was confirmed theoretically using the Foldindex© [22] software to ascertain whether the N terminal SBP segment of the polypeptide conjugate is folded as opposed to the functional *merB* derived segment. Figure 4 clearly indicates the amino sequence containing the SBP is predicted to be unfolded.

This amorphous structure is important because when tethering enzymes to solid substrates, maximum flexibility is required to ensure the target molecule, in this case methylmercury, is not hindered from interacting with the functional domain of the enzyme. The SBP structure allows for greater degrees of freedom to as opposed to direct binding on solid substrates.

As sequencing data indicated successful production of the enzyme with SBP for the N[FZ]SBP+merB variant, a series of silica/zeolite binding assays were performed.

Figure 4. Foldindex© prediction for intrinsically unfolded segment of the N[FZ]SBP+merB variant, indicating the SBP segment is intrinsically unfolded.

3.3. Binding Assays

Solid binding peptides are a valuable tool for recombinant production of immobilized enzymes. The particular SBP used in the current approach is known to have a very high selective affinity for FZ, affording it great potential for use in a variety of contexts [21]. To evaluate the binding of our products to silica and zeolites, a binding assay was performed. Although the binding mechanism has not been fully elucidated, to assess the robustness of the N[FZ]SBP+merB:FZ conjugate, particle bound enzyme were subjected to repeated washing by 30s vortexing in wash buffer and the wash fractions analyzed for unbound enzyme.

As some native *E.coli* proteins are known to bind to silica, both N and C terminal products were purified by HIS-tag and exchange buffer treatments prior to binding. To ensure purity, silica and zeolite particles were prewashed in wash buffer and dried under N₂. No enzyme was detected for the C[FZ]SBP+merB variant after purification. This was not surprising given the lack of detection of the SBP conjugate with MS/MS, troublesome growth issues, and poorly resolved MW data for this variant. Considering the ongoing issues with the C terminal variant, we decided to concentrate on N[FZ]SBP+merB, and all following results are based on that construct.

Our initial use of 60Å silicas and CBV100 showed low or no binding capacity which was expected and is in line with previous research [17,21]. Type Y faujasite zeolite is known to have greater binding capacity for this SBP. Figure 5 and Figure 6 tend to support that notion. Further, we have used a truncated version of the SBP where only 3 repeats of the given amino sequence were used instead of the four used by Nygard *et.al.*, (2002) in their initial work. This was proposed by Sunna *et.al.*, (2013) and seems to be confirmed by our results.

Figure 6 indicates that the N[FZ]SBP+merB variant was successfully bound to FZ type zeolite. The unbound fraction in lane 2 is likely due to loading issues where too much enzyme was mixed with particles and maximum binding capacity was breached. No attempt was taken to optimize binding amounts, but this would obviously be required in scale up. Similarly, the first two wash fractions indicate loss of enzyme, and we surmise this is due to overloading because, by wash 3 fraction, there is little or no enzyme

coming off. The eluted fraction clearly shows that the enzyme had previously been successfully bound to the FZ type zeolite, and so the fact there was no enzyme in the third wash fraction indicates what was bound was bound strongly.

Figure 5. SDS-PAGE gel indicating SBP does not bind to Siliaflash or Sigma-Aldrich 60Å silica gel, or CBV100 zeolite. Lane 1. Ladder (Bio-rad broad range), Lane2. Crude extract, Lane 3. Ni purified, Lane 4. Unbound on Sigma-Aldrich gel, Lane 5. Combined wash fractions for previous, Lane 6. Eluted fraction, Lane 7. Unbound on CBV100 zeolite, Lane 8. Combined wash fractions for previous, Lane 9. Eluted fraction, Lane 10. Siliaflash gel unbound, Lane 11. Combined wash fractions for previous, Lane 12. Eluted fraction

Figure 6. SDS-PAGE gel indicating SBP binds to Type Y Faujasite zeolite. Lane 1. Ladder (Bio-rad), Lane2. Unbound fraction, Lane 3. Wash fraction 1, Lane 4. Wash fraction 2, Lane 5. Wash fraction 3, Lane 6. Eluted fraction showing enzyme was bound, Lane 7. Repeated ladder

Our attempts to assess functionality of the enzyme were not conclusive at this stage. In particular, we are yet to quantify enzymatic activity using methylmercury compounds. In previous work by the author (unpublished) the companion *merA* gene was designed and bound to silica in a similar fashion. There was evidence of retained functionality in that case. One issue with the *merA* enzymatic reaction is that it requires NADPH^+ as a co-factor, making the functionality more difficult and expensive to replicate while the enzyme was immobilized on a solid substrate. However if a suitable co-factor analog can be found then this technology may be more practical. One can envision a co-localised *merA*/*merB* set up where both enzymes could be bound to the same particle providing two stage catalysis resulting in not only degradation but removal of MeHg from contaminated waters.

4. Conclusion

Successful expression and binding to a solid zeolite substrate for this novel recombinant enzyme paves the

way for a methyl mercury filter to be designed and employed to remediate contaminated waters by degrading the compound to its much less toxic form. The benefits of binding with an amorphous peptide linker are that it offers advantages over other traditional binding methods in terms of orientation and steric hindrance problems that are often encountered. Even though we were unable at this stage to show the *merB* derived lyase enzyme remained functional while bound, this technique has been used to bind enzymes that remained functional. Difficulties with the highly toxic nature of methylmercury precluded any further optimization at this preliminary stage. The method used in this work seems to be promising in that both *merB* and *merA* (unpublished) have now been bound to silica nanoparticles using this method. More research is required to address how to implement an efficient and cost effective way to degrade and remove MeHg from aqueous environments.

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Competing Interests Statement

The authors whose names are listed on this manuscript certify that they have NO affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript, and that there are no conflicts of interest to disclose.

Abbreviations

Hg = mercury; MeHg = methylated mercury; SRB = sulfur reducing bacteria; FeRB = iron reducing bacteria; SBP = solid binding peptide; HgR = mercury resistant microbes; OD_{600} = optical density at $\lambda 600$ nm, FZ = faujasite type zeolite.

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Supplementary data

The following are for N terminal variant only.

N8_custom (100%), 34,806.2 Da

N8_custom

8 exclusive unique peptides, 8 exclusive unique spectra, 16 total spectra, 80/317 amino acids (25% coverage)

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M R G S H H H H H H   G M A S M T G G Q Q   M G R D L Y D D D D   K D H P F T V K T Q
A T S R E E P P R L   P S K H R P G V K T   Q A T S R E E P P R   L P S K H R P G V K
T Q A T S R E E P P   R L P S K H R P G V   K T Q T A S K L A P   Y I L E L L T S V N
R T N G T A D L L V   P L L R E L A K G R   P V S R T T L A G I   L D W P A E R V A A
V L E Q A T S T E Y   D K D G N I I G Y G   L T L R E T S Y V F   E I D D R R L Y A W
C A L D T L I F P A   L I G R T A R V S S   H C A A T G A P V S   L T V S P S E I Q A
V E P A G M A V S L   V L P Q E A A D V R   Q S F C C H V H F F   A S V P T A E D W A
S K H Q G L E G L A   I V S V H E A F G L   G Q E F N R H L L Q   T M S S R T P

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MS results following trypsin digest as per Scaffold v 4.8.4. Highlighted are peptide matches.

Scaffold Viewer - Similarity - McCarthy_sp_20180215

File Edit View Experiment Export Quant Window Help

Protein Threshold: 99.0% Min # Peptides: 2 Peptide Threshold: 90%

Alkylmercury lyase OS=Klebsiella pneumoniae GN=merB PE=3 SV=1

Index	Peptide	Exclusive To	Yield	P62225	P77072
1	DGNIIGYGLTLR	Alkylmercury	100%	100%	
2	ETSYVFEIDRR	Alkylmercury	100%	100%	
3	ETSYVFEIDRR	Alkylmercury	100%	100%	
4	HLLQTMSSRP	Alkylmercury	100%	100%	
5	HLLQTMSSRP	Alkylmercury	6%	6%	
6	HQGLEGLAIVSHEAFGLGQE...	Alkylmercury	99%	99%	
7	TNGTADLLVPLLR	Alkylmercury	100%	100%	
8	TTLAGILDWPAER	Alkylmercury	100%	100%	
9	VAAVLEQATSTEYDK	Alkylmercury	100%	100%	
10	VAAVLEQATSTEYDKDGNIG...	Alkylmercury	100%	100%	

Identifications Spectrum Spectrum/Model Error Fragmentation Table

Sequence	Prob	SEQU...	SEQU...	NTT	Modifications	Observed	Actual Mass	Charge	Delta ...	Delta ...	Ret...
(R)TNGTADLLVPLLR(E)	100%	3.59	0.53	2		691.90	1,381.79	2	-0.00015	-0.11	1350
(R)TNGTADLLVPLLR(E)	100%	2.82	0.50	2		691.90	1,381.79	2	-0.000...	-0.072	1360
(R)TNGTADLLVPLLR(E)	94%	1.80	0.41	2		691.90	1,381.79	2	0.000093	0.067	1320
(R)TNGTADLLVPLLR(E)	100%	2.38	0.46	2		691.90	1,381.79	2	0.00012	0.086	1360
(R)TNGTADLLVPLLR(E)	100%	2.13	0.22	2		691.90	1,381.79	2	-0.0015	-1.1	1350
(?)TNGTADLLVPLLR(?)	8%	1.07	0.01	2		691.90	1,381.79	2	-0.00098	-0.71	1320
(R)TNGTADLLVPLLR(E)	100%	2.40	0.34	2		691.90	1,381.79	2	-0.00089	-0.64	1360
(R)TNGTADLLVPLLR(E)	100%	3.28	0.45	2		691.90	1,381.79	2	-0.00049	-0.36	1340
(R)TNGTADLLVPLLR(E)	100%	2.94	0.48	2		691.90	1,381.79	2	-0.000...	-0.070	1350
(?)TNGTADLLVPLLR(?)	37%	1.37	0.21	2		691.90	1,381.79	2	0.00033	0.24	1340
(?)TNGTADLLVPLLR(?)	79%	1.68	0.16	2		691.90	1,381.79	2	0.00098	0.71	1370
(R)TNGTADLLVPLLR(E)	100%	2.55	0.29	2		691.91	1,381.80	2	0.0070	5.0	1390
(R)TNGTADLLVPLLR(E)	100%	2.73	0.47	2		691.91	1,381.80	2	0.0036	2.6	1370

571 Proteins at 99.0% Minimum
2 Min # Peptides
0.0% Prophet FDR
4660 Spectra at 90.0% Minimum
1.63% Prophet FDR

Scaffold output for second expression replicate (gel band excision). 10 unique peptide matches for P77072 (alkyl organomercurial lyase)

Bio View: Identified Proteins (571)

93	Adenosine deaminase OS=Escherichia coli (strain SE11) GN=add PE=3 SV=1
94	Adenylate kinase OS=Escherichia coli O9:H4 (strain H5) GN=adk PE=3 SV=1
95	Aerobic respiration control protein ArcA OS=Shigella flexneri GN=arcA PE=3 SV=1
96	Aldehyde reductase Ahr OS=Escherichia coli (strain K12) GN=ahr PE=1 SV=2
97	Aldehyde reductase YahK OS=Escherichia coli (strain K12) GN=yahK PE=1 SV=1
98	Alkyl hydroperoxide reductase subunit C OS=Escherichia coli O6:H1 (strain CFT073 / ATCC 700928 / UPEC) GN=ahpC PE=3 SV=2
99	Alkylmercury lyase OS=Klebsiella pneumoniae GN=merB PE=3 SV=1
100	Alpha-S2-casein OS=Bos taurus GN=CSN1S2 PE=1 SV=2
101	Annexin A2 OS=Ovis aries GN=ANXA2 PE=1 SV=1
102	Arabinose 5-phosphate isomerase GutQ OS=Shigella flexneri GN=gutQ PE=3 SV=5
103	Arabinose 5-phosphate isomerase KdsD OS=Escherichia coli (strain K12) GN=kdsD PE=1 SV=1
104	Arginine transport ATP-binding protein ArtP OS=Escherichia coli (strain K12) GN=artP PE=1 SV=1
105	Asparagine--tRNA ligase OS=Escherichia coli O157:H7 GN=asnS PE=3 SV=2
106	Aspartate aminotransferase OS=Escherichia coli (strain K12) GN=aspC PE=1 SV=1
107	Aspartate ammonia-lyase OS=Escherichia coli O6:H1 (strain CFT073 / ATCC 700928 / UPEC) GN=aspA PE=3 SV=1

Protein Information: Lookup Accession Number In: NCBI (e.g. |135...)

Gene Ontology:

Sample Information: Biological Sample: Sample Category: Sample Description:

P62225 P77072

[NCBI](#) [Resources](#) [How To](#)

Protein

GenPept

Clipboard

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RecName: Full=Alkylmercury lyase; **AltName:** Full=Organomercurial lyase

UniProtKB/Swiss-Prot: P77072.1

[Identical Proteins](#) [FASTA](#) [Graphics](#) [Item in clipboard](#)

[Go To](#)

LOCUS	MERB_ECOLX	212 aa	linear	BCT 25-OCT-2017
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DEFINITION RecName: Full=Alkylmercury lyase; AltName: Full=Organomercurial lyase.

ACCESSION P77072

VERSION P77072.1

DBSOURCE UniProtKB: locus MERB_ECOLX, accession [P77072](#);
 class: standard.
 plasmid:Inc
 created: Sep 26, 2003.
 sequence updated: Feb 1, 1997.
 annotation updated: Oct 25, 2017.
 xrefs: U77087.1, AAB49639.1, I56L_A, 3F0A_A, 3F0B_B, 3F0P_A,
 3F0P_B, 3F2F_A, 3F2F_B, 3F2G_A, 3F2G_B, 3F2H_A, 3F2H_B, 3FN8_A,
 3FN8_B, 5C0T_A, 5C0T_B, 5C0U_A, 5C0U_B, 5DSF_A, 5DSF_B, 5U79_A,
 5U79_B, 5U7A_A, 5U7A_B, 5U7B_A, 5U7B_B, 5U7C_A, 5U7C_B, 5U82_A,
 5U82_B, 5U83_A, 5U83_B, 5U88_A, 5U88_B
 xrefs (non-sequence databases): PDBsum:I56L, PDBsum:3F0O,
 PDBsum:3F0P, PDBsum:3F2F, PDBsum:3F2G, PDBsum:3F2H, PDBsum:3FN8,
 PDBsum:5C0T, PDBsum:5C0U, PDBsum:5DSF, PDBsum:5U79, PDBsum:5U7A,

```

/experiment="experimental evidence, no additional details
recorded"
/note="{ECO:0000244|PDB:5U7A}."
ORIGIN
    1 mklapyilel ltsvnrtngt adllvpllre lakgrpvsrt tlagildwpa ervaavleqa
    61 tsteydkdgn iigygltlre tsyvfeiddr rlyawcaldt lifpaligrt arvsshcaat
    121 gapvsltvsp seiqavepag mavslvlpqe aadvrqsfcc hvhffasvpt aedwashhqq
    181 leglaivsvh eafglgqefn rhllqtmssr tp
//

```

			Database: McCarthy_N8_20171031.fasta (unknown version, 1 entries)										
			Digestion Enzyme: Trypsin										
			Max Missed Cleavages: 4										
			Probability Model:										
			McCarthy_N8_LM_20171026_custom_minpeptide4_NoRC_JustN8: Peptide Prophet with Delta Mass Correction										
			McCarthy_ID_20180214_custom_minpeptide4_NoRC_JustN8: Peptide Prophet with Delta Mass Correction (f										
Scaffold:	Version: Scaffold_4.8.4												
	Modification Metadata Set: 2334 modifications												
	Source: C:\Program Files\Scaffold 4\parameters\unimod.xml												
	Comment:												
	Protein Grouping Strategy: Experiment-wide grouping with binary peptide-protein weights												
	Peptide Thresholds: 80.0% minimum												
	Protein Thresholds: 99.0% minimum and 2 peptides minimum												
	Peptide FDR: 7.4% (Prophet)												
	Protein FDR: 0.0% (Prophet)												
	GO Annotation Source(s):												
	Alternate ID Source(s):												
Experiment	Biological Sample	Biological sample name	rMS/MS search	Protein names	Protein accession numbers	Database	Protein mass	Protein identity	Exclusive	Exclusive	Exclusive	Percentage identified	Percentage searched
McCarthy_Uncategorized	N8		McCarthy_N8_custor	N8_custor	McCarthy_N8	34,806.20	100.00%	8	8	16	9.70%	25.20%	
McCarthy_Uncategorized	IDband_Feb2018		McCarthy_N8_custor	N8_custor	McCarthy_N8	34,806.20	100.00%	4	4	4	8.33%	16.40%	
END OF FILE													

Excerpt from protein report indicating 100% id for both replicates

Protein alignment
Nucleotide alignment
Web services
Help & Documentation
Feedback

Results for job emboss_needle-I20180220-173316-0110-16771849-p2m

Alignment
Submission Details

View Alignment File

```

#####
# Program: needle
# Rundate: Tue 20 Feb 2018 17:33:20
# Commandline: needle
#
# -auto
# -stdout
# -asequence emboss_needle-I20180220-173316-0110-16771849-p2m.asequence
# -bsequence emboss_needle-I20180220-173316-0110-16771849-p2m.bsequence
# -datafile EBLOSUM62
# -gapopen 10.0
# -gapextend 0.5
# -endopen 10.0
# -endextend 0.5
# -aformat3 pair
# -sprotein1
# -sprotein2
# Align_format: pair
# Report_file: stdout
#####

#=====
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 212
# Identity:      212/212 (100.0%)
# Similarity:    212/212 (100.0%)
# Gaps:          0/212 ( 0.0%)

```

Emboss Needle pairwise sequence results P77072 amino seq vs designed construct (100% match 212 residues).

```

#=====
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 212
# Identity:      212/212 (100.0%)
# Similarity:    212/212 (100.0%)
# Gaps:          0/212 ( 0.0%)
# Score: 1084.0
#
#
#=====

```

EMBOSS_001	1	MKLAPYILELLTSVNRTNGTADLLVPLLRELAKGRPVSRTTLAGILDWPA	50
EMBOSS_001	1	MKLAPYILELLTSVNRTNGTADLLVPLLRELAKGRPVSRTTLAGILDWPA	50
EMBOSS_001	51	ERVAAVLEQATSTEYDKDGNIIIGYGLTLRETSYVFEIDRRLYANCALDT	100
EMBOSS_001	51	ERVAAVLEQATSTEYDKDGNIIIGYGLTLRETSYVFEIDRRLYANCALDT	100
EMBOSS_001	101	LIFPALIGRTARVSSHCAATGAPVSLTVSPSEIQAVEPAGMAVSLVLPQE	150
EMBOSS_001	101	LIFPALIGRTARVSSHCAATGAPVSLTVSPSEIQAVEPAGMAVSLVLPQE	150
EMBOSS_001	151	AADVRSQFCCHVHFFASVPTAEDWASKHQGLEGLAIVSVHEAFGLGQEFN	200
EMBOSS_001	151	AADVRSQFCCHVHFFASVPTAEDWASKHQGLEGLAIVSVHEAFGLGQEFN	200
EMBOSS_001	201	RHLLQTMSSRTP	212
EMBOSS_001	201	RHLLQTMSSRTP	212

```

#-----
#-----

```

Emboss Needle pairwise sequence results P77072 amino sequence vs designed construct confirming mass spec id match 100%.

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Download Sequencing Results [Bottom of Page](#)

Use top table to download all the files together and use the bottom table to download individual files.
Chromatograms may be viewed using freeware such as SnapGene or Technelysium's Chromas.
Check comment column of bottom table for individual sample status such as re-runs etc.

Sequencer Output		Phred Output				Trim Output
Text	Trace	Fasta	Qual	Phred	SCF	Trimmed
Text	Chromat	fasta	qual	phred	scf	Trimmed

To download individual files:
Mac: Hold down Control Key, Click Mouse. Select Save Linked File As...
PC: Right Mouse Click, Save Link As...

Plate Number: 12398

Rows in grey are checked in to be rerun.

Order	Req#	Download	View	SeqId	WID	Sample	Primer	Investigator	UID	Date	Phred Q20	Trimmed	Edited	Comments
26404	873367	Text Chromat	View Text	12398-5	E1	1	Invalid	mccarthy,damien	2805	May 16 2017	phd qual 777 fasta scf	fasta		Results Available
26404	873368	Text Chromat	View Text	12398-6	F1	2	Invalid	mccarthy,damien	2805	May 16 2017	phd qual 624 fasta scf	fasta		Results Available
26404	873369	Text Chromat	View Text	12398-7	G1	3	Invalid	mccarthy,damien	2805	May 16 2017	phd qual 727 fasta scf	fasta		Results Available
26404	873370	Text Chromat	View Text	12398-8	H1	4	Invalid	mccarthy,damien	2805	May 16 2017	phd qual 778 fasta scf	fasta		Results Available
cs	873375	Text Chromat	View Text	12398-83	C11	pGem	CP1	osborne,craig	1437	May 17 2017	phd qual 765 fasta scf	fasta		cs

There are 5 samples.

Genomic centre results

GTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAAC
AAGCGCTCATGAGCCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCA
ACCGCACCTGTGGCGCCGGTGATGCCGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCG
AAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAAC
TTTAAGAAGGAGATATACATATGCGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTG
GACAGCAAATGGGTCGGGATCTGTACGACGATGACGATAAGGATCATCCCTTCACCATGGTTAAAACCCAG
GCAACCAGCCGTGAAGAACCGCCTCGTCTGCCGAGCAAACATCGTCCGGGTGTGAAAACACAGGCCACCTC
ACGCGAAGAACCTCCACGCCTGCCTTCAAACACCGTCTTGGCGTAAAAACGCAGGCGACAAGTCGTGAG
GAACCTCCGCGTTTACCGTCTAAACATAGACCTGGGGTGAAAACCCAGACCGCAAGCAAACCTGGCACCGTA
TATTCTGGAAGTGTGACCAGCGTTAATCGTACCAATGGCACAGCCGATCTGCTGGTTCGGCTGCTGCGTGA
ACTGGCAAACGTCGTCCGGGTTAGCCGTACCACACTGGCAGTATTCTGGATTGGCCTGCAGACGTGTTGC
AG