

A Two-Stage Mass Spectrometry Approach Using PIR Cross-Linkers for Global Protein-Protein Interaction Profiling

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OVERVIEW

- This project aims to develop and apply protein interaction reporters (PIRs)¹ and a two-stage approach using 2D/LC/MS/MS and multiplexed LC/FT-ICR-MS technologies for global protein-protein interaction profiling.
- High mass measurement accuracy (MMA) and unique features of PIR cross-linker are the enabling factors for identification of protein interactions on whole proteome scale.
- This presentation highlights our initial attempt and results of profiling protein-protein interactions on proteome of *Shewanella oneidensis* MR-1.

INTRODUCTION

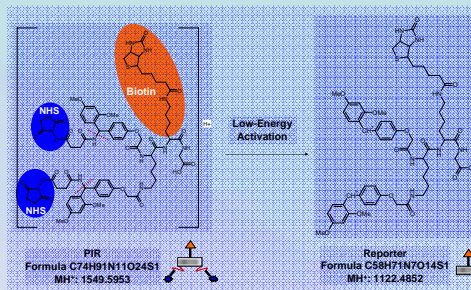
Mapping protein-protein interaction networks at systems level is essential for understanding molecular function in biological organisms. Cross-linking strategies for studying protein interactions are simple in principle; however, present tremendous challenges in reality due to inherent complexity from the reactions and the mass spectra. We aim to develop a novel strategy using special cross-linkers and a two-stage mass spectrometry approach for profiling protein-protein interactions on a global scale. We have reported previously a novel type of cross-linker, called Protein Interaction Reporter (PIR)¹, that includes cleavable bonds with high specificities at low-energy MS/MS to allow release of a reporter tag and intact peptide chains. The second-generation PIR further incorporates a biotin tag for enrichment of cross-linked products. The two-stage mass spectrometry approach has been developed to capitalize on the advanced features of PIR cross-linkers and high performance of FT-ICR-MS instruments. Stage 1 focused on capturing cross-linked products at protein level and construction of a restricted database to be used for searching interacting proteins. Stage 2 involved enrichment of cross-linked peptides to be analyzed by multiplexed LC/FT-ICR-MS with low-energy CID activation on and off at alternating scans. To allow use of reporter ions as lock mass for internal calibration and PIR fragmentation pattern recognition that allows distinguishing inter-, intra-, or dead-end cross-linked peptides, ICR-2LS software and newly developed Excel-based algorithms (XLinks) were applied to analyze and search the data generated from stage 2. The cellular proteome of *Shewanella oneidensis* MR-1 was explored and investigated with this novel strategy.

METHODS

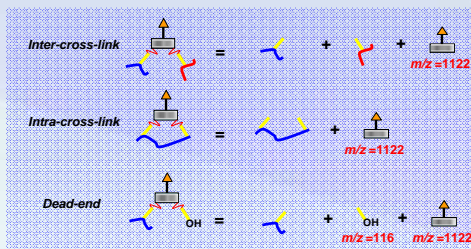
- PIR was made in house based on F-moc solid phase peptide synthesis chemistry.
- *Shewanella oneidensis* MR-1 cells were grown and harvested at mid-log phase. Cells were labeled intact with PIR and then washed and lysed in PBS buffer.
- A monomeric avidin column (Pierce) was used for both stage 1 and stage 2 to enrich cross-linked proteins or peptides.
- Nano LC/MS/MS by ion trap MS (Esquire HCT, Bruker Daltonics) was used for stage 1 analysis and multiplexed LC/FT-ICR-MS (7T Apex-Q, Bruker Daltonics) was used at stage 2 analysis
- ICR-2LS and novel algorithms (XLinks) were used for identification of PIR-labeled peptides and interacting proteins.

RESULTS

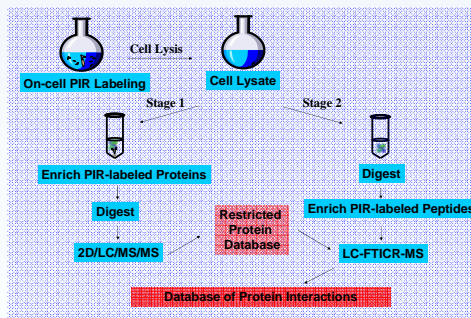
Structures of PIR and Reporter



PIR Differentiation of Cross-linked Peptides

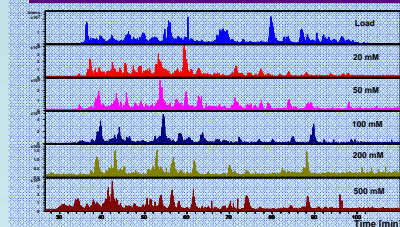


Two-Stage Strategy for Global Protein-Protein Interaction Profiling



Stage 1: Construction of Restricted Protein Database

2D/LC/MS/MS Chromatogram of PIR-labeled Protein Digest

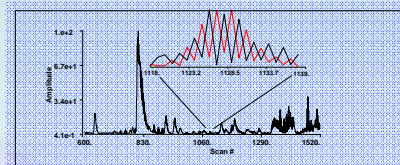


Restricted Protein Database

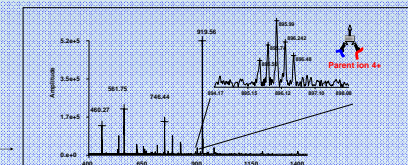
Number	Accession Number	Protein ID	MW (kD)	Physico. Matched	Massol. Score
1	S02275	Transcription elongation factor Tc (TceA)	84	15	4547
2	S02171	Transcription elongation factor Tc (TceB)	84	13	4103
3	S04747	ATP synthase F1, beta subunit (atpD)	56	14	3742
4	S05842	Transcription elongation factor G (PnaA2)	77	18	2996
5	S03336	High-molecular-weight endonuclease (HsdR)	87	11	2713
6	S04748	ATP synthase F1, alpha subunit (atpA)	55	11	1747
7	S02625	DNA-directed RNA polymerase, beta subunit (rpoB)	156	27	1548
8	S01928	cellulose synthase (gluA)	48	8	1443
9	S05836	Nucleic acid phosphorylase (NAP)	37	8	971
10	S02462	transcription elongation factor Tc (TceC)	81	12	848
11	S03514	omnibeta deacetyltransferase, inducible (igaA)	82	17	613
12	S01778	ribulose biphosphate carboxylase (rubA)	85	9	585
136	S03840	ribosomal protein L13 (rplM)	16	2	23
140	S02760	phosphoenolpyruvate carboxyltransferase, cytosolic (pccA)	98	2	22

Also see poster WP27 506 "Protein-protein interaction studies on *Shewanella oneidensis* MR-1" Devi P. Adhikari, Xiaoting Tang, Gerhard R. Munske, James E. Bruce

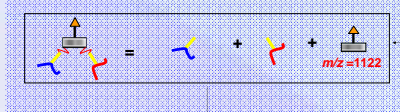
Stage 2: Identification of Protein-Protein Interactions



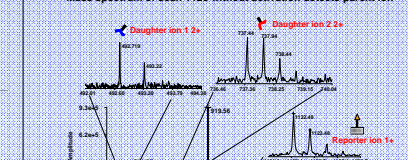
Base peak chromatogram of multiplexed LC/FT-ICR-MS of PIR-labeled peptides with low-energy CID (-18 V) activation on and off at alternating scans. The inset is the extracted ion chromatograms of a PIR labeled parent ion (red trace) and its daughter ion (black trace).



Mass spectrum of scan 1123 without activation detects parent ion



Mass of Daughter Ion	Mass of Peptide	Identified Sequence	Accession Number	Protein ID
983.409	884.377	MPAREYK (N-terminal peptide)	S01168	General secretion pathway protein F (gsrF)
1472.835	1373.800	HVVVYNSDLHK	S02234	Ribosomal protein L2 (rplB)



Mass spectrum of scan 1124 with activation detects reporter ion and 2 daughter ions

CONCLUSIONS

- A novel strategy for global profiling protein-protein interactions has been developed and applied to cellular proteome of *Shewanella oneidensis* MR-1.
- Initial studies resulted in identification of a number of protein-protein interactions as well as some cell surface proteins through dead-end labeling.
- Further validation experiments are underway.

REFERENCE

1. Tang, X., Munske, G. R., Siems, W. F. & Bruce, J. E. "Mass spectrometry identifiable cross-linking strategy for studying protein-protein interactions." *Analytical Chemistry* 77, 311-8 (2005).

ACKNOWLEDGMENTS

This research was supported by the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG02-04ER63924 and the Murdock Charitable Trust.

A reprint of this poster is available at: <http://www.wsu.edu/protomics>