Accurate Mass and PIRs: A New Strategy for Protein Interactions

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OVERVIEW

- Description of Protein Interaction Reporter (PIR) concept
- PIR-derived constraint for accurate mass-based protein ID
- PIR data analysis strategies

INTRODUCTION

We have developed a novel approach for protein interaction profiling that involves a new type of chemical cross-linker. These compounds enable us to encode information at the MS/MS level to allow improved characterization of protein interactions in biological systems. Protein Interaction Reporters, or PIRs can be fragmented with high specificity and produce fragments with diagnostic masses. As a result, PIR ions increase our ability to identify cross-linked proteins with mass spectrometry, since the encoded mass provides information about the chemical functionality of the cross-linker. Furthermore, the specific cleavage of the PIR structure allows the release of intact peptide ions and subsequently, accurate peptide mass-based protein identification to be accomplished. This presentation highlights the PIR approach, the implementation in complex systems and data analysis strategies that are being developed to allow PIR optimization.

METHODS

- PIRs were synthesized using automated F-moc solid phase peptide synthesis chemistry.
- *Shewanella oneidensis* MR-1 cells were grown and harvested at mid-log phase. Intact cells were labeled with PIR and then washed and lysed.
- A monomeric avidin column (Pierce) was used to enrich cross-linked proteins or peptides.
- Nano LC/MS/MS (Esquire HCT, Bruker Daltonics) and multiplexed-LC/FT-ICR-MS (7T Apex-Q, Bruker Daltonics) were used for protein and interaction identification.
- ICR-2LS and novel algorithms (XLinks) were used for identification of PIR-labeled peptides and interacting proteins.
- Calculations of peptide masses were made using the *Shewanella oneidensis MR-1* database downloaded from The Institute for Genome Research (TIGR).

RESULTS

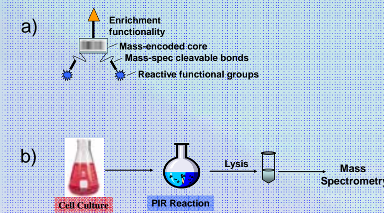

Figure 1 a) Concepts integrated into the PIR strategy. PIR allows cross-linking, affinity purification, and mass spec-cleavable bonds to enable advanced protein interaction studies with MS. **b)** On-cell PIR labeling followed by cell lysis and mass spectrometry.

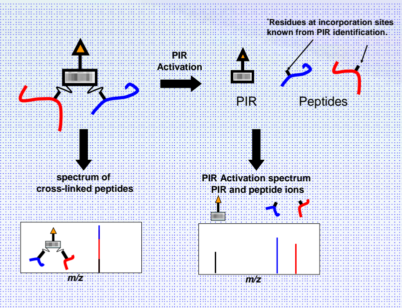

Figure 2. Activation of PIR-labeled peptides results in release of reporter ion and intact peptide ions. The summation of accurate neutral masses can be used to identify protein interactions from complex reaction mixtures.

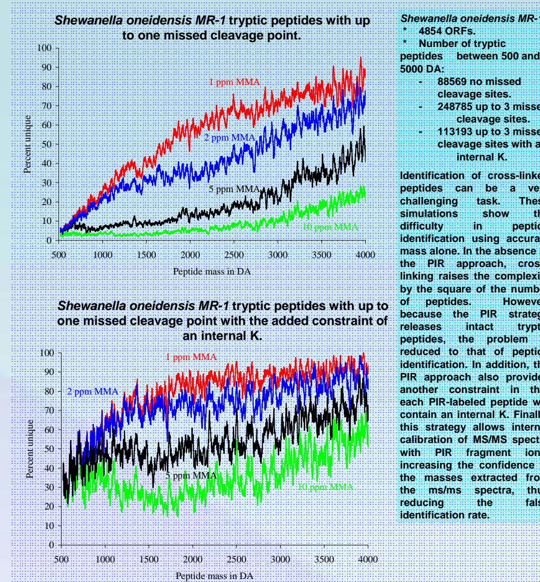

Figure 3 Unambiguous peptide identification using high mass measurement accuracy (MMA) alone is challenging. These simulations show the number of unique peptides vs. MMA in *Shewanella oneidensis MR-1.* These calculations were performed using the protein database from TIGR to determine all peptides and then establish their uniqueness at several MMA values.

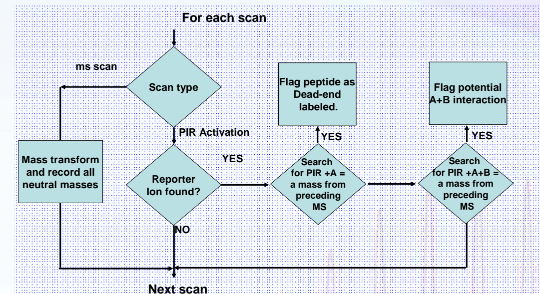

Figure 4. Schematic diagram of algorithm used to identify PIR-peptide pair information.

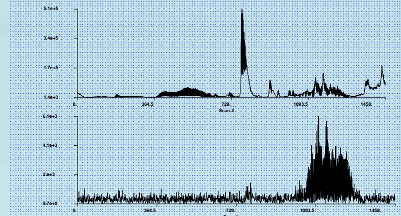

Figure 5. Alternating scan (no activation, PIR activation scan) LC-FTICR-MS data from on-cell PIR studies with *Shewanella oneidensis MR-1.* TOP is the base peak chromatogram. BOTTOM is the extracted ion chromatogram for *m/z* 1122, the 1+ reporter ion. The appearance of increased intensity of the 1122 ions during the PIR activation scans indicates that PIR-labeled products are eluting from the column. The chromatogram region encompassing scans 1000-1400 was further mined to identify PIR-labeled products.

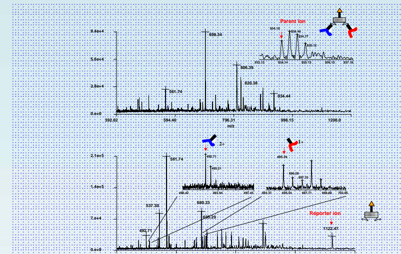

Figure 6. Identification of low intensity PIR-labeled peptide pairs in scans 1054 and 1055 from LC-FTICR data. TOP is low energy scan. $(M+3H)^{3+}$ parent ions observed at *m/z* 934 disappear in PIR activation scan (BOTTOM). The masses of reporter ions together with masses of 2+ and 1+ ions at *m/z* 492 and 695 sum to match that of the precursor, identifying a cross-linked product.

CONCLUSIONS

The PIR strategy can be combined with accurate mass analysis to allow extraction of protein interaction information from biological systems. The cleavage properties of the PIR allow release and measurement of intact peptide ions resultant from cross-linked proteins. This feature and the additional search constraint resultant from the requirement of an internal lysine residue will facilitate improved accurate mass-based peptide identification. The PIR strategy represents a new direction that can allow mass spectrometry to contribute to better understanding of protein interactions in cells.

ACKNOWLEDGMENTS

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