New Approaches for High-Throughput Identification and Characterization of Protein Complexes

Center for Molecular and Cellular Systems

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Team Leaders

Core:

Steven Kennel, Thomas Squire

High Throughput Complex Processing

Mike Ramsey, Karin Rodland

Mass Spectrometry

Greg Hurst, Richard Smith

Molecular and Cellular Imaging

Mitch Doktycz, Steve Colson

Bioinformatics and Computing

Ying Xu, David Dixon

Carol Giometti (ANL) gel electrophoresis
Ray Gesteland (U. Utah) mass spectrometry
Malin Young (SNL) cross-linking
Mike Giddings (U. North Carolina) mass spectrometry

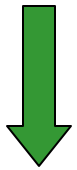
Goal 1

"Identify and Characterize the Molecular Machines of Life"

"…instead of a cell dominated by randomly colliding individual protein molecules, we now know that nearly every major process in a cell is carried out by assemblies … of proteins...Indeed an entire cell can be viewed as a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines."

Bruce Alberts, *"The Cell as a Collection of Protein Machines: Preparing the Next Generation of Molecular Biologists," Cell*, **92**, 291 (1998)

Protein complexes are key to biological function



Understand the network of reactions that occur in sufficient detail to predict, test, and comprehend the responses of a biological system to changes

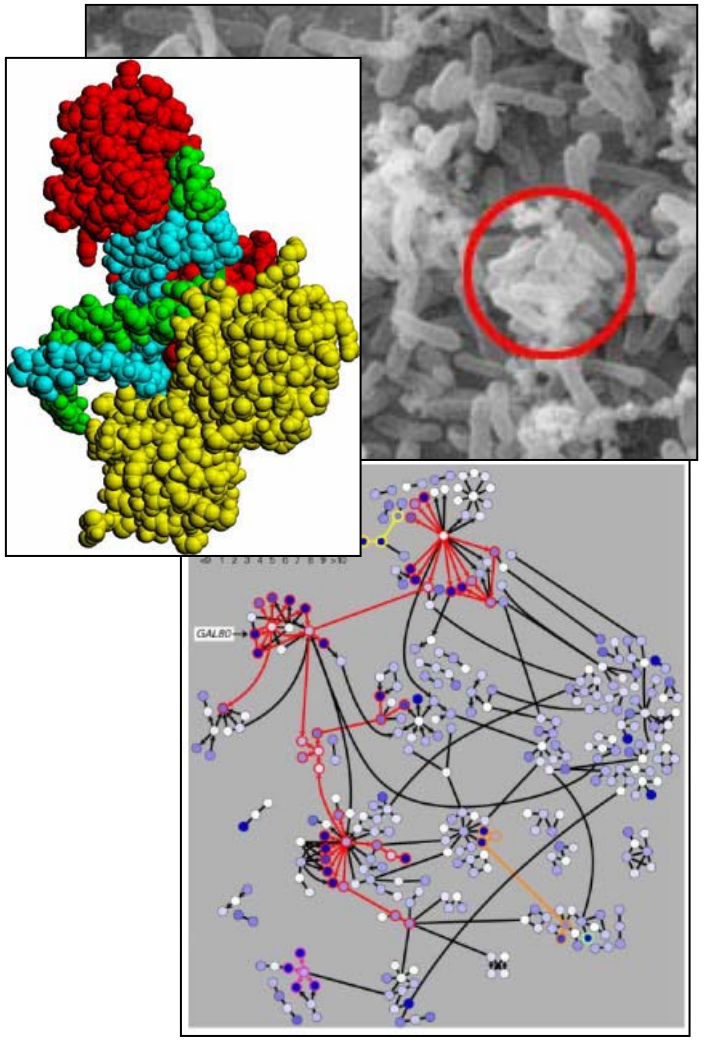
Goal 1 includes three main steps

- Identify complement of protein complexes and their components
- Elucidate function and dynamics of complexes—intermediates, nature of interactions, cellular location, kinetics
- Establish how changes arising from environmental stress, development, etc., affect complex formation and function

which lay the foundation for GTL

Impact of Goal 1

- Molecular level understanding of protein complexes and, ultimately, networks
- Predict/change behavior of organism and community
- Predict function, biological pathways by homology
- Discover new functions



Identification and Characterization of Protein Machines

- New approaches needed for large-scale studies
  - No single tool will provide all required information
  - Computational tools must be integrated from beginning
    - Analyze, compare, predict, share data
    - Quality assessment
    - Guide experimental design and data collection

Develop integrated approach to correlate identified complexes with data from gene expression, protein expression, imaging, and other methods

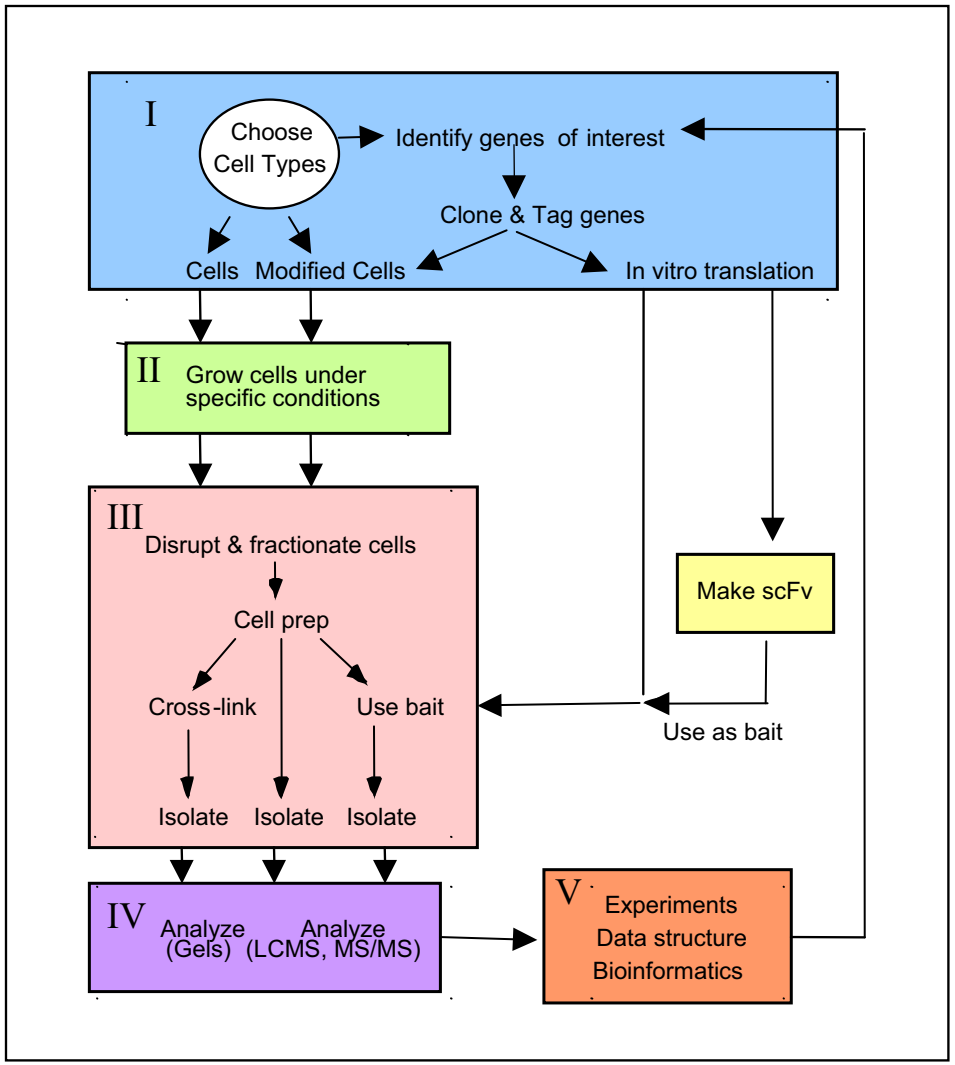
Strategy to Achieve Goal 1

- Initiate protein complex identification using affinity separation combined with mass spectrometry and computational tools
  - Use multiple approaches, non-optimized techniques
  - Focus on targeted complexes
- Evaluate new approaches for high-throughput identification
  - Identify bottlenecks, opportunities for automation
  - Establish dynamic R&D program to develop new, integrated analytical and computational tools
- Incorporate additional tools, data to characterize complexes
  - Imaging tools to characterize complexes in cells
  - Tools to identify interaction interfaces

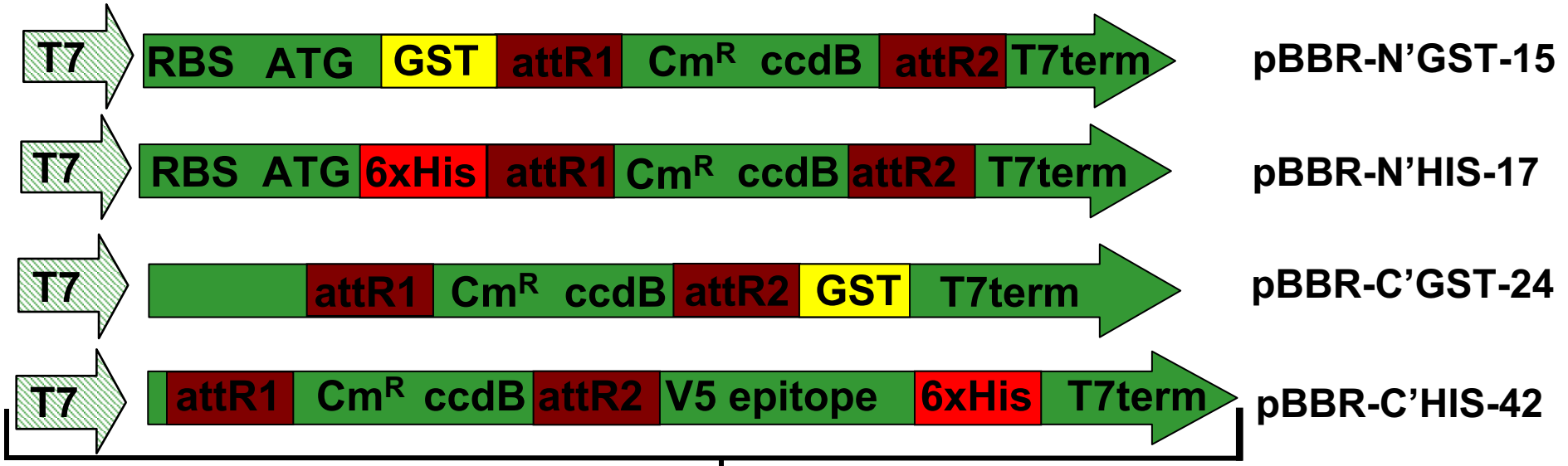
An Approach for High Throughput Identification of Protein Complexes

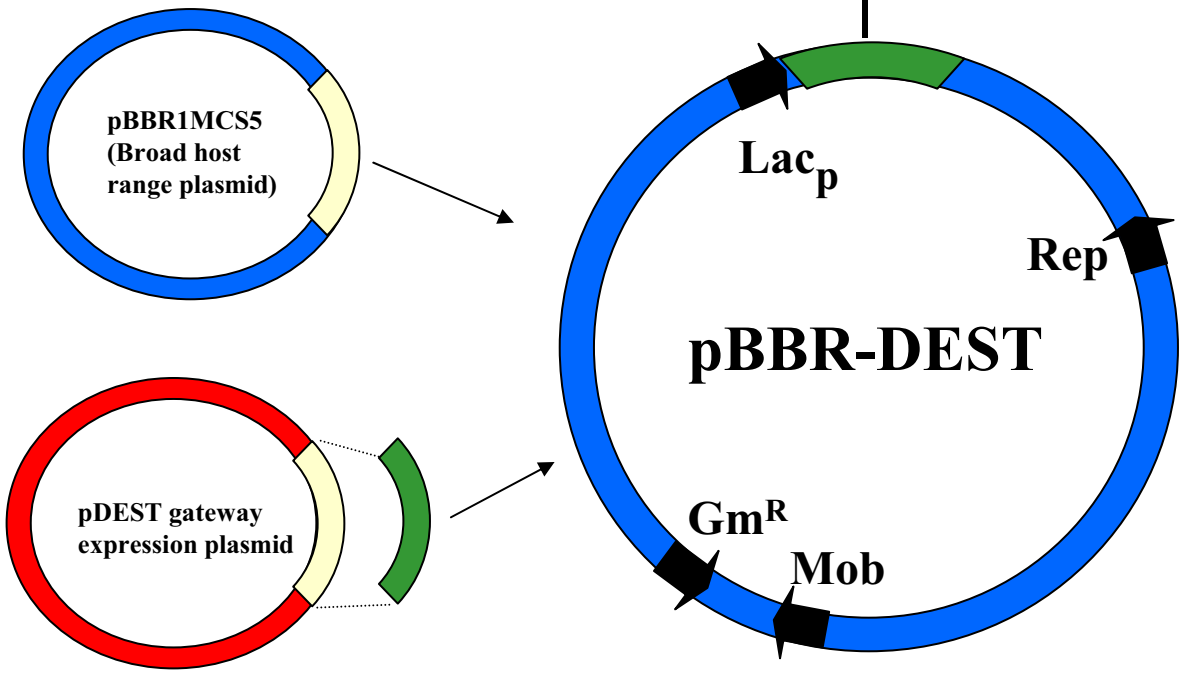
Combine complex isolation, mass spectrometry and data analysis

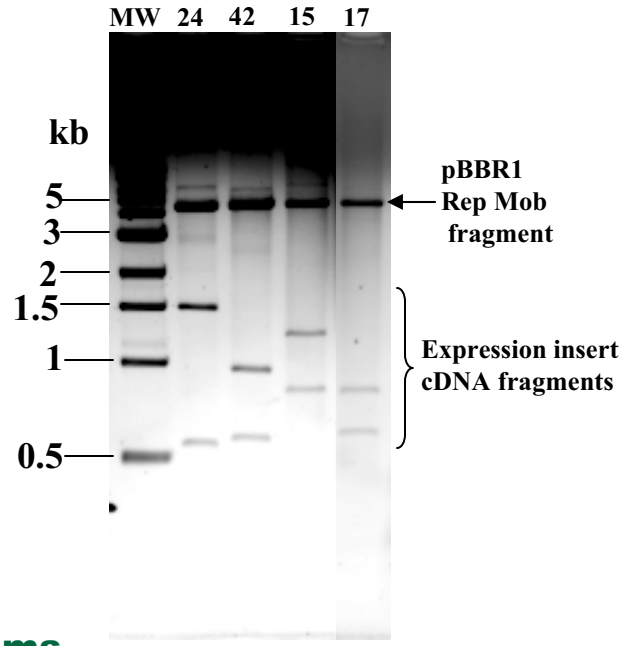
- **Bioinformatics**
- **Cloning, tagging**
- **Controlled cell growth**
- **Affinity isolation**
- **scFv production**
- **Cross-linking**
- **Separation**
- **MS analysis**
- **Data analysis, archival**



Modified pDEST Vectors for Protein Expression in *R. palustris*







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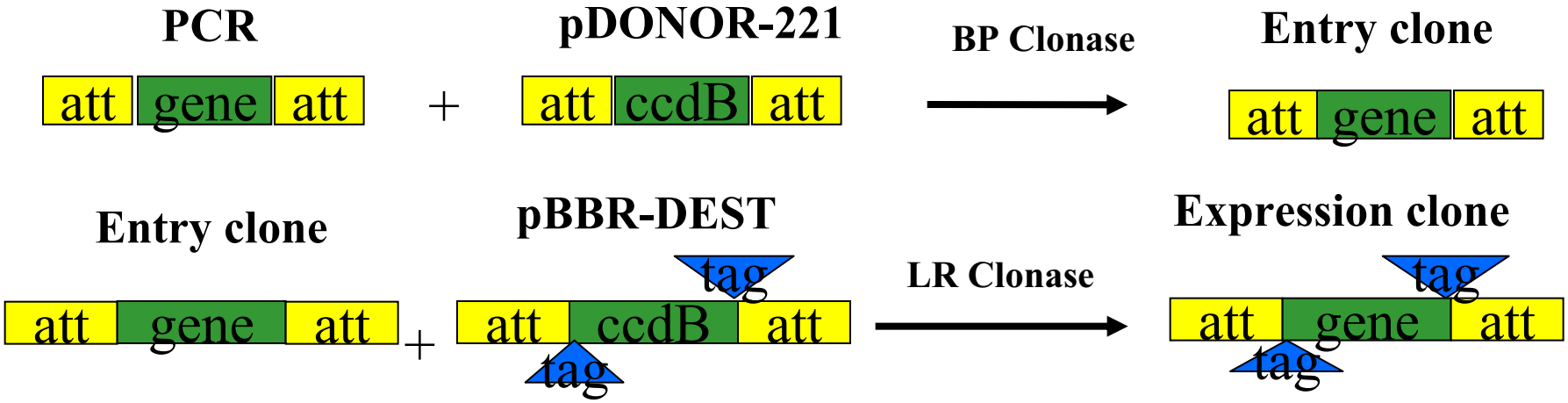
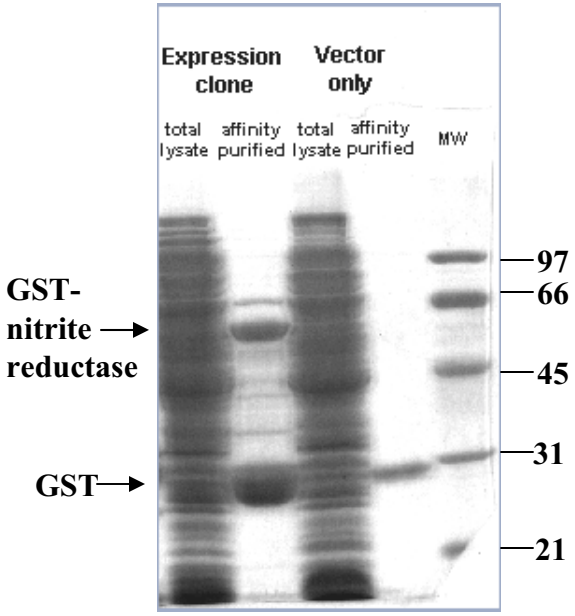
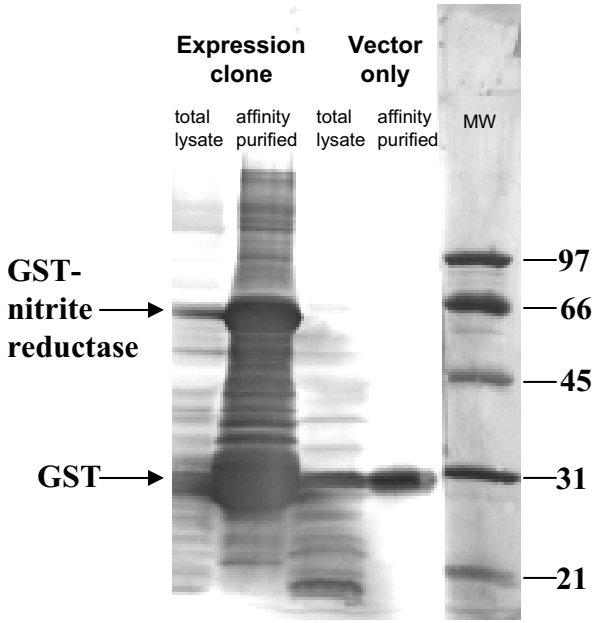
Modified Gateway system for production of affinity tagged *R. palustris* proteins



Protein stain



Western blot



Mass Spectrometry

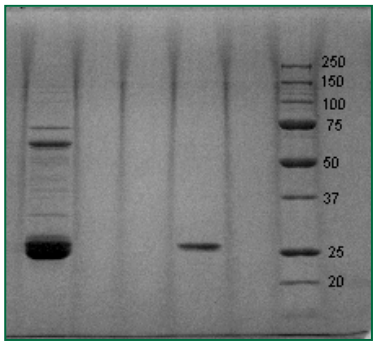
GST-nitrite reductase band

	# of peptides identified	
	MALDI	nano LC MS/MS
GST peptides	5	13
GST/linker peptides	2	1
nitrite reductase peptides	9	11
linker/nit. red. peptides	0	1

GST band

GST peptides	10	22
GST/linker peptides	1	1
nitrite reductase peptides	0	0
linker/nit. red. peptides	0	0

Verification of *R. palustris* Fusion Proteins Expressed in *E. coli*—Two Approaches



Affinity capture of tagged proteins from lysed cells

1D PAGE

whole eluate digestion

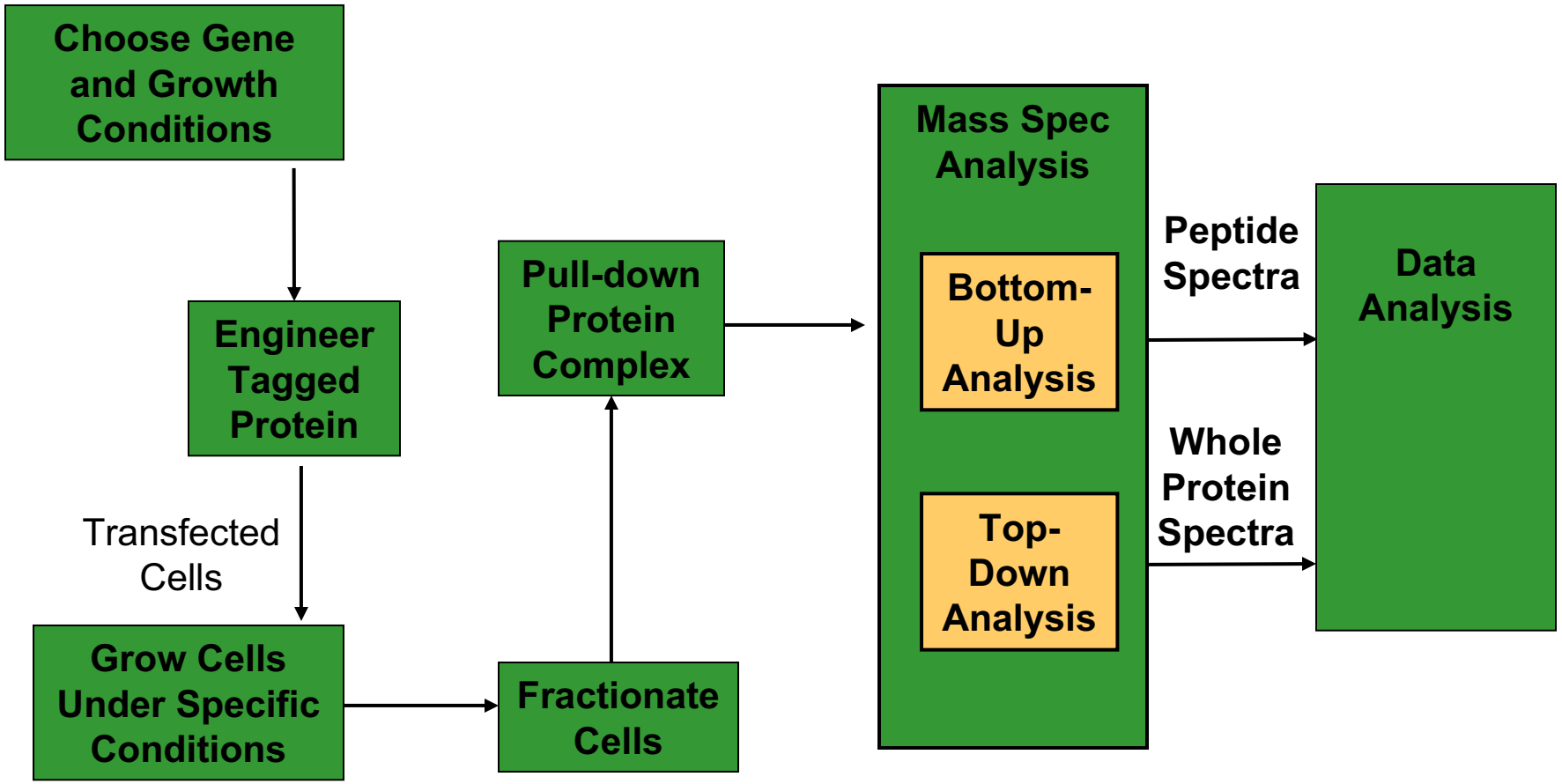
In-gel digestion and mass spectrometric identification of individual gel bands

LC-MS-MS of digest peptides; identification of proteins via SEQUEST

	No. of peptides identified from:		Others
Fusion Protein	**target protein**	**affinity tag**	***identified***
Rpal 4709 + N-terminal GST	45	8	2
Rpal 4709 + C-terminal 6-His & V5 epitope	31	3	19
Rpal 5426 + C-terminal 6-His & V5 epitope	35	3	8

*These are candidate methods for analysis of **protein complexes** isolated via affinity purification*

ORNL GTL Process Flowchart

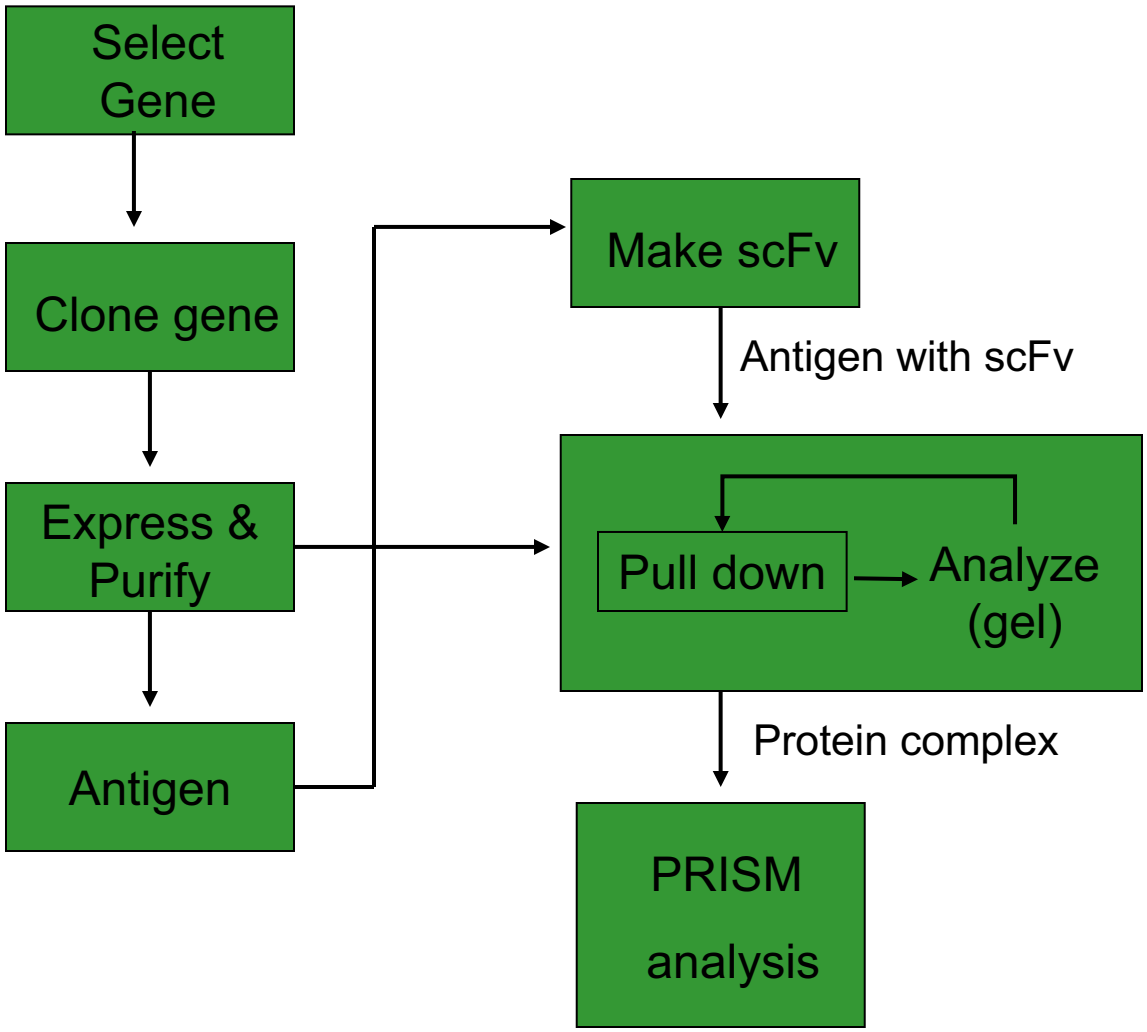


Analysis of expression of affinity tagged *R. palustris* genes

Gene	Function	Affinity Tag	Expression
nirK	Nitrite reductase	N-His	++
		C-His	+
		N-GST	++
		C-GST	0
groEL-2	chaperonin	N-His	++
		C-His	+++
		N-GST	+
		C-GST	+
groEL-1	chaperonin	N-His	+++
		C-His	+++
		N-GST	++
		C-GST	+
soxB	thiosulfate oxidation	N-His	++
		C-His	++
		N-GST	0
		C-GST	0
soxC	thiosulfate oxidation	N-His	++
		C-His	++
		N-GST	+
		C-GST	+
hupS	uptake hydrogenase small subunit	N-His	0
		C-His	++
		N-GST	0
		C-GST	++
hupL	uptake hydrogenase large subunit	N-His	++
		C-His	++
		N-GST	0
		C-GST	++

+++ Excellent ++ Good + Poor 0 None

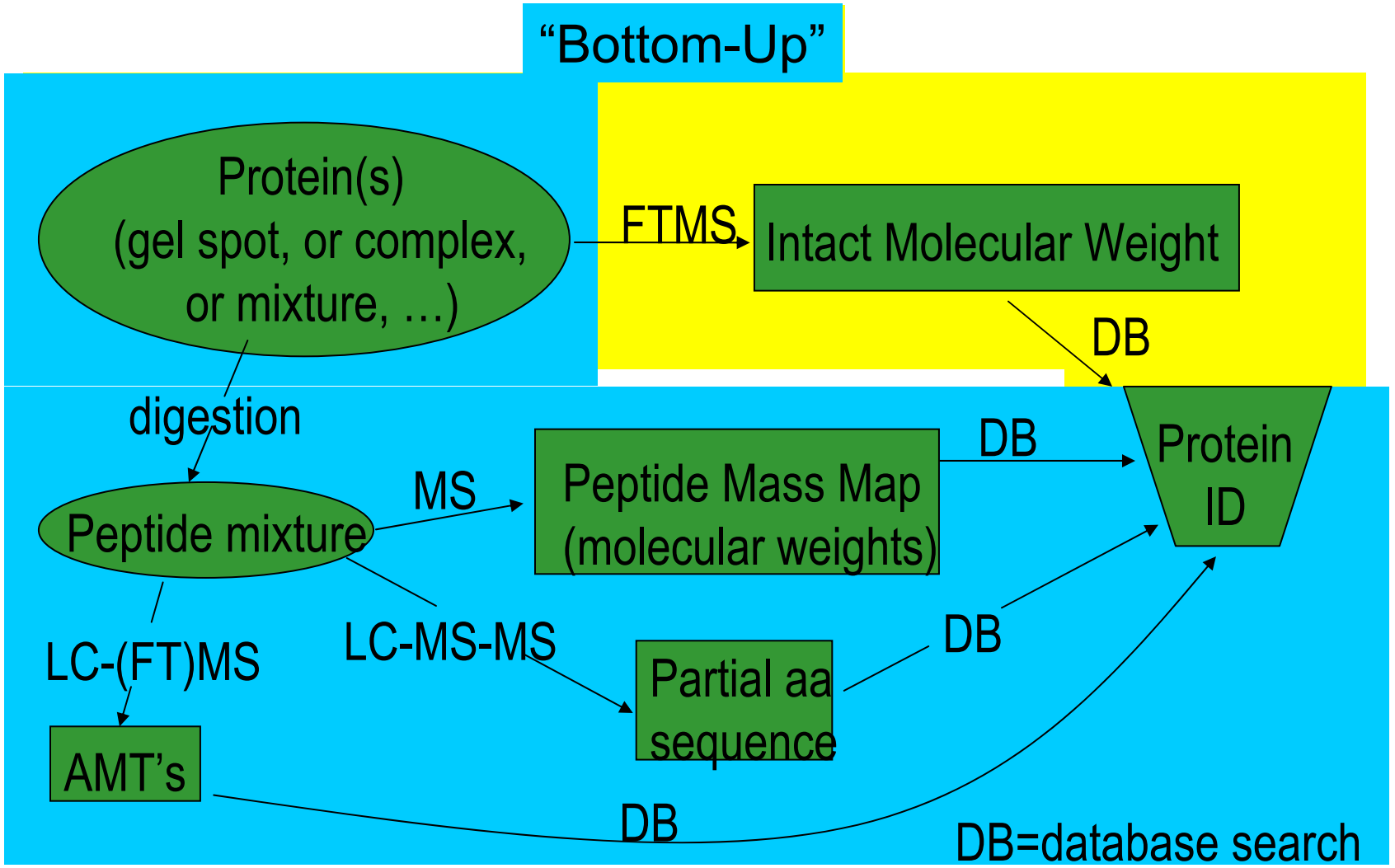
Heterologous Expression



Tagged proteins generated to date for pull-down studies of *S. Oneidensis*

Gene	Description	Annotation
hydB	periplasmic Fe hydrogenase small subunit	SO3921
hydA	periplasmic Fe hydrogenase large subunit	SO3920
napA	periplasmic nitrate reductase	SO0848
omcA	decaheme cytochrome C	SO1779
omcB	decaheme cytochrome C	SO1778
hoxK	Quinone-reactive Ni/Fe hydrogenase small subunit precursor	SO2099
petA	ubiquinol-cytochrome C reductase iron-sulfur subunit	SO0608
	flavocytochrome C flavin subunit	SO3301
	Gfo/Idh/MocA family oxidoreductase	SO3120
	oxidireductase molybdopterin-binding	SO0715
nrfC	formate-dependent nitrite reductase	SO0483
ptpA	phosphotyrosine protein phosphatase	SO2208
ptpB	Tyrosine-specific protein phosphatase	SO3124
cpxP	Spheroplast protein y precursor	SO4476
msrA	methionine sulfoxide reductase (isoform A)	SO2337
msrB	methionine sulfoxide reductase (isoform B)	SO2588
eno	Enolase	SO3440
rnlB	ATP-dependent RNA helicase	SO0407
rpoD	RNA polymerase sigma-70 factor	SO1284
	Cytochrome c3	SO2727
rpoA	DNA-directed RNA polymerase alpha subunit	SO0256
rpoZ	DNA-directed RNA omega subunit	SO0360
hepA	RNA polymerase-associated protein	SO0575

MS for Protein Identification



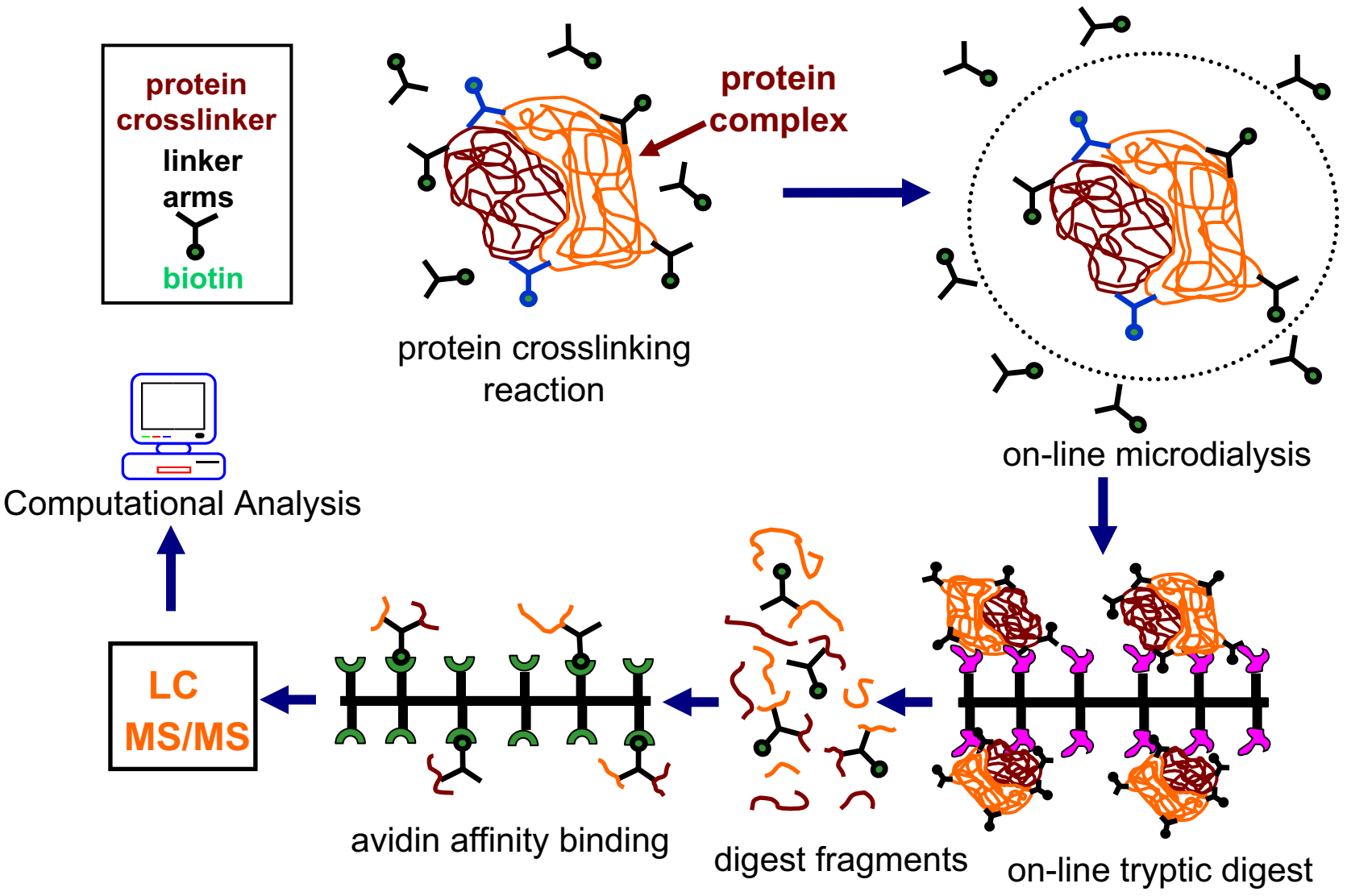
Integrating "Top-Down" and "Bottom-Up" Mass Spectrometric Approaches for Proteomic Analysis of Shewanella oneidensis, N.C. VerBerkmoes, J.L. Bundy, L. Hauser, K.G. Asano, J. Razumovskaya, F. Larimer, R.L. Hettich, and J.L. Stephenson, Jr., J. Proteome Research, in press for Vol 1, issue 3 (estimated June 2002).

Crosslinking and Mass Spectrometry for Protein Complex Analysis

Oak Ridge National Laboratory,
Sandia National Laboratories

- Chemical crosslinking has potential for:
  - Stabilizing “fragile” complexes
  - Providing information on distances between particular residues in proteins or complexes
  - Improving throughput for MS analysis of complexes
- Technical issues currently being addressed:
  - Low abundance of crosslinked products
  - Interpretation of mass spectrometry data

Protein Complex Analysis: Proposed Affinity Crosslinker Approach



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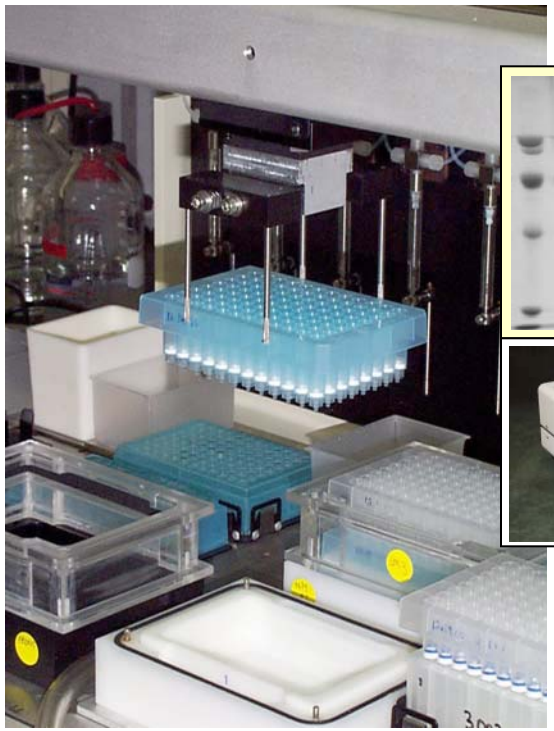
AUTOMATION OF PROTEIN PRODUCTION & ANALYSES

A



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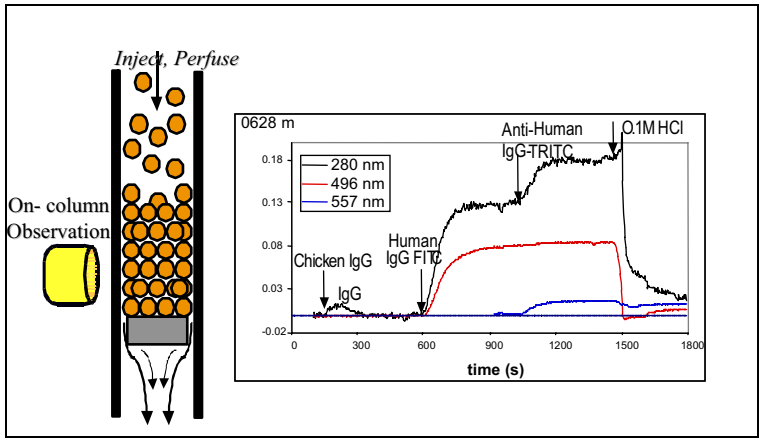

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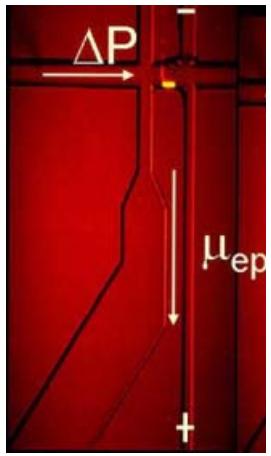


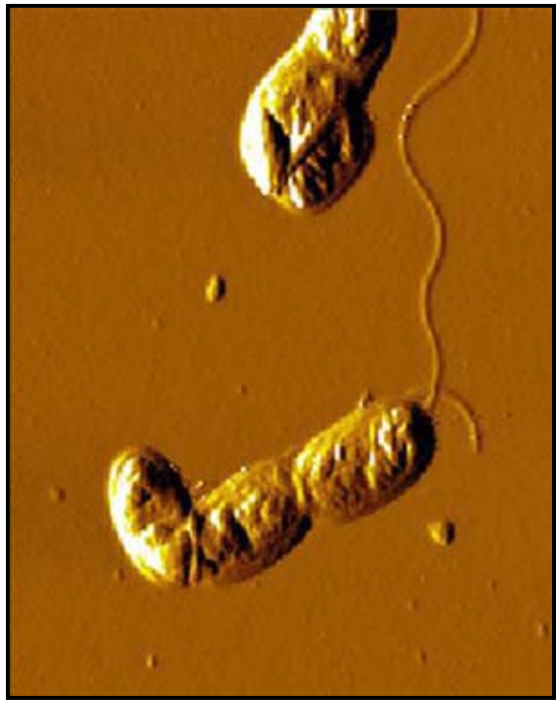
A. Macroscale HT Cloning and Sample Preparation

B. Microscale Sample Production for Mass Spec

C. Lab-On-A-Chip

Emerging approaches for characterizing protein complexes

Molecular and Cellular Imaging Subproject



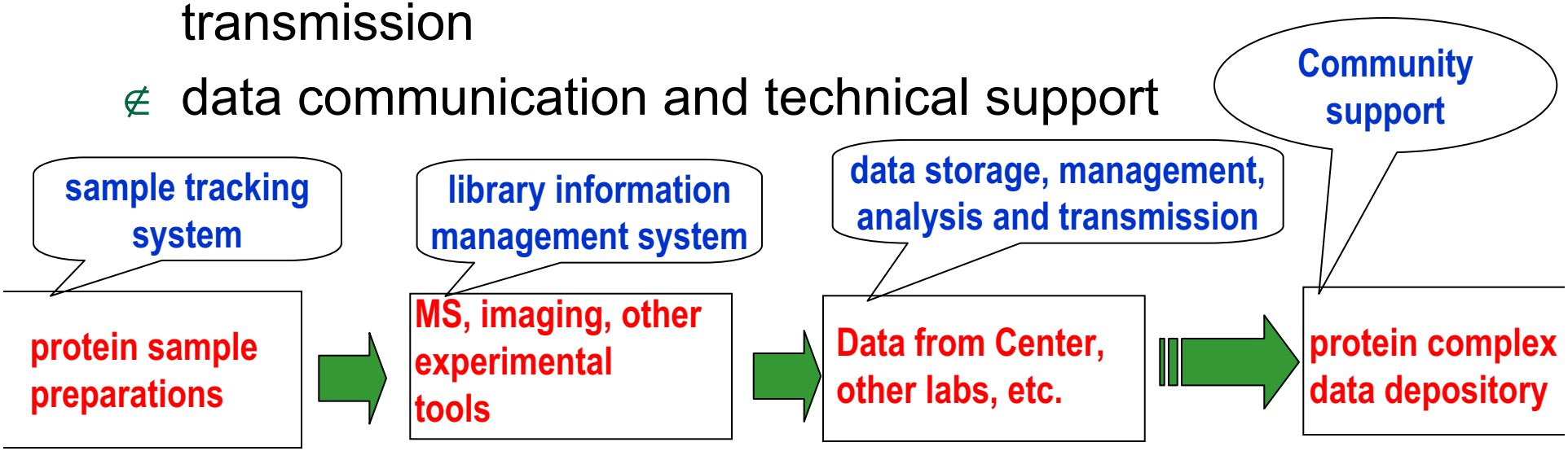
- Characterize protein complexes in isolation, within cells, and on cell surfaces/interfaces
- Employ multimodality approaches to molecular imaging—optical probes, molecular recognition force microscopy, afm/optical, $(optical)^n$
- Validate the composition of protein complexes
- Determine the location of specific complexes at cellular and subcellular locations
- Characterize dynamics, binding forces

Bioinformatics and Computing

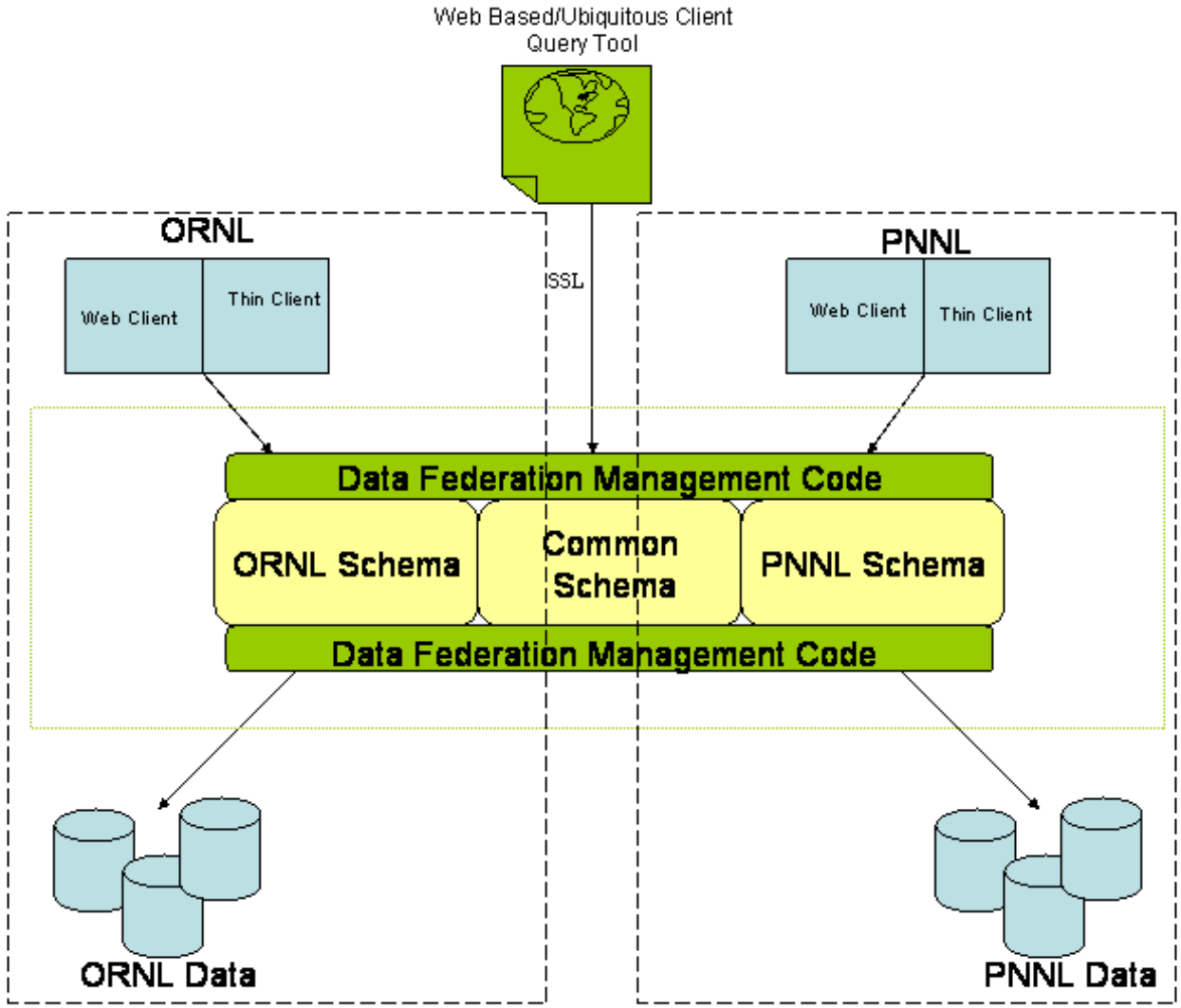
- **Short-term goals**
  - Create infrastructure for sample tracking, data collection and analysis
  - Improve tools for predicting and validating members of protein complexes
  - Build tools for interpreting MS data from cross-linked and modified proteins
- **Long-term goals**
  - Predict protein structures involved in forming complexes
  - Predict function of protein complexes
  - Help build global architecture for integrating data necessary for successful systems biology

Computational Tools Support All Aspects of Center

- sample tracking
- work flow monitoring
- library information management
- data processing, storage, management and transmission
- data communication and technical support



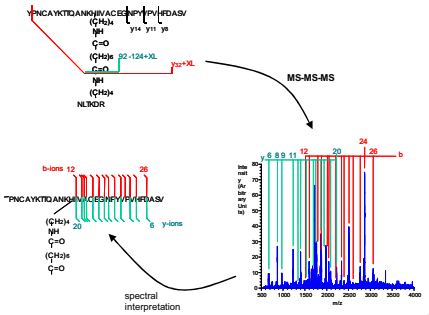
GTL LIMS System Architecture



Computational Characterization of Protein Complexes

Experimental conditions

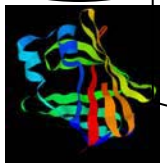
mass spec. data



genomic/proteomic databases

emerging technologies

PDB



structural genomics

data interpretation and modeling

functional characterization, dynamics simulation, etc.....

structural characterization of protein complexes

protein interaction map construction

protein identification and protein complex identification

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Acknowledgements

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