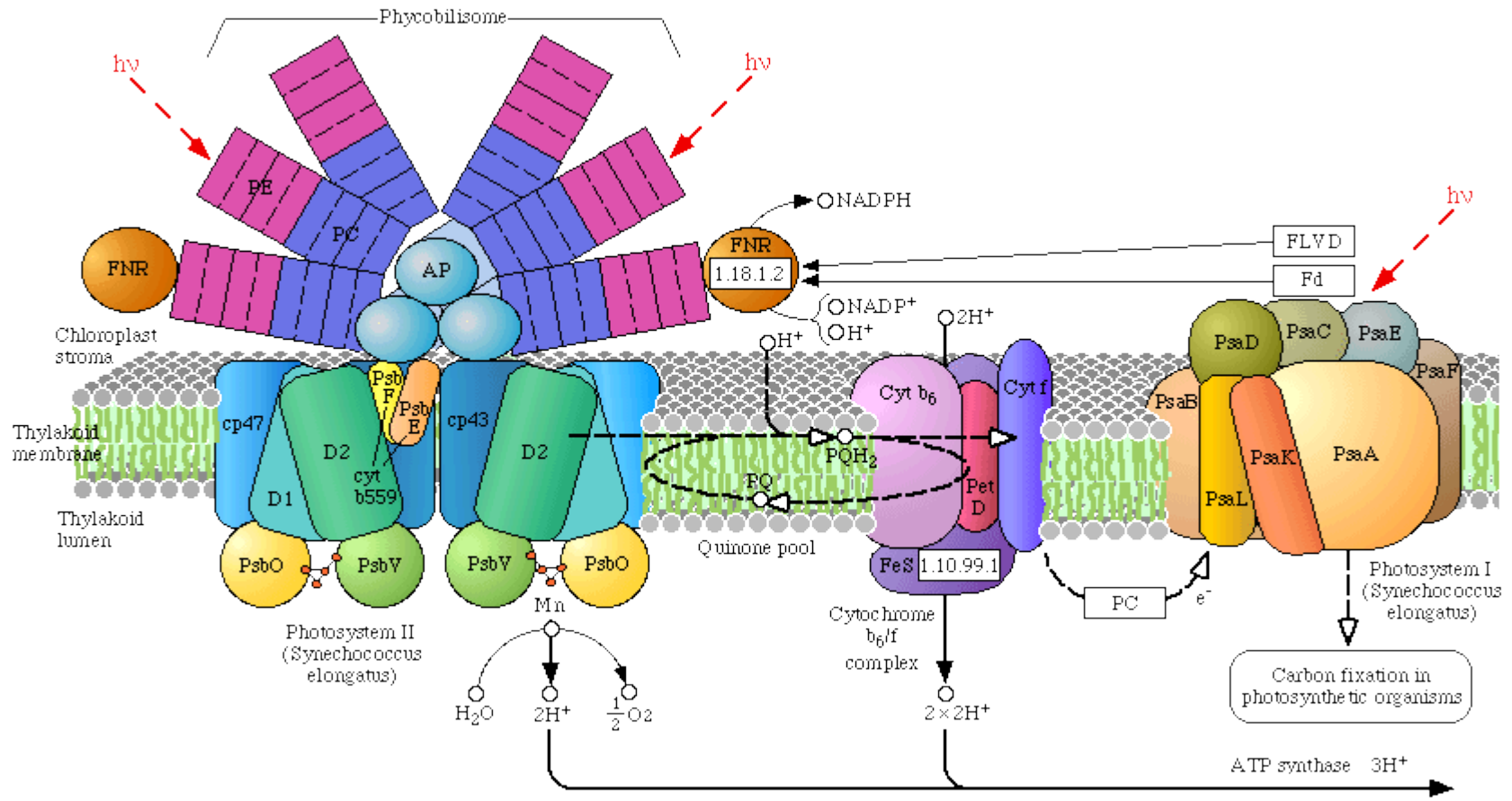


# **Systematically Ranking the Tightness of Membrane Association for Peripheral Membrane Proteins**

**Yingchun Wang**

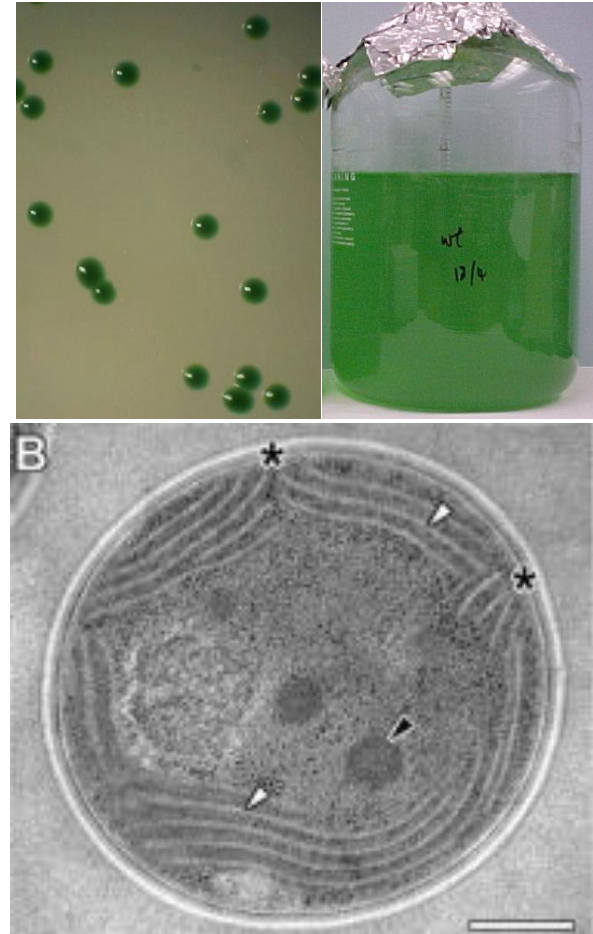
**State Key laboratory of Molecular Developmental Biology  
Institute of Genetics and Developmental Biology, CAS**

# Membrane Proteins



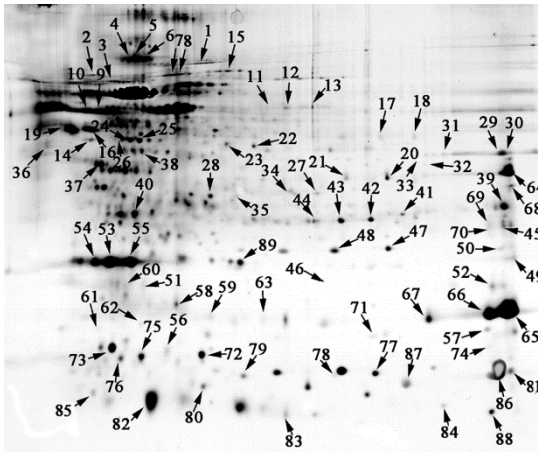
# Model: *Synechocystis*

- Widely used model for photosynthesis and respiration. Many proteins are related with photosynthesis or respiration.
- 3.96 Mb, 3672 protein coding genes.
- Easy to uptake foreign genes and integrate into its own genome through homologous recombination, easy to make mutations.
- Can grow photoautotrophically or heterotrophically using glucose as the sole carbon source.
- Generation of renewable energy and waster water treatment.
- Contains a large fraction of thylakoid membrane.



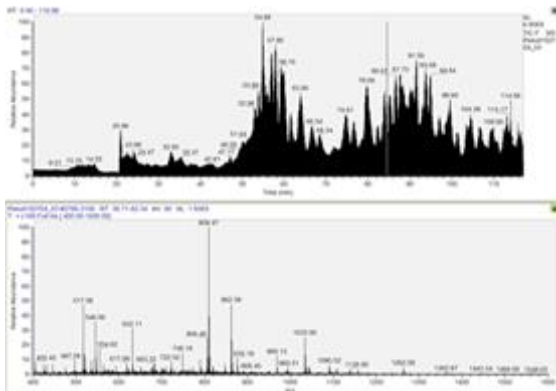
Arch Microbiol (2006) 184: 259–270

# Overview of *Synechocystis* Membrane Proteomics



- N-terminal sequencing (Electrophoresis, 1997; 1999).
- MALDI-TOF (integral and thylakoid fraction)  
➤ (Electrophoresis, 2000; Proteomic science, 2009).
- MALDI-TOF (Outer, plasma, and thylakoid membrane)  
➤ (Mol. Cell. Proteomics, 2002; 2004).

2-DE

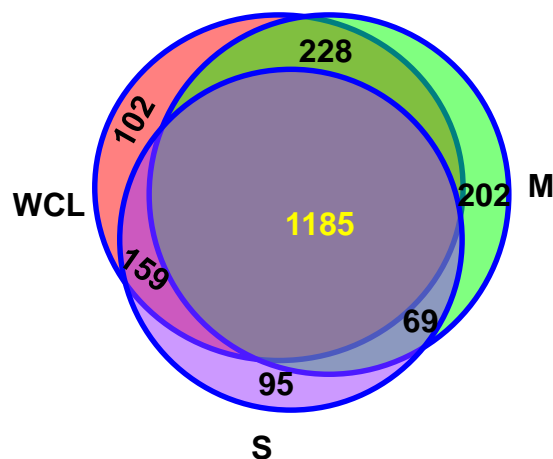


- Proteomics, 2005; 2007;
- J Proteome Res, 2006; 2007;
- J Chromatography A, 2010;

LC-MS

# Functional Proteomic Discovery of Slr0110 as a Central Regulator of Carbohydrate Metabolism in *Synechocystis* Species PCC6803\*

Liyan Gao‡, Chunting Shen‡, Libing Liao§, Xiahe Huang‡, Kehui Liu‡, Wei Wang‡, Lihai Guo¶, Wenhai Jin¶, Fang Huang§, Wu Xu||\*\*, and Yingchun Wang‡\*\*



Identification of membrane and soluble proteins

MCP, 2014, 13:204-19

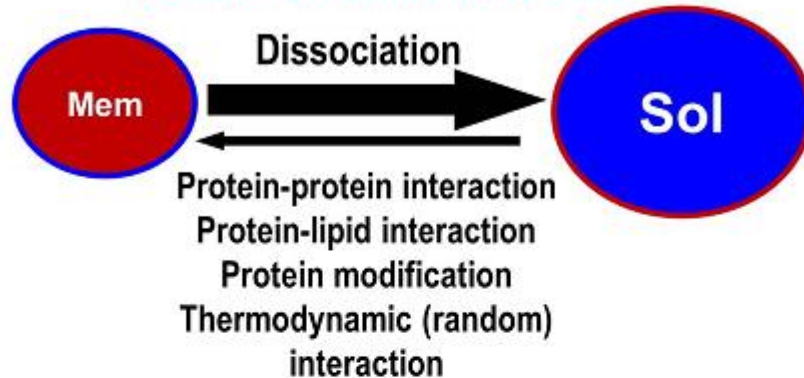
# Questions

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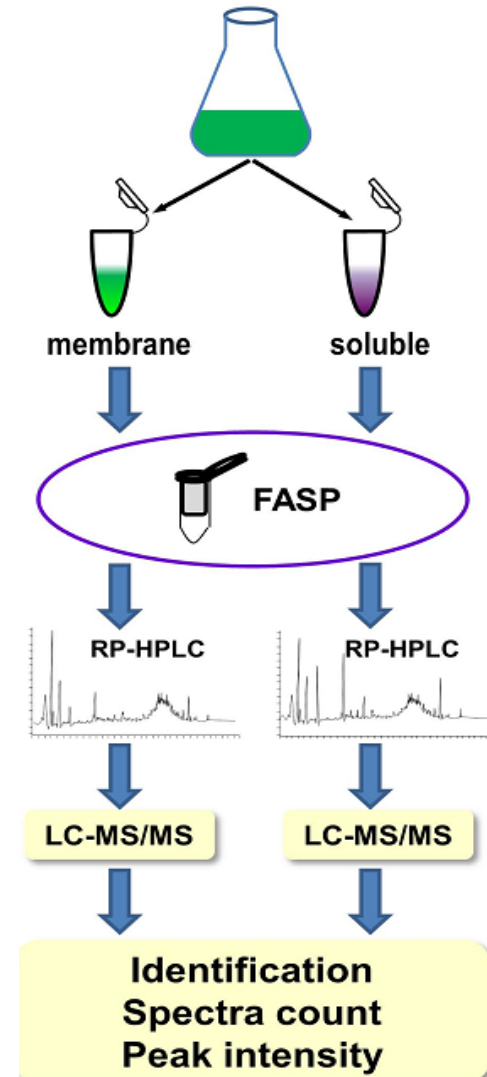
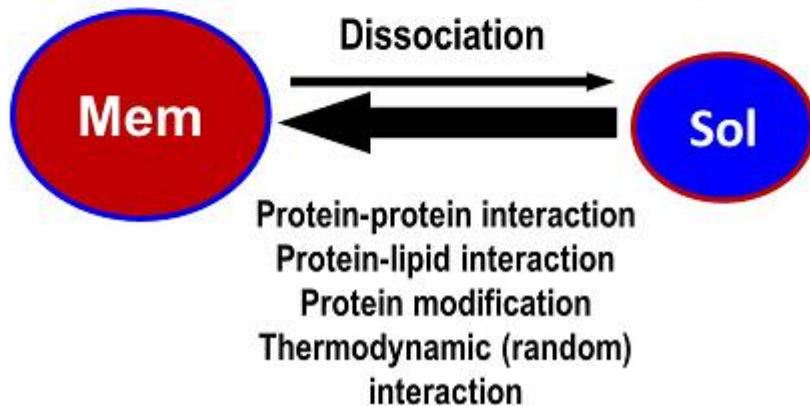
- **How to decide a non-TM containing protein identified from isolated membranes is a peripheral membrane protein or just some carry-over contamination from soluble the fraction.**
- **Are membranes the primary functional places for such a non-TM containing protein.**

# Rational and Experimental Design

## Weaker membrane association

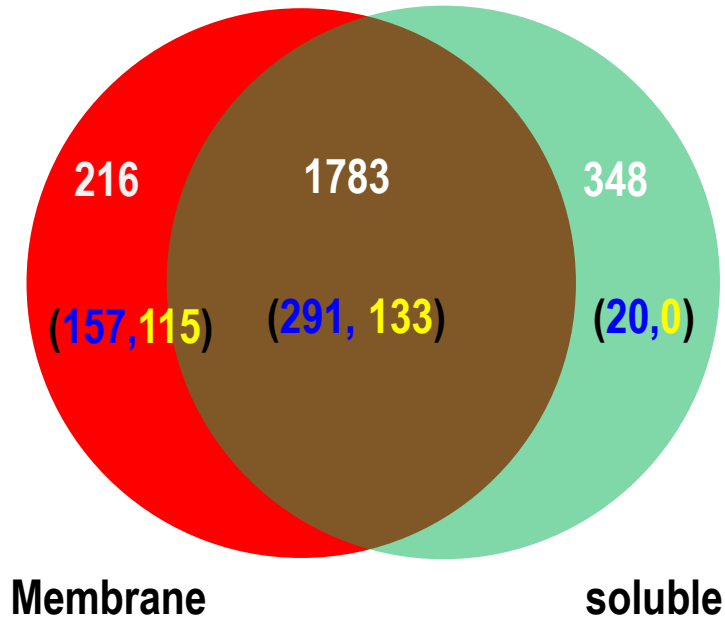


## Stronger membrane association

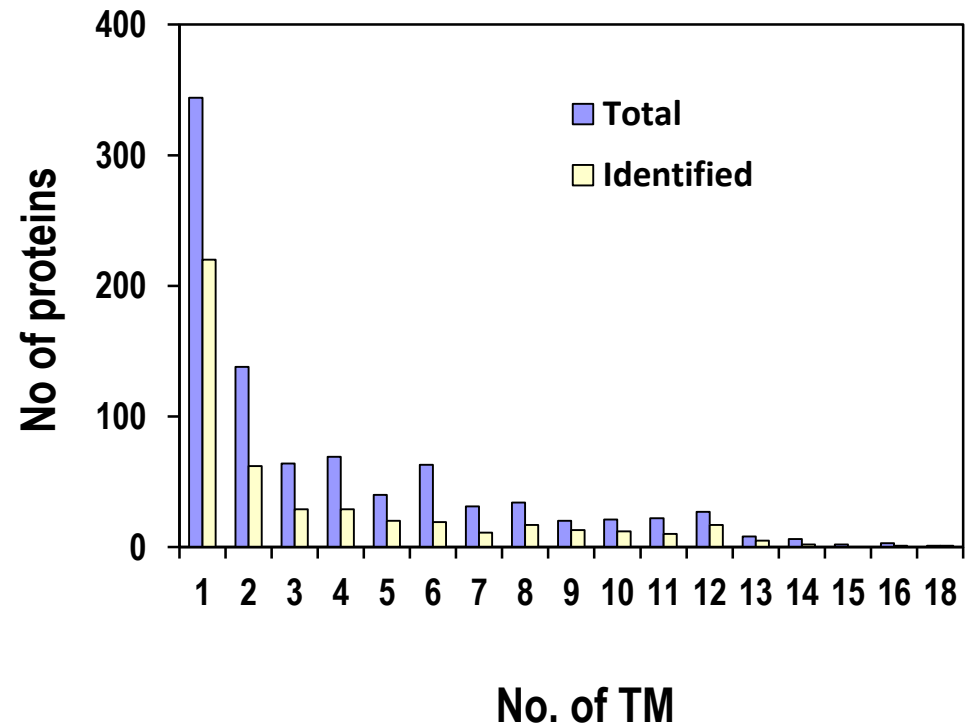


# Identification of *Synechocystis* Proteome

A

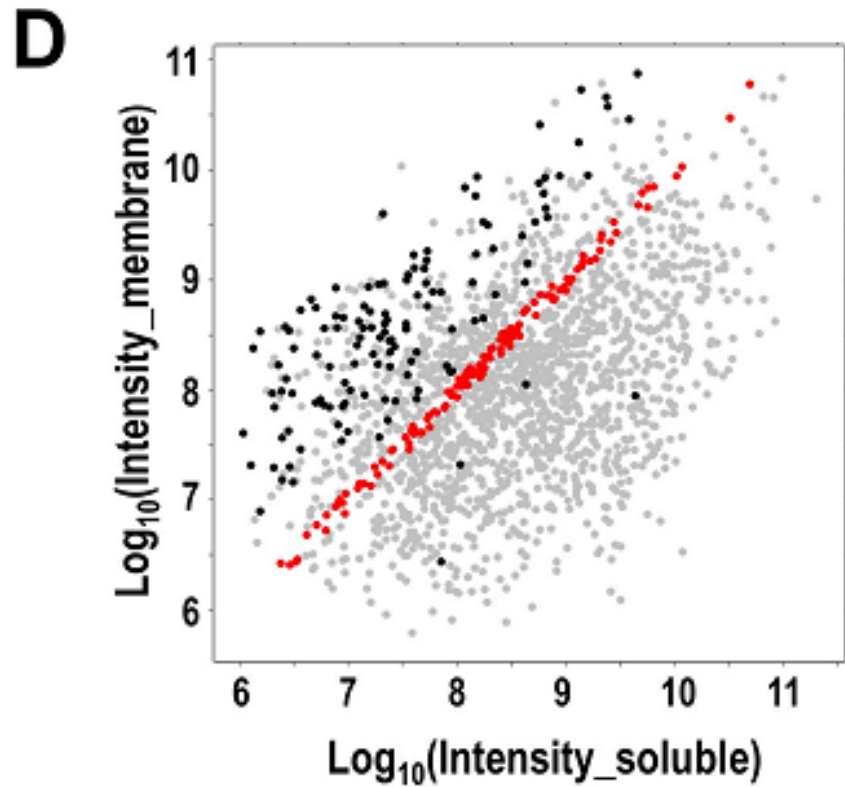
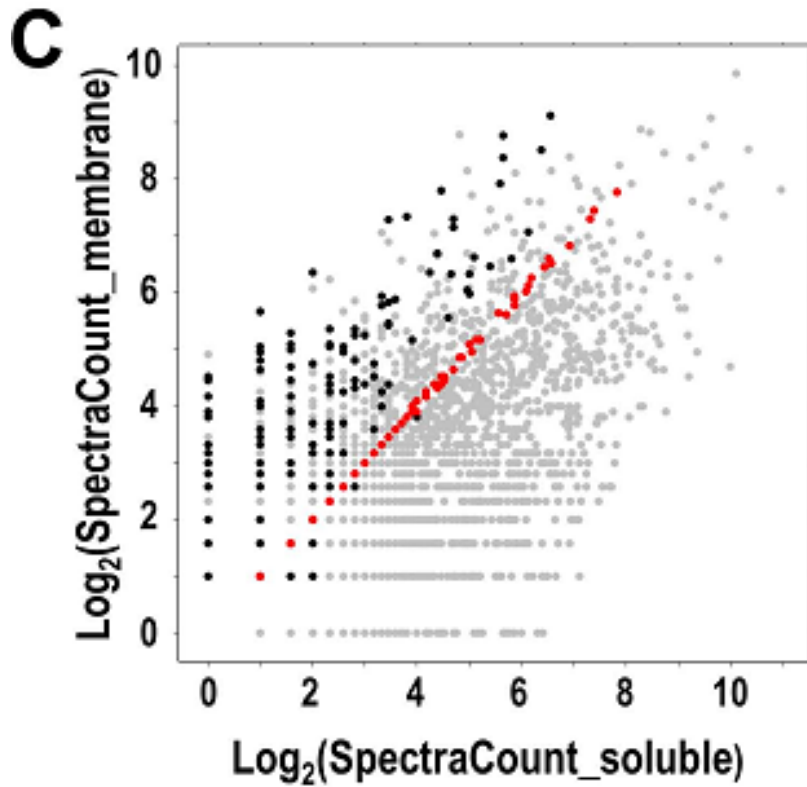


B



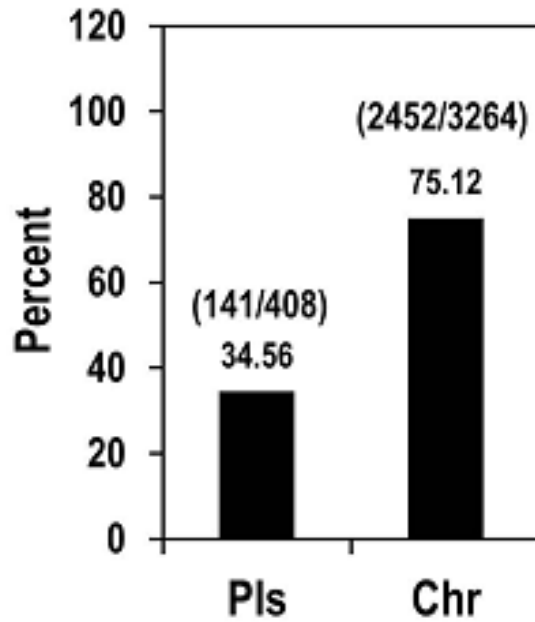


# Separation Efficiency of Membranes

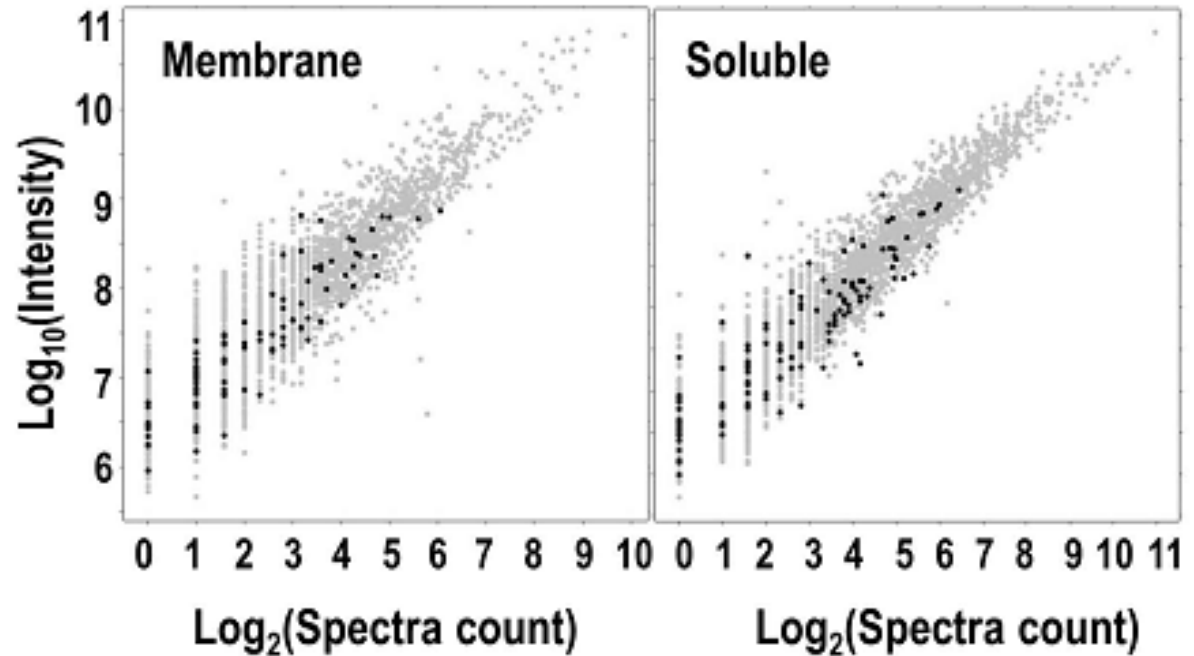


# Proteins Encoded by Chromosome- or Plasmid-Borne Genes

**E**



**F**

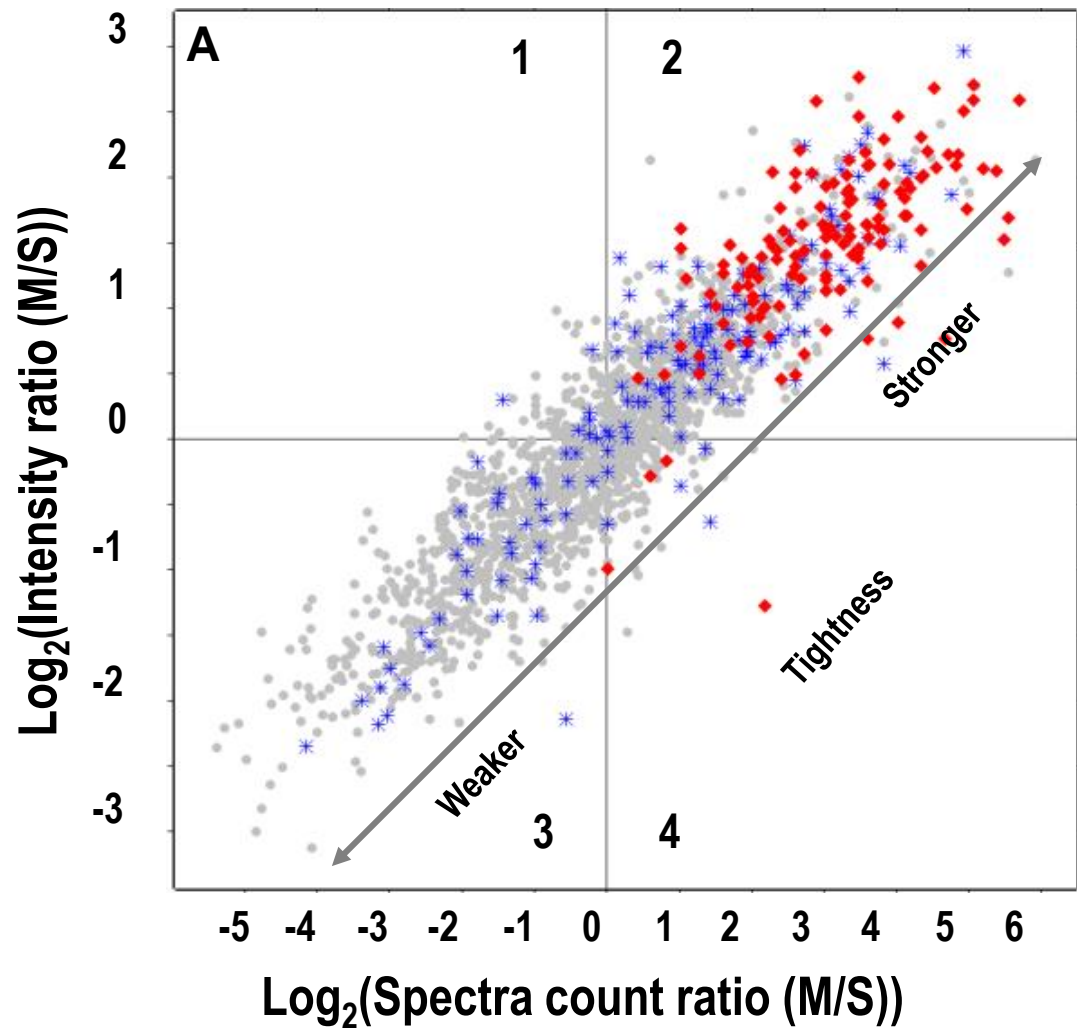
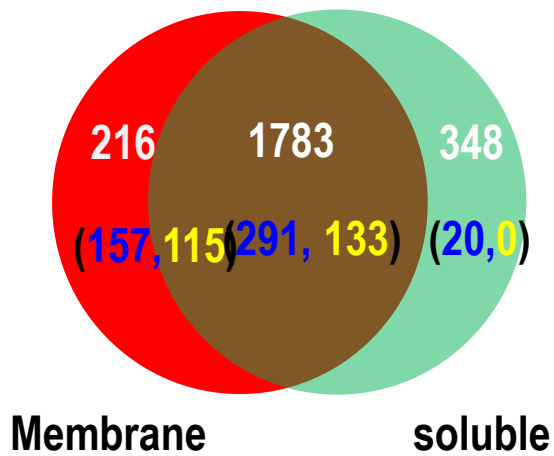


# Summary

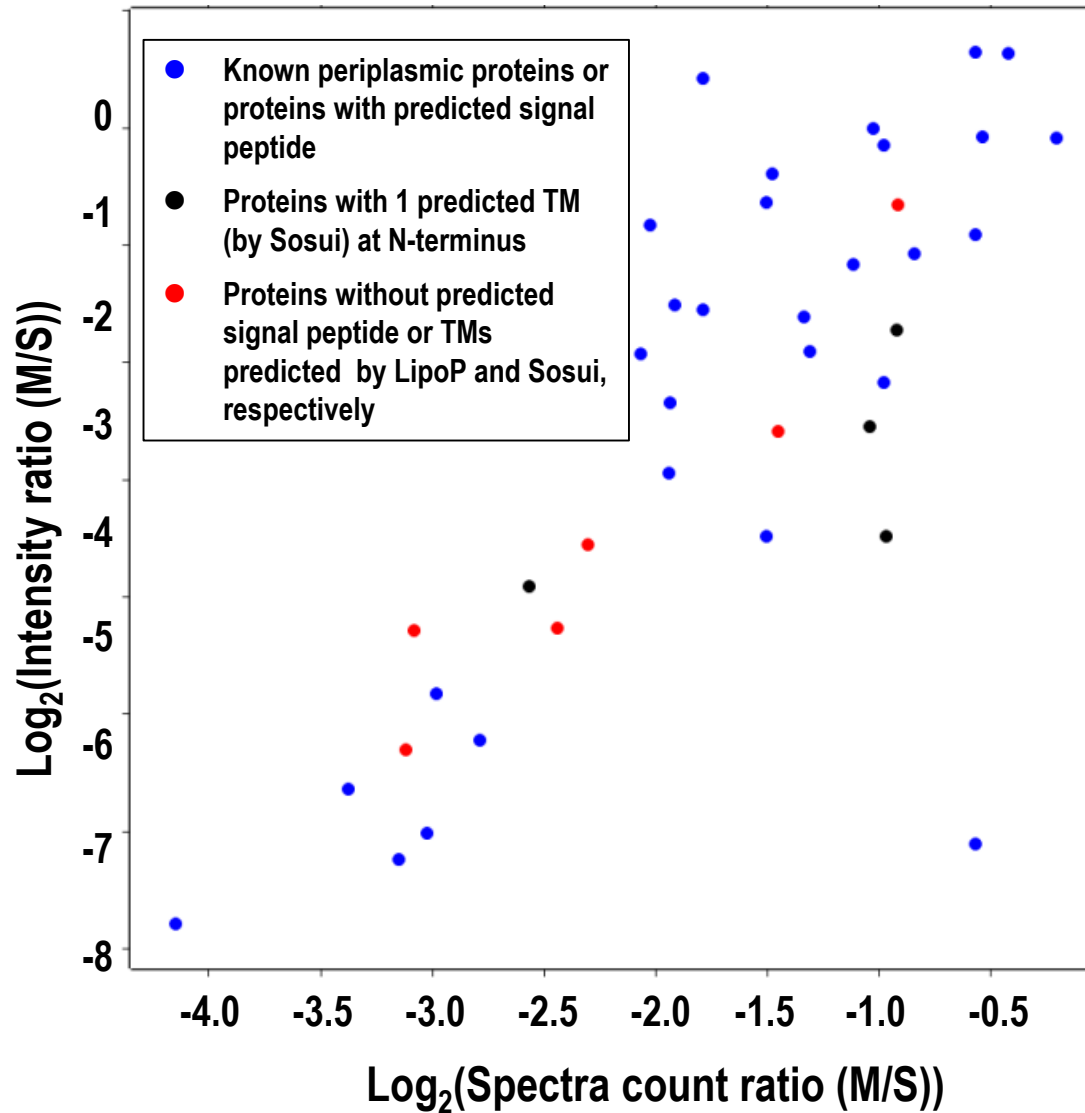
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- **2347 proteins were identified with 2-peptide match (64% of the proteome).**
- **Coverages of identification for TM-containing proteins and the total proteins are both the highest.**
- **Separation of membranes from the soluble fractions in highly efficient.**
- **Proteins encoded by chromosome-borne genes are more likely to be identified than those encoded by plasmid-borne gene.**

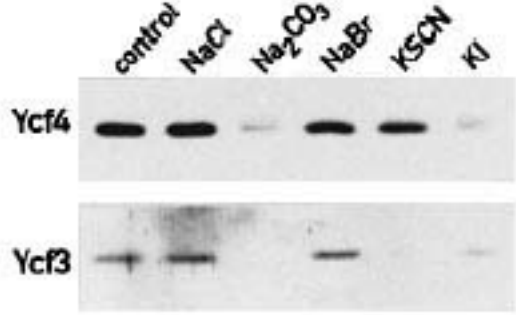
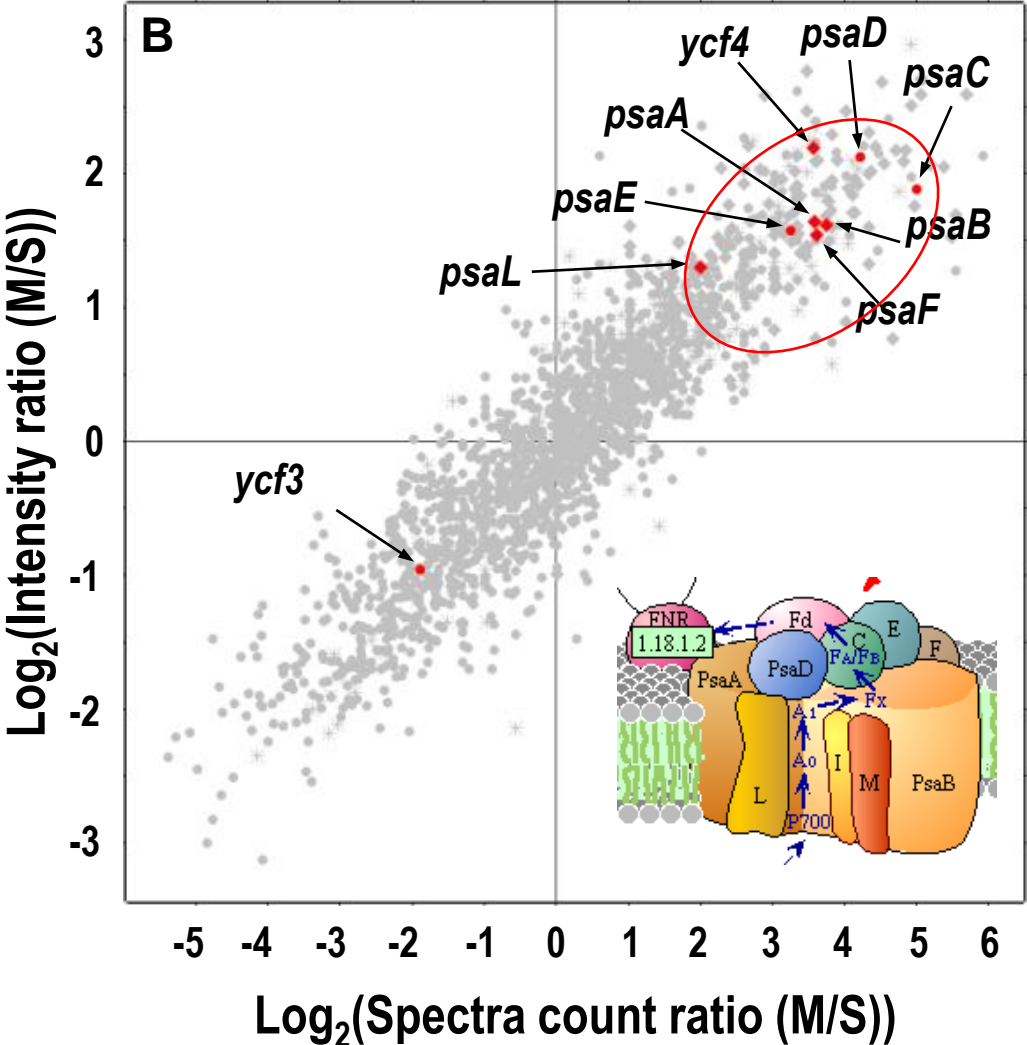
# Tightness of Membrane Association



# Proteins with 1 predicted TM

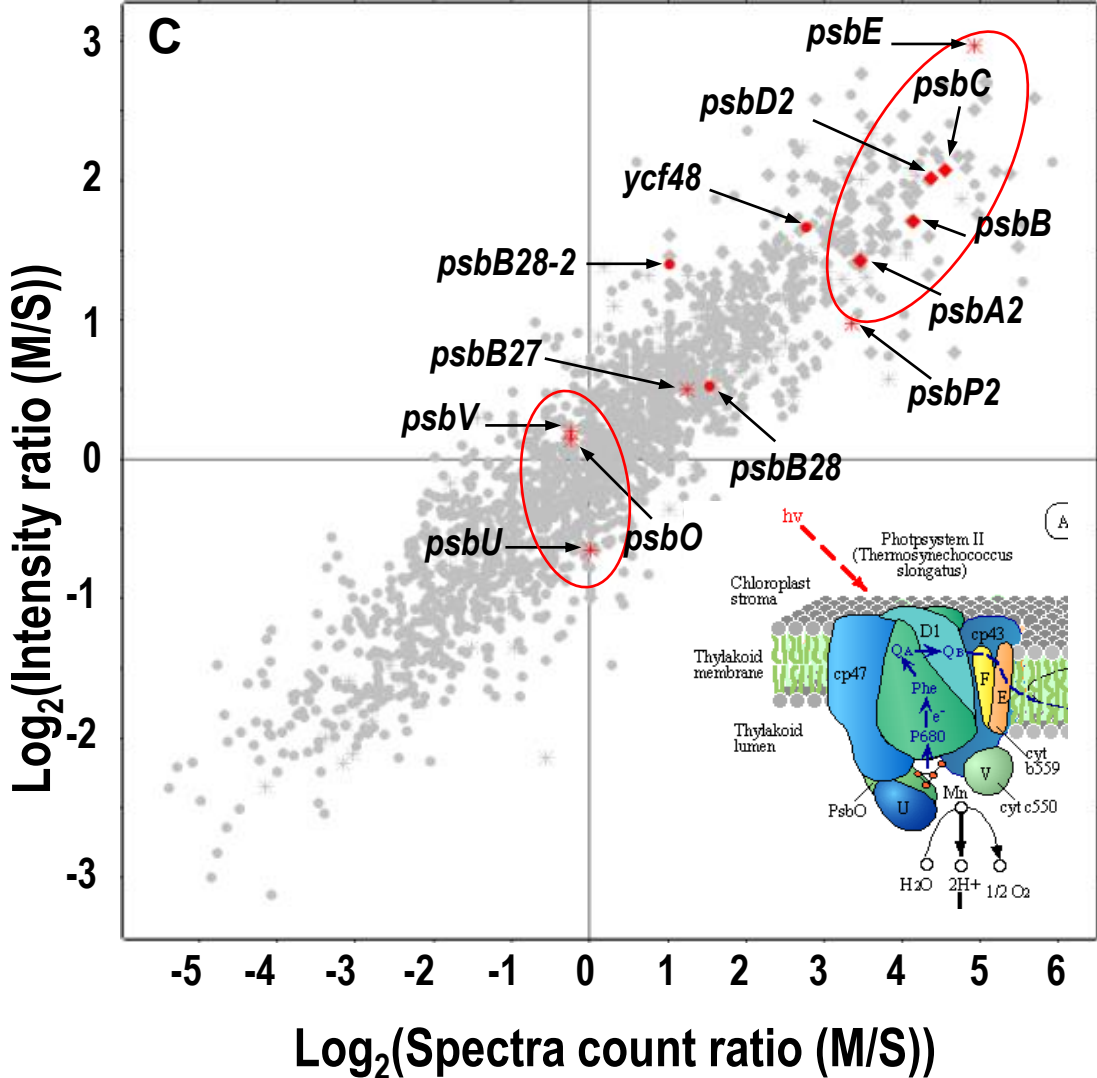


# Tightness of Membrane Association: PSI Subunits

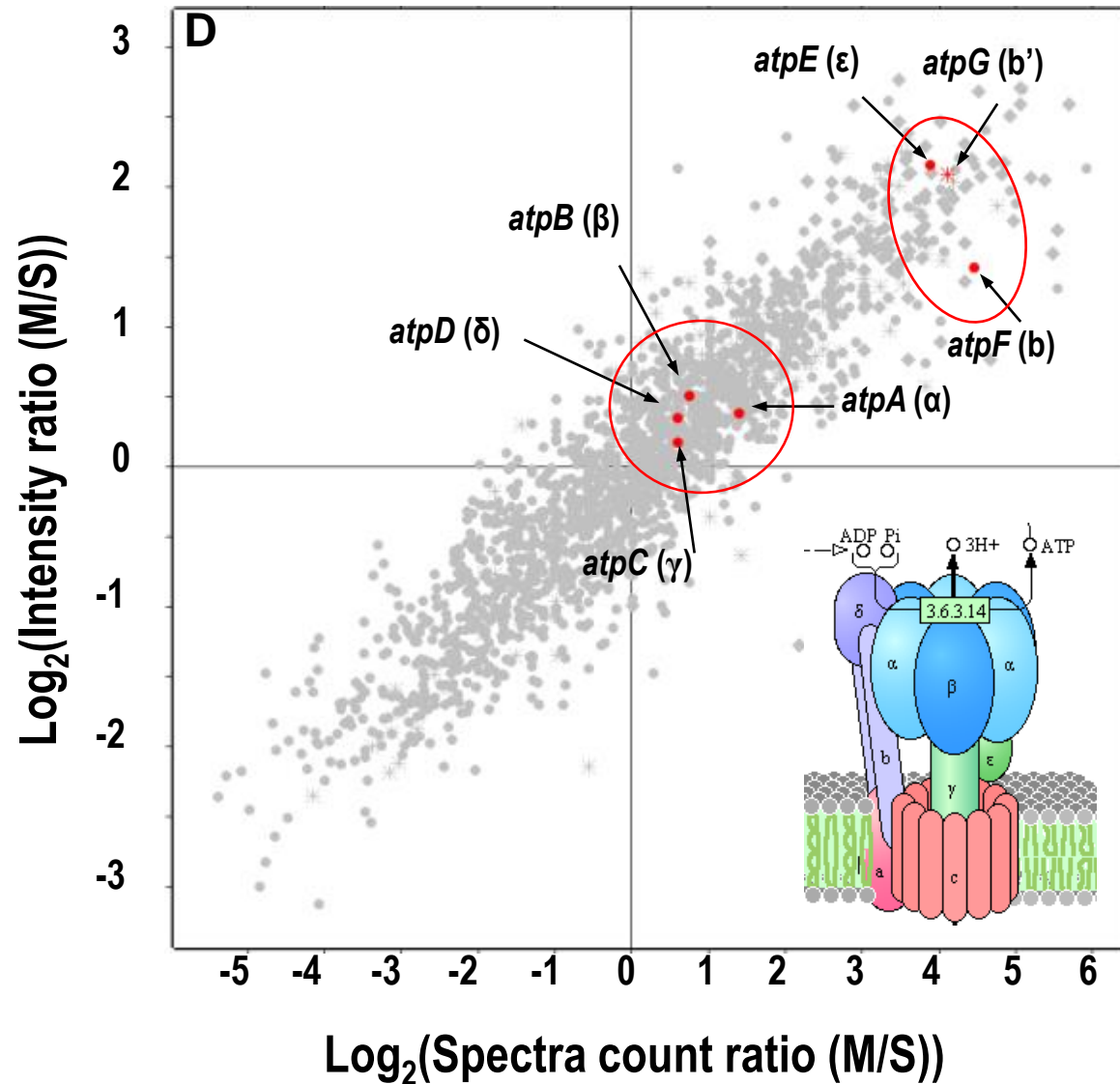


Eric Boudreau, et al, The EMBO Journal, 1997

# Tightness of Membrane Association: PSII Subunits

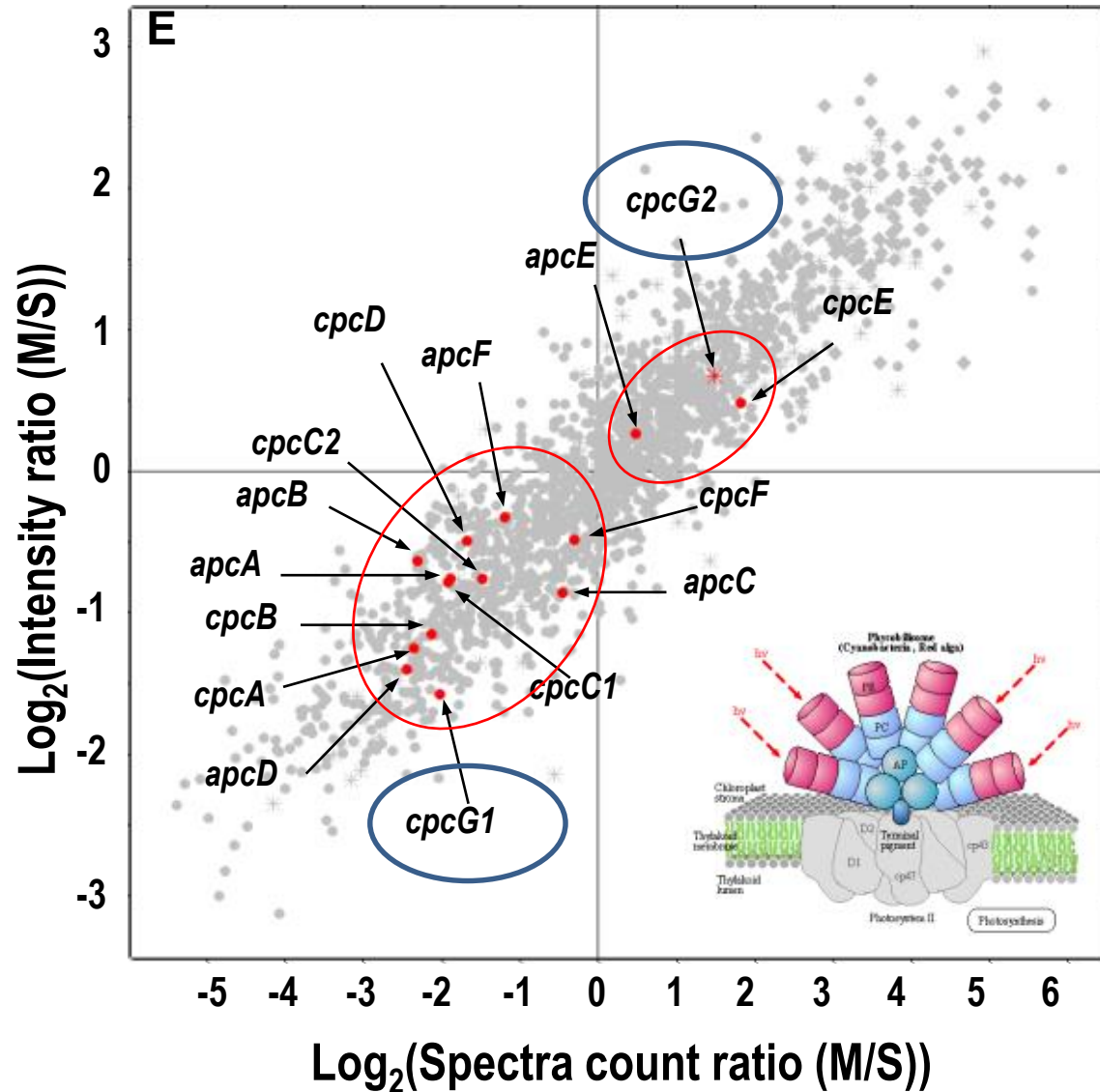


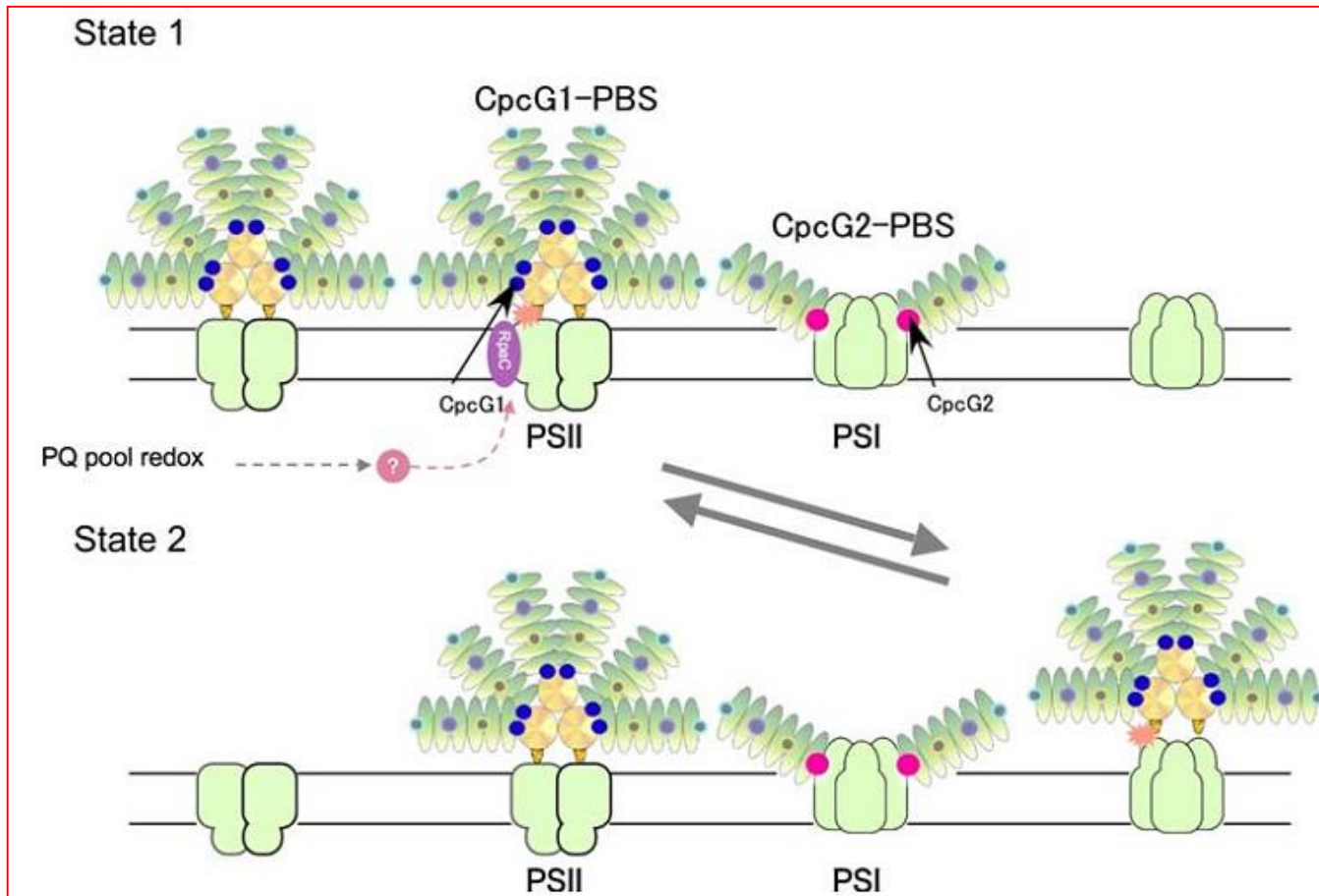
# ATP synthases





# Phycobilisomal Proteins

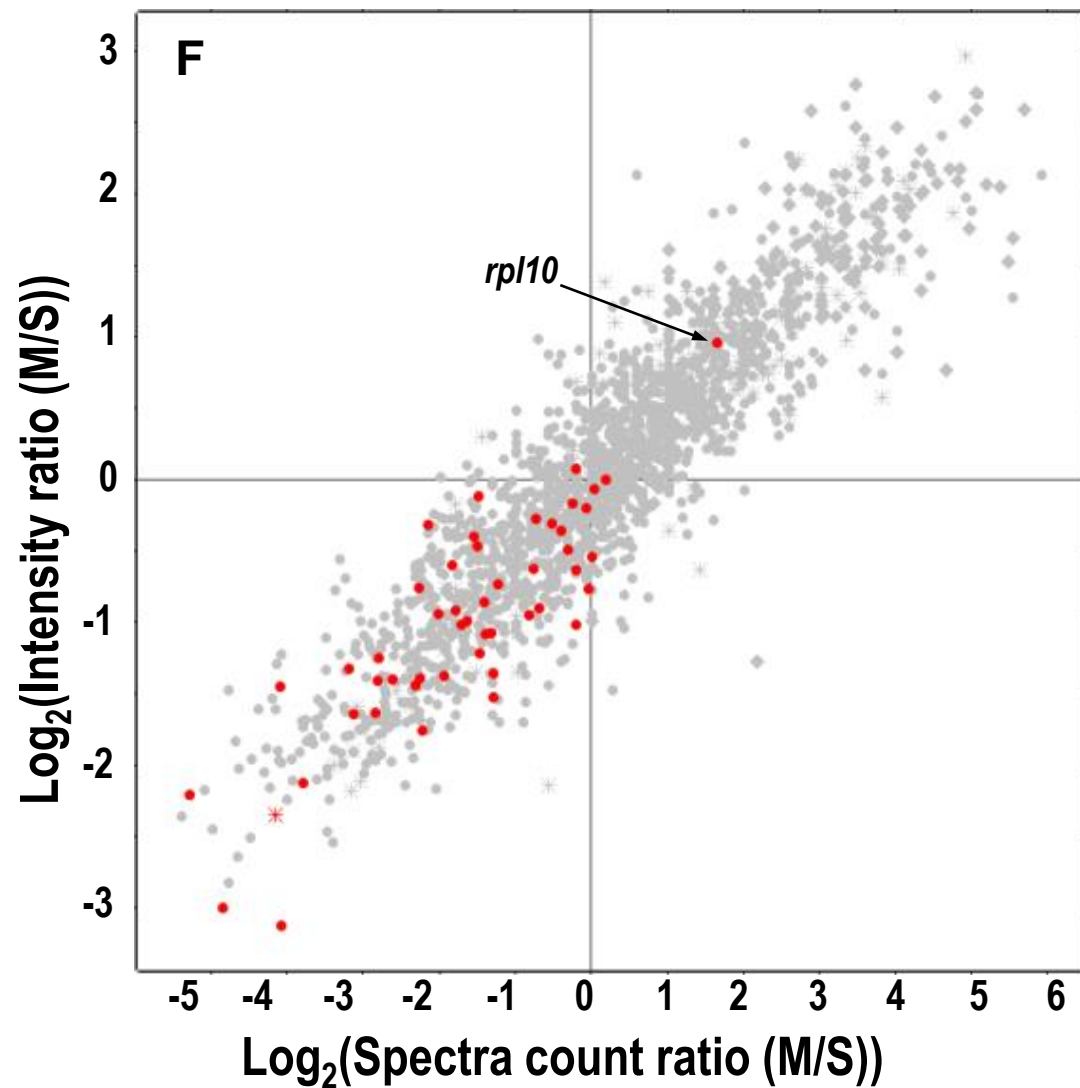




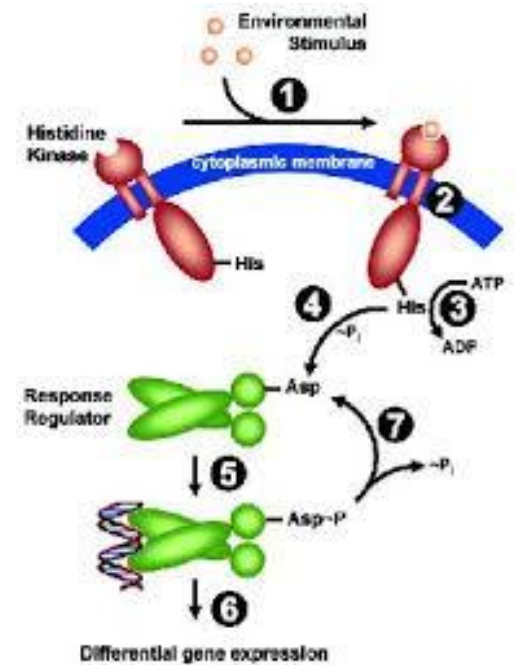
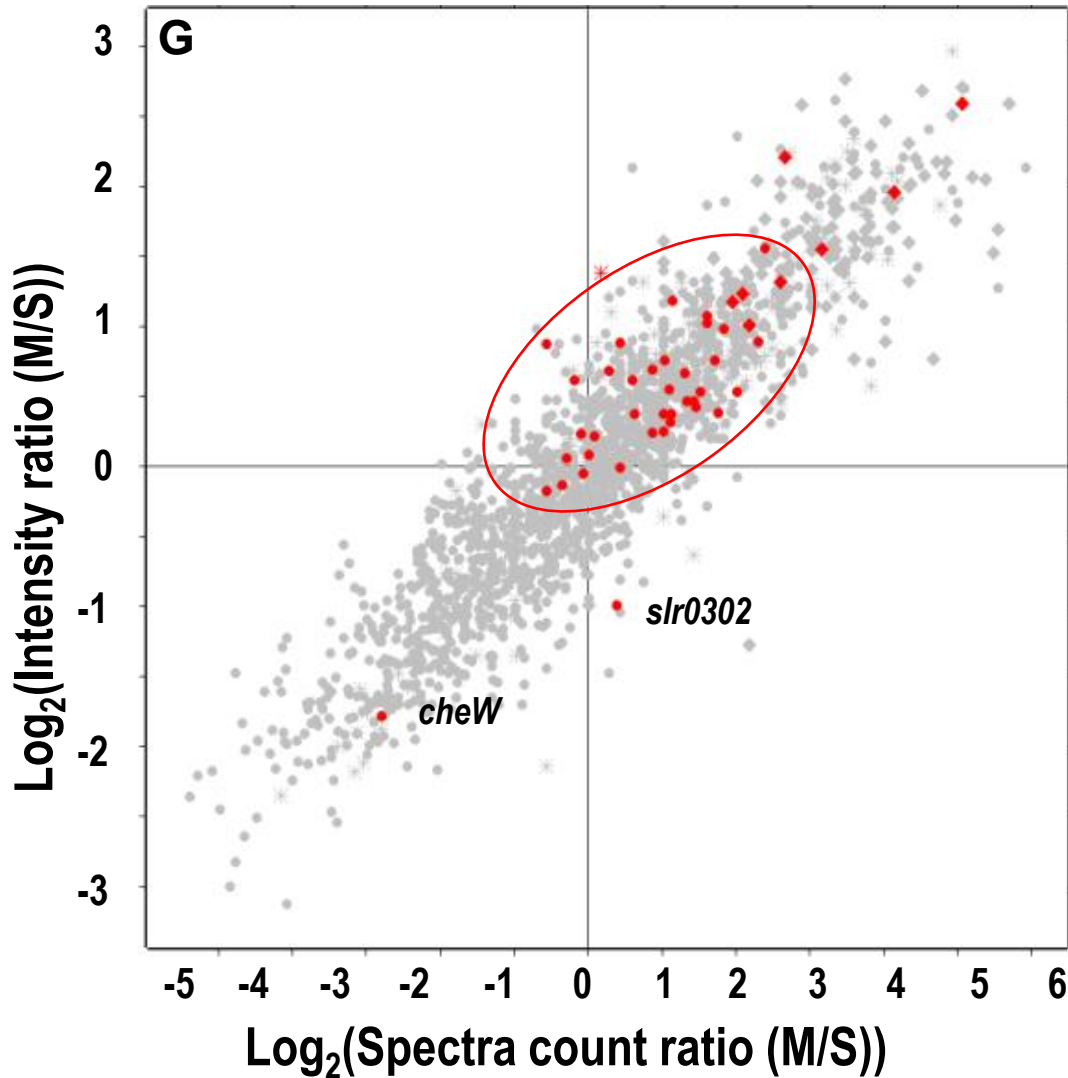
**A working model for the PBS-dependent state transition.**

**Kondo , Photosynth Res, 2009**

# Ribosomal Proteins

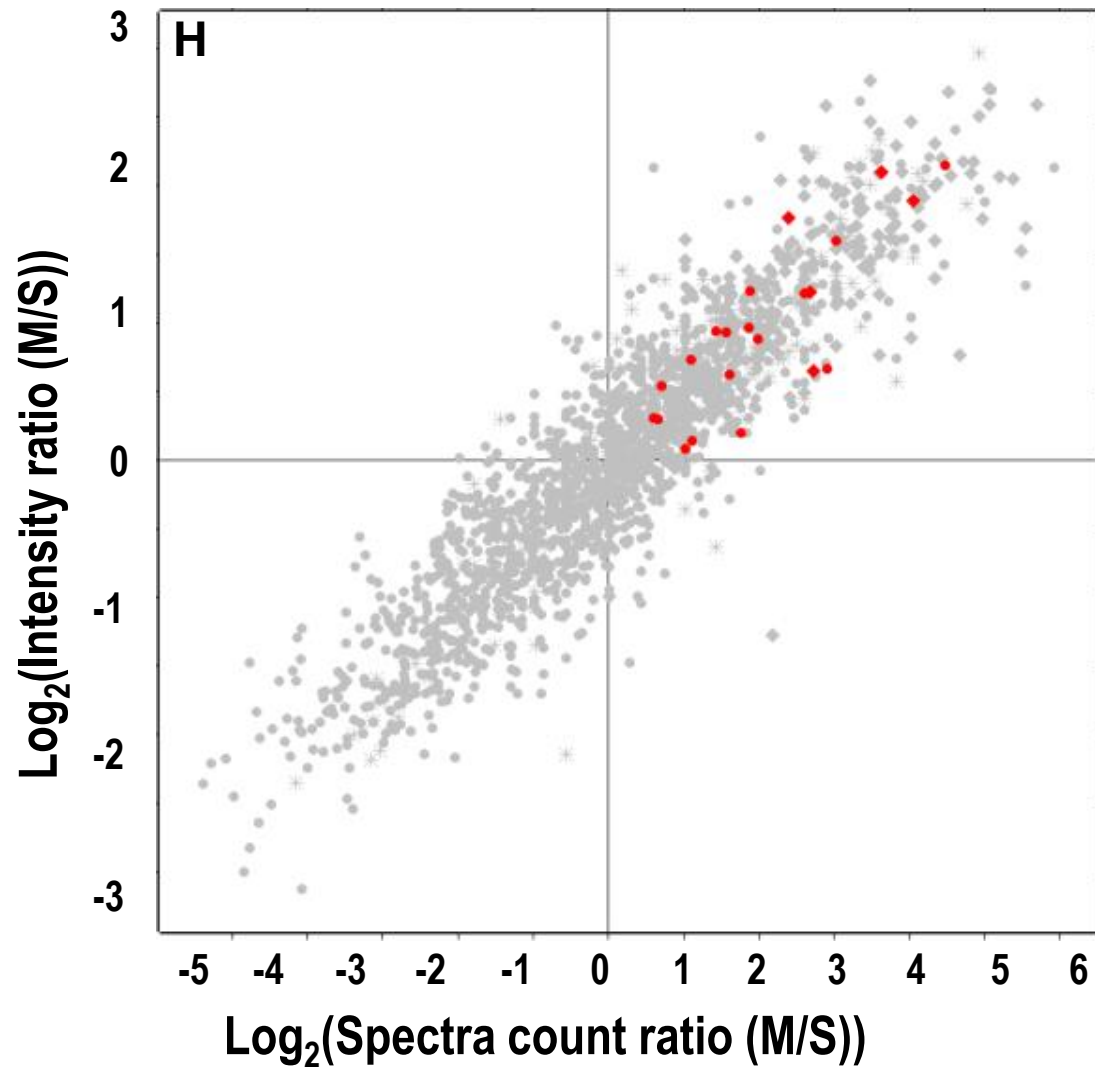


# Signaling Proteins

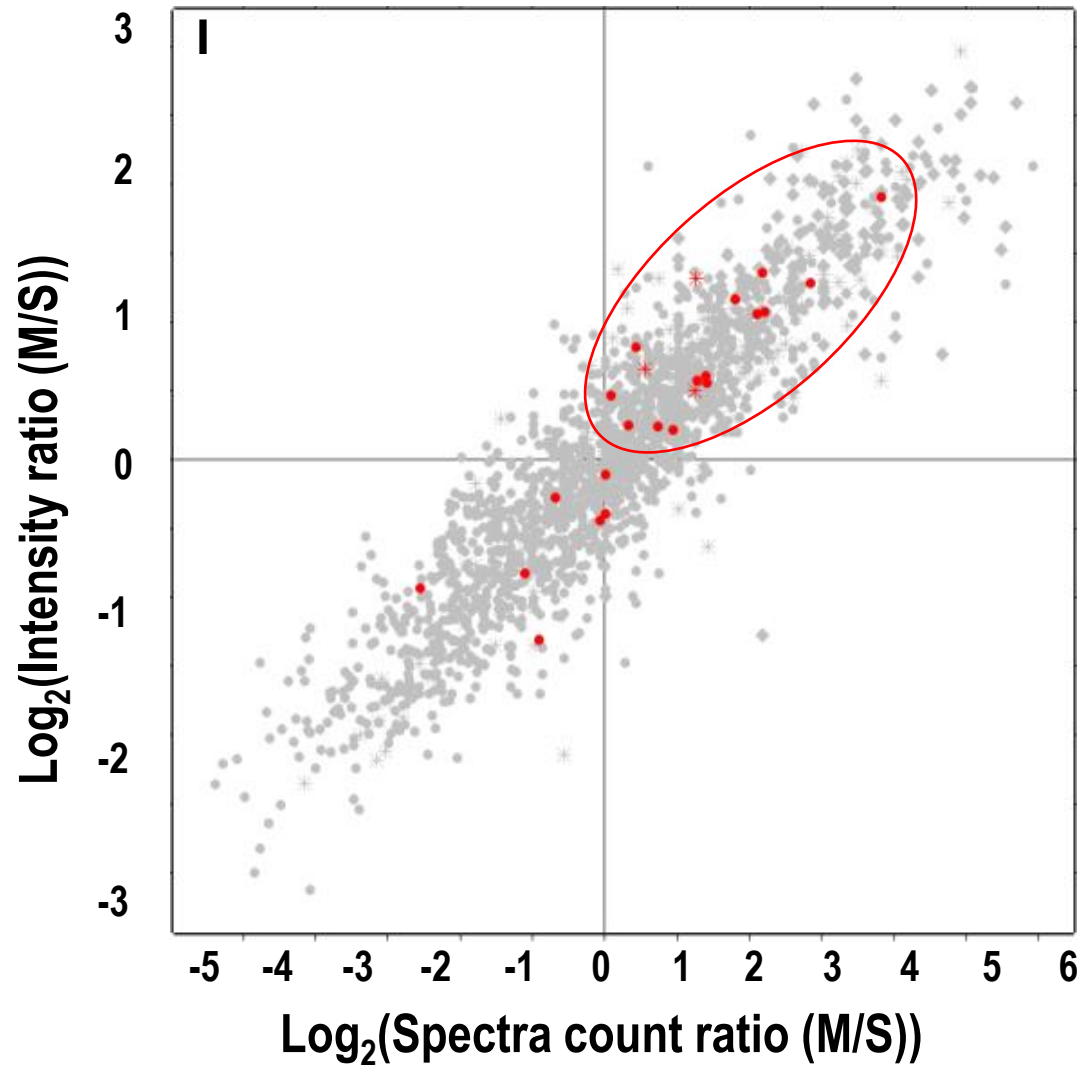


2-component signal transduction systems

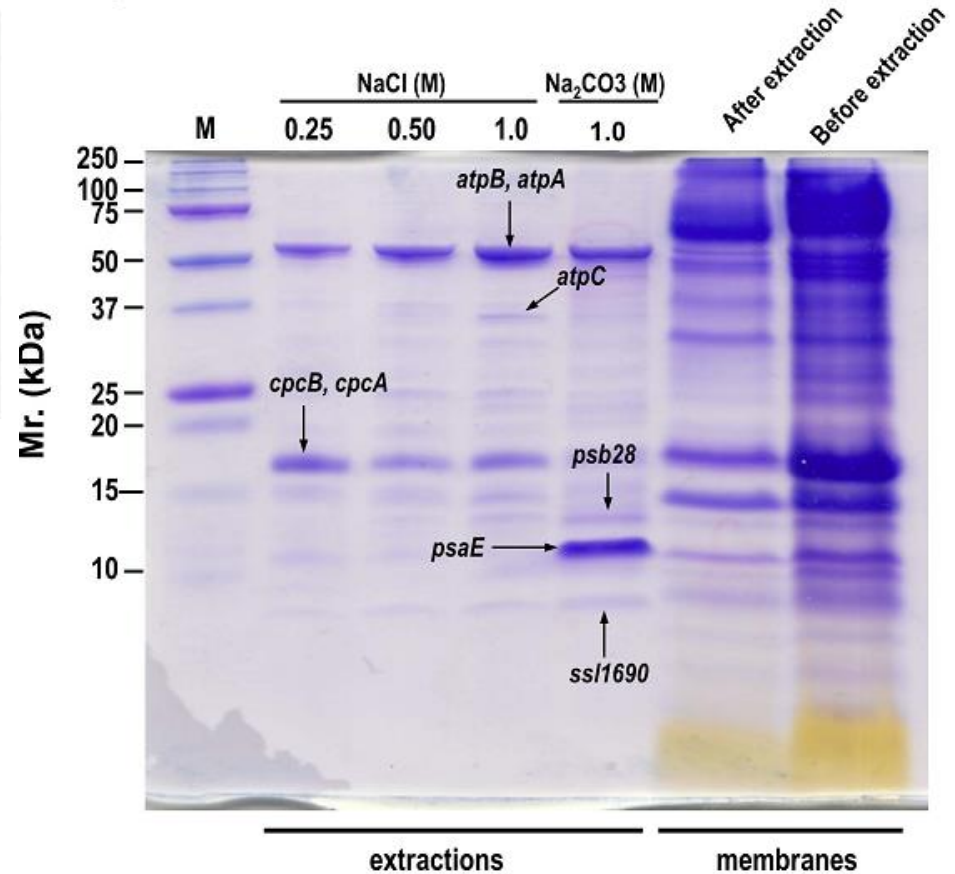
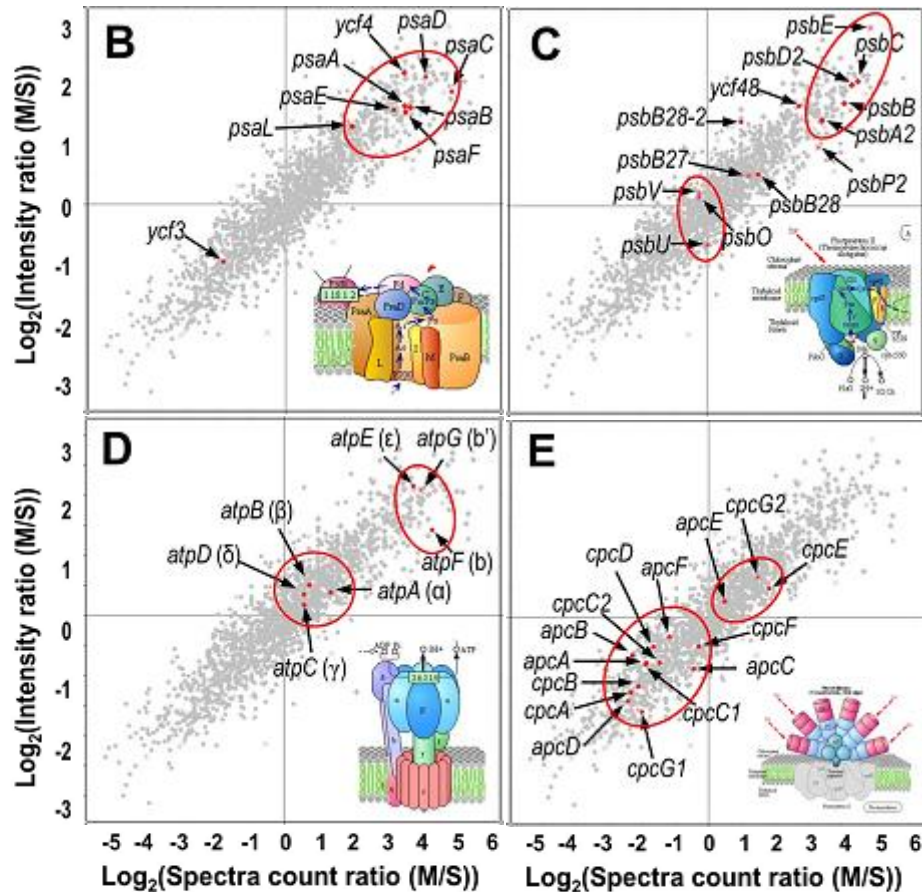
# ABC Transporters



# Lipoproteins

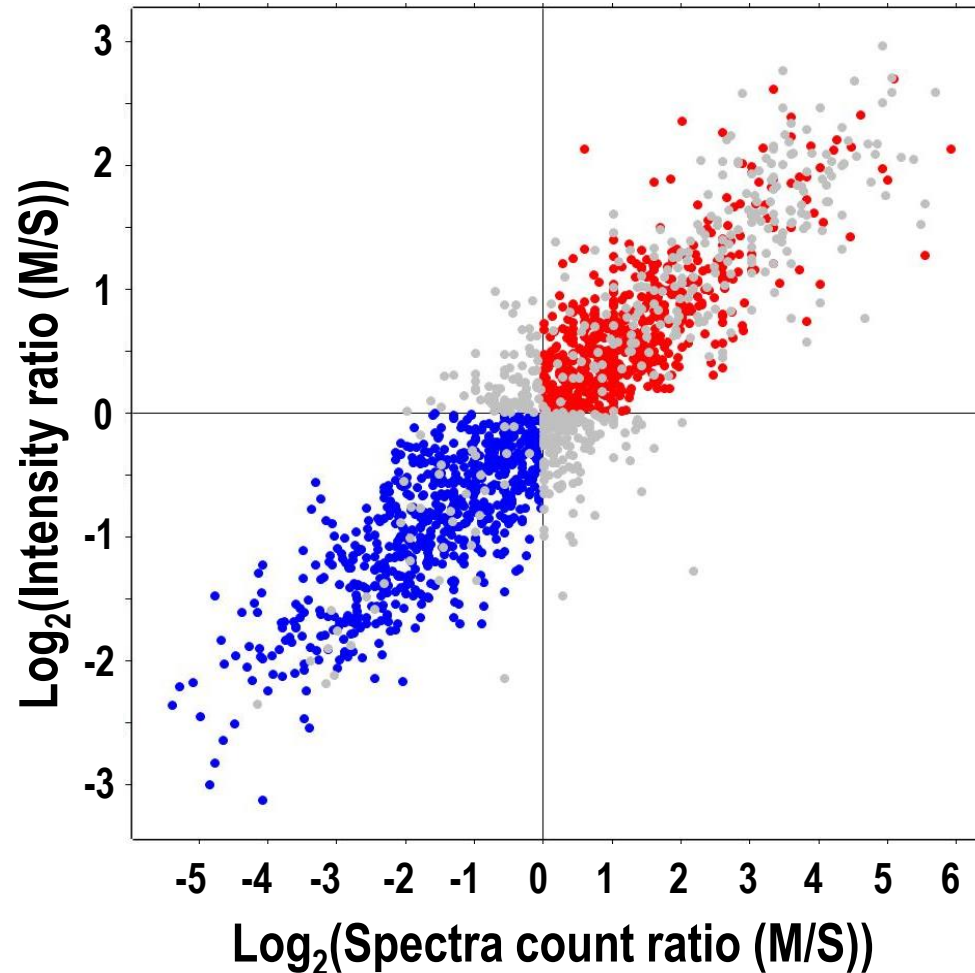


# Validation of The Tightness Measurement





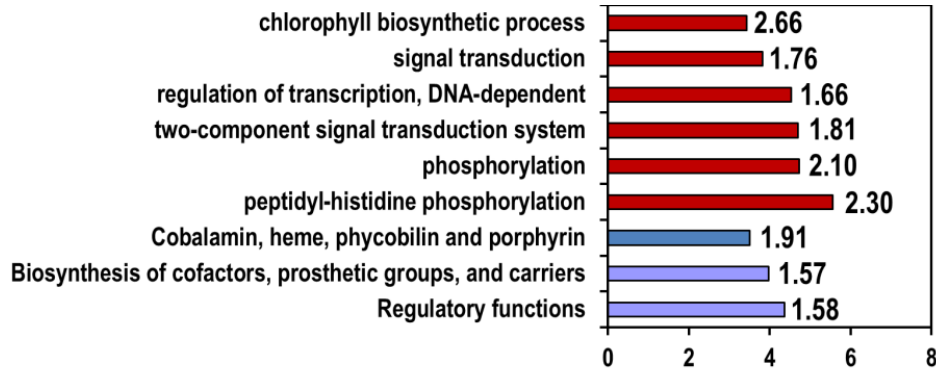
# Enriched Functions of PMPs With Strong or Weak Membrane Association



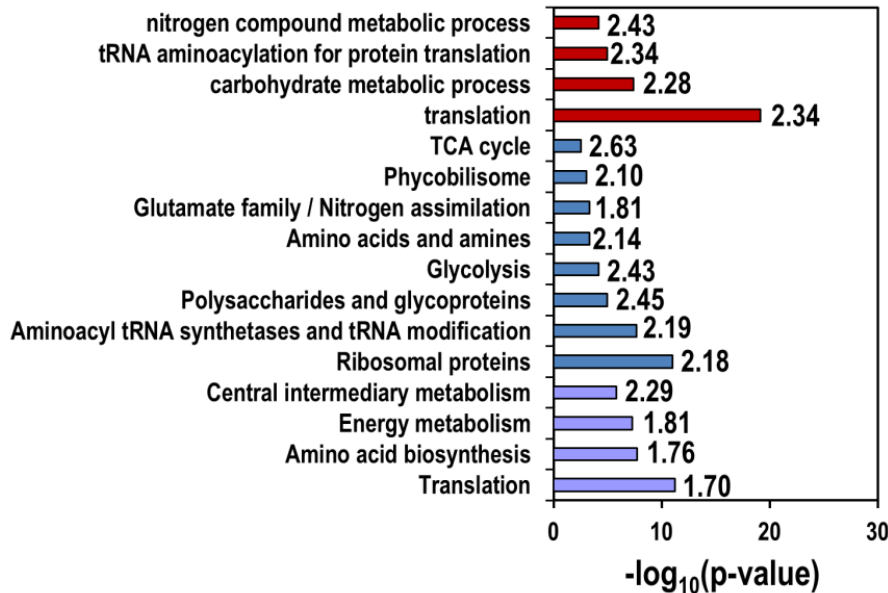
Tight membrane association: M/S ratios > 0 (Red)  
Less tight membrane association: M/S ratios < 0 (Blue)



# Enriched Functions of PMPs With Strong or Weak Membrane Association



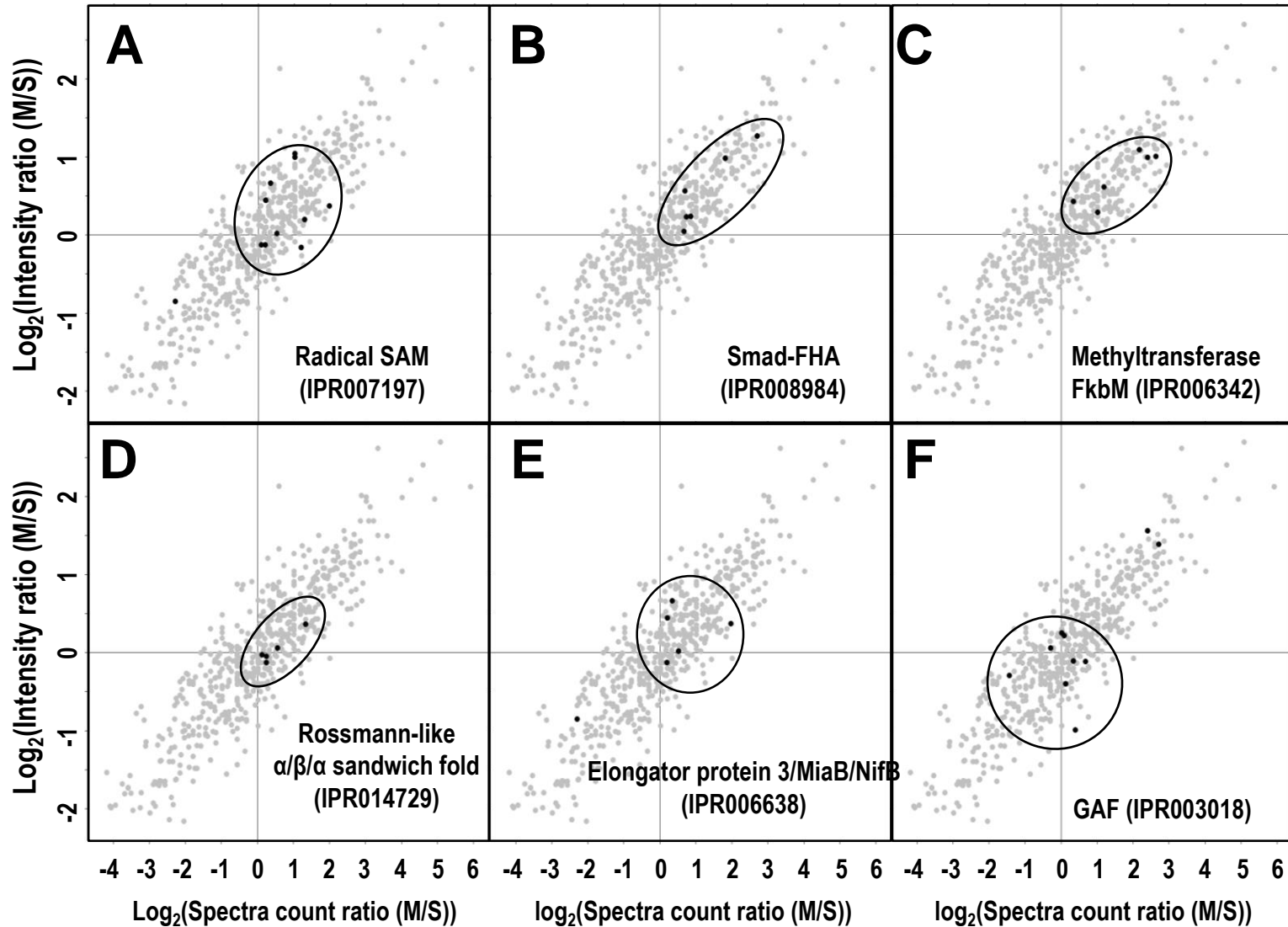
**Tightly membrane-associated proteins**



**Less tightly membrane-associated proteins**

- GO: Biological processes
- CyanoBase: First level function
- CyanoBase: Second level function

# Proteins Sharing the Same Domain Associate the Membranes with Similar Tightness



# Conclusions

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- **This tightness of membrane association for PMPs can be measured using a semiquantitative proteomics approach.**
- **Different tightness of membrane association may be required for performing different functions.**
- **Proteins sharing the same domain tend to associate the membranes with similar tightness.**
- **The method can be extended to all prokaryotic and eukaryotic organisms**
- **This work provides a global view of the structural organization of the membrane proteome with respect to divergent functions, and built the foundation for future investigation of the dynamic membrane proteome reorganization in response to different environmental or internal stimuli.**

# Acknowledgements

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## **Wang Lab at IGDB**

Haitao Ge

Dr. Kehui Liu

Xiahe Huang

Chunting Shen

Yajun Xie

Yuanya Zhang

Chen Bu

Xiaofei Liu

Yujian Wu

Jinglong Wang

Yu Kang

Longfa Fang

## **Institute of Botany, CAS**

Dr. Fang Huang

## **Institute of Hydrobiology, CAS**

Dr. Jindong Zhao

Dr. Xudong Xu

## **University of Louisiana, Lafayette**

Dr. Wu Xu