

稳定同位素代谢标记与 总体蛋白质动力学的数据处理平台

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首届中国计算蛋白质组学研讨会

Dynamic Proteomics

Study Protein Turnover (蛋白周转) on A Proteomic Scale



多数神经退化性疾病与蛋白周转紧密相关



- Alzheimer's disease: accumulation of tau in brain
- Parkinson's disease: accumulation of alpha-synuclein
- Huntington's disease: dysfunction of proteasome pathway
- Prion disease: accumulation of misfolded prion

研究蛋白周转的工具

- 放射性同位素标记

整体或器官蛋白周转

不宜实现每个蛋白的周转

- 稳定同位素标记

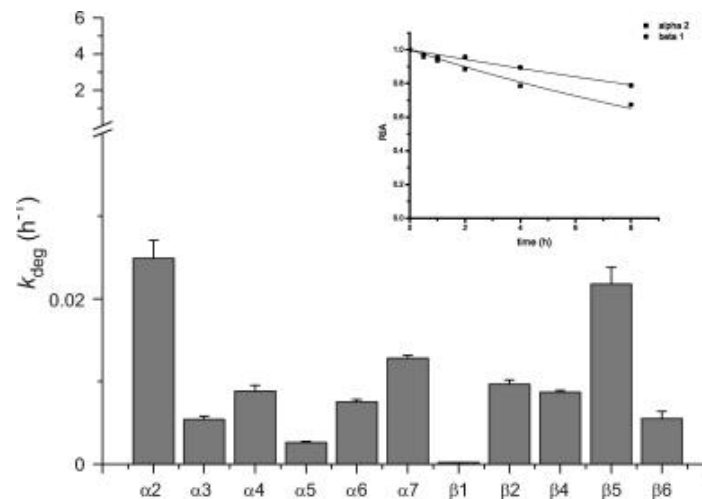
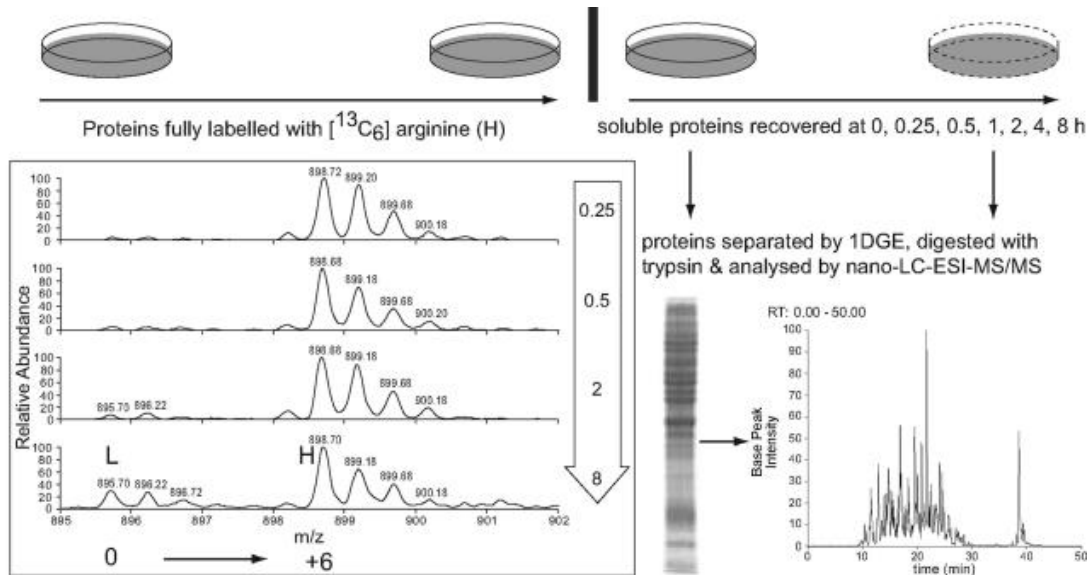
解离蛋白成游离氨基酸

GCMS测同位素丰度

Beynon的动态细胞培养稳定同位素标记技术

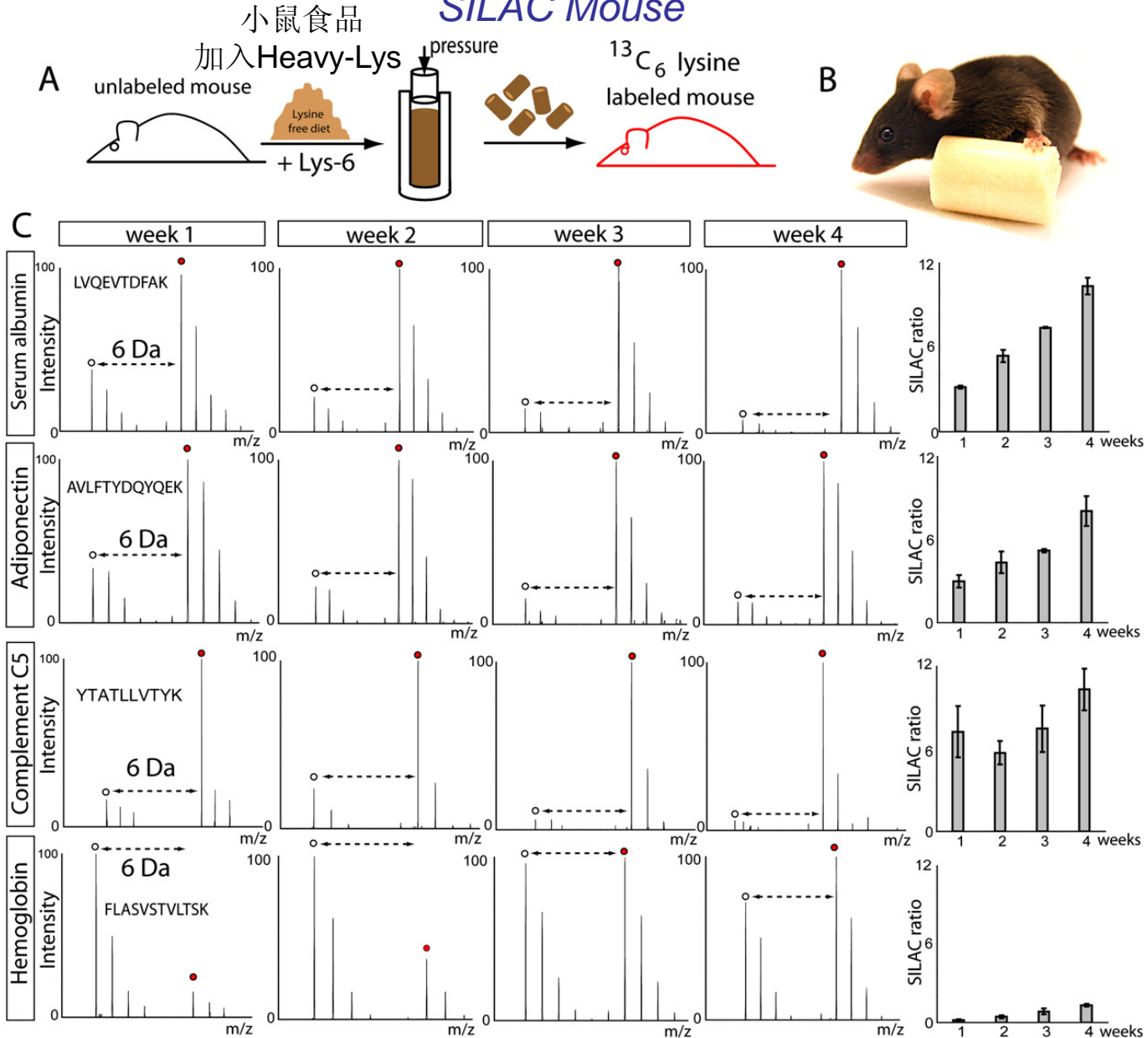
Dynamic SILAC

细胞培养液切换



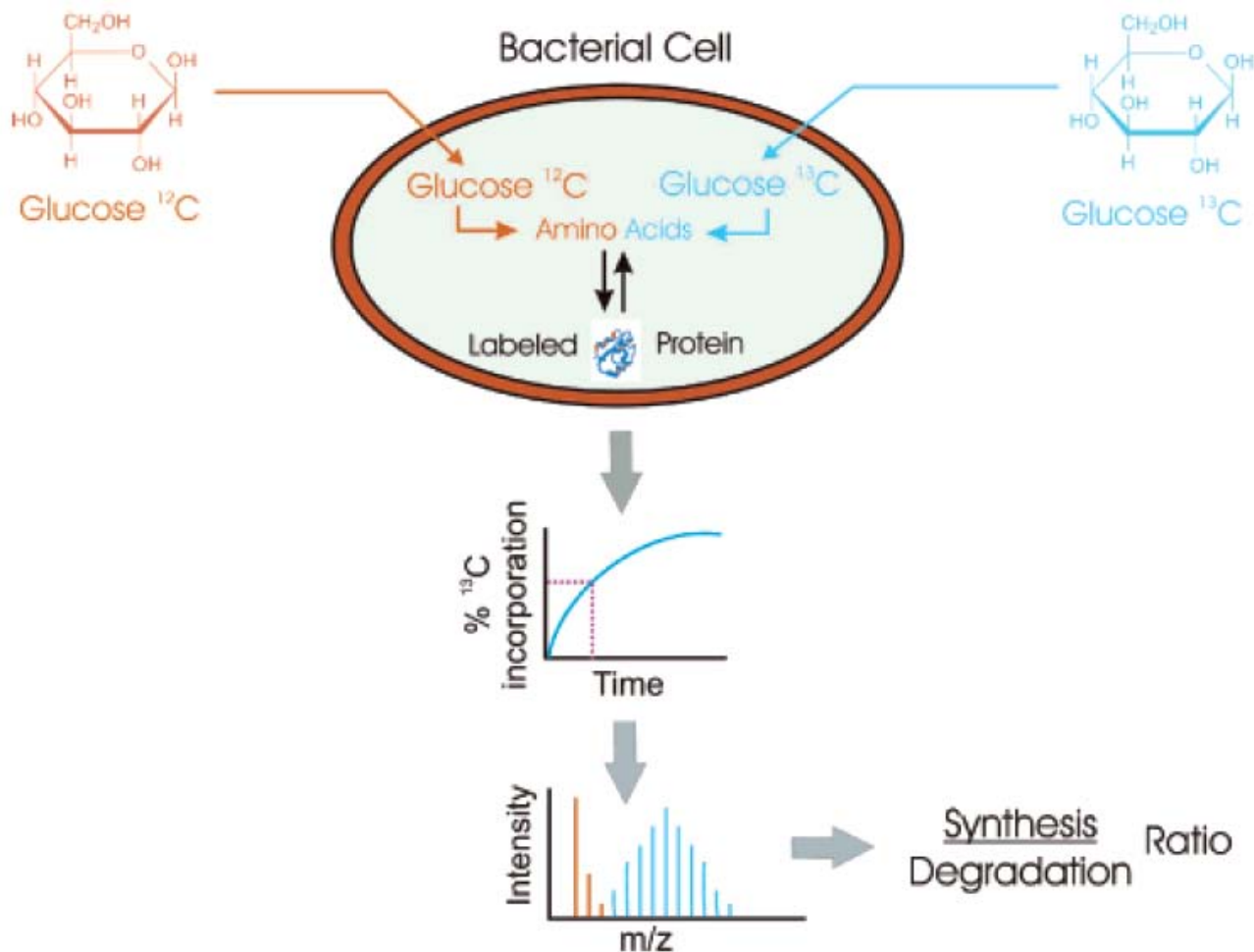
Dynamic Proteomics

SILAC Mouse

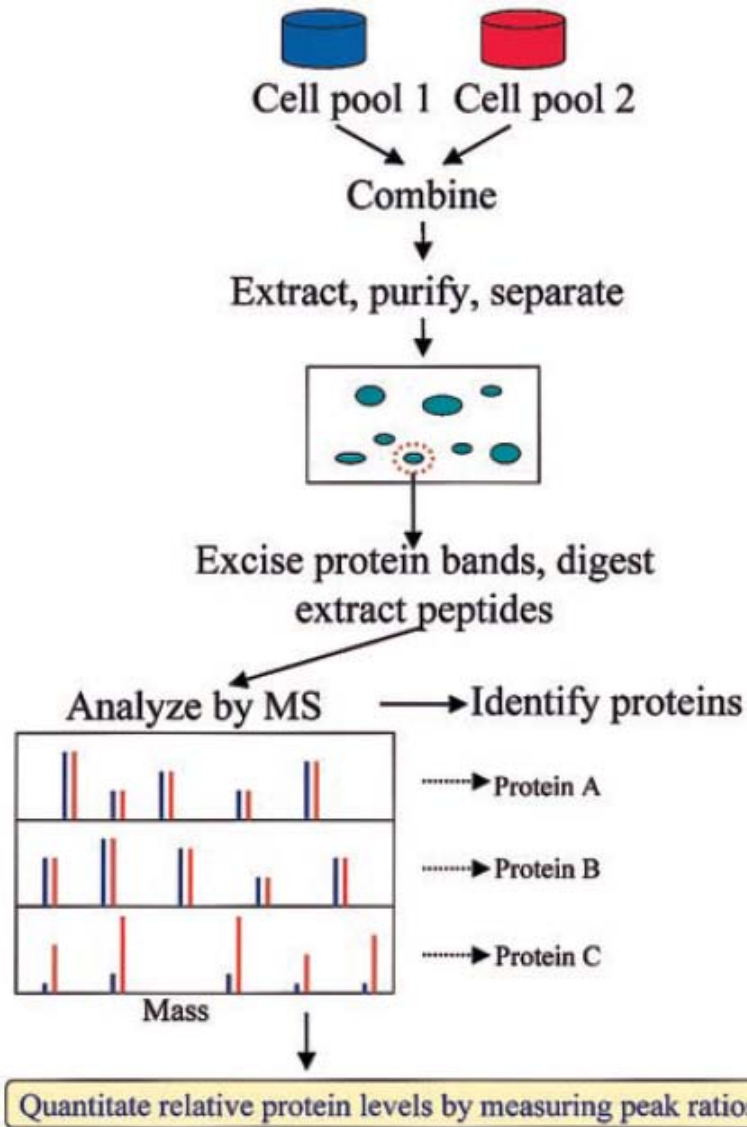


Synthesis/Degradation Ratio Mass Spectrometry

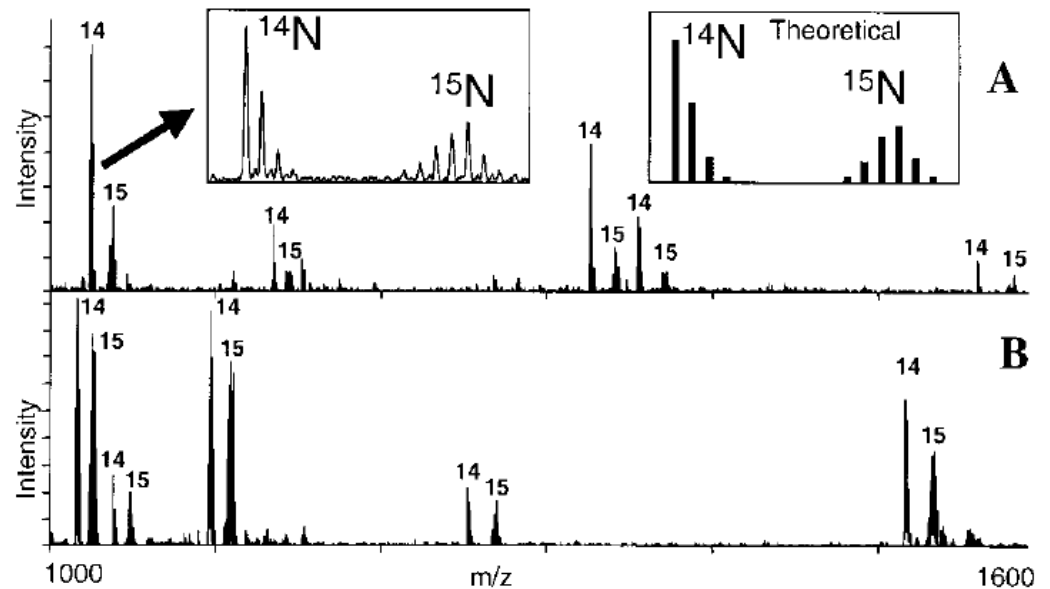
^{13}C 标记



Chait: 氮-15同位素代谢标记及蛋白定量



不像一般的SILAC
氮-15同位素代谢标记所有的氨基酸



Yates:哺乳动物总体蛋白质稳定同位素代谢标记(SILAM)

氮-15标记优点之二：标记食品比较容易制备

Sprague-Dawley rats were fed with ^{15}N enriched algae

(AnalChem2004v76p4951)

44days of labeling

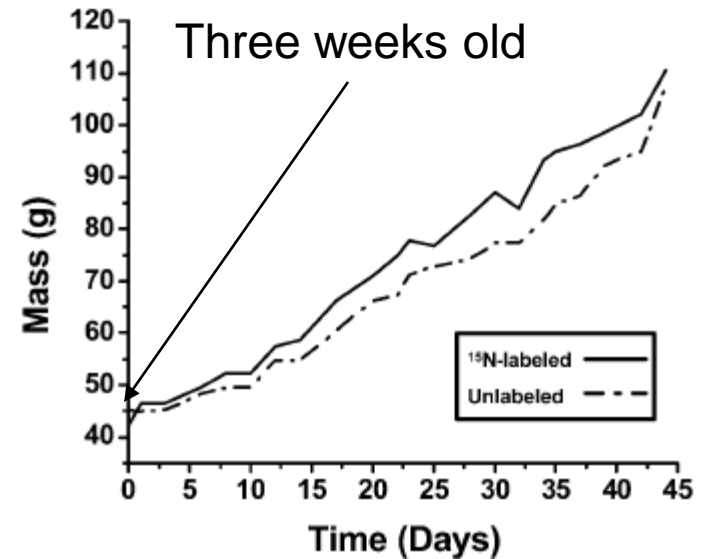
>90% labeling in liver and plasma

~75% in brain

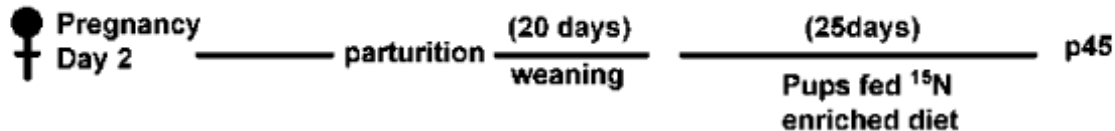
Two generations of labeling

(JProteomeRes2007v6p2005)

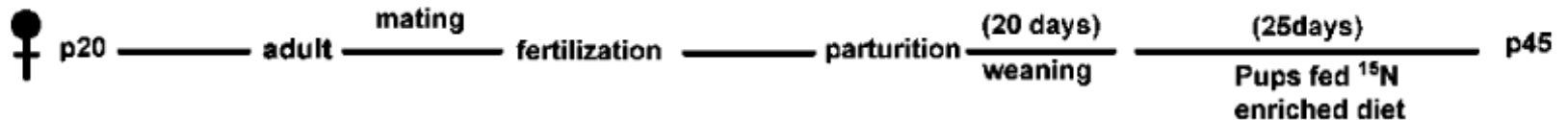
94% labeling in brain



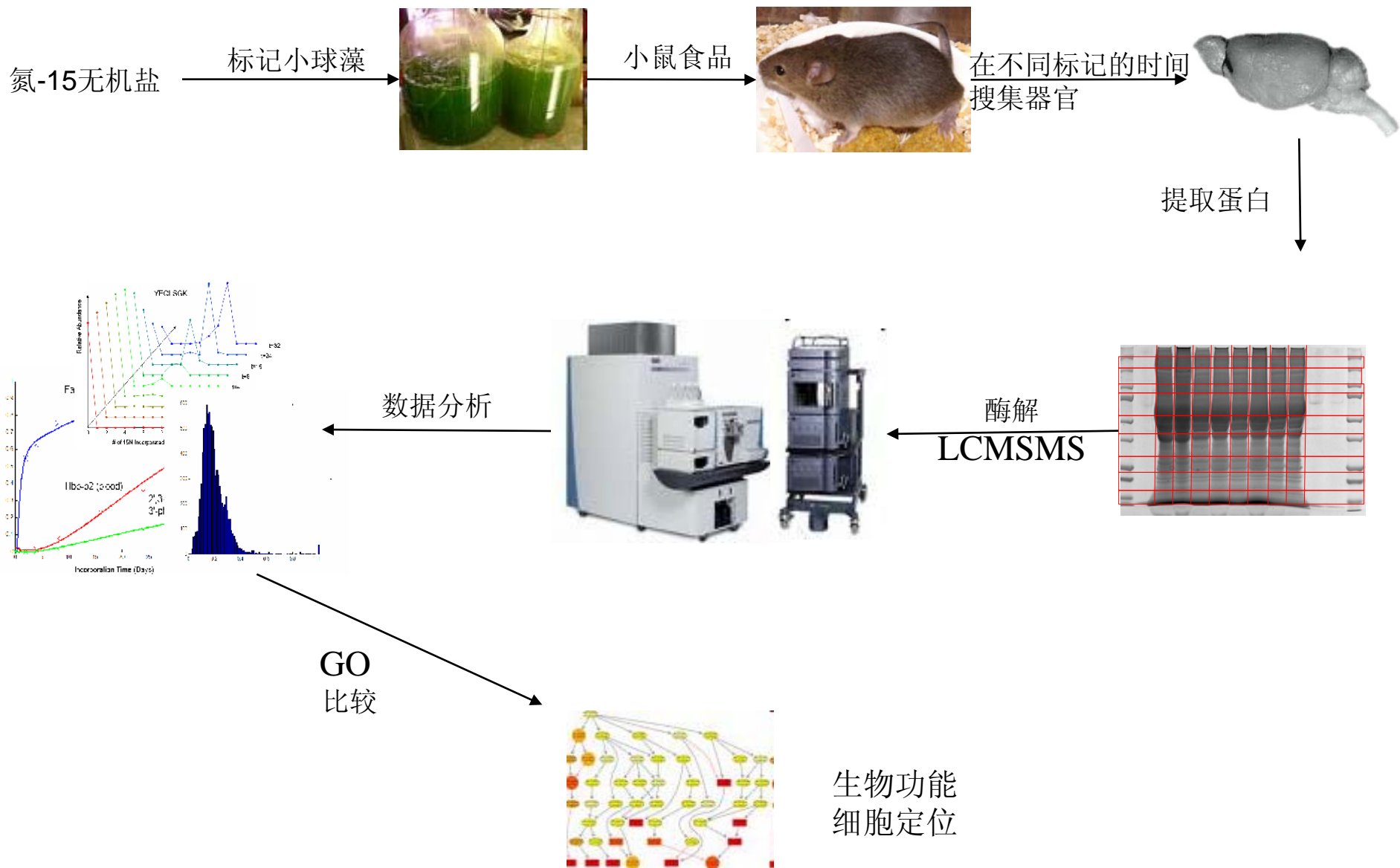
Protocol 1



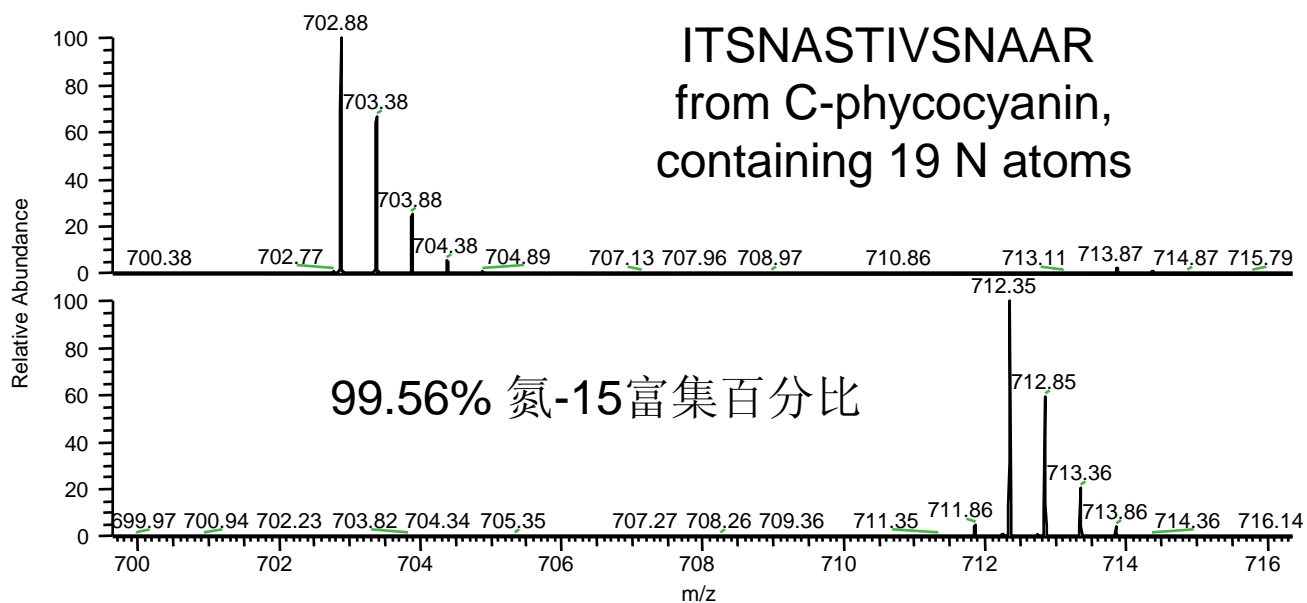
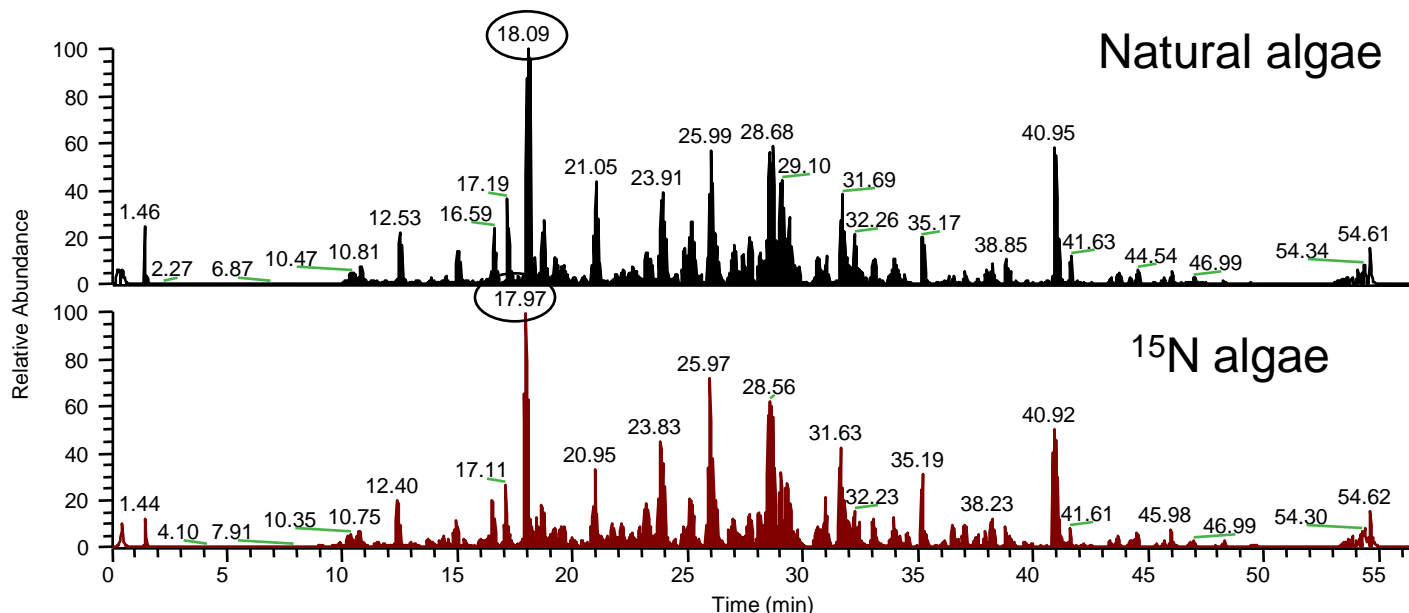
Protocol 2



基于氮-15同位素代谢标记的蛋白组动力学



自然与氮-15小球藻



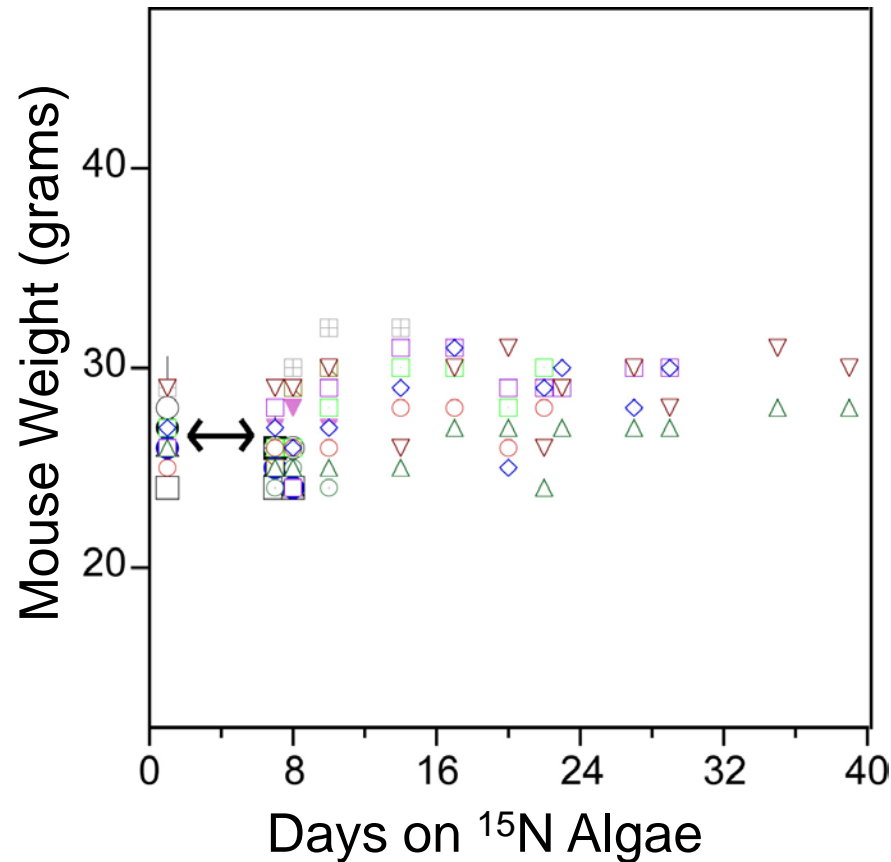
Dynamic Proteomics of Mammals at Homeostasis (动态平衡)

Protein Turnover due to Maintenance of Life

Starting age: 9周 (成年小鼠)

喂自然小球藻 : 1周 (食品适应)

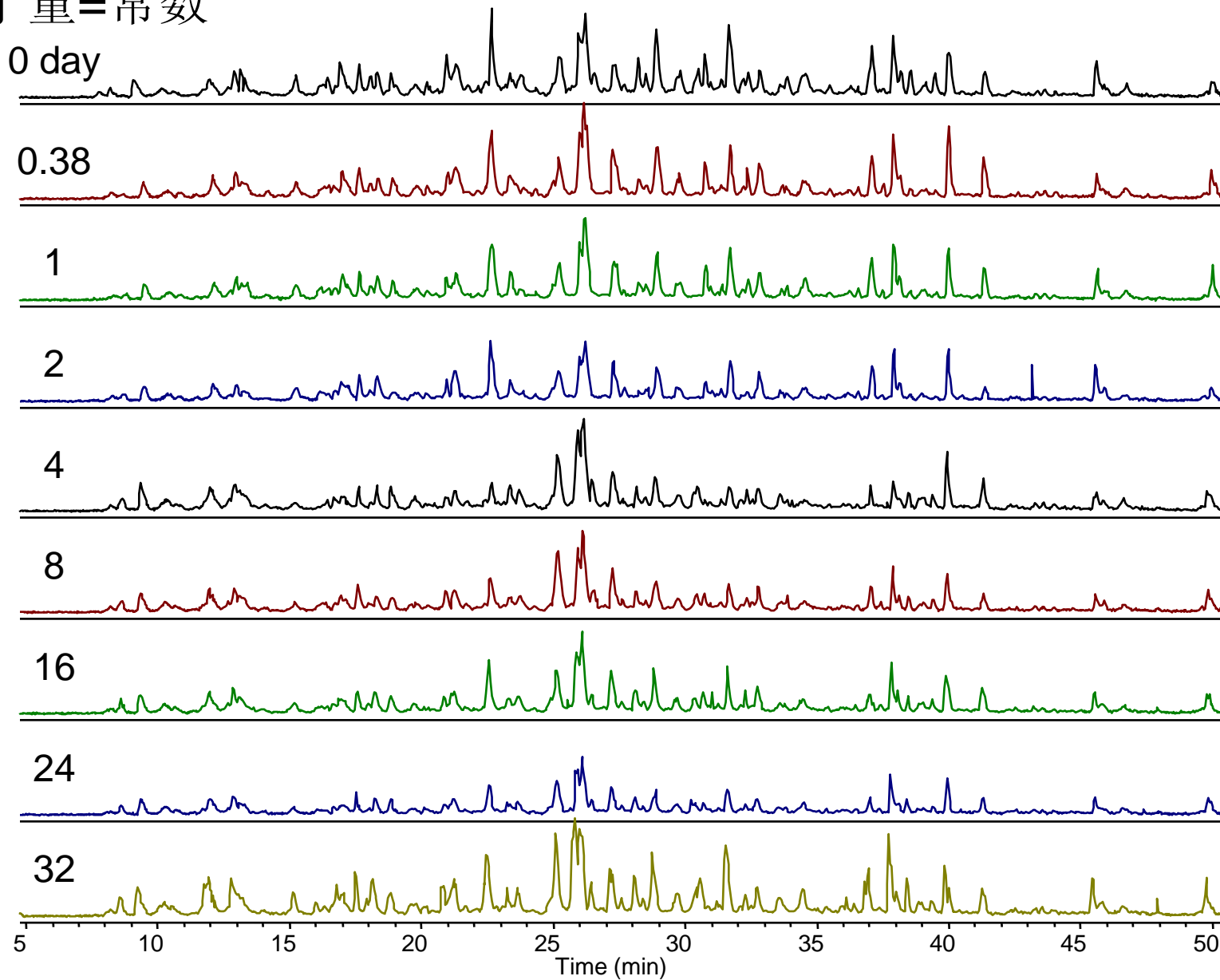
喂氮-15小球藻食品: 0, 0.38, 1, 2, 4, 8, 16, 24, 32 Days



所分析的器官组织: 脑, 肝, 血

不同标记时间的总离子流图

分子量=常数



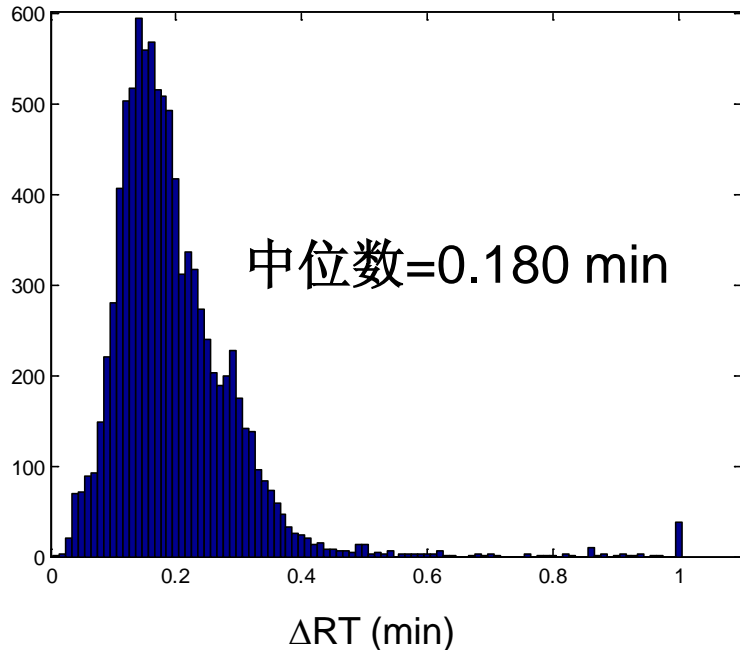
LC保留时间稳定性

Experimental efforts to achieve maximal RT stability

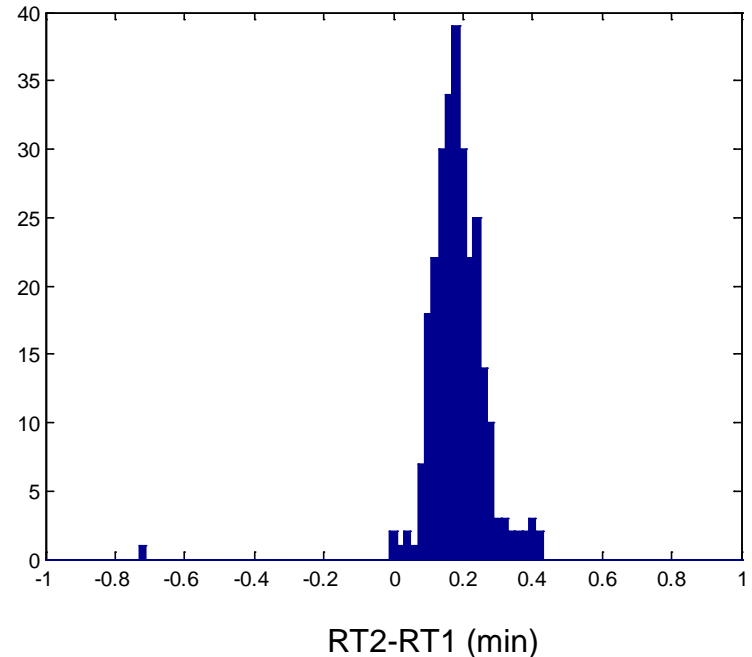
1. “皮实” 的分离柱
2. 恒定的柱温
3. 分离柱老化
4. 注入等量样品

*Two identical samples analyzed 100 hours apart
In between 90 different samples were analyzed continuously*

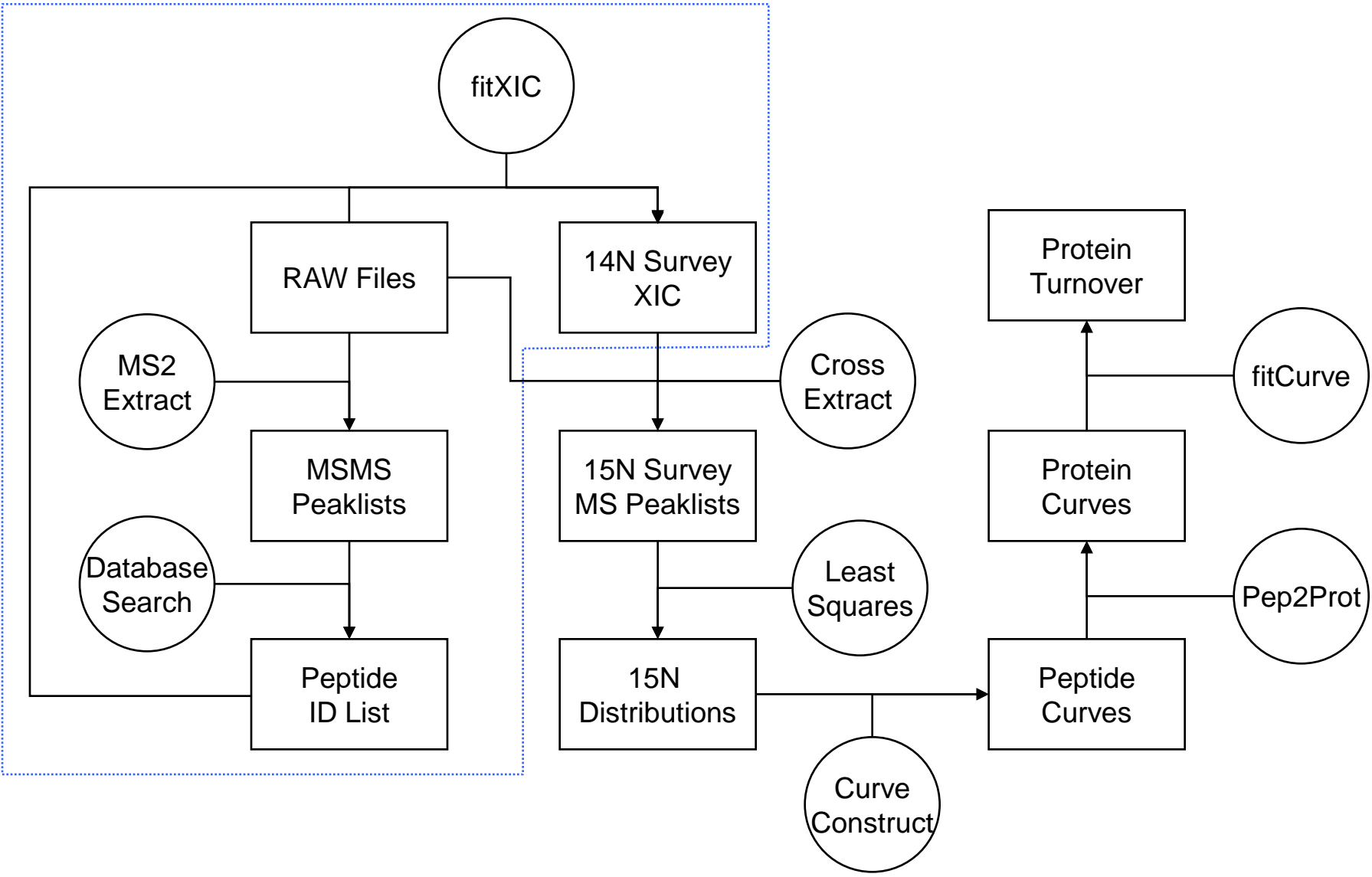
XIC峰宽分布



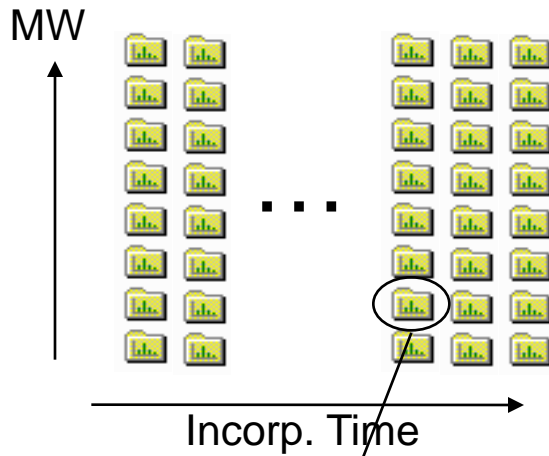
保留时间差值分布



数据分析流程

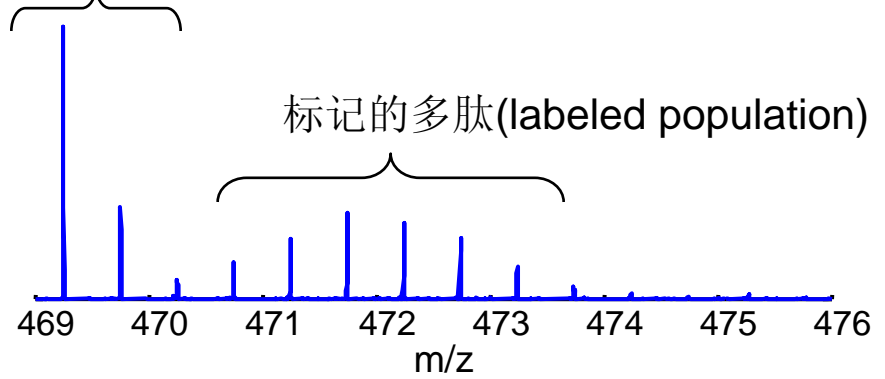


Data Extraction from LCMS Data

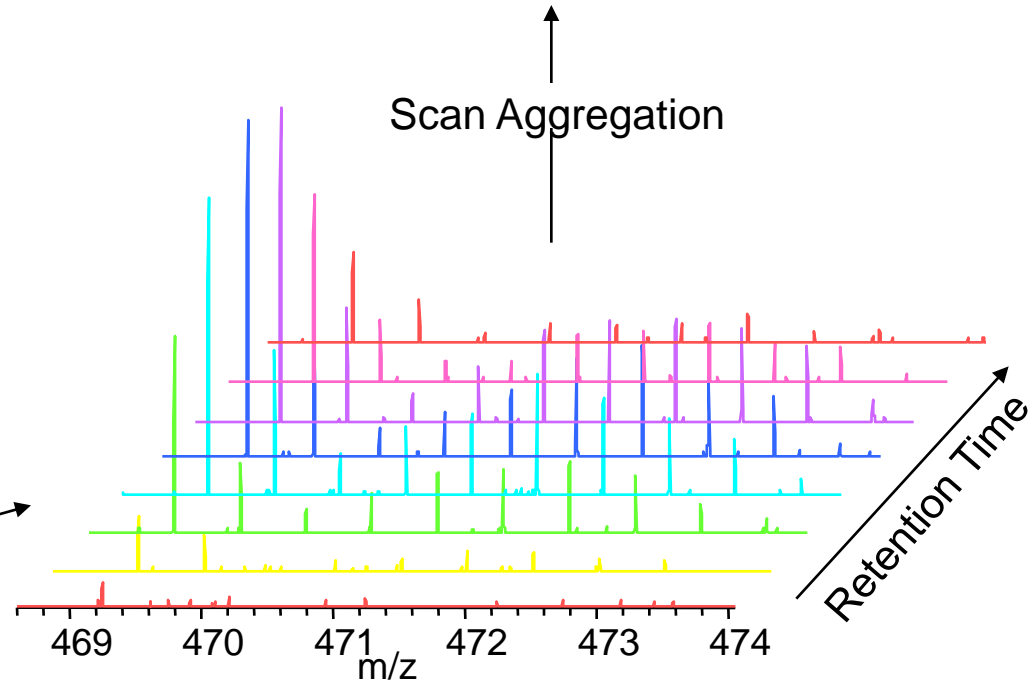
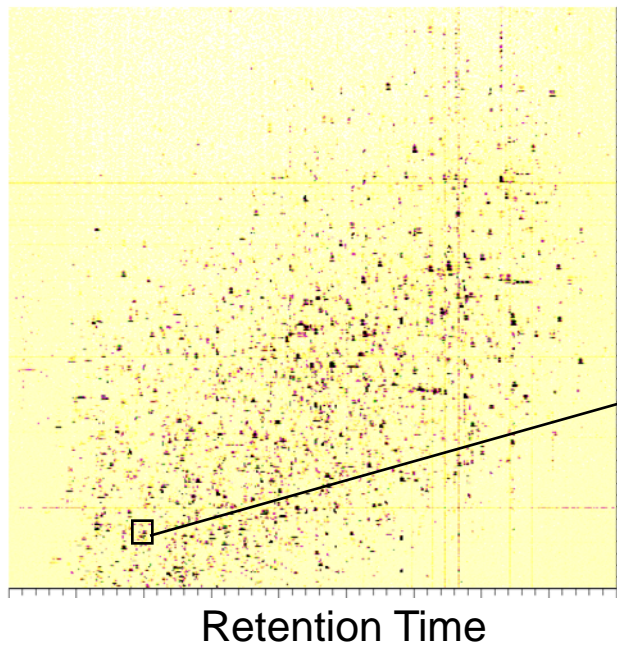


无氮-15的多肽

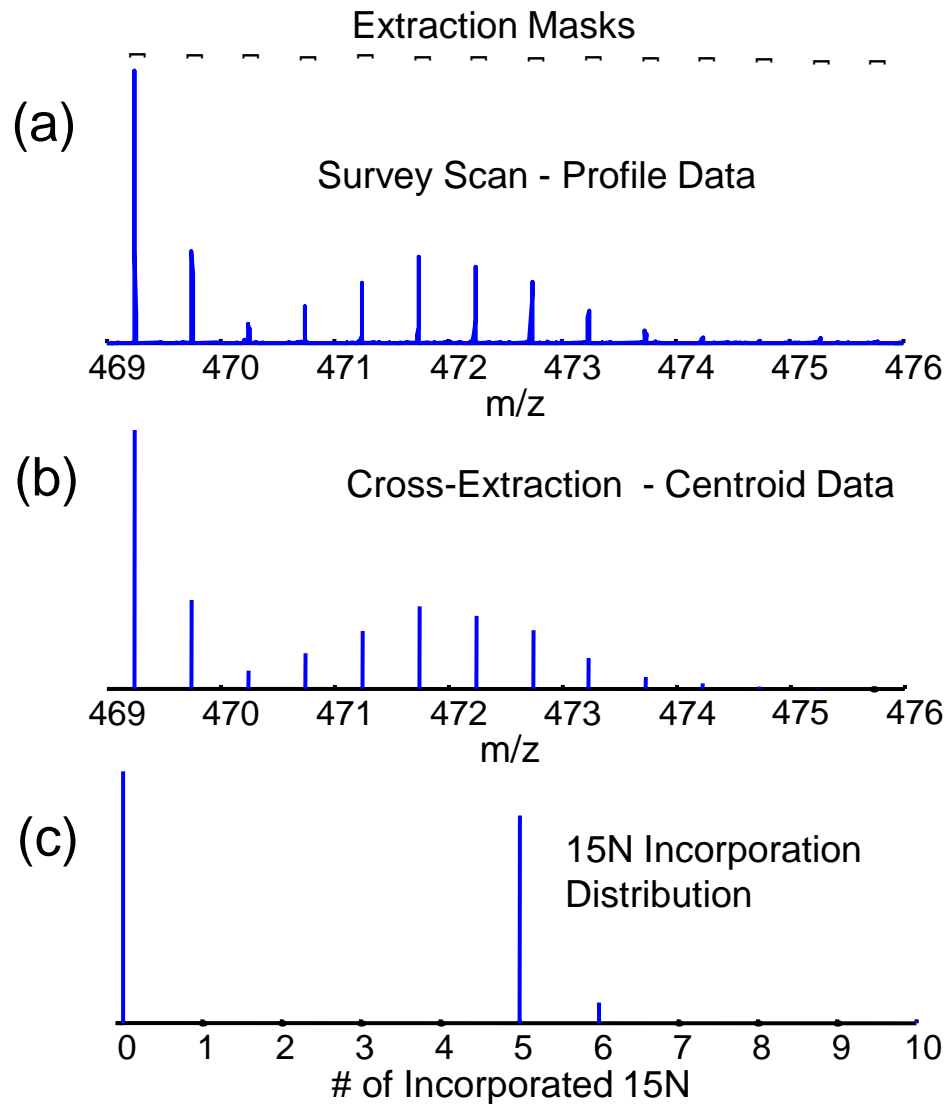
标记的多肽(labeled population)



Scan Aggregation

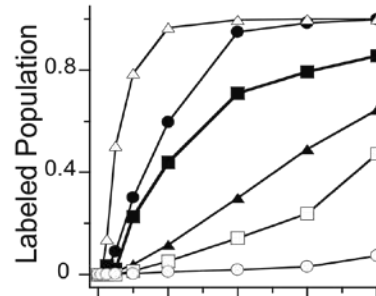
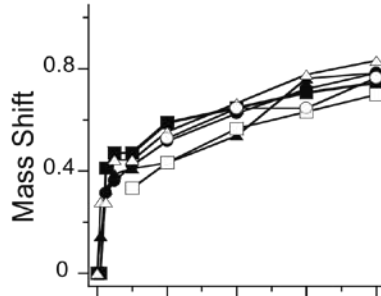


Computing ^{15}N Incorporation Distribution



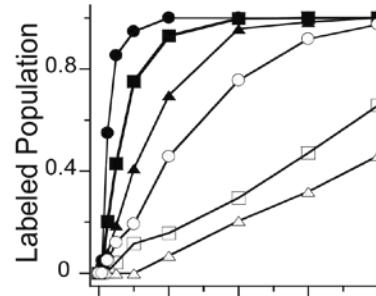
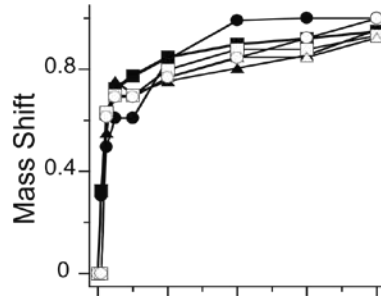
Protein Turnover - Empirical Modeling

Brain



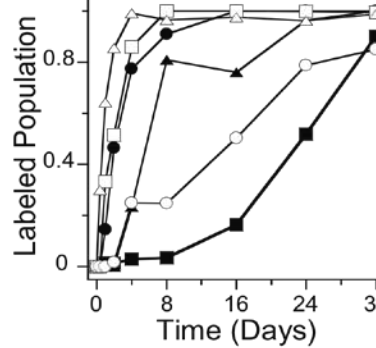
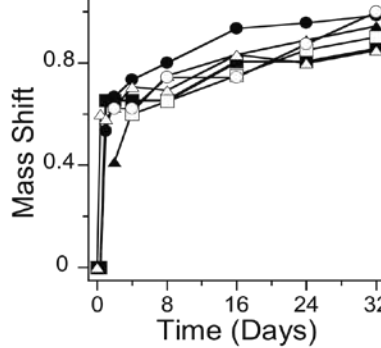
- △ P62991 Ubiquitine
- P18760 Cofilin-1
- P04925 PrP
- ▲ P00405 Cytochrome C Oxidase
- Q9D0M3 Cytochrome C1
- P04370 Mylein Basic Protein

Liver



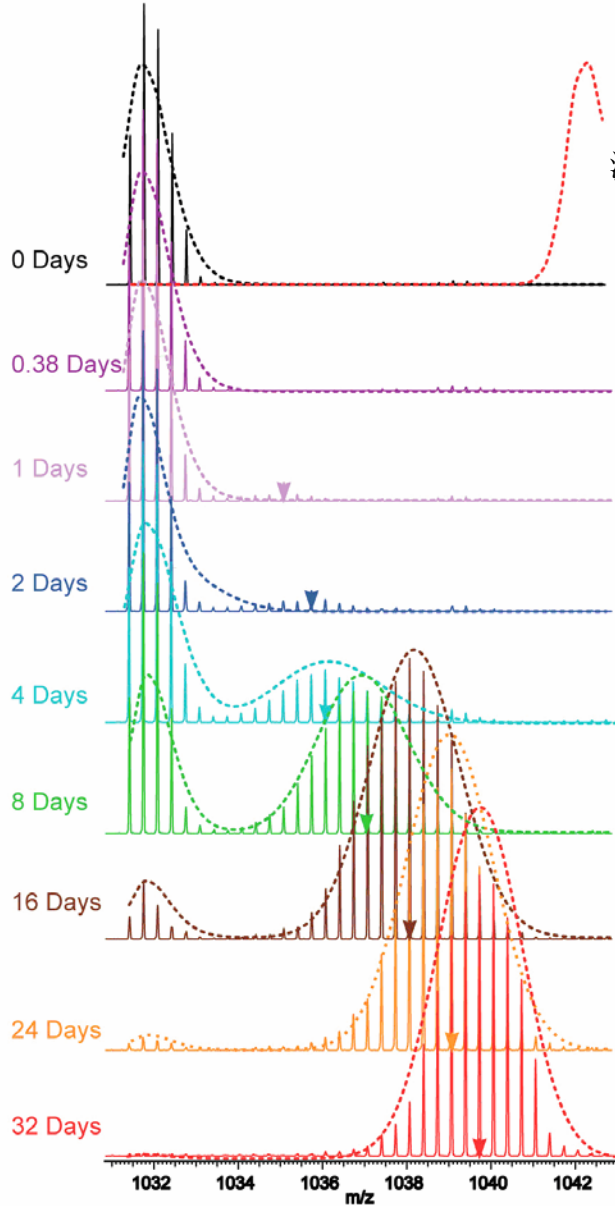
- P16331 Phenylalanine hydrox
- P17742 Prolylisoimerase
- ▲ Q9DOM3 Cytochrome C1
- P62267 Ribosomal S23
- △ P62806 Histone H4
- P10853 Histone H2B

Blood



- △ P11276 Fibronectin
- P28666 Murinoglobulin
- P07724 Serum Albumin
- ▲ Q01853 ER ATPase
- P54116 Erythrocyte Membrane Protein
- P00920 Carbonic Anhydrase

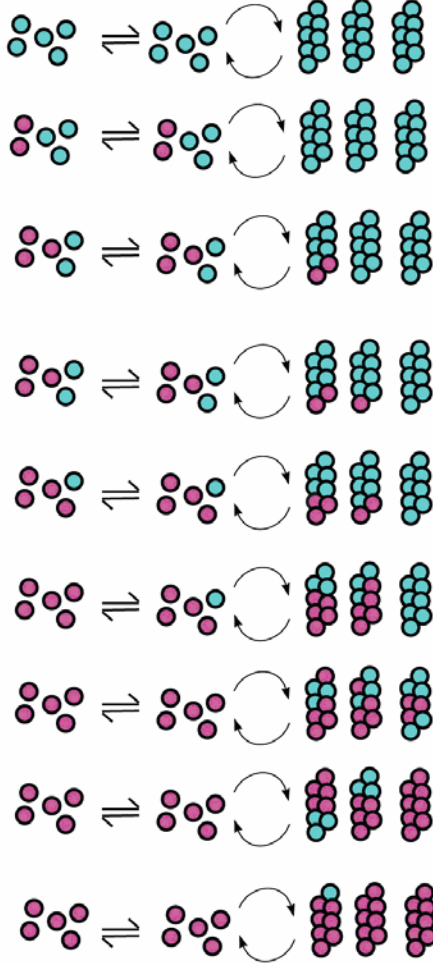
Protein Turnover - Empirical Modeling (经验型的模拟)



血液中的
游离氨基酸

细胞中的
游离氨基酸

蛋白中的
氨基酸

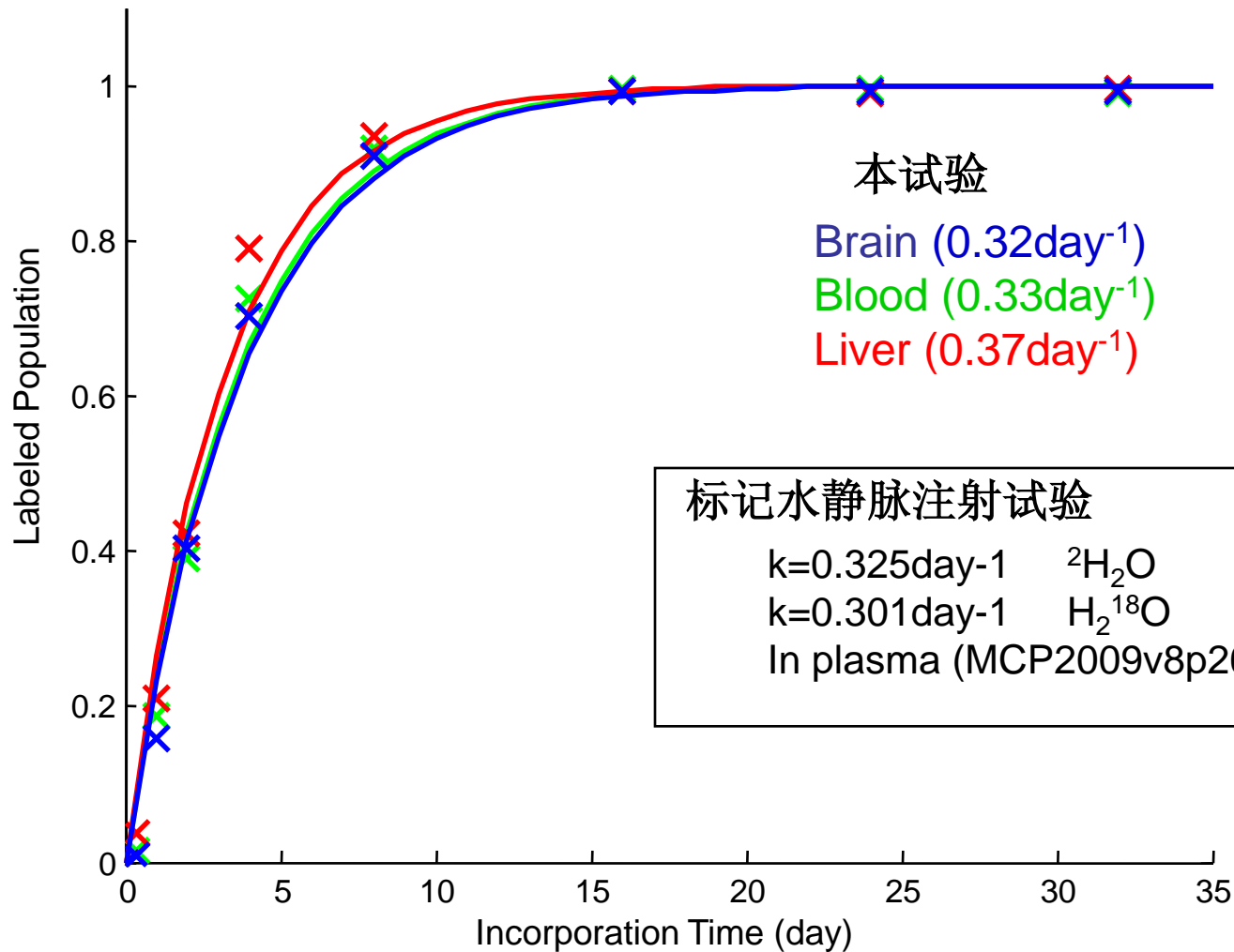


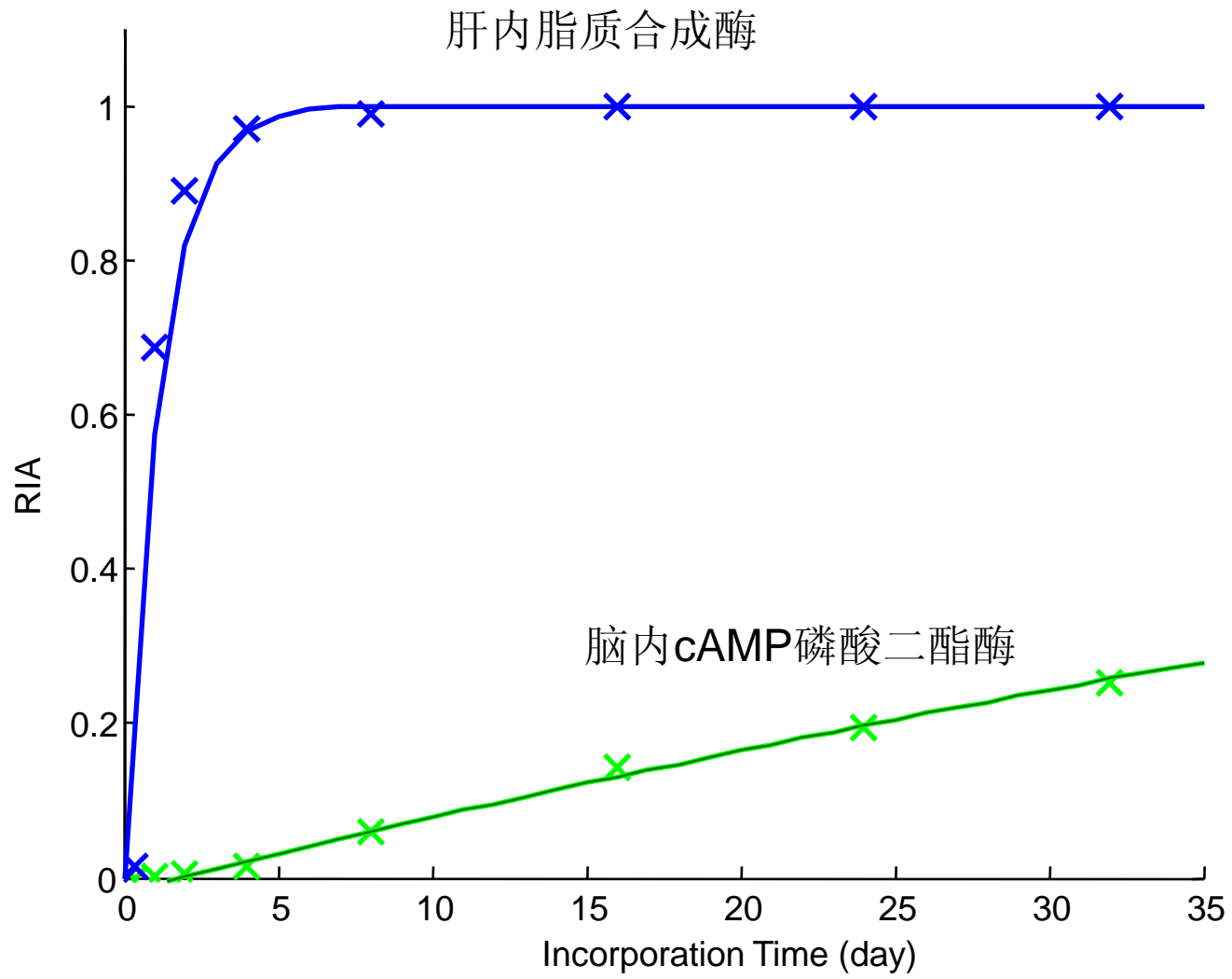
- Mass shift is an independent and fast process

- Incorporation curve may be modeled as a delayed exponential

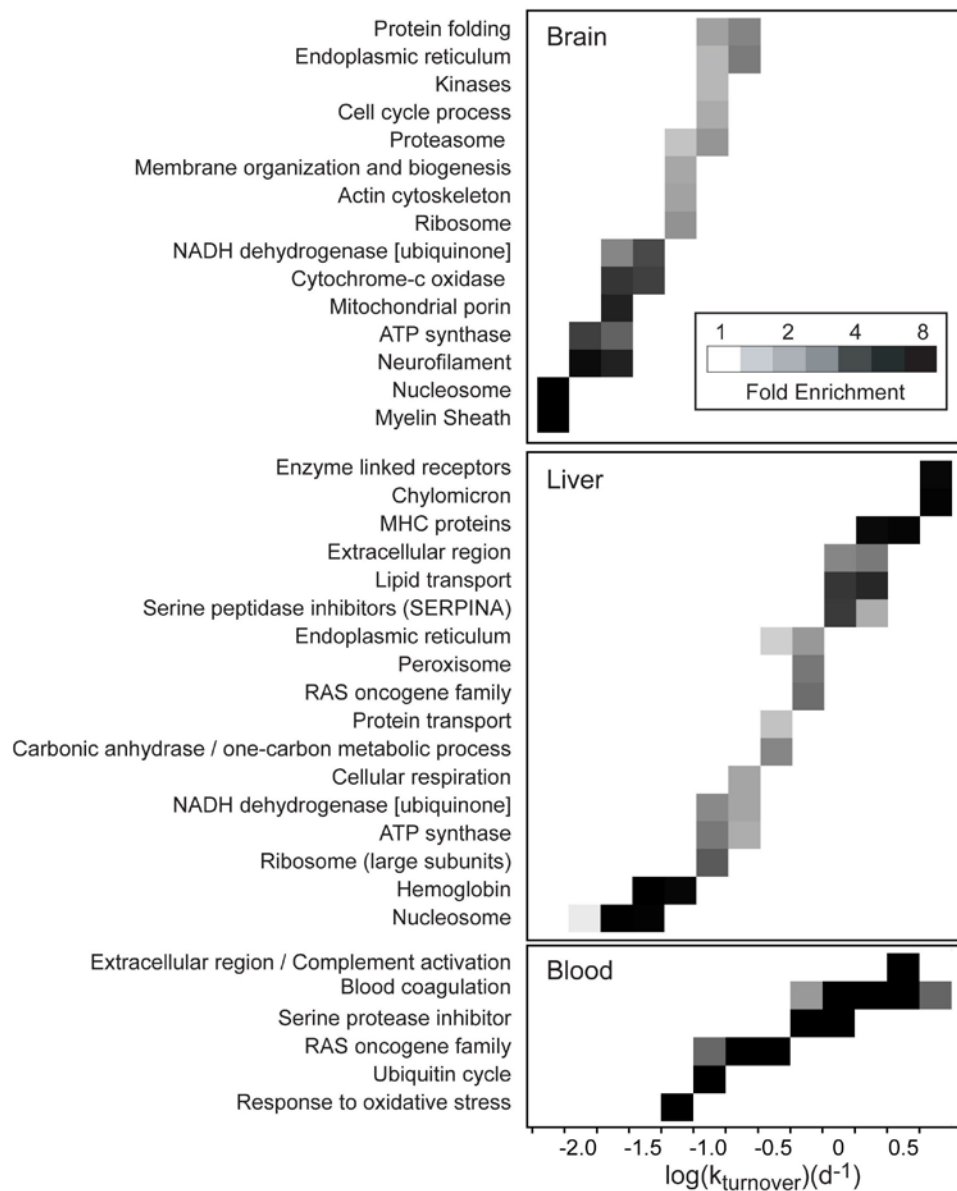
- The model seems universal applicable (to the whole proteomes)

Serum Albumin Turnover in Tissues

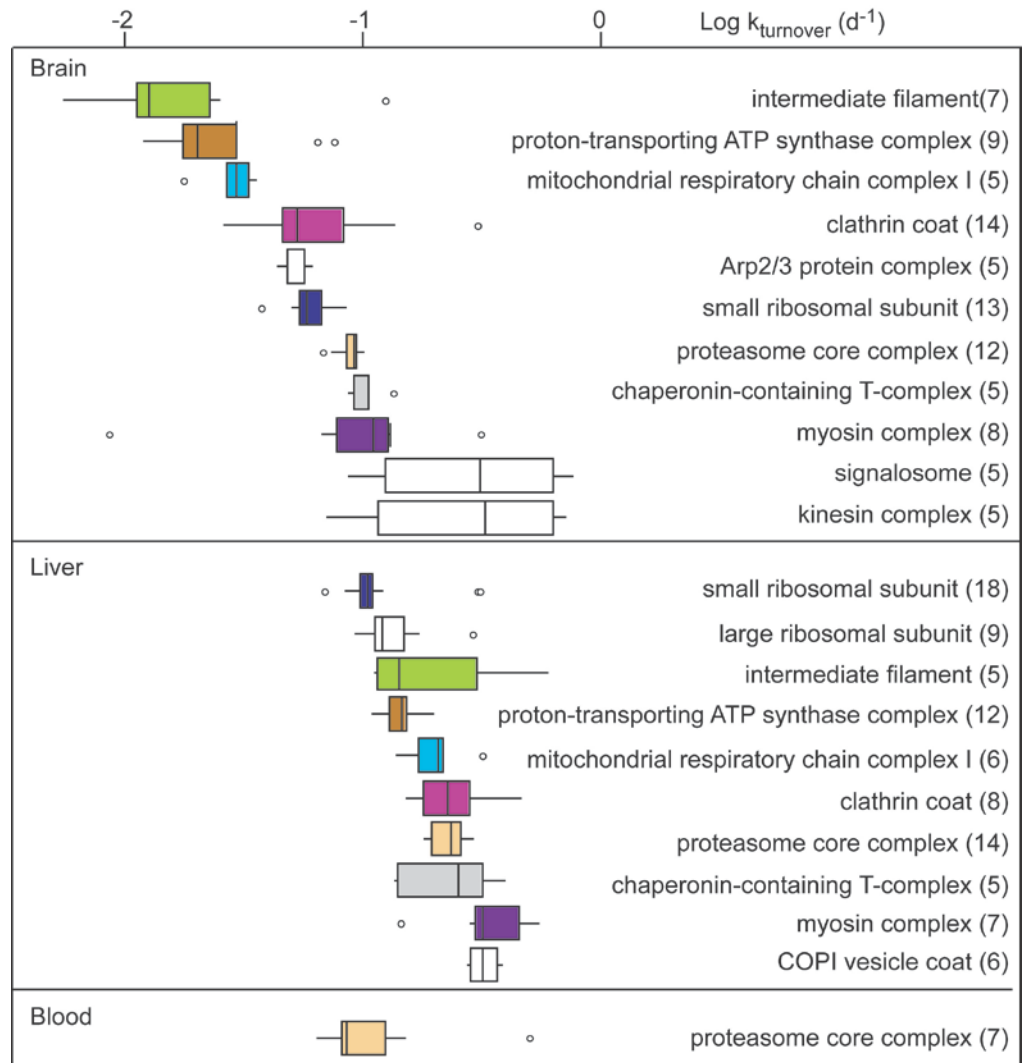




蛋白代谢周转常数与其生物功能的关联



Turnover rates of multi-protein complex subunits



结论

- 测量了小鼠组织中的1000多个蛋白的代谢常数
- 建立了复杂生物体系蛋白代谢周转组动力学的试验和信息处理平台
- 本平台可以用来处理无标定量, **SILAC**, 氘氘交换实验数据

Acknowledgement

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**Price et al, “Analysis of proteome dynamics in the mouse brain”
Proc. Natl. Acad. Sci. USA, 2010, 107, 14508-14513.**

- Coauthors from Institute for Neurodegenerative Diseases and UCSF Dept of Neurology



John C. Price



Sina Ghaemmaghmi



Stanley B. Prusiner

- Coworkers at UCSF Mass Spectrometry Facility



Alma L. Burlingame



David A. Maltby