Detecting and quantitating proteins on gels

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Different stains for different uses

- Visible:
  - Coomassie Brilliant Blue
  - Silver stain
- Fluorescence-based:
  - Sypro Ruby (Molecular Probes)--total protein
  - Deep Purple (GE Healthcare)---total protein
  - Others from Molecular Probes
    Sypro Red, Sypro Orange
  - Pro Q Diamond---phosphorylations
  - Pro Q Emerald----glycosylations

Coomassie Brilliant Blue

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

In acidic conditions, the anion of CBBR combines with the protonated animo groups on proteins via electrostatic interactions.

Inexpensive, image readily acquired by scanning at visible wavelengths

Silver stain

Silver ions (from silver nitrate) are chemically reduced to metallic silver on lysine residues.

This is the most sensitive protein stain, but also the least useful for quantitation because of its low dynamic range.

2D gel image courtesy of the U. Va. Mass Spectrometry Shared Facility, 2006

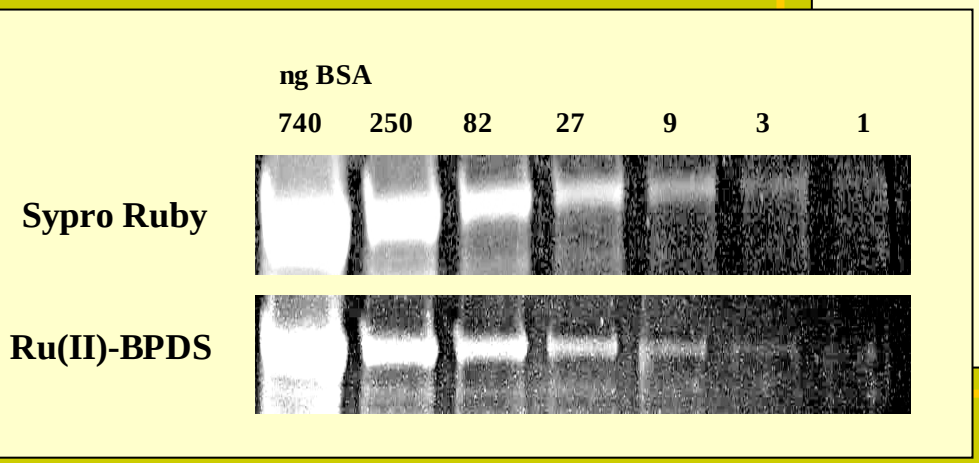
Sypro Ruby, and Ru(II)-BPDS Dye

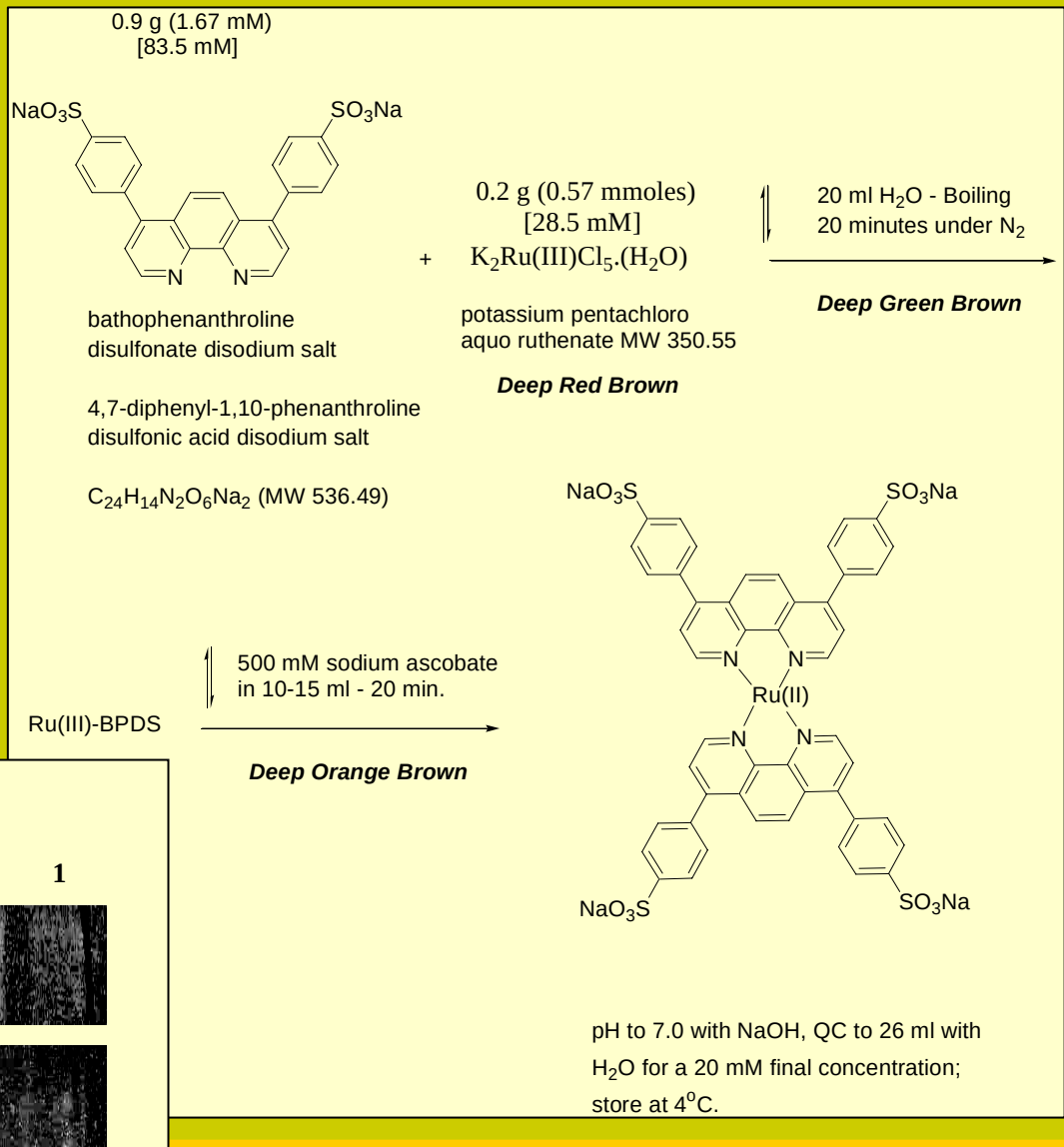
Courtesy of Dr. Jim Mobley

Sypro Ruby (*fluorscent*)

- **Equal to silver stain in sensitivity.**
- **But ~200-500-fold greater dynamic range.**

•Compatible for MS analysis.

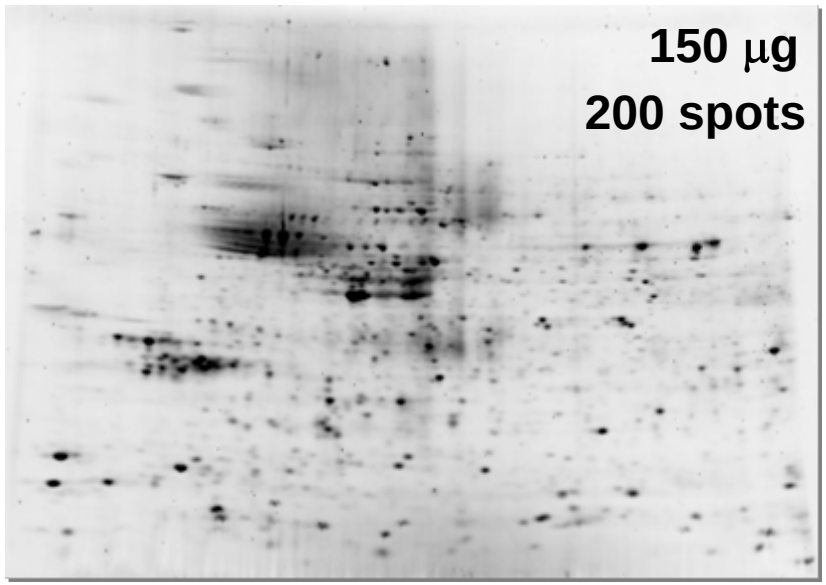




Advantages and limitations of the types of stains

	Sensitivity	Dynamic range	MS- compatible
CBBR	8 ng	10-30 x	yes
Silver	1 ng	< 10 x	Not without special precautions
Fluorescent	2 ng	3 orders of magnitude	yes

Proteins, proteins everywhere, but where's my receptor?



2D gel of rat brain, stained with Sypro Ruby

The genome predicts: 20,000-50,000 polypeptides.

So, 200 spots is <1% of the total proteome.

Conclusion: Even a fluorescently stained 2D gel of an unfractionated sample, only allows detection of the "low hanging fruit."

Central issue in proteome analysis: dynamic range

In cells: protein amounts vary over a wide *dynamic range*:

In blood, albumin is 3.5 g/100 ml (35 g/L = 0.5 mM) (10^{-3} M), whereas cytokines are pM (10^{-12} M)

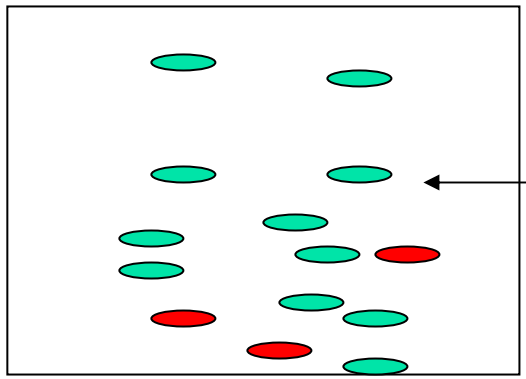
- **This is a difference of nine (9) orders of magnitude.**
- **A 2D gel that is overloaded with respect to an abundant protein, may have *barely detectable* amounts of a low abundance protein……**
  - **If you can't see it, you don't know a protein is there;**
  - **Even if you know it's there, you can't do MS, because there isn't enough protein.**

- **No one stain will detect 9 orders of magnitude differences in abundance of proteins.**

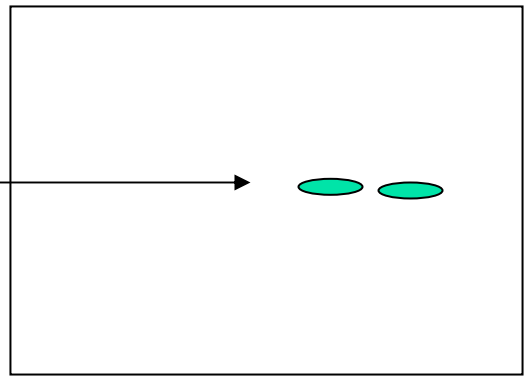
For greatest sensitivity, and the most biological information:

Stain the gel…..

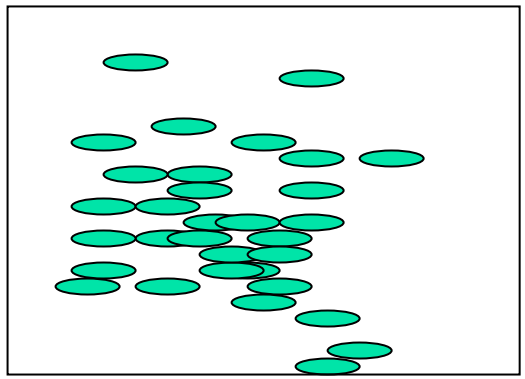
----but do a Western blot or two, with replicate gels:



Western blot for phosphoproteins



Western blot for a particular protein



Stained gel

Deep Purple: the new fluorescence dye from GE

250

80

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

25

10

BLUE: spots on Deep Purple-stained gels;

RED: spots on Sypro Ruby-stained gels;

GREEN: spots matched to every gel

Deep Purple MAY detect more smaller proteins (<25kda).

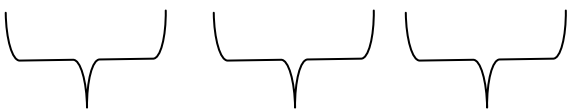
(adapted from Duncan Veal, 2006)

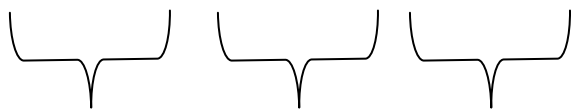
Multiplex Proteomics: ProQ Emerald followed by Sypro Ruby can identify multiple glycosylated proteins at once

ProQ Emerald

Sypro Ruby

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.





(Duncan Veal, Fluorotechnics Pty, Ltd.)

Multiplexing: valuable when sample is scarce or difficult to obtain

Normal liver

Liver tumor

Sypro Ruby: total protein

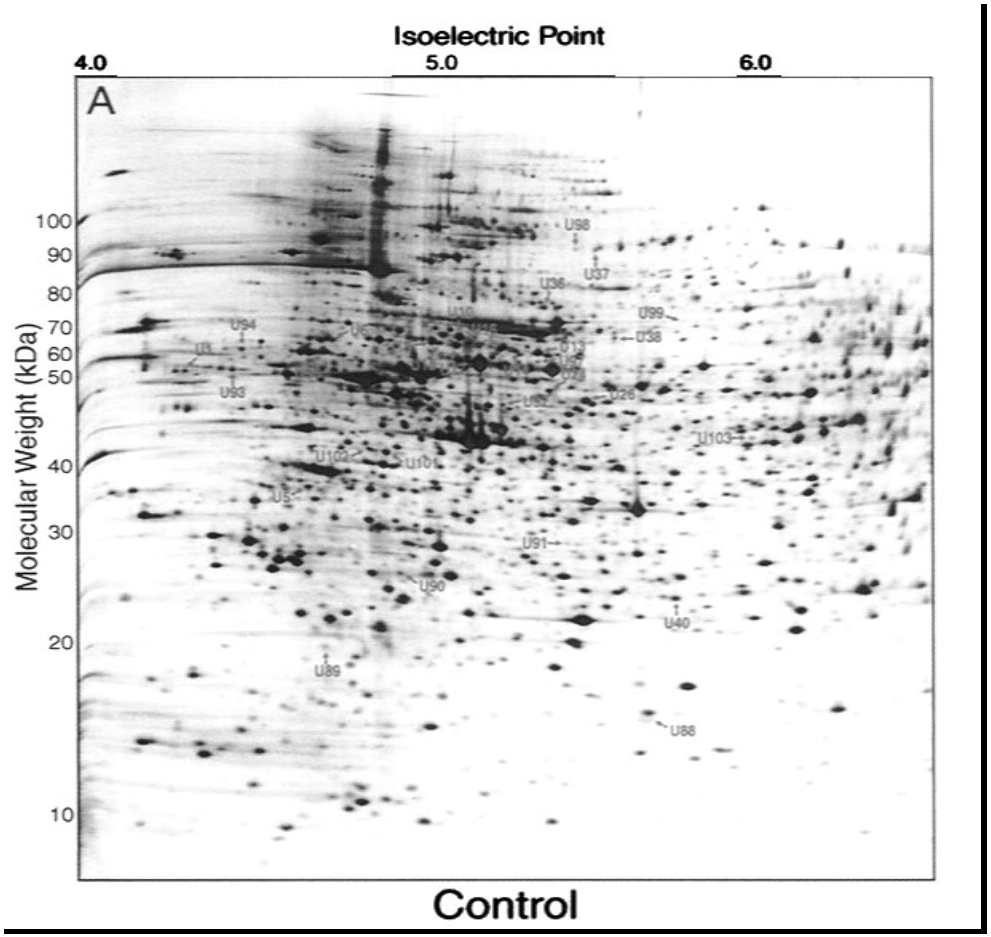
Pro Q Emerald: glycosylated

Pro Q Diamond

The value of fluorescent dyes: each can be detected separately from others on the gel, due to different excitation and emission spectra.

Modified from Duncan Veal, Fluorotechnics Pty, Ltd.

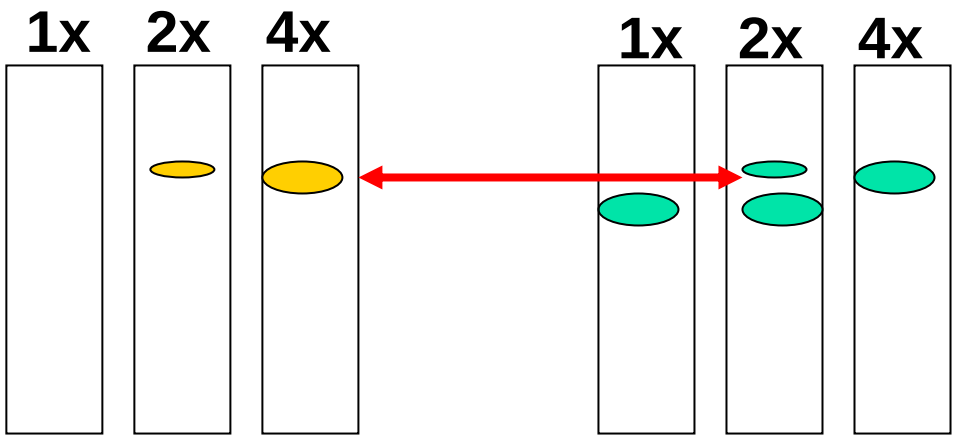
Sometimes, you may not want dynamic range, only sensitivity



With silver-stained gels such as this, **Natalie Ahn's lab** was able to identify 25 new phosphorylations mediated by the MAP kinase pathway.

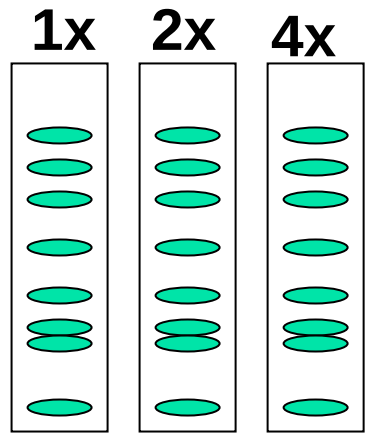
Lewis et al., Cell, 2000

Modernization of conventional approaches: (multiplex) 1D Western blot may give more valuable answers than a 2,000 spot 2D gel



Phospho-epitopes, detected with antibody w/Alexa 800.

Protein X, detected with antibody w/Alexa 700.



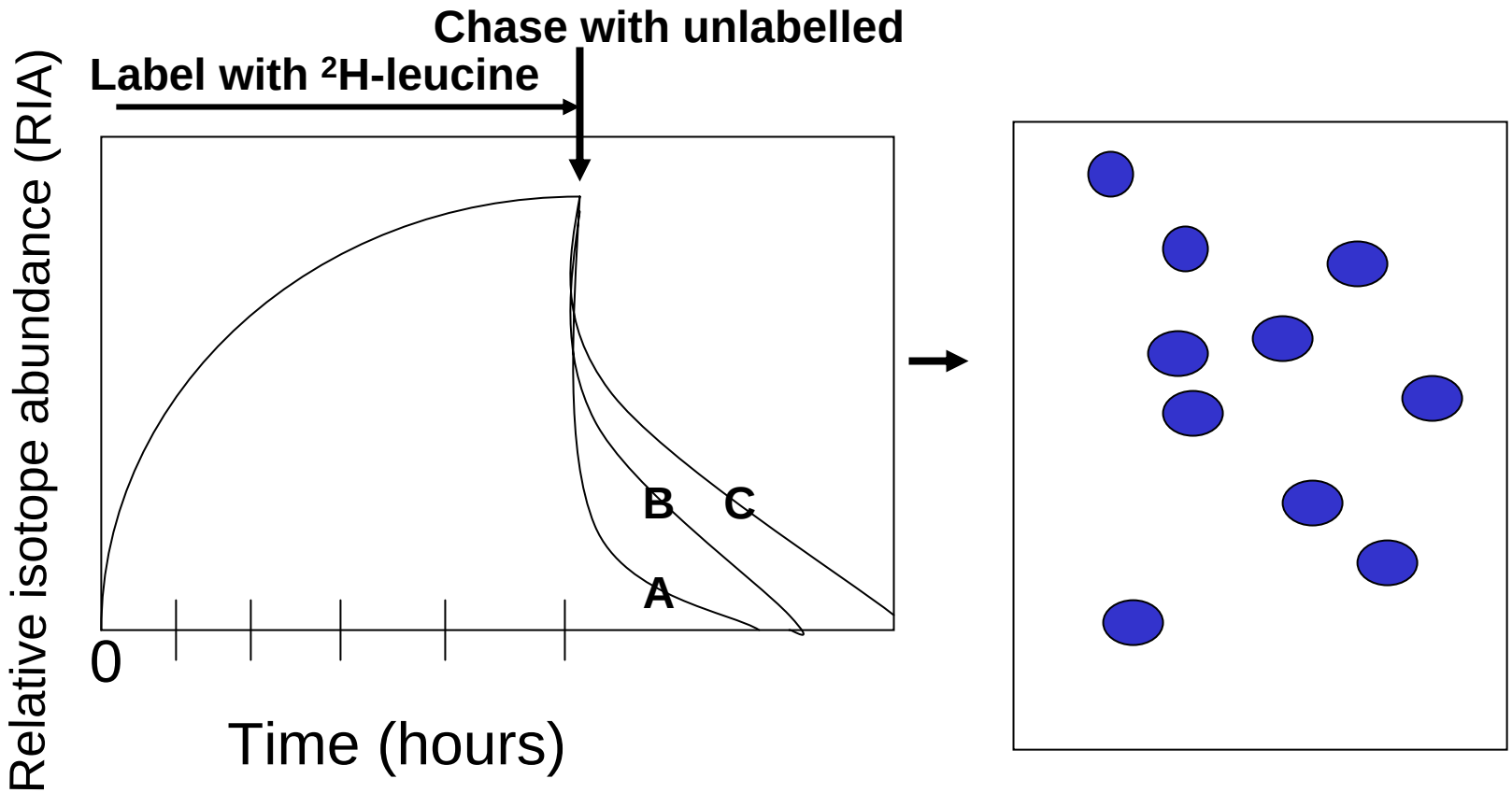
Total protein stain

Conclusion: with fluorescent dyes, all proteins, the one protein and its phosphorylated isoform can be quantified.

2D gels and SILAC: a way to address the dynamic aspect of cellular proteomes

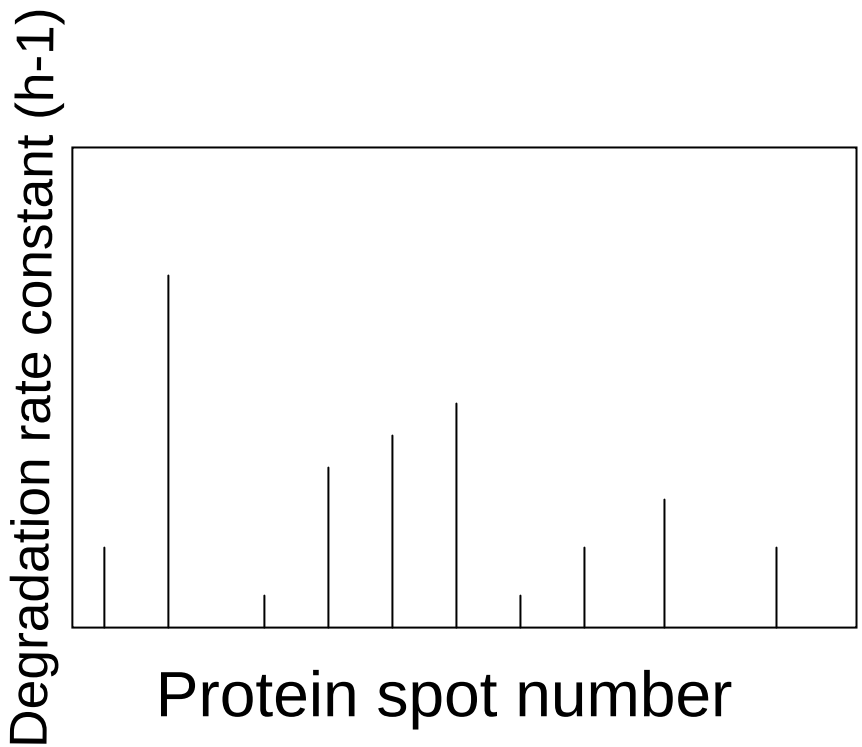
- **Protein quantitation in 2D gels may cover a wide *dynamic* range, but it does not address the *dynamic* nature of the proteome, that proteins are always turning over, at different rates, and these can change in response to stimuli.**
- **SILAC (stable-isotope-labelling-of-cells) where cells are metabolically labelled with a stable-isotope like ^{2}H, can be used to qualitatively enhance the information in 2D gels.**

With SILAC, protein turnover can be quantitated.



Based on Pratt et al., 2002, MCP

MALDI-TOF MS allows tracking of the disappearance of a ^{2}H-peptide from a 2D gel spot (reflecting degradation of that protein)



Among “abundant” proteins, degradation rates varied from 0.01/hr to 0.09/hr. The differences in rates were not predicted by differences in abundance on the gel.

Thus, CBBR-stained 2D gels from a SILAC experiment can yield rich information about protein dynamics.

(based on Pratt et al., 2002, MCP)

SUMMARY

- **Protein stains differ according to**
  - **Sensitivity/Dynamic range/MS-compatibility/Ease of capture of information**
- **The fluorescent dyes offer unparalleled protein analytical capabilities due to the wide dynamic range, and their MS compatibility;**
- **“Multiplexing” allows analysis of subproteomes in the same gel, maximizing use of scarce samples;**
- **Yet, each stain has utility depending on experimental goals.**
- **Stable-isotope-labelling in combination with 2D gels can reveal dynamics of protein turnover, giving added dimension to proteomic analysis.**