Programm Workshop Quantitative Proteomics
Bremen, 11.3.2007

14.00 Konzepte für Quantitative Proteomics Peter R. Jungblut

14.15 Quantitative proteome analysis of 20S proteasome by ICAT-based LC and 2-DE approaches Frank Schmidt

14.35 Quantifizierung von Proteinen mittels MeCAT-Lanthanid-Markierung Christian Scheler

15.00 Protein Expression System Leonhard Pollack

15.15 QconCAT - eine neue Methode zur parallelen absoluten Quantifizierung von Proteinen in der Massenspektrometrie Gerhard Giegerich

15.30 iTRAQ-Reagentien zur Quantifizierung von Proteinen: Prinzip und Anwendungen Christoph Lenz

15.45 Kaffeepause

16.15 Quantitative proteomics based on spectral count and stable isotope dilution techniques Wolfram Weckwerth

16.45 Anwendungsbeispiele für quantitative Proteomics Peter R. Jungblut

Konzepte für Quantitative Proteomics

Peter R. Jungblut

**Max Planck Institute for Infection Biology,
Core Facility for Protein Analysis,
Berlin**

Genomics

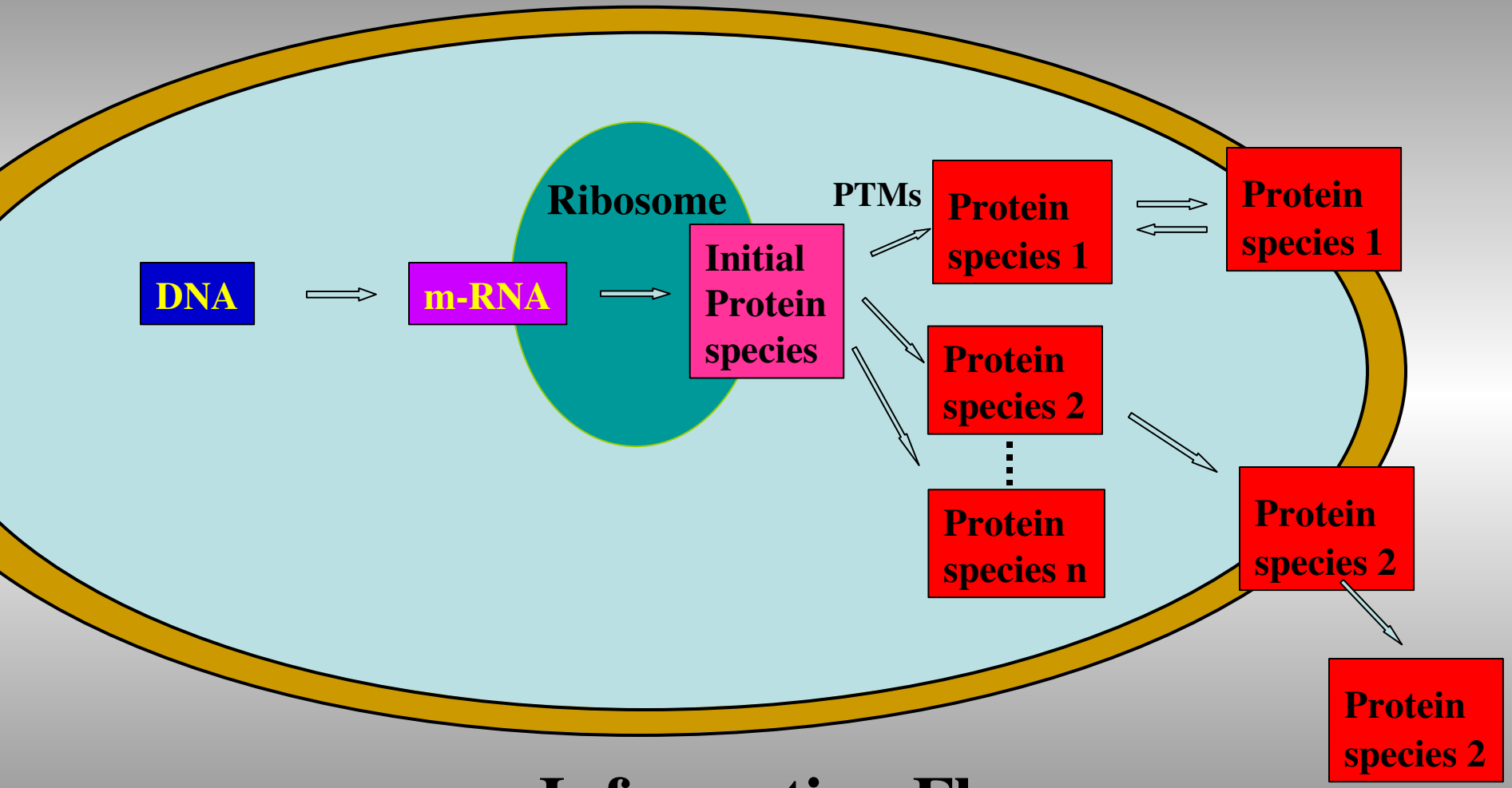
Transcriptomics

Pulse-chase

ICAT-LC/MS

Proteomics

2-DE/MS



Information Flow

Genetics Influence Environment

 Mass Spec Rev.16, 1997, 145-162

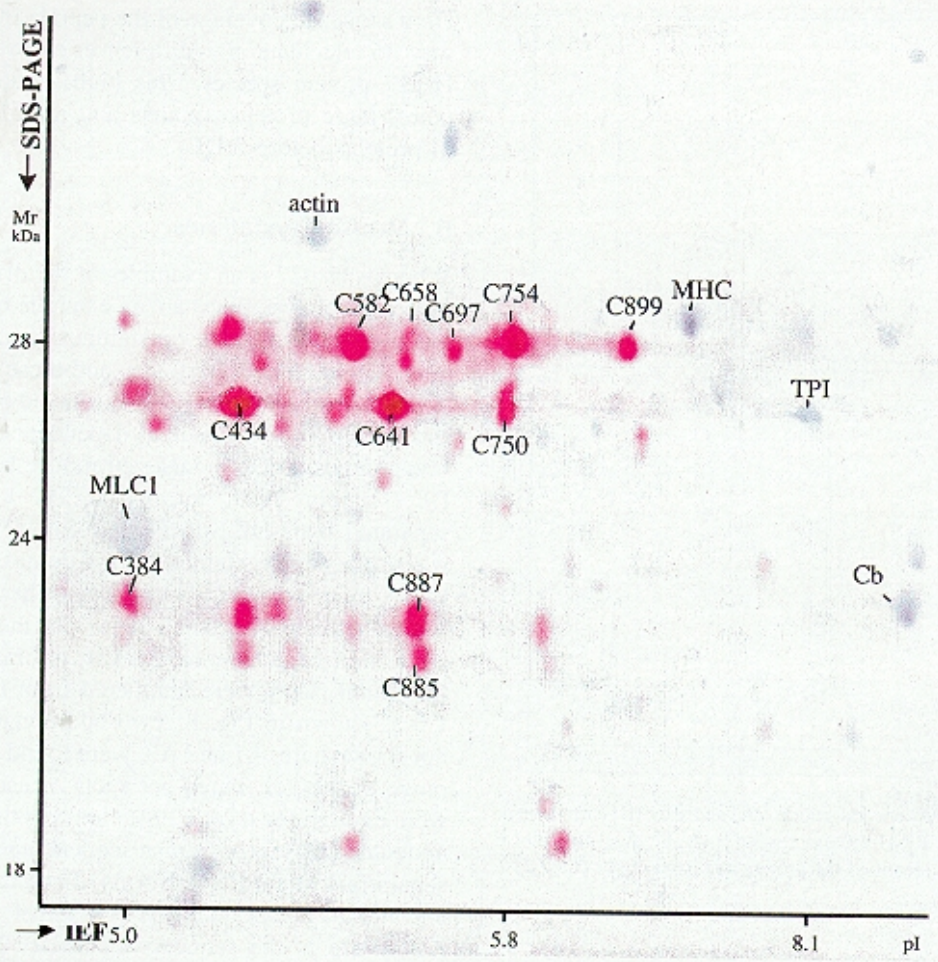
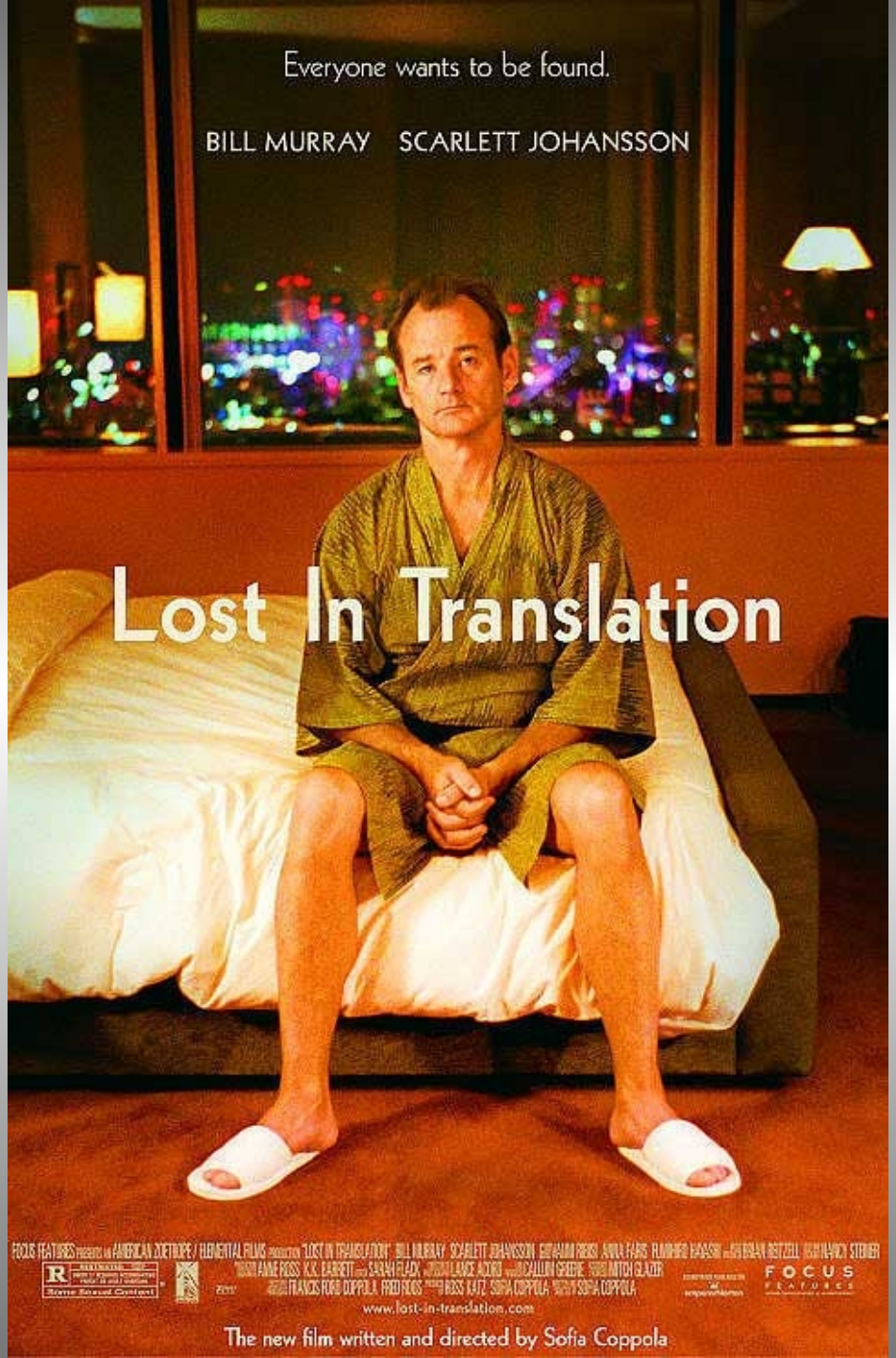


FIGURE 7. Hsp27 region of standard human myocardial protein pattern. The proteins were blotted onto a PVDF membrane, immunostained with an antibody against Hsp27 (red spots), and counterstained with Coomassie Brilliant Blue R250 (blue spots). Spot numbers refer to the human myocardial 2-DE database (http://www.mdc-berlin.de/~emu/heart/). The shown actin is a fragment. MLC1, myosin light chain 1, MHC, myosin heavy chain fragment, TPI, triosephosphate dehydrogenase, Cb alpha-crystallin b chain.

The protein intensity of one spot does not reflect protein expression

one gene ≠ one polypeptide ≠ one spot


Everyone wants to be found.
BILL MURRAY SCARLETT JOHANSSON
Lost In Translation
www.lost-in-translation.com
The new film written and directed by Sofia Coppola

Clear definition of the quantification problem

Protein synthesis
Protein amount
Protein species amount

Relative quantification
Absolute quantification

Comparison of two or more biological situations

Normalisation

For the experiment:
The same protein amount should be applied to the separation method (electrophoresis or liquidchromatography) to have the best chance to measure for most of the proteins within the linear range.

Final normalisation (applied to the final measured values):

- **To the sample mass**
- **To the cell number**
- **To the sample volume**
- **To the total protein amount**
- **To the amount of one or a group of proteins**

Saturation

Measurements within the linear range:
Knowledge of the protein amount/measured value dependency individually for each peptide

Reproducibility

- **At least 3 independent biological replicates for Student T test**
- **At least 4 independent replicates for Mann/Whitney Test**

Quantification

Optical methods

- Optical density of 2-DE separated and stained proteins
- Fluorescence labelling by DIGE

MS

- Isotope labelling
- Metal labelling
- Isobaric tags
- Label-free

Radioactivity

Radioactive labelling

Proteomics 1999

Quantitation of Complex Protein Mixtures using ICAT™ Reagents Kit

1 Cell State - control

mixture 1 (light)

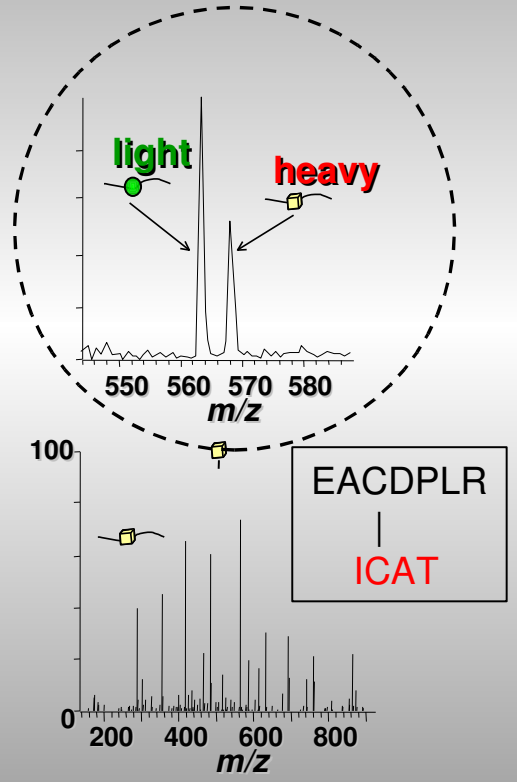
labeled cysteines

mixture 2 (heavy)

combine and proteolyze

purify ICAT peptides

Quantify & Identify by MS



2 Cell State - sample

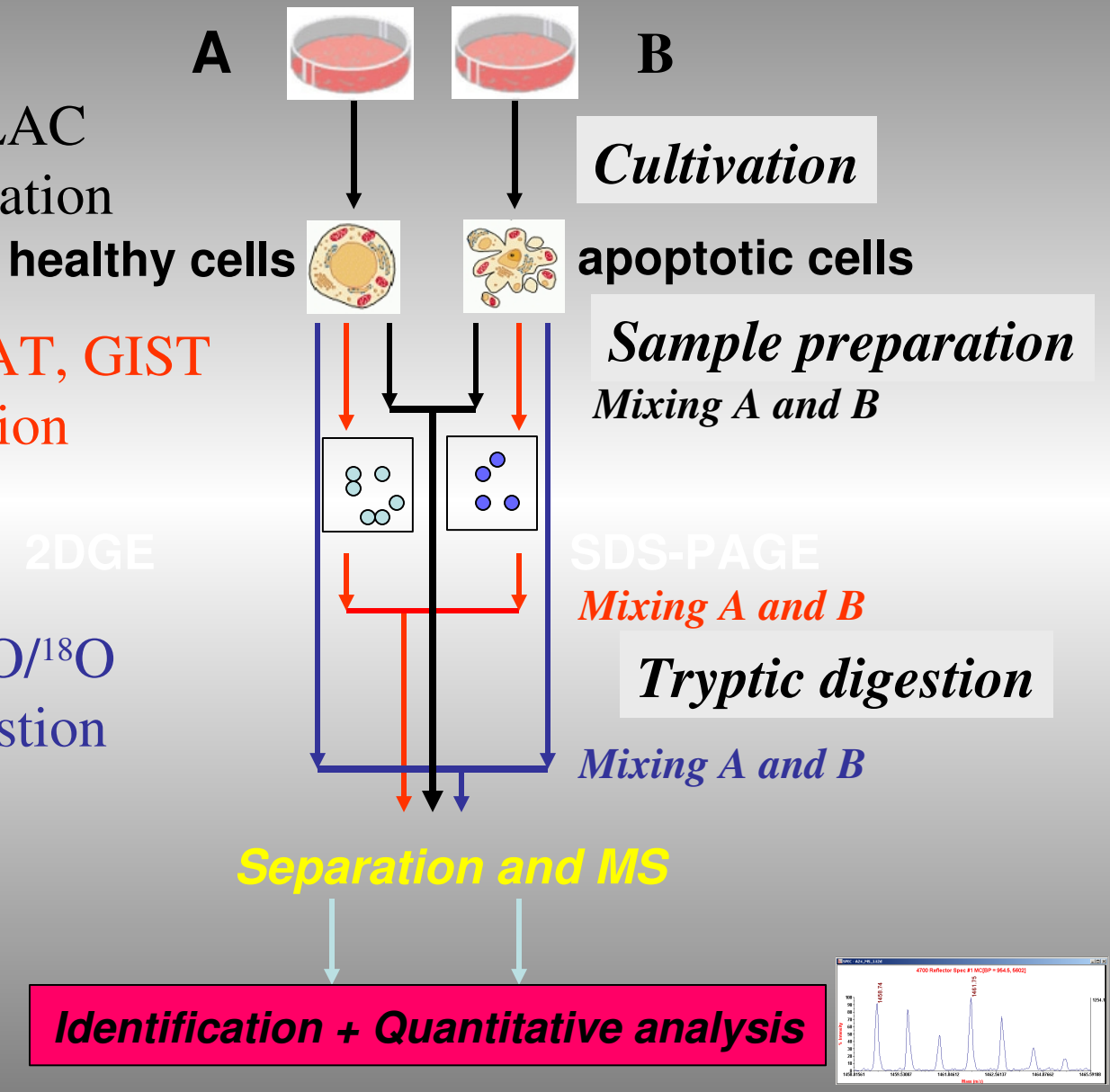
Gygi S.P. et al., Nat Biotechnol 1999, 17, 994-99

Stable Isotope Labeling

Labeling SILAC
during cultivation

Labeling ICAT, GIST
after cultivation

Labeling $^{16}O/^{18}O$
during digestion



Criteria for labeling reagents

- Amino acid which is labeled
- Completeness of reaction
- Size of the labeling reagent
- Shift between the isotopic forms in LC or 2-DE
- Compatibility of the sample, separation and identification technique with the labeling reaction
- Complexity of sample
- Affinity enrichment, without increasing Mr too much
- Multiplexing
- Usability for all kinds of biological material

Proteomics 2007