Protein and Proteome Analysis by Mass Spectrometry

Karsten Boldt

University of Tübingen
Centre for Ophthalmology
Medical Proteome Center
karsten.boldt@uni-tuebingen.de

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What is the proteome?

The proteome

The Proteome (Keith Williams and Marc Wilkins, 1994)

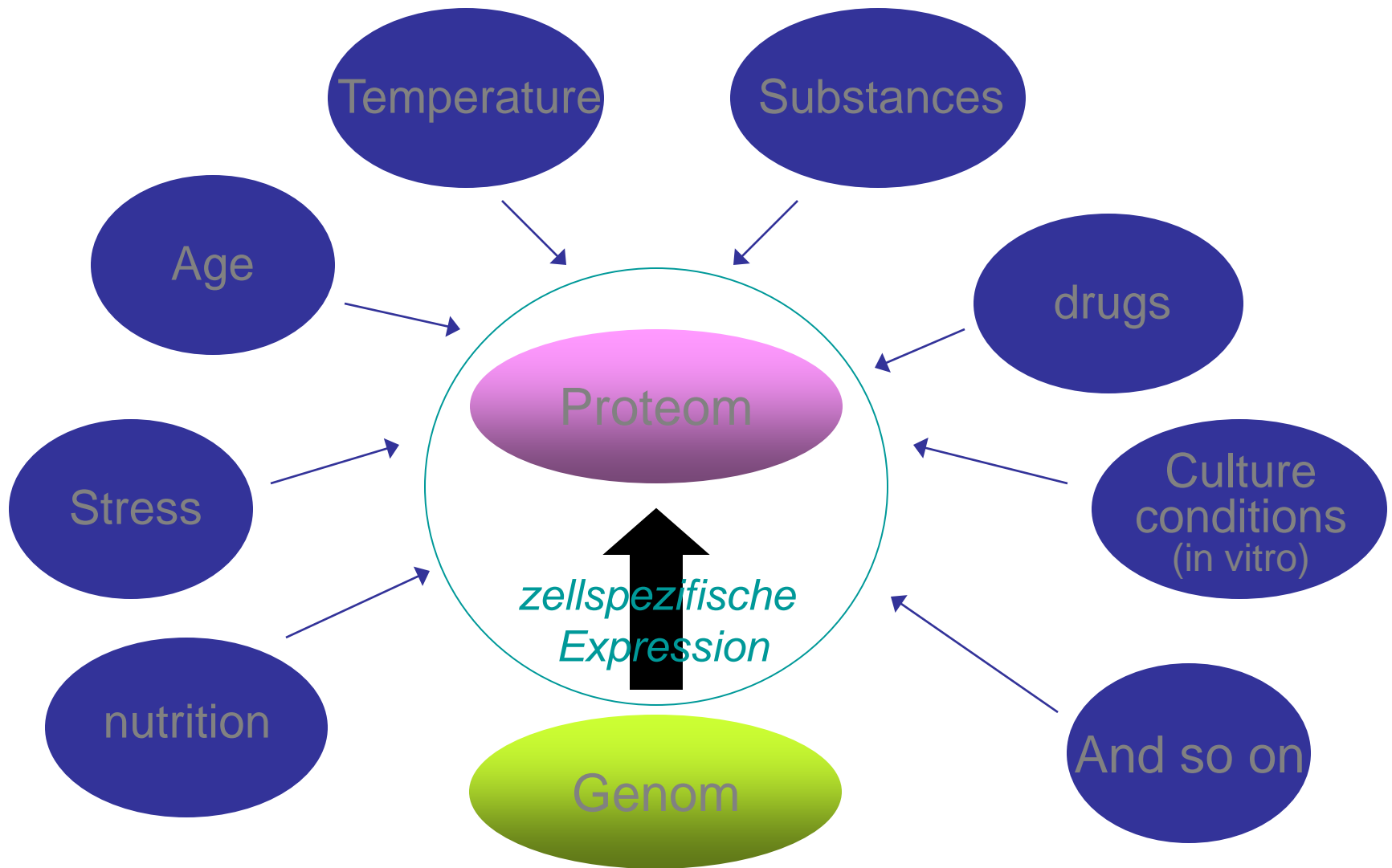*"The protein equivalent to the genome"*

The proteome describes the entirety of proteins of an organism at a **certain point in time and in a certain state**.

*In contrast to the genome, the proteome is not static but **highly dynamic***

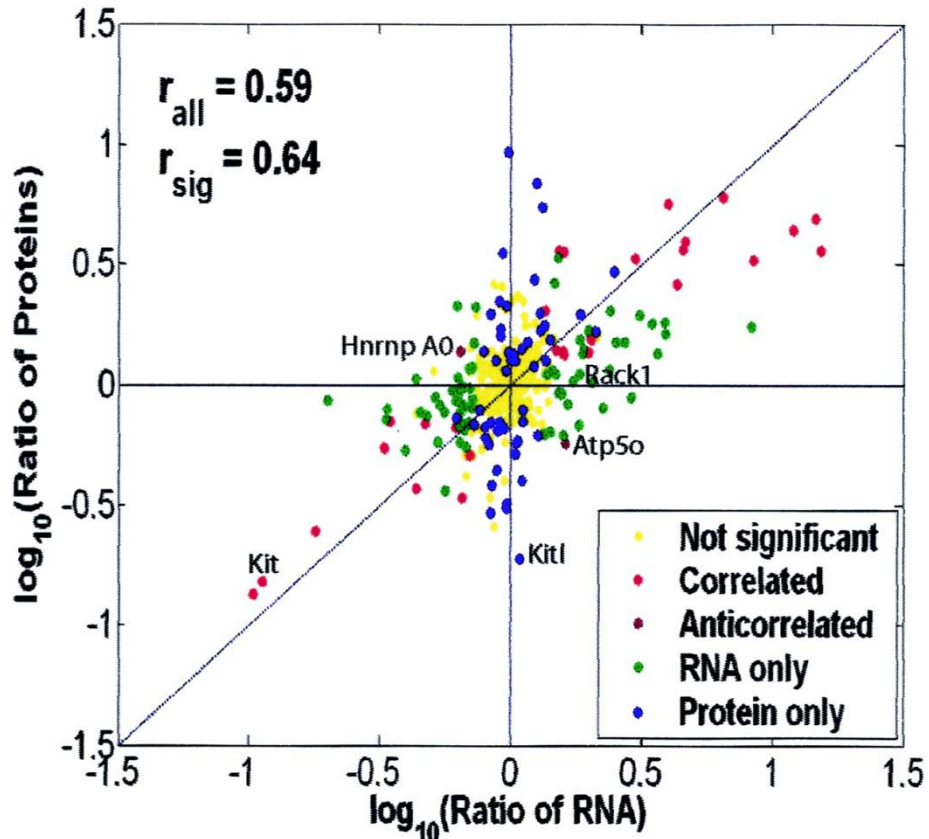*One species,*

the same genome,

different proteome

The proteome influenced by the environment

Why do we need proteome analysis?

Correlation with RNA expression

Scatter plot of mRNA versus cognate protein expression ratios (log10) of MPRO:EML.

Tian Q et al. Mol Cell Proteomics 2004;3:960-969

➤ Protein expression levels are strongly regulated by post-transcriptional regulation

➤ RNA expression levels can only capture around 40% of variation in protein expression

→ **Protein expression analysis can give valuable additional information**

Analysis of sub-proteomes

➢ Proteomics enables the analysis of sub-proteomes like:

  ➢ Proteomes of organelles (mitochondria, cilia, outer segments of photoreceptors,…)

  ➢ Body fluids

  ➢ Protein complexes

  ➢ …

➢ Analysis of post-translational modifications:

  ➢ Phosphorylation

  ➢ Acetylation

  ➢ Sumoylation

  ➢ …

How can we analyse the proteome and what are the challenges?

Analysing the proteome

➢ *Methods for proteome analysis:*

  ➢ *Gel based approaches*

    ➢ *Labour intensive, hard to standardize*

    ➢ *MS necessary for protein identification*

  ➢ *Gel-free, MS-based approaches*

    ➢ *Standardization possible*

    ➢ *Automation possible*

    ➢ *Great improvement in sensitivity, speed and resolution within the last 10 years*

    ➢ *Automated quantification possible*

→ *Gel-free, MS-based approaches are highly suitable for proteome analysis*

Major challenges in proteom analysis

- High complexity:
  - ~ 20 000 genes
  - 50 000 - 100 000 proteins
- Modifications lead to even higher complexity
- Huge dynamic range (10 orders of magnitude)
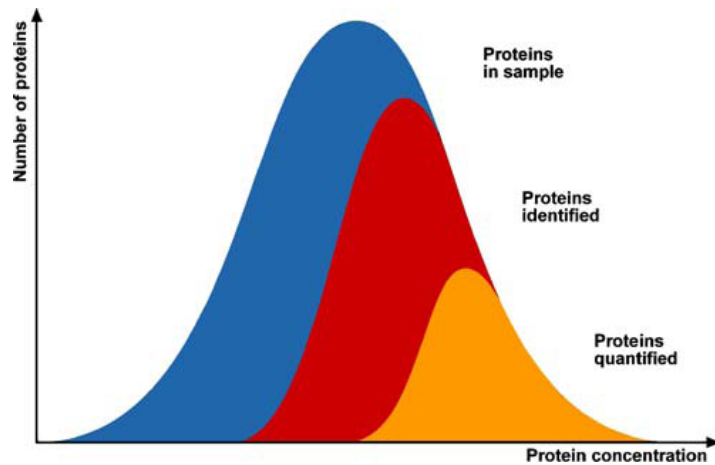
Banscheff et al., Anal. Bioanal. Chem.,2007
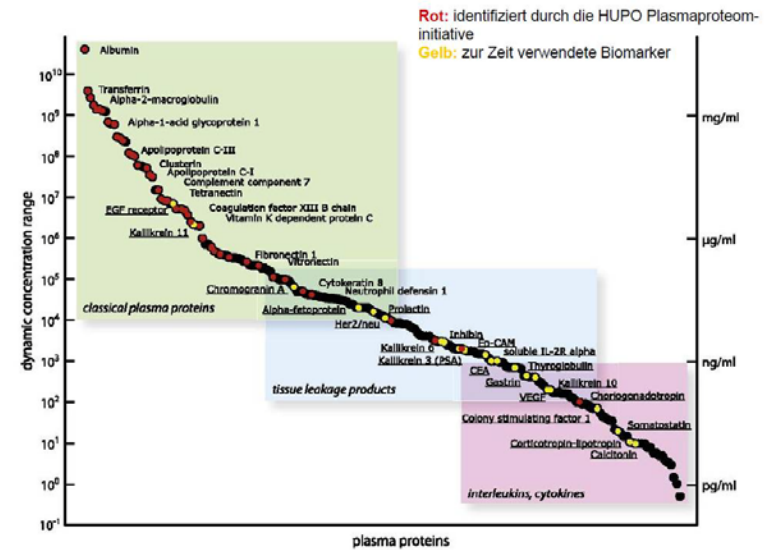
Protein identification by MS: The basics

Bottom-up protein analytics by MS
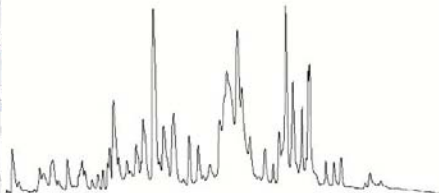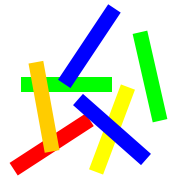
cleavage

Protein identification/quantification

Liquid-Chromatography/ Tandem Mass spectrometry
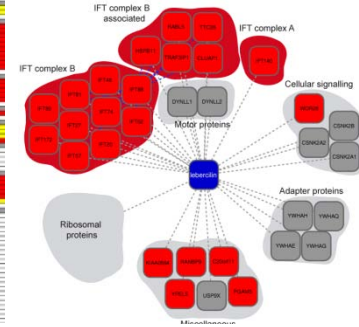
Why do we need proteolytic cleavage

➢ Molecular weight of proteins varies greatly (10-500 kilo Dalton)

➢ MS machines are mainly suited for the analysis of molecules of up to some Dalton

→ Proteolytic cleavage produces peptide fragments making the high throughput analysis and identification by MS possible

→ Major disadvantage: Even higher complexity of the sample

Bottom-up protein analytics by MS
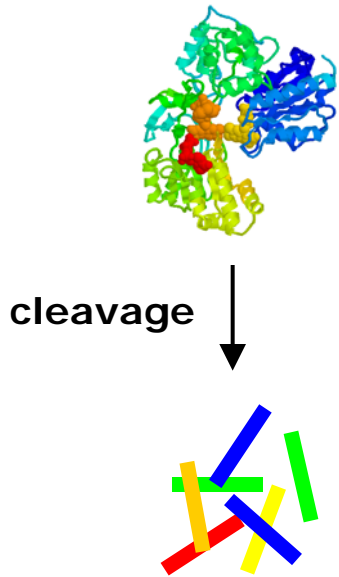
cleavage

Protein identification/quantification

Liquid-Chromatography/ Tandem Mass spectrometry
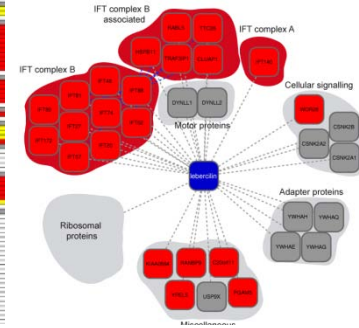
Components of a MS: Ion source

Components of a MS: Ion source
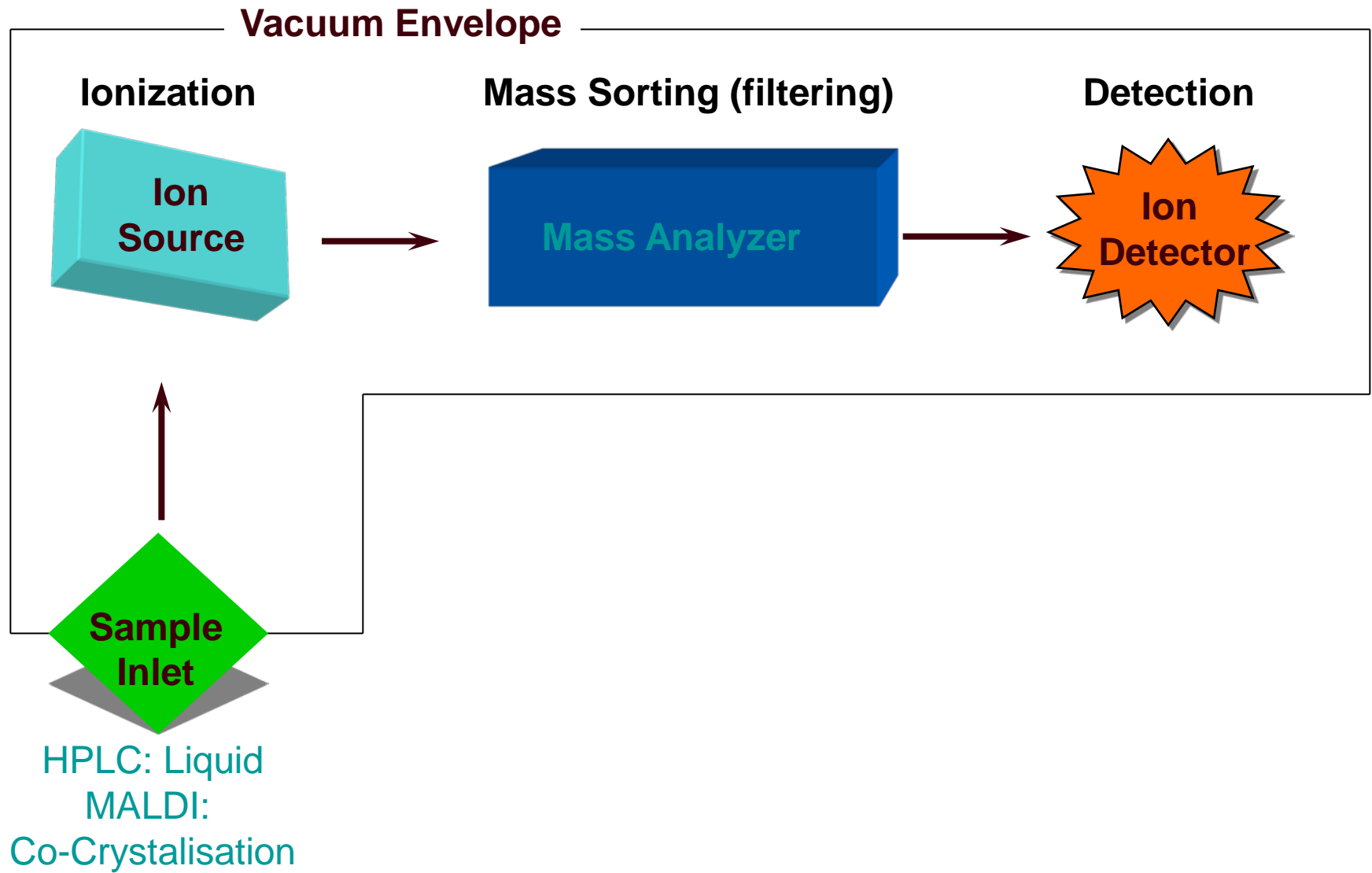
Ion source → Mass analyser → Detector

Electro-Spray Ionization ESI

Liquid chromatography — Spray needle — Nozzle — Sampling cone — Ions
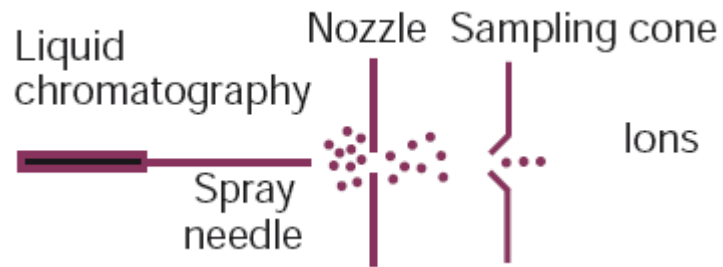
Matrix-Assisted laser desorption/ionization (MALDI)

Pulsed laser — Sample plate — Extraction grid — Ions
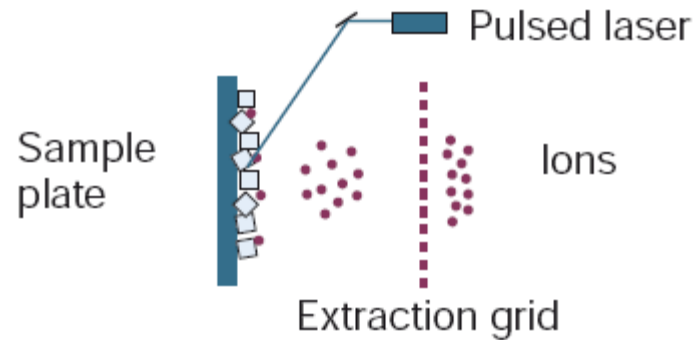
Components of a MS: Mass analyzer

Bottom-up protein analytics by MS

cleavage

Protein identification/quantification

Liquid-Chromatography/ Tandem Mass spectrometry

Identification by Peptide Mass Fingerprint

cleavage

Protein identity

TOF

Theoretical MS

intensity

m/z

Comparison to all possible proteins

MS

intensity

m/z

Identification by fragment ions

MS

cleavage

fragmentation

amino acid sequence

MS/MS

A   K   S   Y

intensity

m/z

Identification by fragment ions

Peptide mixture

1 peptide selected for MS/MS
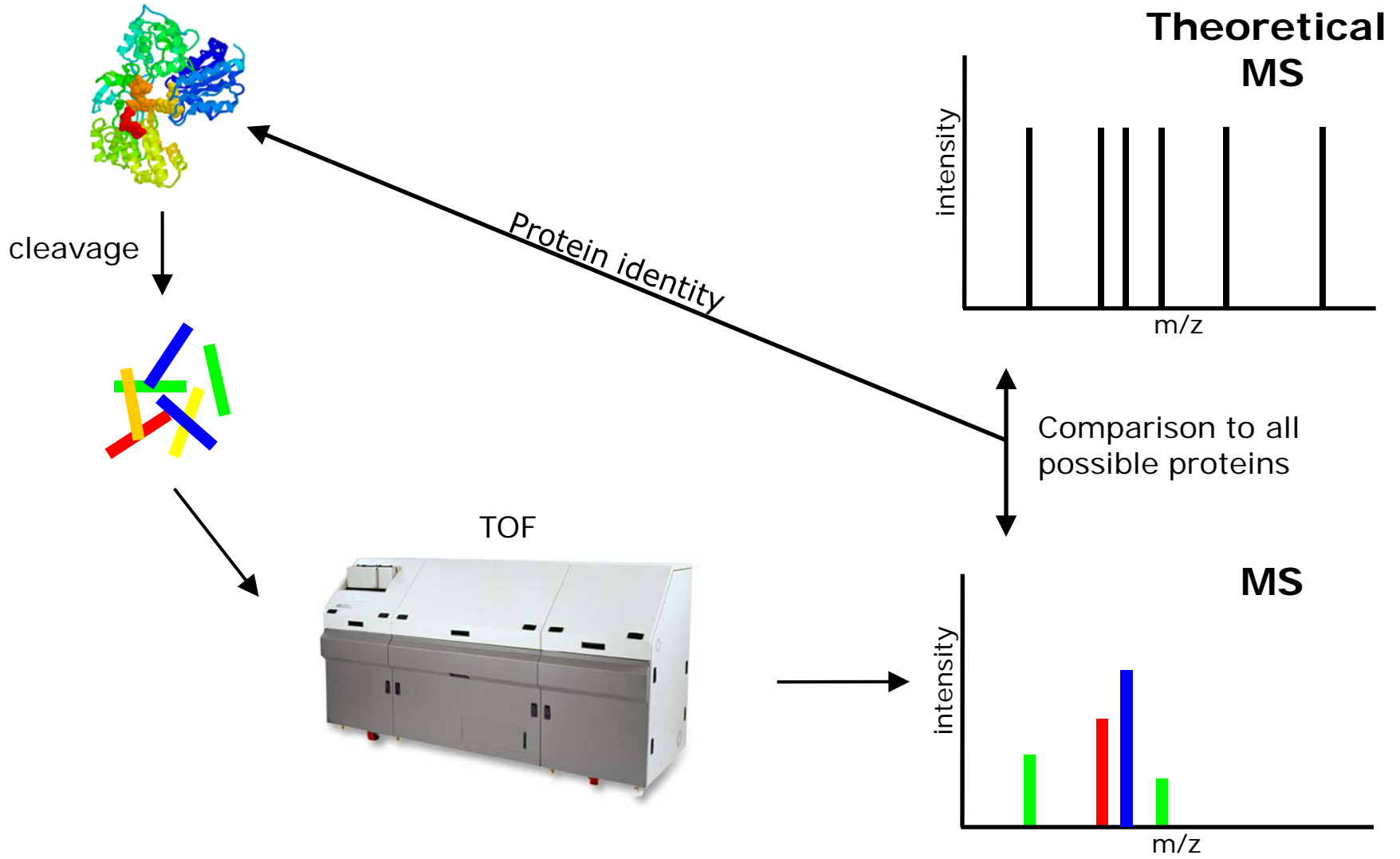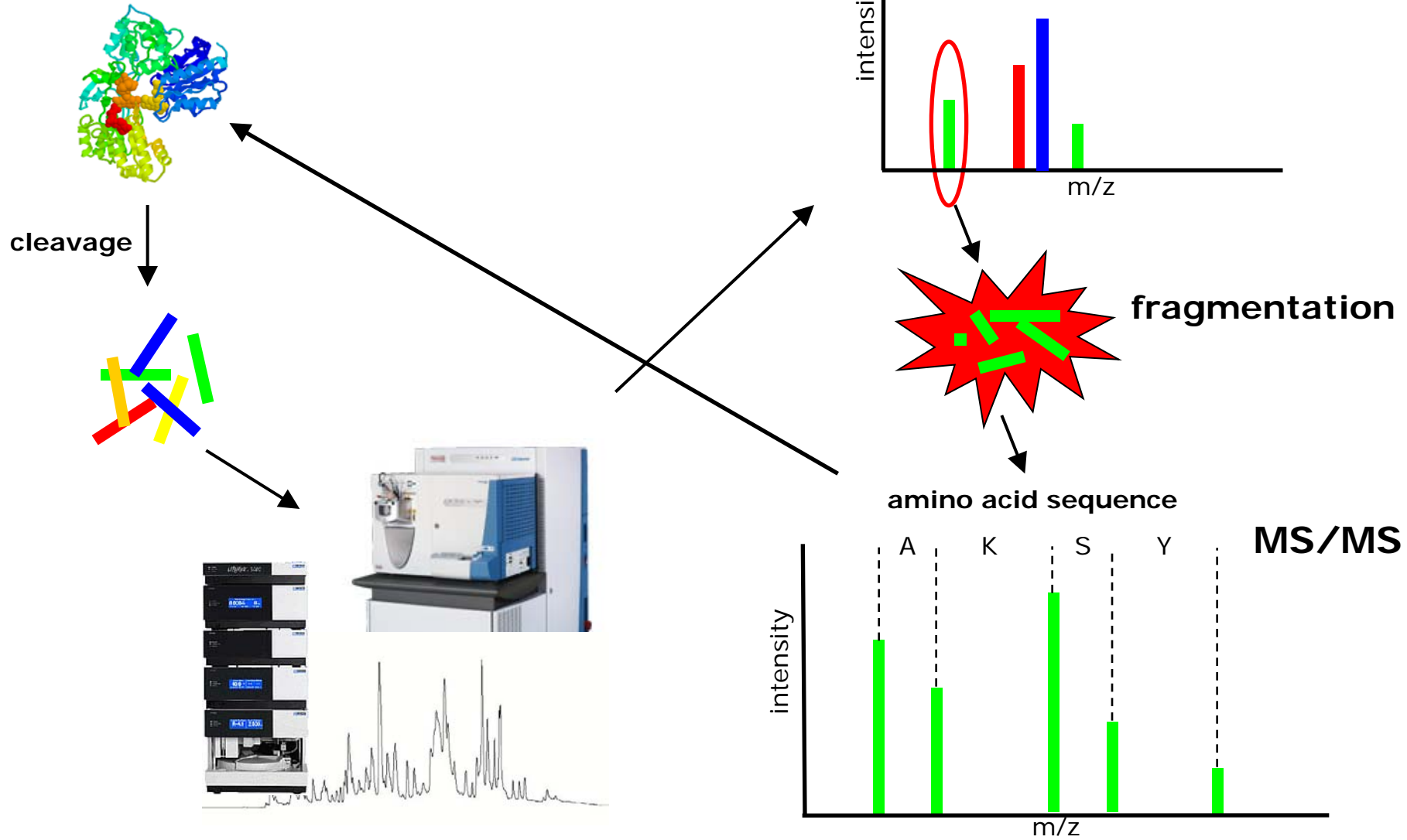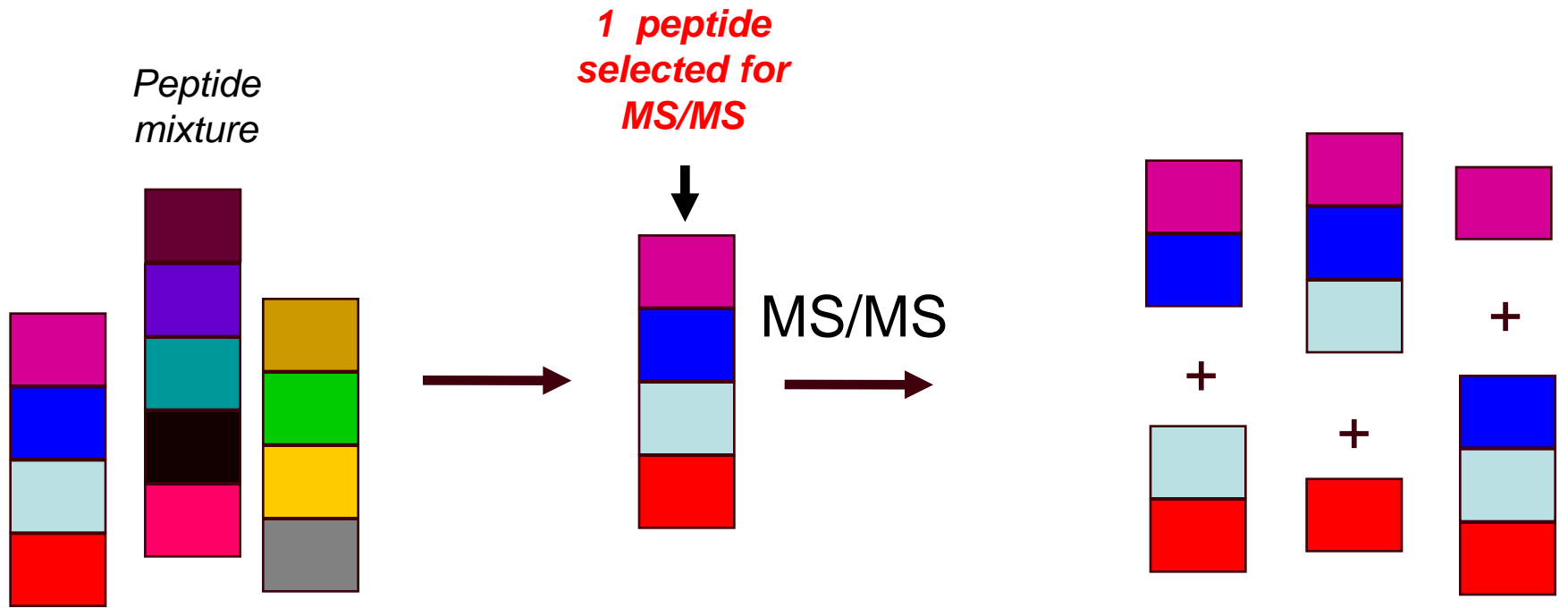
MS/MS

The masses of all the pieces give an MS/MS spectrum

Identification by fragment ions
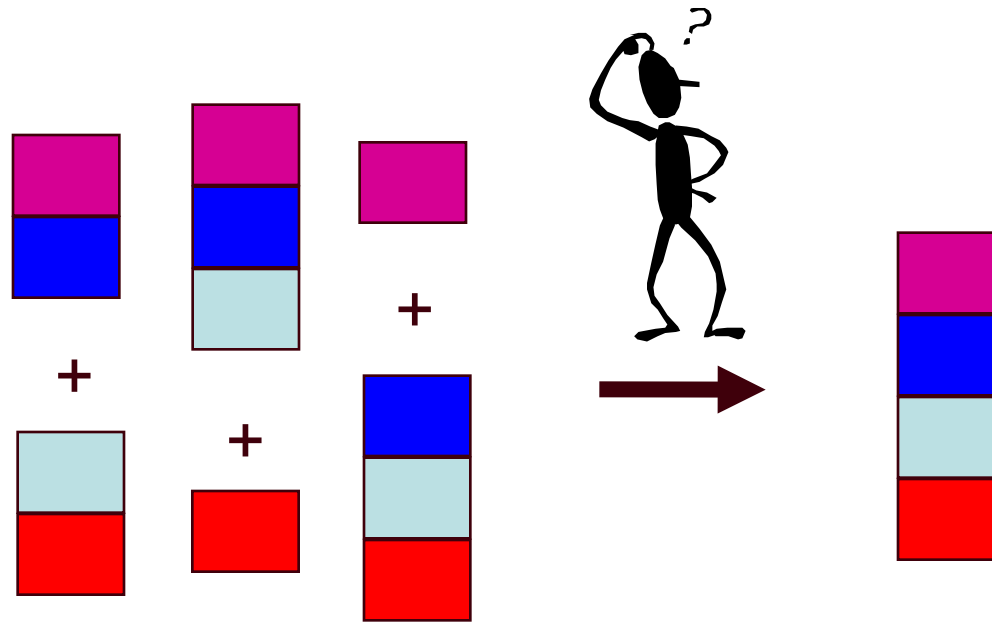
➢ Use the fragment ion masses as specific pieces of the puzzle to help piece the intact molecule back together
➢ Several thousands of peptides/analysis → highly complex job

→ Search algorithms make this job

Identification by fragment ions

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	115.1		98.0		N	2,270.1	1,135.5	2,253.0	2,252.1	19
2	228.1		211.1		I	2,156.0	1,078.5	2,139.0	2,138.0	18
3	357.2		340.2	339.2	E	2,042.9	1,022.0	2,025.9	2,024.9	17
4	470.3		453.2	452.3	L	1,913.9	957.5	1,896.9	1,895.9	16
5	583.3		566.3	565.3	I	1,800.8	900.9	1,783.8	1,782.8	15
6	743.4	372.2	726.3	725.4	C+57	1,687.7	844.4	1,670.7	1,669.7	14
7	871.4	436.2	854.4	853.4	Q	1,527.7	764.4	1,510.7	1,509.7	13
8	1,000.5	500.7	983.5	982.5	E	1,399.6	700.3	1,382.6	1,381.6	12
9	1,114.5	557.8	1,097.5	1,096.5	N	1,270.6	635.8	1,253.6	1,252.6	11
10	1,243.6	622.3	1,226.5	1,225.6	E	1,156.6	578.8	1,139.5	1,138.5	10
11	1,300.6	650.8	1,283.6	1,282.6	G	1,027.5	514.3	1,010.5	1,009.5	9
12	1,429.6	715.3	1,412.6	1,411.6	E	970.5	485.8	953.5	952.5	8
13	1,543.7	772.3	1,526.6	1,525.7	N	841.5	421.2	824.4	823.4	7
14	1,658.7	829.9	1,641.7	1,640.7	D	727.4	364.2	710.4	709.4	6
15	1,755.7	878.4	1,738.7	1,737.7	P	612.4		595.4		5
16	1,854.8	927.9	1,837.8	1,836.8	V	515.3		498.3		4
17	1,967.9	984.5	1,950.9	1,949.9	L	416.3		399.2		3
18	2,096.0	1,048.5	2,078.9	2,077.9	Q	303.2		286.2		2
19	2,270.1	1,135.5	2,253.0	2,252.1	R	175.1		158.1		1

In real life (almost): MSMS on an Orbitrap

The Orbitrap
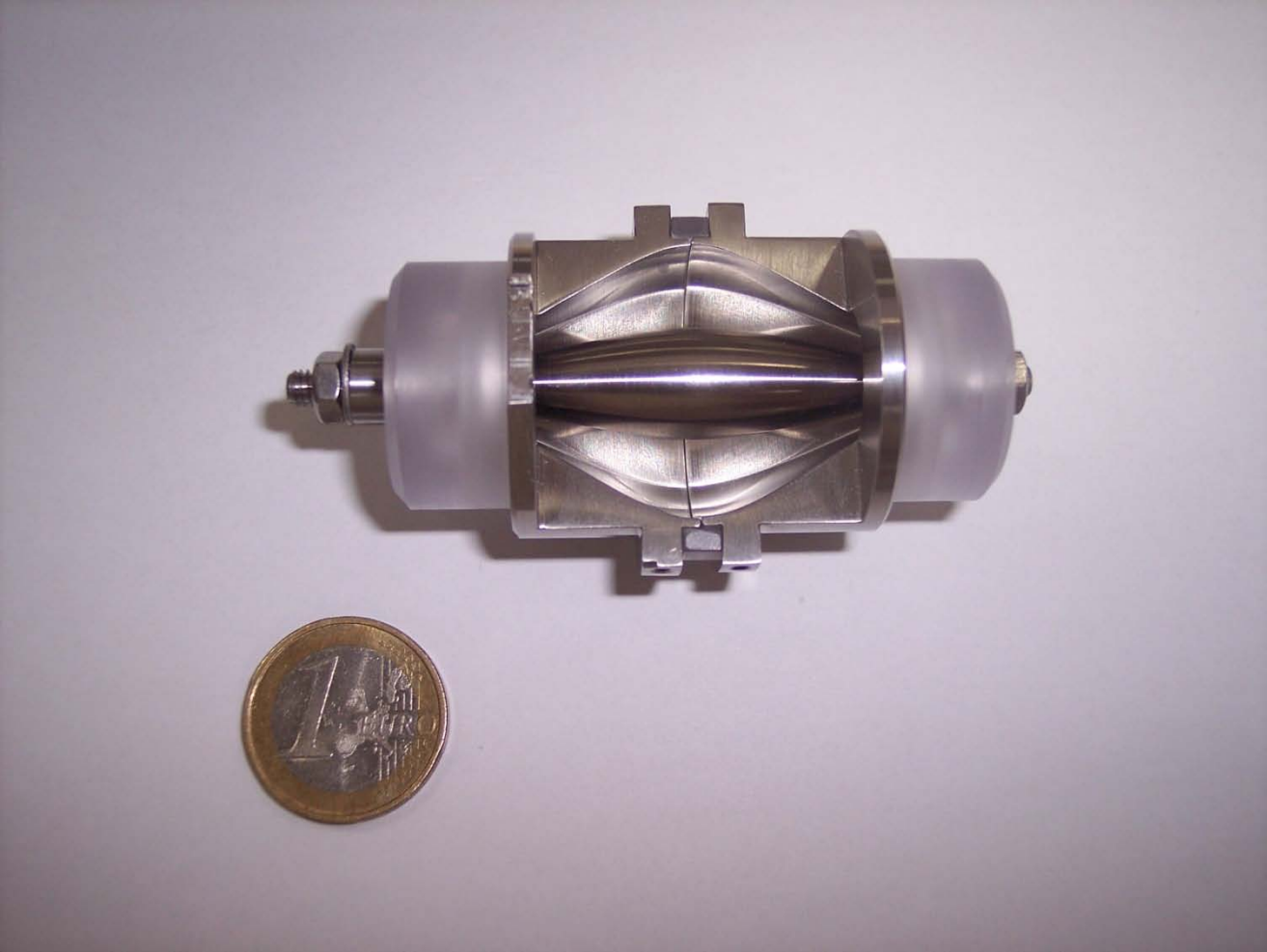
LC-MSMS on an Orbitrap

1. Ions are stored in the Linear Trap
2. …. are axially ejected
3. …. and trapped in the C-trap
4. …. they are squeezed into a small cloud and injected into the Orbitrap
5. …. where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation
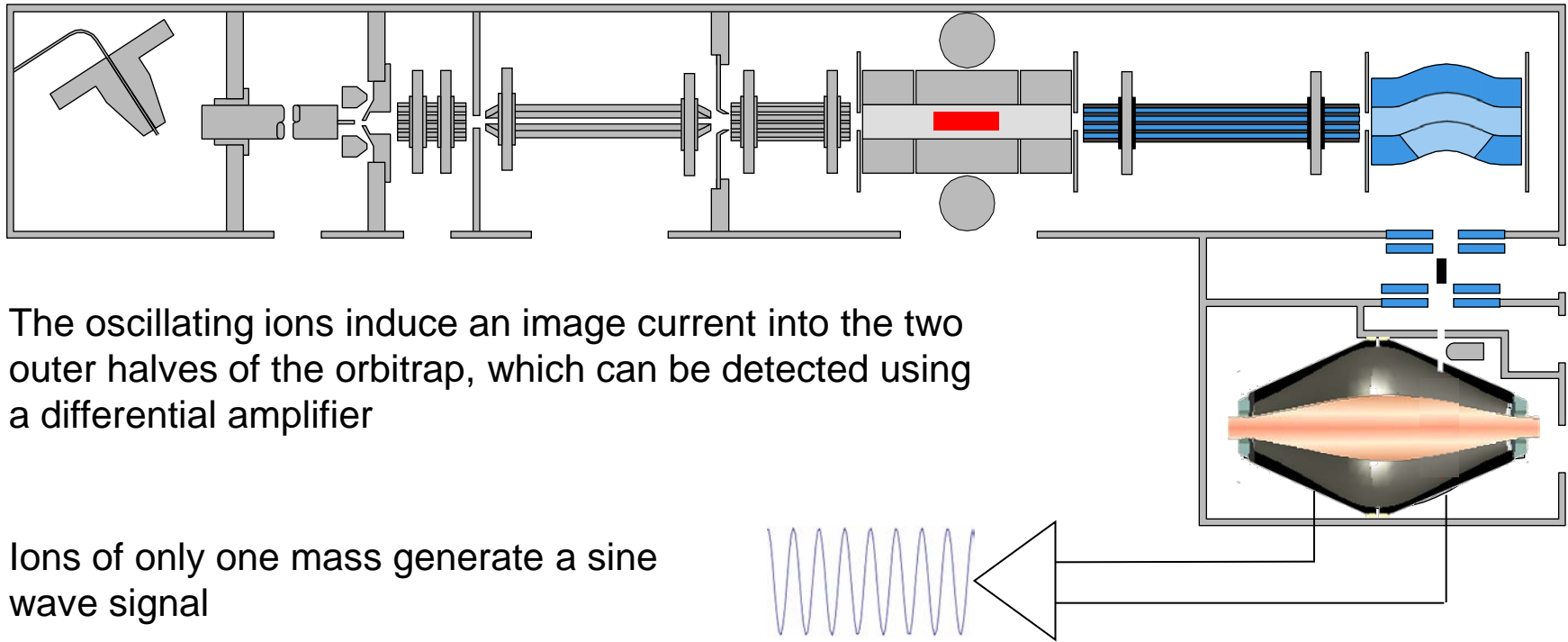
The oscillating ions induce an image current into the two outer halves of the orbitrap, which can be detected using a differential amplifier

Ions of only one mass generate a sine wave signal

Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation

LC-MSMS on an Orbitrap
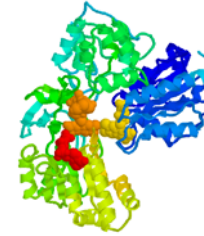

of 1 ion species

LC-MSMS on an Orbitrap

Protein quantification by MS

MS-based quantification: Principle

State A

State B

Introduction of a light and HEAVY label before or after cleavage

light

HEAVY

Mix

Relative quantification

intensity
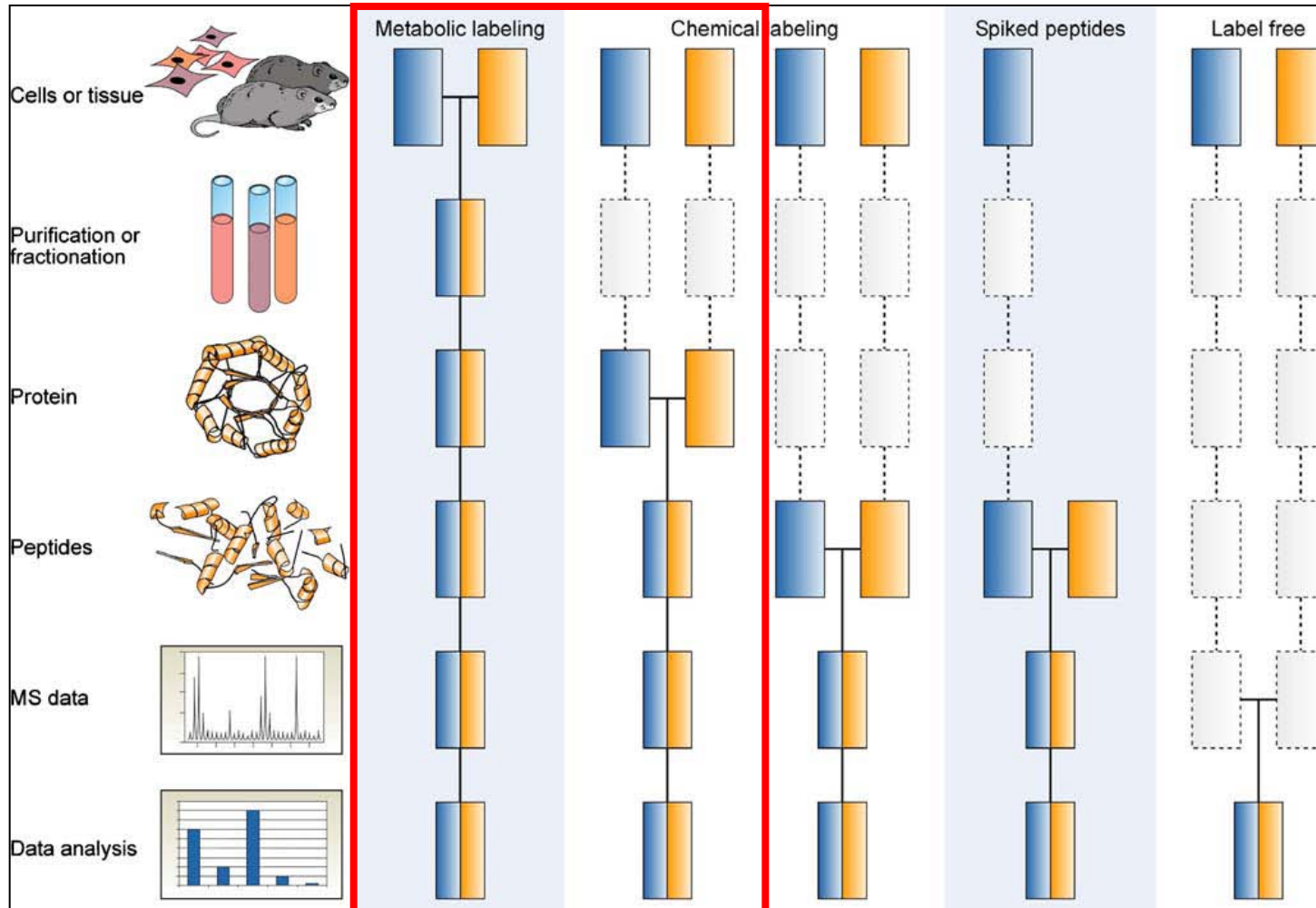
m/z
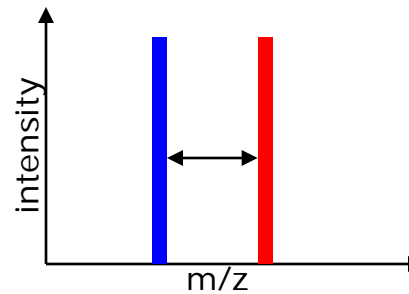
MS-based quantification: an overview

Banscheff et al., Anal. Bioanal. Chem.,2007

MS-based quantification by SILAC
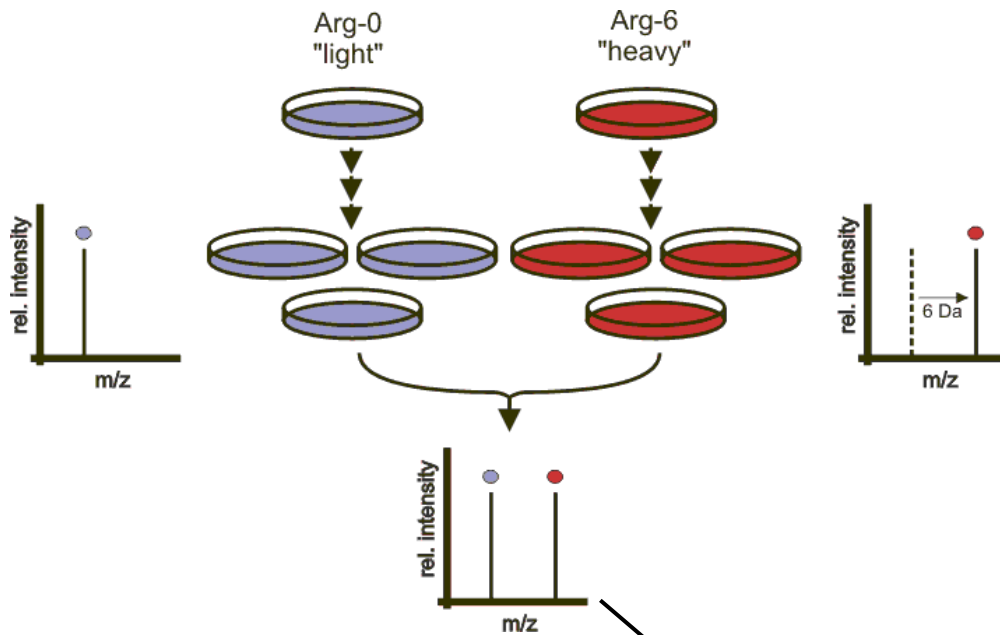
Software based quantification and identification
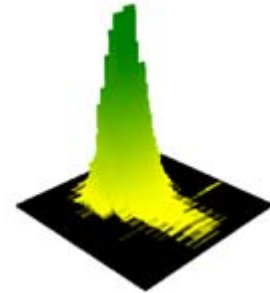
MaxQuant

Cox J, Mann M. Nat Biotechnol. 2008 Dec;26(12):1367-72.

MS-based quantification by ICPL
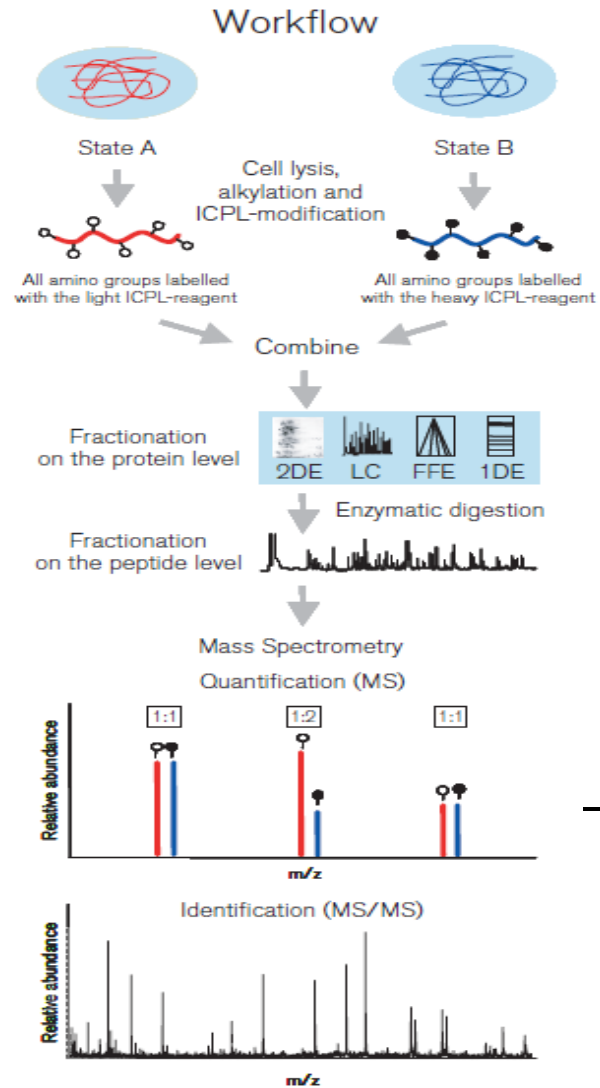
Software based quantification and identification

MSQuant: Mortensen et al. J Proteome Research, 2010

ICPLQuant: Brunner et al. Proteomics, 2010

Proteome Discoverer: ThermoScientific

Andreas Vogt   Norbert Kinkl   Johannes Gloeckner   Ronald Roepman

Protein complex analysis of Lebercilin

Mutations in the lebercilin gene cause LCA

- Autosomal recessive
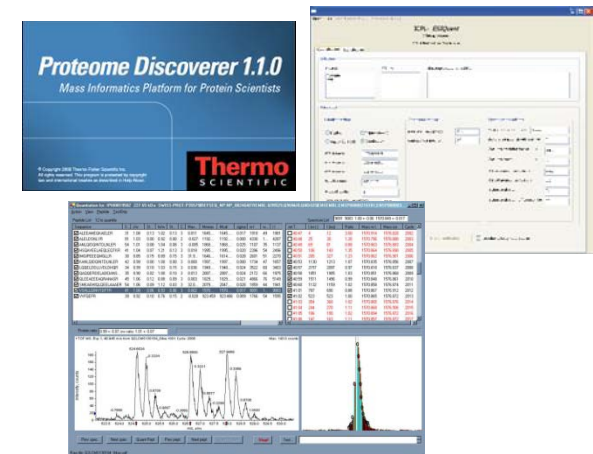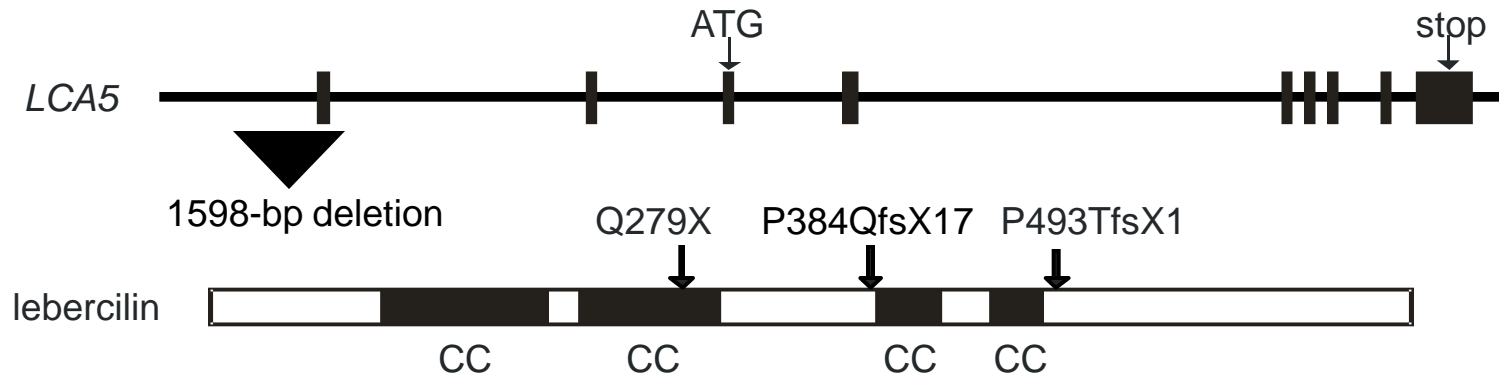
- Severe visual impairment shortly after birth

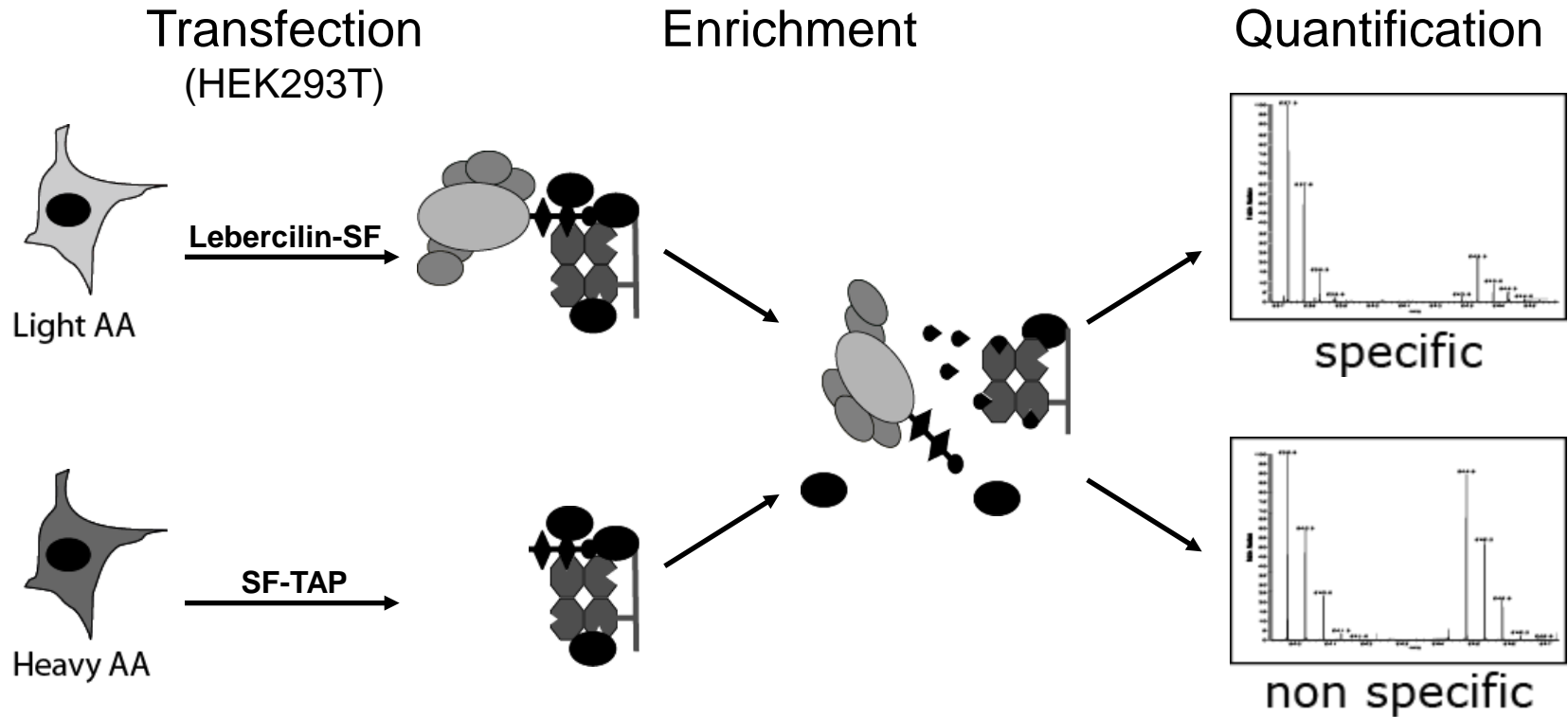- Loss of photoreceptors outer segments
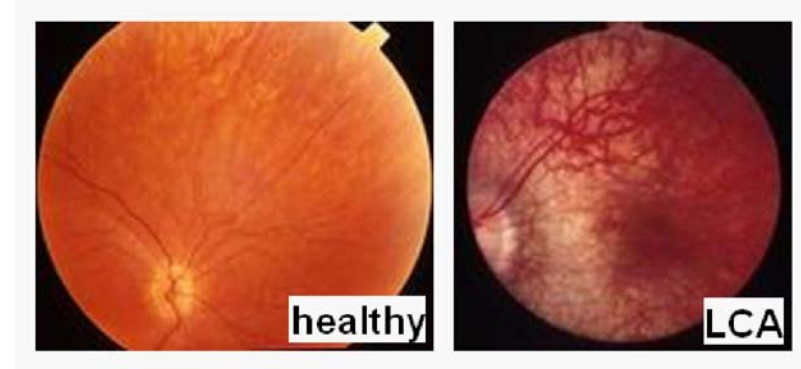
- Ronald Roepman/
  Anneke den Hollander   → P493TfsX1 and Q297X
- Chris Inglehearn   → P384QfsX17
- Irene Maumenee   → 1598-bp Promotor Deletion im *LCA5* Promoter

Den Hollander et al. Nat Genet. 2007 Jul;39(7):889-95.

What does Lebercilin do?

Detection of specific complex components by quantitative protein complex analysis
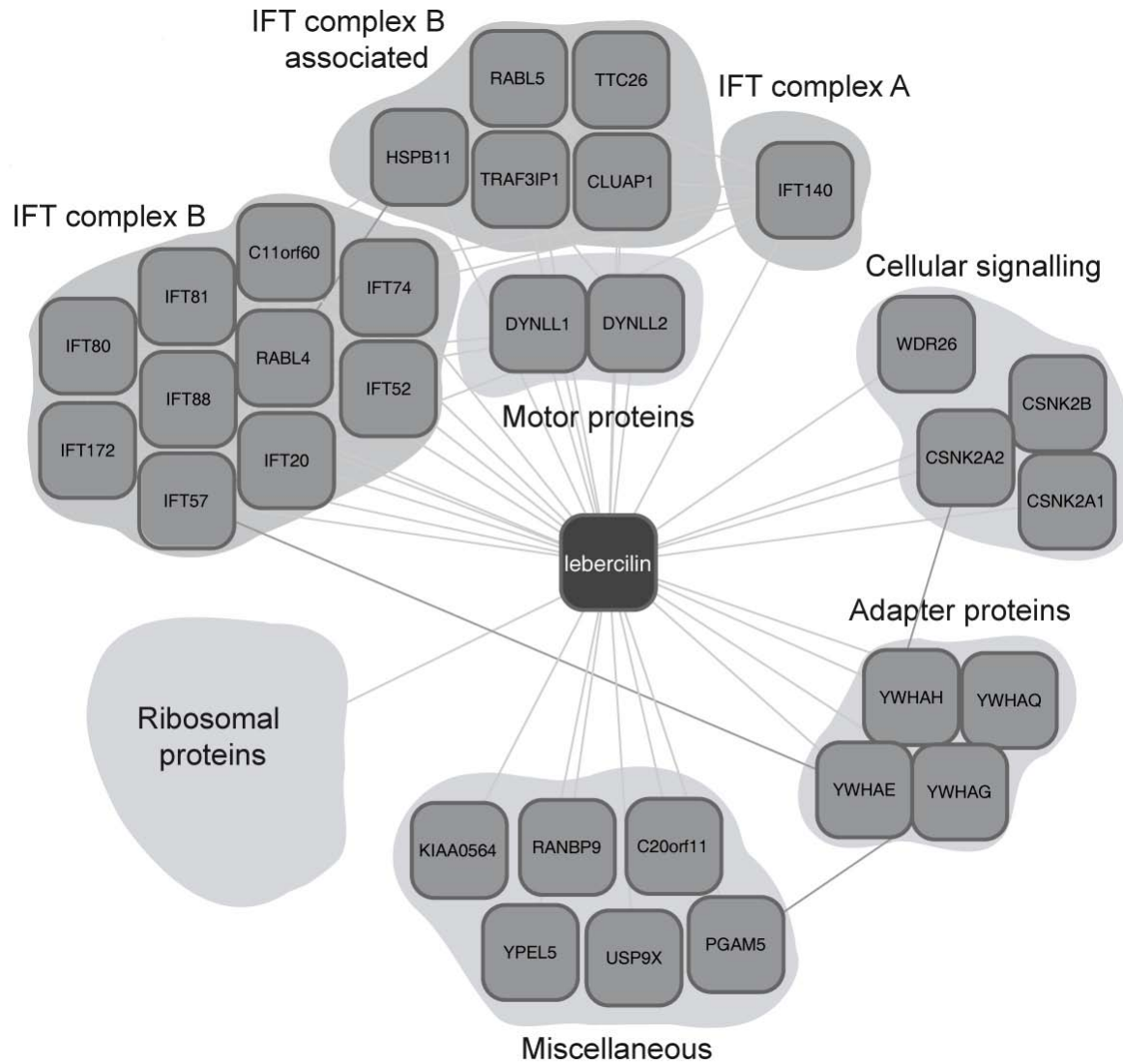
Detection of specific complex components
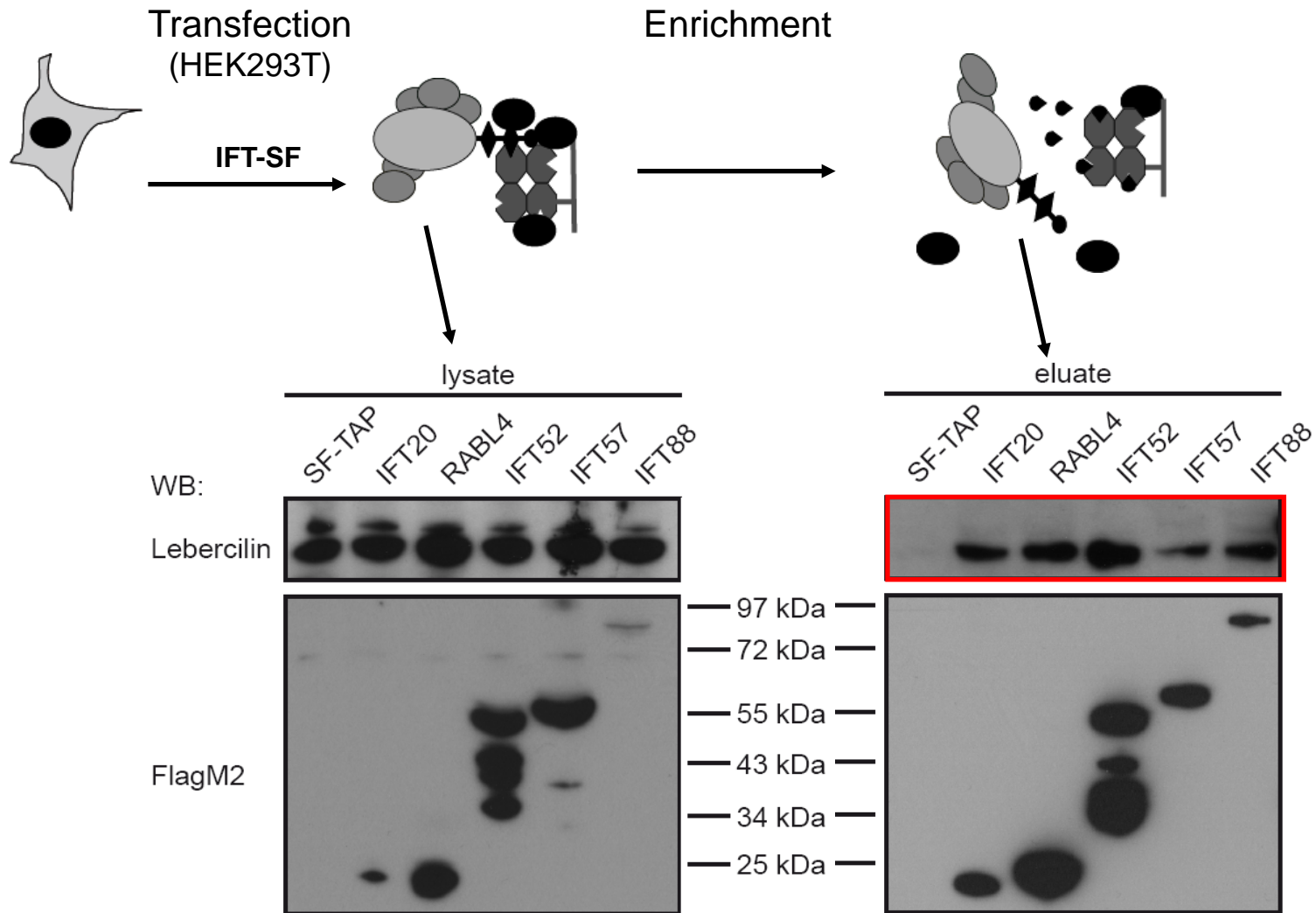

Transfection (HEK293T) — Enrichment — Quantification

Light AA → Lebercilin-SF

Heavy AA → SF-TAP

specific

non specific

MaxQuant

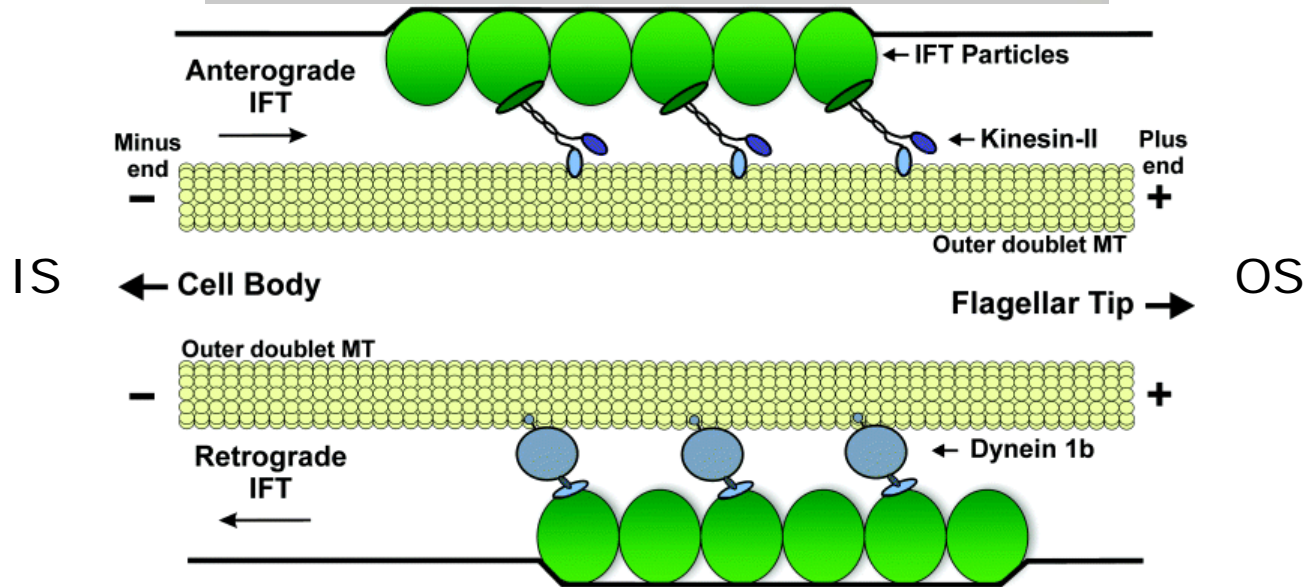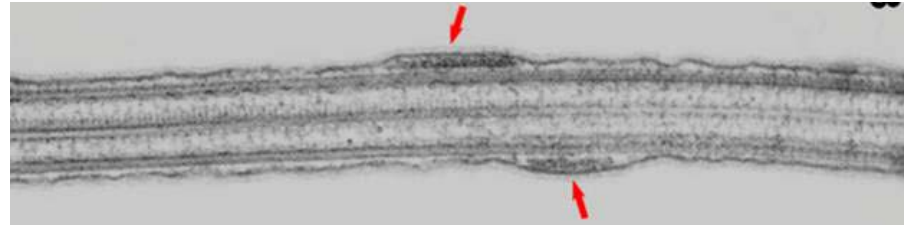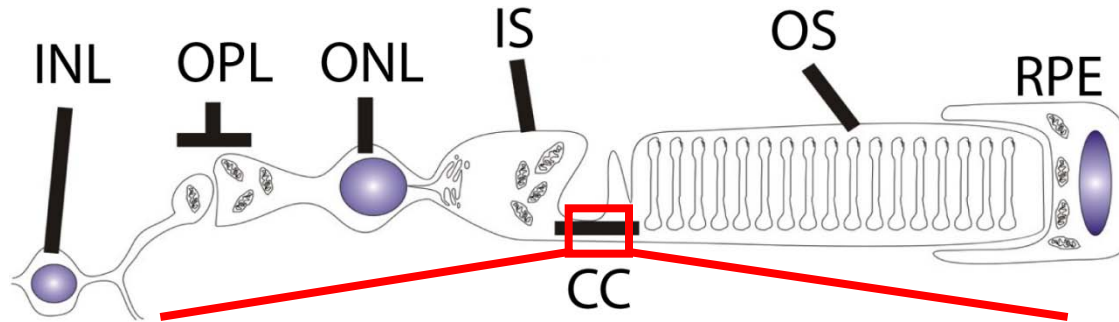Cox J, Mann M. Nat Biotechnol. 2008 Dec;26(12):1367-72.

Enrichment of specific interactors

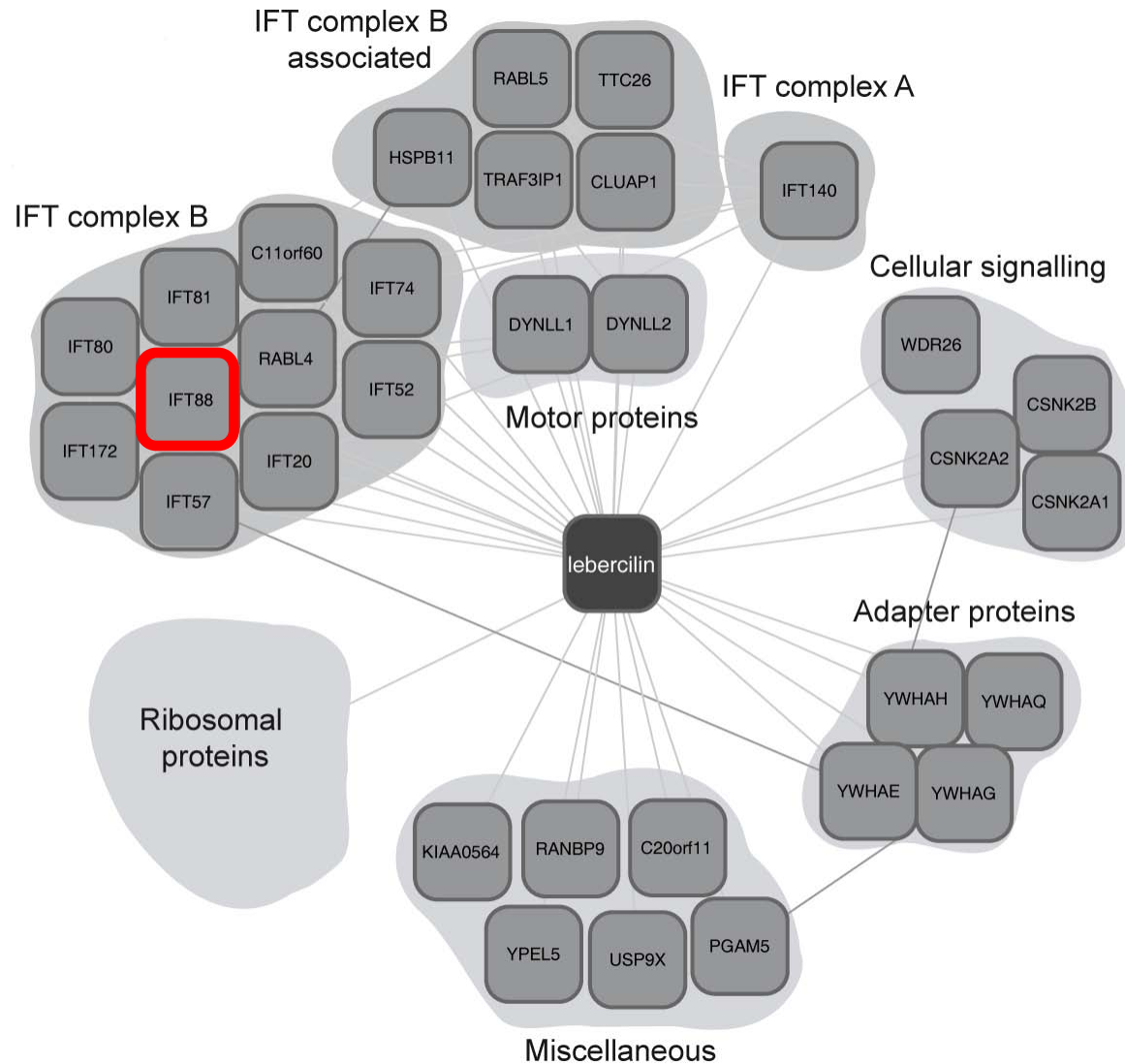# Confirmation of Lebercilin-IFT interaction

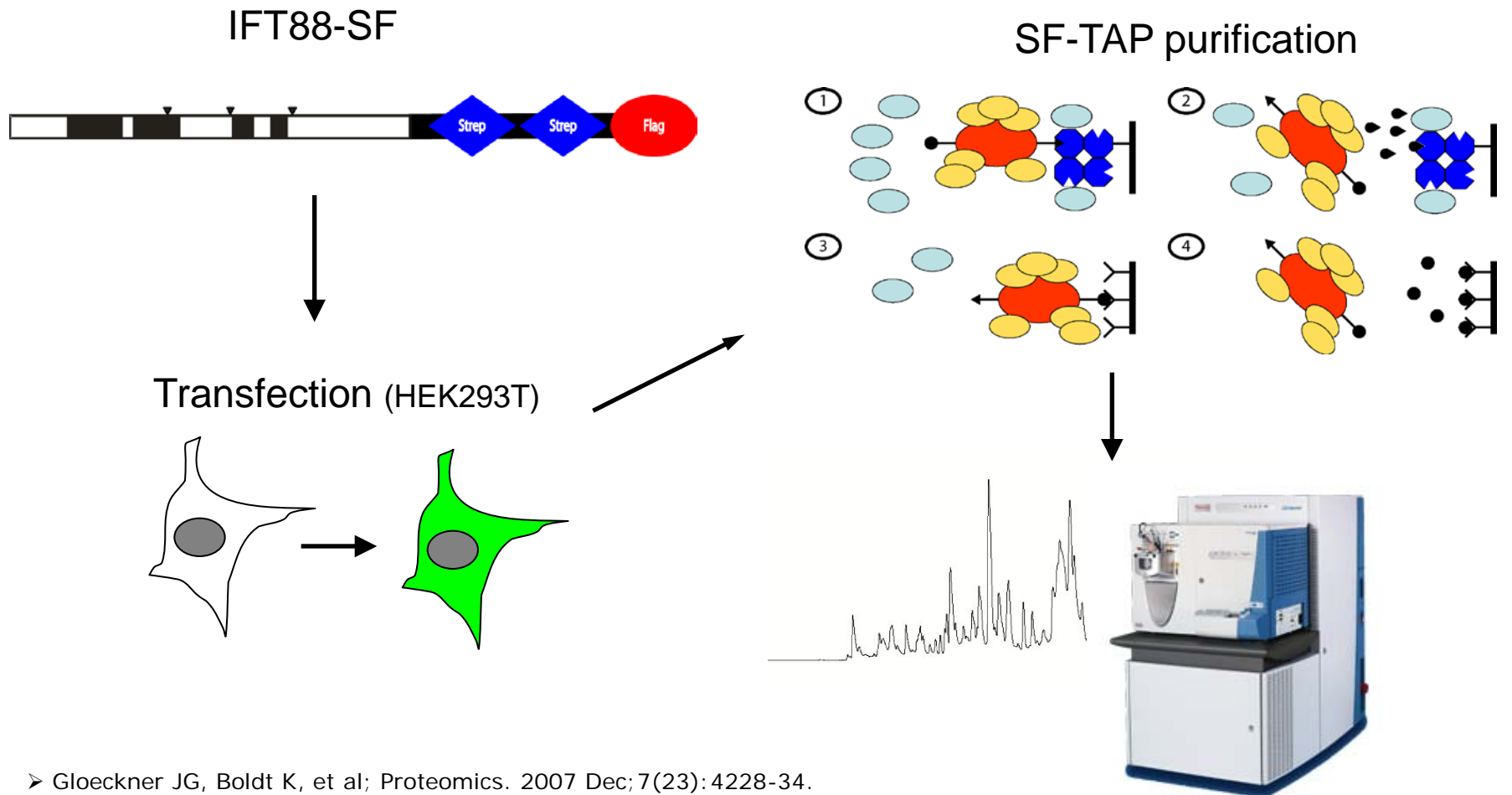# Intraflagellar transport in photoreceptors

Pedersen et al., 2006, Curr. Biol.

Cole, 2003, Traffic

Characterization of the IFT complex B in HEK293T cells

Analysis of the lebercilin complex by SF-TAP
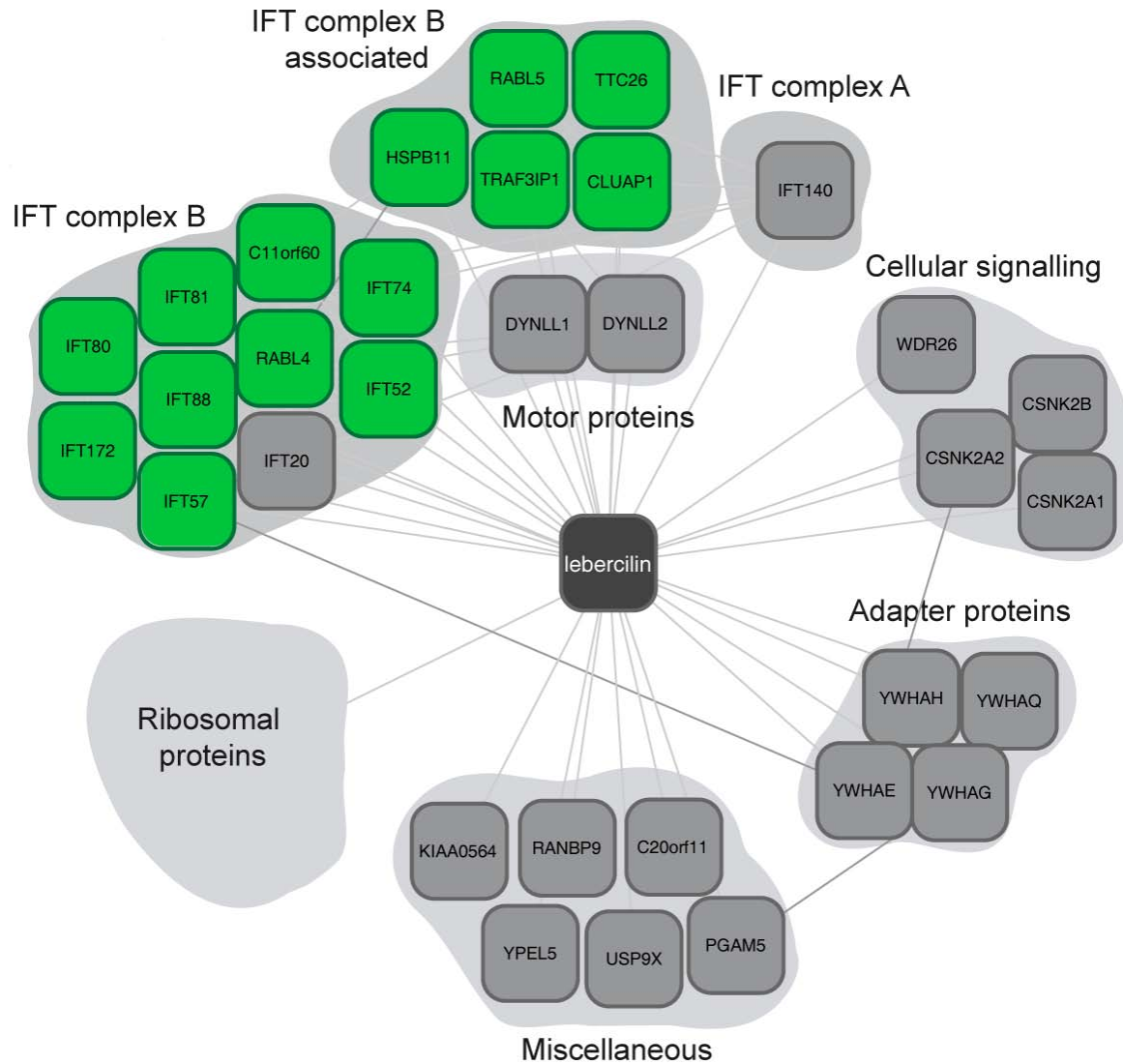
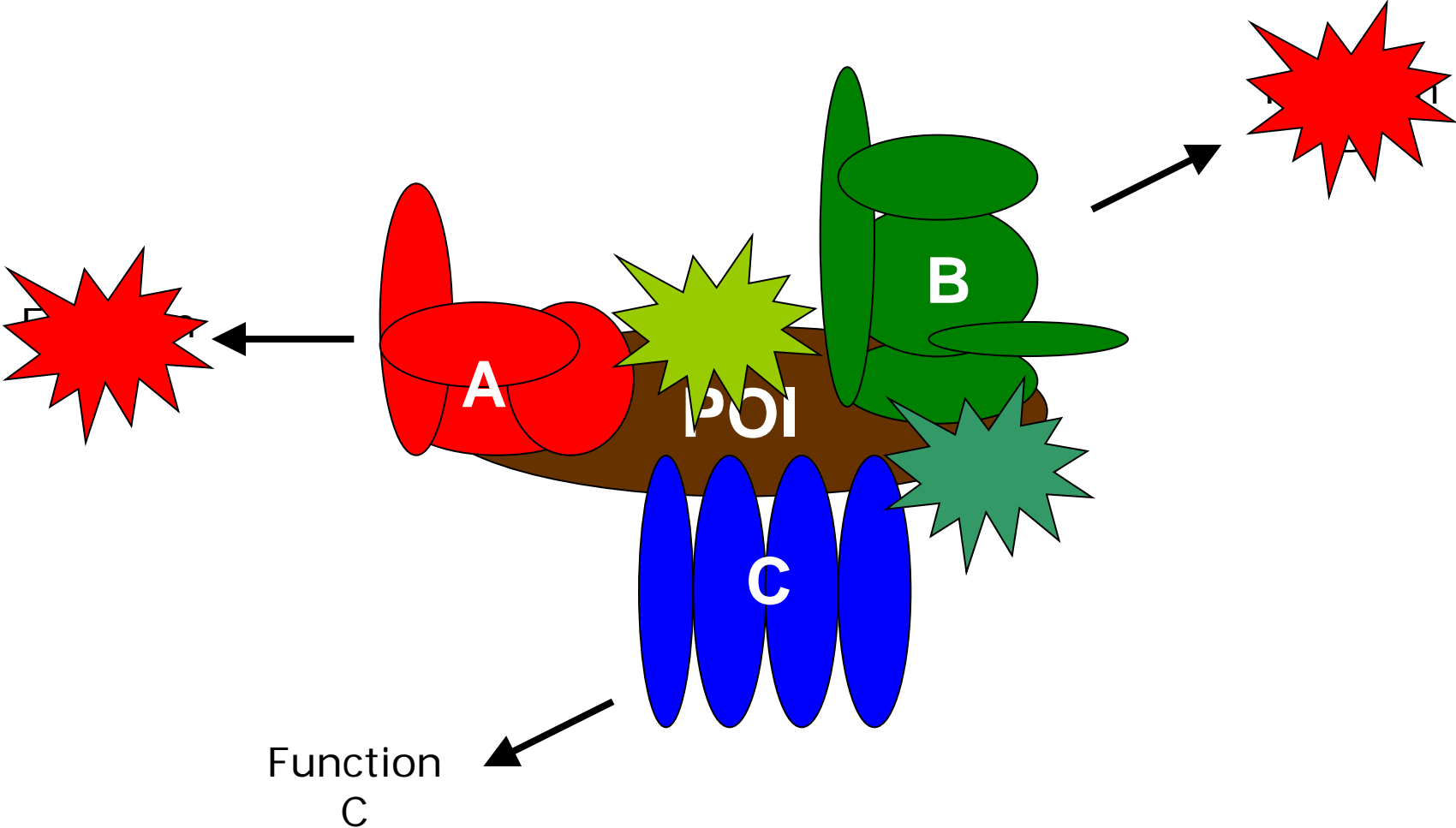IFT88-SF

SF-TAP purification

Transfection (HEK293T)

➢ Gloeckner JG, Boldt K, et al; Proteomics. 2007 Dec;7(23):4228-34.
➢ Den Hollander et.al.; Nat. Genet. 2007 Jul;39(7):889-95
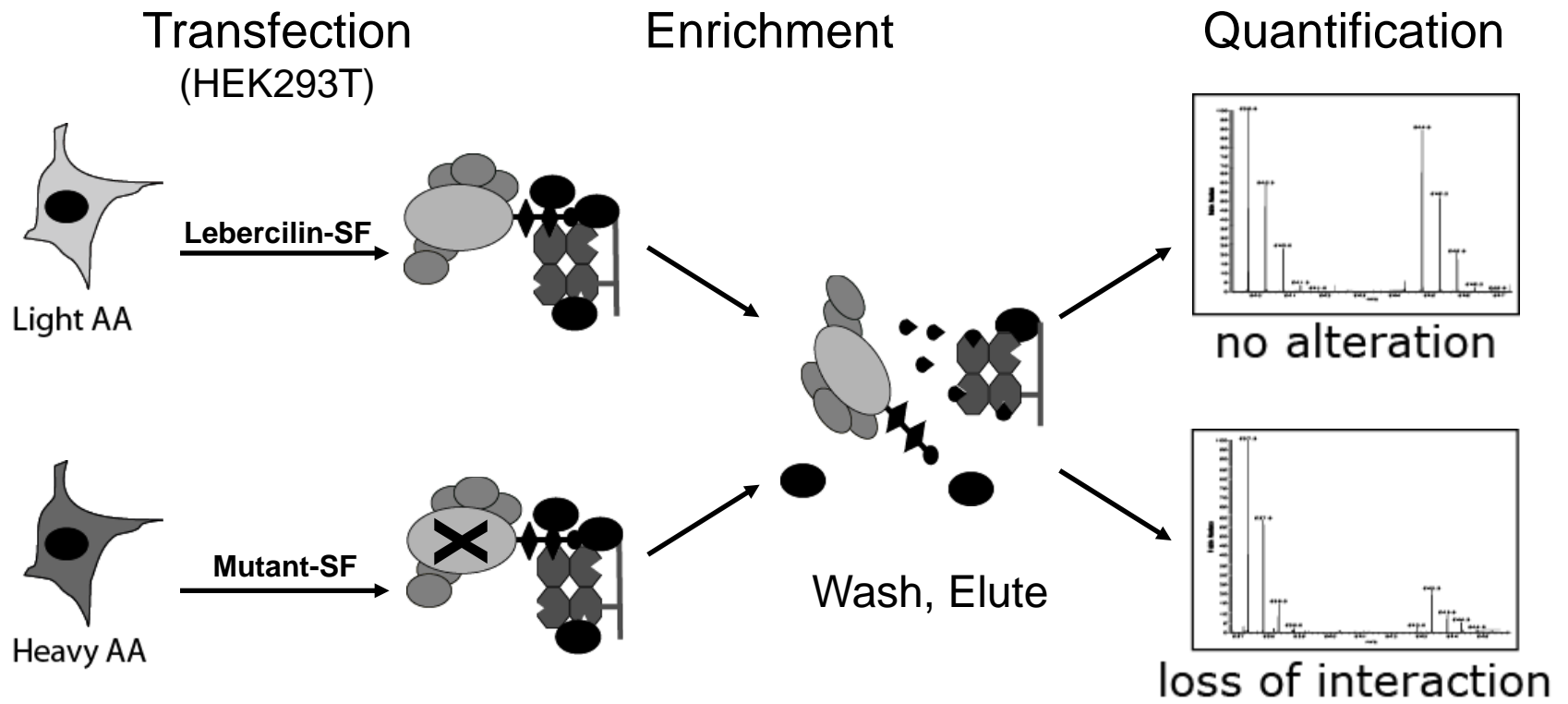
The lebercilin-IFT protein complex

Which effect do mutations have on the complex?

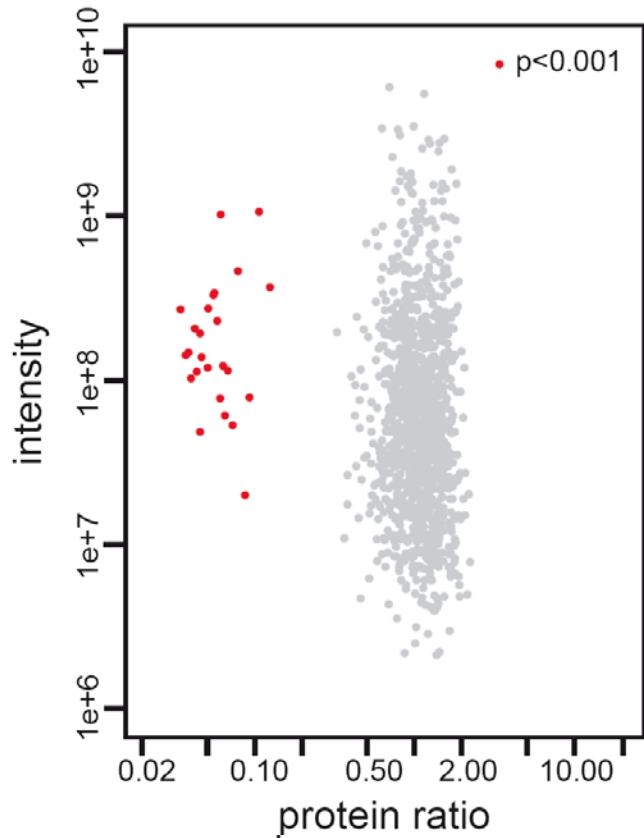# Protein complex alterations due to mutation

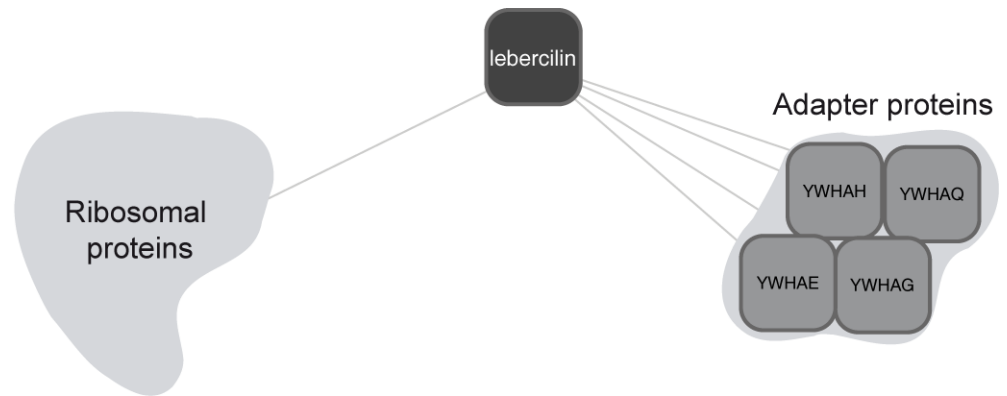# Protein complex comparison

Transfection (HEK293T) · Enrichment · Quantification · Light AA · Heavy AA · Lebercilin-SF · Mutant-SF · Wash, Elute · no alteration · loss of interaction

MaxQuant

Cox J, Mann M. Nat Biotechnol. 2008 Dec;26(12):1367-72.

Impact of mutations on the Lebercilin complex
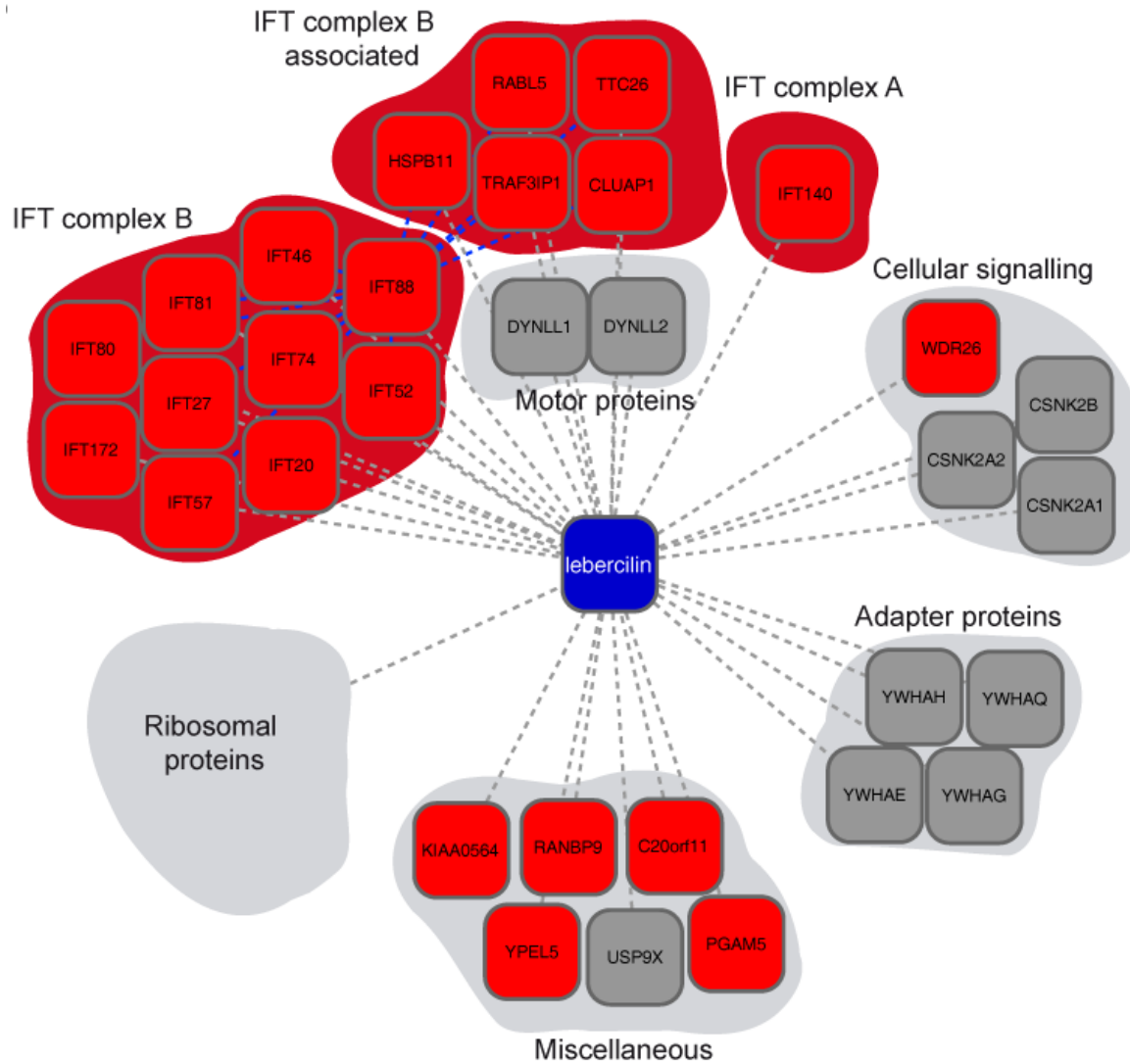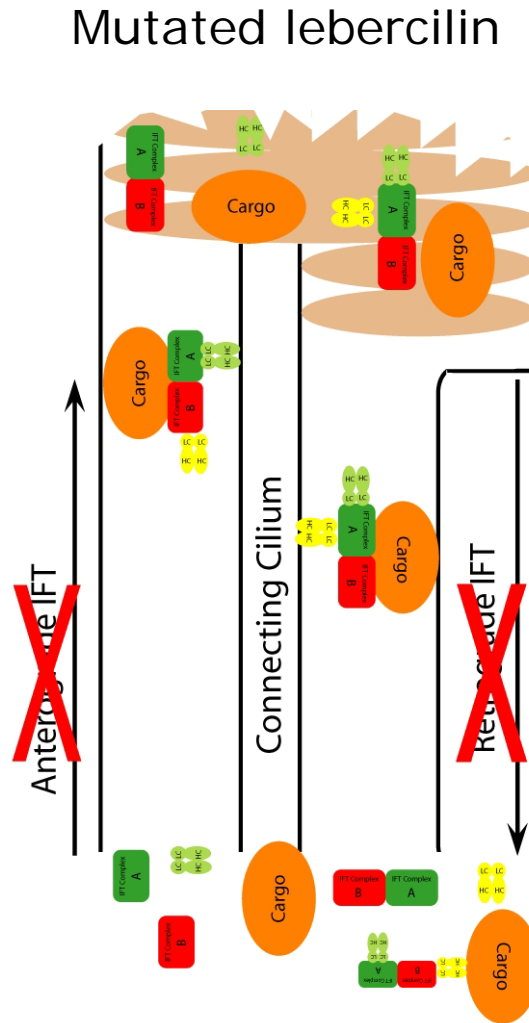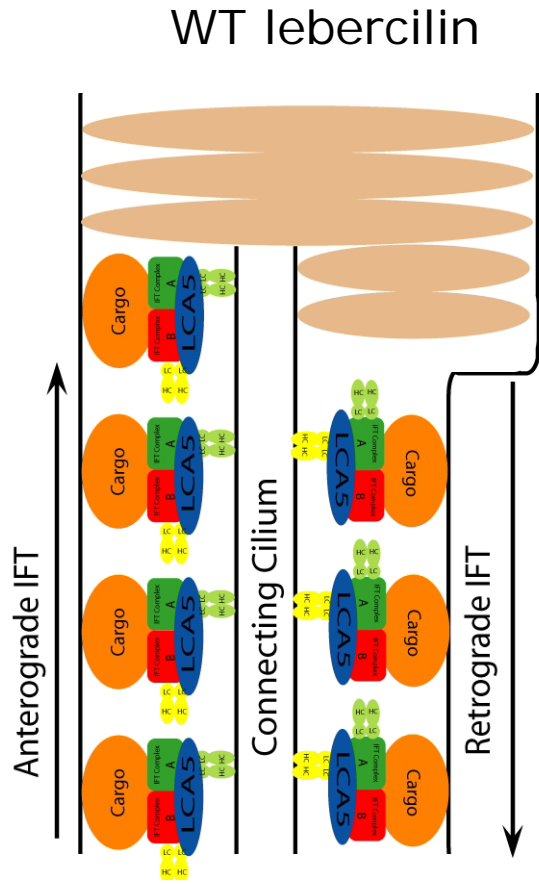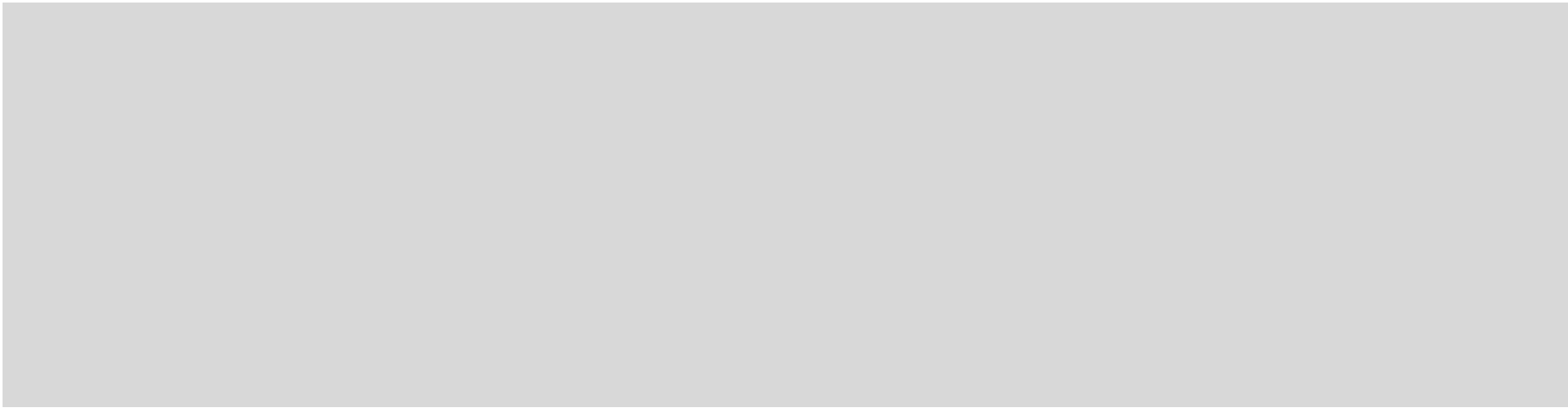
Loss of complex components due to mutation

Boldt et al.
Manuscript in preparation

Loss of Lebercilins's funtion → Impaired IFT → LCA
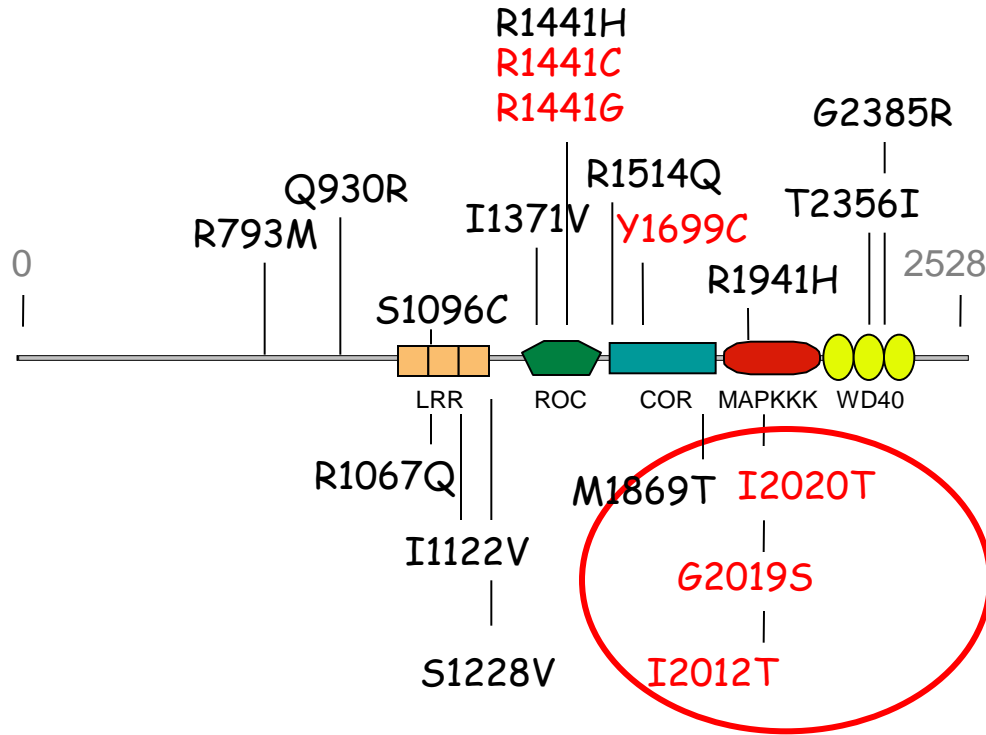
Norbert Kinkl   Andrea Meixner   Johannes Gloeckner

LRRK2 auto-phosphorylation and protein complex analysis
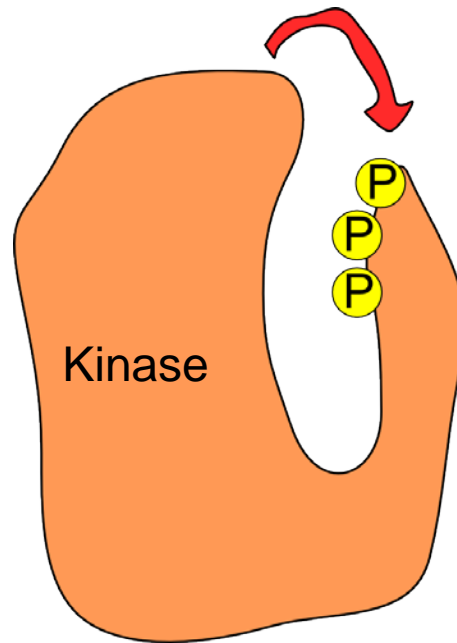
LRRK2 mutations associated with Parkinson's disease

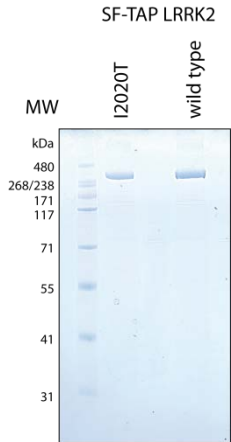(confirmed segregation, red)

kinase domain

For review see: Taylor et al., 2006

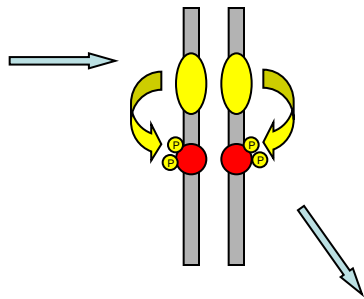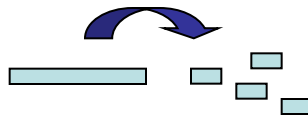# Analysis of LRRK2 autophosphorylation by MS

Analytical strategy

Purification of active LRRK2

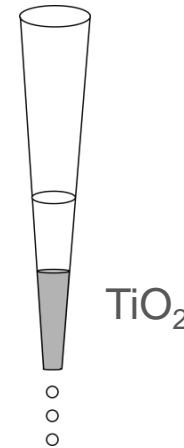**Phosphopeptide enrichment via binding to Titaniumdioxide**

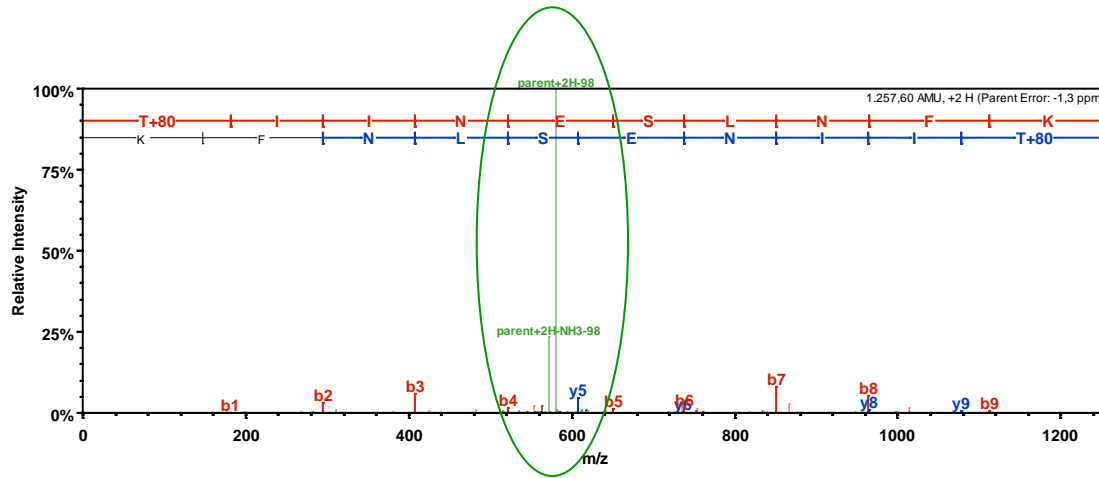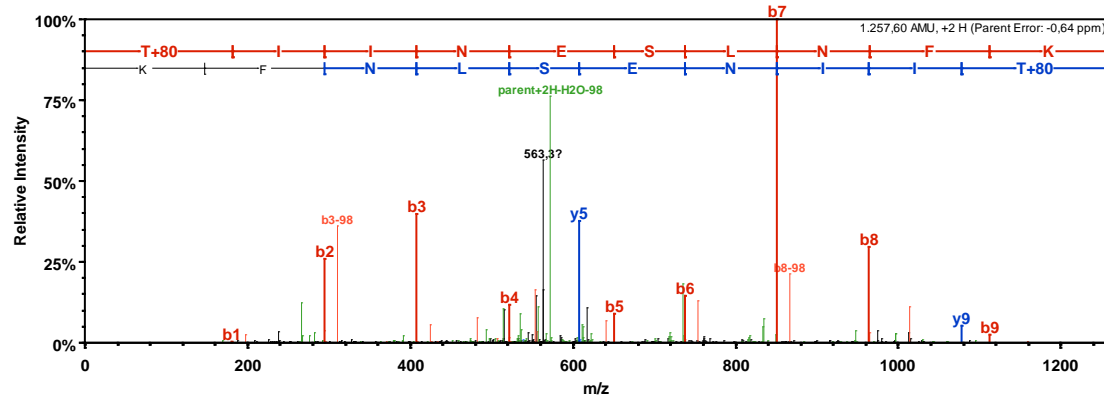Autokinase assay

Mass spectrometry

TiO$_2$

Cleavage
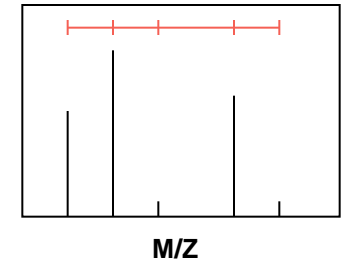
Gloeckner, Boldt et al., J. Proteome Res., 2010
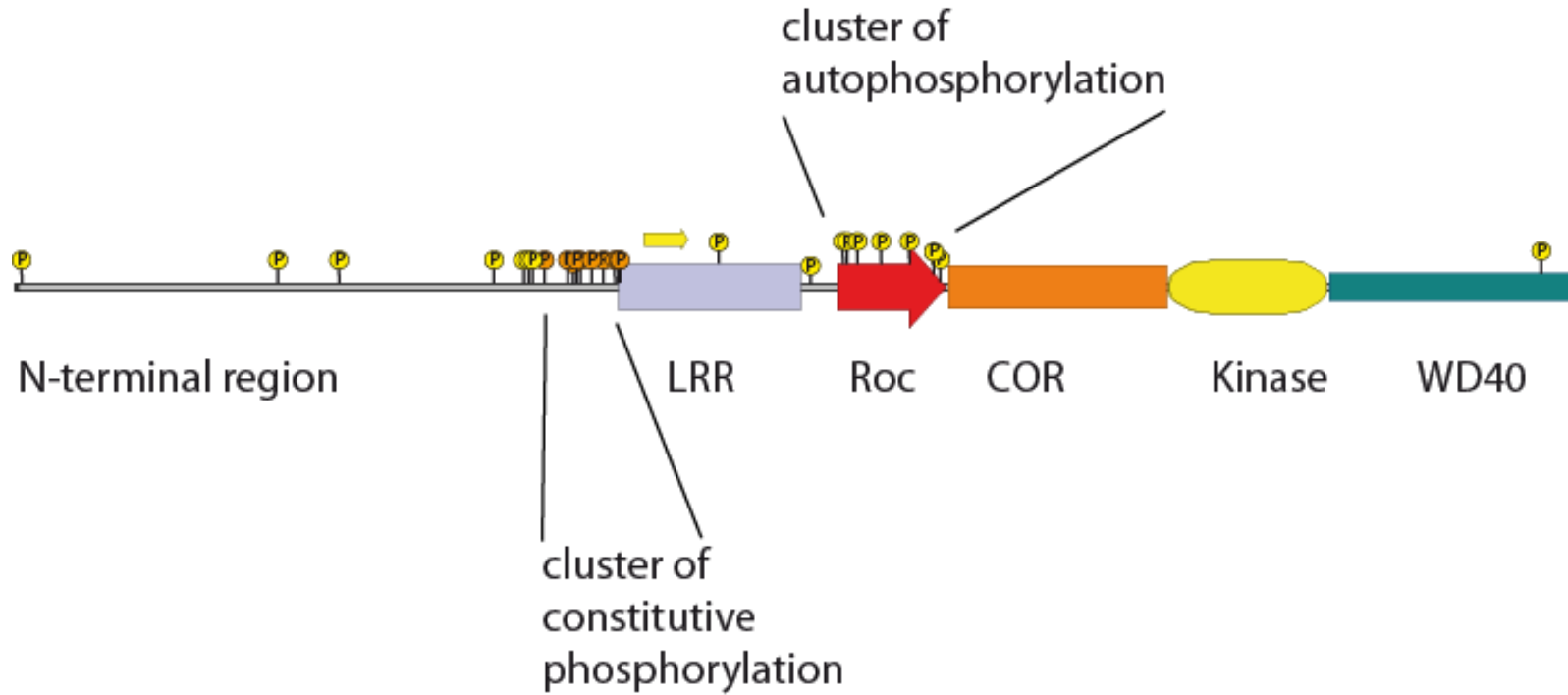
Multi-Stage-Activation for phospho-peptides

MSMS

Typical for phosphopetides:
high neutral loss peak, bad fragmentation
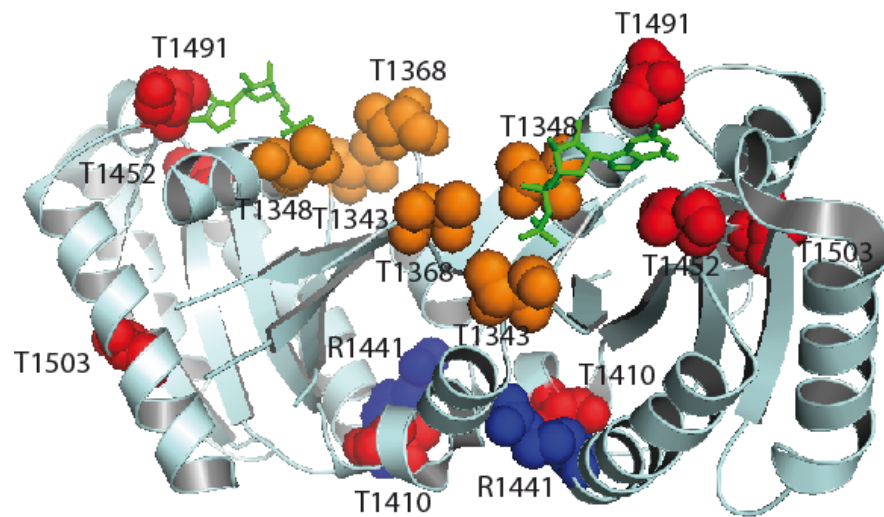→ Isolate the neutral loss fragment and
perform CID

MSA

MSA: better fragment pattern allows mapping of the phosphorylated residue!
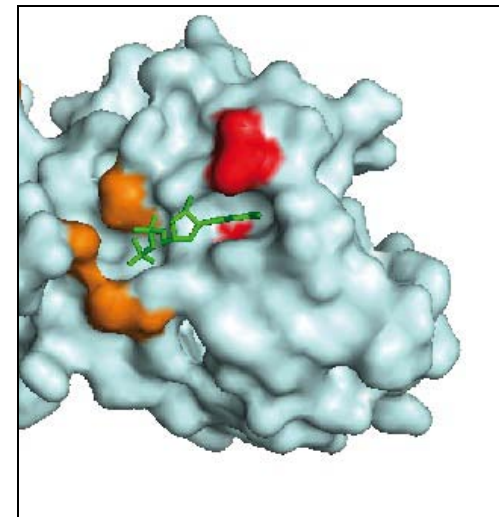
Distinct clusters of phosphorylation

Distinct clusters of phosphorylation at the GTP binding pocket of the Roc domain

GTP binding pocket

● residues mapped unambiguously

● alternative sites (multiphosphorylation possible)

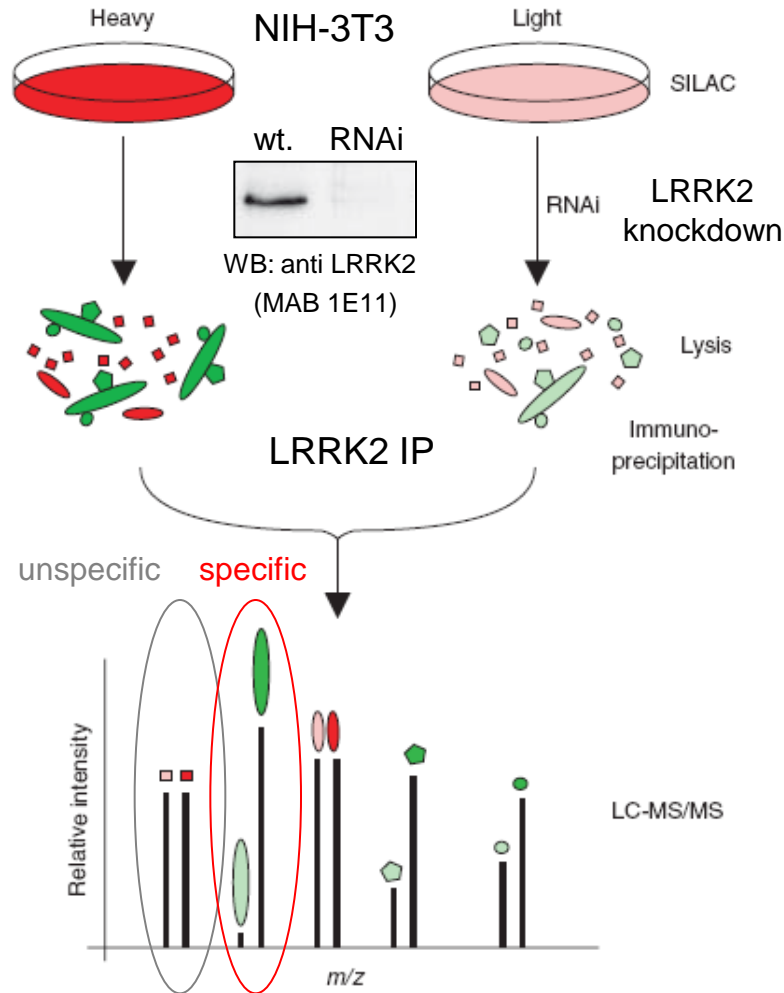● PD-associated mutation R1441C

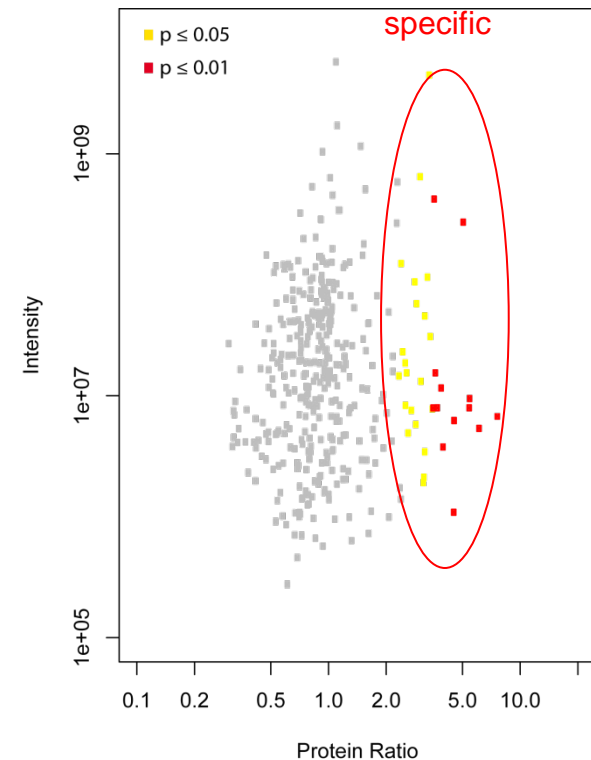# QUICK LRRK2 interaction screen

Quantitative Immunoprecipitation combined with knock-down

Quantitative immunoprecipitation combined with knockdown (QUICK)

Selbach, M., and Mann, M. (2006)
Nat Methods 3, 981-983
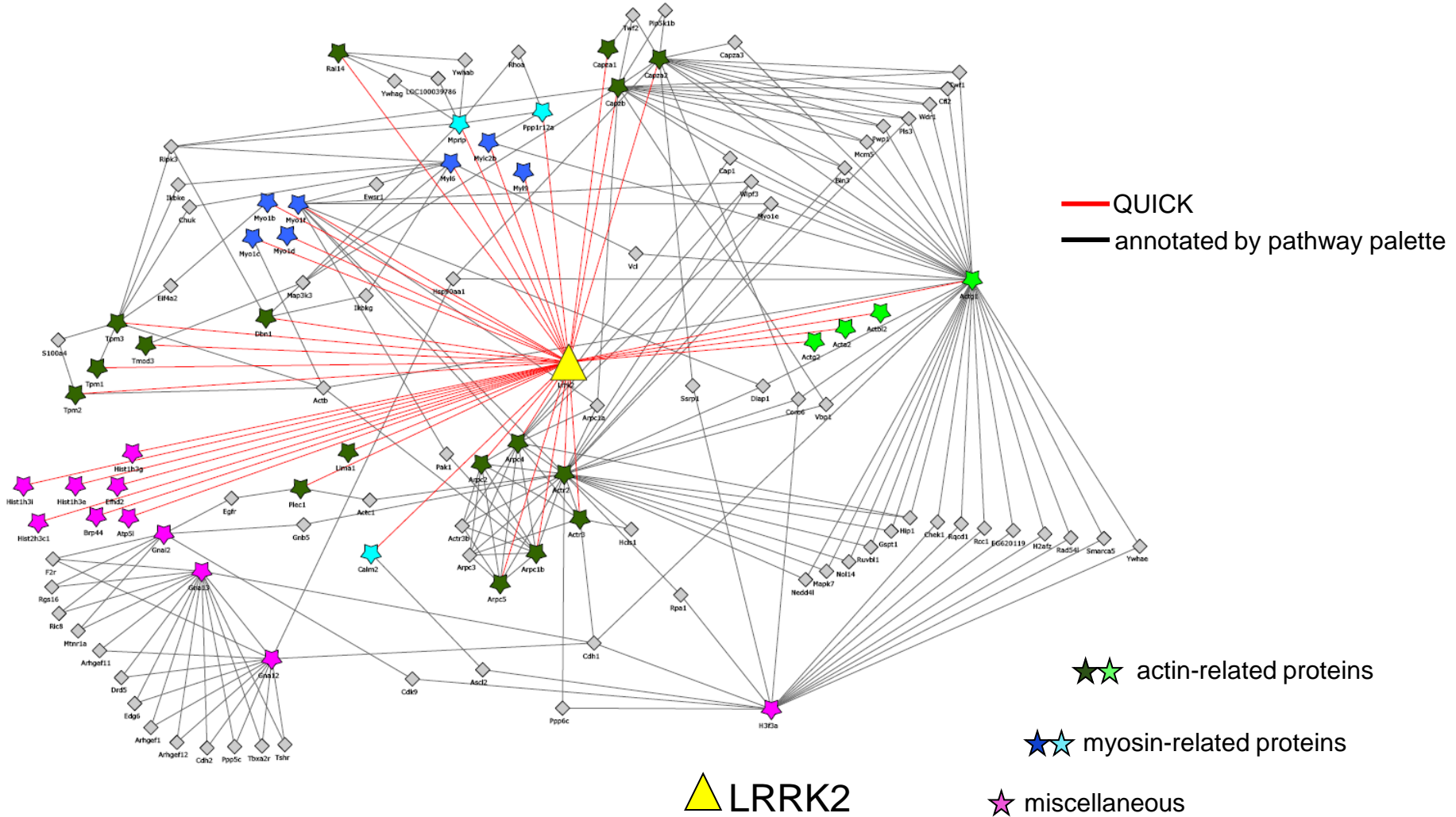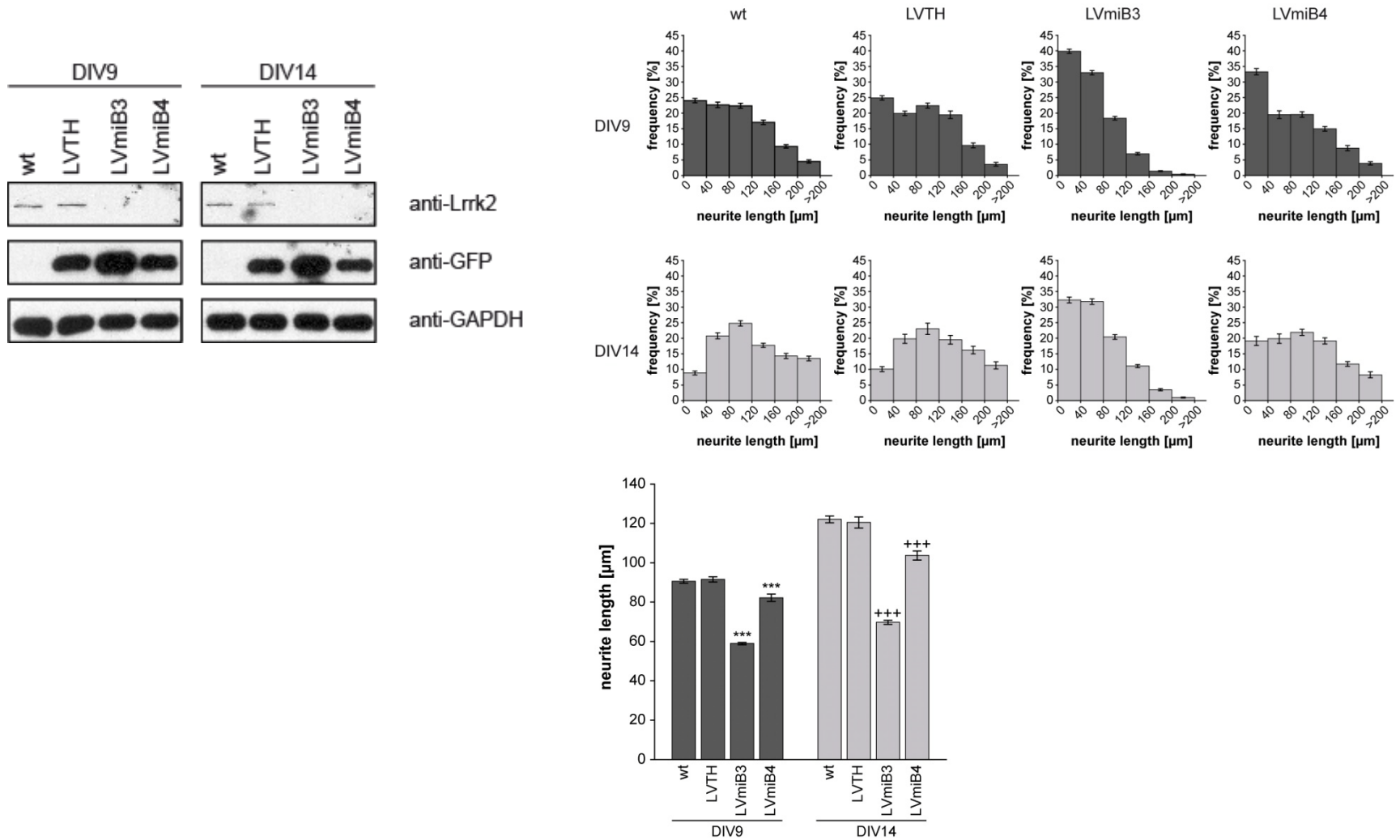
Ratios „heavy/light"

(Meixner, Boldt et al., MCP 2010)

LRRK2 interacts with proteins associated with the actin cytoskeleton

Collaboration with Jarrod A. Mato (Harvard Medical School)

(http://blaispathways.dfci.harvard.edu/palette.html)

Knock down of LRRK2 leads to impaired neurite outgrowth in primary VM cultures

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