# Toward the standardization of mitochondrial proteomics

*Luisa Pieroni*

*Proteomics and Metabonomics*

*IRCCS Fondazione S.Lucia-Rome*

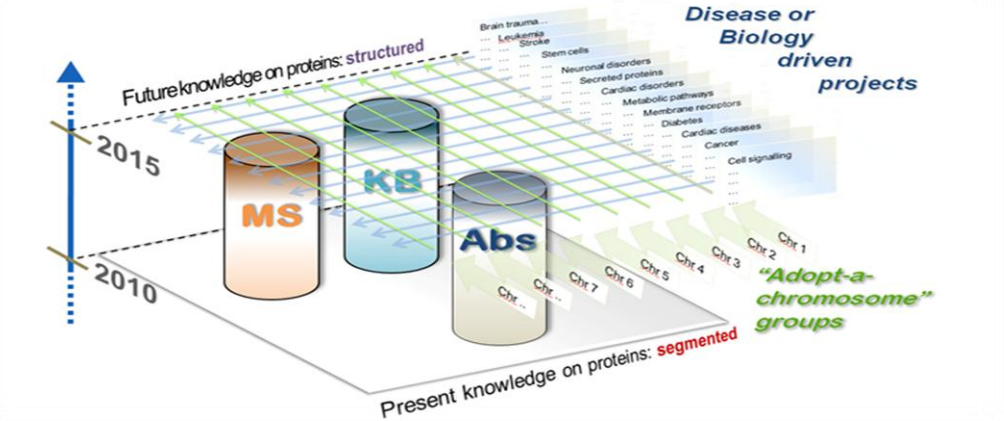
*Italy*

***MiP 2017/MitoEAGLE Conference,Hradec Králové***
***November 17, 2017***

# HUPO 2010

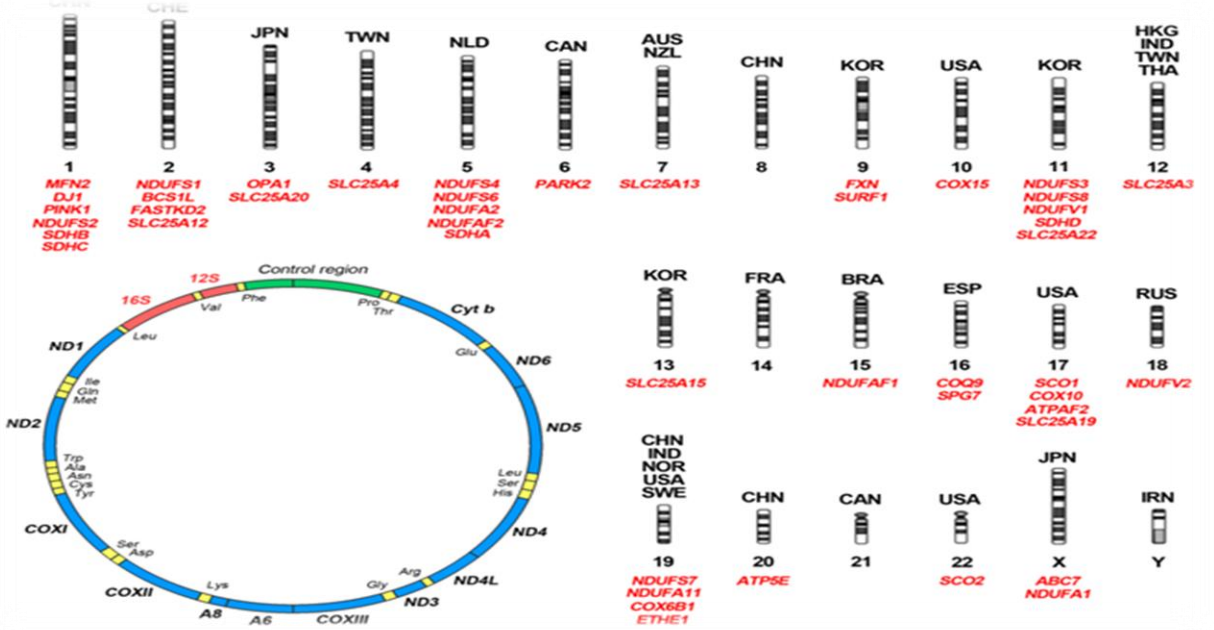
Human Proteome Project

Mapping of the entire human proteome using currently available and emerging techniques




**C-HPP**: Chromosome-Centric

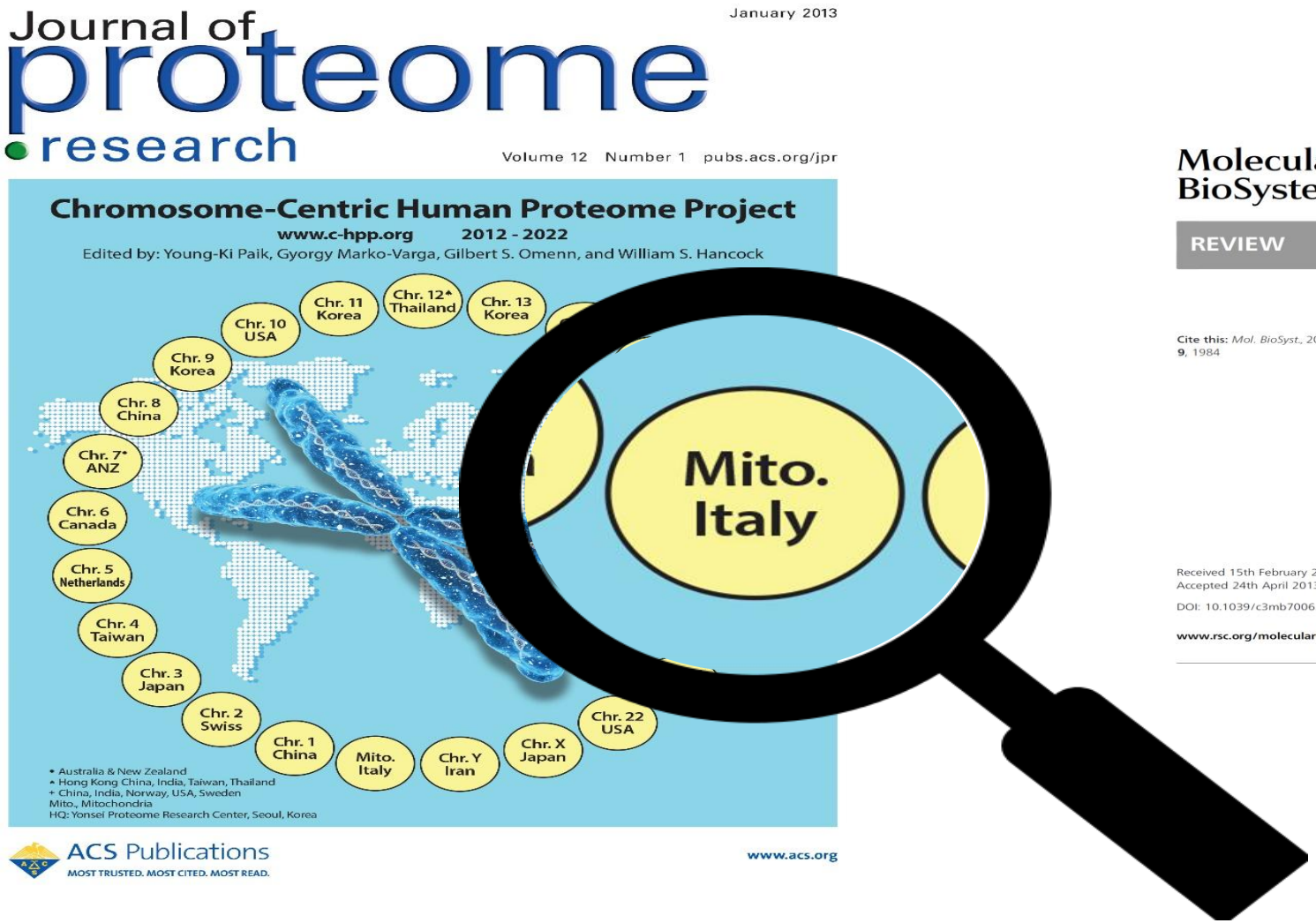
**B/D-HPP**: Biology/Disease




**HUPO 2012**

Human Proteome Project

# Italian initiative of the Human Proteome Project dedicated to mitochondria

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## The mitochondrial Italian Human Proteome Project initiative (mt-HPP)†

Andrea Urbani,*[ab] Michele De Canio,[bc] Ferdinando Palmieri,[d] Salvatore Sechi,[e] Luca Bini,[f] Massimo Castagnola,[g] Mauro Fasano,[h] Alessandra Modesti,[i] Paola Roncada,[cj] Anna Maria Timperio,[k] Luigi Bonizzi,[c] Maurizio Brunori,[l] Francesca Cutruzzolà,[l] Vito De Pinto,[m] Carmine Di Ilio,[no] Giorgio Federici,[p] Franco Folli,[q] Salvatore Foti,[r] Cecilia Gelfi,[st] Davide Lauro,[u] Antonio Lucacchini,[v] Fulvio Magni,[w] Irene Messana,[x] Pier Paolo Pandolfi,[y] Sergio Papa,[z] Piero Pucci,[aa] Paolo Sacchetta[ab] and the Italian mt-HPP Study Group-Italian Proteomics Association (www.itpa.it)

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www.rsc.org/molecularbiosystems

Mitochondria carry maternally inherited genetic material, called the mitochondrial genome (mtDNA), which can be defined as the 25th human chromosome. The chromosome-centric Human Proteome Project (c-HPP) has initially focused its activities addressing the characterization and quantification of the nuclear encoded proteins. Following the last International HUPO Congress in Boston (September 2012) it was clear that however small the mitochondrial chromosome is, it plays an important role in many biological and physiopathological functions. Mutations in the mtDNA have been shown to be associated with dozens of unexplained disorders and the information contained in the mtDNA should be of major relevance to the understanding of many human diseases. Within this paper we describe the Italian initiative of the Human Proteome Project dedicated to mitochondria as part of both programs:

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# Mitochondrial proteomics investigation of a cellular model of impaired dopamine homeostasis, an early step in Parkinson's disease pathogenesis†

Cite this: *Mol. BioSyst.*, 2014, 10, 1332

Tiziana Alberio,[ab] Heather Bondi,[ab] Flavia Colom
Luisa Pieroni,[de] Andrea Urbani*[de] and Mauro Fas

Molecular BioSystems

PAPER

Cite this: *Mol. BioSyst.*, 2015, 11, 1696

# Glucagon-like peptide 1 protects INS-1E mitochondria against palmitate-mediated beta-cell dysfunction: a proteomic study†

Federica Ciregia,[ab] Laura Giusti,[a] Maurizio Ronci,[bcd] Marco Bugliani,[e] Isabella Piga,[a] Luisa Pieroni,[b] Claudia Rossi,[c] Piero Marchetti,[e] Andrea Urbani[bf] and Antonio Lucacchini*[a]

OPEN

Citation: Transl Psychiatry (2016) 6, e904; doi:10.1038/tp.2016.184
www.nature.com/tp

ORIGINAL ARTICLE

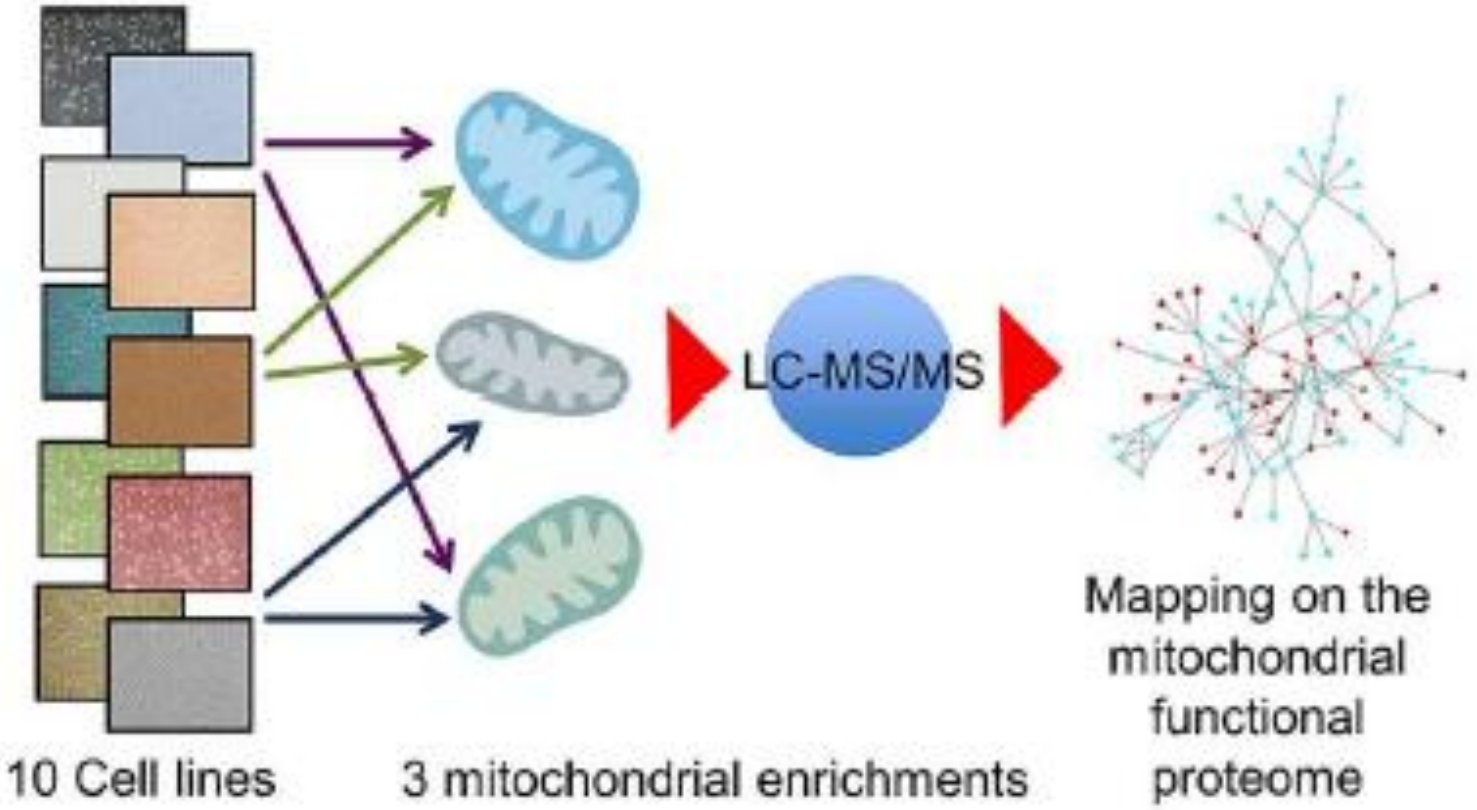
# Bottom-up proteomics suggests an association between differential expression of mitochondrial proteins and chronic fatigue syndrome

F Ciregia[1], L Kollipara[2], L Giusti[1], RP Zahedi[2], C Giacomelli[3], MR Mazzoni[1], G Giannaccini[1], P Scarpellini[4], A Urbani[5,6], A Sickmann[2,7,8], A Lucacchini[1] and L Bazzichi[3]

# We needed a mt-enrichment procedure standardization

- To address the need of our proteomics community to analyse complete mt-proteomes ( including  Matrix, IMM, OMM and IMS proteins)
- To perform reliable comparison  of data from different laboratory

# The study design



# The study design

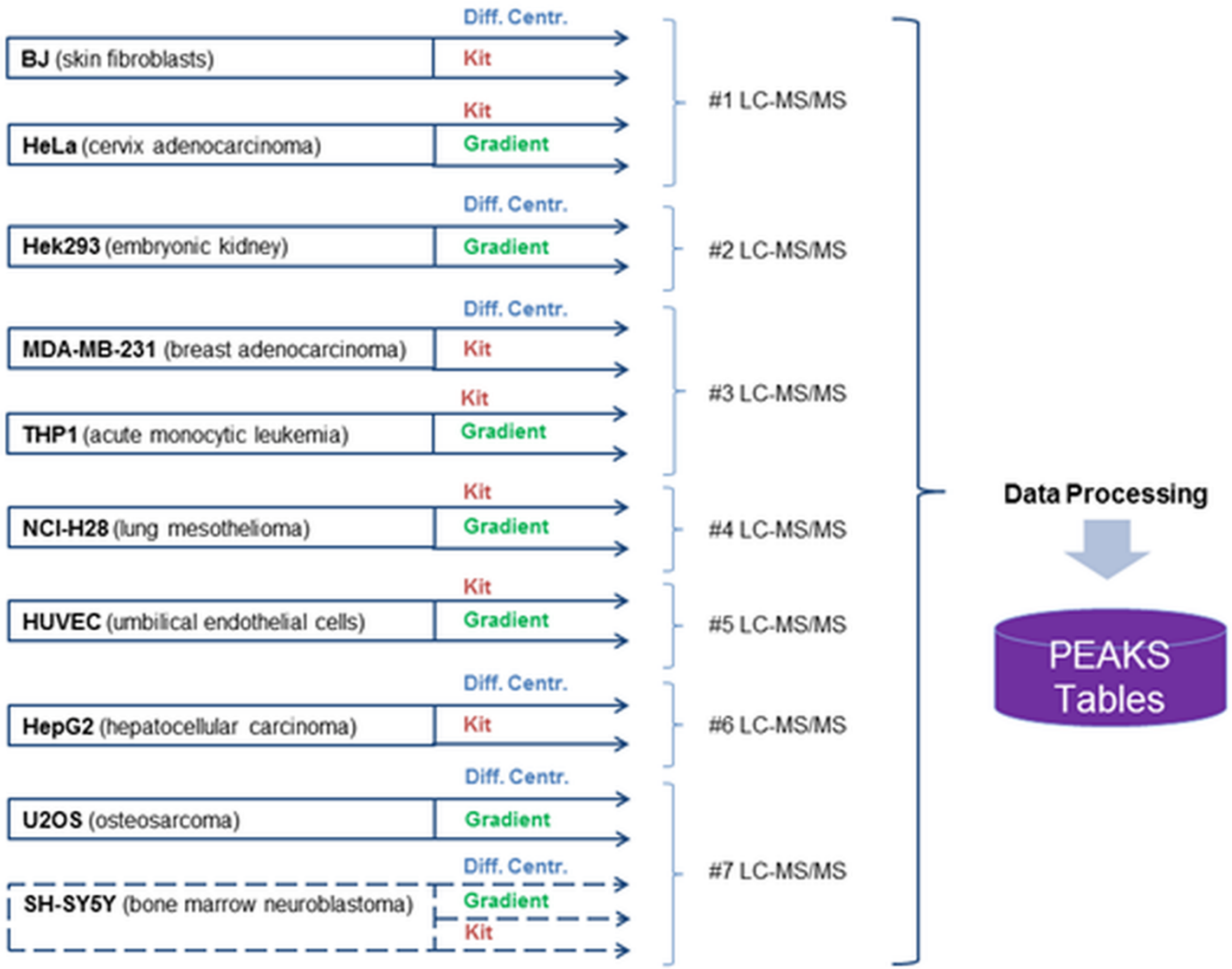
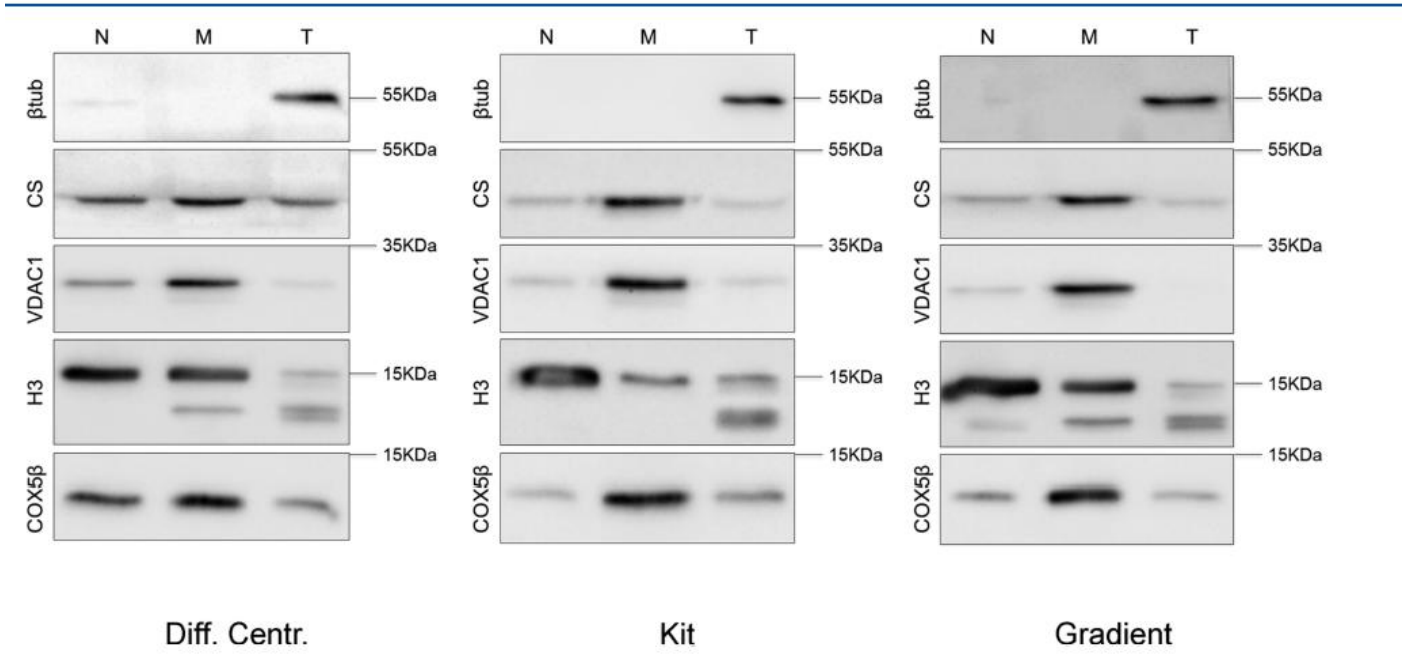


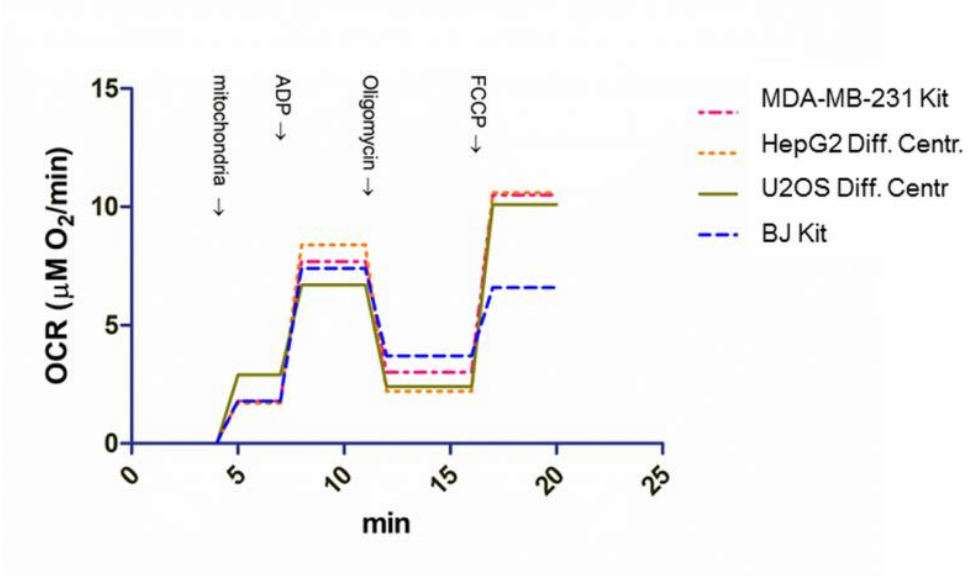

**Table 1. MS and Chromatographic Platforms**

| no. | LC system | column | mass spectrometer | cells |
|---|---|---|---|---|
| 1 | nanoAcquity M Class | Waters HSST3 $C_{18}$ 75 $\mu$m × 15 cm | Synapt G2 Si | BJ, HeLa |
| 2 | Dionex UltiMate 3000 | Thermo Easy-Spray PepMap RSLC $C_{18}$ 75 $\mu$m × 50 cm | Orbitrap Fusion | Hek293 |
| 3 | Dionex UltiMate 3000 | MS Wil GmbH $C_{18}$ 75 $\mu$m × 20 cm | LTQ-Orbitrap- Velos | MDA-MB231, THP1 |
| 4 | nanoEASY II | Nanoseparations $C_{18}$ 100 $\mu$m × 20 cm | LTQ-Orbitrap-XL | NCI-H28 |
| 5 | Dionex UltiMate 3000 | Thermo Easy-Spray PepMap RSLC $C_{18}$ 75 $\mu$m × 50 cm | Bruker Impact HD | HUVEC |
| 6 | Ekspert nanoLC 400 | Thermo Acclaim PepMap 100 75 $\mu$m × 25 cm | TripleTOF 5600+ | HepG2 |
| 7 | nanoEASY II | Thermo Acclaim PepMap 100 75 $\mu$m × 25 cm | Bruker maXis HD | SH-SY5Y, U2OS |

# Mt-enrichment yield and purity check

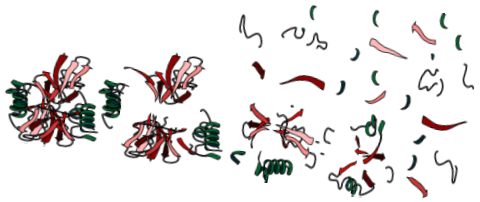


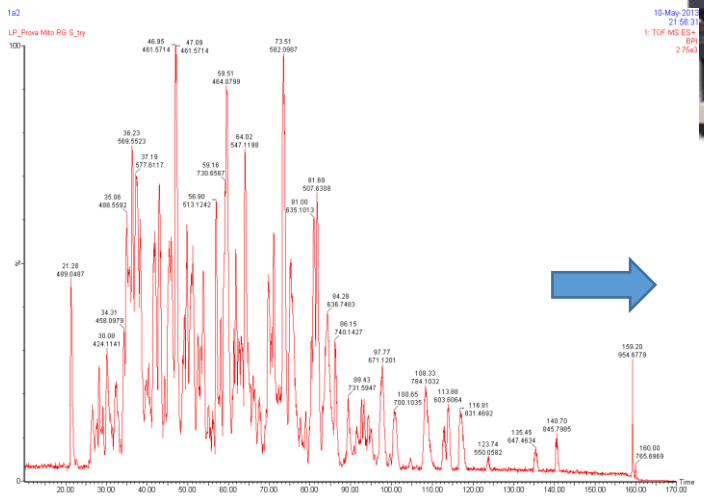

| Cell line/isolation protocol | nmol/min mg prot before lysis | nmol/min mg prot after lysis | % ruptured |
|---|---|---|---|
| HepG2 Diff. Centr. | 18.2 | 253.7 | 7.2 |
| MDA-MB-231 Kit | 17.2 | 137.1 | 12.3 |
| HeLa Kit | 30 | 180 | 16.7 |
| SH-SY5Y Diff. Centr. | 17.3 | 219 | 7.9 |
| BJ Kit | 26.8 | 237 | 11.3 |



# Label free shotgun “bottom up” proteomics

**Proteins Tryptic digestion**





**LC-MS**

**Fusion OrbiTrap**


**maXis HD QTOF**

BRUKER


**TripleTOF™5600**

AB SCIEX

**Synapt G2si QTOF**

Waters

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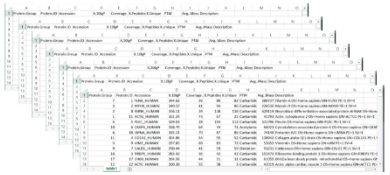
# Dataset processing




Start #1

PEAKS Tables

Read triplicates

Remove single observations

Compute -10LogP/MW

Normalize scores

Remove less representative proteins

Summary tables

Start #2

Read tables

Export UniProtKB IDs

MitoCarta IMPI

Map mitochondrial

Count IDs

Count Table

Count mitoIDs

Count mitoTable

End

End

Lists

Start #3

Read lists

Map subcell. locations

Location table

Count subcell. locations

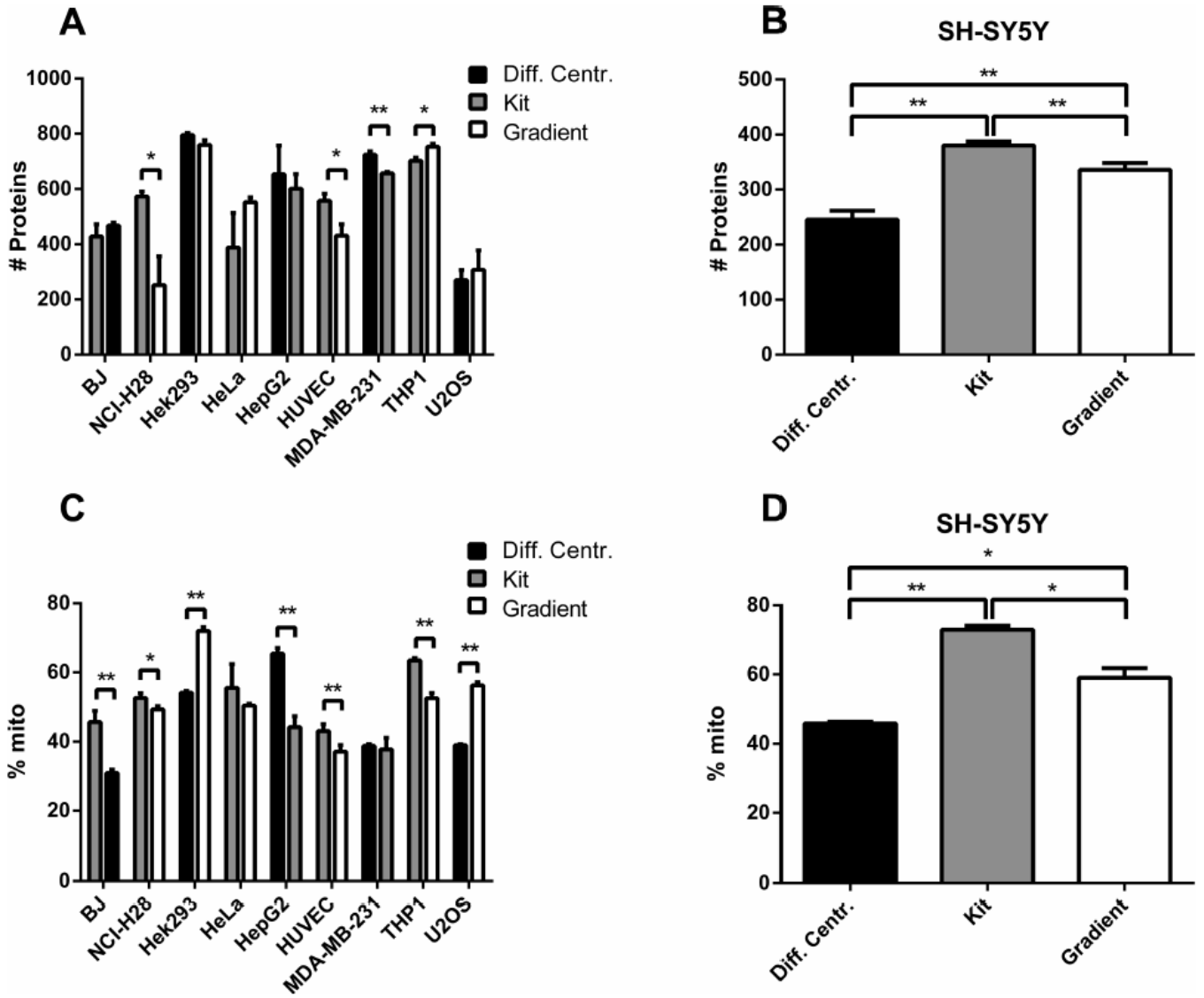
Count submitochondrial locations

Enrichm. by meth.

Fisher's test

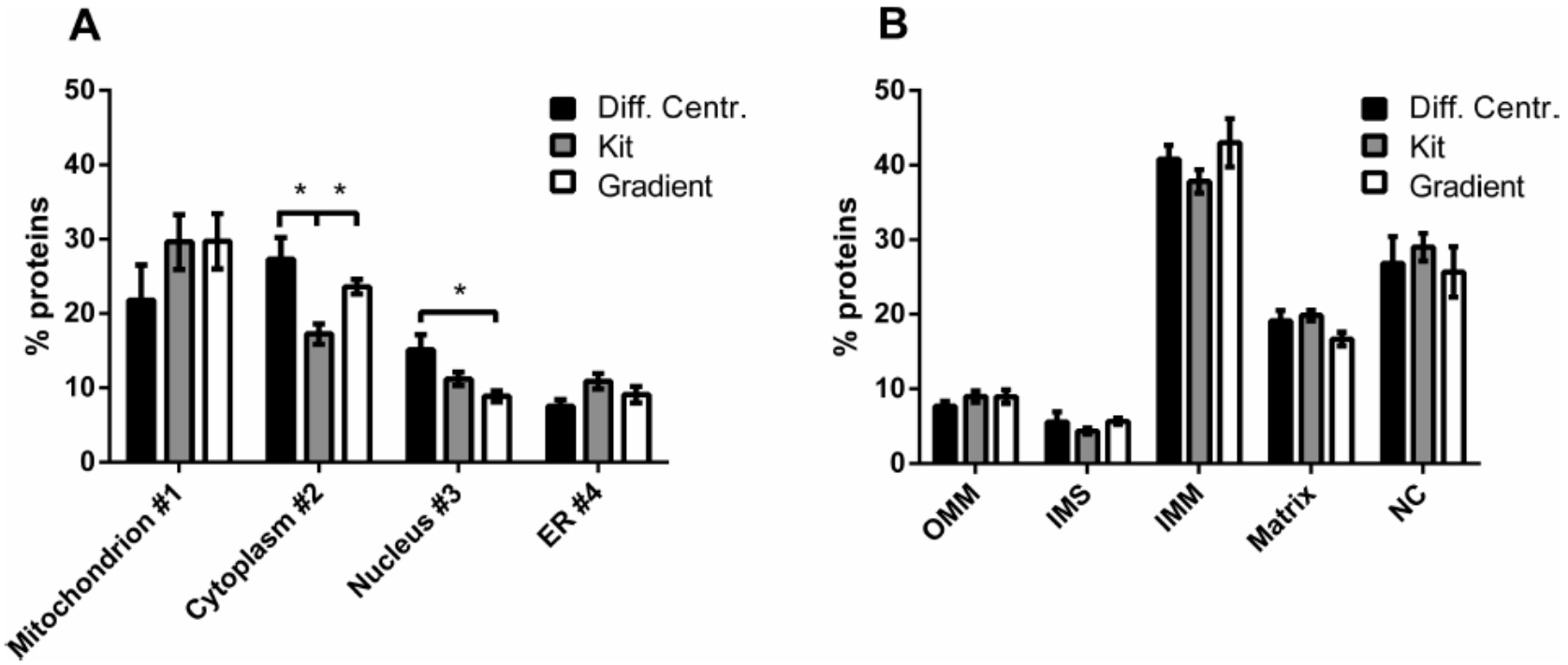
End

# Total and mt proteins [1] per cell line




[1] MitoCarta & IMPI

# Top subcellular locations and submitochondrial enrichment (2)




(2) Primary location in UniProt annotation

# Mapping on the functional mt-proteome

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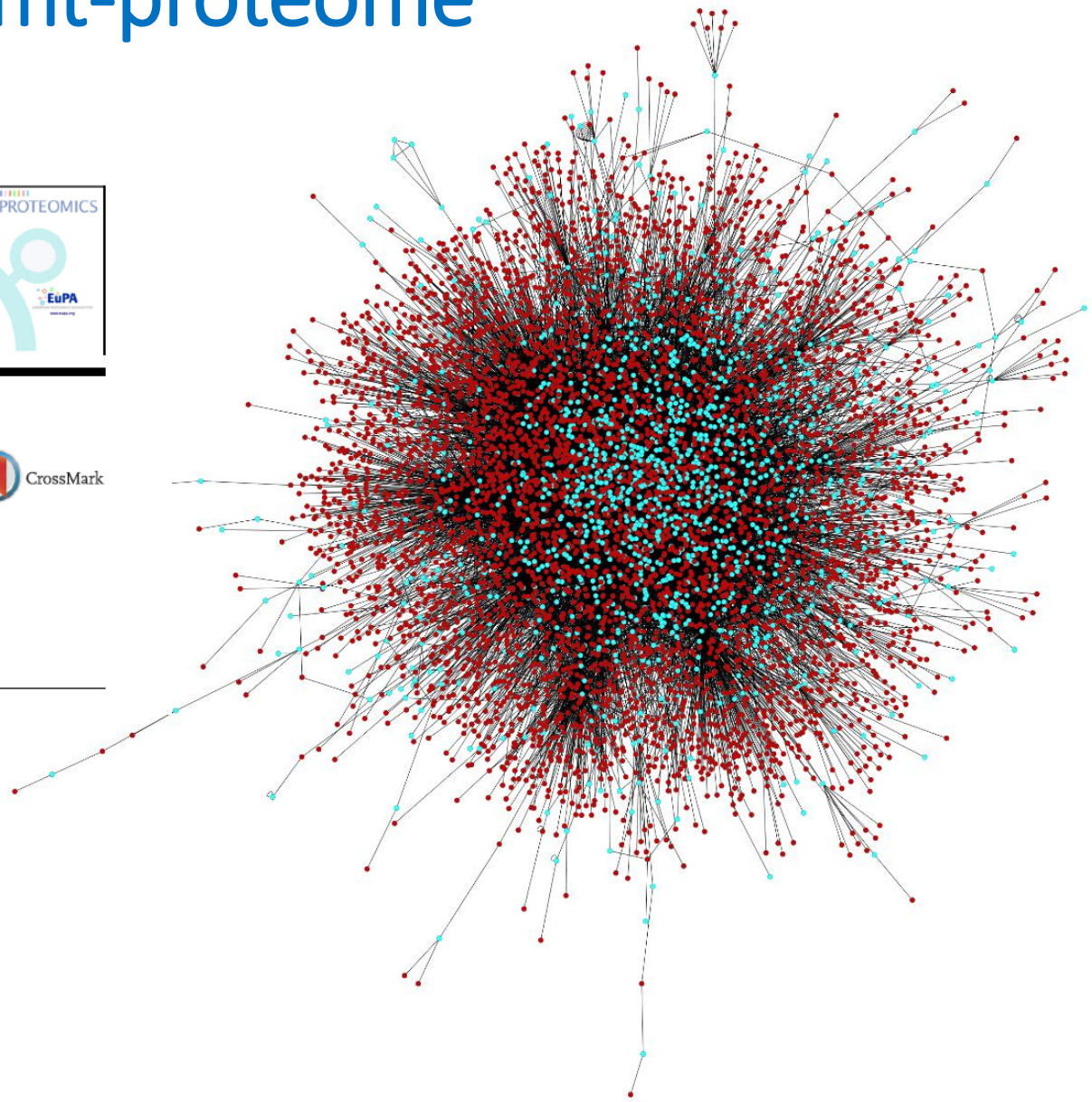
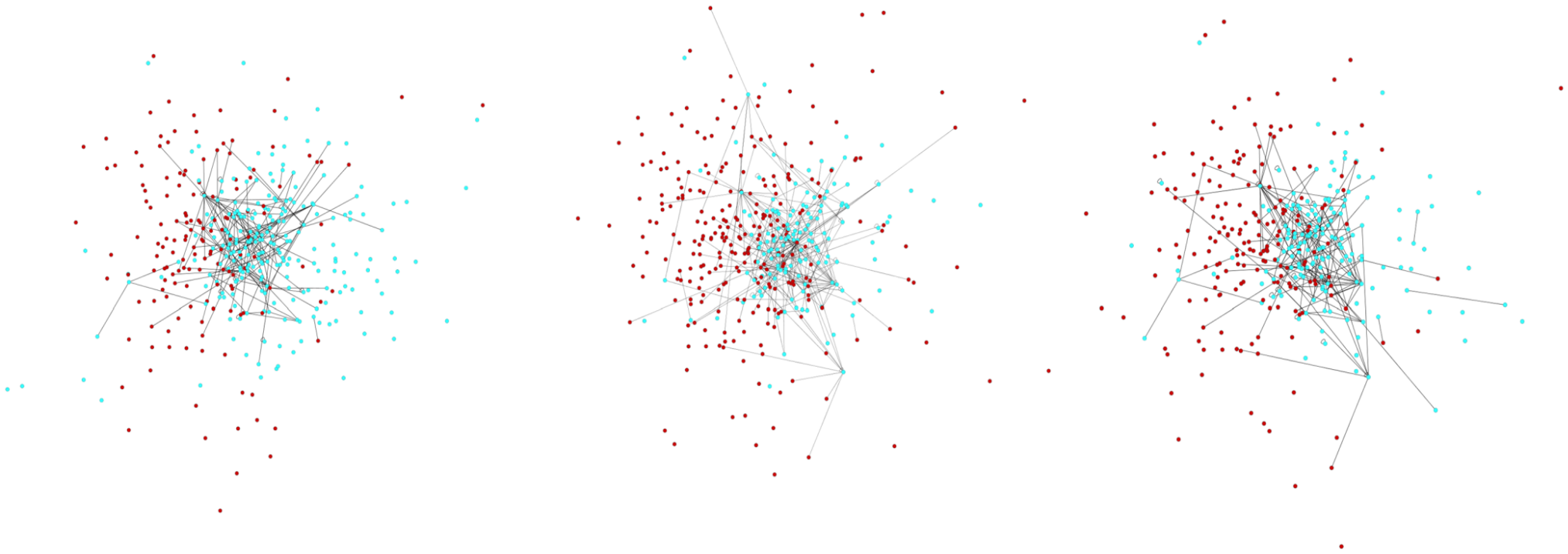
## Towards a functional definition of the mitochondrial human proteome

Mauro Fasano[a,*], Tiziana Alberio[a], Mohan Babu[b], Emma Lundberg[c], Andrea Urbani[d,e]

[a] Division of Biomedical Research, Department of Theoretical and Applied Sciences, and Center of Neuroscience, University of Insubria, I-21052 Busto Arsizio, Italy
[b] Department of Biochemistry, Research and Innovation Centre, University of Regina, Regina, SK, Canada
[c] Science for Life Laboratory, KTH—Royal Institute of Technology, Stockholm, Sweden
[d] Santa Lucia IRCCS Foundation, I-00143 Rome, Italy
[e] Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata", I-00133 Rome, Italy



# Mapping on the functional mt-proteome



Diff. Centr.

Kit

Gradient

# Mapping on the functional mt-proteome

| cell line | method | % mapped | % mitochondrial | % clustered | % mitoclustered |
|---|---|---|---|---|---|
| BJ | kit | 77 | 36 | 22 | 53 |
| | diff. centr. | 84 | 21 | 30 | 43 |
| NCI-H28 | kit | 84 | 46 | 29 | 56 |
| | gradient | 88 | 38 | 28 | 65 |
| Hek293 | diff. centr. | 87 | 44 | 41 | 58 |
| | gradient | 84 | 63 | 38 | 70 |
| HeLa | kit | 82 | 46 | 29 | 70 |
| | gradient | 86 | 41 | 34 | 56 |
| HepG2 | diff. centr | 81 | 57 | 32 | 69 |
| | kit | 78 | 38 | 25 | 65 |
| HUVEC | kit | 79 | 38 | 33 | 56 |
| | gradient | 82 | 30 | 37 | 49 |
| MDA-MB-231 | diff. centr. | 79 | 32 | 38 | 49 |
| | kit | 76 | 36 | 36 | 53 |
| THP1 | kit | 76 | 63 | 36 | 72 |
| | gradient | 78 | 51 | 38 | 62 |
| U2OS | diff. centr. | 83 | 30 | 29 | 53 |
| | gradient | 87 | 45 | 30 | 66 |
| SH-SY5Y | diff. centr. | 89 | 33 | 28 | 54 |
| | kit | 90 | 60 | 26 | 66 |
| | gradient | 89 | 47 | 32 | 64 |

# We do not draw unique consclusions but:

- We compared suitable procedures to achieve effective MS analysis of mitochondrial proteome, defining guidelines for different experimental designs, irrespectively of MS technological platforms available

- Standardization action will contribute to mt-HPP and B/D-HPP

- We added several mitochondrial datasets to specific proteomics KB (e.g. Proteome Xchange) as reference for future proteomics studies

- We integrated proteomic results from the functional point of view

Journal of proteome research

Article

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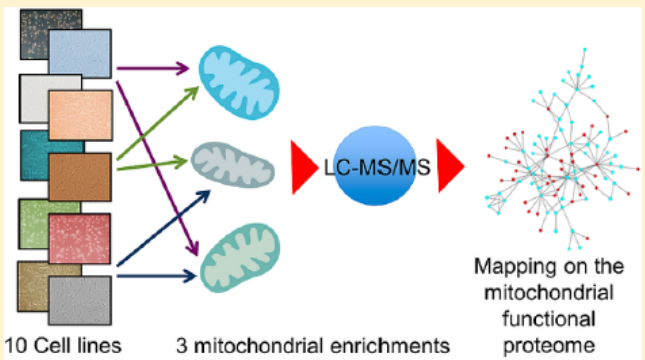
# Toward the Standardization of Mitochondrial Proteomics: The Italian Mitochondrial Human Proteome Project Initiative

Tiziana Alberio,[†,Φ] Luisa Pieroni,[‡,Φ] Maurizio Ronci,[‡,§,Φ] Cristina Banfi,[⊥] Italia Bongarzone,[¶] Patrizia Bottoni,[#] Maura Brioschi,[⊥] Marianna Caterino,[∥,□] Clizia Chinello,[●] Antonella Cormio,[△] Flora Cozzolino,[□,◒] Vincenzo Cunsolo,[■] Simona Fontana,[▼] Barbara Garavaglia,[○] Laura Giusti,[⌀] Viviana Greco,[‡] Antonio Lucacchini,[⌀] Elisa Maffioli,[⬢] Fulvio Magni,[●] Francesca Monteleone,[▼] Maria Monti,[□,◒] Valentina Monti,[○] Clara Musicco,[◪] Giuseppe Petrosillo,[◪] Vito Porcelli,[△] Rosaria Saletti,[■] Roberto Scatena,[#] Alessio Soggiu,[⬢] Gabriella Tedeschi,[⬢,◭] Mara Zilocchi,[†] Paola Roncada,[⊙] Andrea Urbani,[‡,#] and Mauro Fasano*[,†]

**ABSTRACT:** The Mitochondrial Human Proteome Project aims at understanding the function of the mitochondrial proteome and its crosstalk with the proteome of other organelles. Being able to choose a suitable and validated enrichment protocol of functional mitochondria, based on the specific needs of the downstream proteomics analysis, would greatly help the researchers in the field. Mitochondrial fractions from ten model cell lines were prepared using three enrichment protocols and analyzed on seven different LC−MS/MS platforms. All data were processed using neXtProt as reference database. The data are available for the Human Proteome Project purposes through the ProteomeXchange Consortium with the identifier PXD007053. The processed data sets were analyzed using a suite of R routines to perform a statistical analysis and to retrieve subcellular and submitochondrial localizations. Although the overall

*Continued...*

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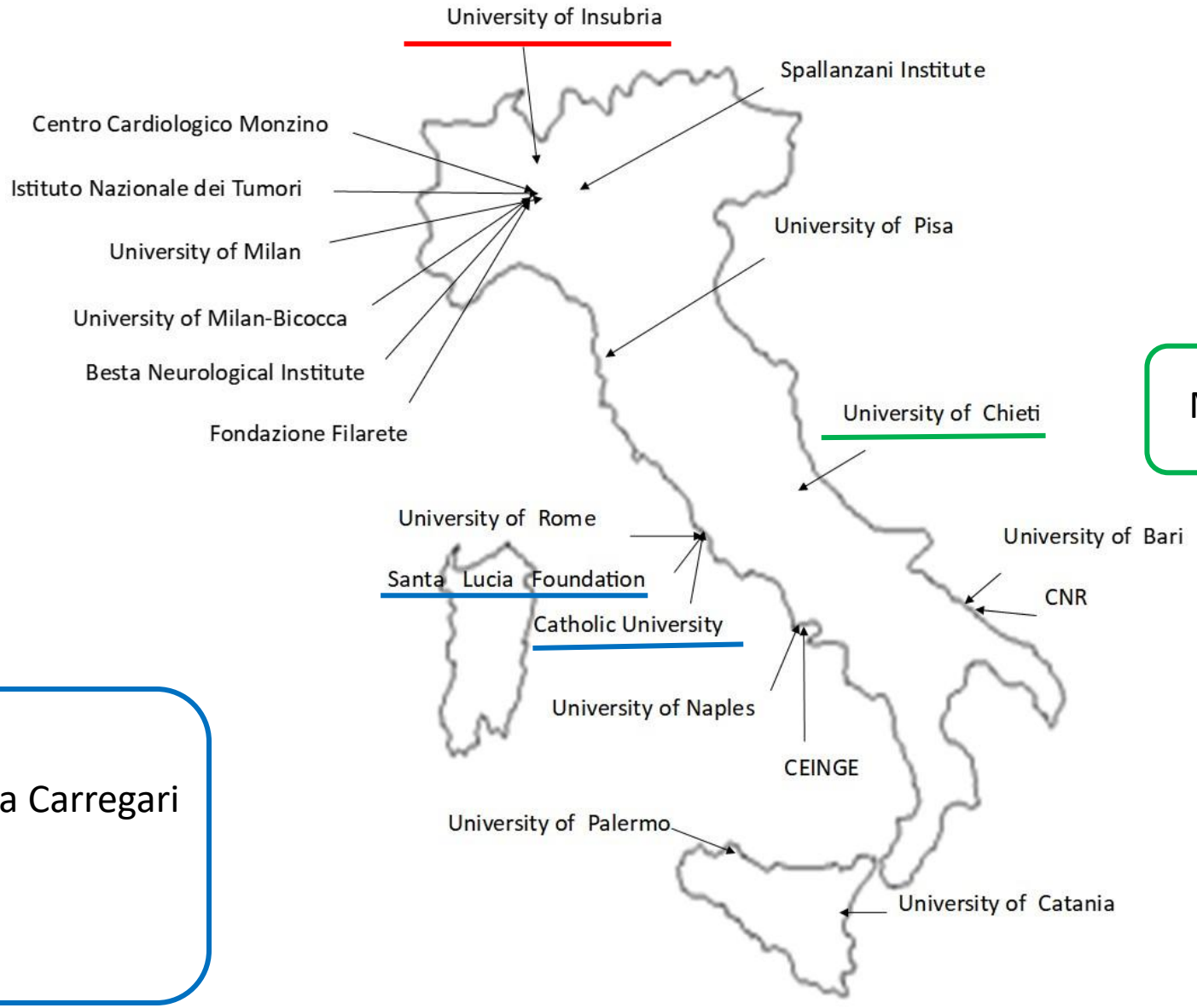


Tiziana Alberio
Mauro Fasano

Maurizio Ronci

Viviana Greco
Victor Corassolla Carregari
Federica Marini

Andrea Urbani