Proteomics of the radiosensitization effect of functionalized AuNPs with protons

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http://research.uv.es/membrana/

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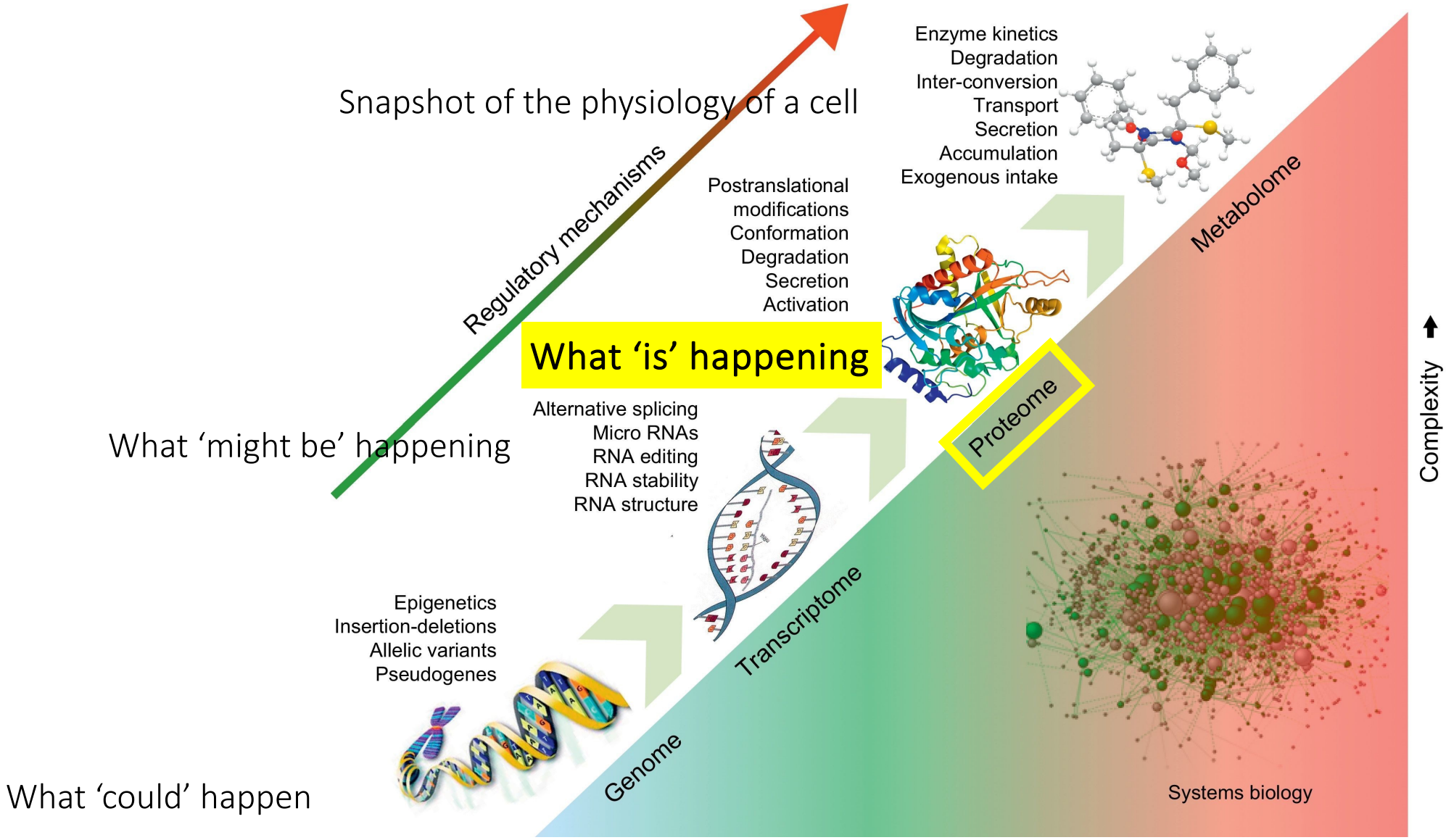
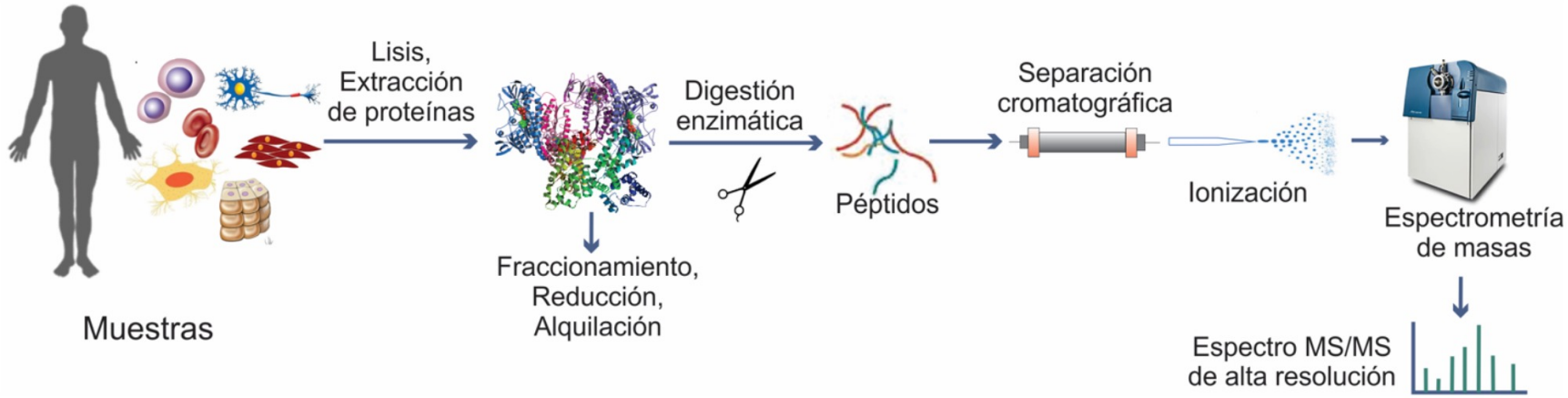
How Do We Study a Biological System? – An Omics Perspective

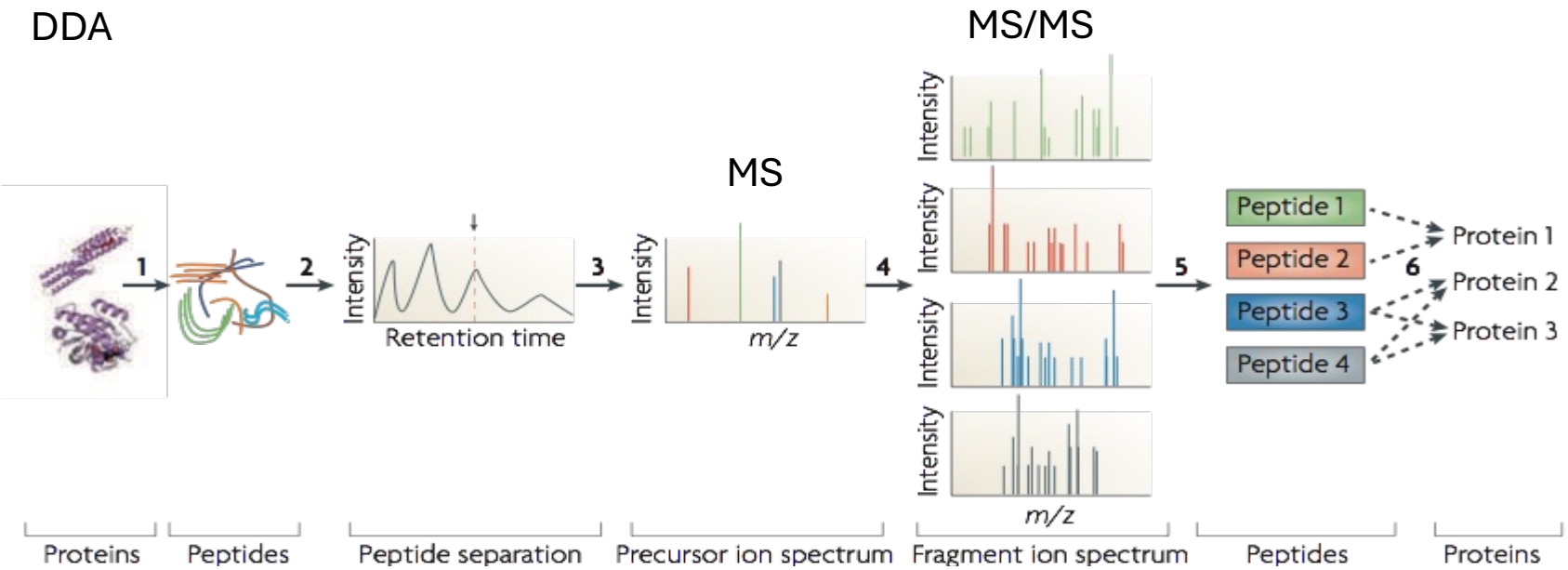


https://doi.org/10.1016/j.rec.2013.04.009

Understanding life at the level of biology will require the study of all of the proteins expressed from a genome

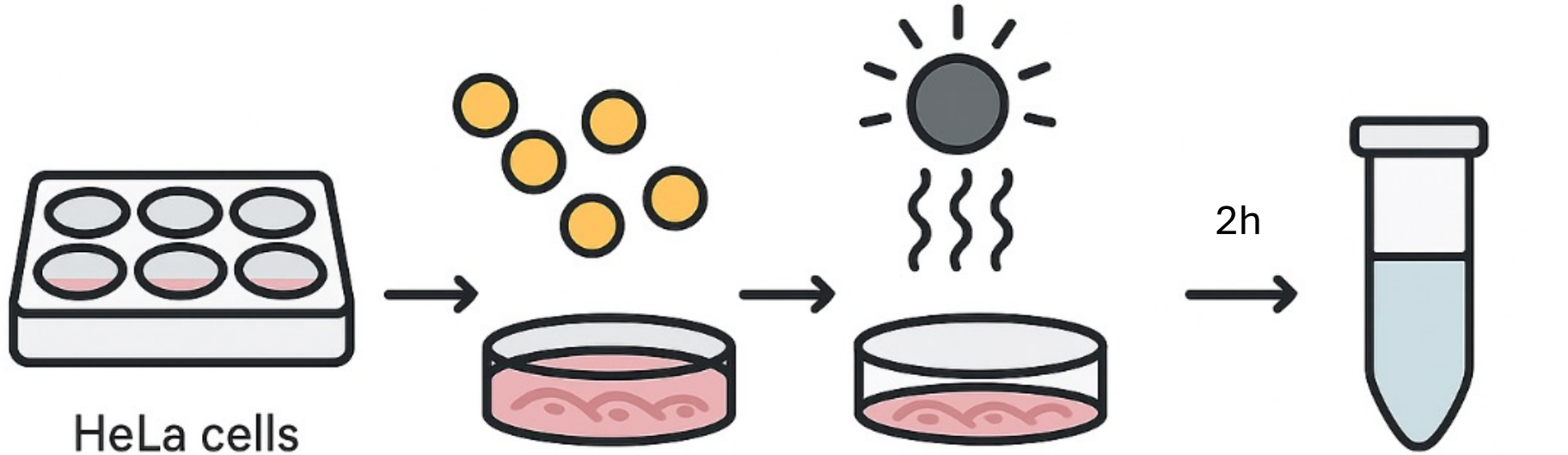
Proteomic workflow





Ahrens, C.H. et al. (2010) Nat Rev Mol Cell Biol 11, 789–801

Preliminar Assay

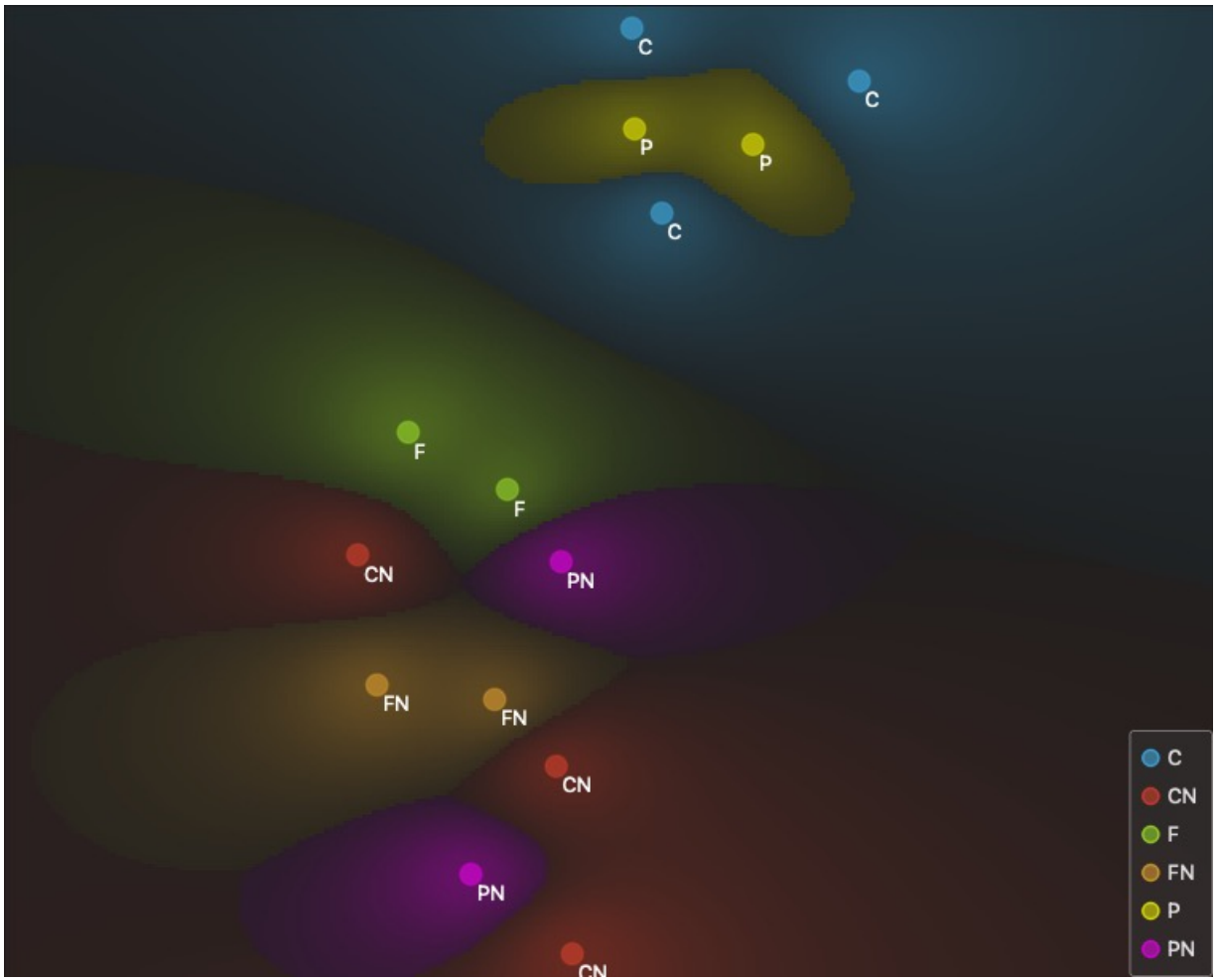


3x Control (C)
3x Control+ NP (CN)
2x Proton 4Gy (P)
2x Proton 4Gy +NP (PN)
2X Photon 4Gy (F)
2X Photon 4Gy + NP (FN)

Preliminar Assay

Insufficient data for statistical analysis

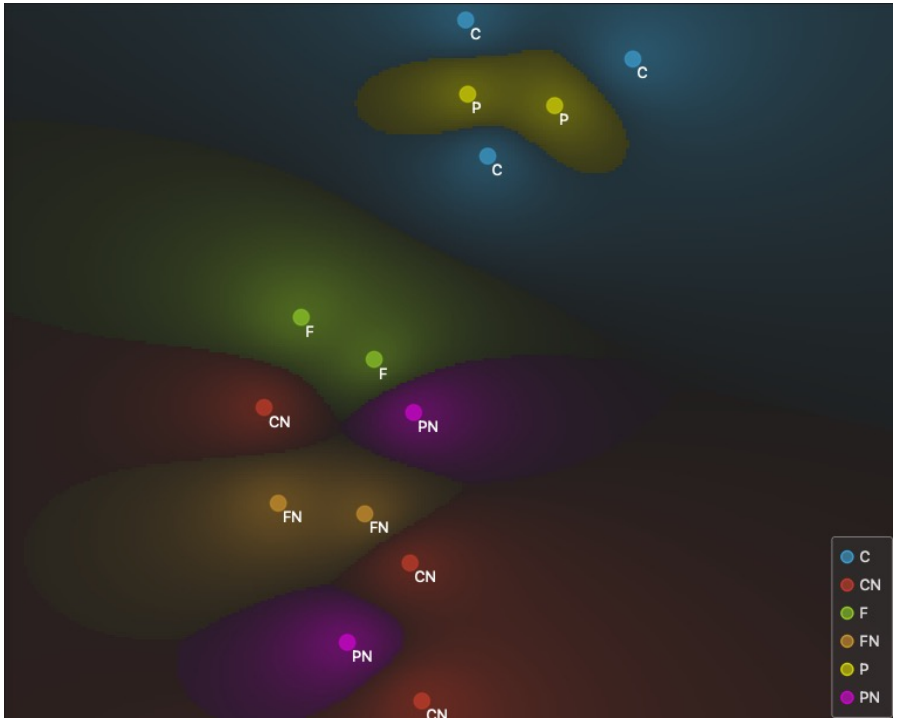
Gold nanoparticles seem to have a stronger biological effect than irradiation alone



t-SNE (t-distributed Stochastic Stochastic Neighbor Embedding) is an unsupervised non-linear dimensionality reduction technique for data exploration and visualisation of high-dimensional data.

Preliminar Assay

Insufficient data for statistical analysis.
Gold nanoparticles seem to have a stronger biological effect than irradiation alone



- +4000 proteins identified
- Comparison: AuNPs vs. Control
  - ↑ 200 proteins upregulated
  - ↓ 800 proteins downregulated
  - Downregulation of proteins involved in:
    - Protein synthesis
    - Protein transport
  - Upregulation of proteins related to:
    - Cell cycle regulation
    - Metalloproteins

Preliminar Assay

Possible causes for the limited differences observed:

Insufficient replicates → limited statistical power.

Medium shielding effect → mixed population of irradiated and non-irradiated cells.

Loss of dead cells during washing → sample no longer fully representative.

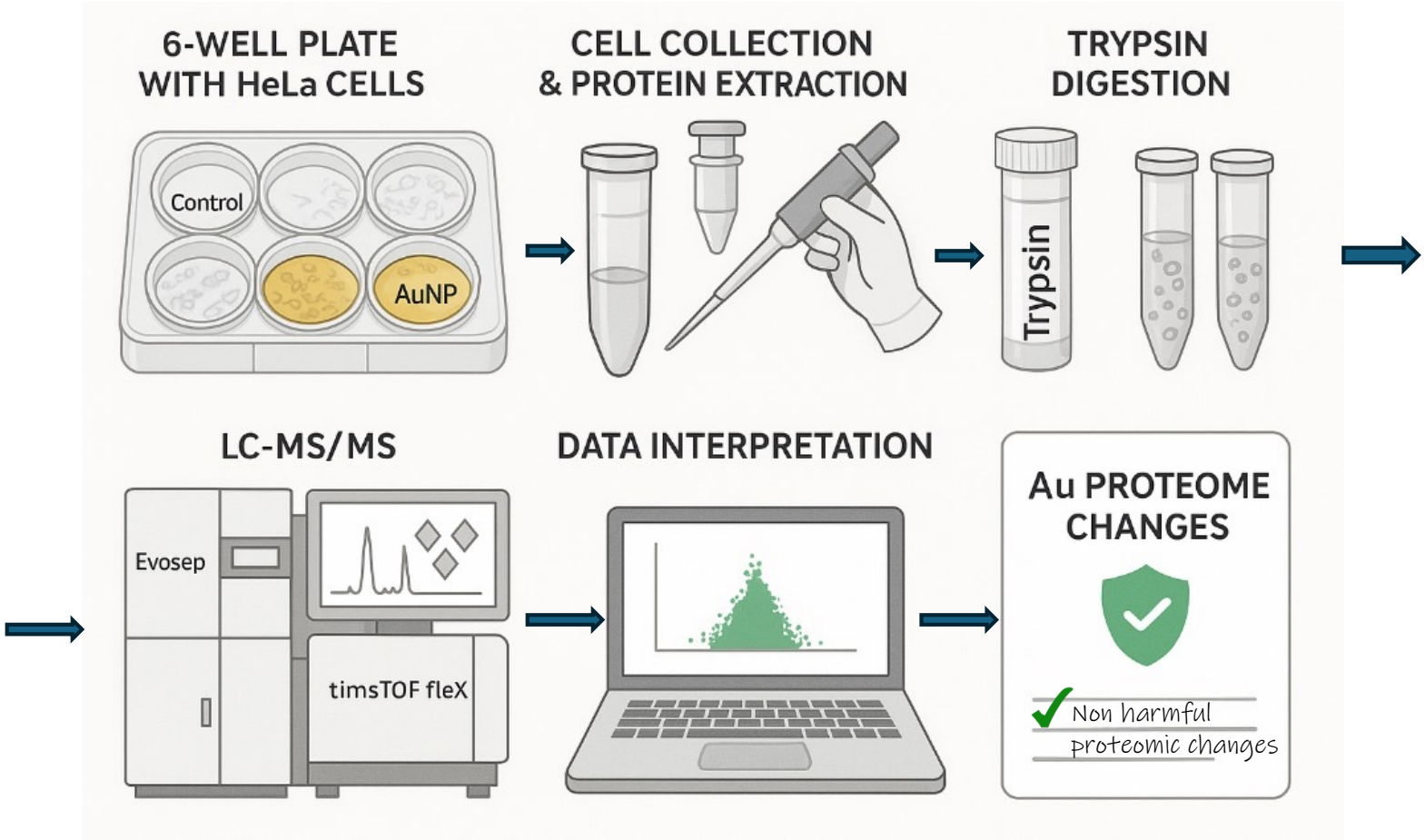
Short recovery time (2 h) → may not be enough for proteomic changes to manifest.

Proteins from “destroyed” cells → not yet degraded, may mask biological signals.

AuNPs proteome changes

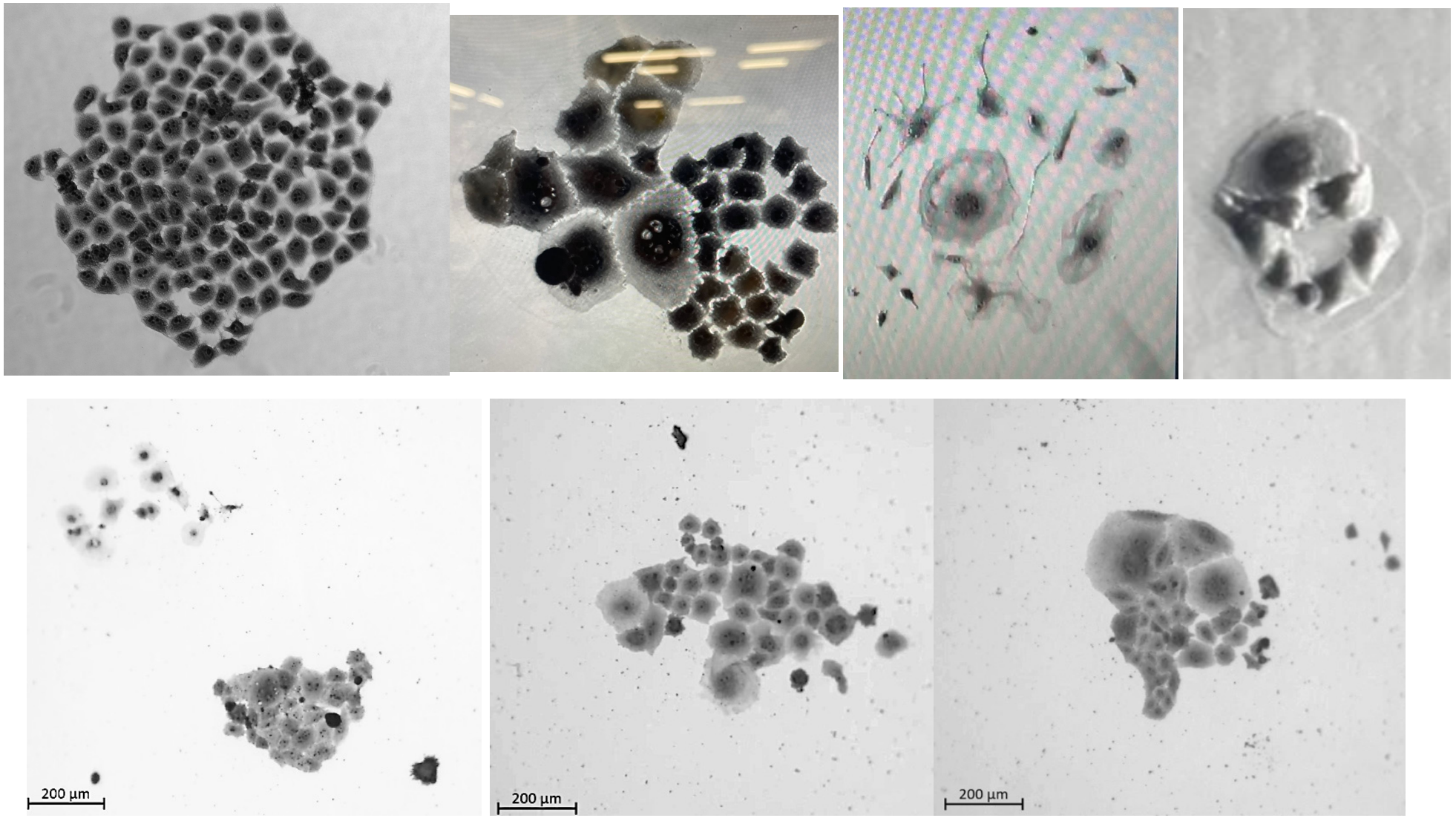
AuNPs proteome changes

Identification of **proteomic alterations** caused by AuNPs.



Proteomic study of irradiated cells with different morphologies

Diverse Colony Morphology After Irradiation



Representative colony morphologies post-irradiation: normal morphology, colonies with abnormally large cells indicative of mitotic catastrophe, and colonies with a reduced number of cells, suggesting impaired cell proliferation.

Proteomic study of irradiated cells with different morphologies

Objective:
To investigate proteomic changes in irradiated cell colonies with diverse morphologies, , aiming to improve our **understanding of the molecular effects of radiation.**
Colony Selection Criteria:
•Normal morphology
•Abnormally large cells (suggesting mitotic catastrophe)
•Small colonies (~few cells, indicating impaired proliferation)
→ *Five colonies (~200 total cells) per condition will be pooled.*

Methodology:
•Imaging & Isolation:
Axio Observer 7 microscope + PALM MicroBeam laser microdissection
•Proteomic Workflow:
Trypsin digestion → LC-MS/MS (NanoElute II + TIMS-TOF, diaPASEF mode)

Expected Outcome:
Comprehensive proteomic profiles of radiation-surviving subpopulations, enabling identification of **radioresistance markers** and **sensitization targets**.

Technological Platform

•Axio Observer 7 microscope +

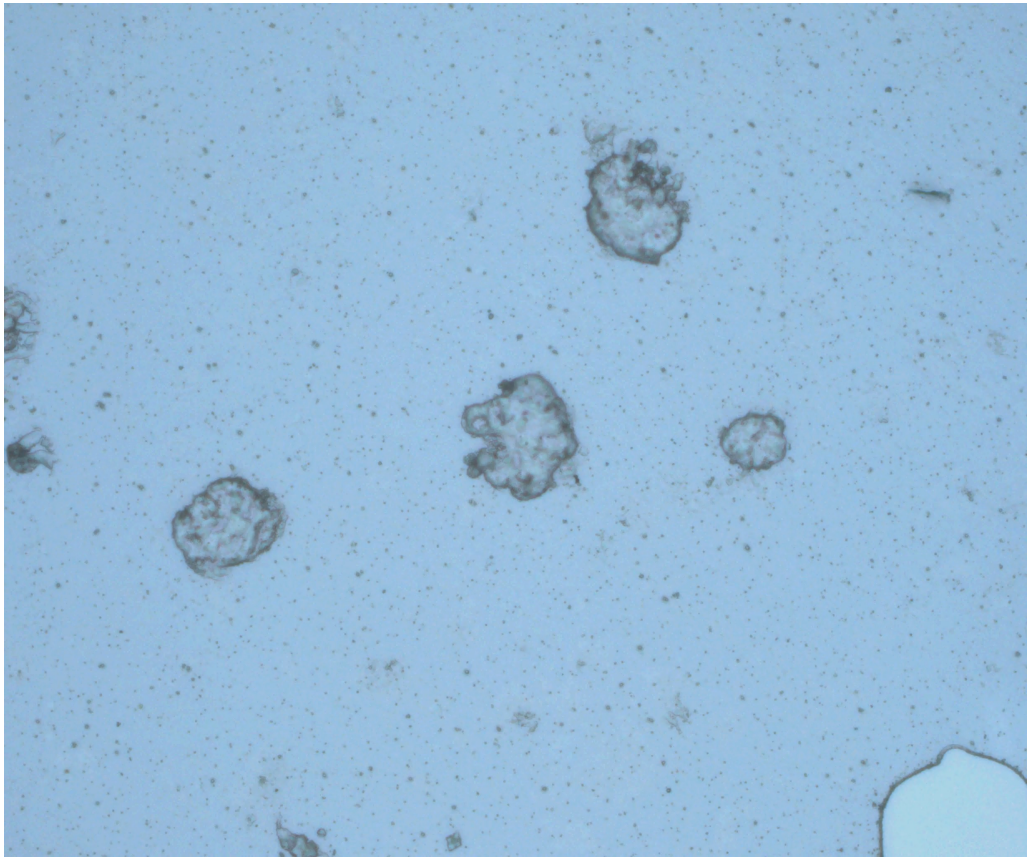
•PALM MicroBeam laser microdissection

TIMS TOF Single Cell Proteomics

- **Hybrid Quadrupole/Time-of-Flight mass** spectrometer, coupled with a highly reproducible NanoElute II chromatography system, which enables operation in nano-flow condition

Technological Platform

Organoids
r ~30-70μm



•Axio Observer 7 microscope + PALM MicroBeam laser microdissection

Technological Platform

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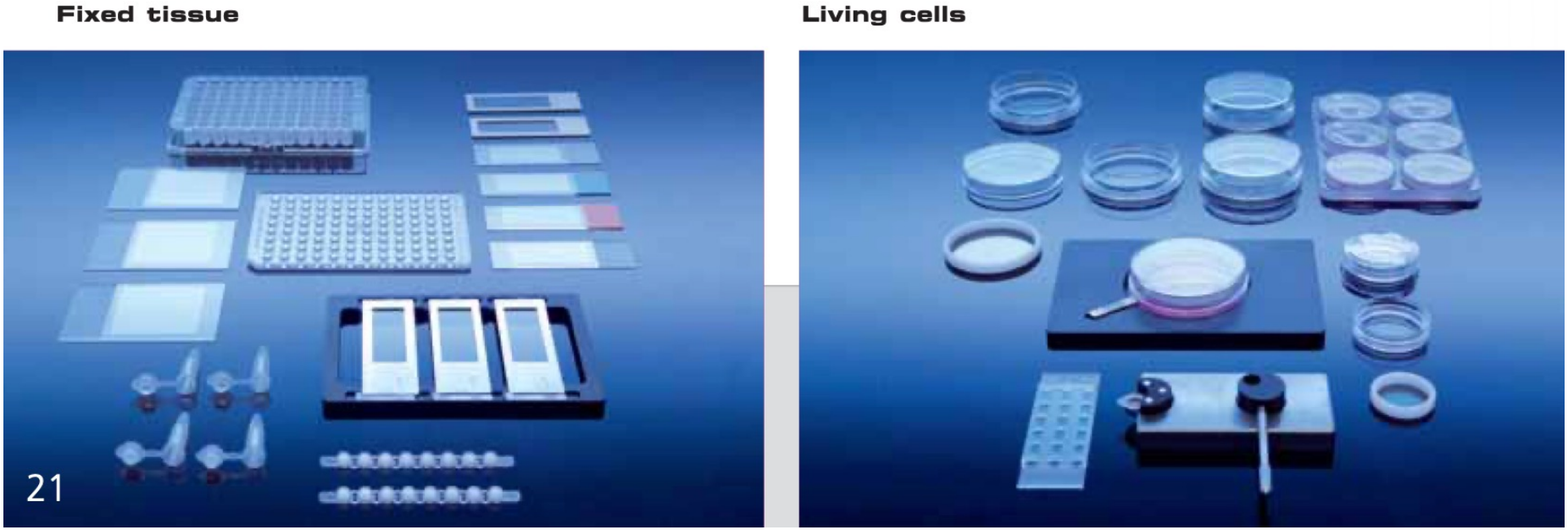


•Axio Observer 7 microscope + PALM MicroBeam laser microdissection

Proteomic study of irradiated cells with different morphologies

Objective:
To investigate proteomic **changes in irradiated cell colonies** with **diverse morphologies**, aiming to improve our understanding of the molecular effects of radiation.

Expected Outcome:
Comprehensive proteomic profiles of radiation-surviving subpopulations, enabling identification of **radioresistance markers** and **sensitization targets**.

Global Analysis of Post-Translational Modifications (PTMs)

Global Analysis of Post-Translational Modifications

- PTMs are key regulators of protein function, stability, localization, and interactions.
- Although specific PTMs have been studied in isolated contexts after radiation exposure, no comprehensive analysis has yet been conducted.
- In this task, we will perform a global analysis of PTMs using mass spectrometry in data-dependent acquisition (DDA) mode.
- Modified peptides will be identified using a localization-aware open search engine, such as MSFragger.

Deliverable:
Comprehensive PTM profiling in irradiated cells, offering novel insights into radiation-induced signaling and potential targets for radiosensitization.

Article

https://doi.org/10.1038/s41467-025-58728-z

MSFragger-DDA+ enhances peptide identification sensitivity with full isolation window search

MSFragger: ultrafast and comprehensive peptide identification in mass spectrometry–based proteomics

Andy T Kong[1,2], Felipe V Leprevost[2], Dmitry M Avtonomov[2], Dattatreya Mellacheruvu[2] & Alexey I Nesvizhskii[1,2]

Nature Communications vol16, : 3329 (2025)

Nature Communications vol 11, 4065 (2020)

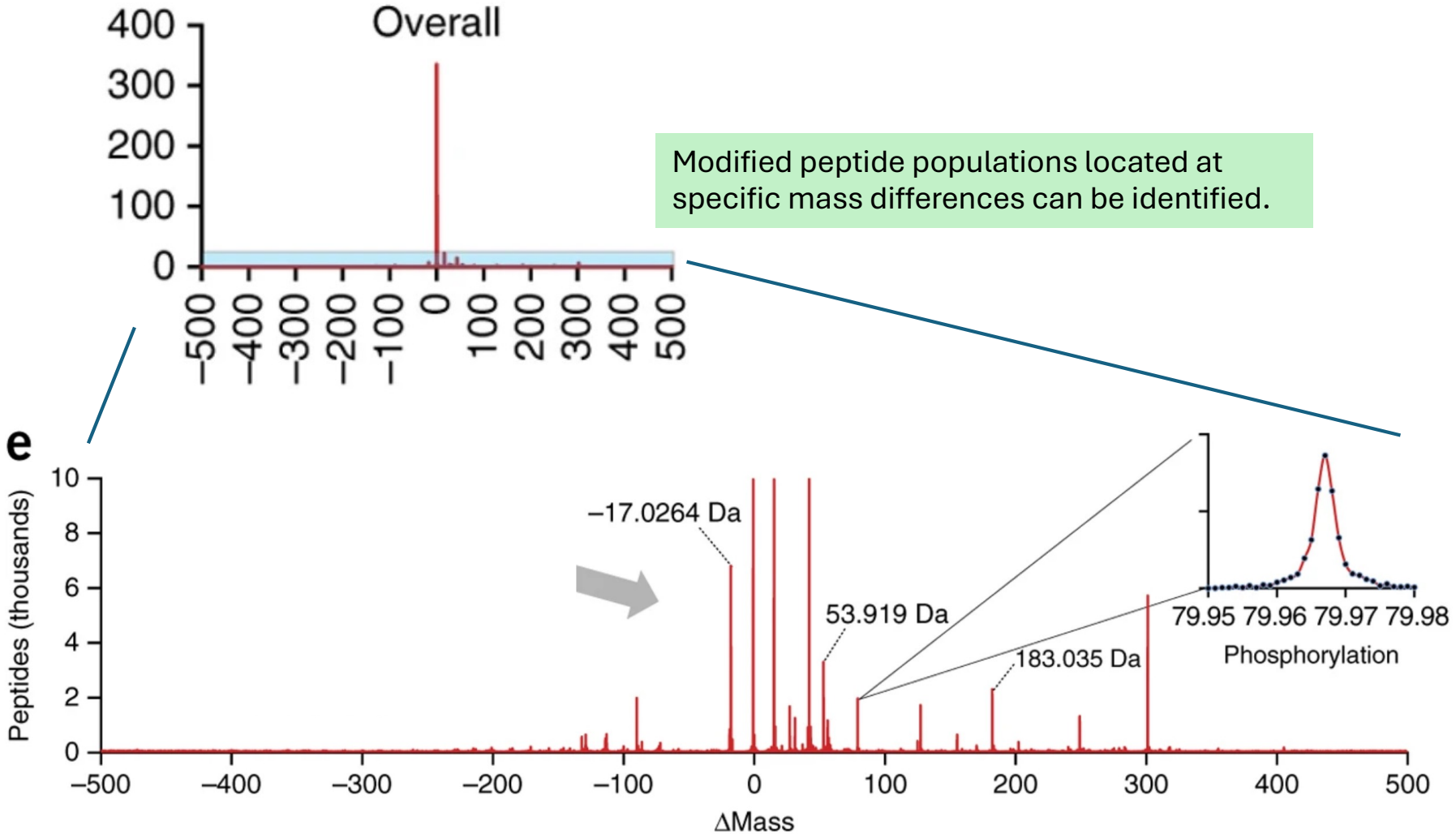
A mass-tolerant database search identifies a large proportion of unassigned spectra in shotgun proteomics as modified peptides

HEK 293 cell data set (24 fractions)

Search	Total peptides	Unique sequences	Proteins	Total MS/MS	Protein FDR (%)	Peptide FDR (%)
Closed (5 p.p.m.)	396,736	110,262	9,513	1,121,145	1.26	0.11
Open (500 Da)	510,139	111,205	9,178	1,121,145	1.12	0.12

Nature Biotechnology **volume 33**, pages 743–749 (2015)

A mass-tolerant database search identifies a large proportion of unassigned spectra in shotgun proteomics as modified peptides



Nature Biotechnology **vol. 33**, pages 743–749 (2015)

PTMs post-irradiation

- **Phosphorilation**
  Activation of DDR kinases (ATM, ATR, p53), leading to extensive phosphorylation of proteins involved in DNA damage signaling and cell cycle control

- **Oxidation and Nitration**
  Overproduction of reactive species (ROS/RNS) after radiation causes oxidation of residues (Met, Cys, Trp, Tyr) and tyrosine nitration (3-nitrotyrosine), affecting protein structure and function.
- **Acetylation / Methylation / Glycosylation**
  PTMs affecting histones and non-histone proteins, contributing to chromatin remodeling and altered protein-protein interactions.
- **SUMOylation / NEDDylation**
  Stress-responsive PTMs modulating transcription, DNA repair, and protein stability under radiation exposure.

- **Ubiquitination**

Radiation alters the ubiquitin-proteasome pathway, regulating protein degradation and recovery from post-irradiation stress

Phosphoproteomic Studies of Irradiation Effects

Phosphoproteome profiling of human skin fibroblast cells in response to low- and high-dose irradiation J Proteome Res 2006 May;5(5):1252-60. doi: 10.1021/pr060028v. En fibroblastos humanos irradiados, se identificaron ~494 fosfopéptidos, con aumentos de 1.9x o 3.6x en fosforilación según la dosis de radiación

Deciphering the Acute Cellular Phosphoproteome Response to Irradiation with X-rays, Protons and Carbon Ions (2017) doi: 10.1074/mcp.M116.066597 Usando SILAC y enriquecimiento de fosfopéptidos, identificaron 181 sitios de fosforilación regulados tras irradiación y demostraron diferencias específicas según la calidad de la radiación (X-rays vs protons)

Proteomics insights into DNA damage response and translating this knowledge to clinical strategies Proteomics 2016 Dec 12;17(3-4):1600018. doi: 10.1002/pmic.201600018 Review **:** Activation of DDR kinases (ATM, ATR, p53), leading to extensive phosphorylation of proteins involved in DNA damage signaling and cell cycle control.

Proteomic Studies of Irradiation Effects

- **Proteomic Analysis of Proton Beam Irradiated Human Melanoma Cells** PLoS One- 2014 Jan 2;9(1):e84621. doi: 10.1371/journal.pone.0084621 BLM cells were exposed to 3 Gy proton irradiation and analyzed 28–35 days later. They found significant changes: 13 proteins upregulated, 4 downregulated—mainly related to DNA repair, stress response and metabolism

- **Proteomic Analysis of Energy Metabolism and Signal Transduction in Irradiated Melanoma Cells** Int J Ophthalmol. 2013 Jun 18;6(3):286–294. doi: 10.3980/j.issn.2222-3959.2013.03.06 Tras irradiación con X-rays en células de melanoma 92-1, se cuantificaron 29 proteínas con expresión alterada relacionadas con metabolismo, control del ciclo celular y muerte celular

- **Comparative Proteomic Profiles across Species (Human, Rodent, NHP)** https://doi.org/10.1667/RADE-21-00182.1 Utilizing SomaScan aptamer-based assays post total-body irradiation, identified shared and species-specific protein expression changes, inflammation, DNA repair markers, and potential biomarkers

- **Analysis of the Proteomic Profile in Serum of Irradiated Nonhuman Primates Treated with Ex-Rad, a Radiation Medical Countermeasure** https://pubs.acs.org/doi/10.1021/acs.jproteome.2c00458 In mice treated with radioprotective gamma-tocotrienol, several radiation-induced serum protein changes were reversed, revealing potential restorative effects via proteomic modulation

- **Proton Compared to X-Irradiation Induces Different Protein Profiles in Oral Cancer Cells and Their Derived Extracellular Vesicles** Int J Mol Sci. 2023 Nov 30;24(23):16983. doi: 10.3390/ijms242316983 Compared proteomes of oral squamous carcinoma cells post low/high-LET protons vs. X-rays (4 & 8 Gy). Proton irradiation led to distinct modulation in DNA damage, apoptosis, adhesion, migration, and metabolism pathways

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