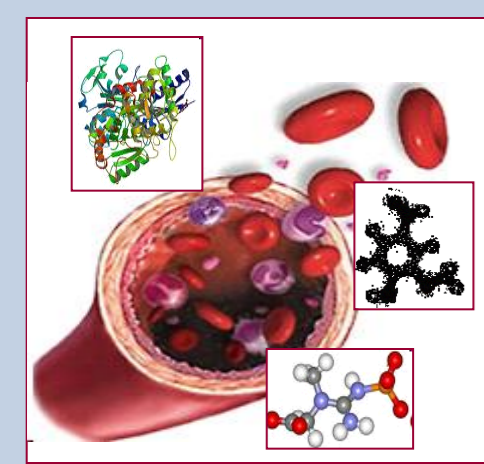


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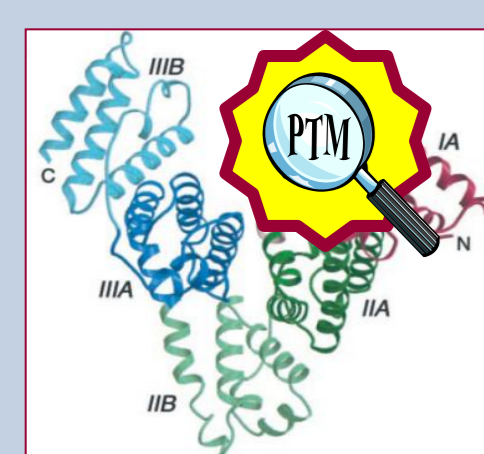


1. INTRODUCTION: Human exposure to exogenous compounds

It is established that the human body is exposed to **food additives**, **drugs** and **toxic chemicals**. These exogenous compounds interact with each other and with our body's molecules. Such unpredictable interactions can contribute to potential diseases.

By its constant perfusion in the body, **blood** registers 24 hours a day traces of passage of chemicals and acts as a reservoir of useful information. Moreover, those exogenous compounds and their metabolites can potentially interact and bind irreversibly to blood proteins, inducing permanent **post-translational modifications (PTMs)**.

Identifying and monitoring modifications of blood proteins may provide biomarkers that would prove essential in many clinical applications. In particular, they would assist in the diagnostic and prevention of some adverse drug reactions (ADRs) or intoxications. Additionally, they would contribute to the understanding of molecular mechanisms involved in the damage itself.



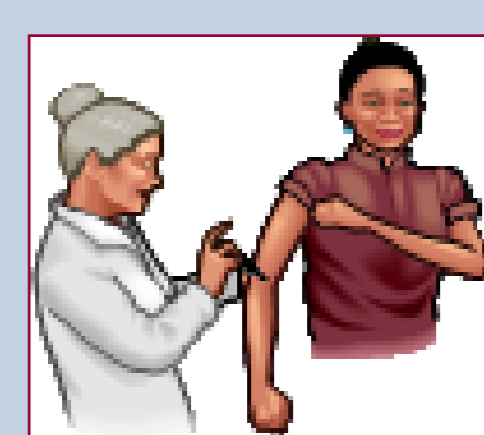
2. EXPERIMENTAL SET-UP: searching for PTMs on blood proteins

Searching for unknown modifications on blood proteins is a challenging task which requires the **development of appropriate technique of protein/peptide separations** prior to the MS/MS-MS data acquisition. The final step involves data analysis using dedicated **bioinformatics tools**.

Model

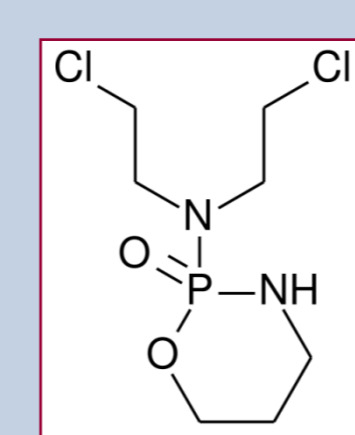
Patients with lymphoma treated with a cytostatic drug: cyclophosphamide.

Four blood samples taken from each patient at four different times:

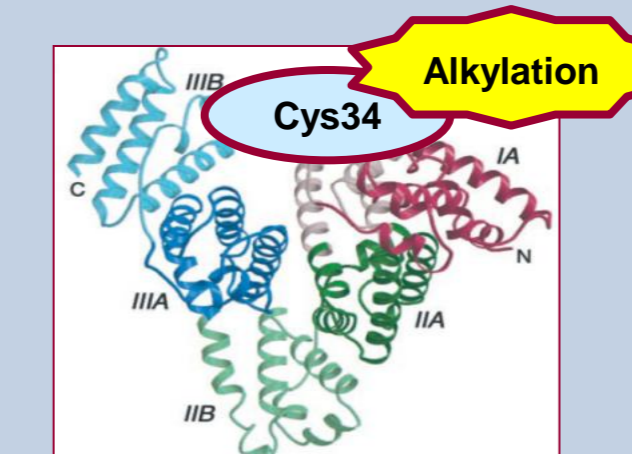


J00 → 1 day before treatment (control sample)
J02 → 1 day after treatment
J10 → 10 days after treatment
J20 → 20 days after treatment

Cyclophosphamide potentially reacts to serum **albumin** by alkylating cysteine at position 34 (1) on the **ALVLIAFAQLYQQCPFEDHVK** peptide (control)

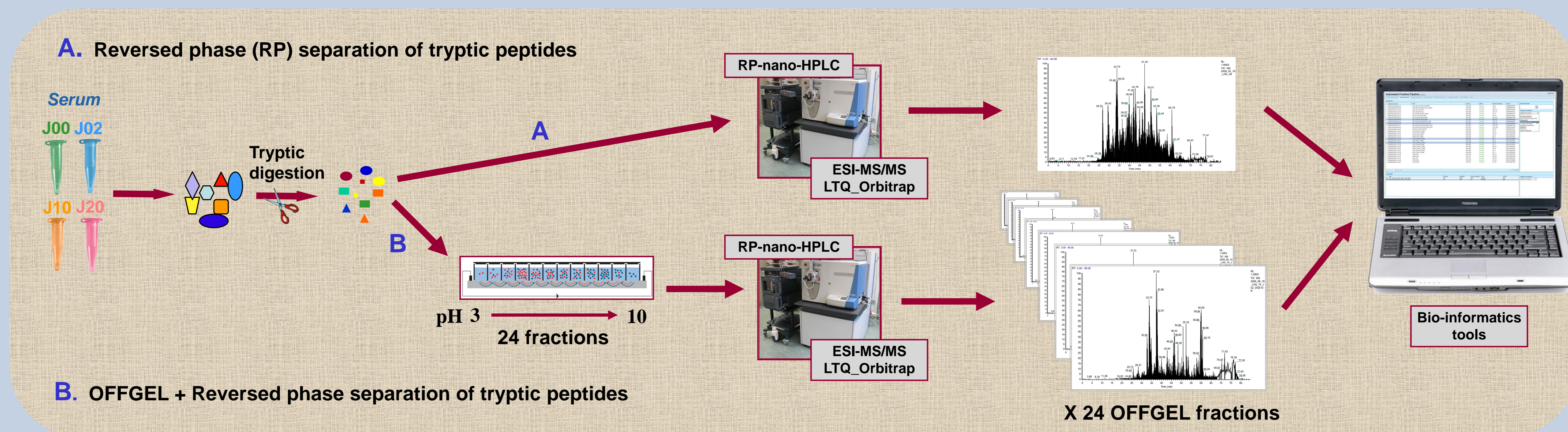


Cyclophosphamide



Human serum albumin

3. WORKFLOW OF POST-TRANSLATIONAL MODIFICATIONS (PTMs): analysis in albumin



4. EXPERIMENTAL CONDITIONS: LC-MS/MS

Nano-HPLC		
	Trapping	Analytical
Mode	Isocratic	Gradient
Time	12 minutes	85 minutes
Flow rate	3 µ/min	220 nL/min

LTQ-Orbitrap	
MS1	Full scan in the orbitrap Mass range: 400-1600 Th
MS2	Ion selection: 3 most abundant Dynamic exclusion: 1 minute

Analytical Gradient		
Minutes	A	B
0	95 %	5 %
1	95 %	5 %
55	65 %	35 %
65	20 %	80 %
67	20 %	80 %
69	95 %	5 %
85	95 %	5 %

A: H₂O, 0.1% FA
 B: AcN, 0.1% FA

5. RESULTS

MS/MS Spectra Comparison	A. method	B. method (OFFGEL)
Albumin	270	700
Control peptide	35	50
Total	820	6000+
Alkylations on control peptide	None	On Cys34

6. PERSPECTIVES & CONCLUSIONS

- Optimizing the OFFGEL separation to maximize peptides recovery.
- Exploring the combination of CID and HCD fragmentation modes on the LTQ-Orbitrap. Although the quality of MS-MS spectra acquired by Collision Induced Dissociation (CID) fragmentation is excellent, adding other fragmentation strategies may improve data quality. High energy Collision Dissociation (HCD) fragmentation is of prime interest as it may detect ions not visible otherwise. Therefore, combination of CID and HCD could be useful for better PTMs identification.
- Improving proteins and/or peptides separation. Due to the matrix complexity, separation steps are mandatory: as illustrated, OFFGEL peptide separation prior to LC separation resulted in 7 times more MS/MS spectra.

References

(1) Noort D, Hulst AG, Jansen R (2002) Covalent binding of nitrogen mustards to the cysteine-34 residue in human serum albumin. *Arch Toxicol* 76:83–88