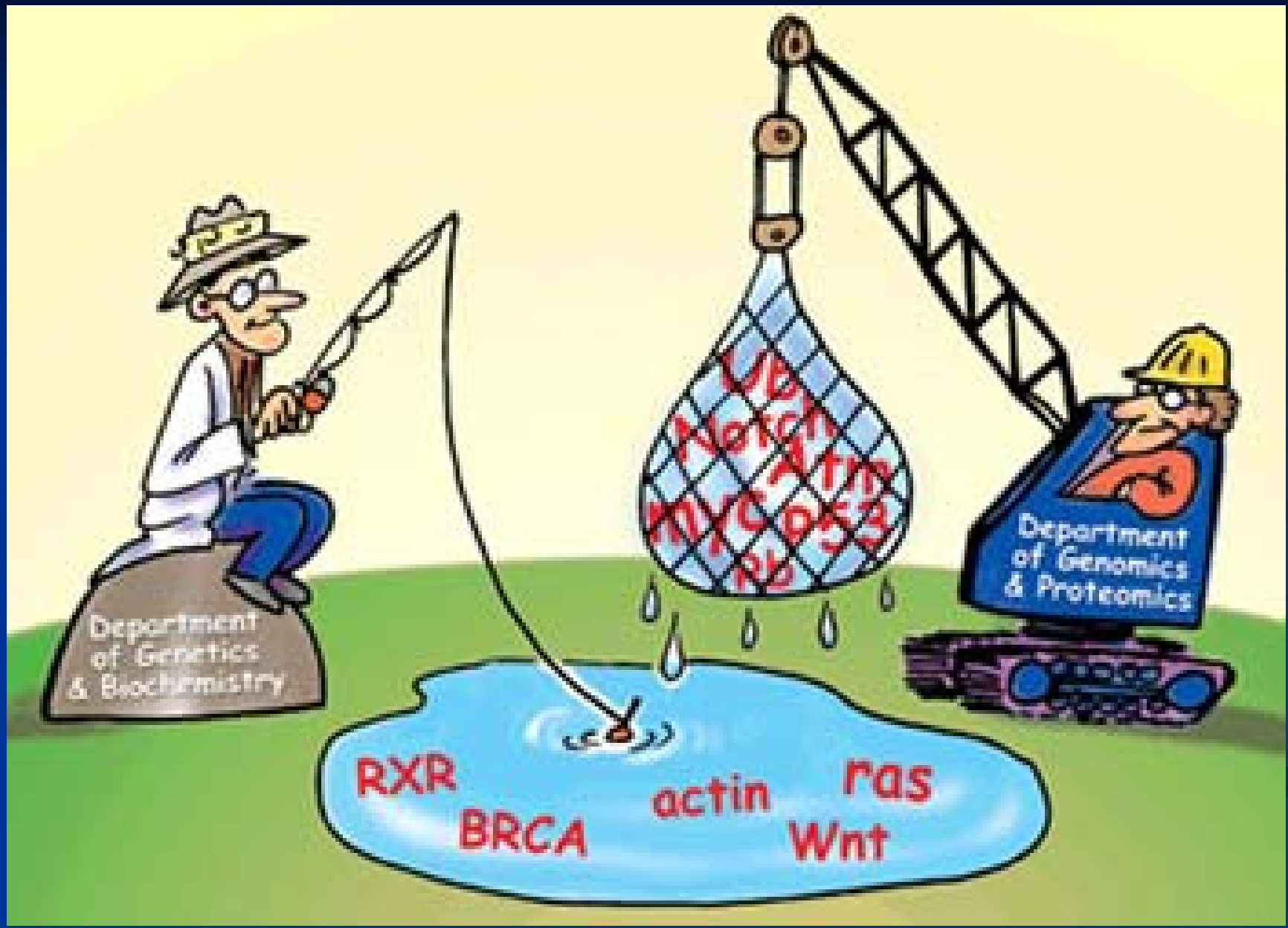
ADVANCING CANCER BIOTHERAPY WITH PROTEOMICS



Science 291: 1221, 2001

INFLAMMATORY PROTEIN PROFILE DURING SYSTEMIC HIGH DOSE INTERLEUKIN-2 ADMINISTRATION

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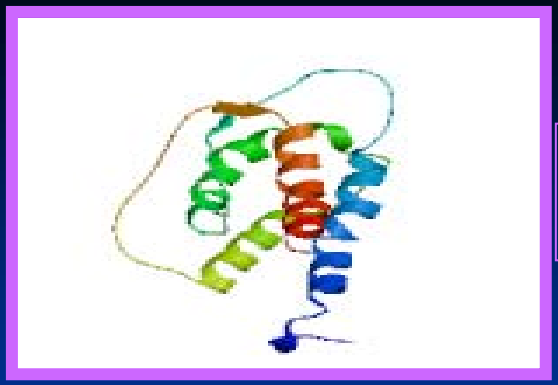
Section of Immunogenetics
NIH

ALEXANDRIA

iSBTC

10-13 November 2005



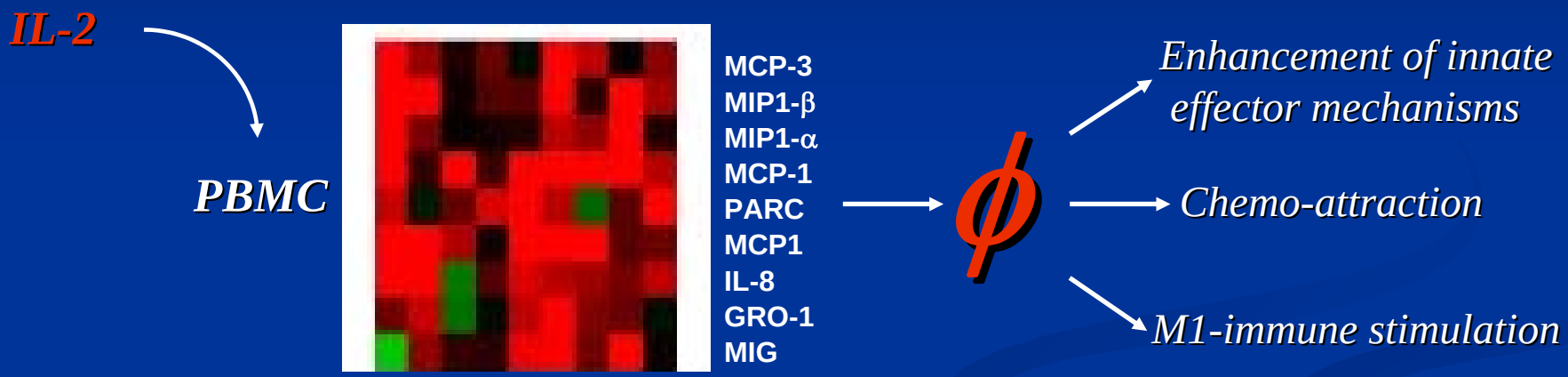
ENIGMATIC ROLE OF INTERLEUKIN-2 IN CANCER THERAPY

- Systemic IL-2 can effectively treat metastatic renal cell cancer and melanoma and plays an essential role during active-specific immunization against cancer by increasing the frequency of tumor regressions

- Modulatory effects of IL-2 on various pathways of cellular immune responses have been extensively described

- The use of high dose IL-2 treatment is limited by the occurrence of several side effects and severe toxicity

- The mechanisms of IL-2 mediated cancer regression remain today largely unknown. Recent data suggest that IL-2 acts through the activation of momonuclear phagocytes at the tumor site.

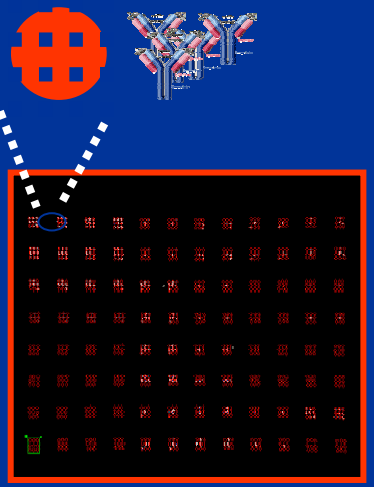

IL-2
PBMC
MCP-3
MIP1-β
MIP1-α
MCP-1
PARC
MCP1
IL-8
GRO-1
MIG
ϕ
Enhancement of innate effector mechanisms
Chemo-attraction
M1-immune stimulation

We characterized the protein profile of sera obtained from **ten** patients with **RCC** undergoing **high dose** (720,000 IU/kg intravenously every 8 hours) **IL-2 therapy**.

PROTEOMIC ANALYSIS OF SERUM IN PATIENTS UNDERGOING IL-2 THERAPY

SERUM

Multiprotein array platforms



SELDI

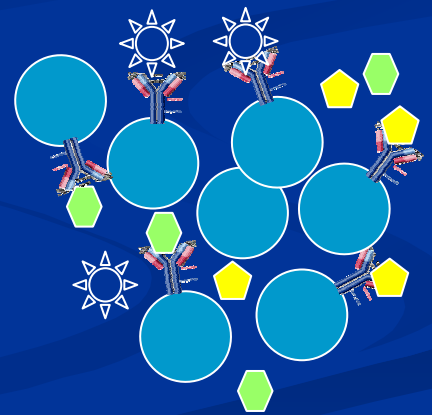
H4

SAX2

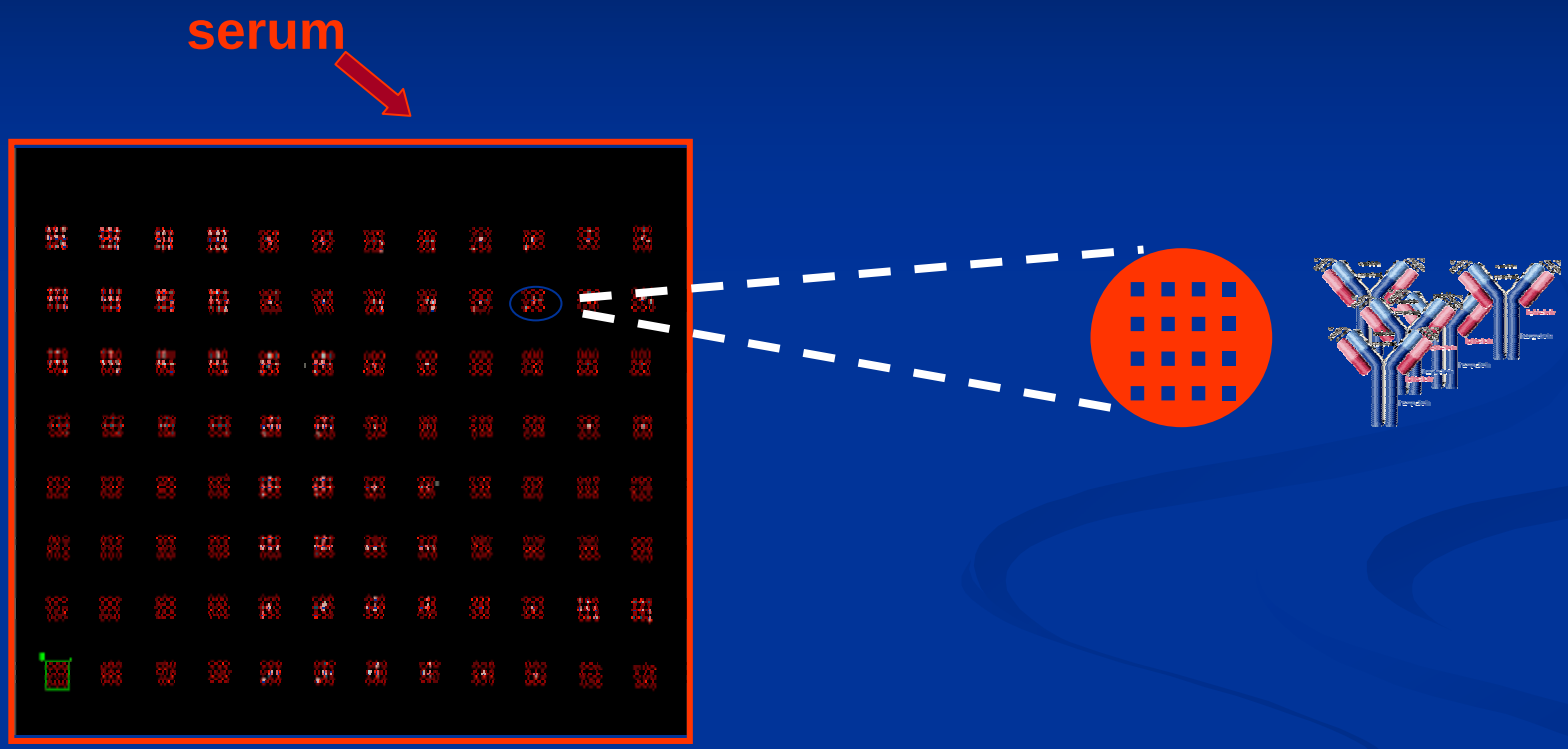
WCX2

IMAC

Immuno-nephelometry

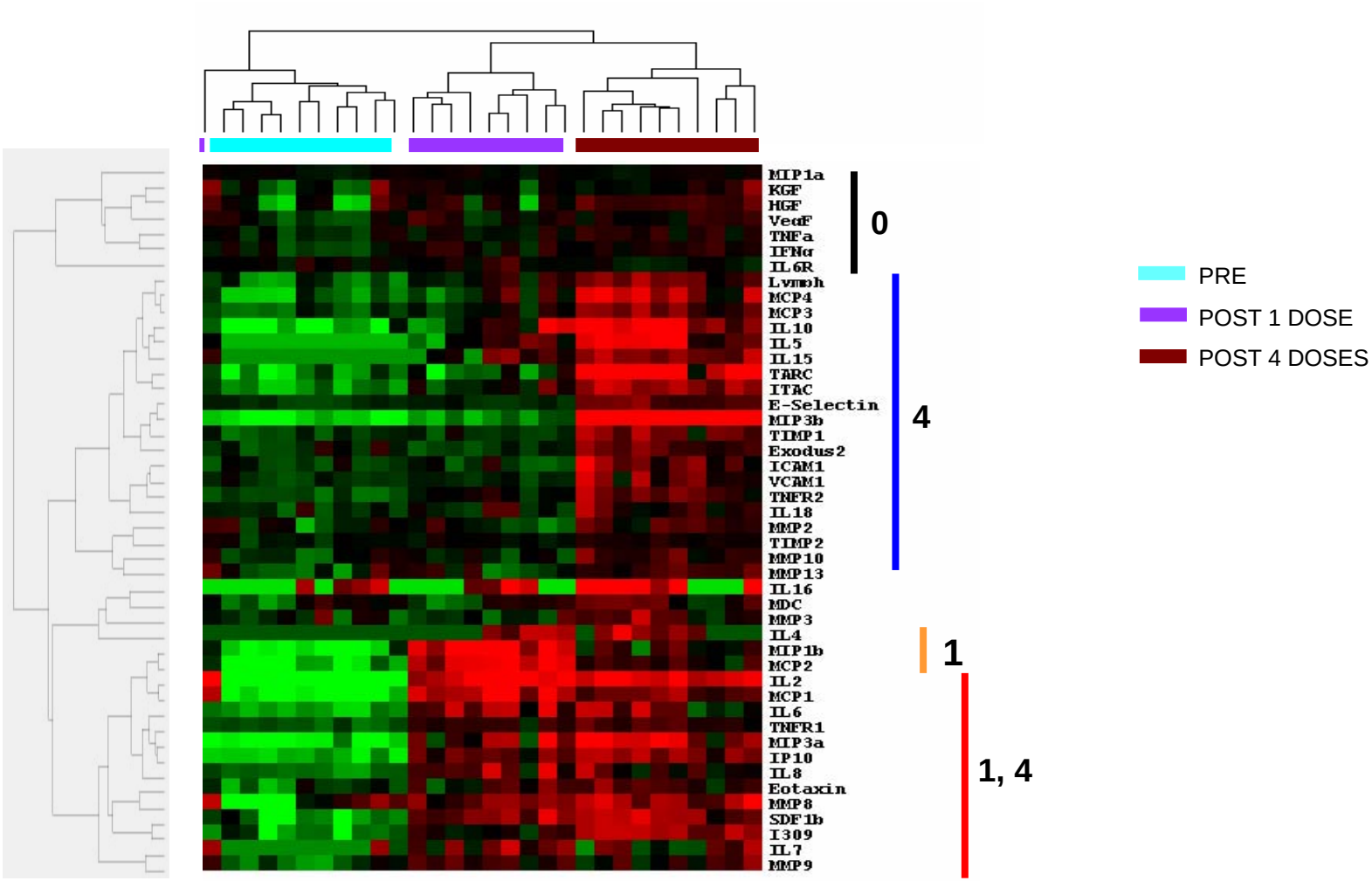


MULTIPLEXED PROTEIN ARRAY PLATFORM



UNSUPERVISED HIERARCHICAL CLUSTERING OF SERUM SAMPLES FROM RCC PATIENTS OBTAINED PRE, POST 1 AND POST 4 DOSES OF IL-2 (720,000IU/KG).

IL-2 treatment is able to induce a very complex cytokine storm



=soluble factor minimally changing from pre to post 4 doses

=soluble factor expression enhanced at 4 doses only

= soluble factor expression enhanced post 1 dose

= soluble factor expression enhanced at 1 and 4 doses

Panelli et al., Journal of Translational Medicine 2004,2:17

Storm of chemokines, cytokines and soluble factors increasing in the circulation in response to IL-2

Many soluble factors that increased during IL-2 administration are also increased in **inflammatory conditions**

Soluble forms of adhesion molecules

Chemoattractant factors

Matrix metalloproteinases and their inhibitors

TNF-alpha and soluble TNFR1

FROM PROTEIN ARRAYS TO SELDI ANALYSIS OF IL-2 INDUCED PROTEINS

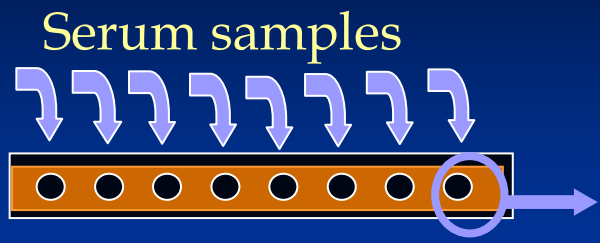
Multiplexed protein array platforms

sensitive tools which allows the detection of proteins at very low concentration in serum

Discovery limited by the number of capture antibody selected for protein detection.

Analysis Extended to **S**urface **E**nhanced **L**ased **D**esorption **I**onization **T**ime of **F**light **M**ass **S**pectrometry (SELDI-TOF-MS).

SURFACE ENHANCED LASED DESORPTION IONIZATION TIME OF FLIGHT MASS SPECTROMETRY (SELDI-TOF-MS).

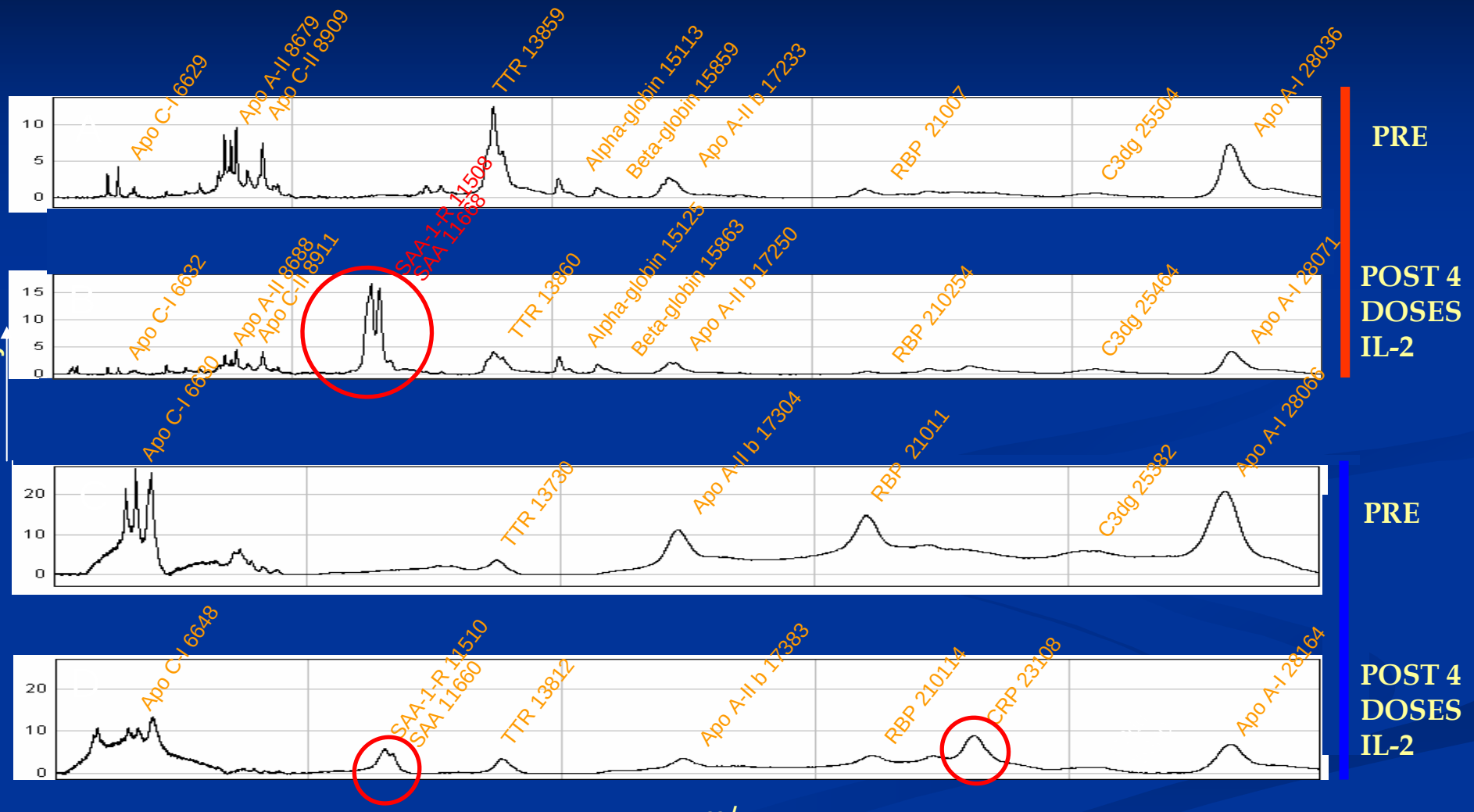


Different matrix are available to allow the preferential binding of proteins on the basis of their specific chemical or biological characteristics

SELDI ADVANTAGES

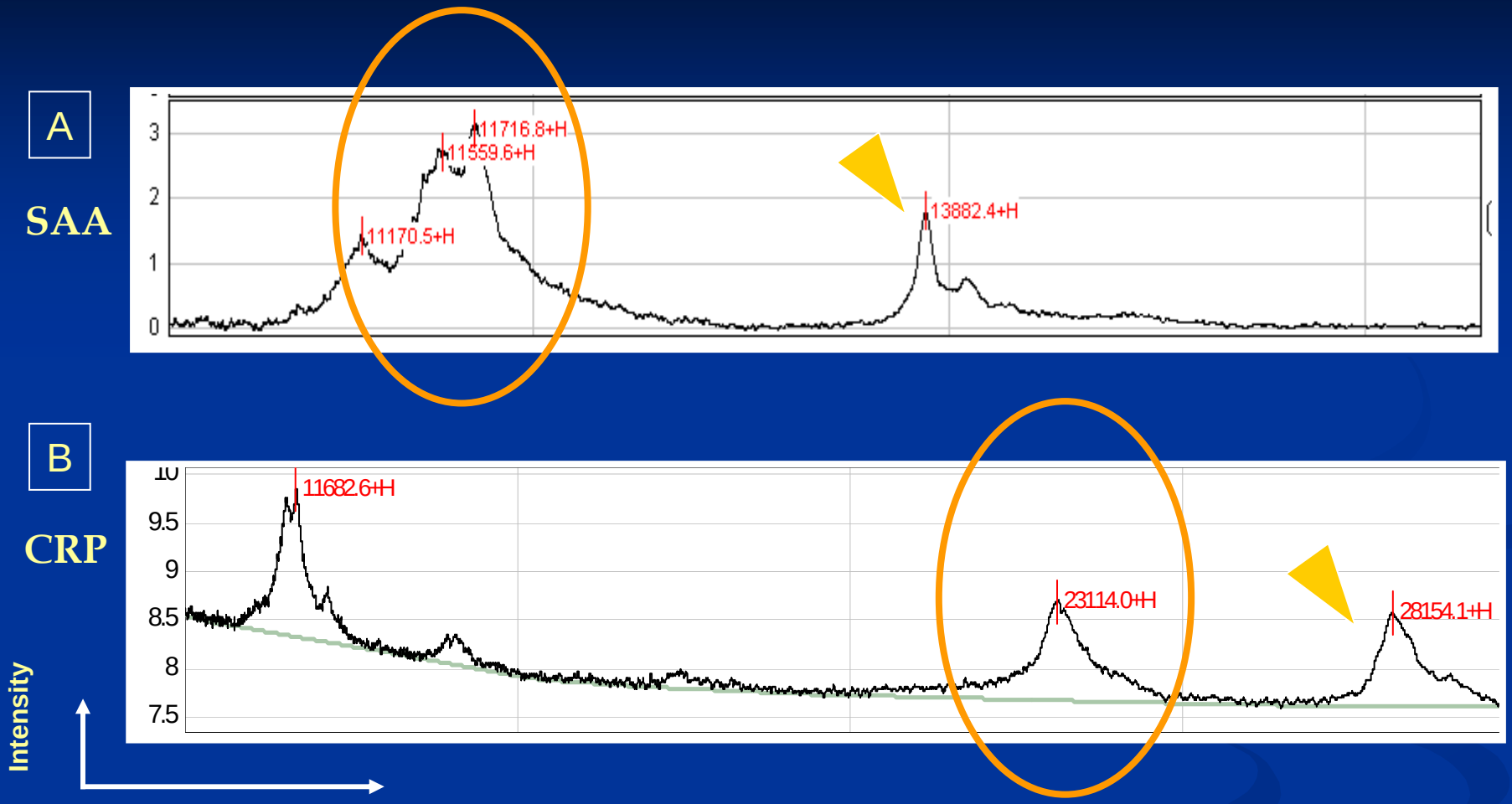
- fast and easy
- very reduced amount of samples are necessary to perform the analysis
- accurate information about the molecular weight
- possibility to discriminate between isoforms
  (phosphorilation, glycosilation, truncated forms)
- possible capture of co-precipitating proteins

SELDI ANALYSIS OF RCC SERUM
SAX2



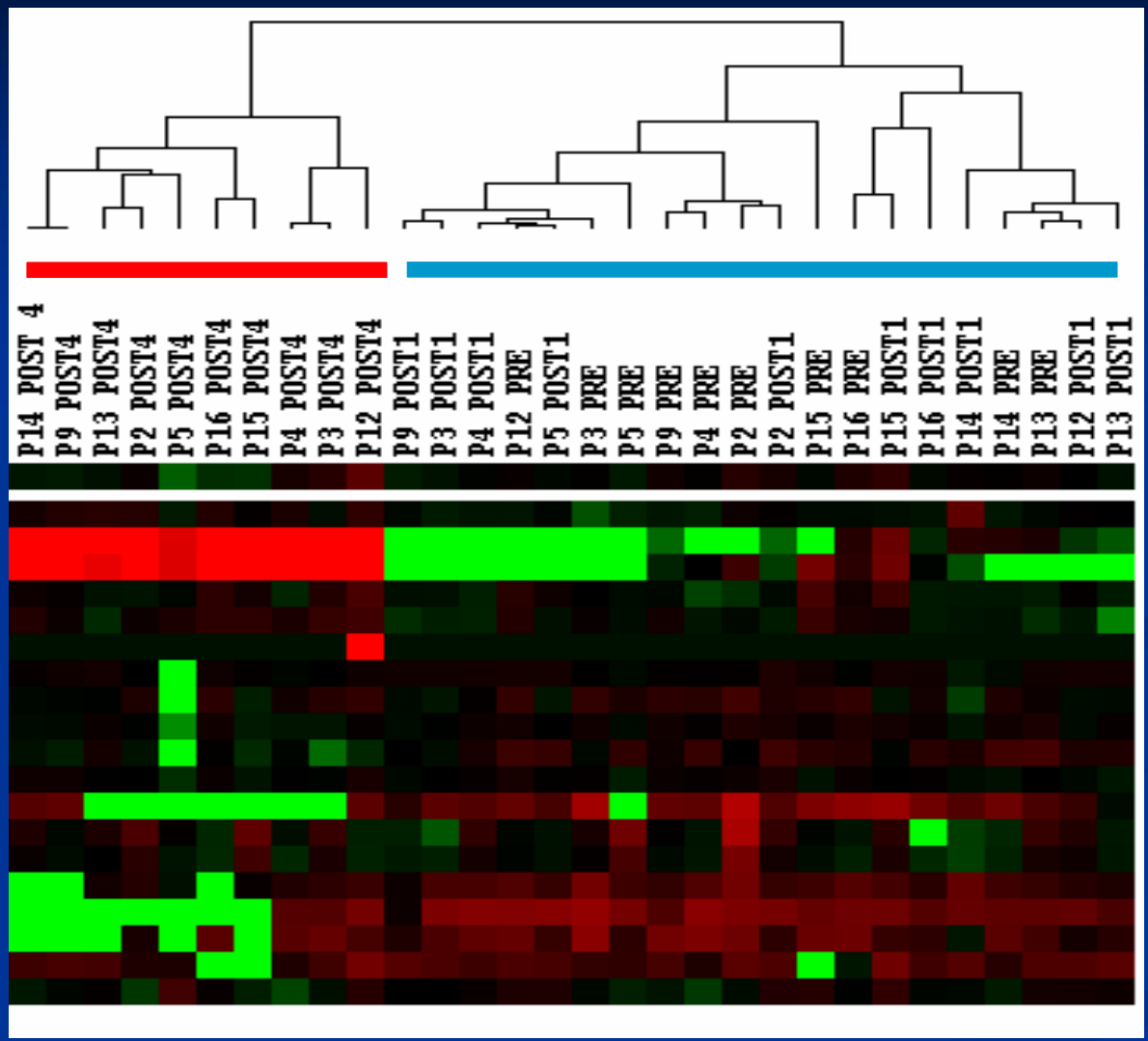
Rossi L. et al., Proteomics 2005 in press

SELDI IMMUNOAFFINITY CAPTURE OF SAA AND CRP



Rossi L. et al., Proteomics 2005 in press

UNSUPERVISED HIERARCHICAL CLUSTERING OF RCC SERUM SAMPLES OBTAINED BEFORE AND AFTER 1 AND 4 DOSES OF IL-2 (SELDI M/Z AREA).



- Increase in SELDI m/z
- Decrease in SELDI m/z
- No change in SELDI m/z

- Immunochip/SELDI
- SELDI literature
- Nephelometry

39,624 Da
SAA
SAA1-R
Alpha1 antitrypsin
25,476 Da
SAA-1-RS

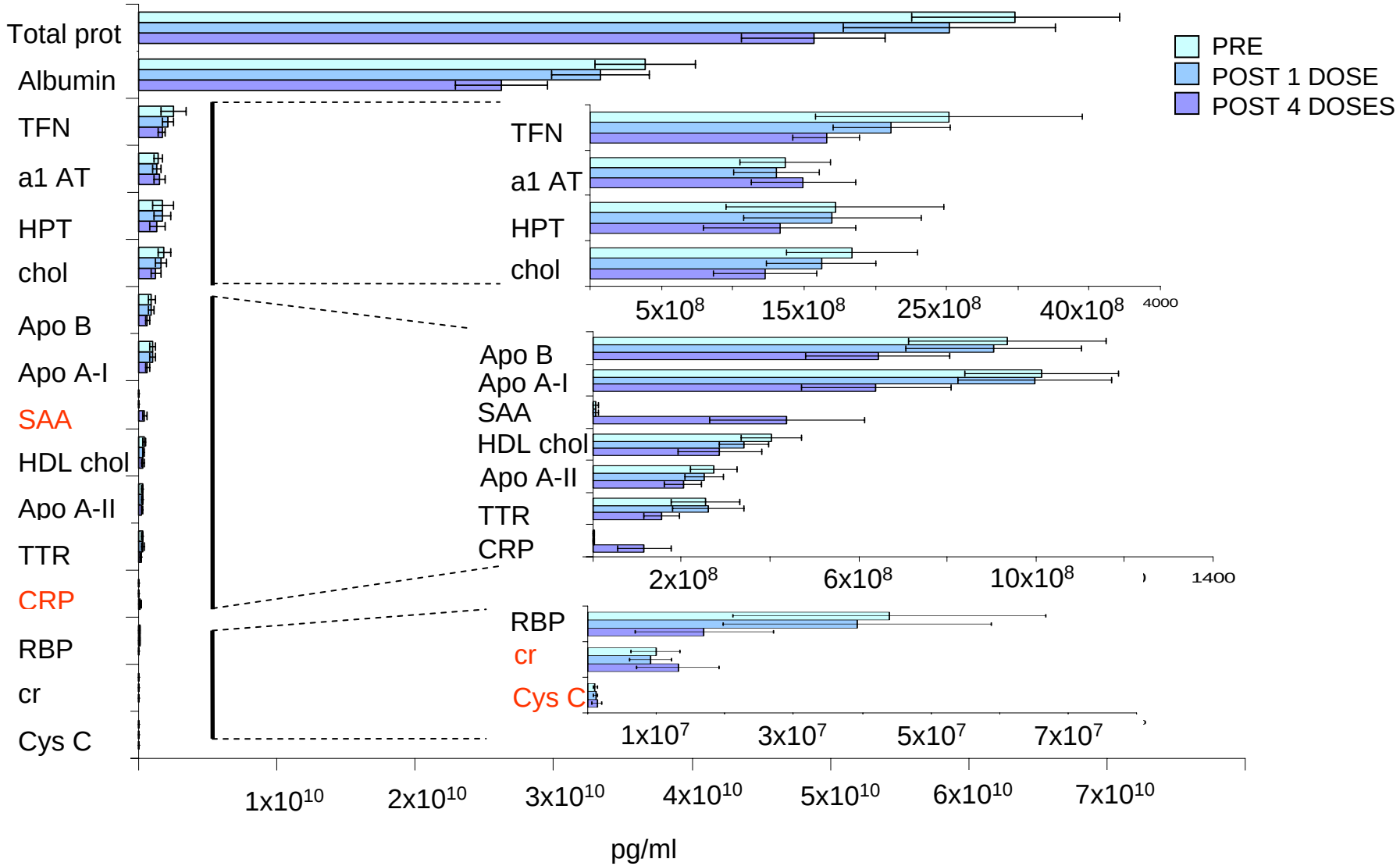
I

Albumin
ApoA-II
Apo A-I
Apo C-I
Apo-A IIb
Truncated form of TTR
Beta globin
Alpha globin
TTR
RBP
Apo CII
Beta2 GPI
Hemopexin

II
Negative acute phase reactants

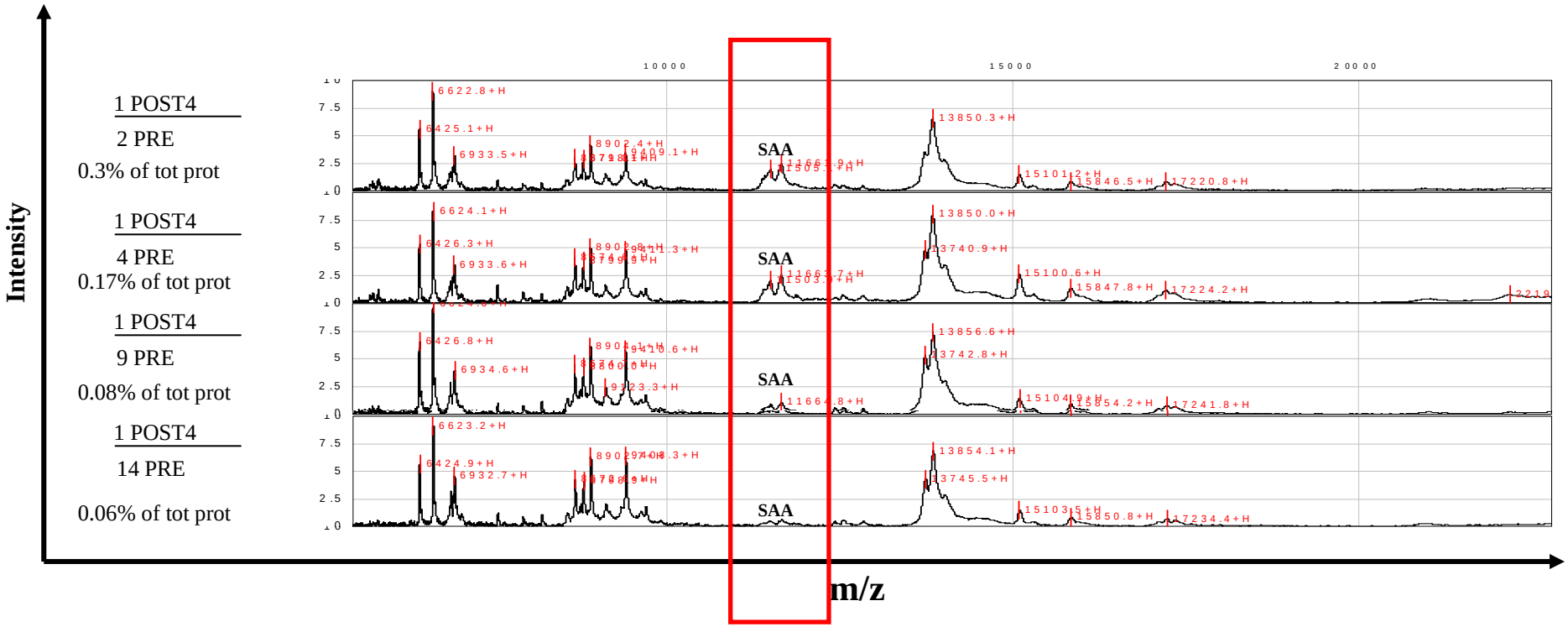
Rossi L. et al., Proteomics 2005 in press

QUANTIFICATION OF SERUM FACTORS CONCENTRATION BY IMMUNONEPHELOMETRY

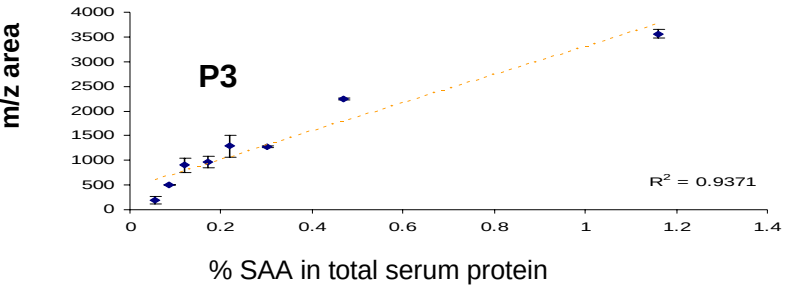


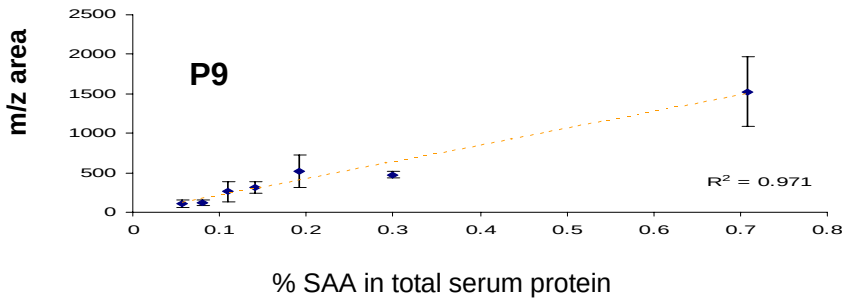
Rossi L. et al., Proteomics, 2005 in press

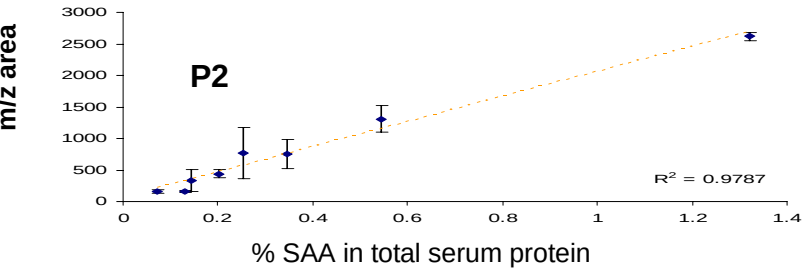
SEQUENTIAL DILUTION OF ONE POST 4 SAMPLE WITH THE CORRESPONDING PRE SAMPLE.

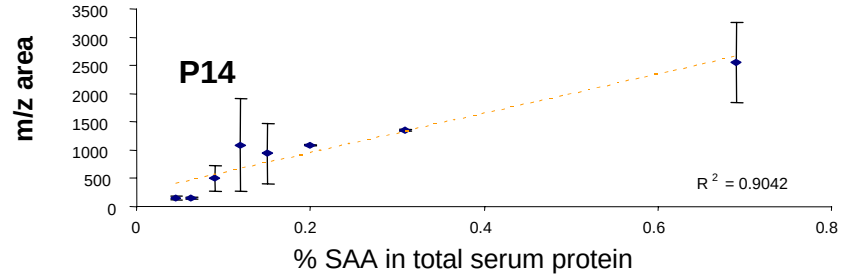


CORRELATION BETWEEN % SERUM SAA IN TOTAL SERUM PROTEIN AND THE RELATIVE M/Z AREA IN PATIENT SERA









Rossi L. et al., Proteomics, 2005 in press

The interplay between different platforms could provide a variety of information

Critical aspect: collection of good quality material

1) Simultaneous detection of the expression levels of several factors

2) Bio-functional suggestions → isoforms
→ Protein to protein interaction

3) Discovery of new factors

SUMMARY

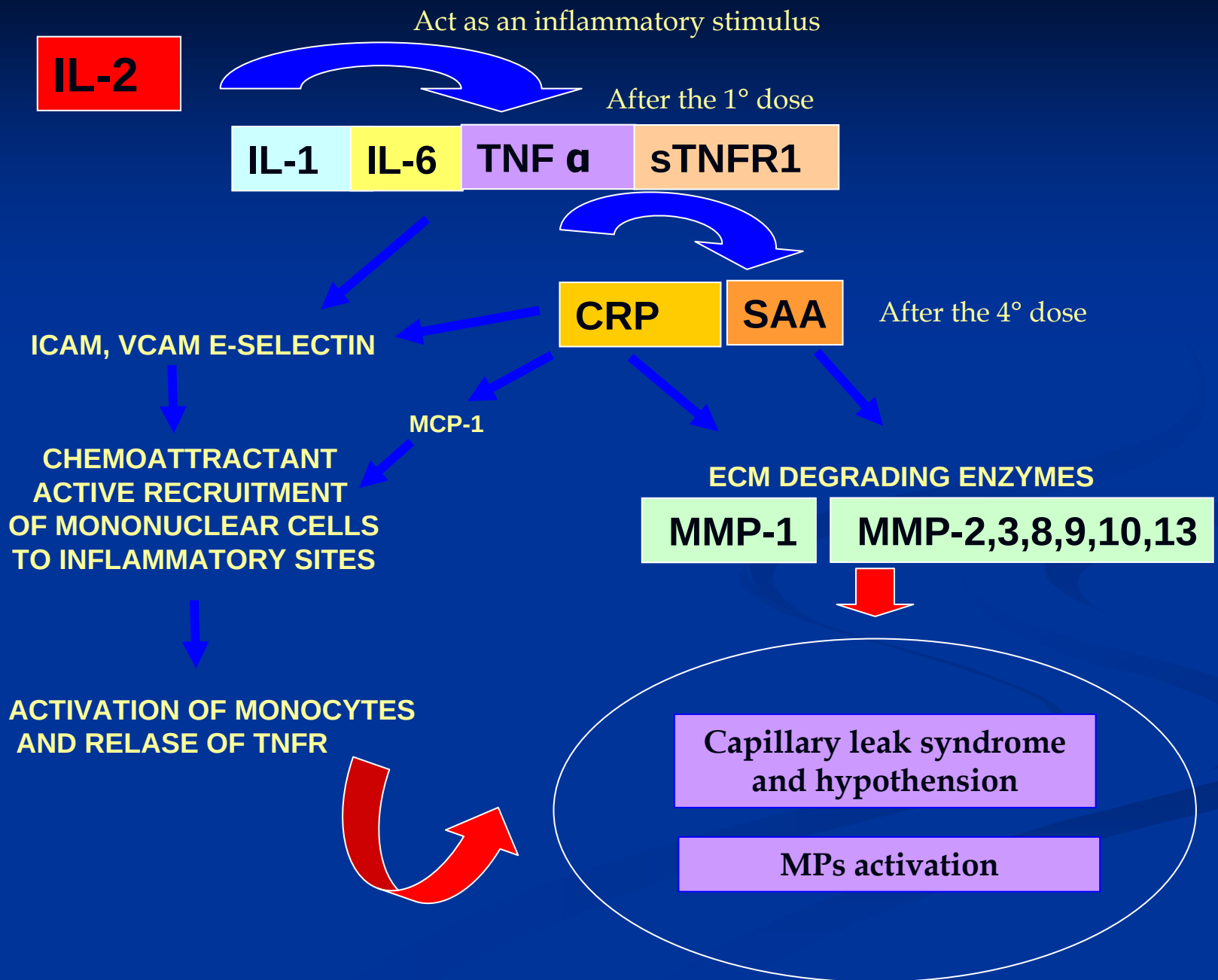
1. We observed an outburst of a multitude of proteins increasing/reducing in the circulation in response to IL-2:

2. Changes became more dramatic with increasing doses

3. Subclasses of soluble factors displayed different kinetics

4. All these data well correlate with a systemic inflammatory profile

PROTEIN INTERPLAY FOLLOWING HIGH DOSE IL-2 THERAPY



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