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Decoding the signaling pathways modulated by phosphatases of intracellular pathogens.

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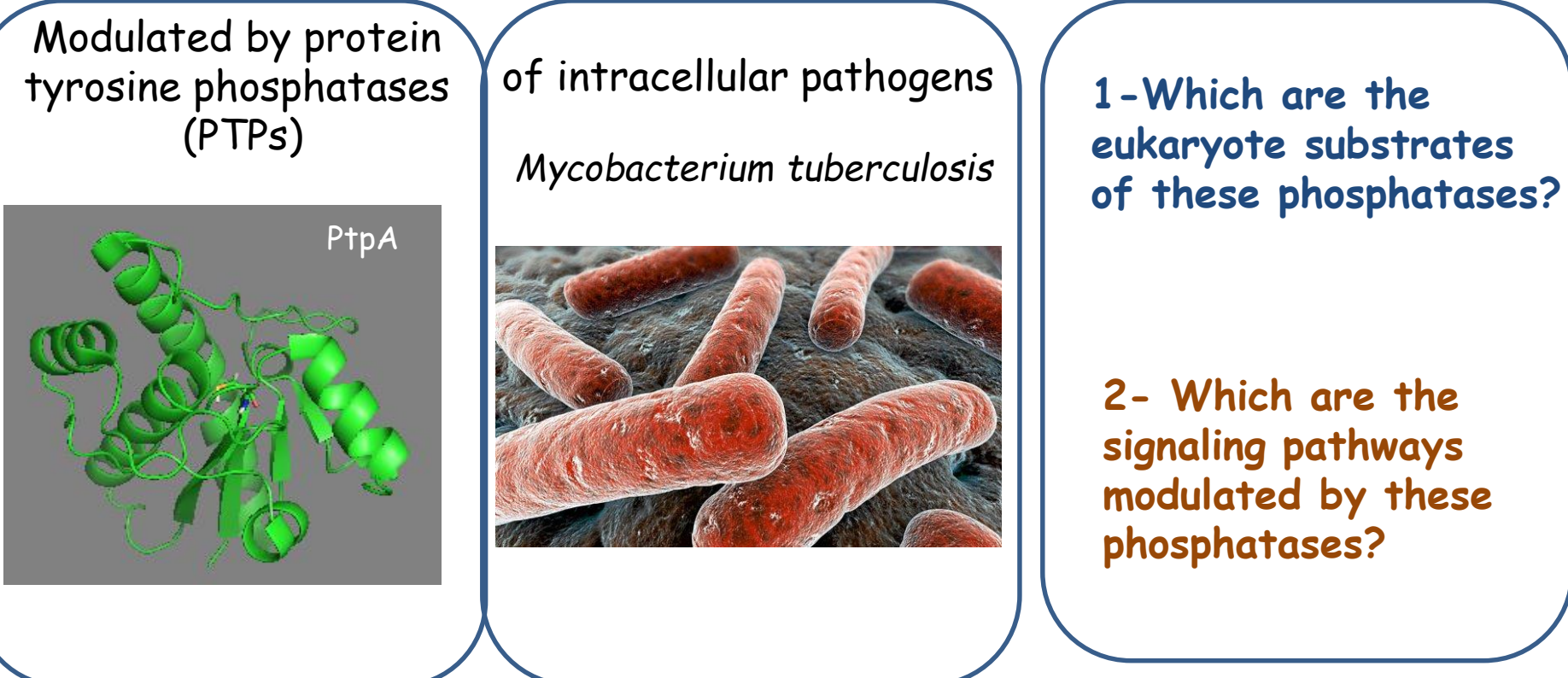
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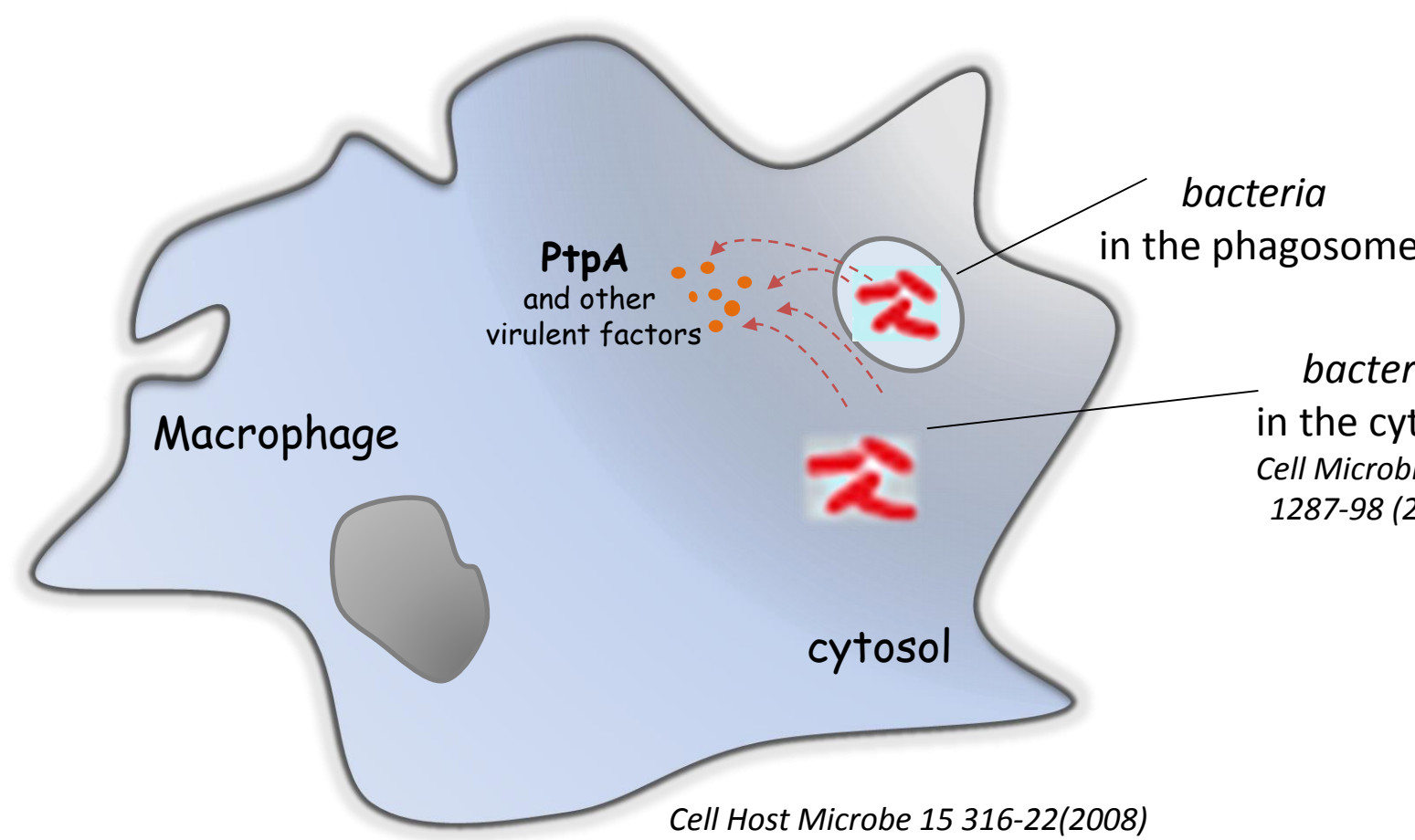
Decoding the signaling pathways modulated by phosphatases of intracellular pathogens.

Mariana Margenat, Danilo Segovia, Dario Porley, Vivian Irving, Ana Ramón, Gwénaëlle André-Leroux, Ana María Ferreira, Mabel Berois, **Andrea Villarino**.

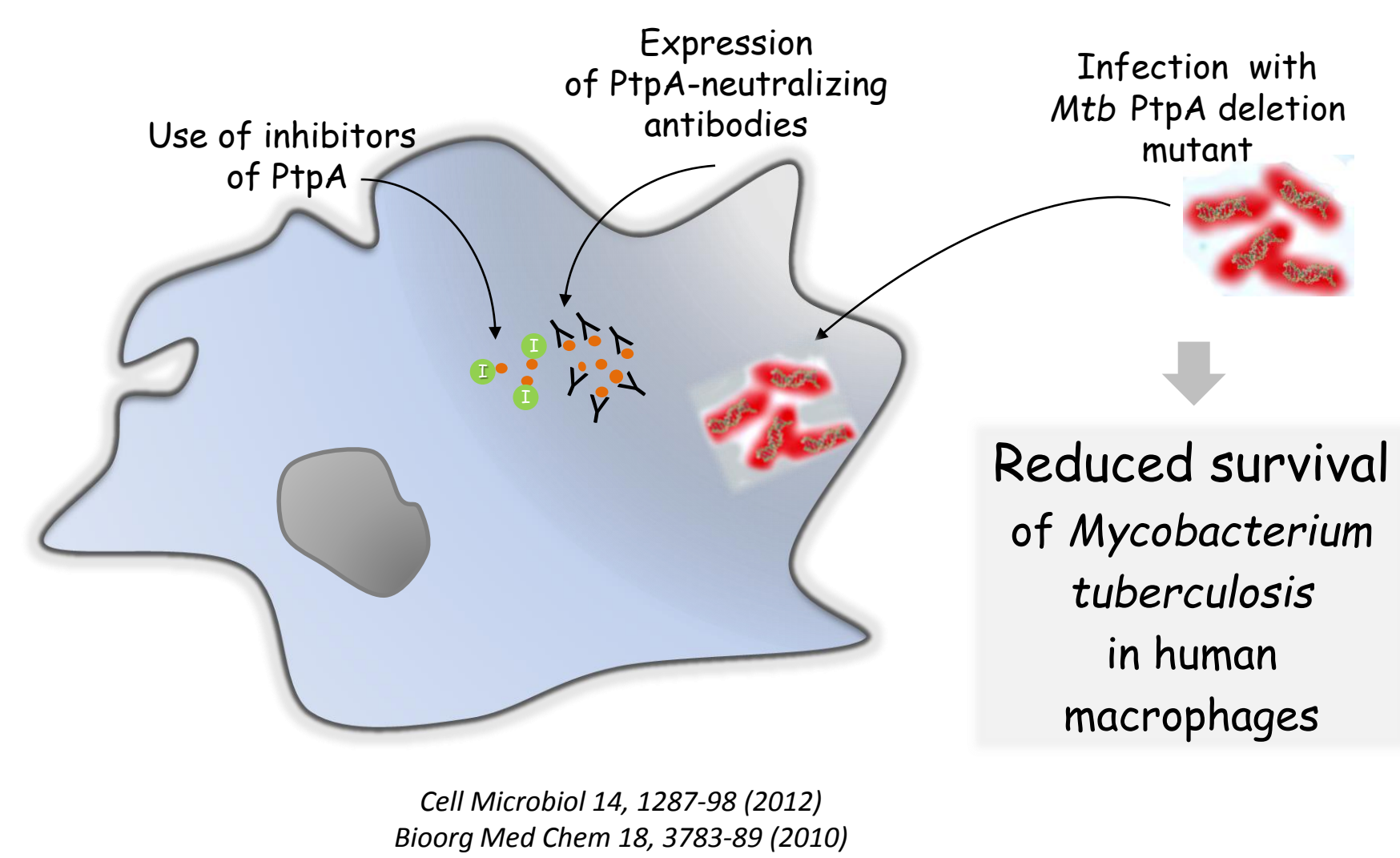
Decoding the eukaryotic signaling pathways



✓ The tyrosine phosphatase PtpA from *Mycobacterium tuberculosis* has been detected in the cytosol of infected macrophages



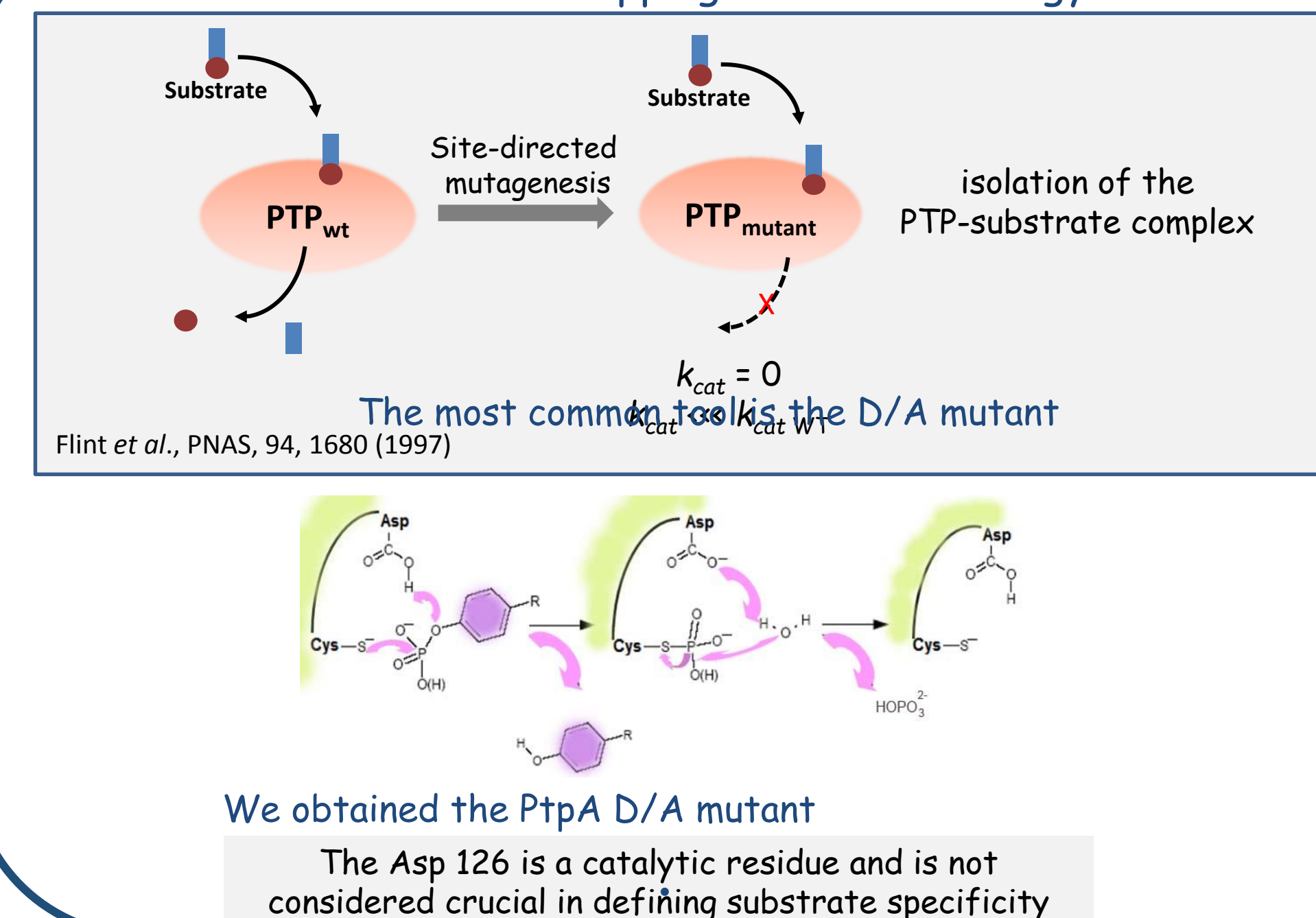
✓ PtpA is a key virulent factor



✓ Important role of mycobacterial phosphatases in the colonization of spleen and lung

MtbΔmms (PtpA, PtpB, SapM genes disrupted)
 Plos ONE, 8, e77930 (2013)
 No bacilli were recovered in spleens and lungs of guinea pigs after 10 weeks of inoculation

"PTP substrate trapping mutants strategy"

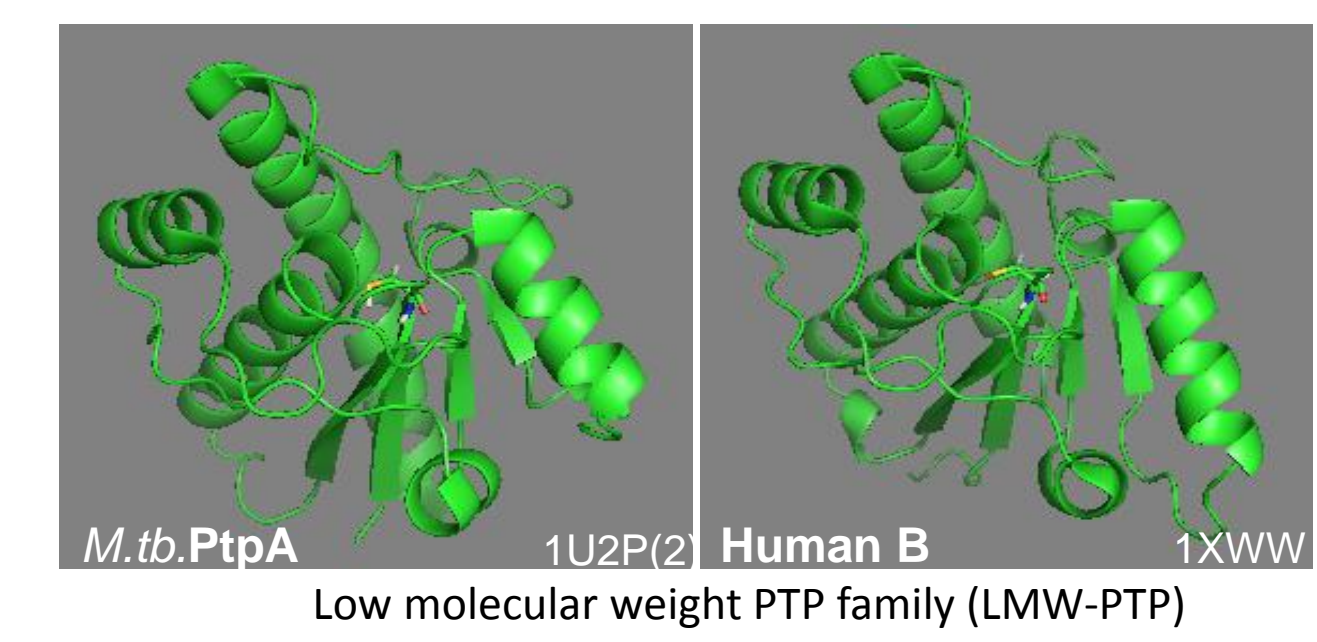


1- Which are the eukaryote substrates of these phosphatases?

"Improved PTP substrate trapping strategy"

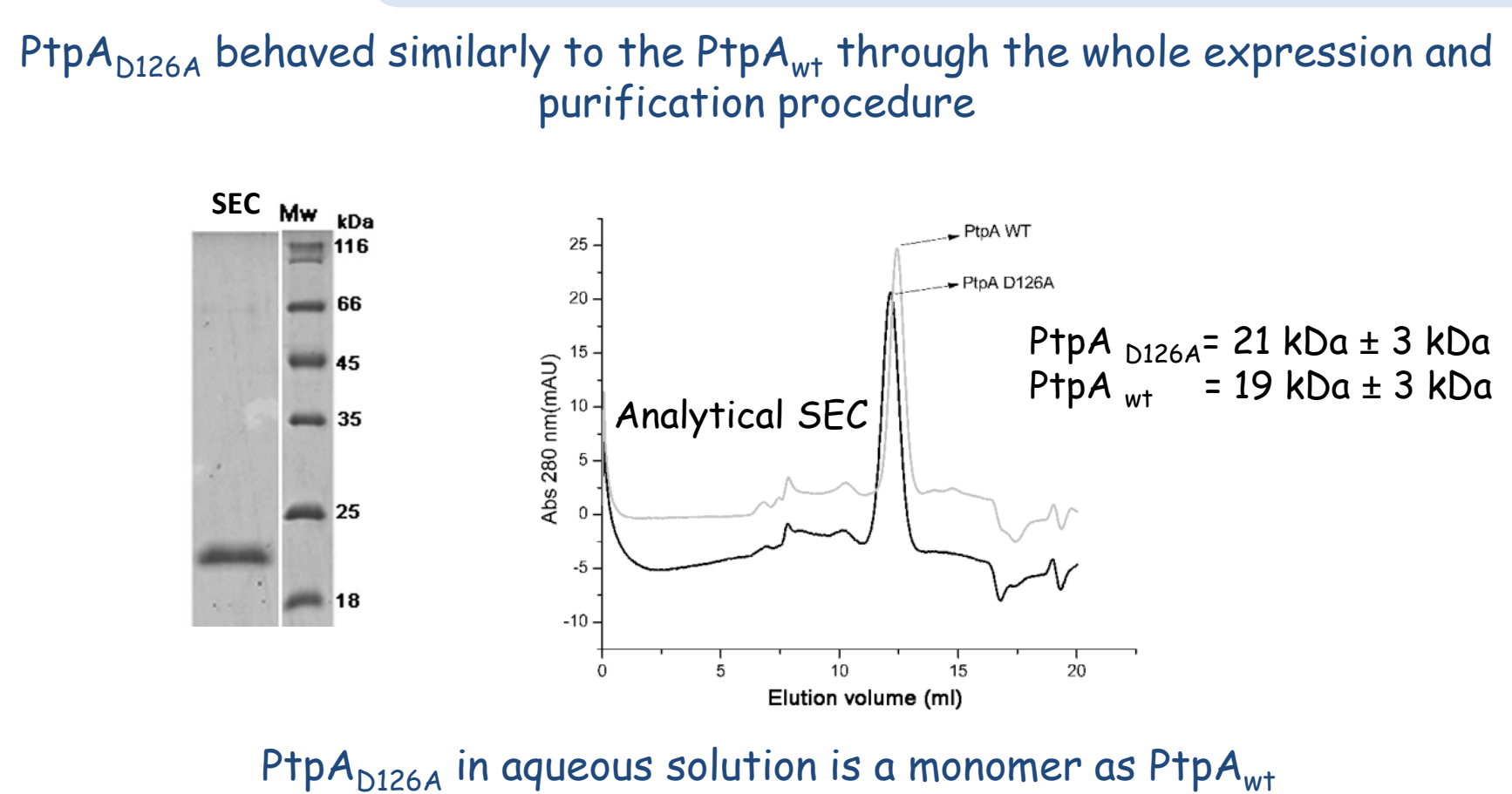
- Verification of the structural and biochemical properties of the PtpA D126A mutant to ensure its adequacy for substrate trapping
- Preparation of extracts of human-macrophage-like THP-1 cells preserving phospho-tyrosine (P-Y) modifications
- Analysis by SPR of the interaction between the phosphatase and the macrophage protein extracts, selection of conditions for washing and elution steps.
- Substrate trapping assay using stringent experimental conditions defined by SPR to reduce non-specific interactions
- Validation of protein candidates as substrates of PtpA

PtpA shows 37% of sequence identity and high structural similarity to its human orthologue HCPTPB.

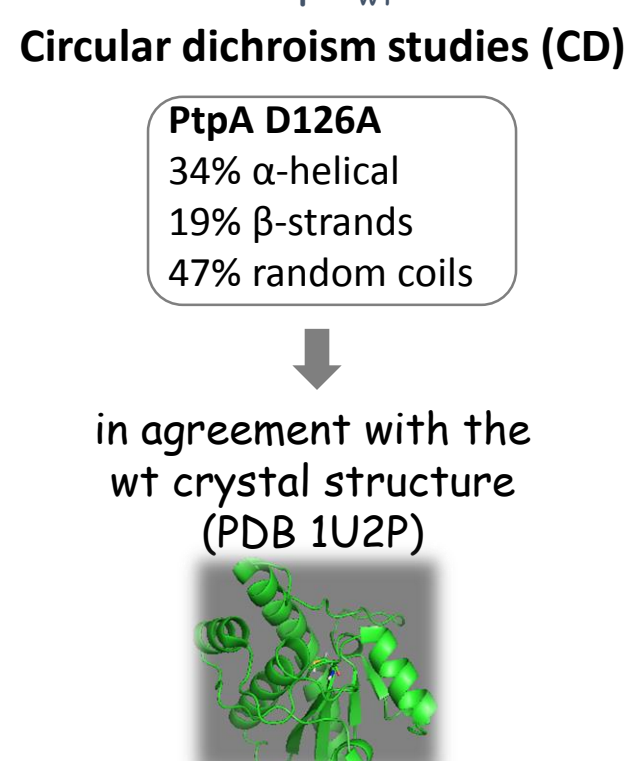


The human genome encodes for 107 PTPs but only one belongs to the PtpA family. Call 117, 699-711 (2004)

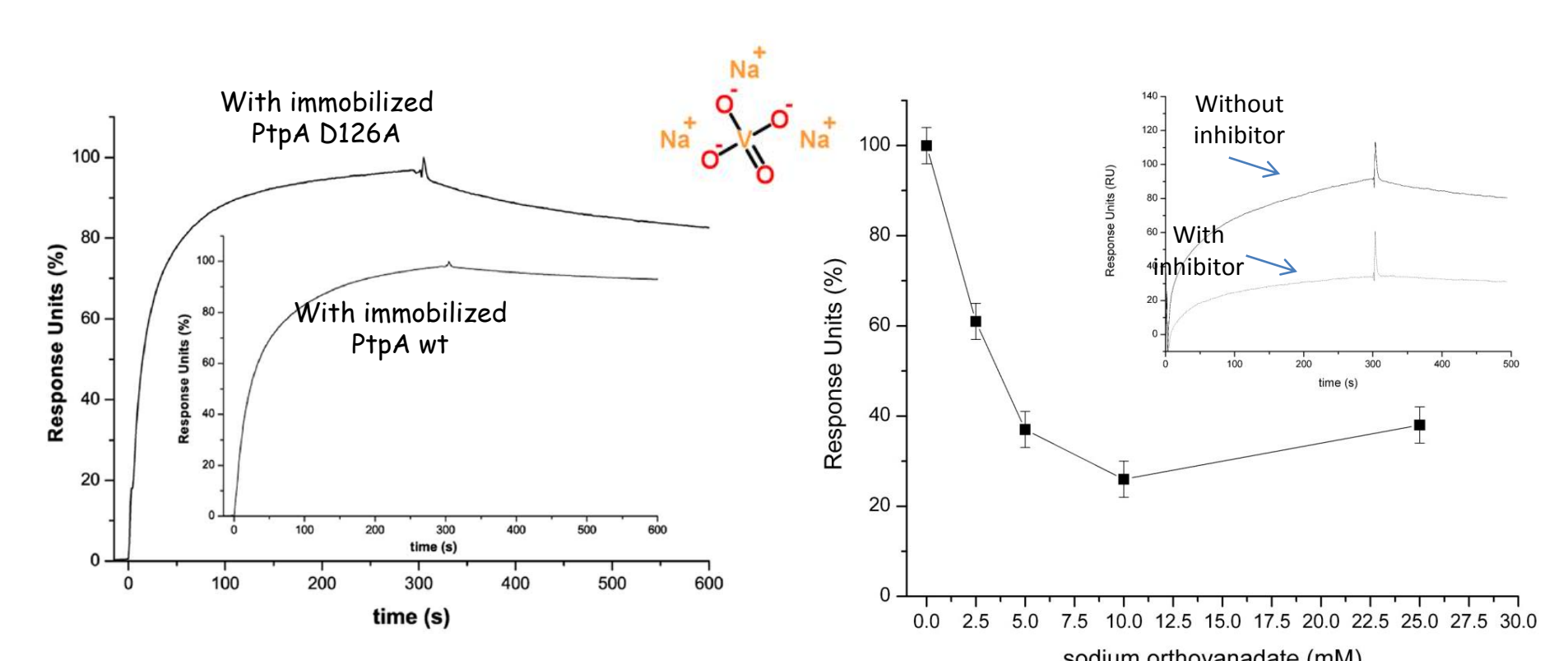
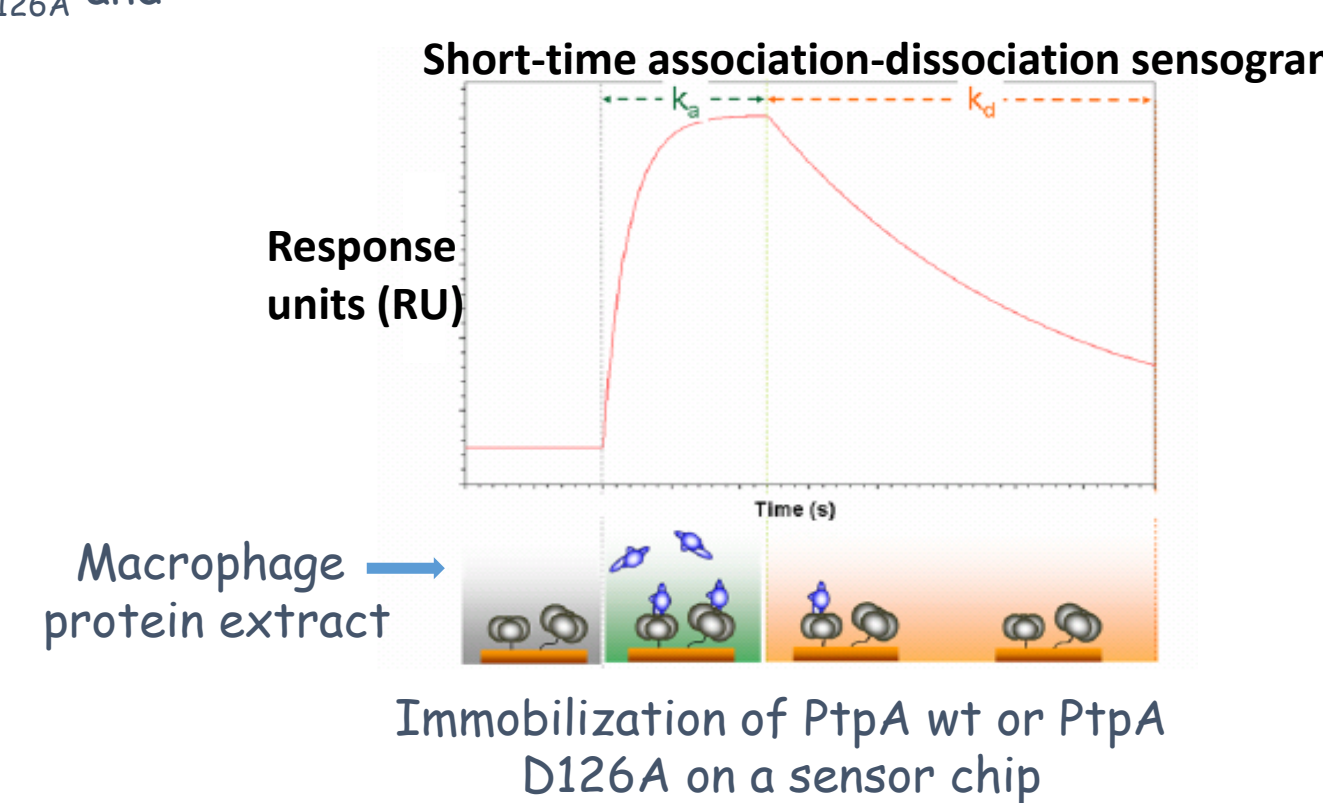
Verification of the structural and biochemical properties of the PtpA D126A mutant to ensure its adequacy for substrate trapping



The secondary structure of PtpA_{D126A} and PtpA_{wt} are basically equal

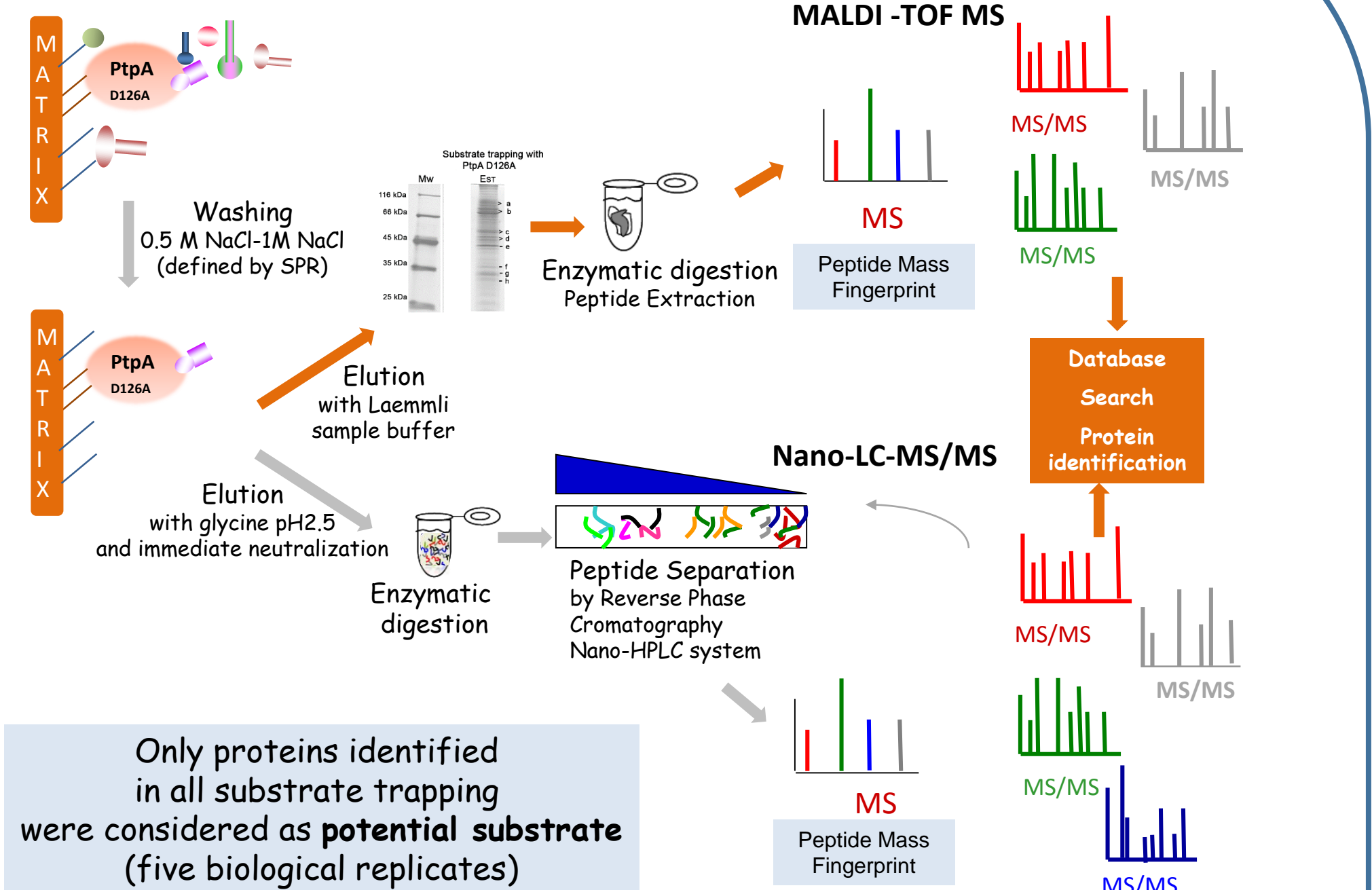


Study by Surface Plasmon Resonance (SPR) how PtpA interacts with macrophage protein components



The macrophage extract contained components which were capable of binding to PtpA mutant through the active site

Substrate trapping assay



Only proteins identified in all substrate trapping were considered as potential substrate (five biological replicates)

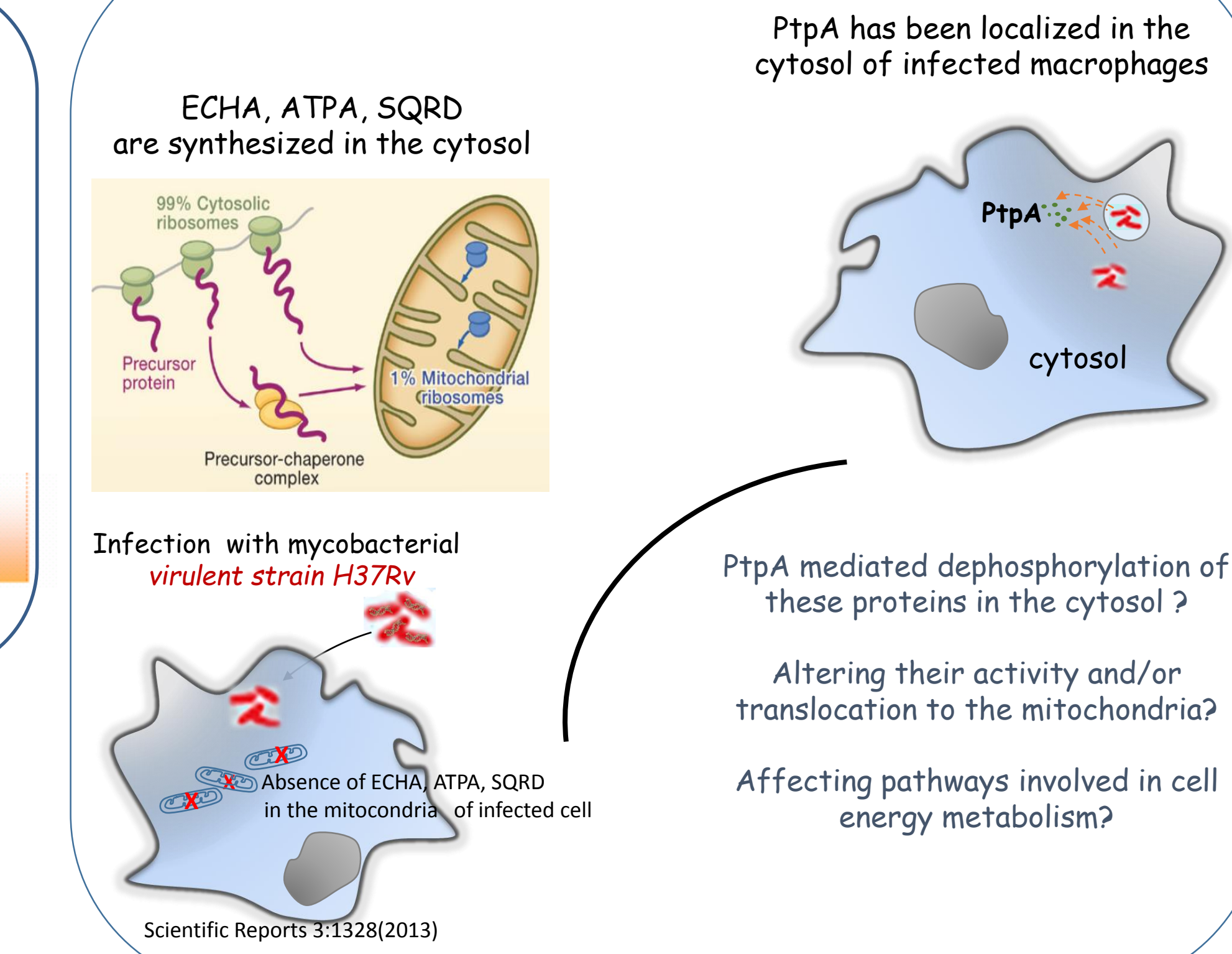
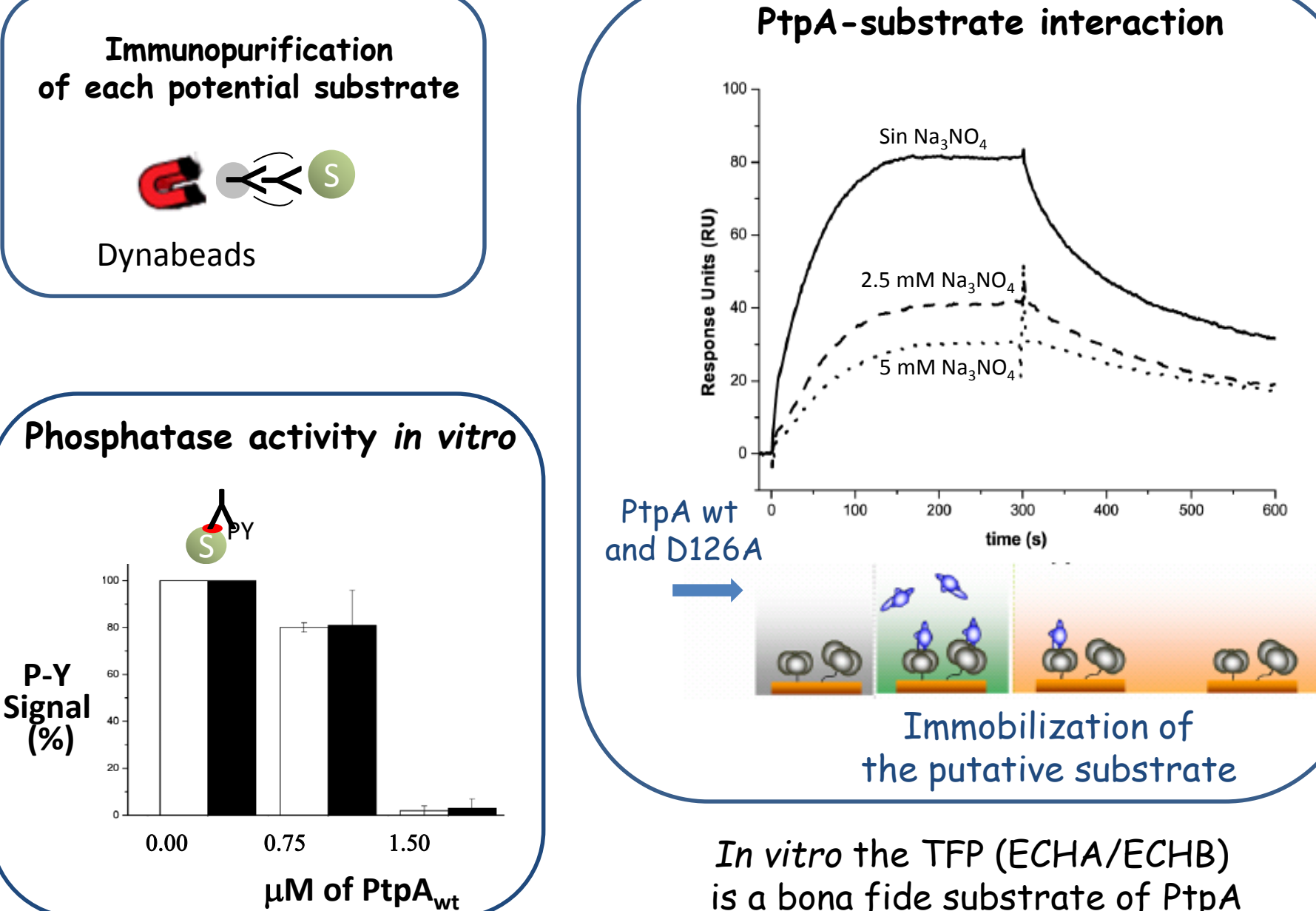
Four novel putative PtpA substrates

| Biological process* | Protein accession | Protein name | Mascot score ^b | Mass (Da) | No. of matched ions ^a | No. of peptide sequences ^a |
|----------------------------------------------------------|-------------------|---------------------------------------------------------|---------------------------|-----------|----------------------------------|---------------------------------------|
| Lipid metabolism, fatty acid beta-oxidation | ECHA_HUMAN | Trifunctional enzyme subunit alpha, mitochondrial | 2422 | 82947 | 63 | 23 |
| Sulfide oxidation, using sulfide: quinone oxidoreductase | SQRD_HUMAN | Sulfide:quinone oxidoreductase, mitochondrial | 705 | 49929 | 28 | 10 |
| Respiratory electron transport chain | ATPA_HUMAN | ATP synthase subunit alpha, mitochondrial | 531 | 59714 | 16 | 7 |
| Glycolysis | K6PP_HUMAN | Glyceraldehyde 3-phosphate dehydrogenase, platelet type | 922 | 85542 | 47 | 15 |

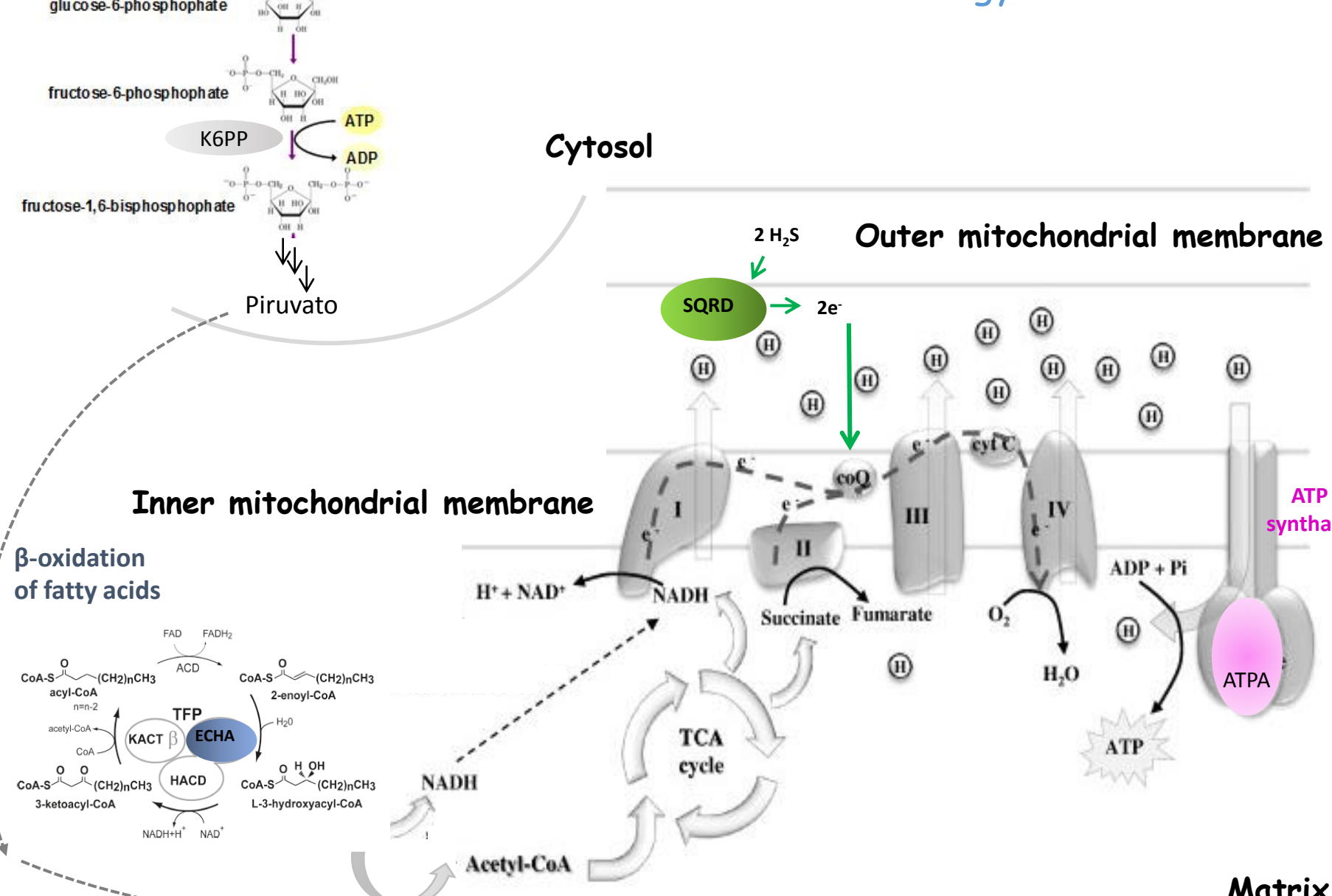
* Only the main biological processes with traceable author statement are shown. from UniProt (http://www.uniprot.org/ database (released on January 2014)).
^a The value of score, number of matched ions and peptide sequences is the best value obtained.

High salt concentration seems to be a good choice for washing steps to reduce non-specific binding and low pH would be useful for eluting the proteins bound to PtpAD126A.

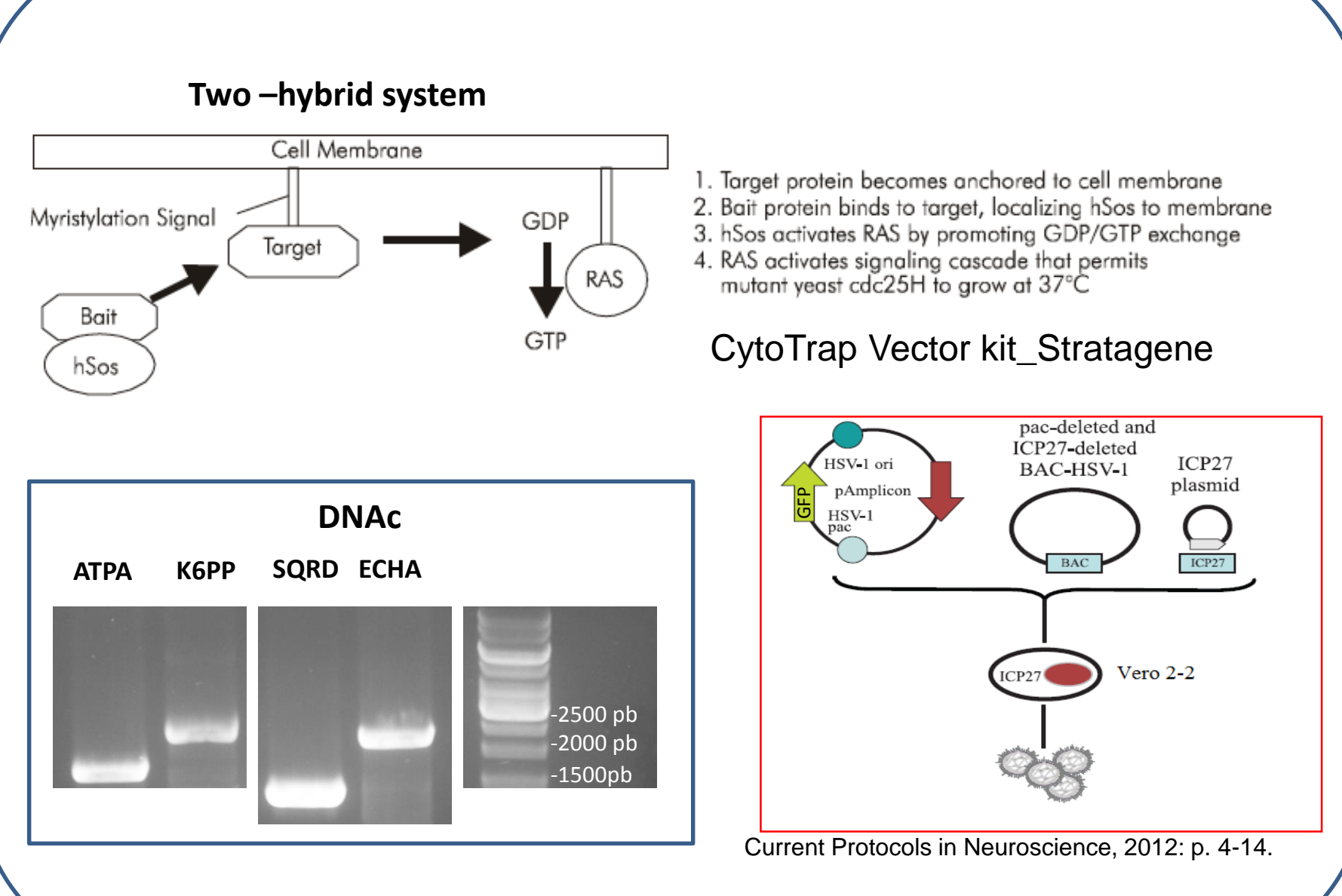
By different approaches we are addressing the validation of these proteins candidates as PtpA substrates.



All putative substrates related to energy metabolism



Interaction at a cellular level



Decoding the eukaryotic signaling pathways

