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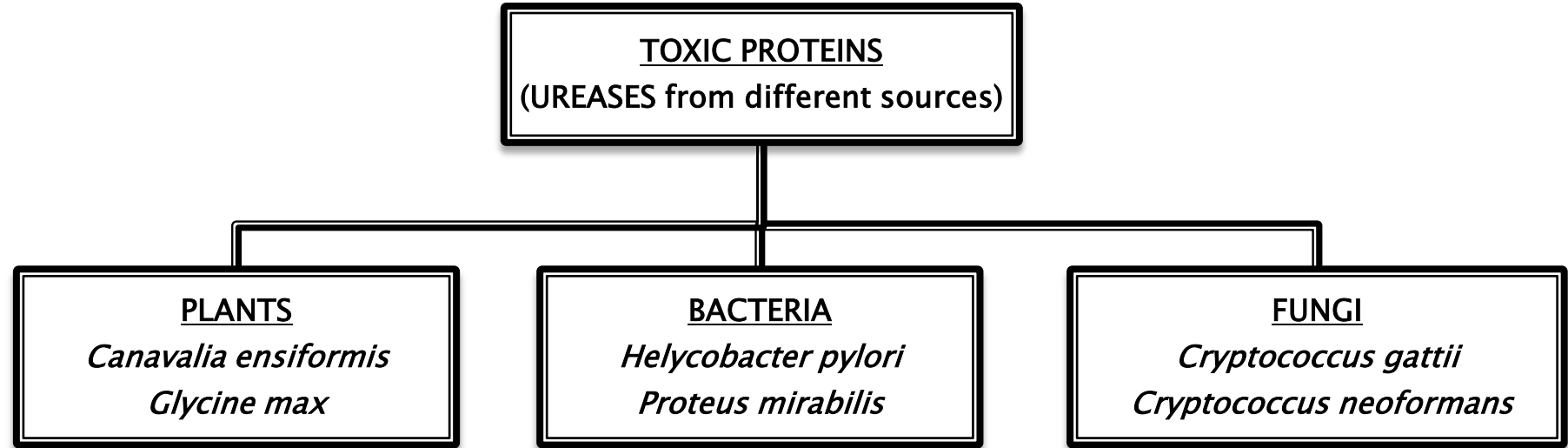
***PLATELET AGGREGATION UNDER A QUANTITATIVE
PROTEOMICS POINT OF VIEW:
ADP, COLLAGEN AND UREASES AS AGONISTS***

Dr. Diogo Ribeiro Demartini - Postdoctoral Fellow

Laboratory of Toxic Proteins

Department of Biophysics and Center of Biotechnology

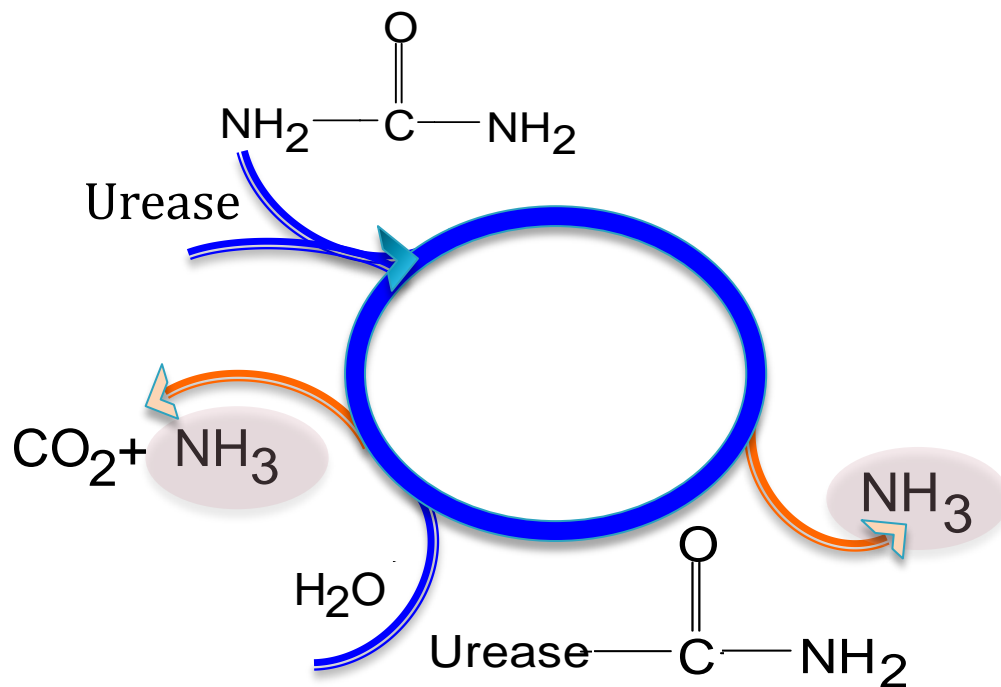
UFRGS- Porto Alegre, RS. Brazil



Prof. Célia Carlini
ccarlini@ufrgs.br

1) Ureases – introduction

Ureases (EC 3.5.1.5) are nickel-dependent enzymes



Synthesized by:

Plants
Fungi
Bacteria
(not by animals)

Physiological roles:

Nitrogen metabolism
Adaptation to acidic environment
Defense
Communication

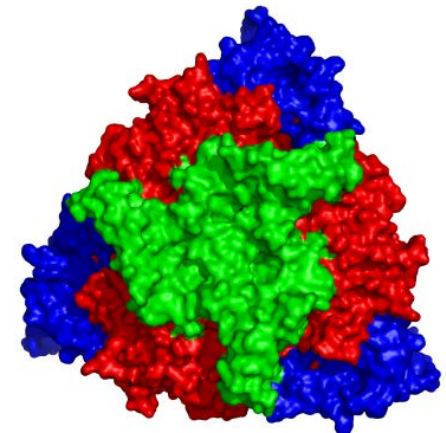
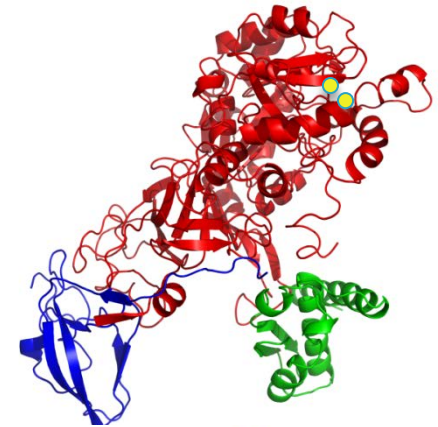
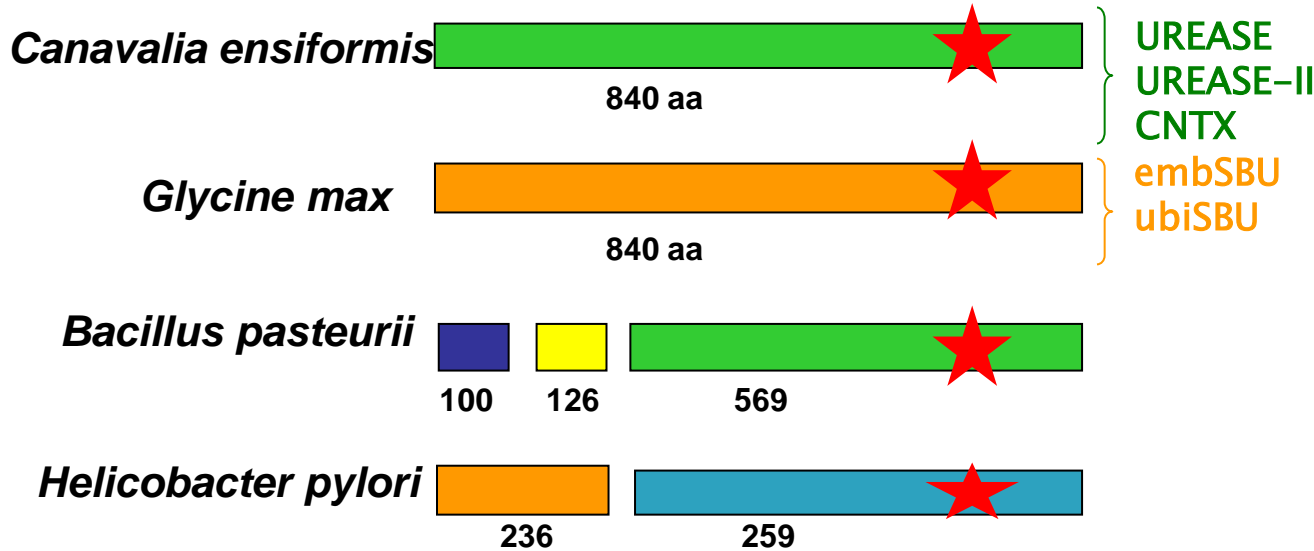


1) Ureases – introduction



Ureases (EC 3.5.1.5) from different sources share ~ 50 % identity

Isoforms



Differences in
quaternary structure

Dimer
Trimer
Hexamer
Dodecamer

Klebsiella aerogenes
urease monomer X trimer

2) Ureases – effects unrelated to the enzymatic activity

Canavalia ensiformis
UREASE – UREASE
(2 x 90 kDa)



Insecticidal
Fungytoxic
Platellet aggregation

Staniscuaski, F. et al. (2005). *Toxicon* **45**, 753-760.
Postal, M. et al. (2012). *Peptides* **38**, 22-32.
Carlini, C. R. et al. (1985). *Br J Pharmacol* **84**, 551-560

Helicobacter pylori
UREASE - HPU
($\beta\alpha$)₃₄



**Gastric cancer
ulcers**

Platellet aggregation (collagen)
Neuhtophil activation

Wassermann, G. E. et al. (2010). *J Cell Mol Med* **14**, 2025-2034.
Uberti, A. F. et al. (2013). *Toxicon* **69**, 240-249.

Proteus mirabilis
UREASE - PMU
($\beta\alpha\gamma$)₃



**Severe urinary
tract infection**

Fungytoxic
Platellet aggregation (ADP)

Broll, V. et al., under preparation0

3) *Helicobacter pylori*

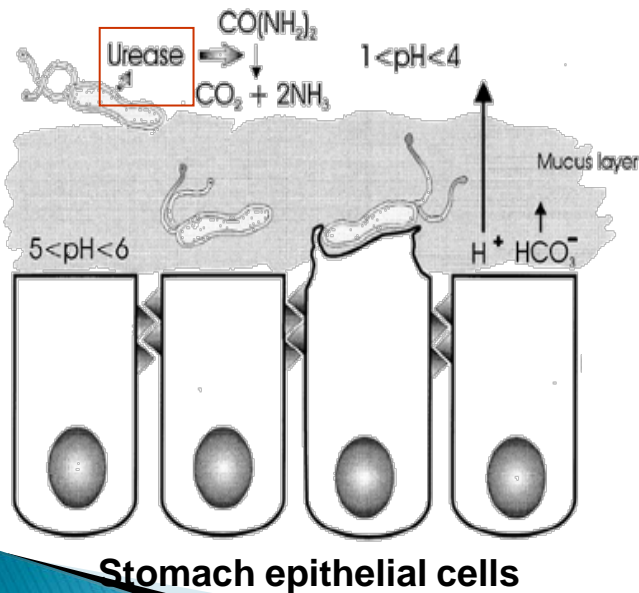


INFECTION AND STOMACH DISEASE

SEVERAL PATHOGENIC FACTORS ARE KNOWN

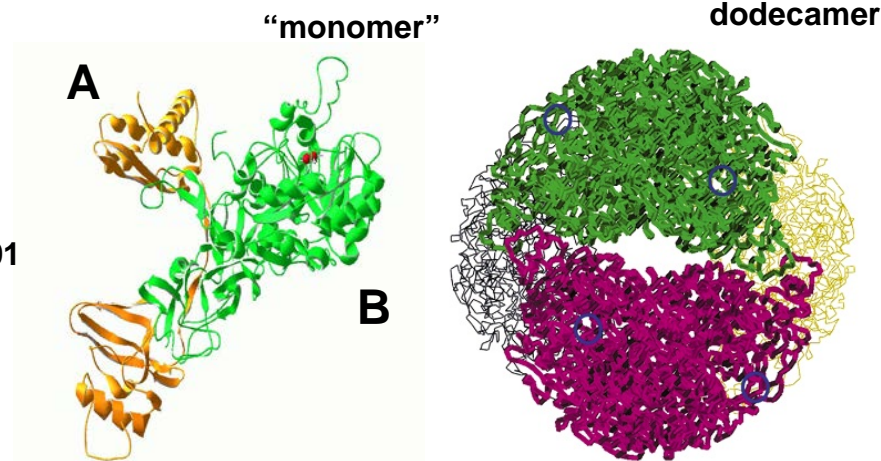
- Vacuolating cytotoxin VacA;
- *cagA* pathogenic island: modified flagella that enables bacterium to “inject” genetic material into the host cells;
- **Urease: (up to 10% total cell protein): important for colonization (alkalinization and binding to mucus glycoconjugates);**

Montecucco et al. 1999.



Chain A: 238 aa
Chain B: 569 aa

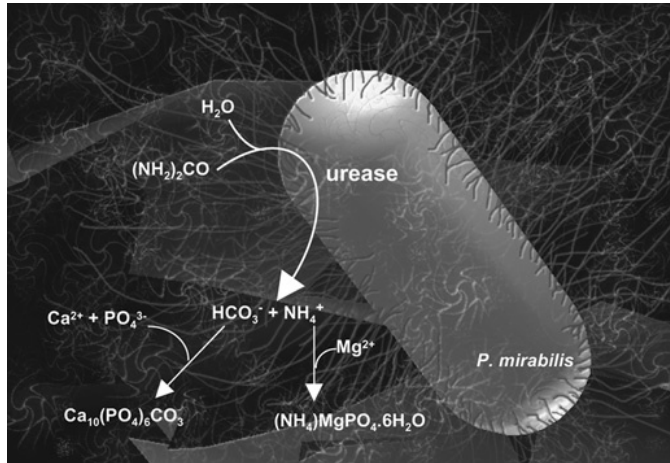
Ha et al., 2001



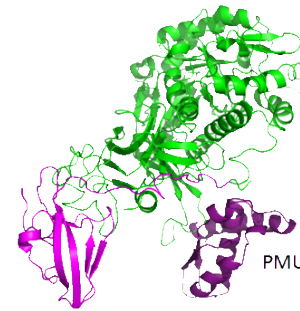
4) *Proteus mirabilis*



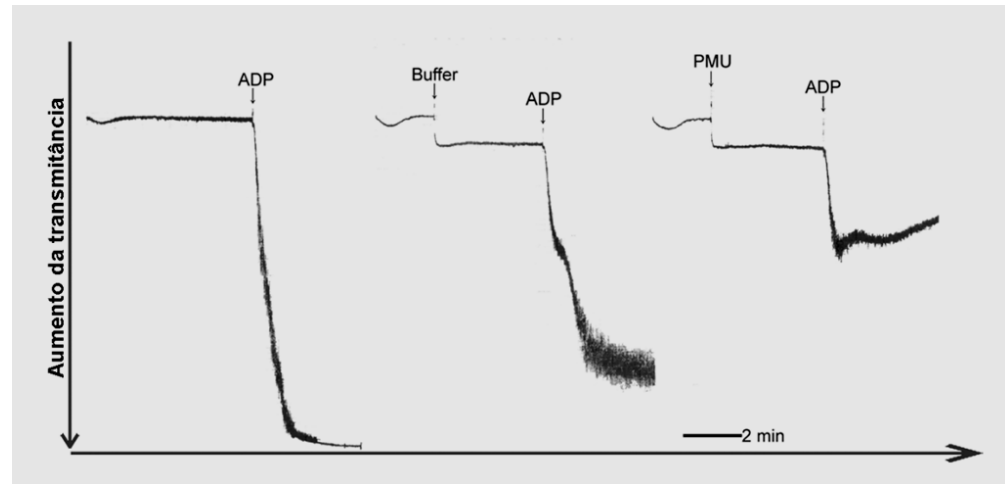
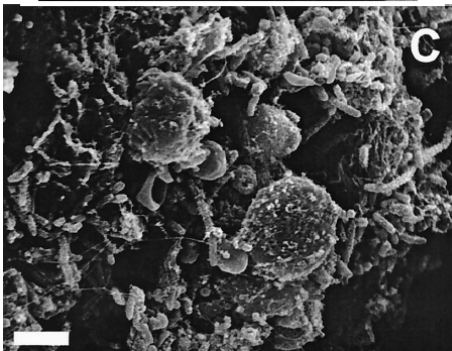
Proteus mirabilis and severe urinary tract infections



J Clin Pathol 2010;63:424e430



$(\alpha\beta\gamma)_3$



Valquiria Broll, Ph.D. student

5) Platelets

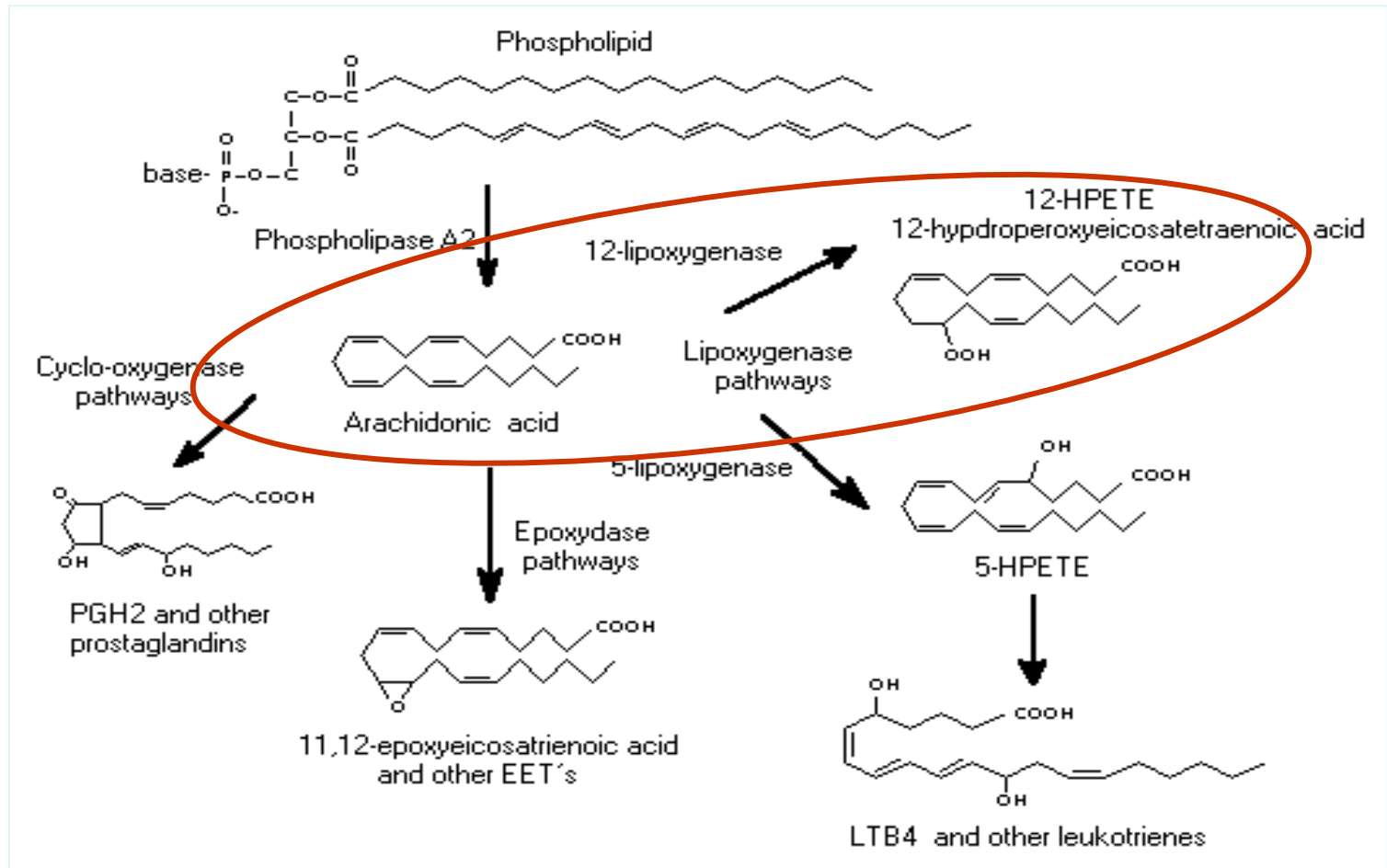


- Platelets play a key role in hemostasis: aggregation;
- When stimulated, platelets undergo exocytosis (release reaction): vasoactive amines, Ca^{2+} , **ADP**, coagulation factors;
- Physiological agonists: **ADP**, **collagen**, thrombin, thromboxane A_2 , PAF-acether, serotonin.
- Platelets respond to stimuli through multiple signalling pathways, which can be characterized pharmacologically using a panel of different antagonist/inhibitors.

5) Platelet aggregation triggered by ureases

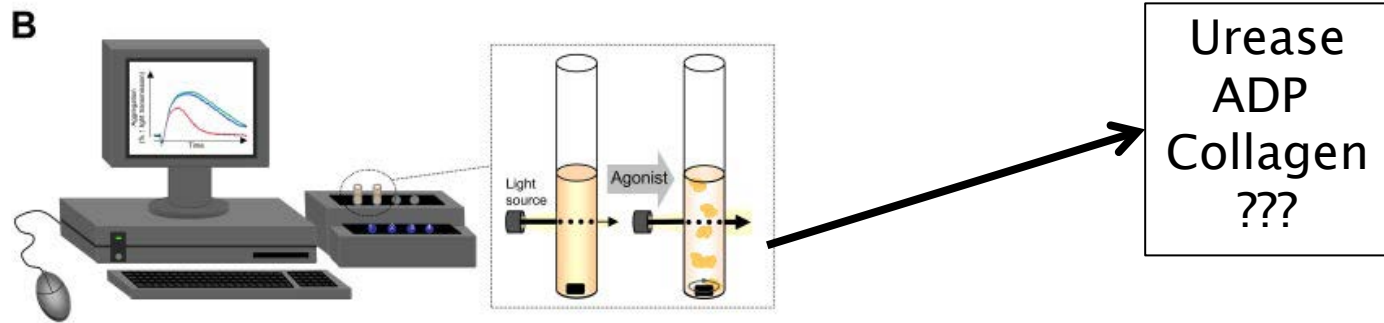
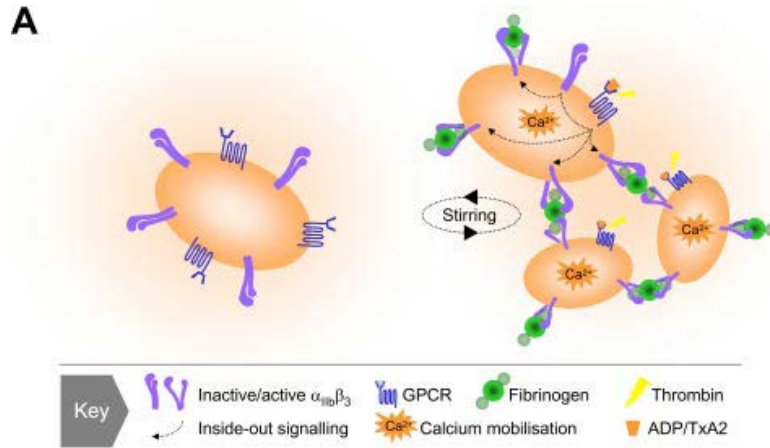


Eicosanoids pathways



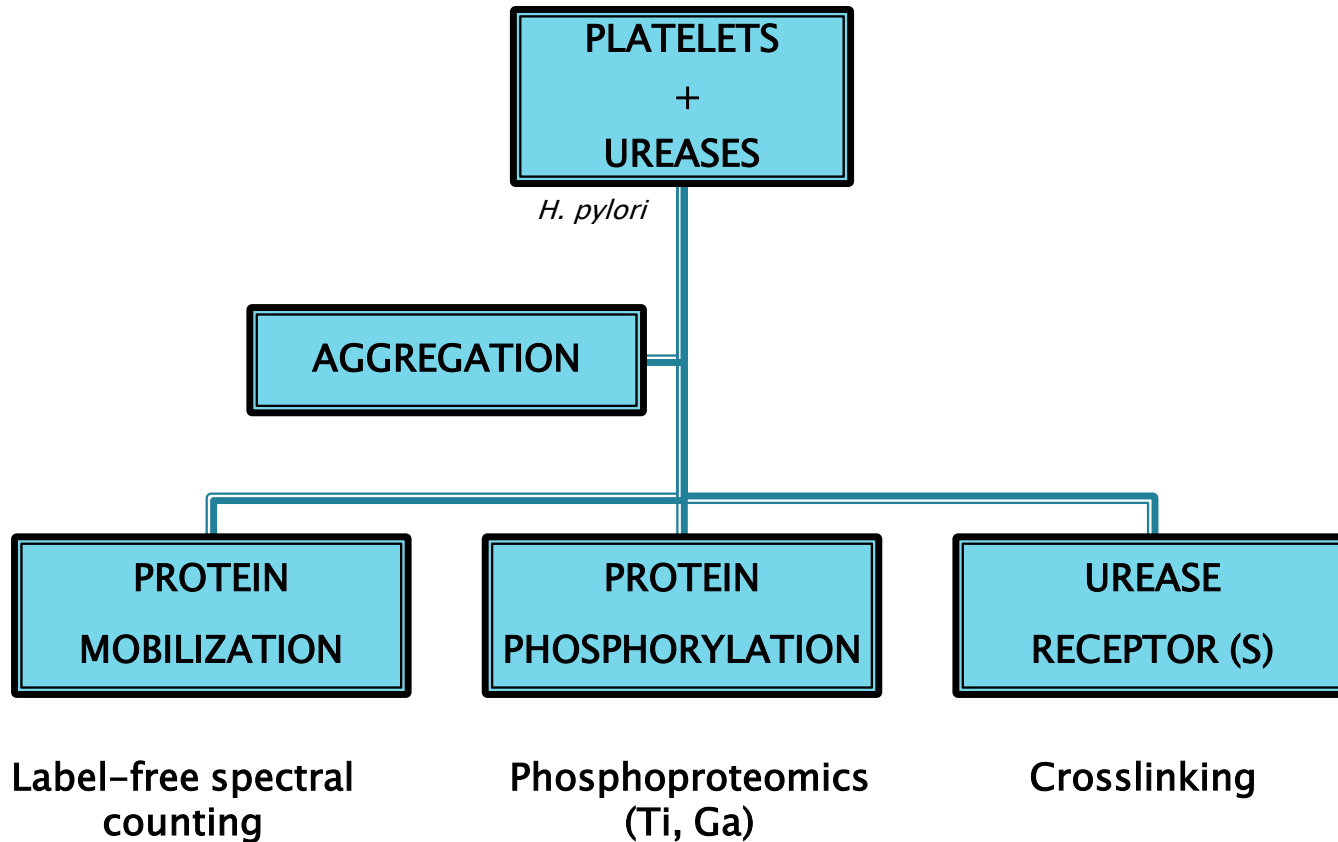
Reviewed in Olivera-Severo et al., 2006.

5) Platelets –the aggregation assay



BLOOD. 2007 109: 5087-5095

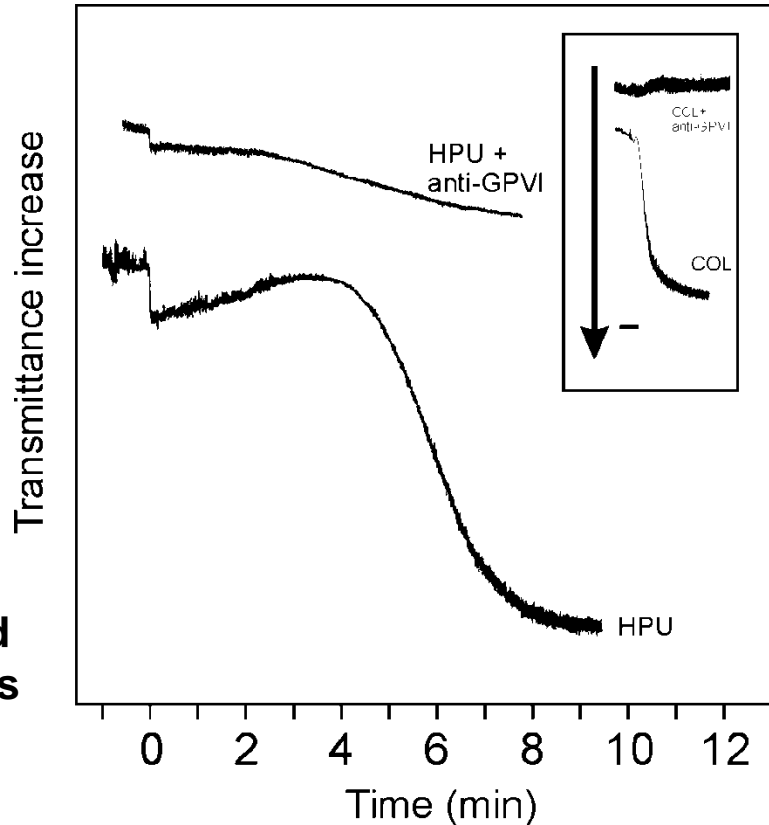
6- What are we looking for?



7- Why ADP and Collagen??



In case of collagen...



Anti-GPVI blocks HPU-induced aggregation of rabbits platelets

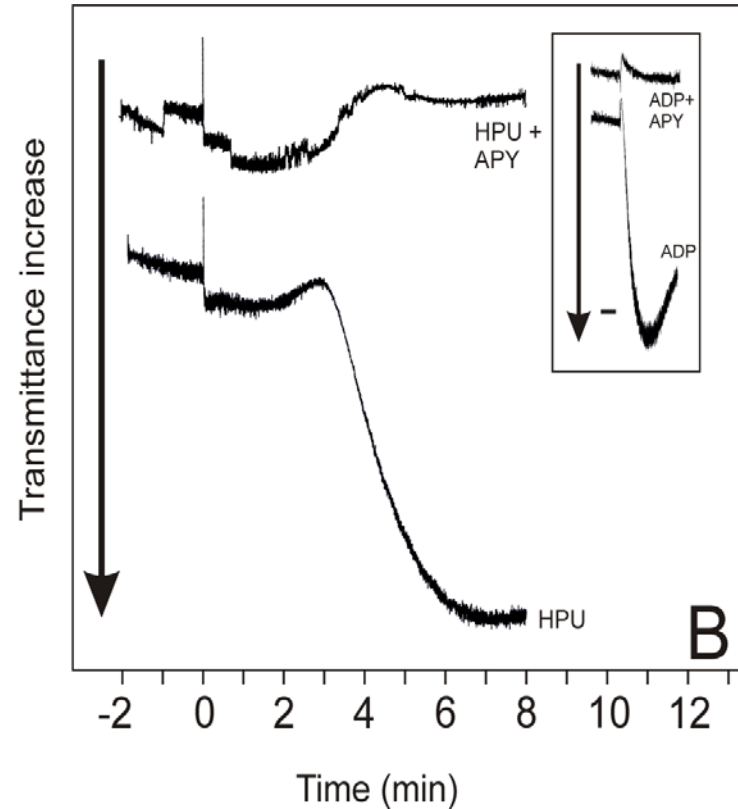
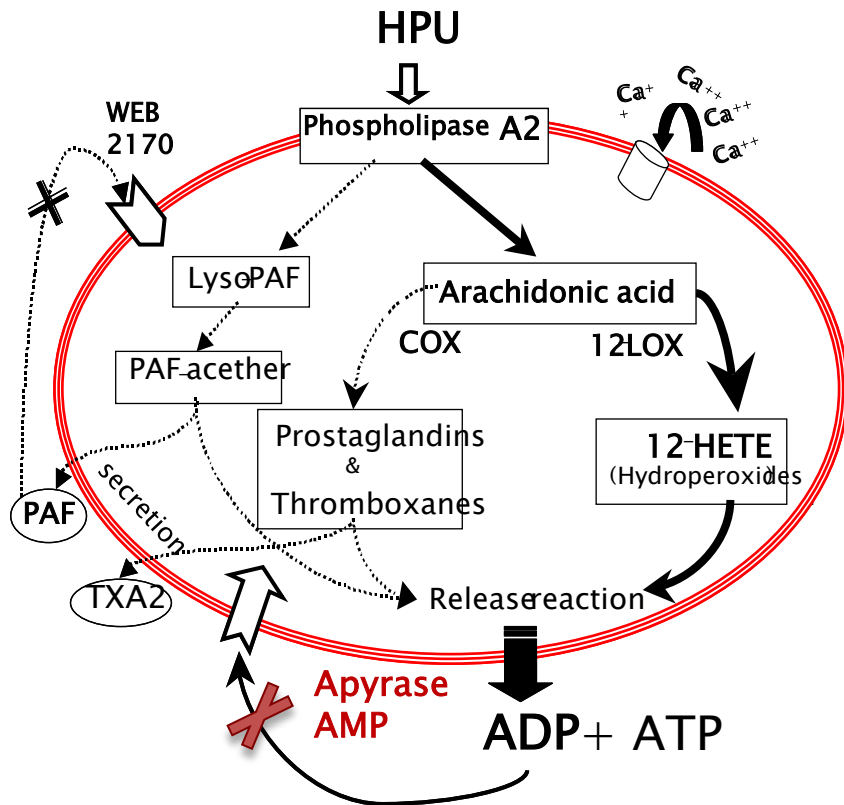
Platelets were pre-incubated with anti-GPVI (500 ug/mL, Santa Cruz) for 10 min, r.t. without stirring and then exposed to 300 nM HPU or 20 ug/mL collagen (inset).

7- Why ADP and Collagen??



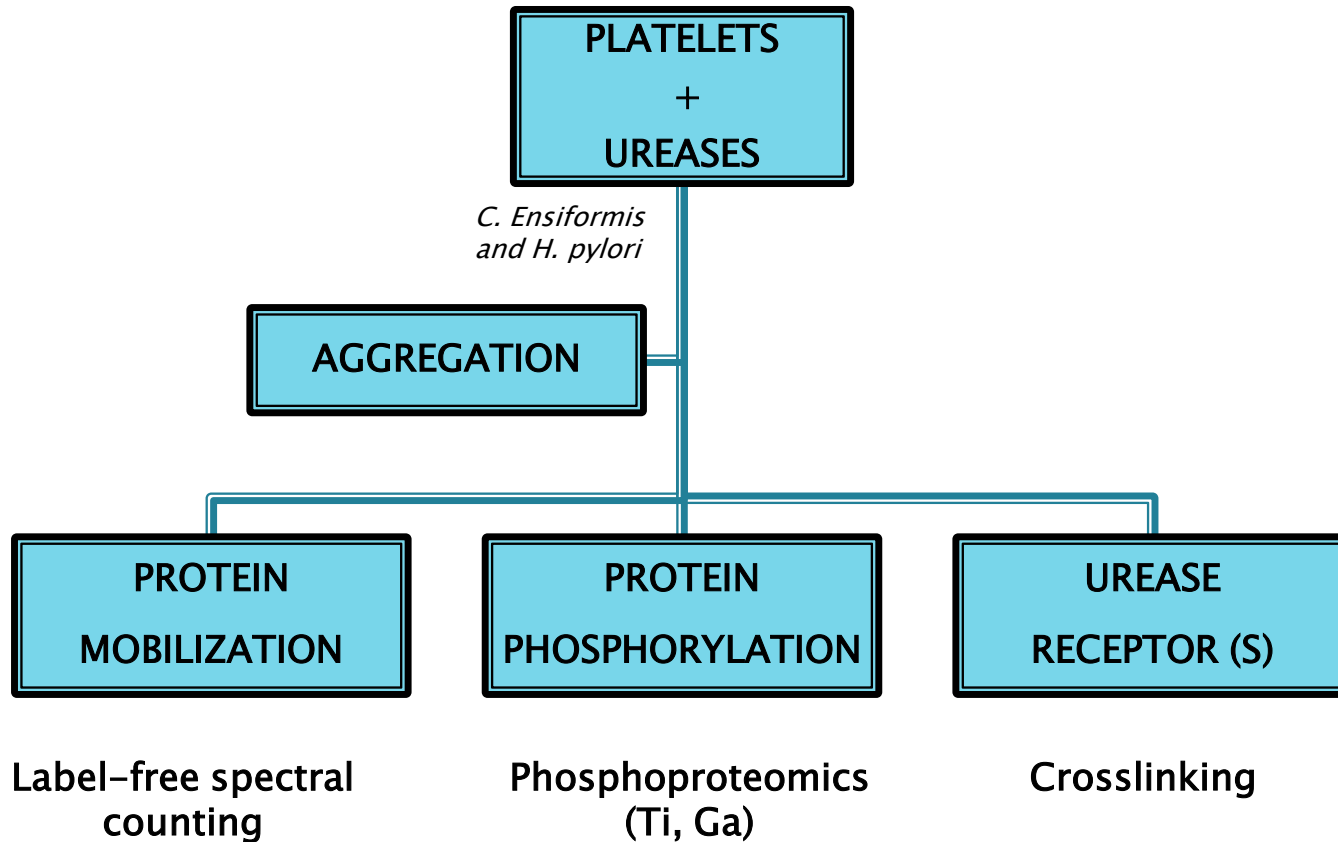
Helicobacter pylori urease induced platelet aggregation x eicosanoids

In case of ADP

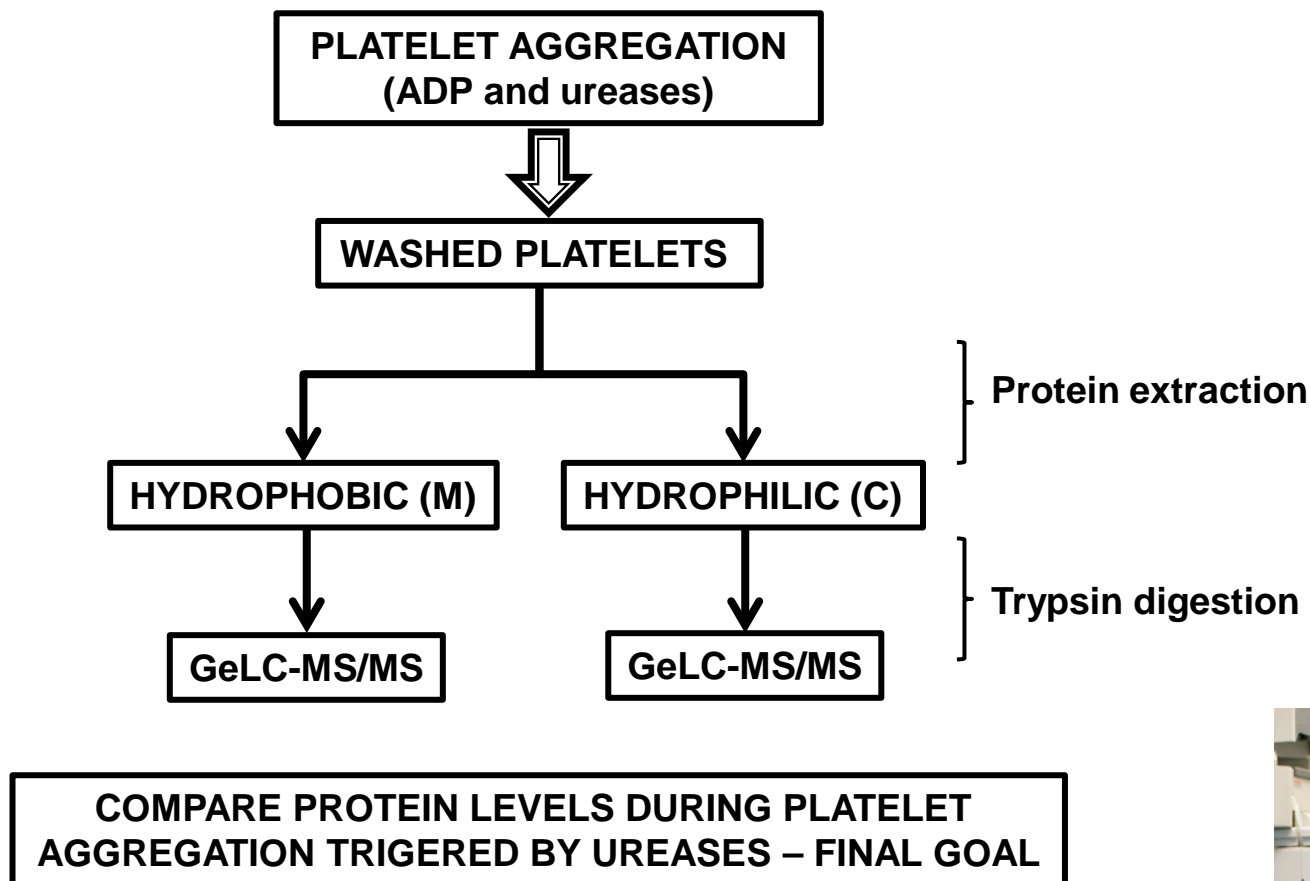


Wassermann, Olivera-Severo,, Uberti, Carlini, (2010)
J. Cell. Mol. Medicine 14, 2025-2034

8- What are we looking for? Again....



9- Experimental approach – first round!



ESI (LTX-XL) 90 min run - top 5;
+1 (*Xcorr* 1.5), +2 (*Xcorr* 2.0), +3 (*Xcorr* 2.5)
BioWorks 3.3.1;
Scaffold 3.0

Database:
Homo sapiens (taxid 9600 – UNIPROT)

Dr. Diogo Ribeiro Demartini
192th OMICS Conference –Chicago/Northbrook – Aug 4–6



Dr. Jay Thelen
University of Missouri
Columbia, MO

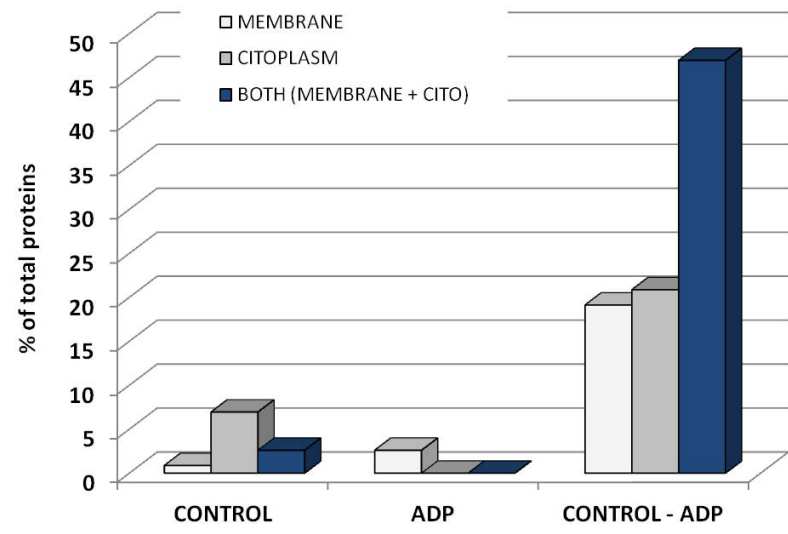
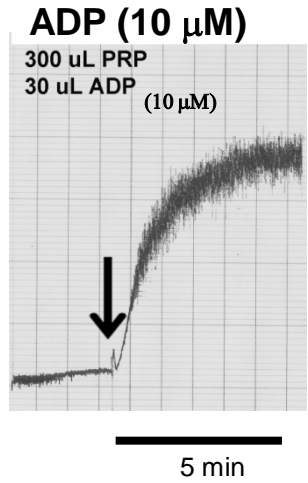
10/1/2014

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9- ADP and Canavalia ensiformis urease

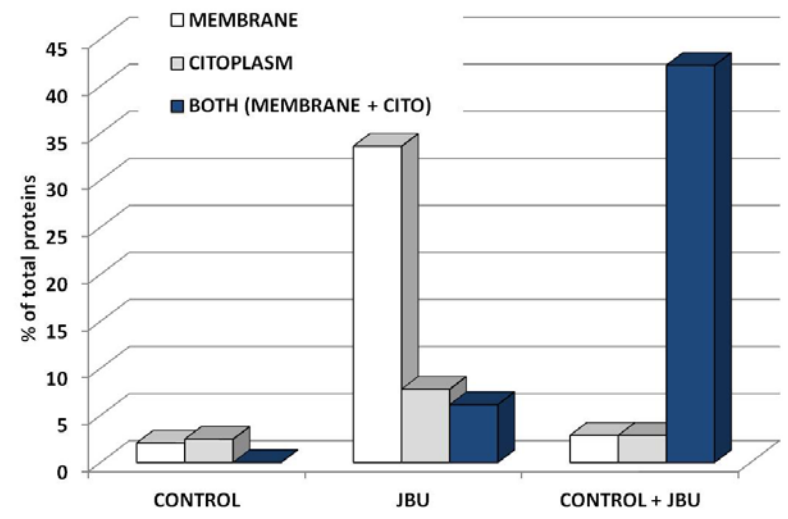


Transmittance increase



Transmittance increase

UREASE



9- ADP and Canavalia ensiformis urease



Some key proteins detected either in control and experimental condition

Protein	Acession	kDa	Ratio	Condition
14-3-3 PROTEIN ZETA/DELTA			2,87	UREASE/CONTROL
	P63104	28 kDa	1,46	ADP/CONTROL
COFILIN-1			2,01	UREASE/CONTROL
	P23528	19 kDa	0,77	ADP/CONTROL
INTEGRIN BETA-1			2,24	UREASE/CONTROL
	P05556	88 kDa	2,48	ADP/CONTROL
INTEGRIN BETA-3			0,92	UREASE/CONTROL
	P05106	87 kDa	0,87	ADP/CONTROL
GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE			6,00	UREASE/CONTROL
	P04406	36 kDa	0,70	ADP/CONTROL
FRUCTOSE-BISPHOSPHATE ALDOLASE A			1,86	UREASE/CONTROL
	P04075	39 kDa	1,12	ADP/CONTROL

* Number of total acquired spectra JBU/Control or ADP/control

- ATPase complex ($\alpha, \beta, \gamma, \delta$): detected only in UREASE condition;
- Adhesion and cytoskeletal proteins are prevalent in both conditions;
- Contaminants are present: we did not remove abundant proteins;

9- ADP and Canavalia ensiformis urease



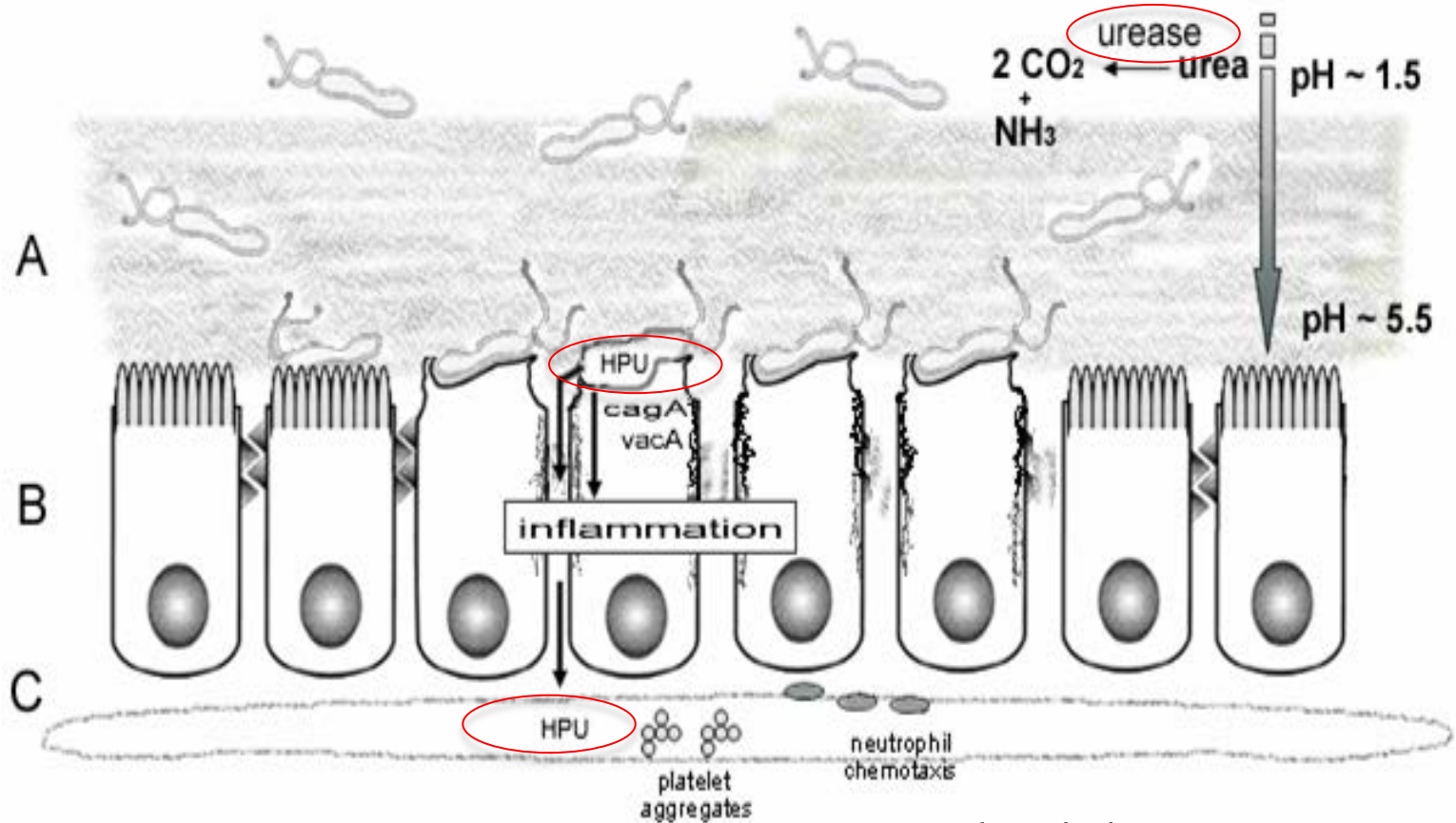
PROTEIN	PLATELET ?	ACCESSION	FRACTION	CONTROL	JBU	RATIO	CONDITION
Pleckstrin	Y	P08567	BOTH	4	18	4,5	CTRL + JBU
Calponin-2	Y	Q99439	BOTH	3	12	4,0	CTRL + JBU
F-actin-capping protein subunit alpha-1	?	P52907	BOTH	2	11	5,5	CTRL + JBU
Vinculin	Y	P18206	BOTH	157	513	3,3	CTRL + JBU
14-3-3 protein beta/alpha	Y	P31946	BOTH	2	12	6,0	CTRL + JBU
14-3-3 protein zeta/delta	Y	P63104	BOTH	19	65	3,4	CTRL + JBU
Fibronectin	ACTIVATION	P02751	CITO		10		JBU ONLY
14-3-3 protein epsilon	Y	P62258	CITO		2		JBU ONLY
Pyruvate kinase isozymes M1/M2	Y	P14618	MEMBRANE		11		JBU ONLY
Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	Y	Q93084	MEMBRANE		10		JBU ONLY
Coronin-1C	?	Q9ULV4	MEMBRANE		10		JBU ONLY
Complement C3	Y	P01024	MEMBRANE		19		JBU ONLY
14-3-3 protein gamma	Y	P61981	MEMBRANE		2		JBU ONLY

PROTEIN	PLATELET ?	ACCESSION	FRACTION	CONTROL	ADP	RATIO	CONDITION
P-selectin	Y	P16109	BOTH	2	15	7,5	CTR + ADP
Fibrinogen alpha chain	Y	P02671	BOTH	19	9	0,5	CTR + ADP
Chloride intracellular channel protein 1	Y	O00299	BOTH	8	6	0,8	CTR + ADP
Tubulin beta-1 chain	Y	Q9H4B7	BOTH	3	6	2,0	CTR + ADP
Gelsolin	Y	P06396	BOTH	8	2	0,3	CTR + ADP
Platelet glycoprotein V	Y	P40197	MEMBRANE	14	25	1,8	CTR + ADP
Platelet endothelial cell adhesion molecule	Y	P16284	MEMBRANE	2	15	7,5	CTR + ADP
Platelet glycoprotein 4	Y	P16671	MEMBRANE	2	9	4,5	CTR + ADP

10) Next step....(already being done..)

- 1) Platelet aggregation with *Proteus mirabilis* and *Helicobacter pylori* ureases (lessons form the control experiment);
- 2) Platelet aggregation using collagen (low number of proteins detected at the control experiment);
- 3) Phosphopeptide enrichment (all conditions);
- 4) Quantitative and qualitative approaches;

11) Potential contributions of *H. pylori* urease to gastritis and stomach cancer



Carlini and Polacco, 2008. Crop Science



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D – Ph.D.
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