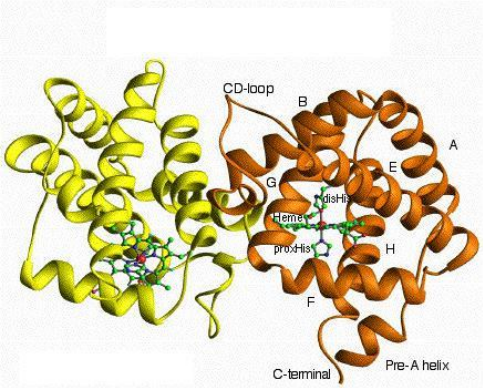
Bioactive peptides from vegetable proteins



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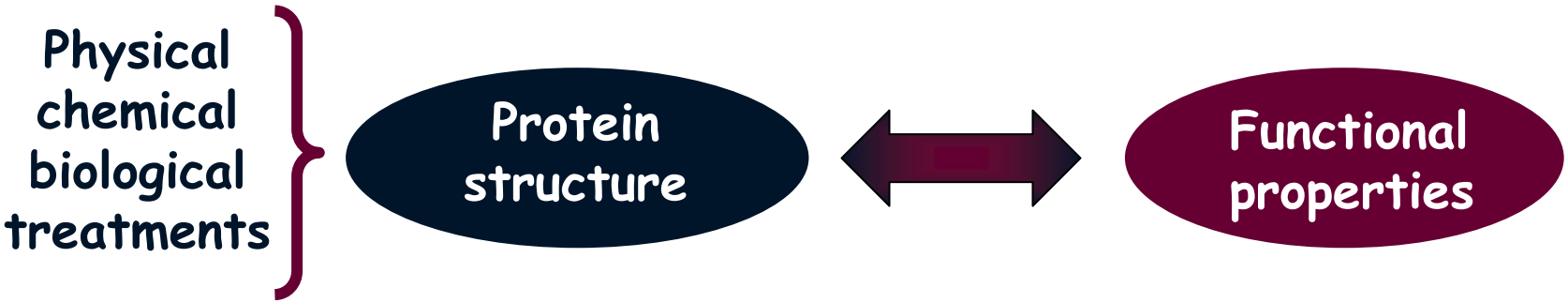
Previous research lines

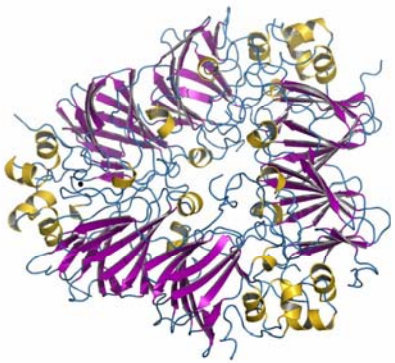
Analysis of the relationship between structural - physicochemical characteristics of vegetable proteins, in particular soybean and amaranth proteins, and their functional properties



- ✓ hydration properties: solubility, WIC, WHC, etc.
- ✓ capacity to form gels and films. Characterization of matrix gel, rheologial propeties, etc.
- ✓ foaming and emulsifing capacity. Interfacial and rheological behaviour, stability, etc.

Objective of our research line

The main objective of our research line is to evaluate the potentiality of amaranth as a novel source of bioactive compounds, particularly peptides, for using as food ingredients and/or in the development of functional foods.



Amaranth

Pseudoceral – Amaranthaceae family

Autochthonous from Central America

Desirable agricultural properties

Seed storage proteins

- ✓ 15- 17% protein content
- ✓ well-balanced amino acid composition
- ✓ Main protein factions: albumins, 11S globulins, P-globulins and prolamins

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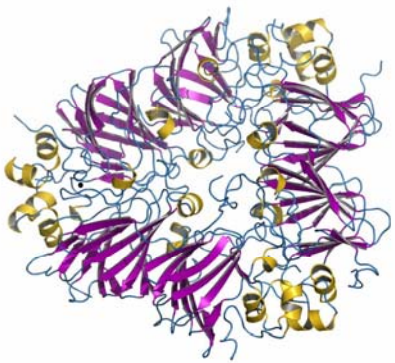
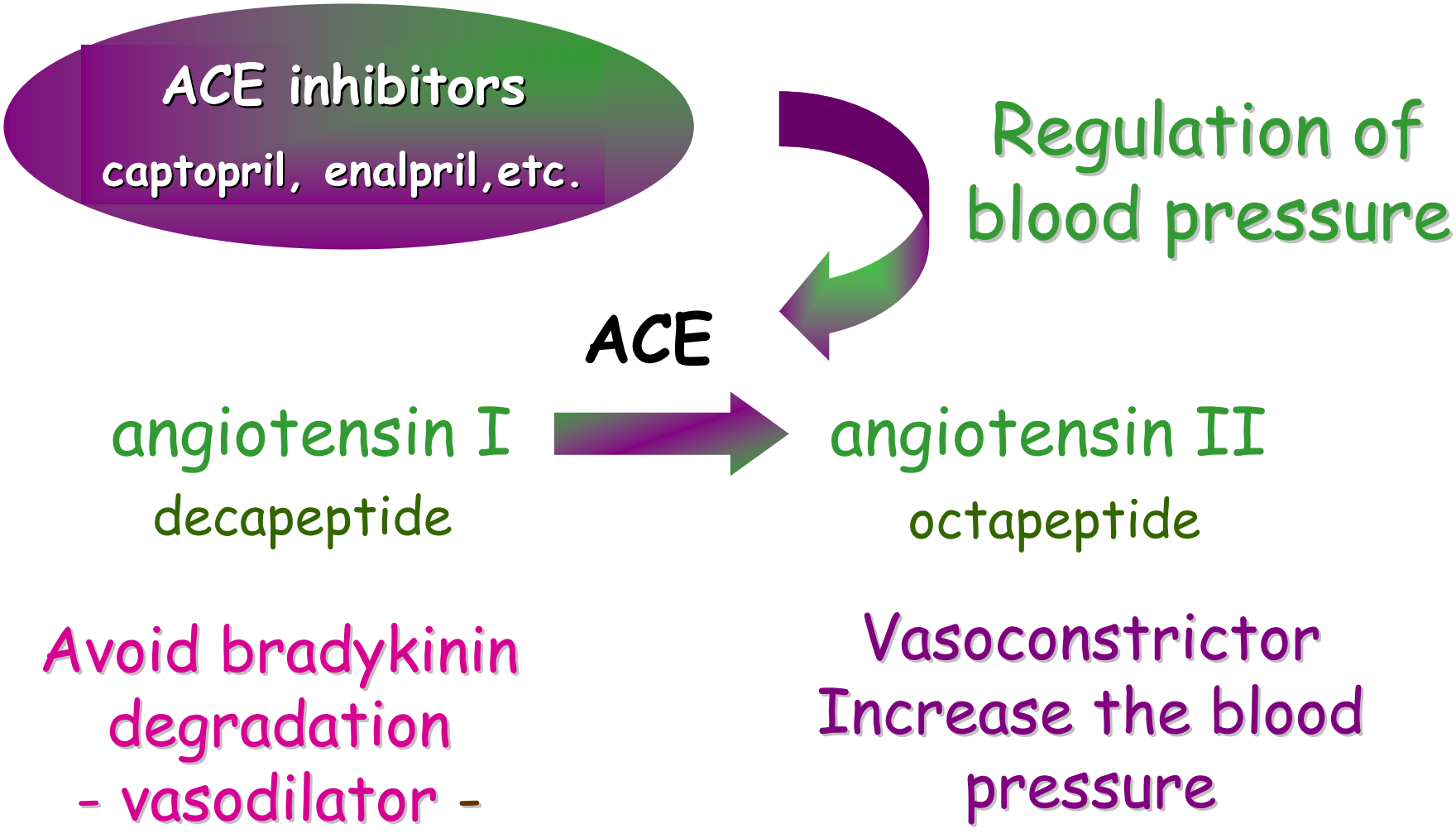
Antihypertensive activity

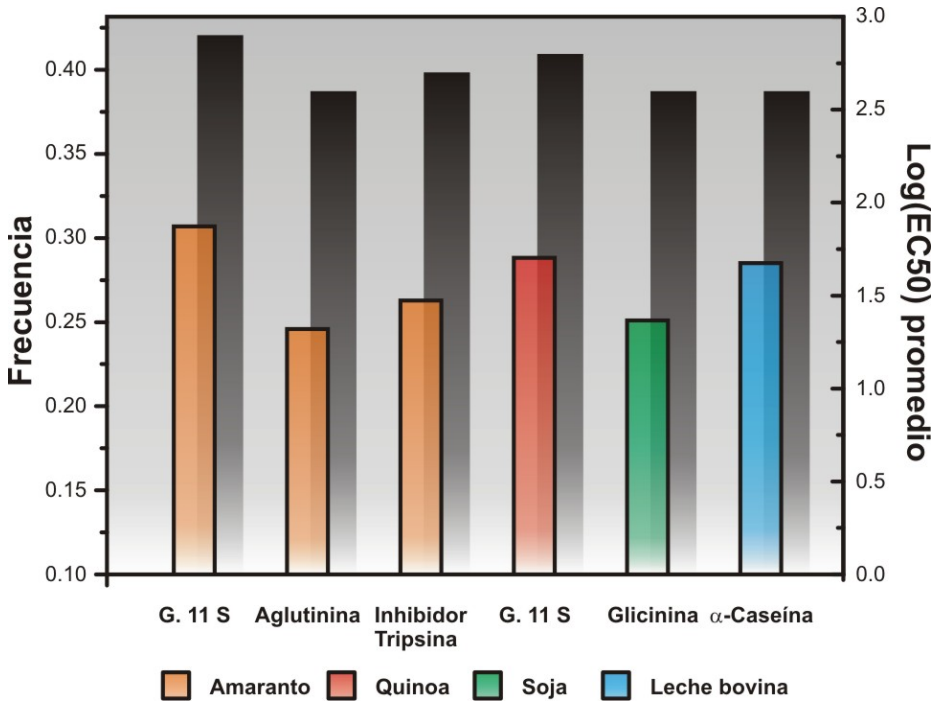


ACE – Angiotensin converting enzyme



ACE inhibitory peptides

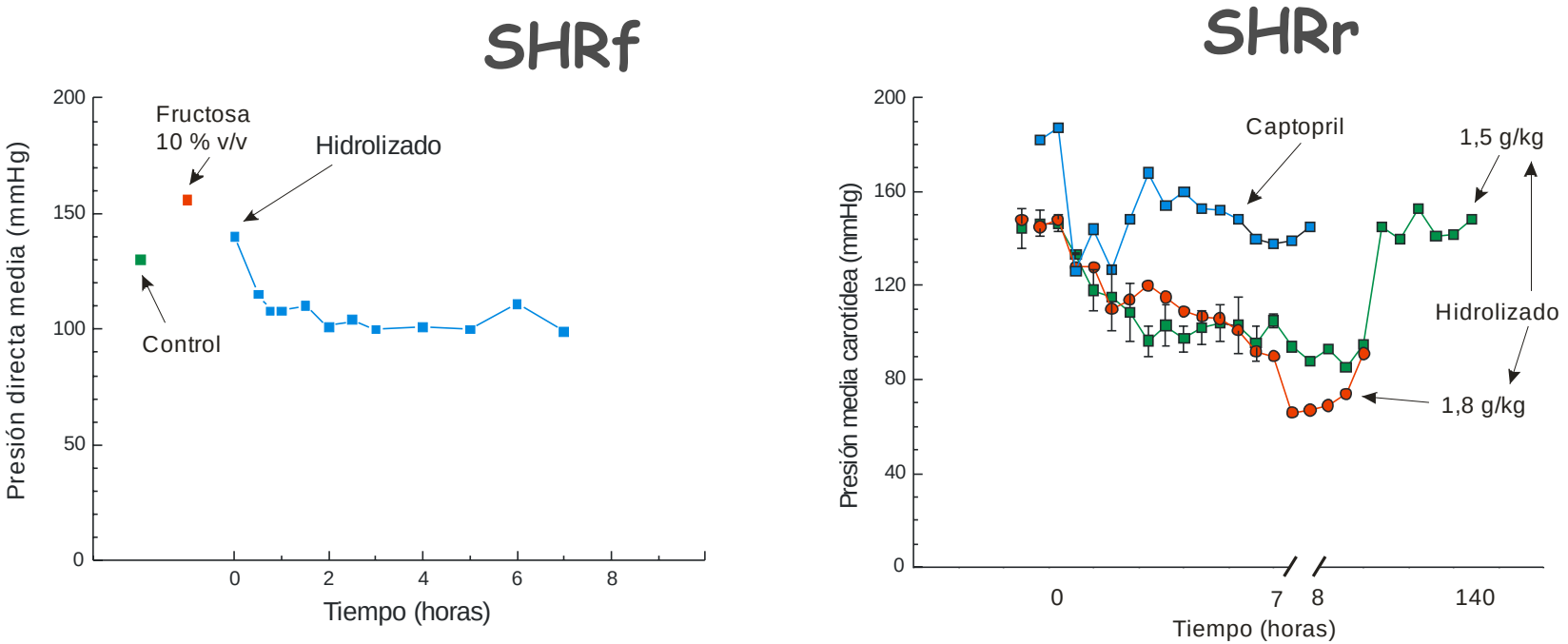
We have identified 154 possible inhibitory peptides in the 11S globulin fraction



STHASGFFFFHPTKMAKSTNYFLISCLLFVLFNGCMGEGR
FREFQQGNECQIDRLTALEPTNRIQAERGLTEVWDSNEQ
EFRCAGVSVIRRTIEPHGLLLPSFTSAPELIYIEQGNGITG
MMIPGCPETYESGSQQFQGGEDERIREQGSRKFGMRGD
RFQDQHQKIRHLREGDIFAMPAGVSHWAYNNGDQPLVAV
ILIDTANHANQLDKNFPTRFYLAGKPQQEHSGEHQFSRES
RRGERNTGNIFRGFETRLLAESFGVSEEIAQKLQAEQDD
RGNIVRVQEGLHVIKPPSRAWEEREQGSRGSRYLPNGVE
ETICSARLAVNVDDPSKADVYTPEAGRLTTVNSFNLPILR
HLRLSAAKGVLYRNAMMAPHYNLNAHNIMYCVRGRGRIQ
IVNDQGQSVFDEELSRGQLVVVPQNFAIVKQAFEDGFEW
VSFKTSENAMFQSLAGRTSAIRSLPIDVVSNIYQISREEAF
GLKFNRPETTLFRSSGQGEYRRKISIA

It is possible to obtain antihypertensive peptides from amaranth storage proteins

Effect of the hydrolysate administration



The blood pressure decreased in a dose-dependent way as hydrolysate increased.

The hypotensive effect was maximal 1.5h after the administration

Possible mechanism

In vitro assay

Isolated aortic smooth muscle + norepinephrine or norepinephrine and amaranth hydrolysate

↓

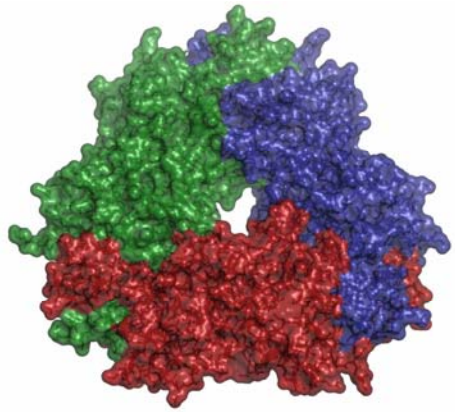
Contractile responses were recorded

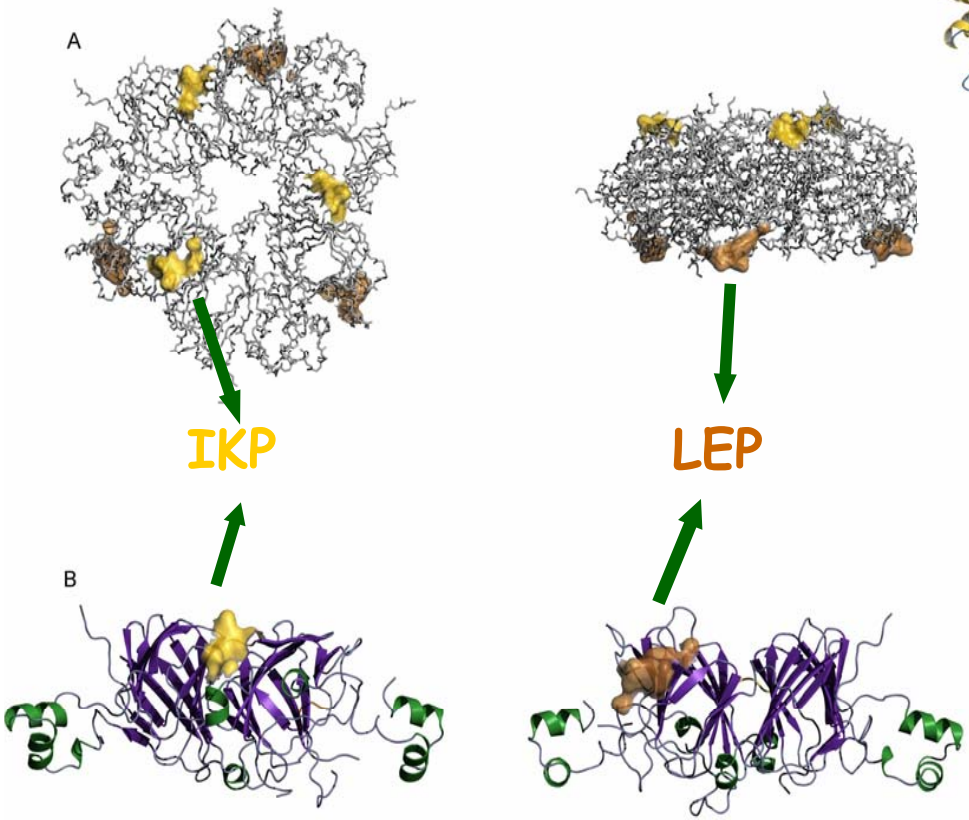
Results

- Peptides act as a non-competitive antagonist
- 65% GH shown a vasodilator effect.

In silico simulation of the interaction between ACE and novel potential peptide inhibitors

Molecular modelling of Amaranth 11S globulin





Evaluation of exposed surface and IC_{50}, candidates

Phytochem. **70:** 864-870 (2009)

Virtual library screening by automated docking

gi|122726601|gb|ABM66807.1| 11S globulin [Amaranthus hypochondriacus]

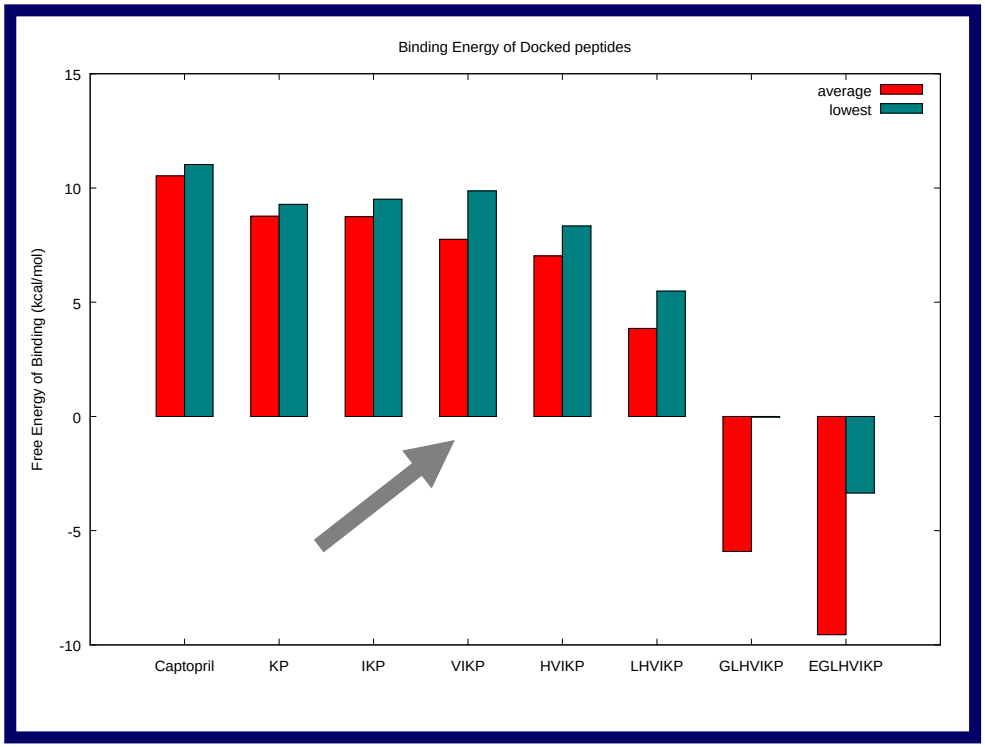
MAKSTNYFLISCLLFVLFNGCMGEGRFREFQQGNECQID**RLTALEP**TNRIQAEAGL
TEVWDSNEQEFRCAGVSVIRRTIEPHGLLLPSFTSAPELIYIEQGNGITGMMIPACP
QTYESGSQQFQGGEDERIREQGSRKFGMRGDRFQDQHQKIRHLREGDIFAMPA
GVFHWAYHNGDHPLVPVILIDTANHANQLDKNFPTRSYLAGKPQQEHSGEHQFS
RESRRGERNTGNIFRGFETRLLAESFGVSEEIAQKLQAEQDDRGNIVRVQ**EGLHVI**
KPPSRAWEEREQGSRGSRYLPNGVEETICSARLAVNVDDPSKADVYTPEAGRLT
TVNSFNLPILRHLRLSAAKGVLYRNAMMAPHYNLNAHNIMYCVRGRGRIQIVNDQ
GQSVFDEELSRGQLVVVPQNFAIVKQAFEDGFEWVSFKTSENAMFQSLAGRTSAI
RSLPIDVVSNIYQISREEAFGLKFNRPETTLFRSSGQGEYRRKISIA

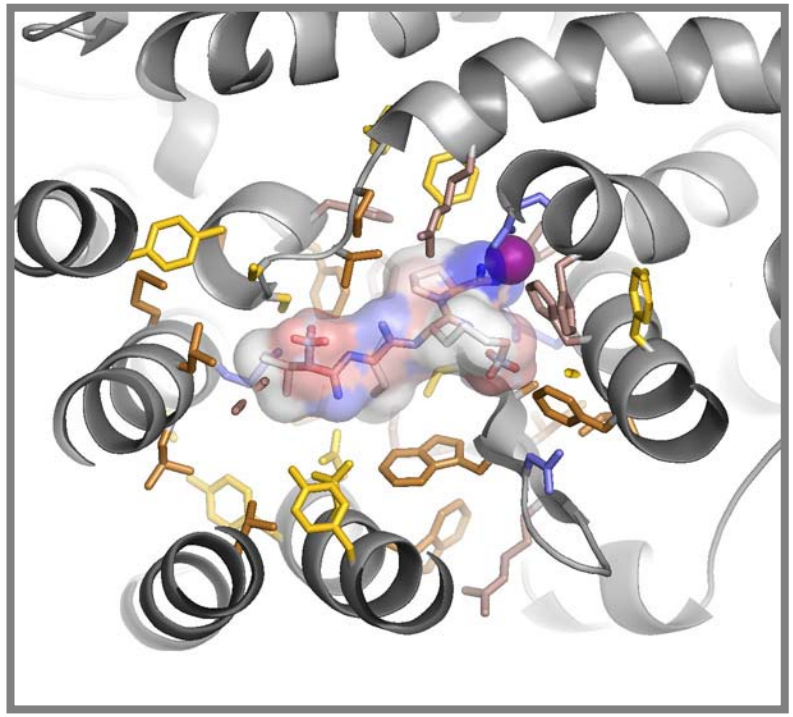
KP
IKP
VIKP
HVIKP
LHVIKP
GLHVIKP
EGLHVIKP

EP
LEP
ALEP
TALEP
LTALEP
RLTALEP

Phytochem. **70:** 864-870 (2009)

Calculated free energy for the formation of the ACE-peptide complex

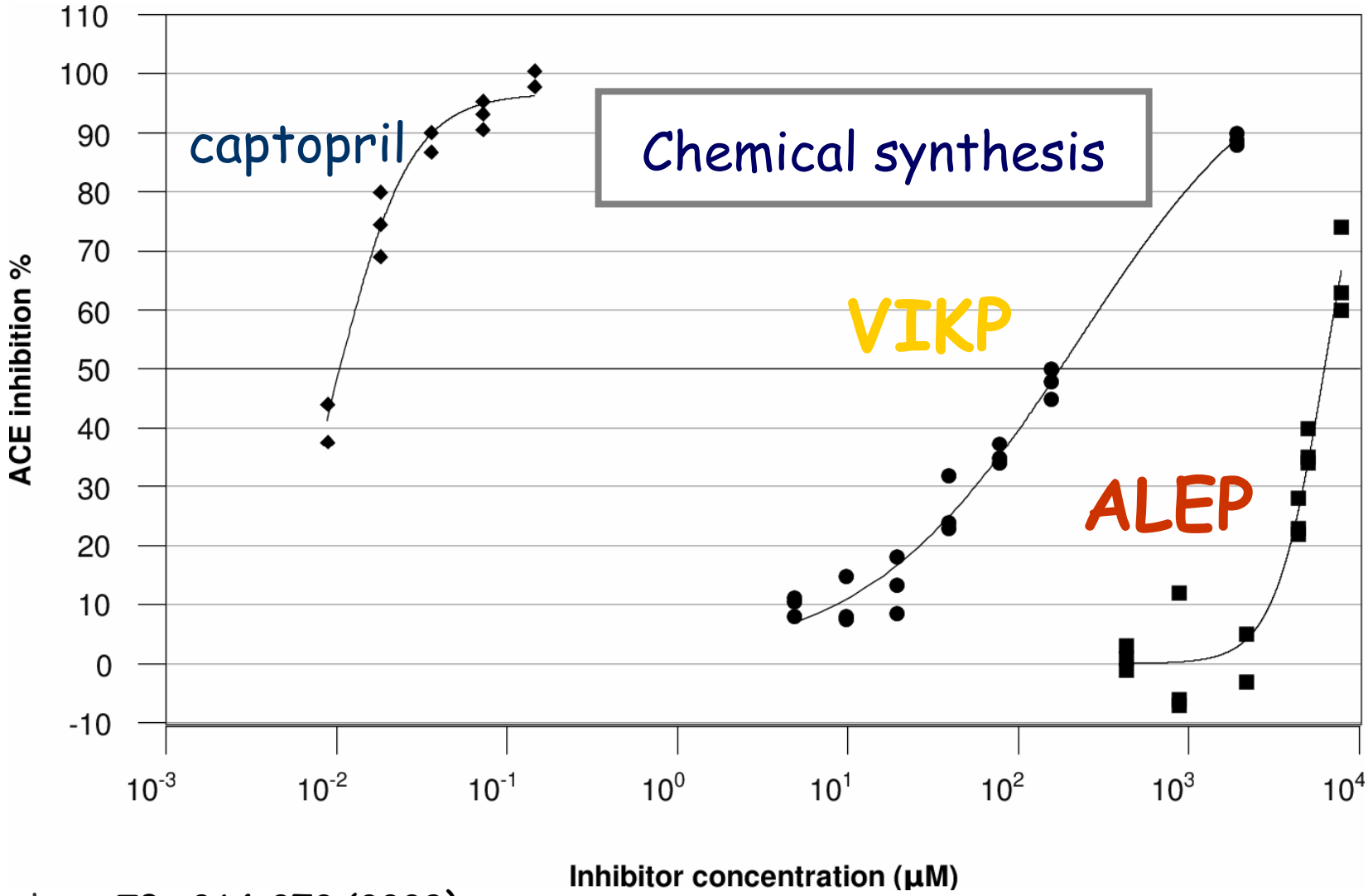




VIKP
Ki ~ 700 nM
50% electrostatic
50% vdW + hydrophobic + desolvatation

Phytochem. **70:** 864-870 (2009)

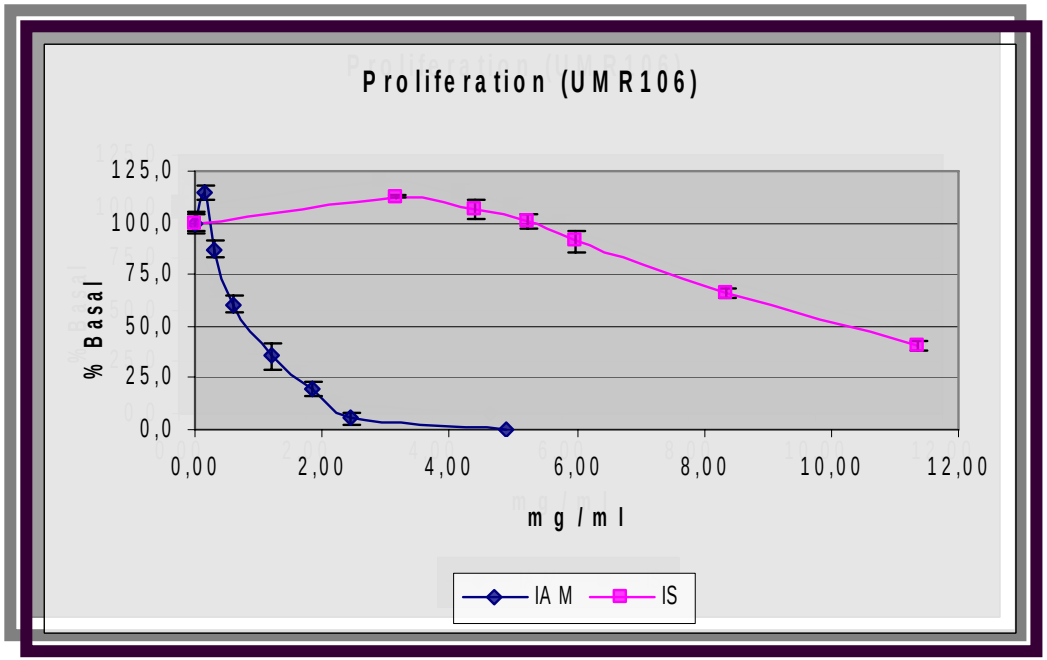
Inhibition of ACE activity
Synthetic peptides –*in vitro* assay



Phytochem. **70**: 864-870 (2009)

Antitumor activity

Inhibition of cell proliferation



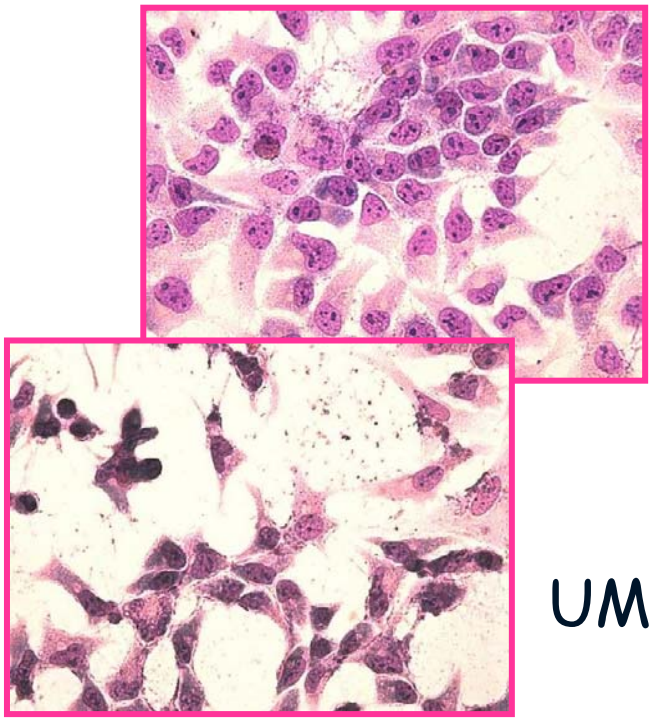
Different sensitivities to the API were observed for the four cell lines.

Proteolytic hydrolysis improved the inhibitory effect

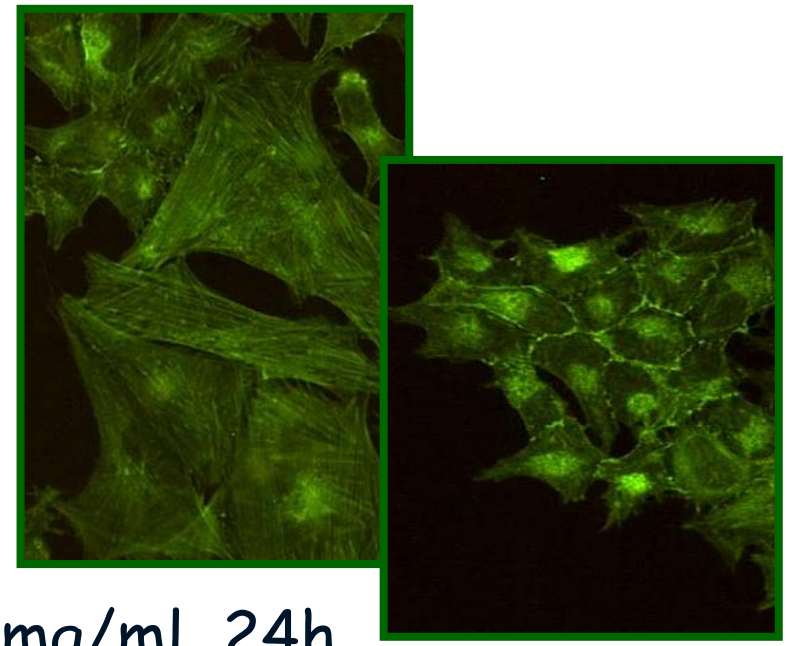
APIDH30
UMR106
IC50: 0.5 mg/ml

	Cellular lines			
	UMR106 (IC_{50}) [mg/ml]	MC3T3-E1 (IC_{50}) [mg/ml]	Caco-2 (IC_{50}) [mg/ml]	TC7 (IC_{50}) [mg/ml]
API	**1.0 ± 0.05**	**2.5 ± 0.06**	**1.5 ± 0.1**	**2.5 ± 0.08**
SPI	**10.0 ± 0.1**	**> 25**	**-**	**-**
BSA	**Non inhibition**			
BBI	**Non inhibition**			

Changes in cell morphology and cytoskeletal proteins



UMR106



UMR106 + API 1mg/ml 24h

The cells exhibited a dense nucleus surrounded by a very small and highly condensed cytoplasm after incubation

A partial disorganization of the actin filaments as well as an alteration in the shape of the cells was observed

Possible mechanism of cell death

Flow-cytometry analysis

After 24h incubation, the API increased the proportion of apoptotic cells in a dose-dependent fashion

LDH – Necrotic marker

LDH activity increased for API concentrations higher than 0.5 mg/ml

API inhibited cell adhesion in a dose-dependent manner

Conclusions

- APIs exhibit anti-hypertensive and a potential antitumor properties. Both effects were enhanced by protease treatment.

- In "in vitro" experiments we have demonstrated an important effect of the amaranth hydrolysates as inhibitors of ACE. We have also identified using in silico simulation two novel tetrapetides encrypted exclusively in amaranth 11S globulin with high power to inhibit ACE.

- We also detect a significant effect in lowering blood pressure in rats that we suspect is primarily due to peripheral vasodilatation. We assume that the amaranth hydrolysates would be acting at the level of the local Renin-angiotensin-system.

- The mechanism of action of the antiproliferative activity appears to involve an inhibition of cell proliferation and cell adhesion along with the production of cell damage resulting in a permanent loss of cell viability. The processes of apoptosis and necrosis might be involved in the mechanism of cell death.

- Cytostatic and cytotoxic effects exerted by the API on tumor cells would point to its use as a potential ingredient in functional food in order to decrease the risk of human diseases such as cancer, or even prevent such pathology altogether.

Argentine groups working in functional foods

- CERELA - CONICET – NUTucuman.

– Application of lactic bacteria in functional food formulation, - Food design and novel dietary supplements using starters and lactic probiotic, - Peptide production and isoflavone bioconversion, - Characterization of active peptides, - Conjugated linoleic acid production , - Biopolymer production and hydrolysis of allergenic proteins

- School of Exact Sciences and School of Pharmacy and Biochemistry – UBA – Bs.As.

– Different nutrition aspects of carbohydrates, vitamins, minerals, etc.

– Vegetable processing and use of waste. Formulation of functional foods.

- NU Comahue – Neuquen

– Process design for the production of functional foods based on fruits

- NU Rio Negro – Viedma

– Bioactive ingredients for food development

- NU Quilmes – Quilmes, Pcia. Bs.As.

– Multi-components obtaining from soybean and yeast as potential functional foods ingredients

- NU Córdoba – Córdoba

- Physicochemical and functional properties of baking products

- CIDCA – CONICET – NU La Plata – La Plata, Pcia. Bs.As

– Development of jams with fiber addition, - Dairy functional foods, - Encapsulation of bioactive compounds, - Bioactive peptides from vegetable proteins

- INTA

- INTI

CIDCA (UNLP-CONICET)

Calle 47 y 116 – La Plata, Pcia. Bs.As.

Thank you for your attention

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