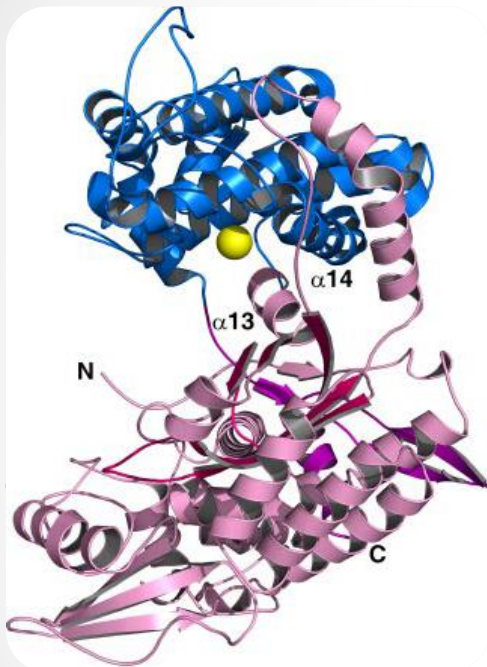


Characterization and therapeutic potential of human dipeptidyl peptidase III (hDPPIII)

Shalinee Jha

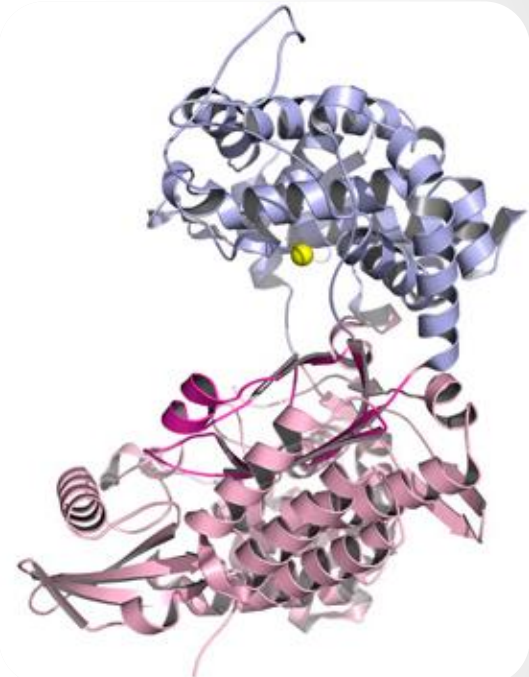
Group: Prof. Peter Macheroux
Institute of Biochemistry, TU Graz

What is dipeptidyl peptidase III ?



yeast DPP III

- Cleaves di-peptides from N- termini of short chain opioid peptides.
- Zinc-dependent metallopeptidase.
- Contains zinc binding motif **HEXXGH** and **EECRAE** in the upper lobe.
- Expressed in several tissues, mostly cytosolic .



human DPP III

Pathological importance of DPPIII

Spinorphin as an Endogenous Inhibitor of Enkephalin-degrading Enzymes: Roles in Pain and Inflammation

Author(s): Y. Yamamoto, H. Ono, A. Ueda, M. Shimamura, K. Nishimura and T. Hazato

Affiliation: Department of Medical Biology, The Tokyo Metropolitan Institute of Medical Science, Bunkyo-ku, Tokyo 113-8613, Japan



Pergamon

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Original Paper

Dipeptidyl Peptidase III in Malignant and Non-malignant Gynaecological Tissue

Š. Šimaga,¹ D. Babić,³ M. Osmak,² J. Ilić-Forko,³ Lj. Vitale,¹ D. Miličić⁴ and M. Abramić¹

¹Rudjer Bošković Institute, Department of Organic Chemistry and Biochemistry; ²Rudjer Bošković Institute, Department of Molecular Genetics, Bijenička 54, 10000 Zagreb; ³Department of Gynaecological and Perinatal Pathology; and ⁴Department of Obstetrics and Gynaecology, University Hospital and School of Medicine Zagreb, Zagreb, Croatia

OPEN ACCESS Freely available online



MicroRNA Regulation of Human Protease Genes Essential for Influenza Virus Replication

Victoria A. Meliopoulos, Lauren E. Andersen, Paula Brooks, Xiuzhen Yan, Abhijeet Bakre, J. Keegan Coleman, S. Mark Tompkins, Ralph A. Tripp*

Department of Infectious Diseases, University of Georgia, Athens, Georgia, United States of America



Research article

Peptidases released by necrotic cells control CD8⁺ T cell cross-priming

Jaba Gamrekelashvili,^{1,2} Tamar Kapanadze,^{1,2} Miaojun Han,¹ Josef Wissing,³ Chi Ma,¹ Lothar Jaensch,³ Michael P. Manns,² Todd Armstrong,⁴ Elizabeth Jaffee,⁴ Ayla O. White,⁵ Deborah E. Citrin,⁵ Firouzeh Korangy,¹ and Tim F. Greten¹

Published OnlineFirst February 4, 2013; DOI: 10.1158/0008-5472.CAN-12-4400

Molecular and Cellular Pathobiology

Cancer Research

Proteomic Analysis of Ubiquitin Ligase KEAP1 Reveals Associated Proteins That Inhibit NRF2 Ubiquitination

Bridgid E. Hast¹, Dennis Goldfarb², Kathleen M. Mulvaney¹, Michael A. Hast⁴, Priscila F. Siesser¹, Feng Yan¹, D. Neil Hayes³, and Michael B. Major^{1,2}

Pain

Cancer

Oxidative stress



the FEBS Journal

Ets-1/Elk-1 is a critical mediator of dipeptidyl-peptidase III transcription in human glioblastoma cells

Abhay A. Shukla, Misti Jain and Shyam S. Chauhan

Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India

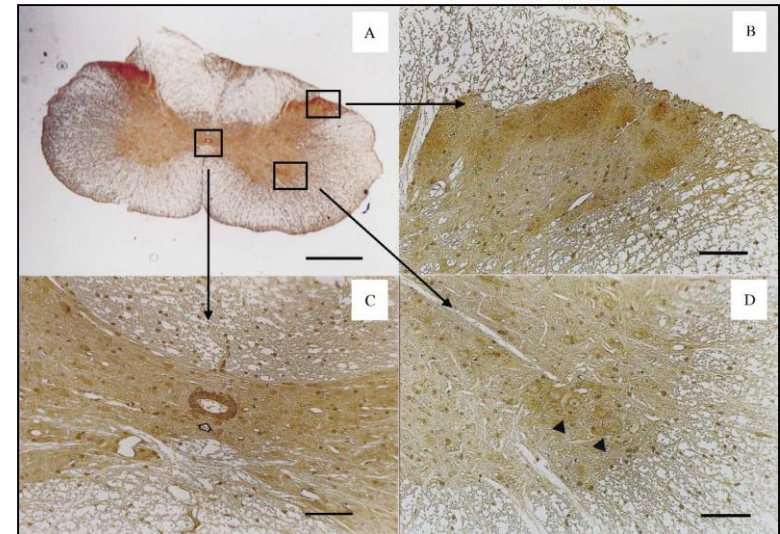
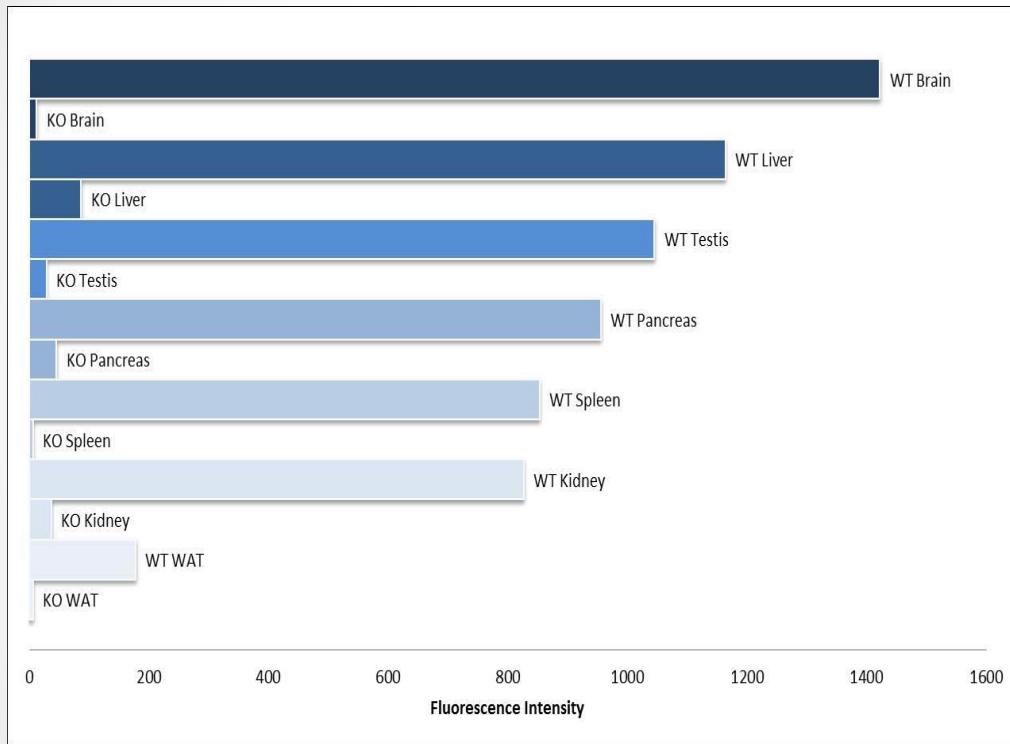
Influenza

Immune Response

Protein Turnover

Experiments with tissue lysates

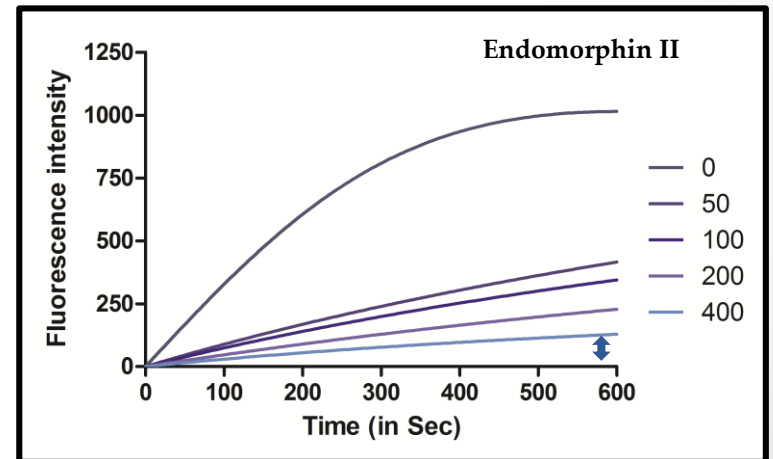
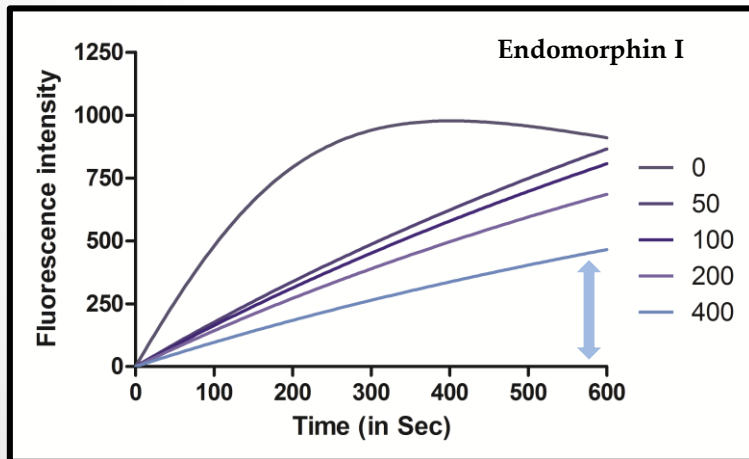
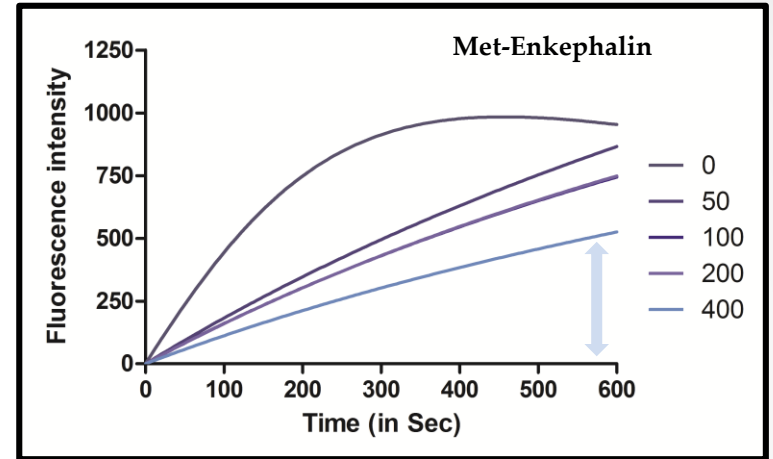
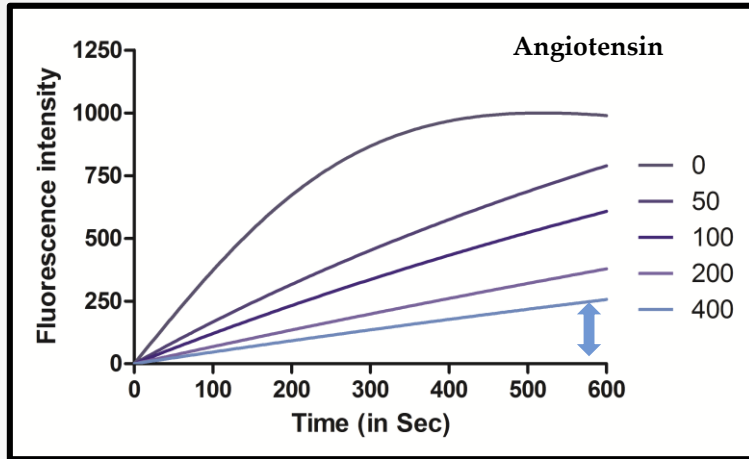
Comparative expression of DPP III in wild-type and knockout mice tissues



Positive immunostaining is seen with intense reactivity in superficial laminae and ependymal cells surrounding the central canal and with moderate reactivity in ventral horn cells.

Localisation of DPP III in areas of brain and spinal cord gives clues for its possible role in nociception

Substrate competition assay

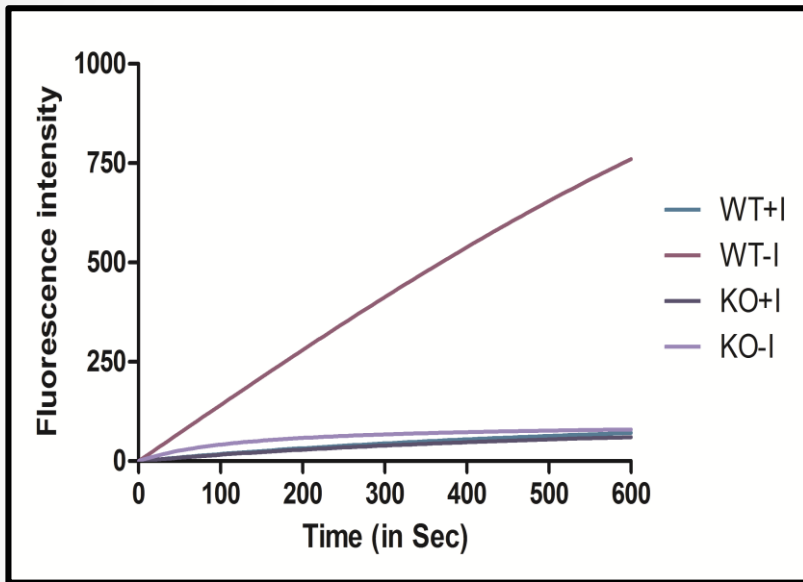


● Percent reduction in activity :

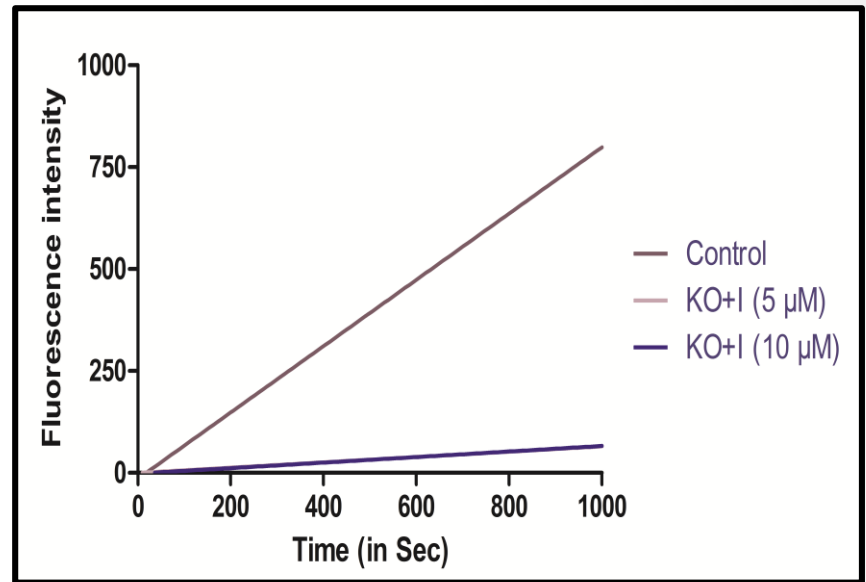
↓ 8 ↓ 15 ↓ 21 ↓ 30

●

Comparison of DPPIII activity in presence of IVYPW



Activity of DPPIII in wild type and and knock-out brain tissue lysate in + and – of IVYPW

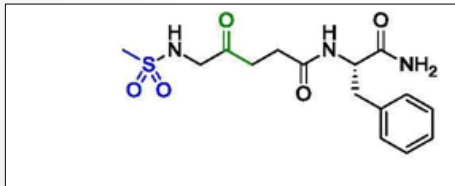
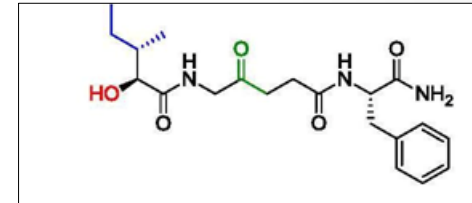
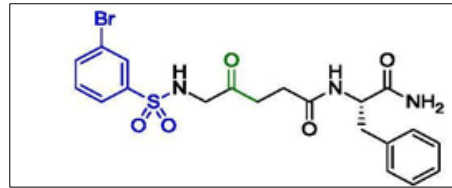
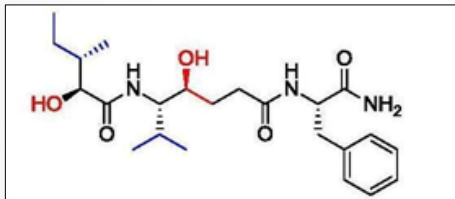
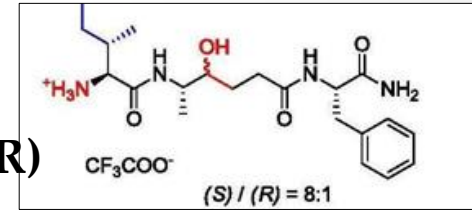
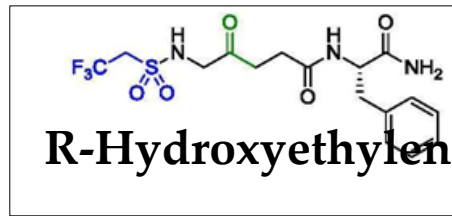
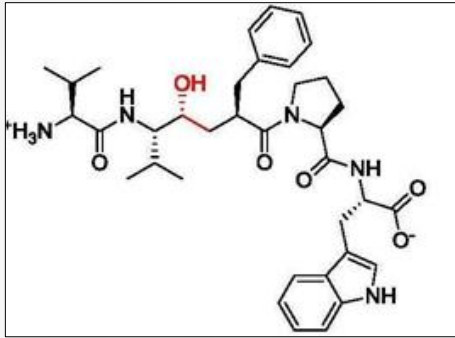


Activity of DPPIII in wild type brain tissue lysate with:

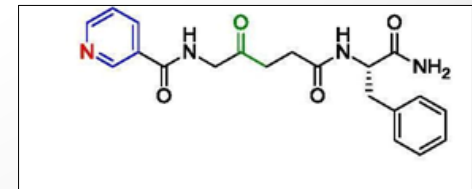
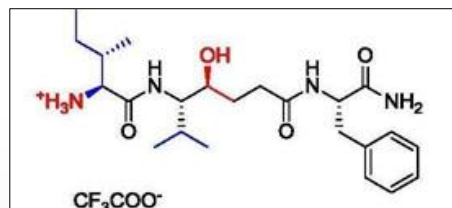
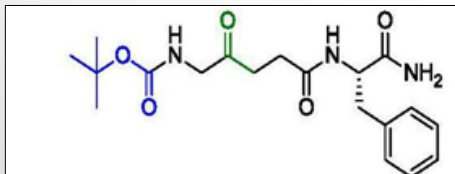
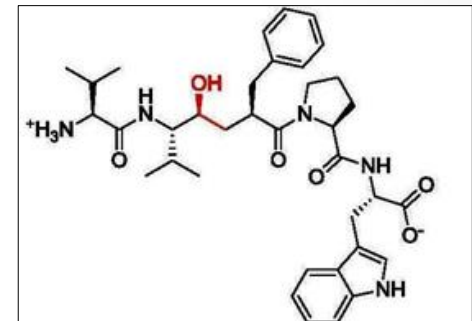
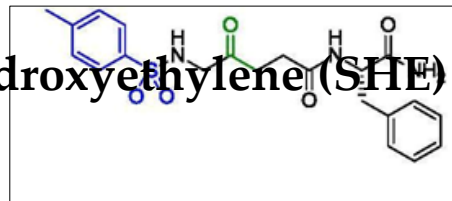
- No IVYPW
- 5 μ M IVYPW incubated with knock-out brain tissue lysate
- 10 μ M IVYPW incubated with knock-out brain tissue lysate

Hydroxyethylene Transition State

Peptidomimetic Inhibitors

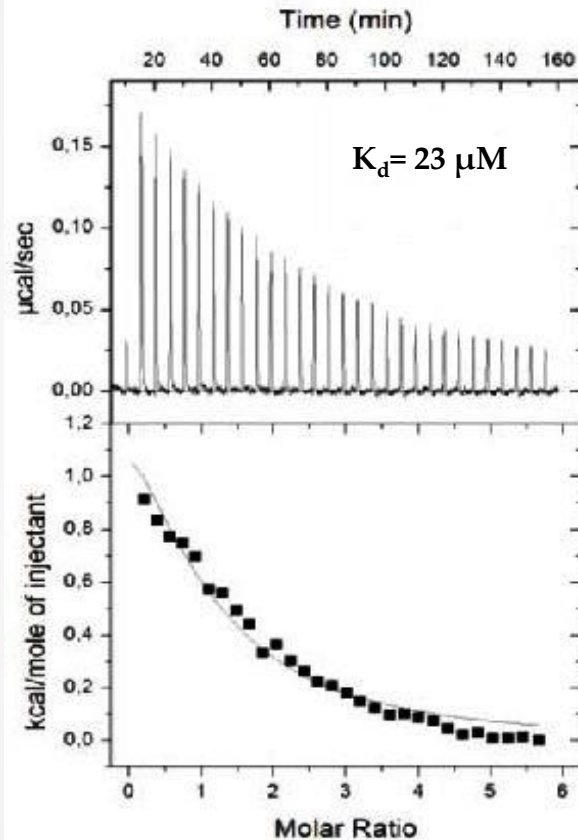


S-Hydroxyethylene (SHE)



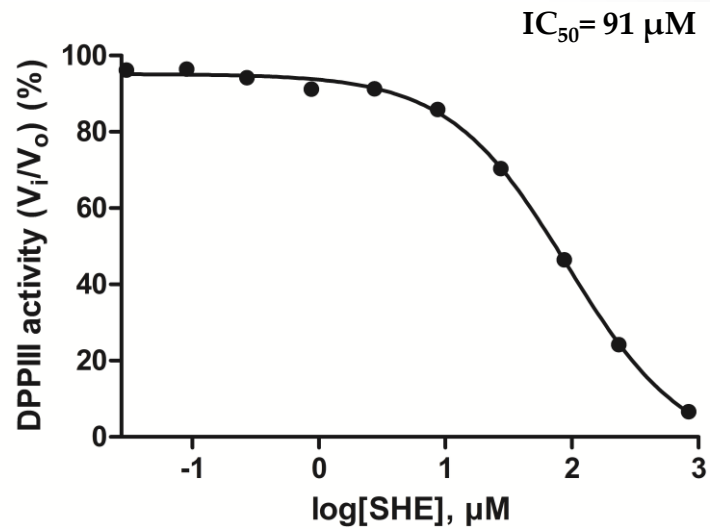
Kinetics with SHE

ITC



[hDPP3]= 20 μM
[SHE]= 500 μM

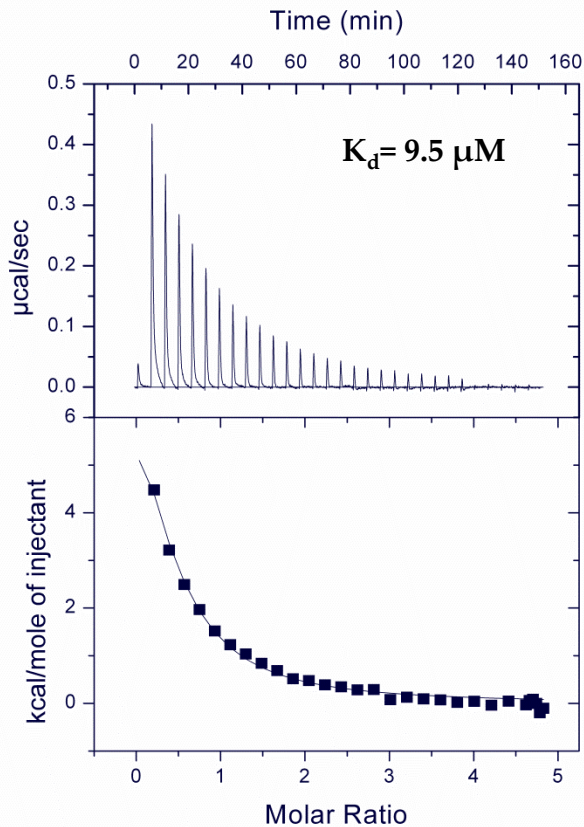
Fluorescence-based assay



[hDPP3]= 50 nM
[SHE]= 0-500 μM
[AA₂NA]= 200 μM

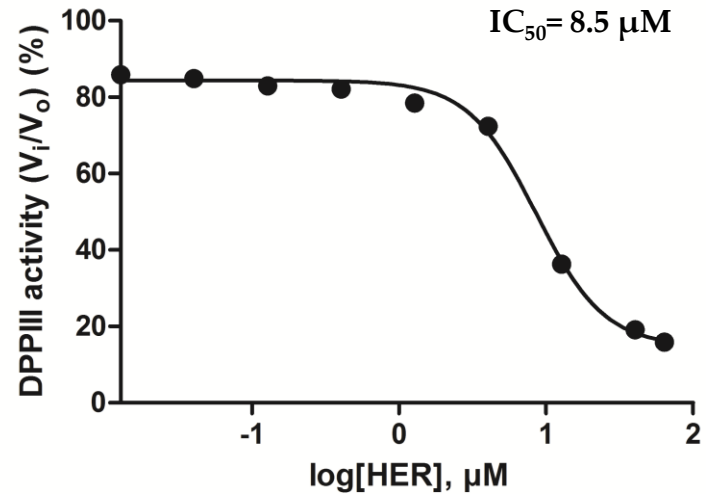
Kinetics with HER

ITC



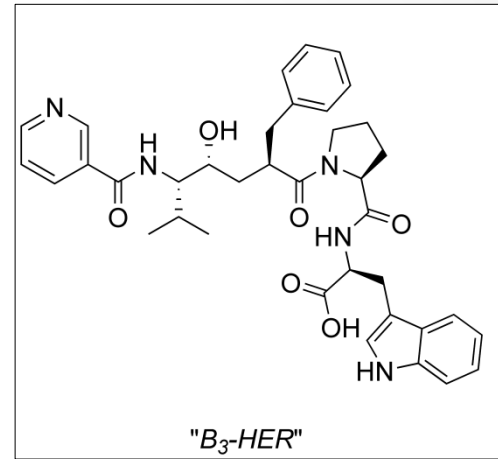
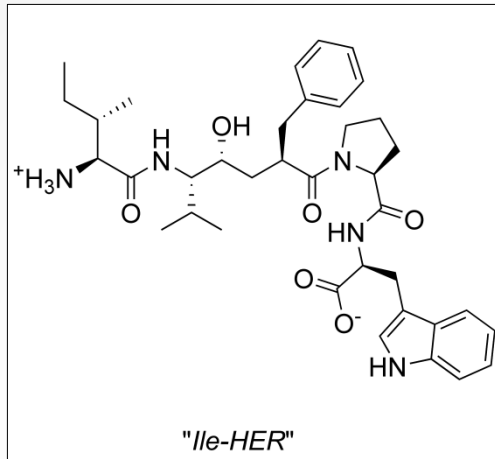
[hDPP3]= 20 μM
[HER]= 500 μM

Fluorescence-based assay

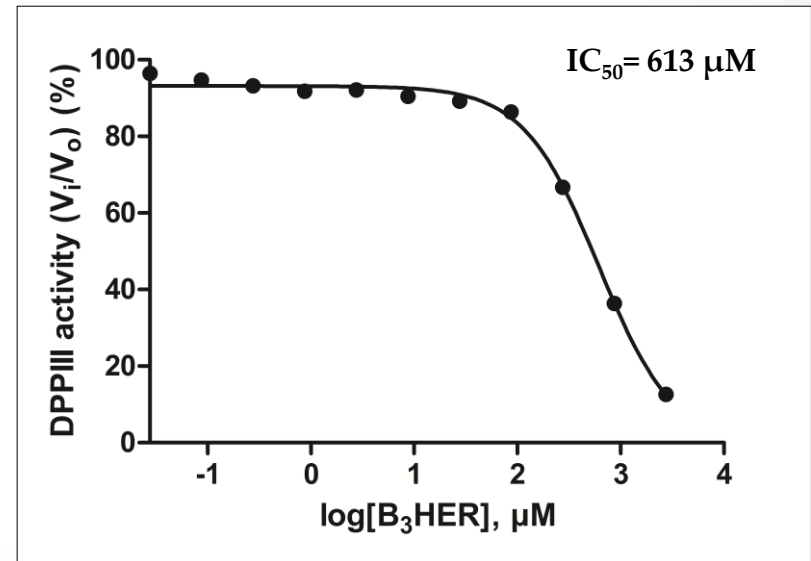
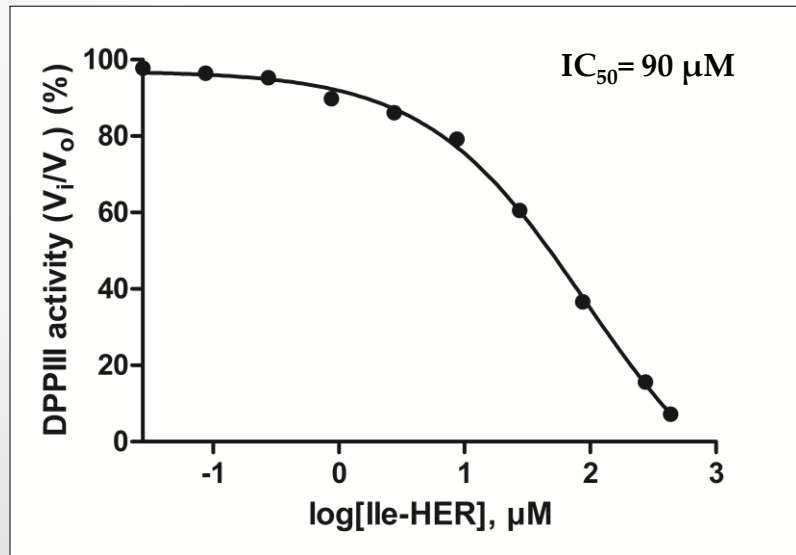


[hDPP3]= 50 nM
[HER]= 0-500 μM
[AA₂NA]= 200 μM

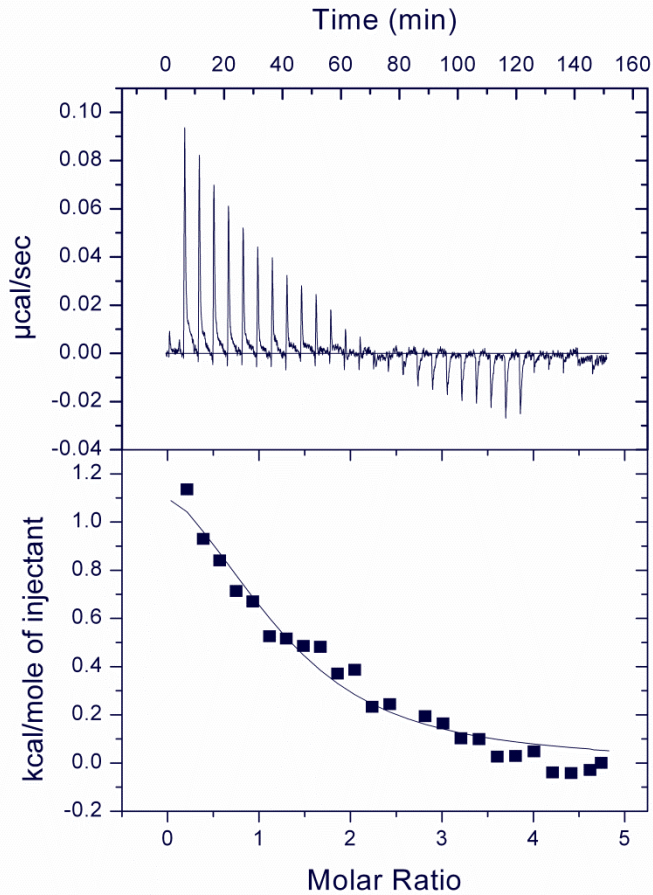
HER derivatives



Fluorescence-based assay



ITC with Ile-HER



$K_d = 10 \mu\text{M}$
 $[\text{hDPP3}] = 20 \mu\text{M}$
 $[\text{Ile-HER}] = 500 \mu\text{M}$

SHE/HER versus IVYPW

- Peptide substrate mimetic inhibitors like tynorphin (VVYPW) or IVYPW have a scissile peptide bond.
- Susceptible to degradation by DPPIII itself.
- Hydroxyethylene pseudopeptide inhibitors would be much more potent in a time-based manner.

Summary

- DPPIII activity was found to be highest in brain.
- Endomorphin II and Angiotensin bind more strongly to DPPIII *in vivo*.
- *HER, SHE and Ile-HER* successfully inhibited hDPP3 at low micromolar concentrations.
- The first demonstration of efficient inhibition of a metalloprotease by a hydroxyethylene pseudopeptide.

Outlook

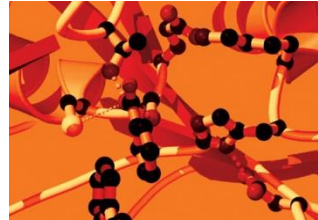
Realistic

- Multiple turnover experiments without and with inhibitors (HER and IVYPW/VVYPW) to address the degradation of tynorphin and the stability of HER
- Inhibition of DPP3 by HER in brain lysates of wild type and KO mouse
- Co-crystal structure of HER and hDPP3

Futuristic

- Intracellular Peptide Profiling :To find out potential substrates and products of DPPIII
- Immunoassays : To determine the level of opioid peptides in tissue extracts
- Nociceptive assays in mice
- Role of DPPIII in blood pressure regulation

Acknowledgement



Prof. Peter Macheroux

AGM members

Prof. Karl Gruber

Prashant Kumar

Prof. Rolf Breinbauer

Jakov Ivkovic

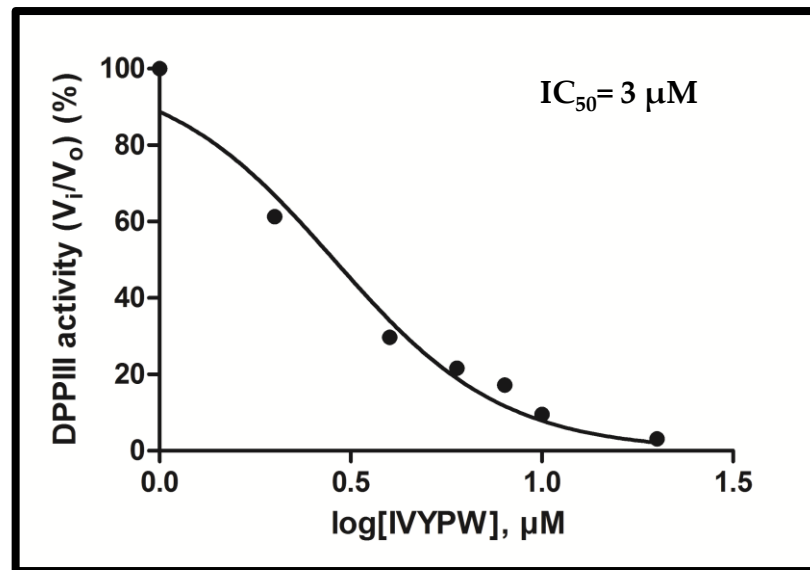
Christian Lembacher-
Fadum

Prof. Robert Zimmerman

Ulrike Taschler

Thank You!!!

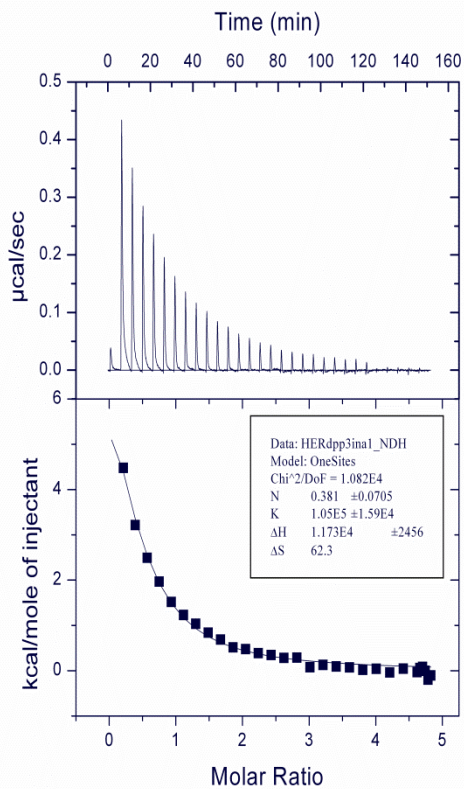
Assay with IVYPW



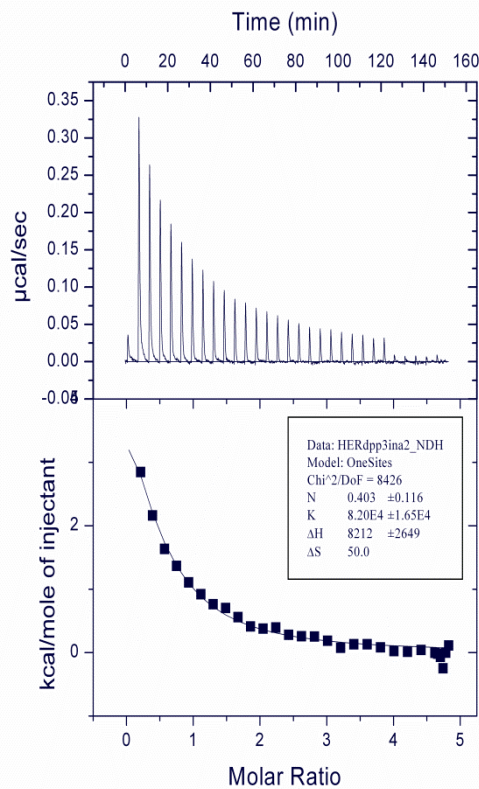
ITC with HER

[hDPP3]= 20 μM

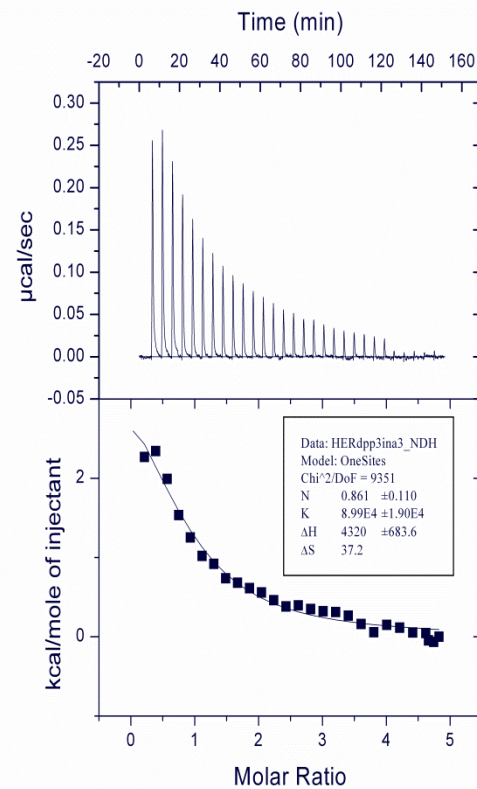
[HER]= 500 μM



$K_d = 9.5 \mu\text{M}$



$K_d = 12.1 \mu\text{M}$



$K_d = 11.1 \mu\text{M}$

ITC with Ile-HER

