PANACHE

Production of next generation modulators of pannexins and connexins as novel therapeutics in the treatment of inflammatory cardiovascular, hepatic and joint diseases

Funded by the Horizon 2020 Framework Programme of the European Union

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01/03/2020 – 28/02/2025

Deliverable number:	D1.18 (D18)
Deliverable title:	Workshop “Pannexin-derived and connexin-derived peptidomimetics as novel therapeutic tools”
Work package:	WP1
Leading partner:	VUB
Participating partners:	VUB/UNIGE/PROTOQSAR
Due date:	28/02/2025
Submission date:	11/11/2024
Dissemination level:	public

1. Introduction

The third PANACHE workshop, initially entitled "Pannexin-derived and connexin-derived peptidomimetics as novel therapeutic tools", but ultimately renamed "Channeling energy into drug development", was organized by the *In Vitro* Toxicology Group (IVTD) and the Research Group of Organic Chemistry (ORGC) of the Vrije Universiteit Brussel (VUB) on behalf of the PANACHE consortium on 7 and 8 October 2024 as a full online event. The program consisted of 2 sessions (attachment 1). Session 1 (7 October 2024) was publicly accessible and included a number of state-of-the-art lectures. Session 2 (8 October 2024) was attended exclusively by members of the PANACHE consortium and industry representatives, and focused on the exploitation of results. The workshop was promoted *via* the PANACHE website, the PANACHE-dedicated social media platforms as well as through the internal communication channels of the different members of the PANACHE consortium (attachment 2). A leaflet containing information related to the PANACHE project, including general information, vision and objectives, was prepared to promote session 2 of the workshop among relevant companies (attachment 3). Furthermore, in preparation of session 2, an online questionnaire comprising questions about the workshop topic was distributed in advance to all registered industry representatives. The online questionnaire could be accessed *via* PANACHE online questionnaire.The results of this online questionnaire were utilized to tailor the content of session 2 of the workshop and to stimulate the discussion with the industry representatives. For the session 1 of the workshop, a total of 99 online registrations were received from more than 20 different universities, institutes and companies across Europe, the Middle East, Africa and Asia (attachment 4). For session 2 of the workshop, a total of 16 internal participants of the PANACHE consortium and representatives from 5 different international pharmaceutical and consultancy companies were registered online (attachment 4).

2. Session 1

Session 1 consisted of 4 keynote lectures presented by experienced researchers from 4 different countries, followed by 6 flash presentations delivered by young researchers from the 3 research groups of the PANACHE consortium. The session was chaired by Mathieu Vinken (VUB). The keynote lecture presentations and flash presentations are available in attachments 5 and 6, respectively. It should be noted that the presentation of Prof. Volodymyr Korkhov was not included in attachment 5 due to the confidential and unpublished nature of the data presented during his lecture.

a. Keynote lectures

Steven Ballet

Steven Ballet is a full Professor at the Vrije Universiteit Brussel, Belgium, and serves as the head of the Research Group of Organic Chemistry (https://orgc.research.vub.be/). His research group primarily focuses on the peptide and peptidomimetic fields, specifically their synthesis based on non-canonical amino acids and their application as therapeutic molecules and functional materials.

Title: The rise of peptides and peptidomimetics in drug discovery and development.

Summary: His presentation addressed several aspects of the peptides and peptidomimetics field, including an introduction and historical background of both peptides and peptidomimetics, several facts and figures regarding the use of peptides as therapeutics, and a brief overview of the process employed for the development of peptides and peptidomimetics. Furthermore, Steven incorporated several examples of peptides utilized as therapeutics.

Silvia Penuela

Silvia Penuela is an Associate Professor at the Schulich School of Medicine and Dentistry, University of Western Ontario, Canada, and serves as the chair of the basic science research group The Penuela lab (https://www.schulich.uwo.ca/penuelalab//index.html). Her research group focuses on various aspects of the Pannexin proteins, with particular emphasis on the role of these proteins in a range of pathological disorders, including diverse types of cancer and other diseases such as osteoarthritis.

Title: Pannexin channels in cancer, inflammation, and cell death.

Summary: Her presentation elucidated the pathophysiological role of Pannexin1 channels in various inflammatory and cell death mechanisms, as well as the specific role of these channels in the pathogenesis of melanoma. The latter was substantiated by several research studies and results generated by her research group.

Volodymyr Korkhov

Volodymyr Korkhov is an Associate Professor at the Federal Institute of Technology Zurich, Switzerland, and group leader of the laboratory Mechanisms of Signal transduction at the Biomolecular Research of the Paul Scherrer Institute, Switzerland (https://www.psi.ch/en/lbr/people/volodymyr-korkhov). His research group focuses on elucidating the structure-function relationships of membrane proteins and protein complexes involved in various aspects of cellular signaling by employing multidisciplinary approaches such as membrane protein biochemistry, biophysics, and structural biology methods.

Title: Structural studies of connexin gap junction channels and hemichannels.

Summary: His presentation focused on structural biology aspects of connexin gap junctions and hemichannels. Specifically, the presentation summarized the latest findings in the field, with particular emphasis on the Connexin43 and 32 isoforms. In this context, he showed several unpublished novel data from their research team.

Thomas Hartung

Thomas Hartung is a full Professor at the Bloomberg School of Public Health, Johns Hopkins University, USA, and director of the Johns Hopkins Center for Alternatives to Animal Testing (https://caat.jhsph.edu/about-caat/). His research focuses on replacing animal-based toxicological studies by developing alternative animal-free methods based on *in vitro* organoid cultures and artificial intelligence (AI).

Title: The scAInce of drug development and toxicology.

Summary: His presentation focused on the potential use of AI as an alternative animal-free testing tool for drug development and toxicology studies. During his presentation, he summarized current figures of animal-based testing methodologies in drug development and the potential advantages of using AI as an alternative animal-free tool. Additionally, he presented several results related to 2 AI-driven research projects in which he is currently participating, namely the Human Exposome Project and the ONTOX project.

b. Flash presentations

Presenter: Carmen Ortiz-González, ProtoQSAR SL, Spain.

Title: QSAR modeling for the prediction of pharmacokinetics and bioactivities of therapeutic peptides.

Abstract: In the last years, peptides have emerged as promising candidate compounds in numerous drug discovery studies due to their high degradation rate and the reduction of toxic metabolites release, among other advantages. These peptides can be formed from 2 up to, in some cases, even 50 amino acids, presenting a more voluminous structure than the most commonly used small organic molecules. Advances in computational methods and the increasing of the use of artificial intelligence in recent years have supposed a revolution in many fields, including drug discovery. In computational-aided drug design, diverse techniques are used to study pharmacokinetic and pharmacodynamic properties. One such technique is Quantitative Structure-Activity Relationship (QSAR), which involves the development of statistical models, often-based on machine learning algorithms to predict molecular properties of new molecules, using known data from a training dataset. Several QSAR models and tools (commercial and non-comercial) are available to predict ADMET (absorption, distribution, metabolism, excretion and toxicity) properties aiding in the assessment of the druggability of molecules. In this talk, we will emphasize on the utility of generating

QSAR models specifically for the study of peptides as novel therapeutic candidates. This approach may facilitate more rapid hit identification when working with this kind of biomolecules in drug Discovery.

Presenter: Daan Peeters, Vrije Universiteit Brussel, Belgium.

Title: Nanobody-based Panx1 channel inhibitors reduce inflammation in acute liver injury.

Abstract: The opening of pannexin1 channels is considered as a key event in inflammation. The release of adenosine triphosphate through pannexin1 channels triggers inflammasome signalling and activation of immune cells. By doing so, pannexin1 channels play an important role in several inflammatory diseases. Although pannexin1 channel inhibition could represent a novel clinical strategy for treatment of inflammatory disorders, therapeutic pannexin1 channel targeting is impeded by the lack of specific, potent and/or in vivo-applicable inhibitors. In this respect, our group identified 3 cross-reactive nanobodies that showed affinity for both murine and human pannexin1 proteins in the nanomolar range. Moreover, the pannexin1-targeting nanobodies were demonstrated to block pannexin1 channel-mediated release of adenosine triphosphate *in vitro*. Additionally, the pannexin1-targetting nanobodies were found to display anti-inflammatory effects *in vitro* through reduction of interleukin 1 beta levels. This anti-inflammatory outcome was further confirmed *in vivo* using a human-relevant mouse model of acute liver disease relying on acetaminophen overdosing. More specifically, the pannexin1-targetting nanobodies reduced serum levels of inflammatory cytokines and attenuated liver damage. These effects were associated with alteration of the expression of several NLRP3 inflammasome components. In conclusion, this study introduces for the first time specific, potent and *in vivo*-applicable nanobody-based inhibitors of pannexin1 channels. As demonstrated for the case of liver disease, the pannexin1-targeting nanobodies hold great promise as anti-inflammatory agents, yet this should be further tested for extrahepatic inflammatory disorders.

Presenter: Malaury Tournier, University of Geneva, Switzerland.

Title: A new stable Panx1 peptidomimetic for the prevention of myocardial ischemia/reperfusion injury.

Abstract: Myocardial infarction needs a fast reperfusion to rescue the ischemic myocardium. However, reperfusion paradoxically accelerates cardiomyocyte death and amplifies the inflammatory response. ATP release through pannexin1 (Panx1) channels triggers leukocyte recruitment to the site of injury. Thus, inhibition of Panx1 channels might reduce myocardial ischemia/reperfusion (I/R) injury. Existing Panx1 channel inhibitors display low specificity or *in vivo* stability. Previously, we developed SBL-PX1-42, a stapled peptidomimetic analog of ^{10}Panx1, the best known Panx1 channel inhibiting peptide. This peptidomimetic exhibited Panx1 channel-dependent anti-inflammatory properties *in vitro* and a plasma stability of >60 min (30-fold higher than ^{10}Panx1). Here, we investigated the potential of SBL-PX1-42 to prevent myocardial I/R injury *in vivo*. Wild-type (WT) hearts were used to assess cardiotoxicity of SBL-PX1-42 in an *ex vivo* Langendorff perfusion assay. 0.9% NaCl was used as a vehicle control.

SBL-PX1-42 did not affect heart rate, left ventricular developed pressure, contractility and relaxation, suggesting absence of cardiotoxicity of the compound. Next, we induced 30 min ischemia by ligation of the left anterior descending coronary artery followed by 24h reperfusion in WT and *Panx1*$^{-/-}$ mice. A single intravenous injection of 90µM SBL-PX1-42 at 5 min before reperfusion decreased the infarct size in 70% of the WT mice. SBL-PX1-42 did not reduce infarct area in *Panx1*$^{-/-}$ mice indicating its specificity for Panx1. In conclusion, SBL-PX1-42 is a promising specific and stable Panx1 peptidomimetic to prevent myocardial I/R injury in mice.

Presenter: Batuhan Yıldız, Vrije Universiteit Brussel, Belgium.

Title: Silymarin and its major components as a potential naturally occurring Panx1 channel inhibitor.

Abstract: Pannexin1 (Panx1) channels are essential mediators of cellular communication, and their abnormal opening has been linked with various pathological conditions through the promotion of inflammation and cell death. The development of novel pharmacological inhibitors for these channels represents a promising therapeutic approach. Silymarin, the biologically active polyphenolic complex mixture derived from the milk thistle seed extracts of *Silybum marianum L*. Gaertn, has been extensively reported for its therapeutic action against several Panx1 channel-associated diseases. Silymarin contains several major bioactive flavonolignans, namely silybin, silychristin, and silydianin, with Panx1-related anti-inflammatory activities, making them a potential promising new source of Panx1 channel inhibitors. Through *in vitro* and *in silico* approaches, this study aimed to investigate the potential inhibitory effect of the silymarin mixture and its major bioactive components, silybin, silychristin, and silydianin on Panx1 channel activity. *In vitro* testing demonstrated that silybin, silychristin, and silydianin inhibited Panx1 channel activity by reducing the extracellular adenosine triphosphate levels without altering the Panx1 protein expression. Additionally, silybin reduced interleukin-1β levels and decreased NRLP3 inflammasome-linked cell death. *In silico* studies confirmed the *in vitro* results by identifying the binding affinity of silybin, silychristin, and silydianin with the first extracellular loop of the Panx1 channel, as well as their ability to reduce the Panx1 channel pore diameter. Overall, the current *in vitro* and *in silico* studies provide substantial evidence for the Panx1 channel inhibitory properties of silybin, silychristin, and silydianin and suggest a novel alternative anti-inflammatory mechanism of these compounds based on the inhibition of Panx1 channel activity.

Presenter: Rita Ortega-Vallbona, ProtoQSAR SL, Spain.

Title: DockTox: molecular docking for in silico screening of small molecules targeting molecular initiating events.

Abstract: Introducing DockTox, an online molecular docking tool designed to predict chemical interactions with key proteins involved in toxicological pathways. It enables the automated docking of small molecules onto pre-processed protein structures relevant to

Molecular Initiating Events (MIEs) of adverse outcome pathways in the liver, kidney and developing brain. DockTox stands out with its unique features. It not only generates conformers from input molecules and calculates binding energies, but also provides detailed 2D/3D interaction maps and visualization plots. Its most distinctive capability is the calculation of an 'interaction fraction', which compares the interactions of a query ligand with those of reference ligands. This metric offers a more accurate and informative measure of protein-ligand affinity than binding energy alone. This feature significantly enhances the understanding of how a molecule interacts with its target protein, setting DockTox apart from other docking tools. With over 20 pre-processed proteins associated with MIEs and a user-friendly interface, DockTox facilitates the virtual screening of small molecules to detect interactions with key proteins implicated in toxicity pathways, offering valuable insights into the potential toxicological effects of chemical compounds.

Presenter: Filippo Molica, University of Geneva, Switzerland.

Title: Cold Exposure Rejuvenates the Metabolic Phenotype of *Panx1*$^{-/-}$ Mice.

Abstract: Pannexin1 (Panx1) ATP channels are important in adipocyte biology, potentially influencing energy storage and expenditure. We compared the metabolic phenotype of young (14 weeks old) and mature (20 weeks old) wild-type (WT) and *Panx1*$^{-/-}$ mice exposed or not to cold (6°C) during 28 days, a condition promoting adipocyte browning. Young *Panx1*$^{-/-}$ mice weighed less and exhibited increased fat mass, improved glucose tolerance, and lower insulin sensitivity than WT mice. Their energy expenditure and respiratory exchange ratio (RER) were increased, and their fatty acid oxidation decreased. These metabolic effects were no longer observed in mature *Panx1*$^{-/-}$ mice. The exposure of mature mice to cold exacerbated their younger metabolic phenotype. The white adipose tissue (WAT) of cold-exposed *Panx1*$^{-/-}$ mice contained more small-sized adipocytes, but, in contrast to WT mice, white adipocytes did not increase their expression of Ucp1 nor of other markers of browning adipocytes. Interestingly, Glut4 expression was already enhanced in the WAT of young *Panx1*$^{-/-}$ mice kept at 22°C as compared to WT mice. Thus, Panx1 deletion exerts overall beneficial metabolic effects in mice that are pre-adapted to chronic cold exposure. *Panx1*$^{-/-}$ mice show morphological characteristics of WAT browning, which are exacerbated upon cold exposure, an effect that appears to be associated with Ucp1-independent thermogenesis.

3. Session 2

Session 2 was structured into 2 parts, both chaired by Mathieu Vinken (VUB). The first part of session 2 consisted of 9 presentations delivered by members of the PANACHE consortium. These presentations aimed to provide an overview of the PANACHE project and a summary of the expertise and results obtained by each PANACHE consortium member. The second part of session 2 focused on the exploitation aspects of the PANACHE project. Specifically, the PANACHE exploitation strategy was presented followed by a discussion with the industry

representatives. A presentation containing the questions from the online questionnaire sent to the companies, along with their responses, was used to facilitate the discussion with the attendees.

Attachment 1

PANACHE

VRIJE UNIVERSITEIT BRUSSEL

3rd PANACHE WORKSHOP

Channeling energy into drug development

Organized by the Vrije Universiteit Brussel-Belgium

7-8 October 2024 | Virtual meeting | Free of charge

7 October: public access (register here)
8 October: only for companies (register here)

Click here for more practical information

This project has received funding from the European Union's Horizon 2020 Future and Emerging Technologies programme under grant agreement number 858014

3rd PANACHE workshop
Channeling energy into drug development

Monday 7 October 2024 (Central European Time)

Public access
Registration is free of charge, but mandatory. Please register here

13h00 - 13h05	**Welcome** Mathieu Vinken, Vrije Universiteit Brussel-Belgium
13h05 - 16h30	**Keynote lectures**
13h05 - 13h50	**The rise of peptides and peptidomimetics in drug discovery and development** Steven Ballet, Vrije Universiteit Brussel-Belgium
13h50 - 14h35	**Structural studies of connexin gap junction channels and hemichannels** Volodymyr Korkhov, Paul Scherrer Institute-Switzerland and Federal Institute of Technology Zurich-Switzerland
14h35 - 14h45	**Break**
14h45 - 15h30	**Pannexin channels in cancer, inflammation and cell death** Silvia Penuela, University of Western Ontario-Canada
15h30 - 16h15	**The scAInce of drug development and toxicology** Thomas Hartung, Johns Hopkins University-USA
16h15 - 16h30	**Break**
16h30 - 17h00	**Flash presentations**
16h30 - 16h35	**QSAR modeling for the prediction of pharmacokinetics and bioactivities of therapeutic peptides** Carmen Ortiz, ProtoQSAR SL-Spain
16h35 - 16h40	**Nanobody-based Panx1 channel inhibitors reduce inflammation in acute liver injury** Daan Peeters, Vrije Universiteit Brussel-Belgium
16h40 - 16h45	**A new stable Panx1 peptidomimetic for the prevention of myocardial ischemia/reperfusion injury** Malaury Tournier, University of Geneva-Switzerland
16h45 - 16h50	**Silymarin and its major components as a potential naturally occurring Panx1 channel inhibitor** Batuhan Yildiz, Vrije Universiteit Brussel-Belgium
16h50 - 16h55	**DockTox: molecular docking for *in silico* screening of small molecules targeting molecular initiating events** Rita Ortega, ProtoQSAR SL-Spain
16h55 - 17h00	**Cold exposure and Panx1 deletion reduce the progression of atherosclerosis** Filippo Molica, University of Geneva-Switzerland
17h00	**Wrap-up** Mathieu Vinken, Vrije Universiteit Brussel-Belgium

3rd PANACHE workshop
Channeling energy into drug development

Tuesday 8 October 2024 (Central European Time)

Only for companies
Registration is free of charge, but mandatory. Please register here

13h00 - 13h05	**Welcome** Mathieu Vinken, Vrije Universiteit Brussel-Belgium
13h05 - 14h50	**PANACHE presentations**
13h05 - 13h15	**Overview of the PANACHE project** Mathieu Vinken, Vrije Universiteit Brussel-Belgium
13h15 - 13h25	**Expertise of the Research Group of Organic Chemistry of the Vrije Universiteit Brussel-Belgium** Thomas Barlow, Vrije Universiteit Brussel-Belgium
13h25 - 13h35	**PANACHE results obtained by the Research Group of Organic Chemistry of the Vrije Universiteit Brussel-Belgium** Steven Ballet, Vrije Universiteit Brussel-Belgium
13h35 - 13h45	**Expertise of the *In Vitro* Toxicology Group of the Vrije Universiteit Brussel-Belgium** Mathieu Vinken, Vrije Universiteit Brussel-Belgium
13h45 - 13h55	**PANACHE results obtained by the *In Vitro* Toxicology Group of the Vrije Universiteit Brussel-Belgium** Andrés Tabernilla, Vrije Universiteit Brussel-Belgium
13h55 - 14h05	**Expertise of the Connexin Group of the Department of Pathology and Immunology of the University of Geneva-Switzerland** Brenda Kwak, University of Geneva-Switzerland
14h05 - 14h15	**PANACHE results obtained by the Connexin Group of the Department of Pathology and Immunology of the University of Geneva-Switzerland** Brenda Kwak, University of Geneva-Switzerland
14h15 - 14h25	**Expertise of ProtoQSAR SL-Spain** Rafael Gozalbes, ProtoQSAR SL-Spain
14h25 - 14h35	**PANACHE results obtained by ProtoQSAR SL-Spain** Laureano Carpio, ProtoQSAR SL-Spain
14h35 - 14h50	**Break**
14h50 - 16h00	**PANACHE exploitation**
14h50 - 15h10	**PANACHE exploitation strategy** Freddy Van Goethem, Vrije Universiteit Brussel-Belgium
15h10 - 16h00	**Discussion of PANACHE exploitation with attendees**
16h00	**Wrap-up** Mathieu Vinken, Vrije Universiteit Brussel-Belgium

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UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

ProtoQSAR
Chemoinformatics and modeling solutions to optimize your chemicals

This project has received funding from the European Union's Horizon 2020 Future and Emerging Technologies programme under grant agreement number 858014

Attachment 2


3rd PANACHE
WORKSHOP
Channeling energy into
drug development
Organized by the
Vrije Universiteit Brussel-Belgium
7-8 October 2024 | Virtual meeting | Free of charge


Third PANACHE workshop
Written by PANACHE
Categorised Events


Mathieu Vinken · 1st
Professor/in vitro toxicologist
4mo ·
You are invited to attend the third online workshop of the European PANACHE project focused on the development and testing of novel anti-inflammatory ...see more
workshop · 4 pages
PANACHE
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3rd PANACHE
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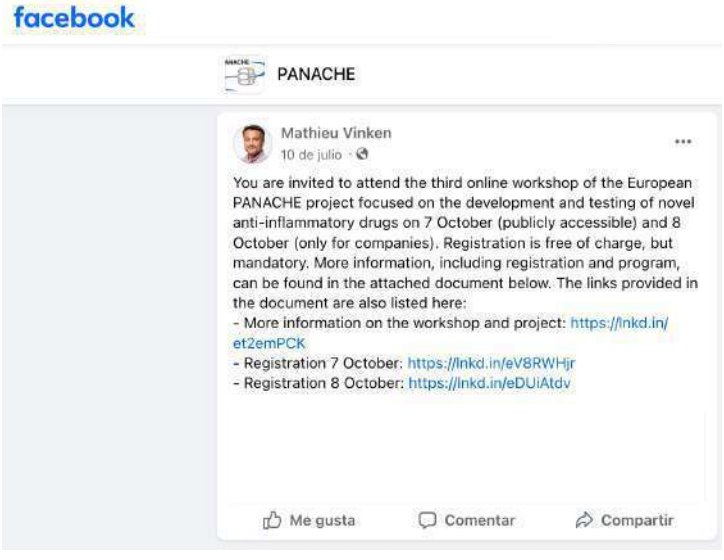
22
7 reposts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PANACHE
Mathieu Vinken
10 de julio ·
You are invited to attend the third online workshop of the European PANACHE project focused on the development and testing of novel anti-inflammatory drugs on 7 October (publicly accessible) and 8 October (only for companies). Registration is free of charge, but mandatory. More information, including registration and program, can be found in the attached document below. The links provided in the document are also listed here:
- More information on the workshop and project: https://lnkd.in/et2emPCK
- Registration 7 October: https://lnkd.in/eV8RWHjr
- Registration 8 October: https://lnkd.in/eDUiAtdv
Me gusta
Comentar
Compartir

Attachment 3

ABOUT PANACHE

PANACHE is a multidisciplinary collaborative project funded by the European Union's Horizon 2020 Future and Emerging Technologies (FET) programme that aims at the development of new anti-inflammatory drugs.

5 years

1 March 2020 - 28 February 2025

3.5 million €

3.503.628,75€ granted by the EU

4 partners

1 industrial and 3 academic partners

3 countries

Belgium, Spain, Switzerland

Project coordinator

Research Group of *In Vitro* Toxicology (IVTD)
Vrije Universiteit Brussel (Belgium)

Partners

Research Group of Organic Chemistry (ORGC)
Vrije Universiteit Brussel (Belgium)

ProtoQSAR S.L. (PROTOQSAR)
ProtoQSAR 2000 S.L. (Spain)

Research Group of Connexins in Cardiovascular Disease (UNIGE)
Université de Genève (Switzerland)

VUB VRIJE UNIVERSITEIT BRUSSEL

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PANACHE

Production of next generation modulators of pannexins and connexins as novel therapeutics in the treatment of inflammatory cardiovascular and hepatic diseases

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PANACHE

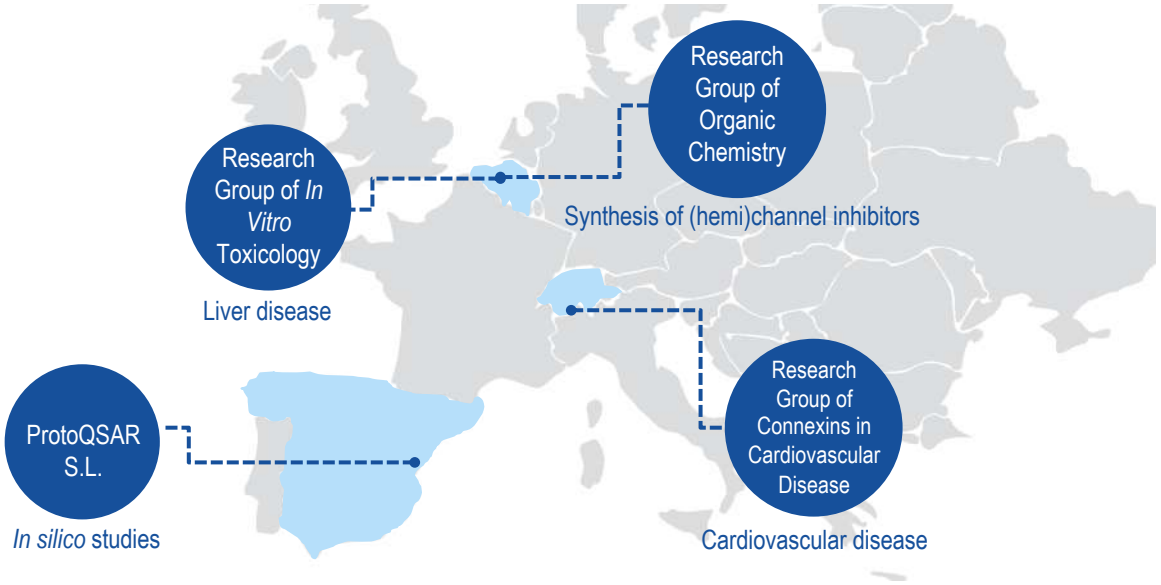
This project has received funding from the European Union's Horizon 2020 Future and Emerging Technologies programme under grant agreement number 858014

THE PROJECT

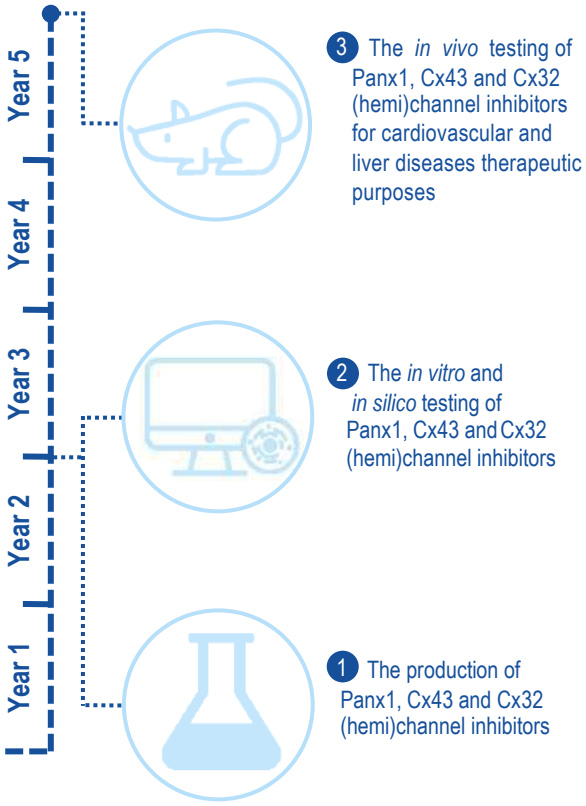
The modulation of membrane-bound proteins by drugs is receiving increasing attention from both academia and industry. Among such proteins are pannexin1 (Panx1), connexin (Cx) 43 and Cx32 that form (hemi)channels at the plasma membrane surface. These (hemi)channels mediate cellular communication and have emerged as key players in inflammation. This carries translational relevance, as (hemi)channel inhibition could represent an innovative strategy for the treatment of a plethora of diseases. However, a hurdle in clinical exploration is the lack of appropriate (hemi)channel inhibitors.

PANACHE therefore is a timely project, since it will generate a novel generation of (hemi)channel inhibitors as potential drugs. This will be accomplished by joining academic and industrial scientists from the chemical, chemo-informatics and biomedical fields as well as by relying on *in vitro* and *in silico* studies, animal experimentation and testing human material.

PANACHE will allow taking a leap forward to the realization of its long-term vision, namely the production of metabolically robust and selective (hemi)channel inhibitors that can be used for the establishment of a generic approach to synergize current therapy of hard-to-treat inflammatory diseases.



OUR OBJECTIVES



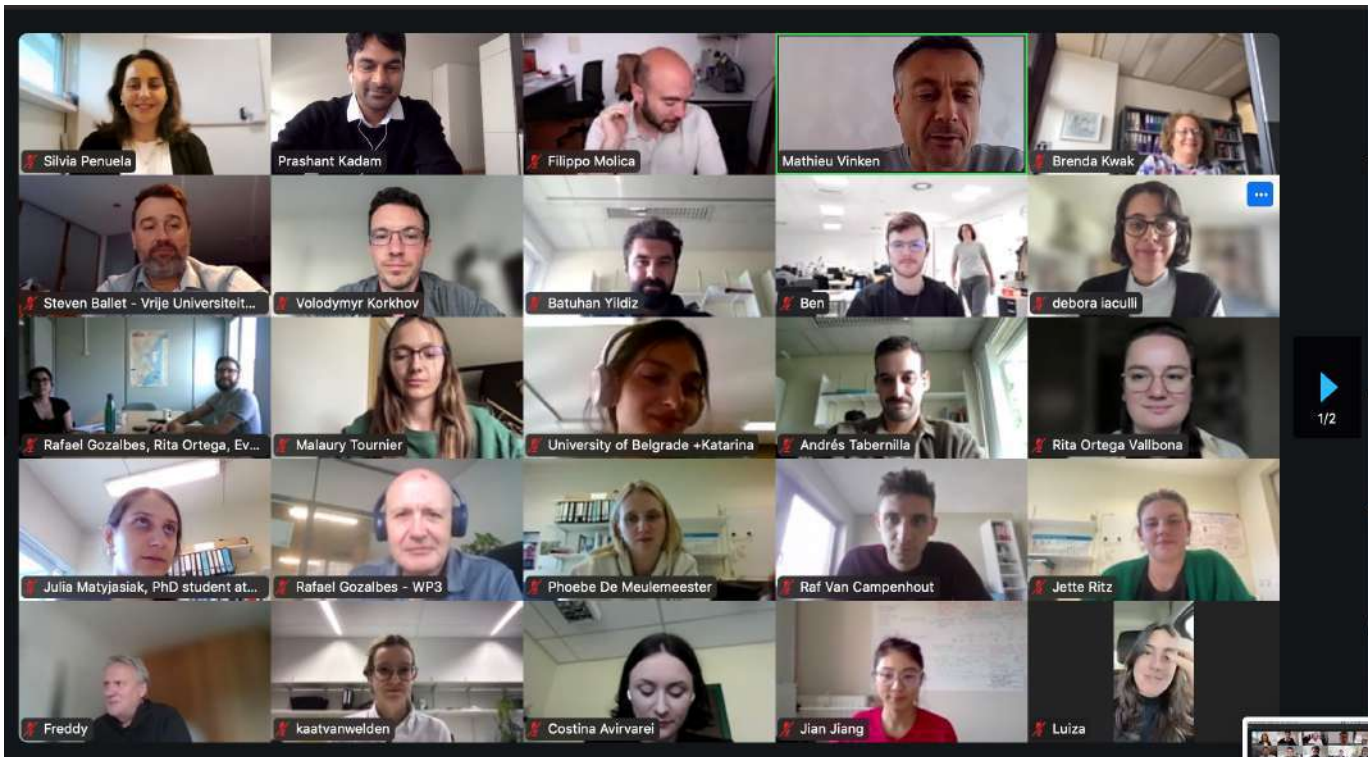
OUR VISION

The long-term vision of PANACHE is the production of an unprecedented set of (hemi)channel inhibitors that can be used for the establishment of a mechanistically anchored and generic approach to synergize current therapy of hard-to-treat inflammatory diseases. For proof-of-concept purposes, focus will be put on inflammatory disorders in the cardiovascular system and the liver.

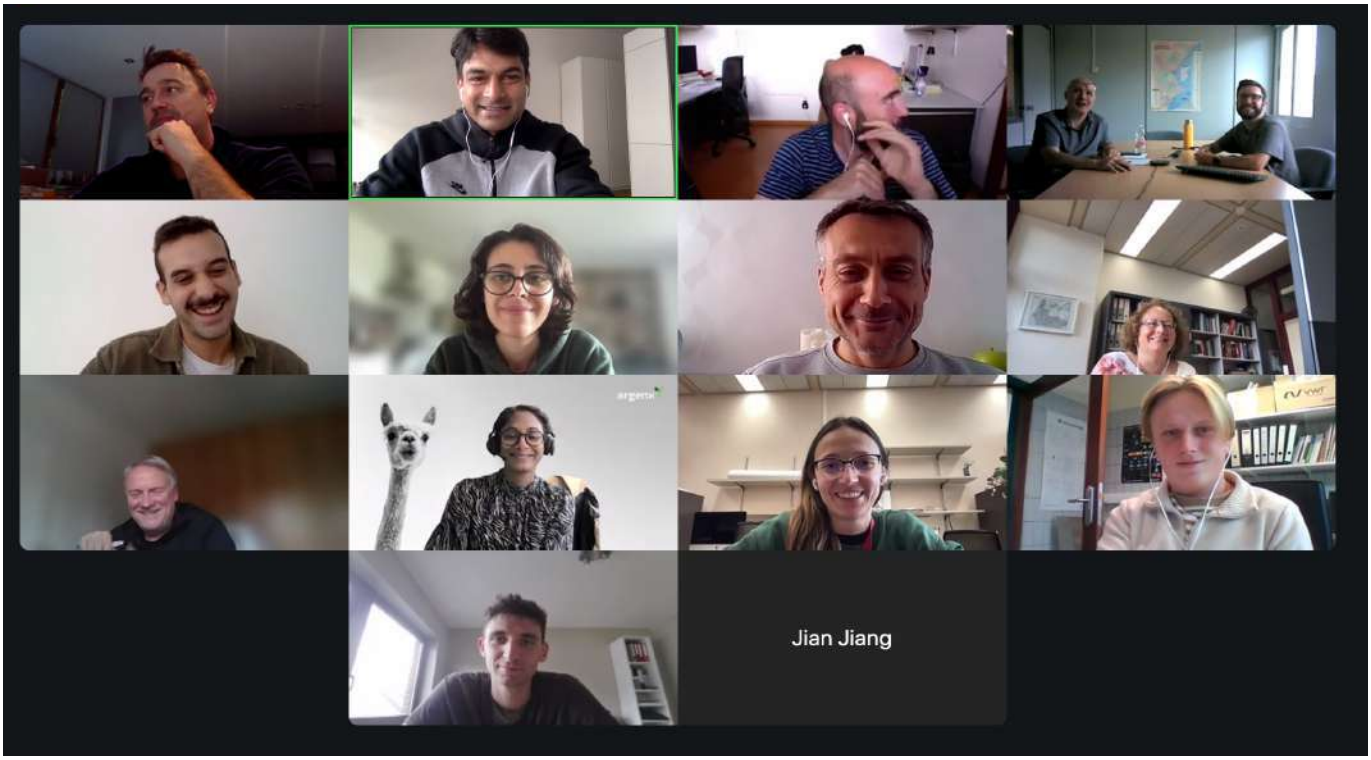
The scope of PANACHE is, however, much broader, as these innovative (hemi)channel inhibitors are anticipated to be equally applicable for the therapy of a number of other inflammatory disorders in which Panx1, Cx43 and Cx32 are known to be involved. Such applications will be tested in follow-up initiatives of PANACHE, thereby realizing its long-term vision.

Attachment 4

Session 1



Session 2



Attachment 5

3rd PANACHE WORKSHOP

The Rise of Peptides and Peptidomimetics in Drug Discovery and Development

Prof. Steven Ballet

ORGANIC CHEMISTRY RESEARCH GROUP

Prof. Steven Ballet

Head of Research Group of Organic Chemistry (ORGC) at the Vrije Universiteit Brussel

Research interests:

- Peptides and therapeutic molecules
- Peptides as functional materials
- The synthesis of non-canonical (especially constrained) amino acids

VUB ORGC ORGANIC CHEMISTRY RESEARCH GROUP

https://orgc.research.vub.be

Contents

Introduction and Historical Background

Facts and Figures of Peptides as Therapeutics

Peptides and Peptidomimetics

Examples of Peptide Therapeutics

Historical Background

- Advances in **molecular pharmacology** after World War II made it possible to express the biological activity of a compound as quantifiable molecular properties.
- Scientists began to manipulate various parts of the molecules and observe the resulting changes in their biological activities.

  => Structure-activity relationship (SAR) studies
- Our repertoire of potential drug candidates has expanded to larger modalities.
  - mAbs, Nbs, etc.
  - peptides

VUB ORGC ORGANIC CHEMISTRY RESEARCH GROUP

Historical Background

- Peptides can use natural pathways in our bodies.
- Several peptide drugs are essentially "*replacement therapies*".
- **Peptides isolated from natural sources, such as insulin and adrenocorticotropic peptide (ACTH), provided life-saving medicines in the first half of the 20th century.**

A Renaissance of Peptides as Therapeutics

Peptides were not considered viable therapeutic molecules.

More recently, a broader and more nuanced appreciation of the potential of peptide therapeutics has emerged.

Previous liabilities no longer problematic because chemistry now exists to circumvent most issues characteristic of peptides

Disadvantages
Metabolic instability
Poor membrane permeability
Poor oral bioavailability
Poor solubility
Rapid clearance
High manufacturing cost

Advances in the Synthesis of Peptides

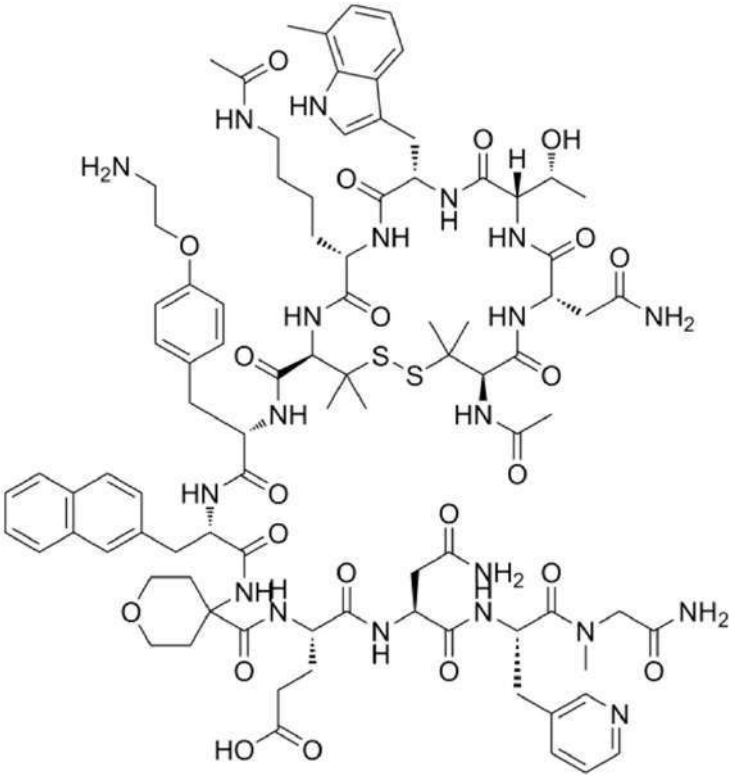
Bruce Merrifield
1921–2006

- Polypeptides were first chemically synthesized in 1954 (**Vincent du Vigneaud**), recognized with the Nobel Prize in Chemistry a year later.

- Another leap forward was **Bruce Merrifield**'s visionary idea to automate peptide synthesis **by assembling amino acids on a 'solid phase'**, leading to the invention in 1963 ***of solid-phase peptide synthesis (SPPS)***

  => Also recognized with the Nobel Prize in Chemistry (1984).

Current Status of Peptides as Therapeutics



JNJ-77242113

- Peptide drug candidates are now being generated against a very wide range of molecular targets that reach beyond historically-dominant extracellular hormone receptors.
  - disrupt protein-protein interactions
    - E.g., **Keap1–Nrf2 protein–protein interaction**
  - target cytokine receptors
    - E.g., **JNJ-77242113** against IL-23 receptor
  - inhibit intracellular targets
    - E.g., **KS-58** inhibitor of $KRAS^{G12C}$

So far, more than 80 peptide drugs have been approved in the United States, Europe, and Japan.

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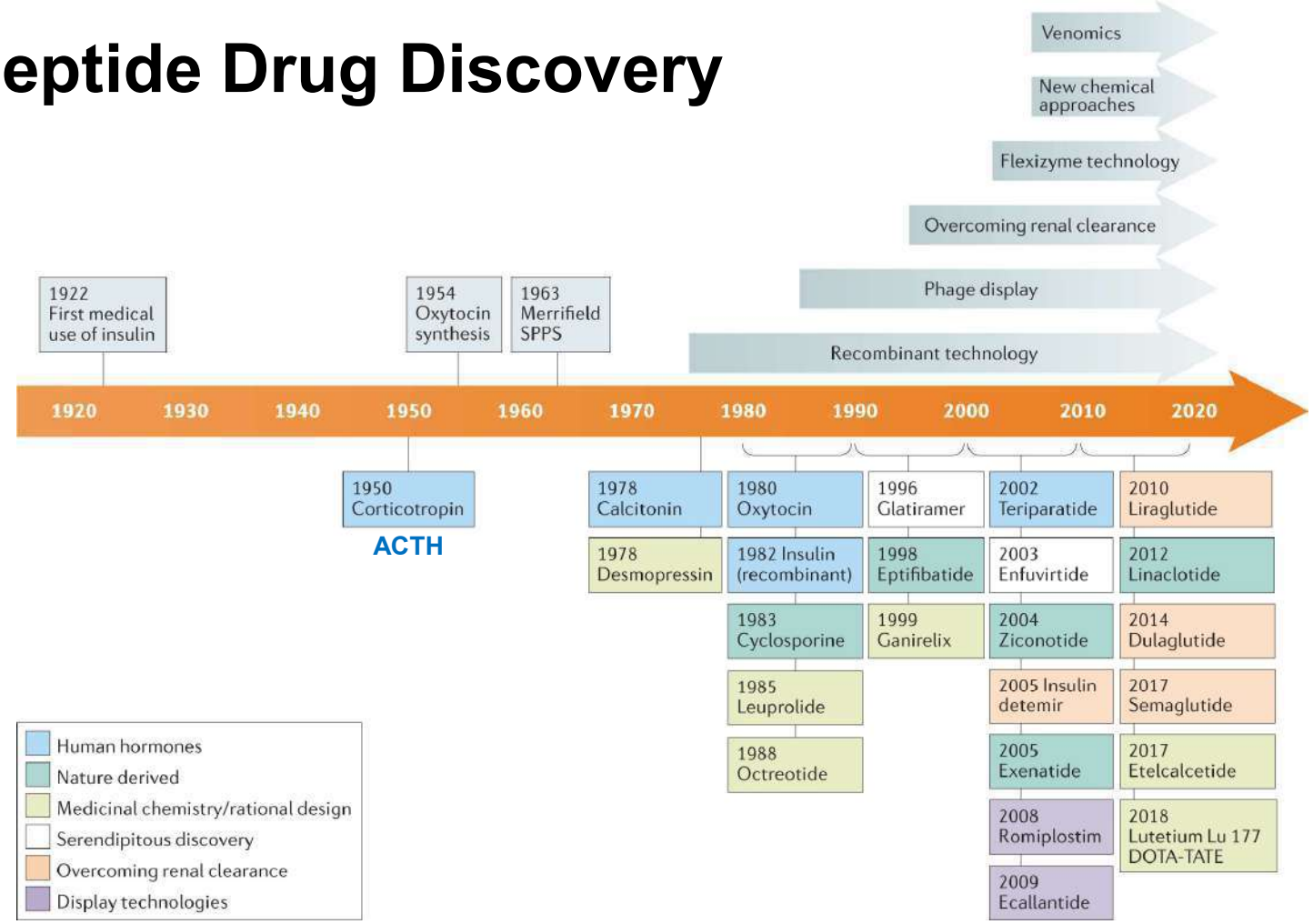
Timeline of Peptide Drug Discovery



Nature Reviews Drug Discovery, **2021**, 20, 309–325

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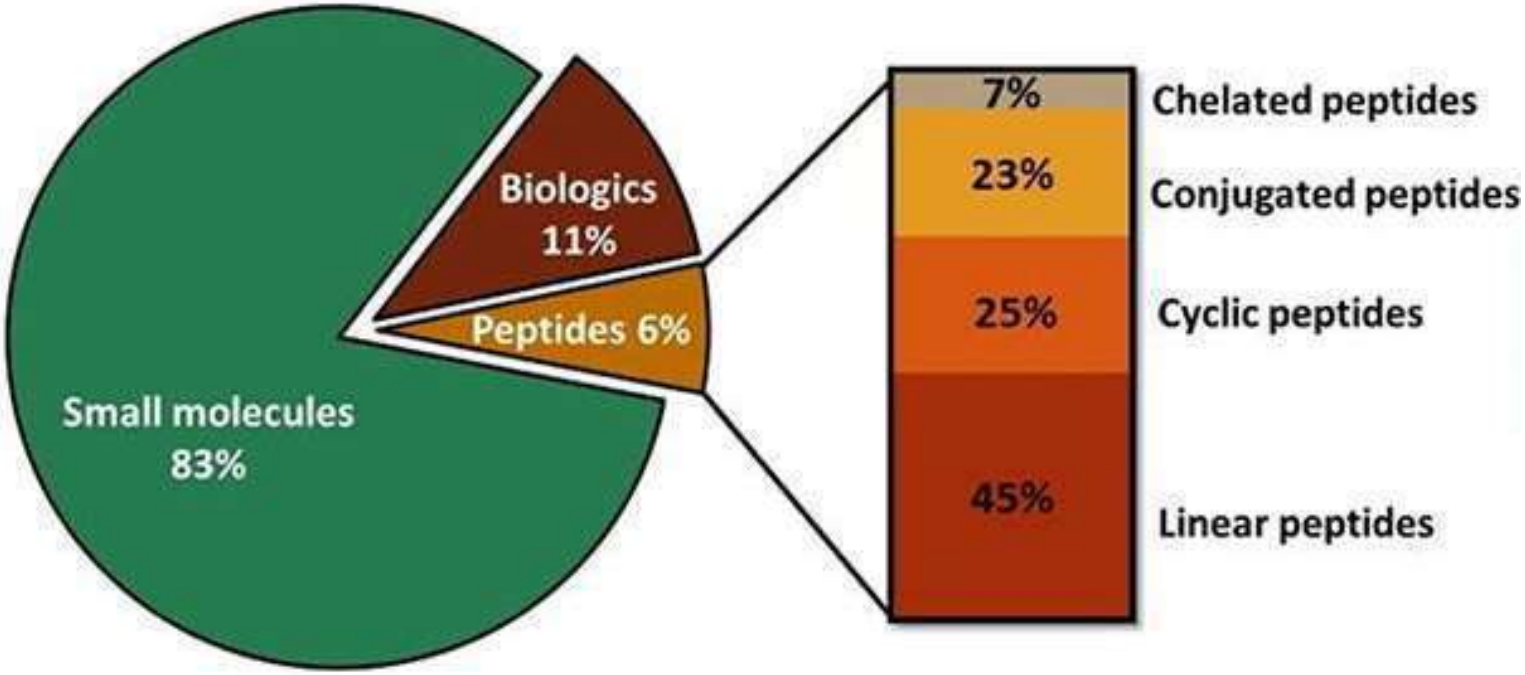
Blockbuster Peptides

Peptide Drugs Global Sales in 2020 (Million in unit $)			
Name	**Company**	**Sales (Million $)**	**Indication**
Trulicity (Dulaglutide)	Eli Lilly	5056	Type 2 diabetes, obesity
Victoza (Liraglutide)	Novo Nordisk	3961	Type 2 diabetes, obesity
Ozempic (Semaglutide)	Novo Nordisk	3755	Type 2 diabetes, obesity
Sandostatin (Octreotide)	Novartis	1439	Acromegal, diarrhea associated with metastatic carcinoid tumors and vasoactive intestinal peptide tumors
Copaxone (Glatiramer acetate)	Teva	1337	Multiple sclerosis
Forteo (Teriparatide)	Eli Lilly	1046	Osteoporosis
Zoladex (Goserelin)	AstraZeneca	888	Breast and prostate cancer
Lupron (Leuprorelin)	AbbVie	752	Breast and prostate cancer

Proportion of Peptide Therapeutics



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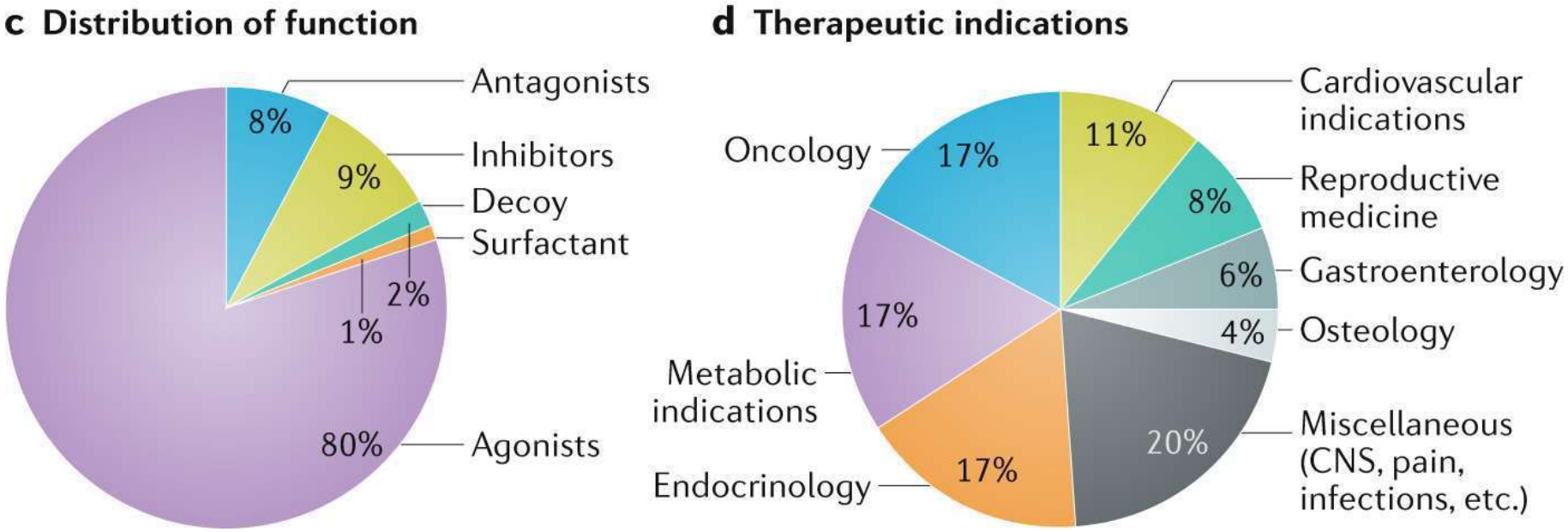
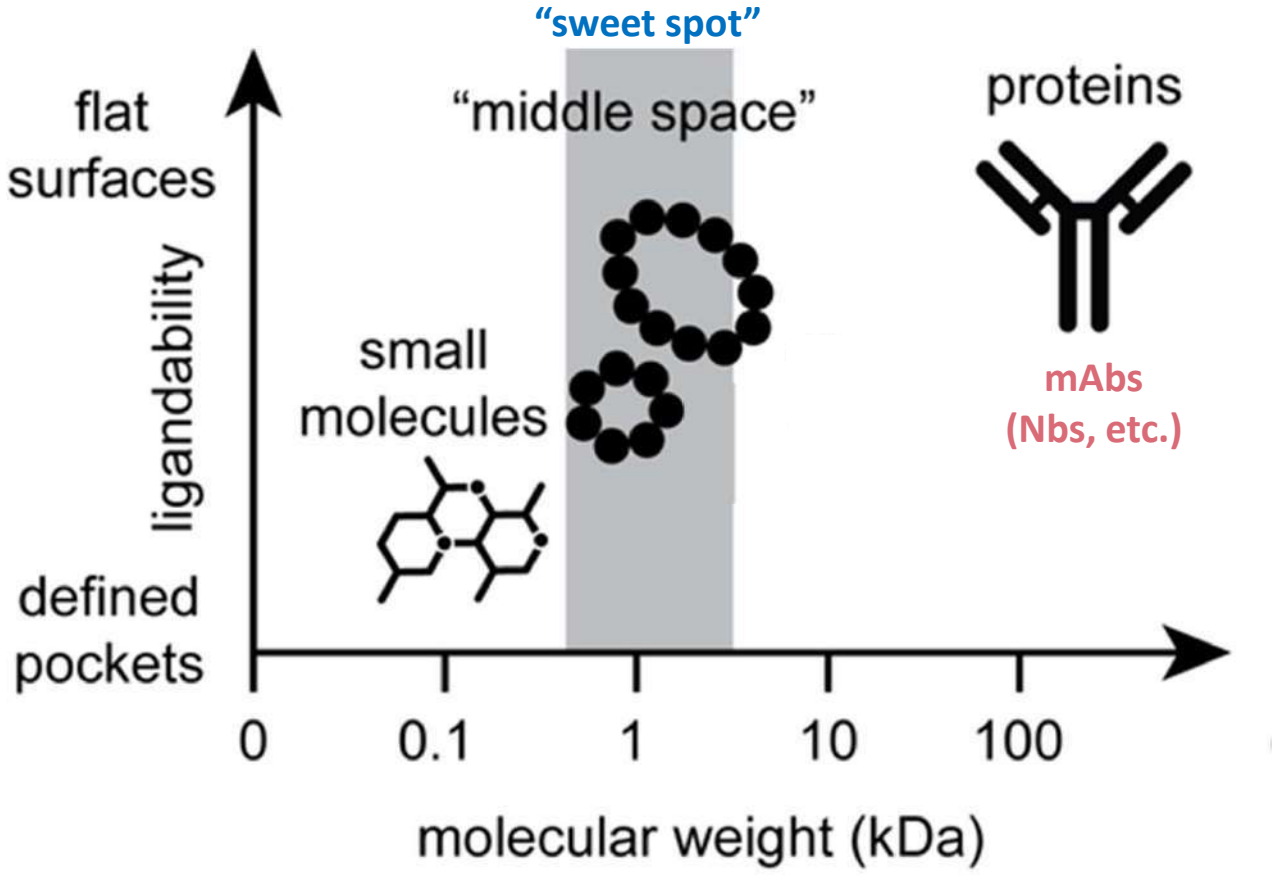
Drug Discovery Today, **2023**, 28, 103464

What are these like?



Nature Reviews Drug Discovery, **2021**, 20, 309–325

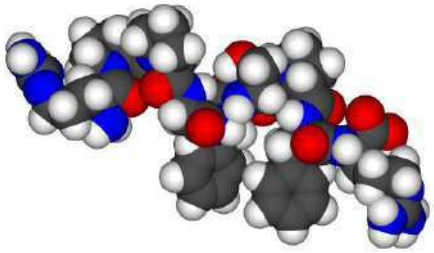
Peptides as Therapeutics



Comparison of Small Molecules and Peptides

- high oral bioavailability
- metabolic stability
- high numbers of biological targets
- small dimensions → cavities

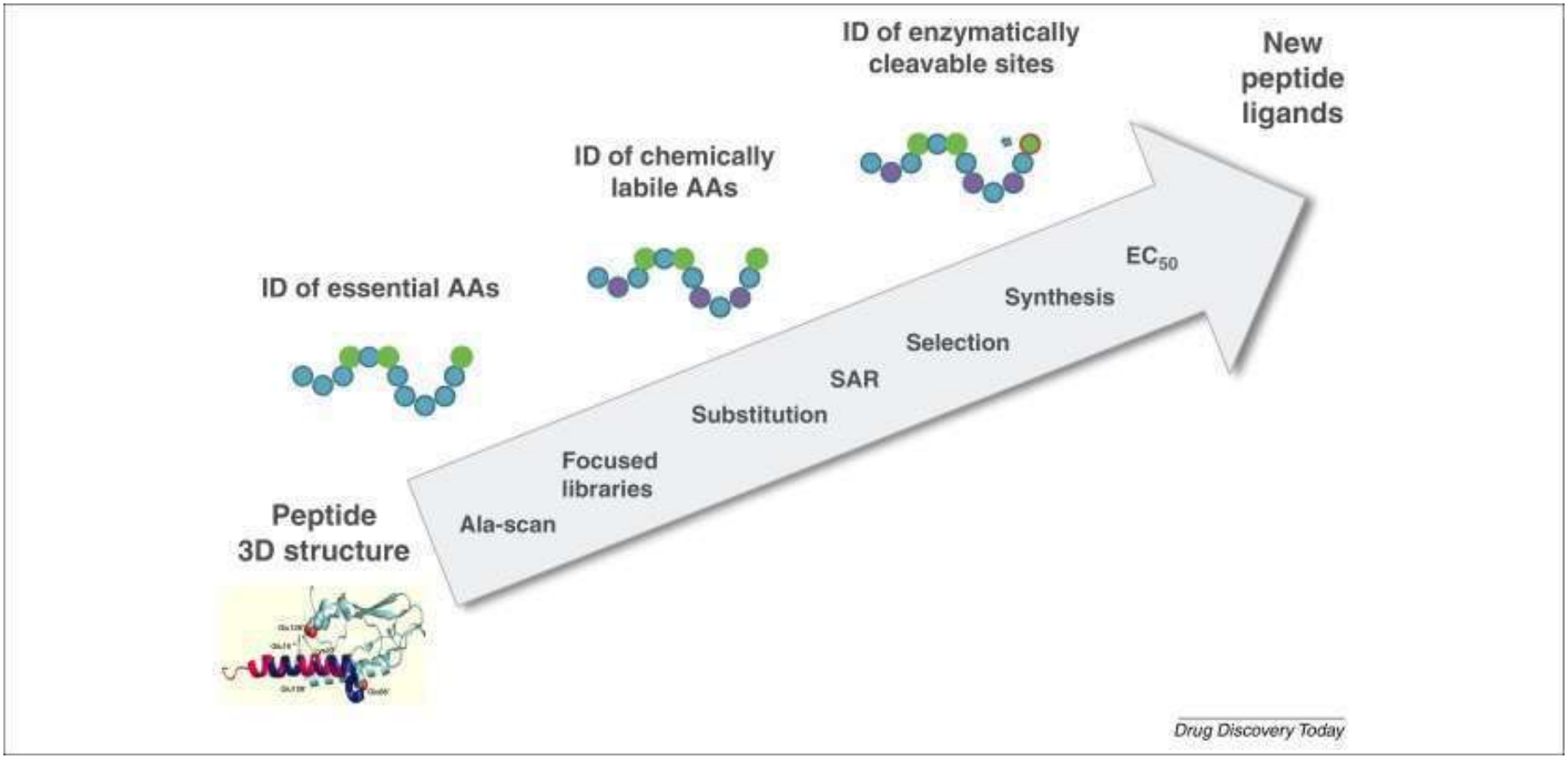
- poor selectivity
  - many side-effects
  - high toxicity



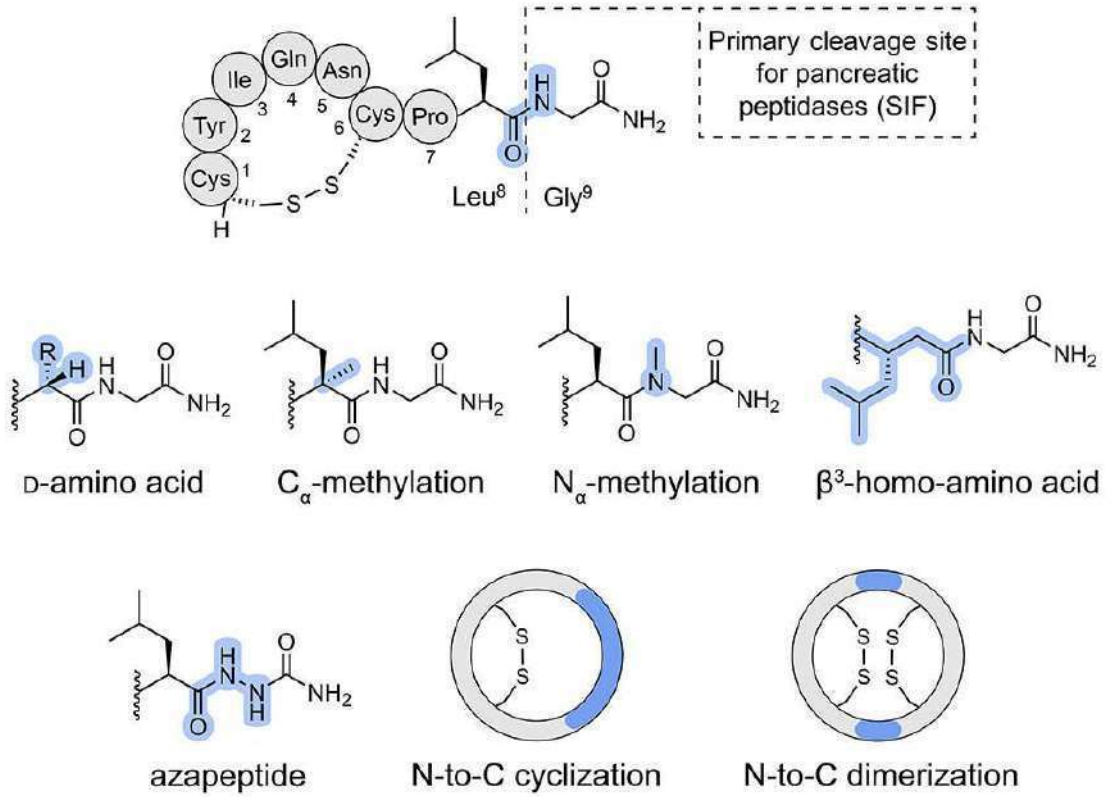
- high potency, large surface area
- high selectivity
- low toxicity
- high chemical and biological diversity

- poor membrane permeability
- poor oral bioavailability and rapid clearance

Development of Peptide Therapeutics



Development of Peptide Therapeutics



- Inherent susceptibility of peptides to enzymatic degradation in the gastrointestinal tract is a key bottleneck in oral peptide drug development.
- Chemical strategies exist to overcome this problem.
- A selection of these is shown

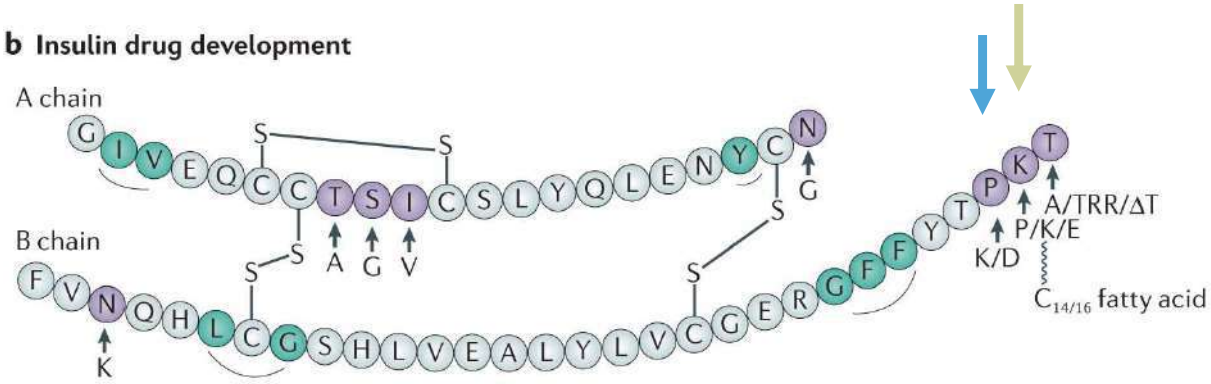
J. Med. Chem, **2022** 65, 6191-6206

Insulin-derived Peptides: AA substitutions & lipidation

Green residues are crucial for receptor binding, demonstrating why it is difficult to develop a small-molecule drug that can directly activate the insulin receptor.

The **purple residues** and the table highlight point modifications in insulin drug analogues.

fast-acting analogues on a blue background and long-acting analogues on an olive background



Analogue	A chain	B chain	Approval
Porcine		T30A	1966
Bovine	T8A, I10V	T30A	1966
Sheep	T8A, S9G, I10V	T30A	NA
Human			1982
Lispro		P28K, K29P	1996
Aspart		P28D	2000
Glargine	N21G	+31R, +32R	2000
Glulisine		N3K, K29E	2004
Detemir		K29K (C_{14} fatty acid)	2005
Degludec		ΔT30, K29E (C_{16} fatty acid)	2015

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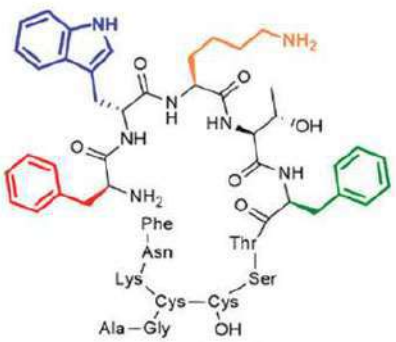
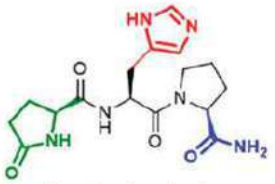
Peptides vs. Peptidomimetics

Sequence composed of proteinogenic amino acids with limited **'natural'** modifications/stabilizations

Peptides



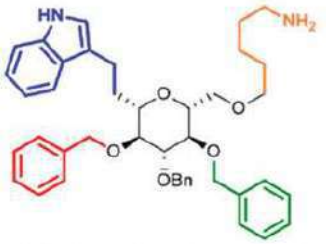
somatostatin-14



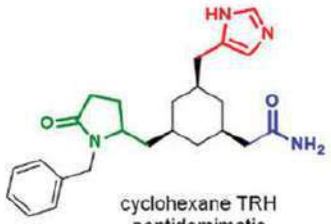
thyrotropin-releasing hormone (TRH)

- Limited stability towards proteolysis
- Rapid excretion, poor cell permeability
- Interaction with multiple targets

Peptidomimetics



β-D-glucose based somatostatin-14 peptidomimetic



cyclohexane TRH peptidomimetic

- Higher stability towards proteolysis
- Better transport properties
- Selectivity against non target receptors

More extensive modifications to alter pharmacologic and physicochemical profile

Use of a 'scaffold'

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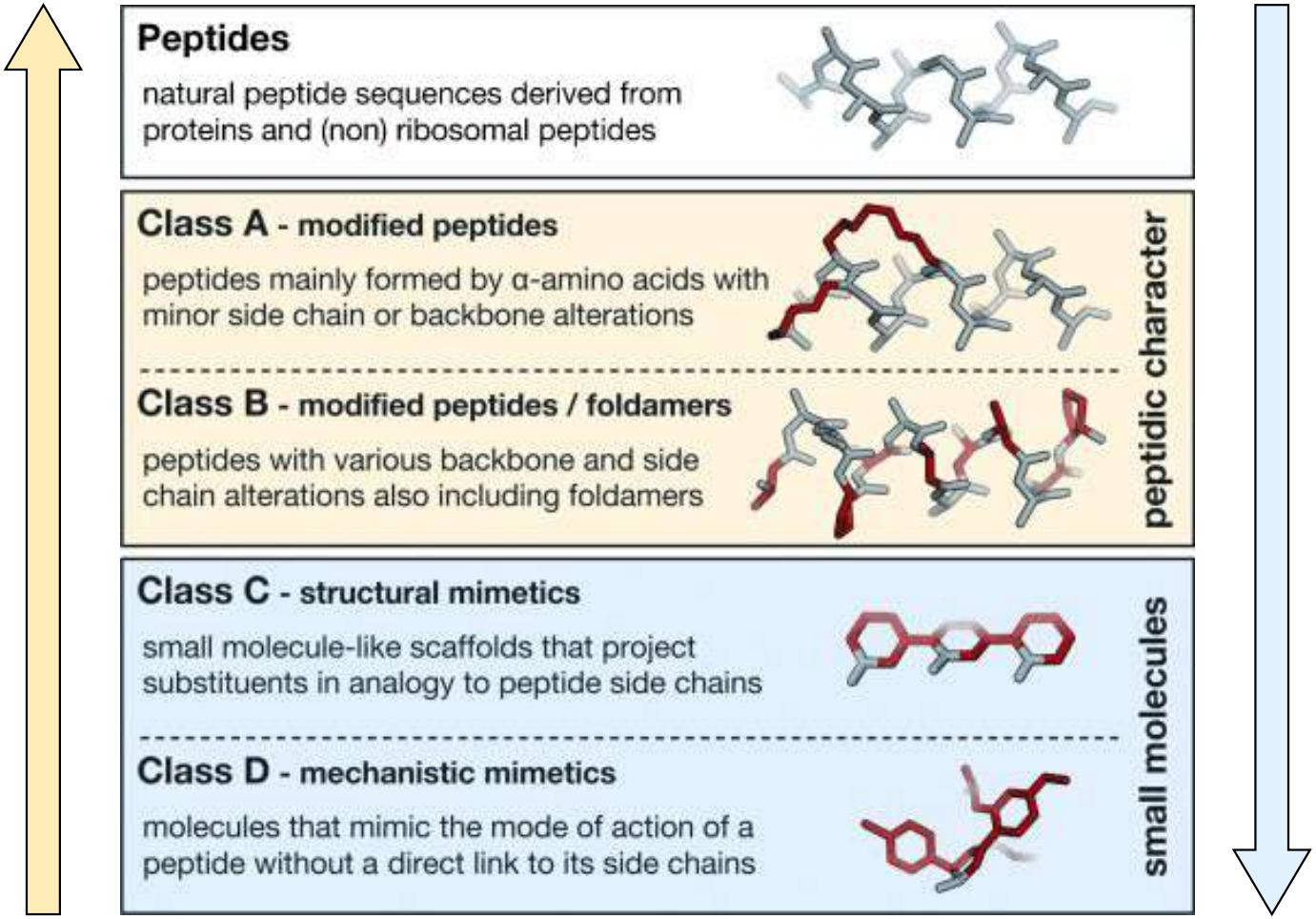
Chem. Soc. Rev., **2020**, 49, 3262-3277

More peptide character



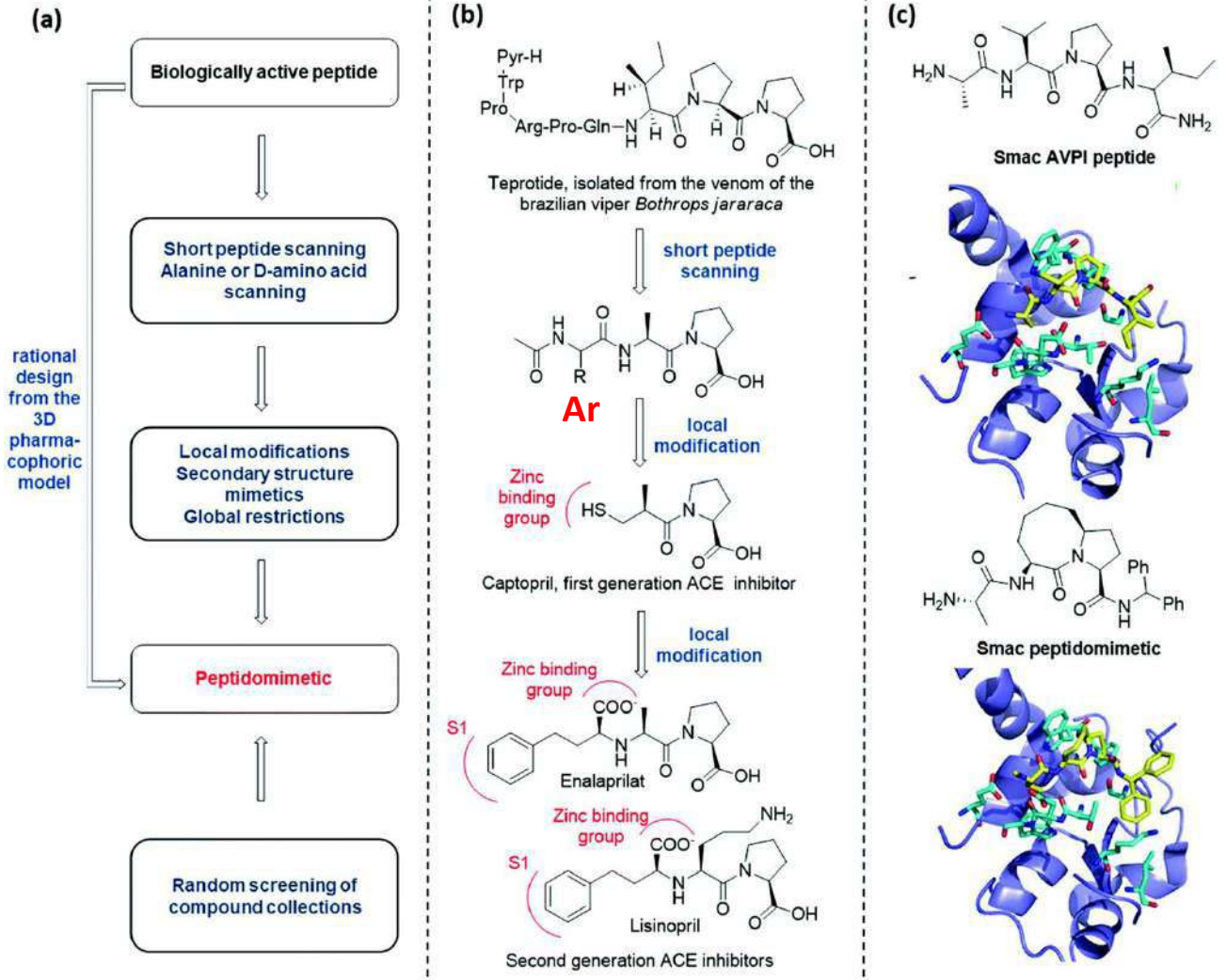
More small molecule character

Angew. Chem. Int. Ed., **2015**, 54, 8896 – 8927

Development of Peptide Therapeutics

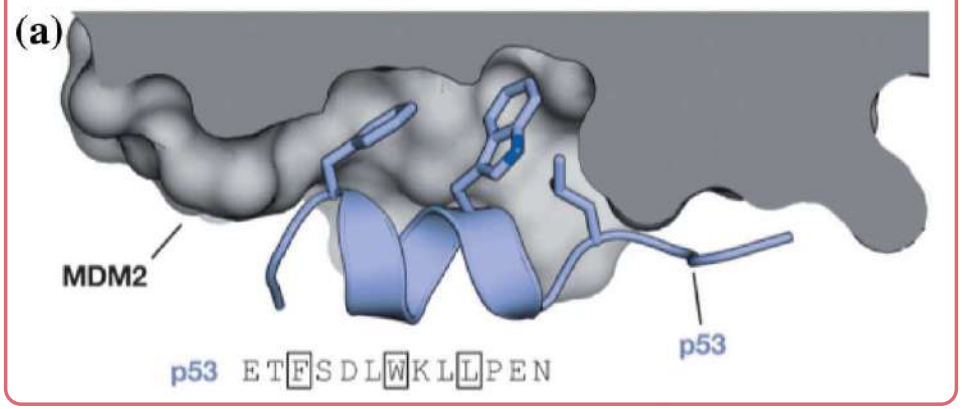
Standard steps that are performed to develop peptide therapeutics

(b) Pure peptide development
(c) Development of peptidomimetics



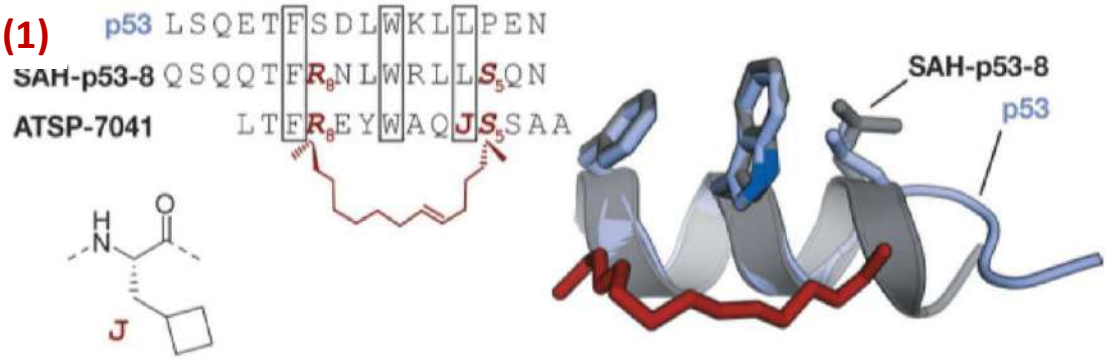
Chem. Soc. Rev., **2020**, 49, 3262-3277

MDM2–p53 interaction

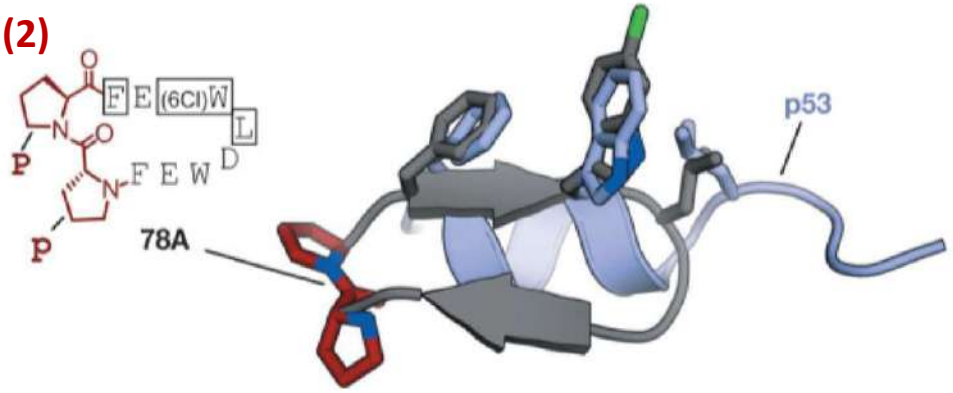


Stapled helical peptide

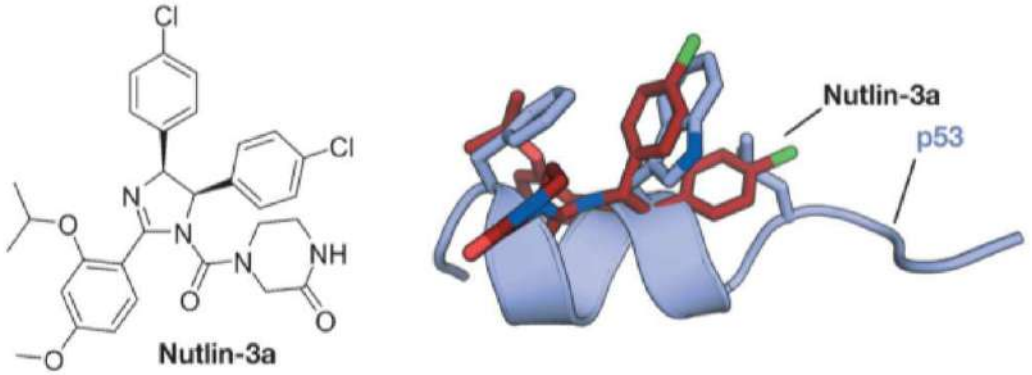
(1)



(2)



(3)



Change of secondary structure element

Translation into small molecule

1. *Int. J. Pept. Res. Ther*. **2020**, 26, 225–241
2. *Frontiers Oncology*, **2016,** 6, 7.
3. *Lancet Oncology*, **2012**, 13, 1133–1140

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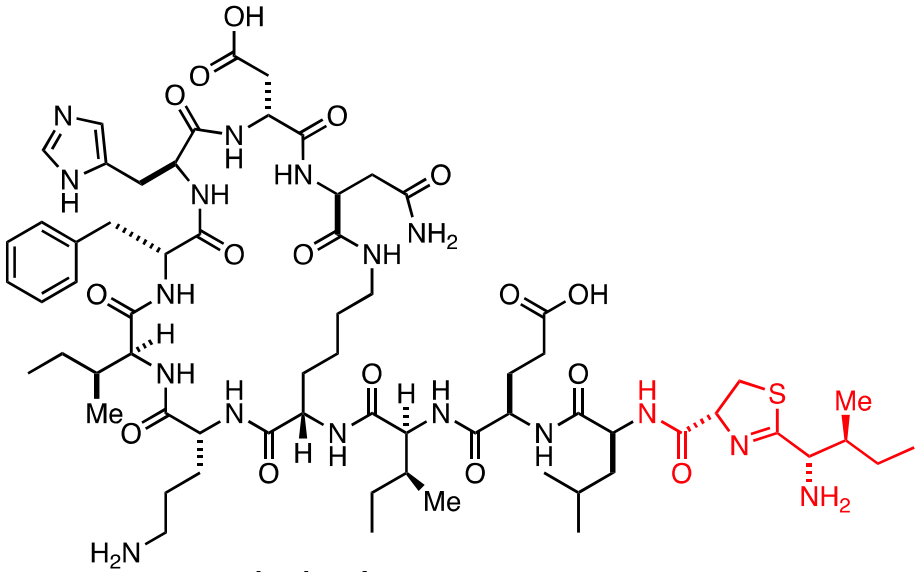
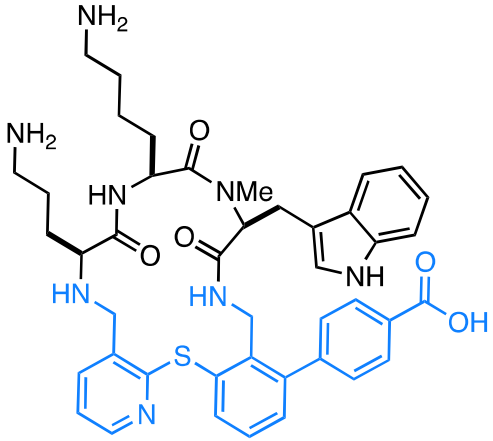
Antimicrobial Peptides



bacitracin



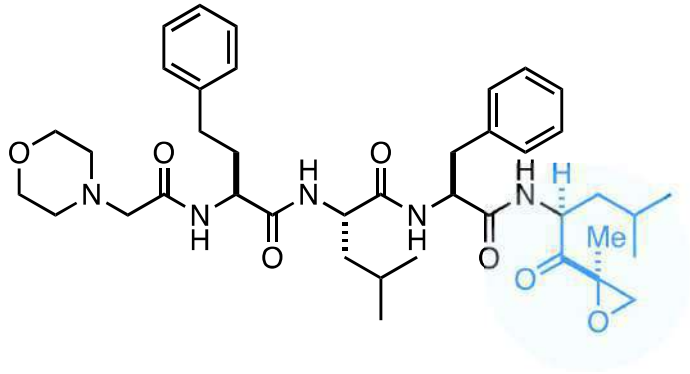
zosurabalpin

- Serendipitous discovery in 1945
- interfering with cell wall and peptidoglycan synthesis in Gram negative bacteria
- used as a topical preparation, renotoxicity when used systemically

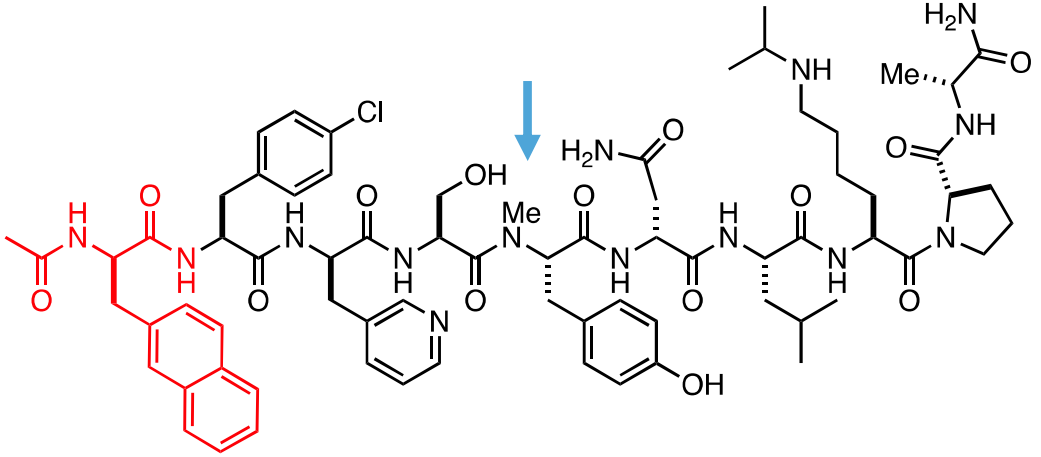
- Belongs to novel class of tethered macrocyclic peptide (MCP) antibiotics
- In clinical trials against highly drug-resistant bacteria
- **Novel mechanism of action**

1. *PNAS USA,* **2024**, 121, e2315310121
2. *Nature*, **2024,** 625, 566–571

Antineoplastic Peptides



carfilzomib



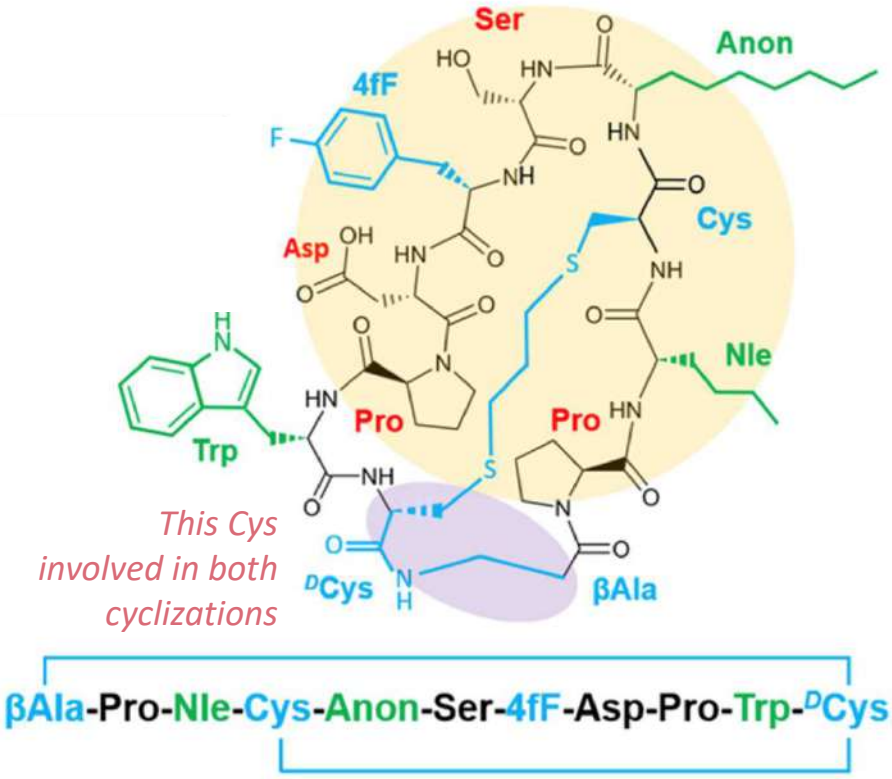
abarelix

- Approved by the EMA as an orphan medicine against multiple myeloma in 2008.
- inhibits proteasome-mediated proteolysis by selectively binding to *N*-terminal threonine-containing sites of the 20S proteasome in a covalent and irreversible manner ("covalent warhead").

- Approved in 2005 in the EU.
- Antagonist of gonadotropin-releasing hormone antagonist
- used in oncology to reduce testosterone production in advanced prostate cancer patients.
- Naphthyl group likely introduced to improve affinity for the target receptor.

1. *Nat. Prod. Rep.*, **2013,** 30, 600-604
2. *Ther. Adv. Urol.*, **2011**, 3, 127–140

Anti-KRAS Peptide KS-58



KRAS is a critical protein in all cells but is very frequently mutated in many cancers, especially pancreatic cancer.

KS-58 is the first peptide that inhibits the $KRAS^{G12D}$ anti-cancer activity in vivo

Asp^{8} is critical for binding to $KRAS^{G12D}$ and the molecule's activity but also renders the molecule water soluble.

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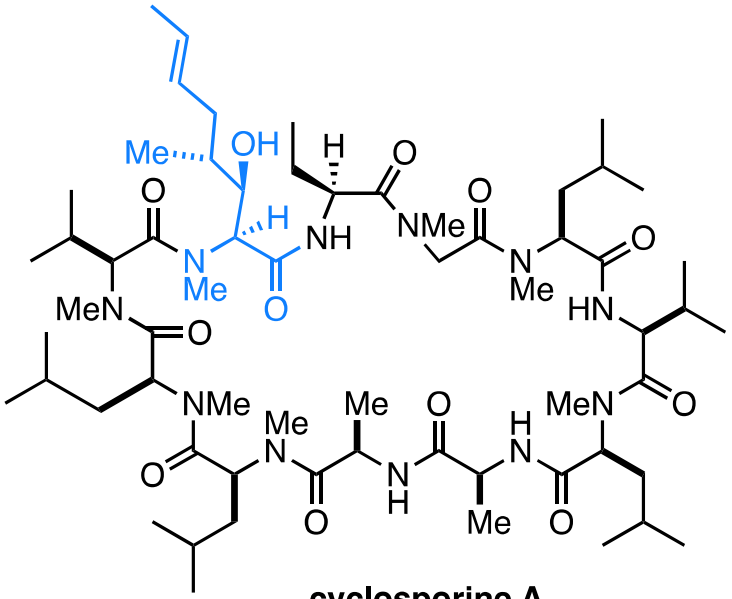
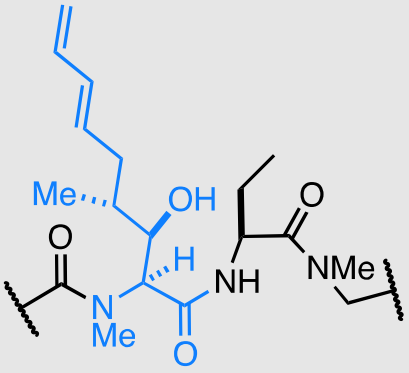
Scientific Reports, **2020**, 10, 21671

Immunosuppressive Peptides



cyclosporine A



voclosporin

- Isolated from a fungus in 1971
- The immunosuppressive effect determined in 1972
- chemical structure determined in 1976

- an immunosuppressant medication **taken orally** or intravenously for rheumatoid arthritis, psoriasis, Crohn's disease, nephrotic syndrome, eczema, and in organ transplants to prevent rejection

The unsaturated α-amino, β-hydroxy acid **MeBmt** is a key structural feature of cyclosporin A and many other natural products.

Single-carbon extention ensures even more potent inhibition of calcineurin

- **Voclosporin** is a novel agent approved in 2021 in for treating and managing lupus nephritis.

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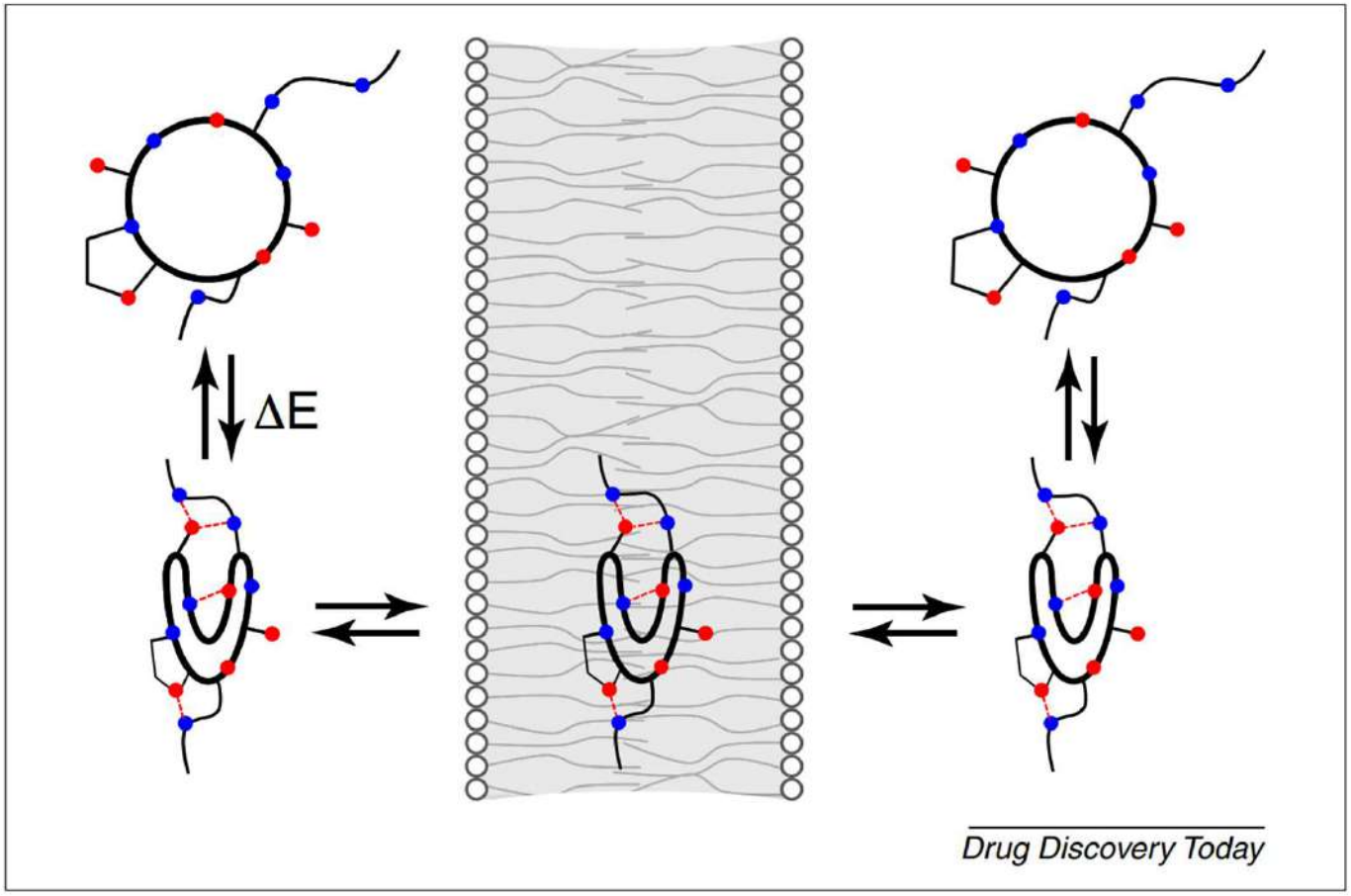
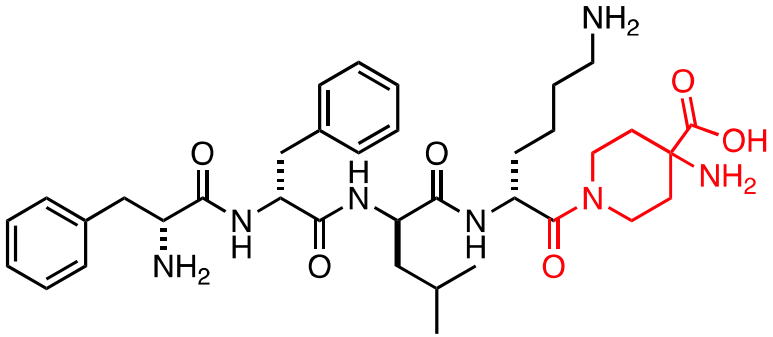
1. *Curr. Med. Chem.*, **2021**, 28, 3925-3934
2. *Expert. Opin. Pharmacother.*, **2018**, 19, 1613-1621

Immunosuppressive Peptides

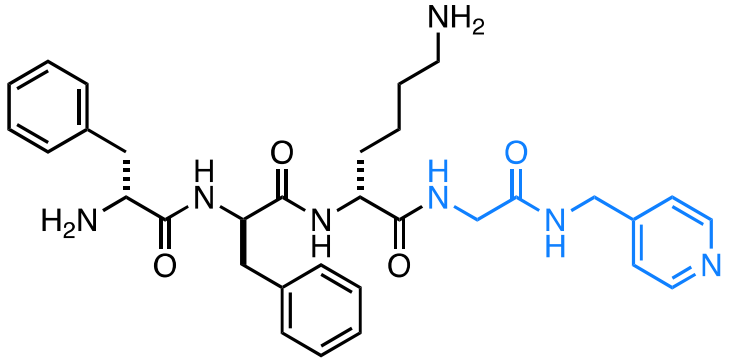


Drug Discovery Today, 21, 5, 2016

Antipruritic Peptides



difelikefalin



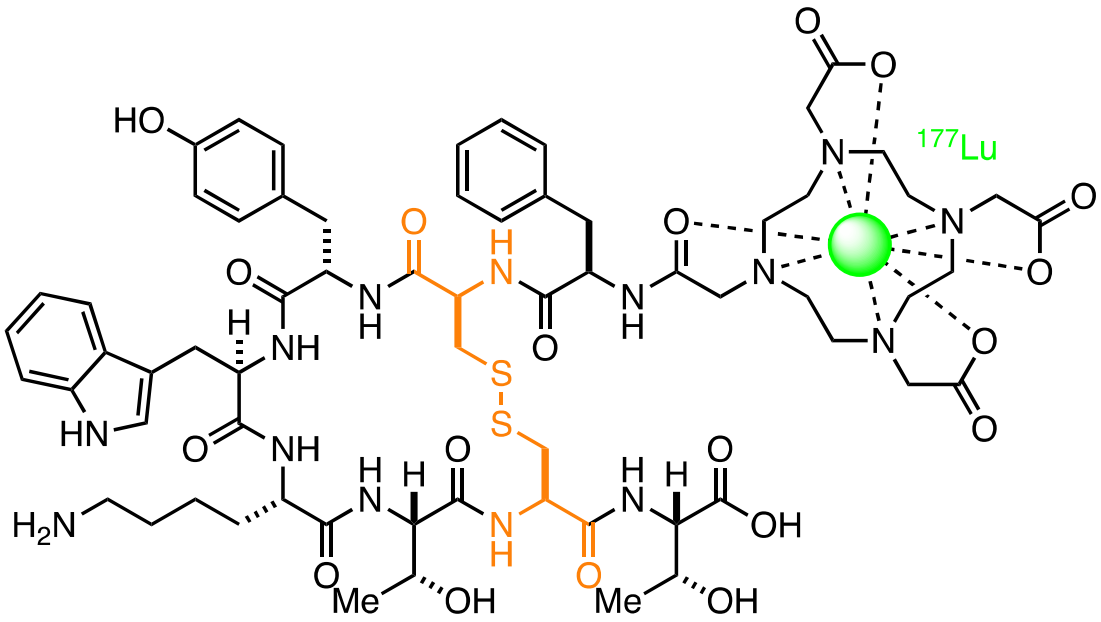
JT09

- acts as a peripherally-restricted, **highly selective agonist of the κ-opioid receptor**
- approved for medical use in the USA in 2021
- Difelikefalin injection is in Phase III trials for the treatment of itching (*pruritus*) in patients on hemodialysis

- >33,400 fold selectivity for κ-opioid receptor over other opioid receptors
- approximately as efficacious as morphine in alleviating peripheral pain, without other CNS-mediated side-effects associated with morphine (addiction, sedation, dysphoria, tolerance, addiction)

1. *The New England Journal of Medicine*, **2020**, 382, 222–232
2. *Eur. J. Pharmacol.,* 2019, 856, 172396

Imaging Agents

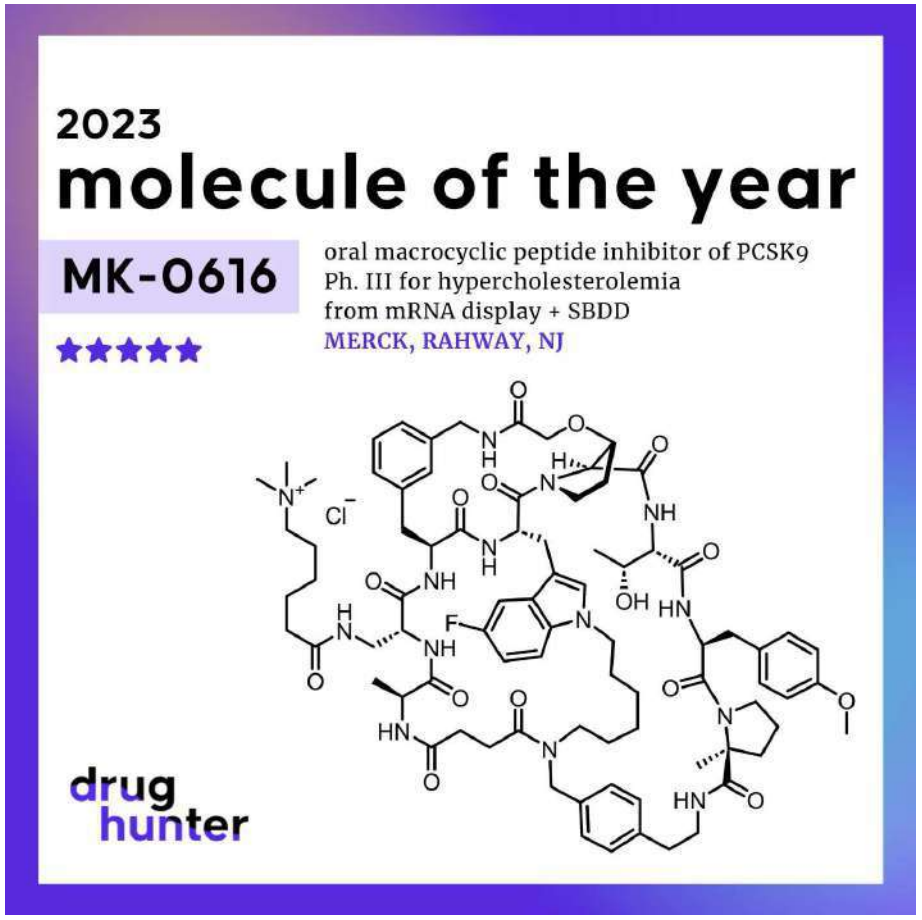


Lutathera

Lutathera marks first FDA Approval for a Peptide Receptor Radionuclide Therapy (PRRT).

Approved in 2018 for the treatment of somatostatin receptor-positive gastroenteropancreatic neuroendocrine tumors.

After lutathera binds to the SSTR2 receptors, it is designed to enter into the cell. It then kills the cancer cells through the release of beta minus (β-) radiation.



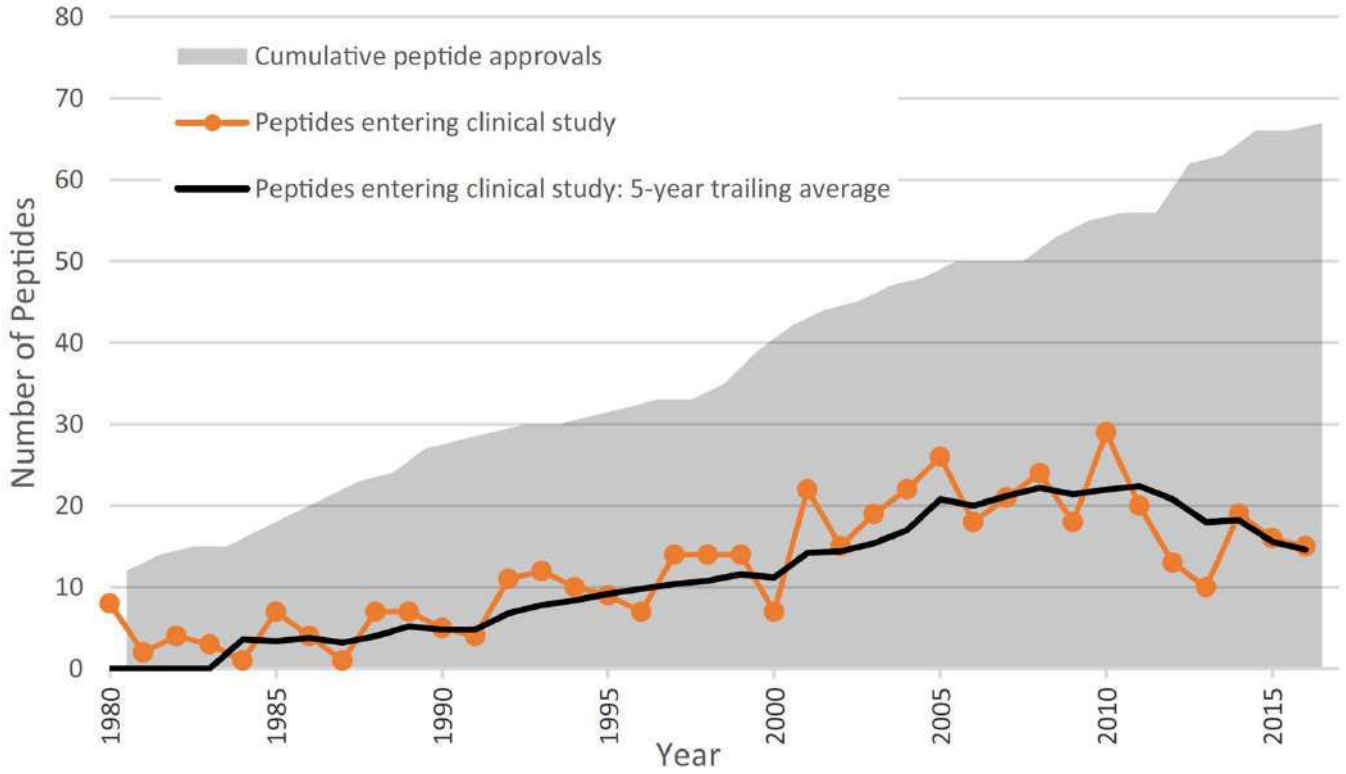
MK-0616 is an orally available inhibitor of the enzyme PCSK9 and is currently in Phase III trials for the treatment of high cholesterol and atherosclerosis.

In Phase II trials, reduction in LDL levels was comparable to existing antibody therapies (evolocumab).

"It is a truly innovative molecule which has **proved the potential of macrocyclic peptide[s] as oral drug[s]** as well as the prospective capability of mRNA display in drug discovery."
–Seong Heon Kim, Head of Drug Discovery at HyundaiPharm

The Rise of Peptides

J.L. Lau, M.K. Dunn / Bioorganic & Medicinal Chemistry 26 (2018) 2700–2707



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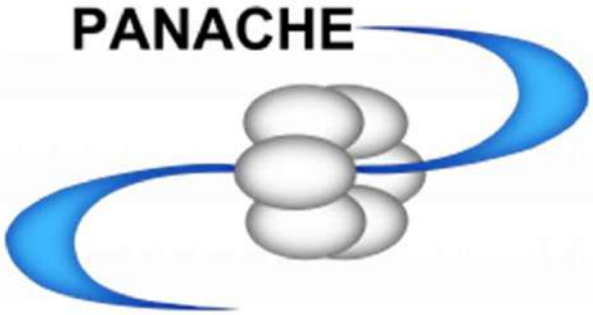


This project has received funding from the European Union's Horizon 2020 Future and Emerging Technologies programme under grant agreement number 858014

Funded by the Horizon 2020 Framework Programme of the European Union

Pannexin channels in cancer, inflammation and cell death

Silvia Penuela, Ph.D.
Associate Professor
Associate Chair – Research
Department of Anatomy and Cell Biology

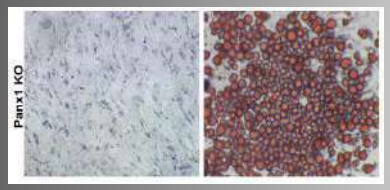
Chair- Basic Science Research Group
Schulich School of Medicine and Dentistry

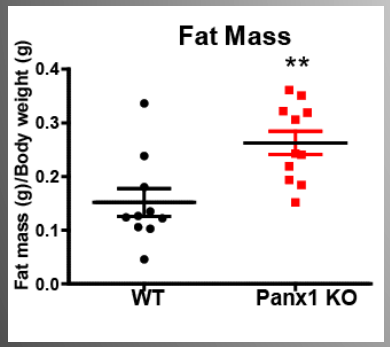
Department of Oncology, Experimental Oncology Division
University of Western Ontario
London, Ontario. Canada

Penuela Lab – Pannexin Research

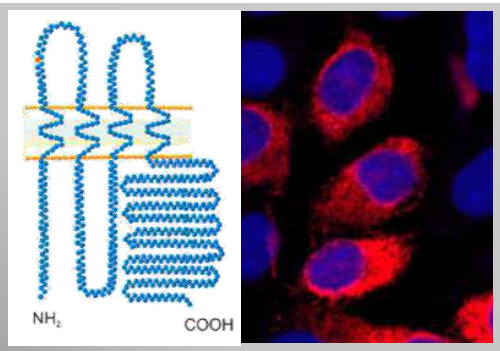
THE PENUELA LAB
EST. 2014

Pannexins in fat and inflammation



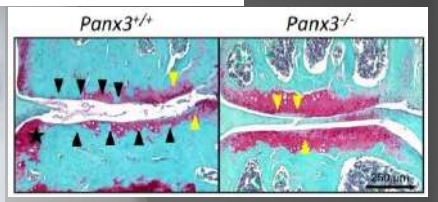


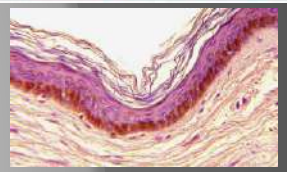
Panx2 in skin cell death



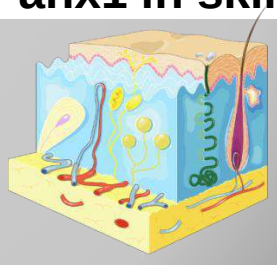
Interacting connexins in skin and skin cancer

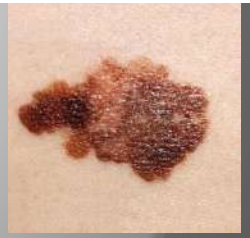
Panx3 in osteoarthritis and skin cancer



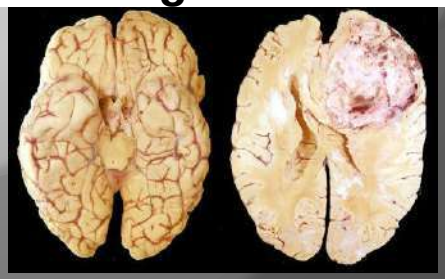


Panx1 in skin and melanoma



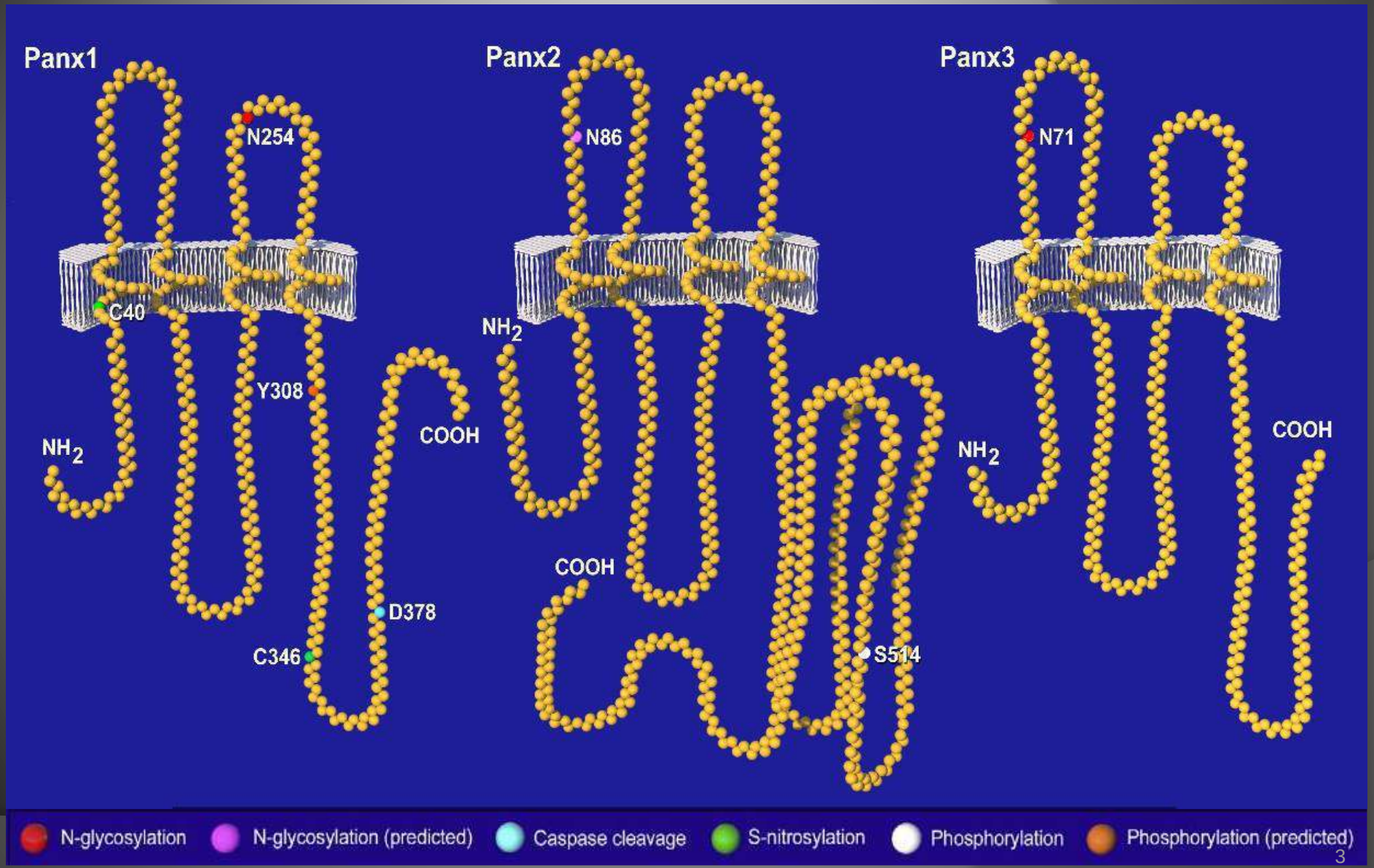


Panx1 in glioblastoma

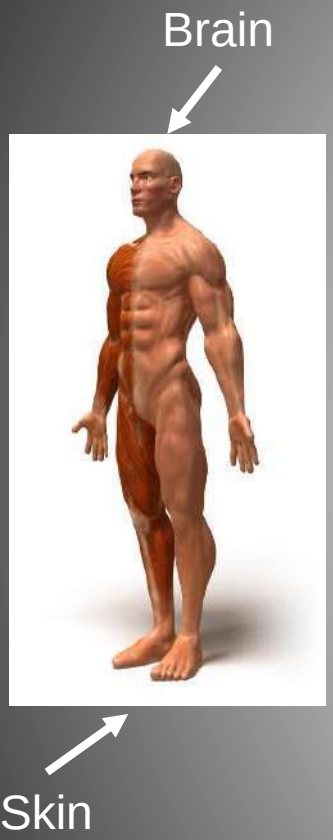


Pannexin Channels

Penuela, Simek and Thompson. *FEBS Letters*, 2014



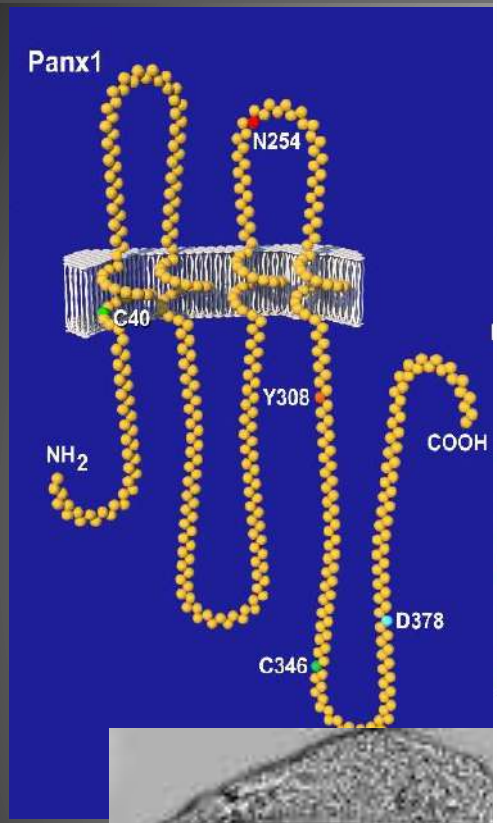
Pannexins in different tissues and diseases

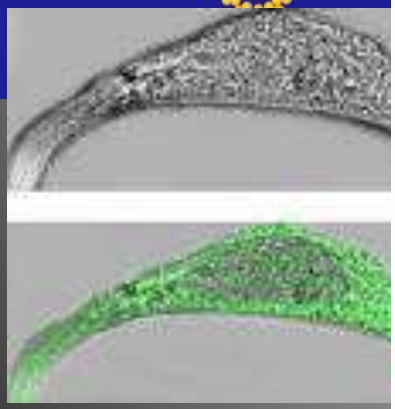


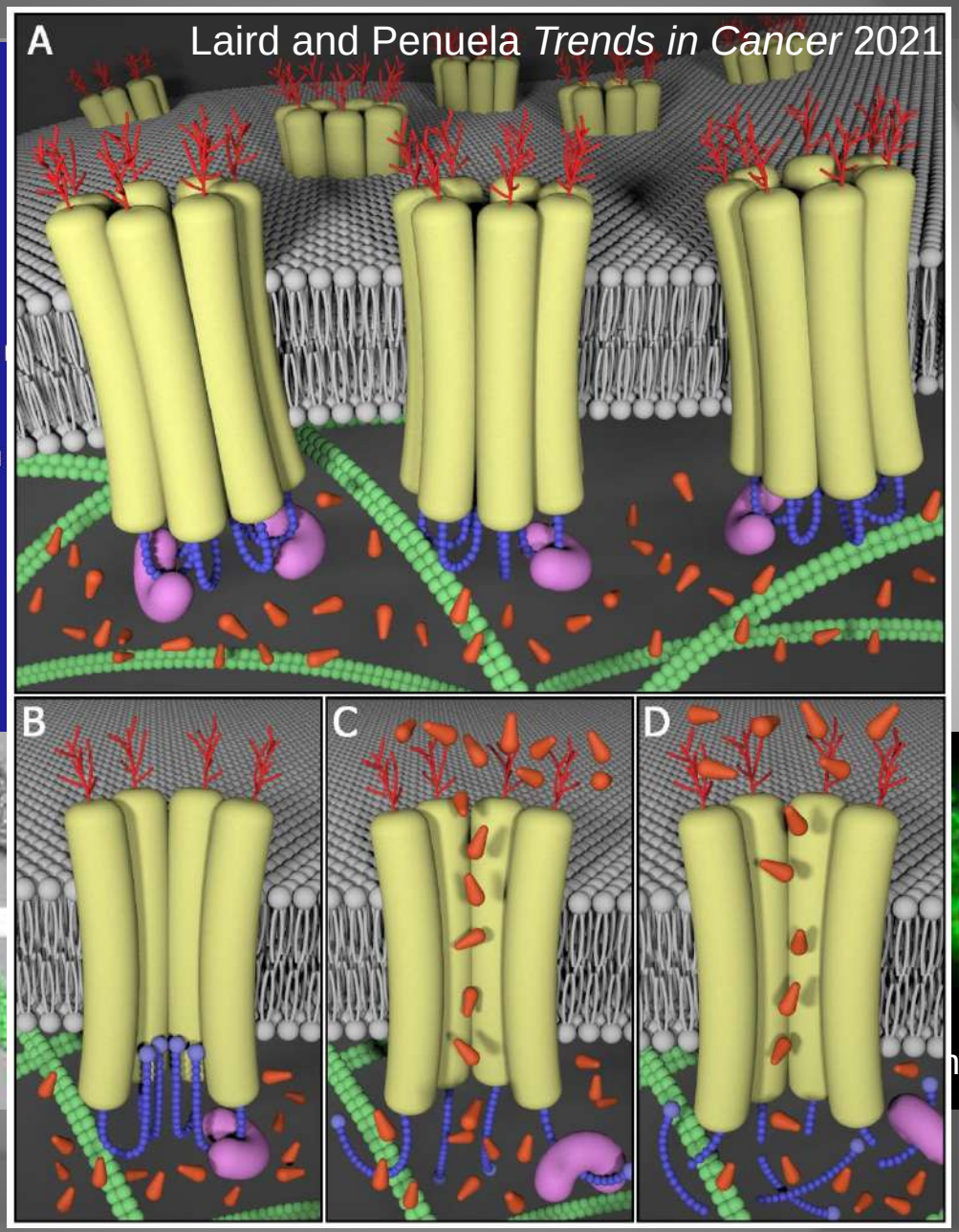
Panx1	Panx2	Panx3
Ubiquitous expression	Brain Cerebellum Spinal Cord GI tract Kidney Testis Eye Skin Liver Muscle	Skin Cartilage Bone Mammary Gland Intestine
Melanoma		
Glioma		
Rhabdomyosarcoma		
Ischemia/Stroke		
Epilepsy		
Glaucoma		
Overactive Bladder		
Microbial Infection		
HIV/AIDS	**Glioma**	
Migraine	**Ischemia/Stroke**	**Osteoarthritis**
MS/Encephalomyelitis		**Osteosarcoma**
Hypertension		
Crohn's		
Alzheimer's		
Diabetes		

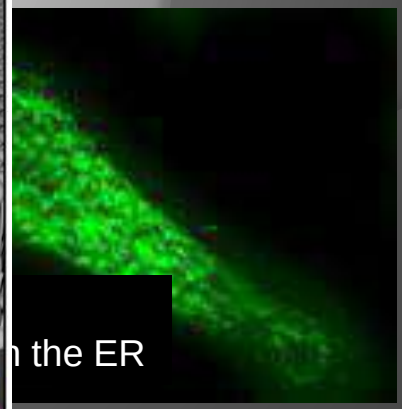
Penuela et al., *Biochem. J.*, 2014

Pannexin 1 Channels

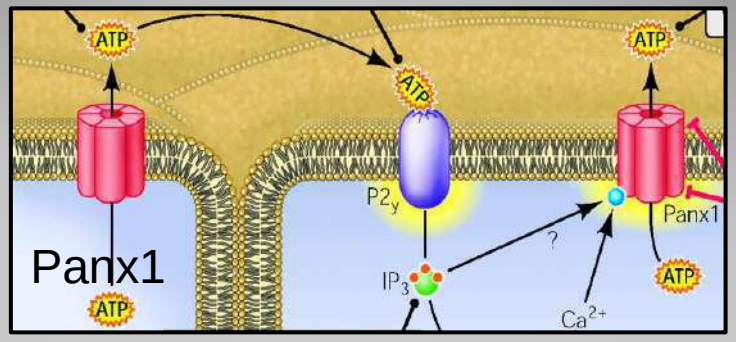








Physiological & Pathological Significance of Pannexin1



Barbe et al. 2006

nature International weekly journal of science

Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis

Faraaz B. Chekeni, Michael R. Elliott, Joanna K. Sandilos, Scott F. Walk, Jason M. Kinchen, Eduardo R. Lazarowski, Allison J. Armstrong, Silvia Penuela, Dale W. Laird, Guy S. Salvesen, Brant E. Isakson, Douglas A. Bayliss & Kodi S. Ravichandran

Science AAAS

Ischemia Opens Neuronal Gap Junction Hemichannels

Roger J. Thompson, Ning Zhou, Brian A. MacVicar*

Science AAAS

Activation of Pannexin-1 Hemichannels Augments Aberrant Bursting in the Hippocampus

Roger J. Thompson,[1*†] Michael F. Jackson,[2] Michelle E. Olah,[2] Ravi L. Rungta,[1] Dustin J. Hines,[1] Michael A. Beazely,[2] John F. MacDonald,[2] Brian A. MacVicar[3†]

PNAS

Pannexins in ischemia-induced neurodegeneration

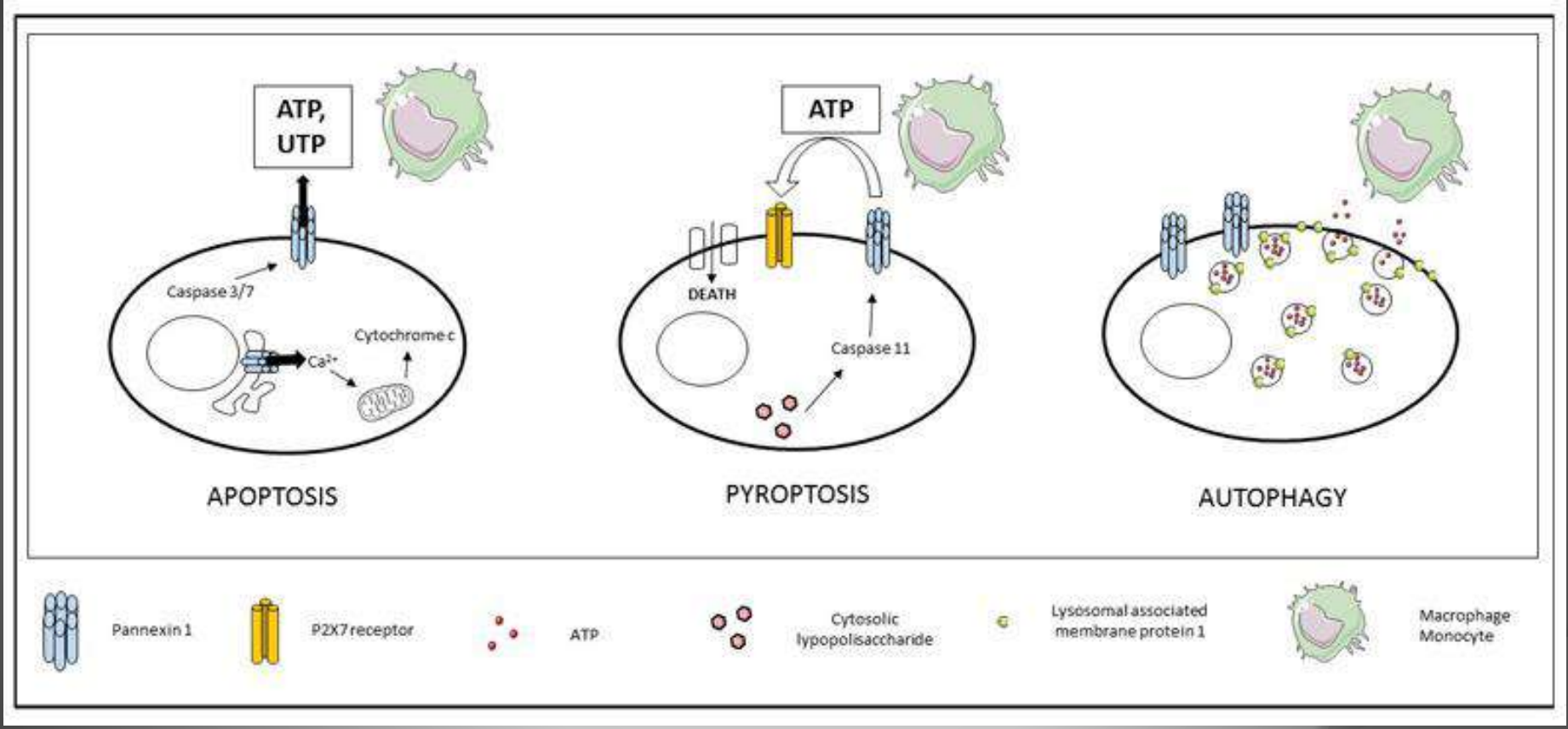
Panagiotis Bargiotas[a,b,c,1,2], Antje Krenz[b,2,3], Sheriar G. Hormuzdi[c,d], Dirk A. Ridder[a], Anne Herb[c,e], Waleed Barakat[b], Silvia Penuela[f], Jakob von Engelhardt[c,e], Hannah Monyer[c,e,4,5], and Markus Schwaninger[a,b,4,5]

nature medicine

Activation of neuronal P2X7 receptor–pannexin-1 mediates death of enteric neurons during colitis

Brian D Gulbransen[1–3], Mohammad Bashashati[1–3], Simon A Hirota[3–6], Xianyong Gui[7], Jane A Roberts[8], Justin A MacDonald[3,5,6], Daniel A Muruve[3,4], Derek M McKay[2,3], Paul L Beck[3,4], Gary M Mawe[8], Roger J Thompson[1,9] & Keith A Sharkey[1–3]

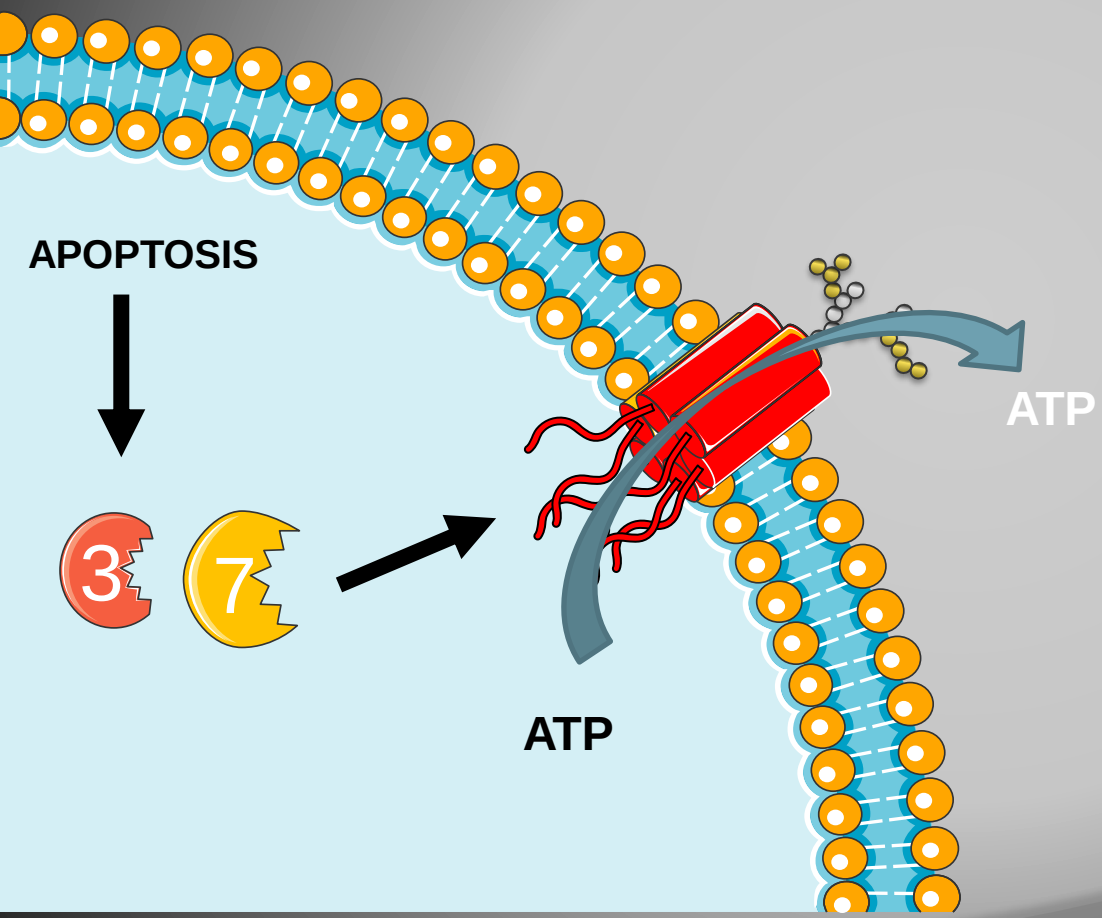
Pannexin 1 in cell death

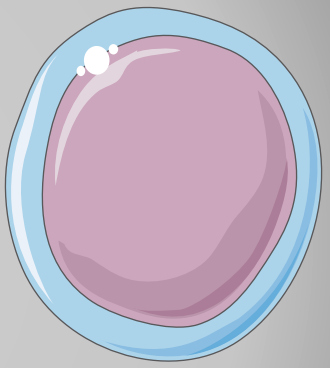


Yanguas Crespo et al, 2017

Communicating through Panx1

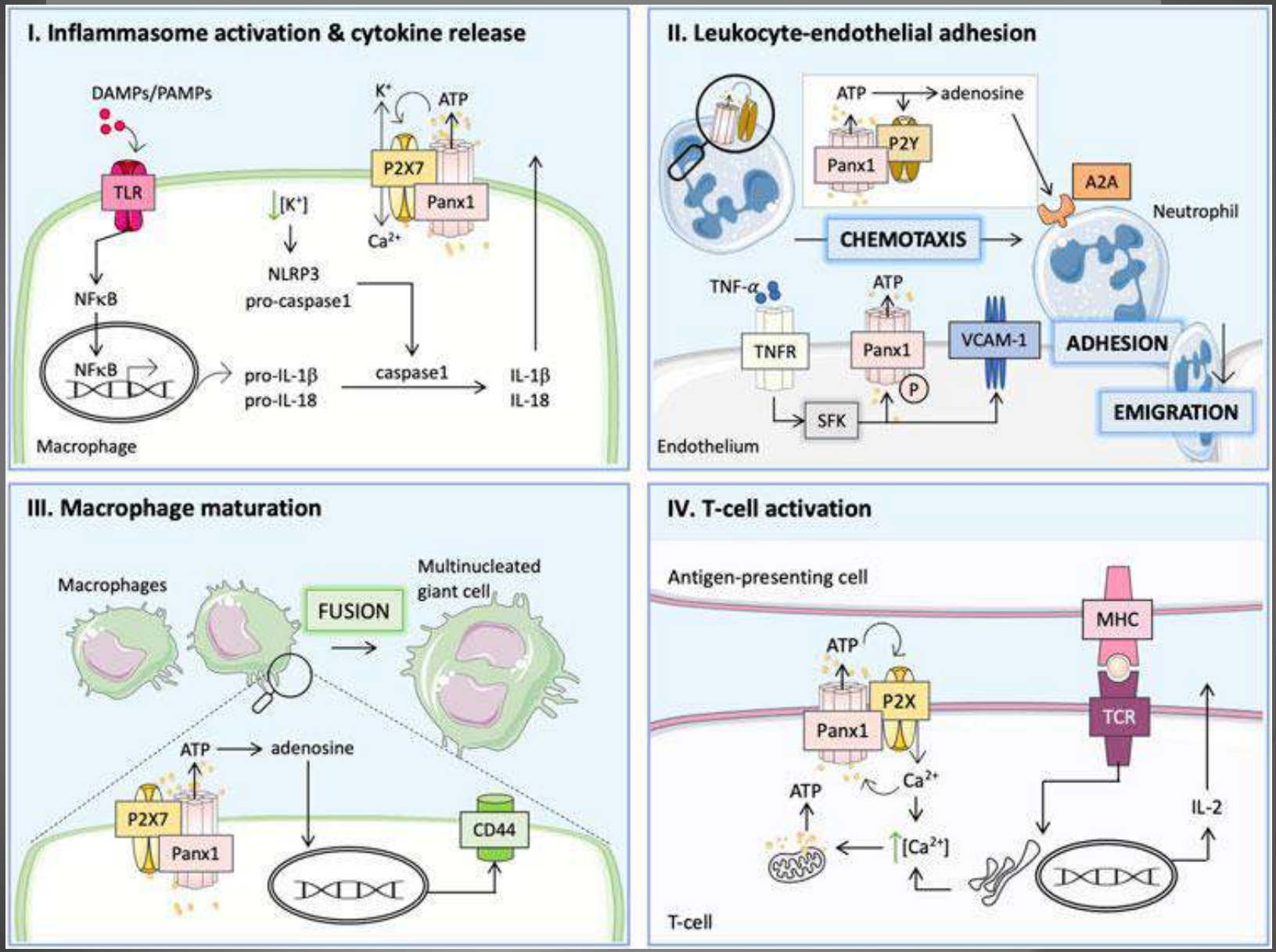
Chekeni *et al.*, Nature 2010





THP-1 monocytes

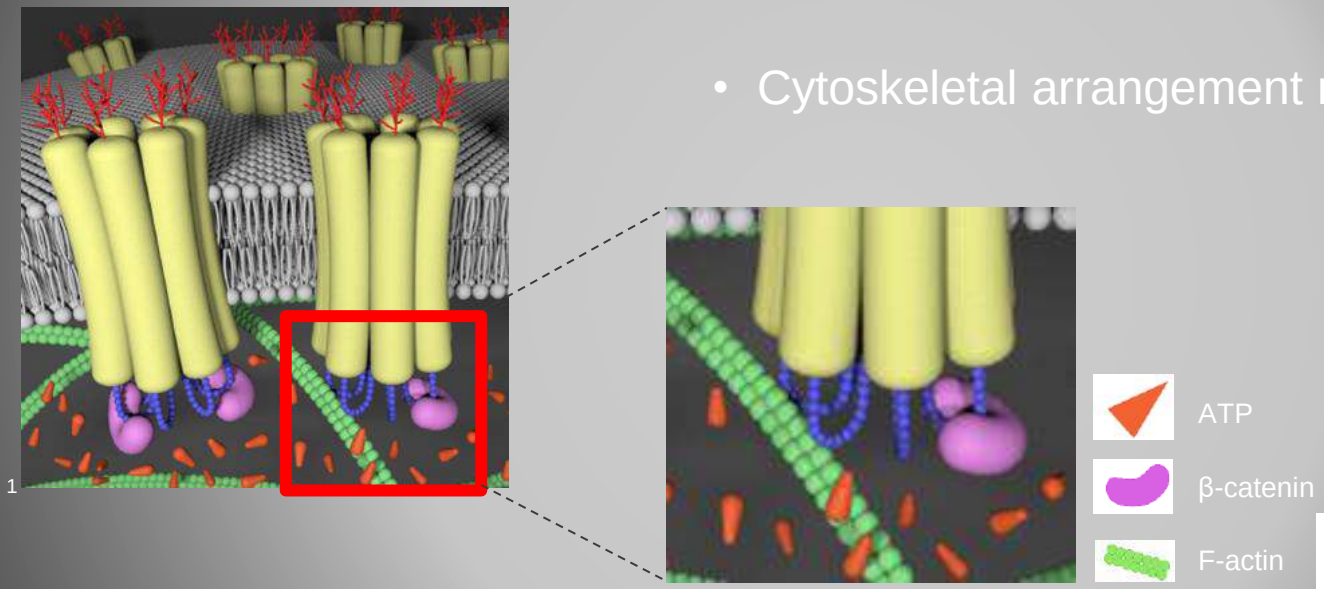
Pannexin 1 in inflammation

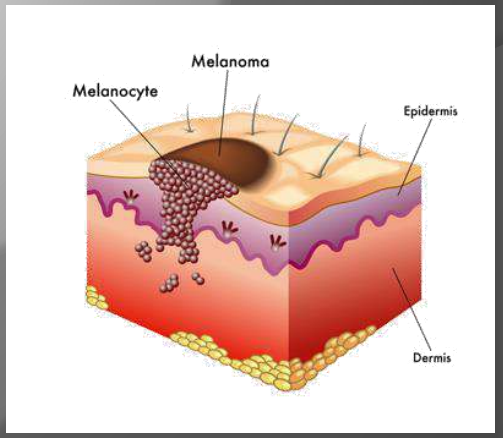


Rusiecka et al, 2022

PANX1 may regulate cytoskeletal organization and metabolism

- Modulation of Wnt/β-catenin pathway
- Cytoskeletal arrangement modulation





1. Adapted from Laird D. and Penuela S. (2021) with BioRender

Pannexin 1 in Cancer

ONCOGENE

→ **Breast Cancer*:*** Gain of function mutation in PANX1 increases metastasis (Furlow *et al. 2015*)

→ **Human Glioma U87-MG Cells:** PANX1 siRNA reduces proliferation (Wei *et al. 2015*)

→ **Rat C6 Glioma Cells:** Panx1 over-expression has a tumour- suppressive role. (Lai et al, 2007).

→ **Leukemia:** PANX1 up-regulated in leukemic cells compared to T-cells (Boyd-Tressler *et al. 2014*)

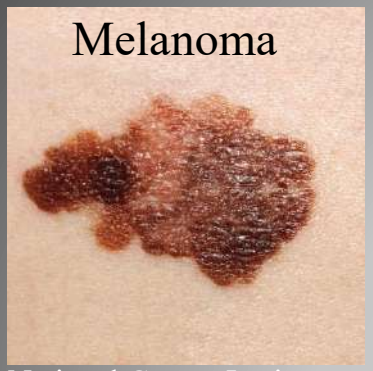
➢ **Melanoma**: Loss of Panx1 attenuates melanoma progression (Freeman et al,2019; Penuela et al, 2012)

TUMOUR SUPPRESSOR

- **Rhadbomyosarcoma:** PANX1 expression decreased in tumours.
- **Human BCC and SCC:** Lower PANX1 in keratinocytic skin cancer (Cowan *et al*, 2012)

Laird and Penuela, Trends in Cancer 2021; Jiang and Penuela, BMC Cell Biology 2016

Cutaneous Melanoma Development

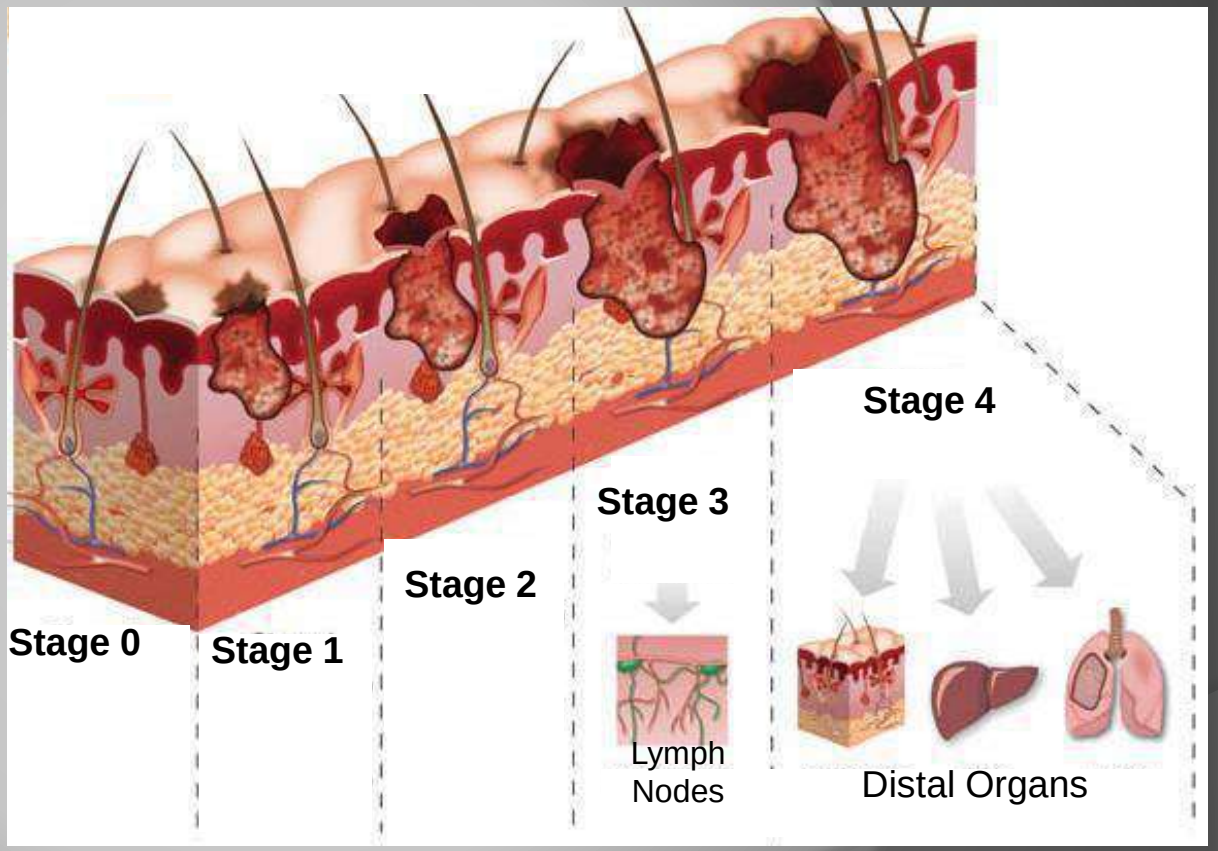


National Cancer Institute

Deadliest of all skin cancers (75% of deaths)

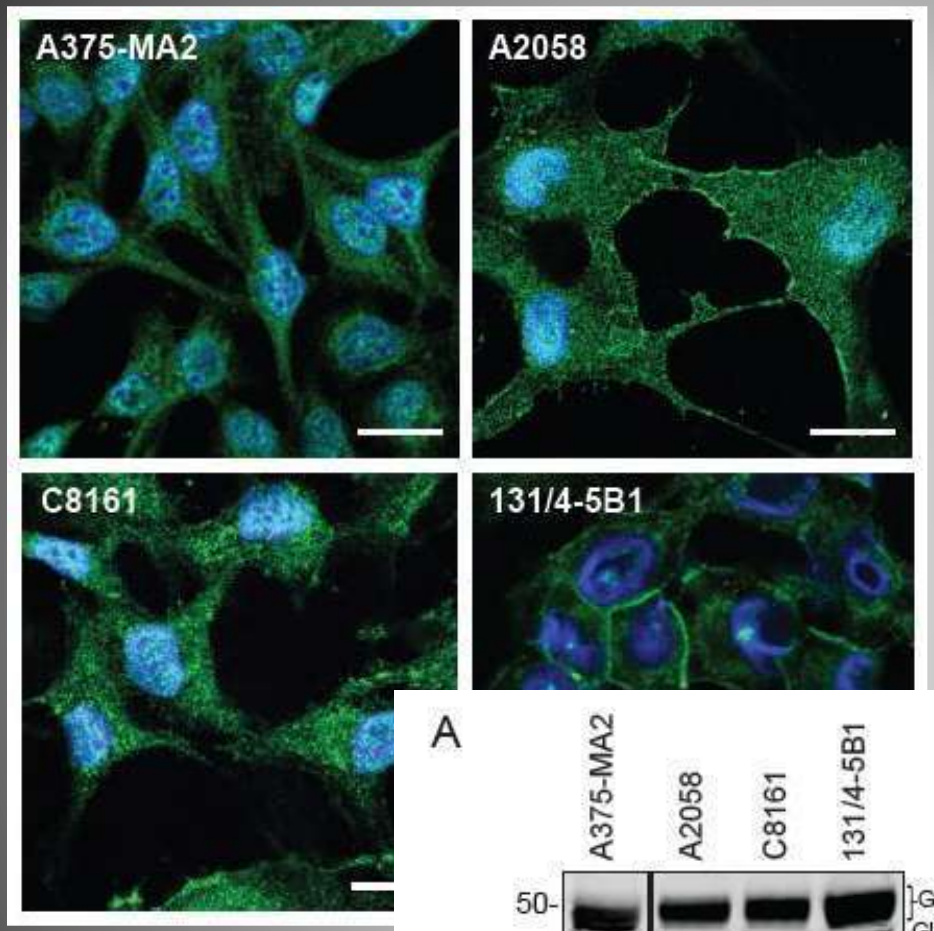
Limited treatment options

Increasing in incidence

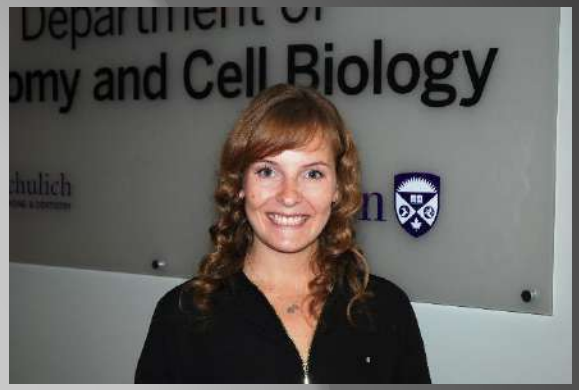


http://dyersburgskinandallergyclinic.com

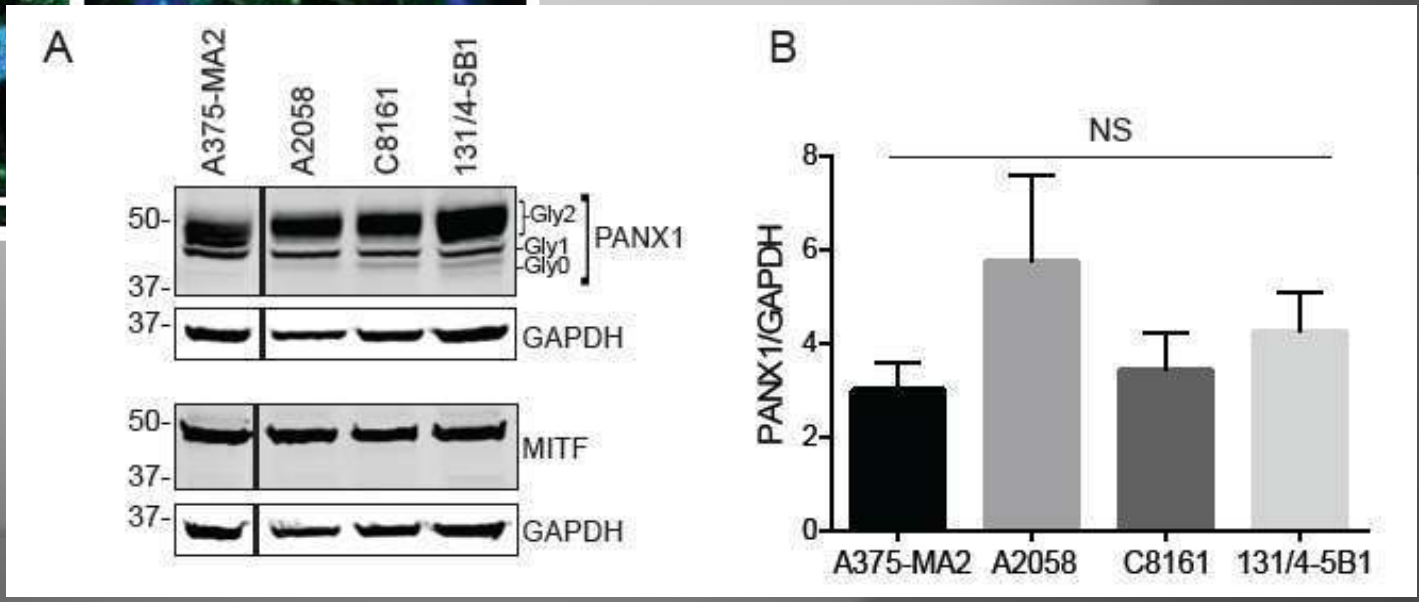
PANX1 is Highly Expressed in Human Melanoma Cell Lines



Freeman et al. *Cancers* 2019



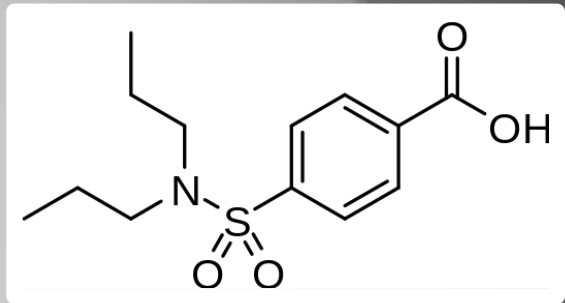
Taylor Freeman



PANX1 Channel Inhibitors

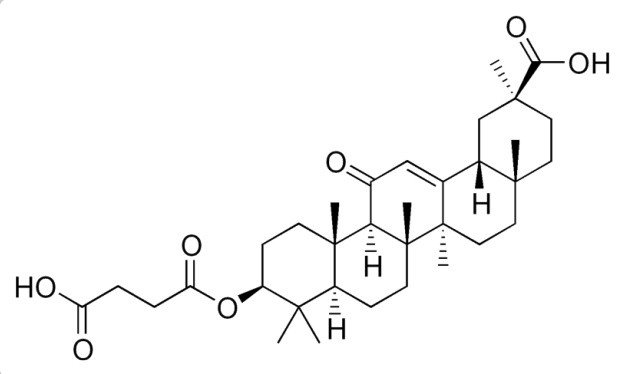
Probenecid (PBN)

(Silverman et al., 2008, Am J Physiol Cell Physiol)
(Ransford et. al., 2007, Am. J. Respir. Cell. Mol. Biol.)



Carbenoxolone (CBX)

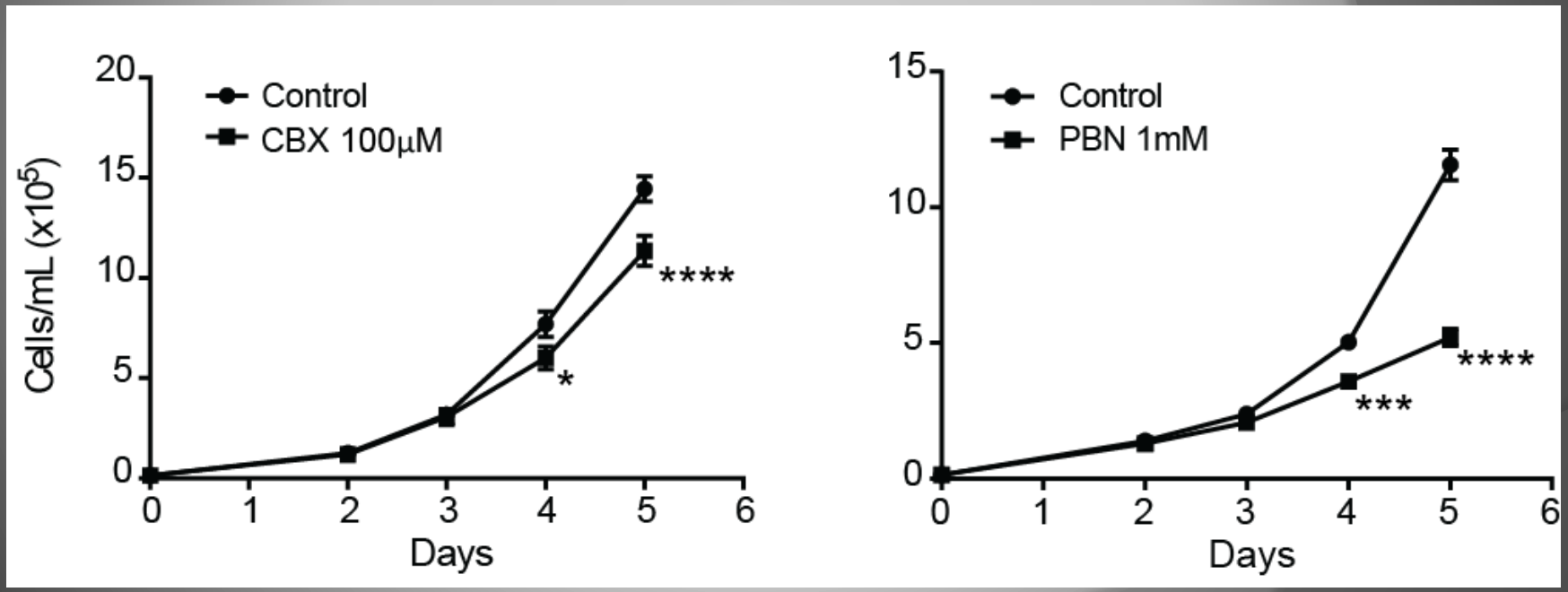
(Barbe et. al., 2006, Physiology)
(Patel et. al., 2014, FEBS Lett.)



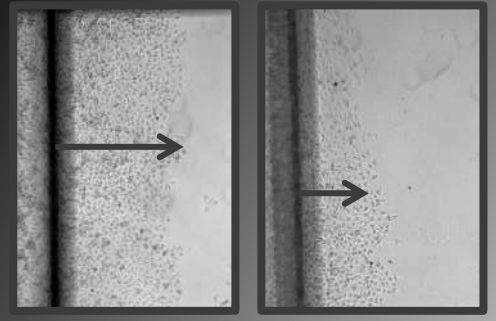
Spironolactone (SPIR)

(Good et al, 2018)
(Dunaway et al, 2022)

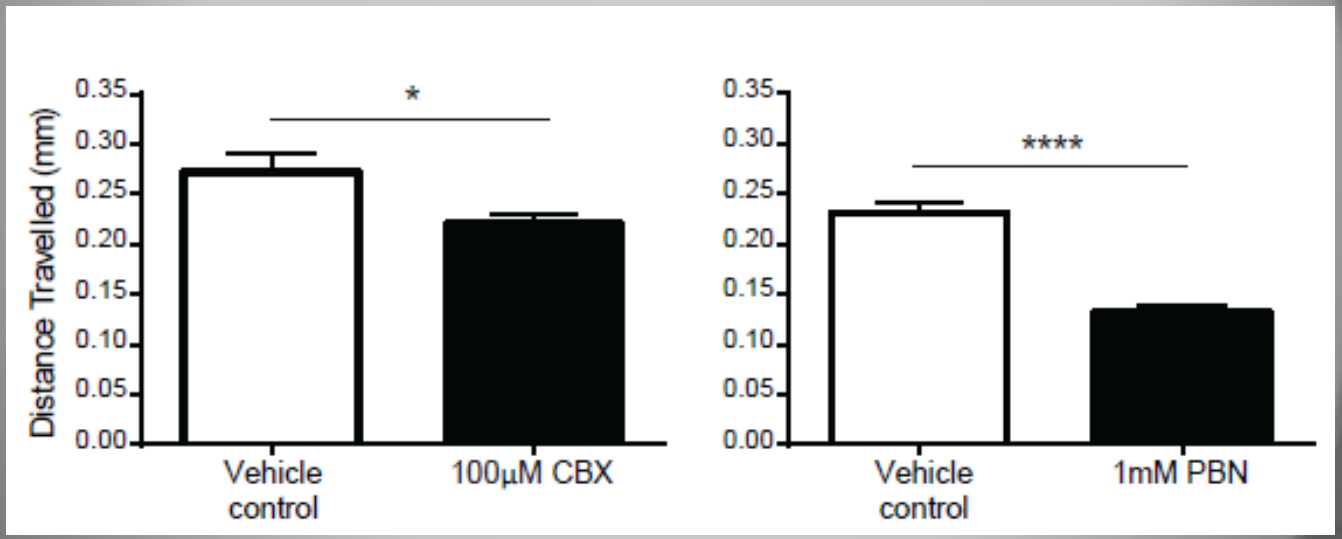
CBX and PBN significantly reduce A375-MA2 cell growth



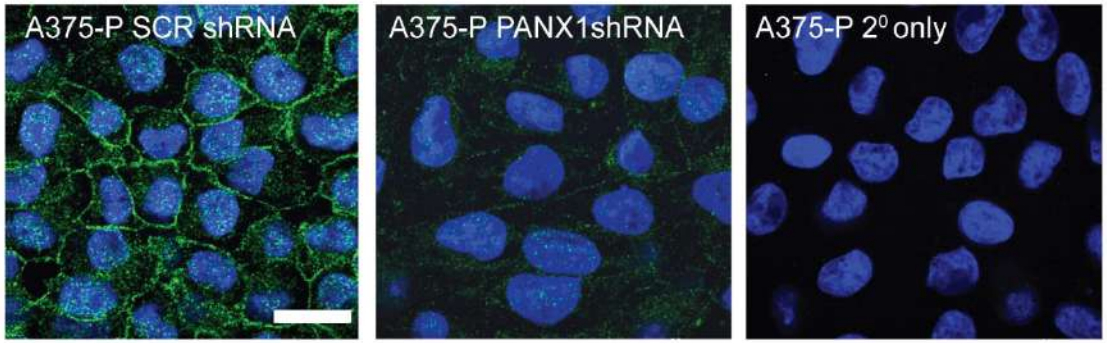
PANX1 channel blockers significantly reduce A375-MA2 cell migration

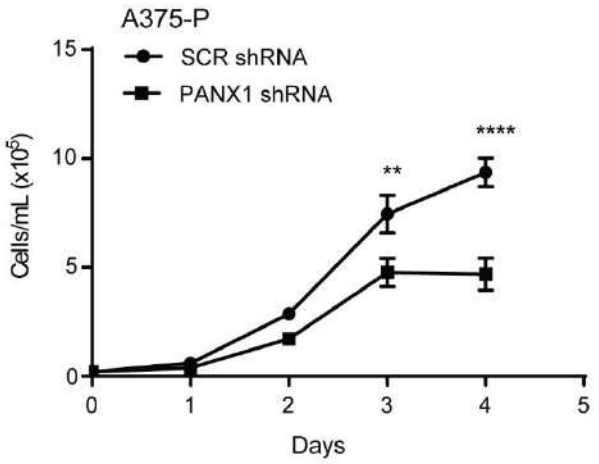


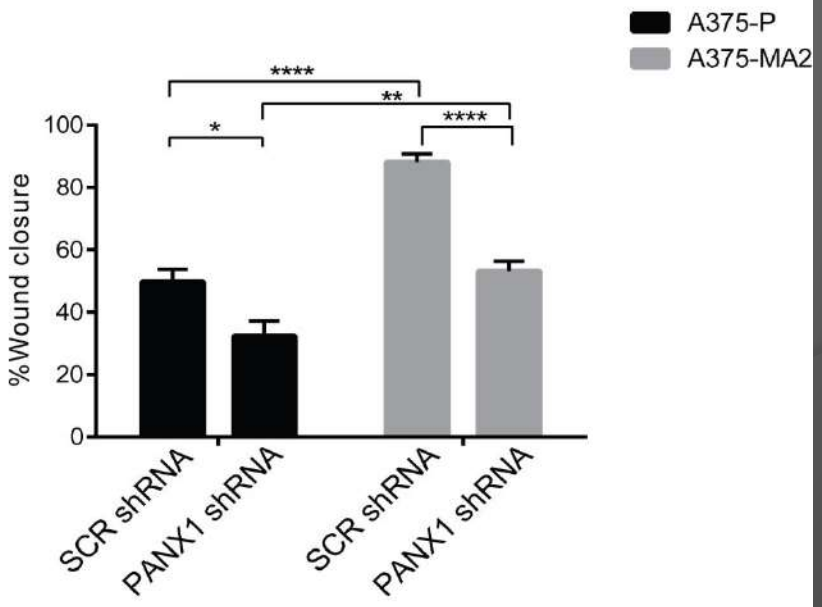
Control Panx1 Blocker



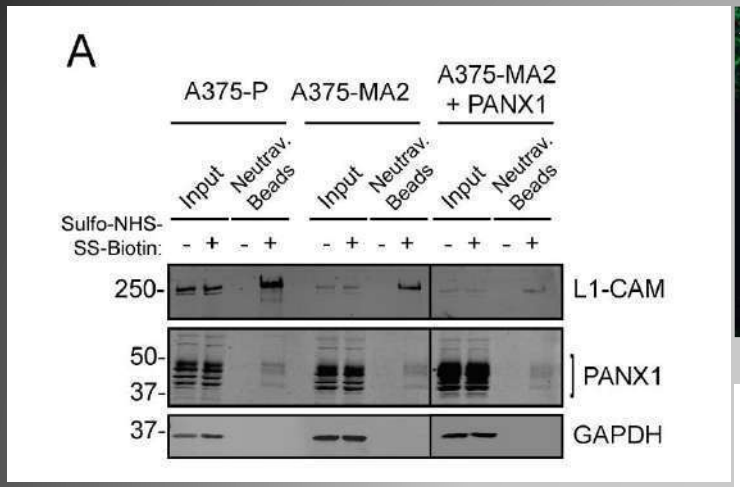
shRNA knockdown of PANX1 reduces growth and cellular migration of melanoma cells

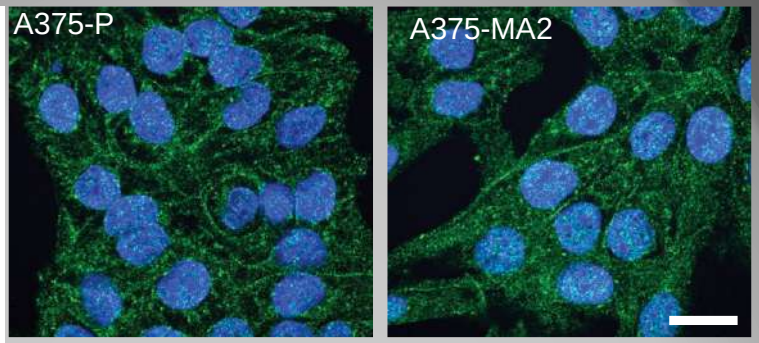


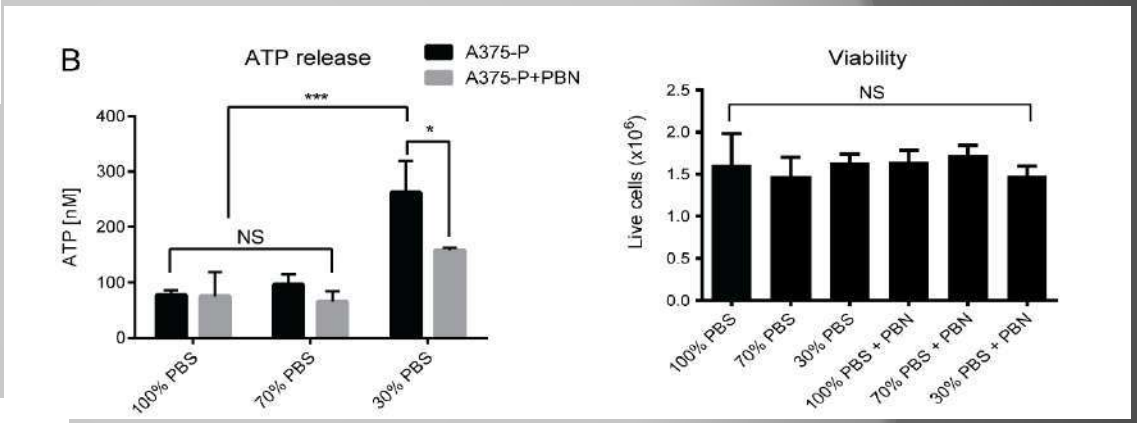


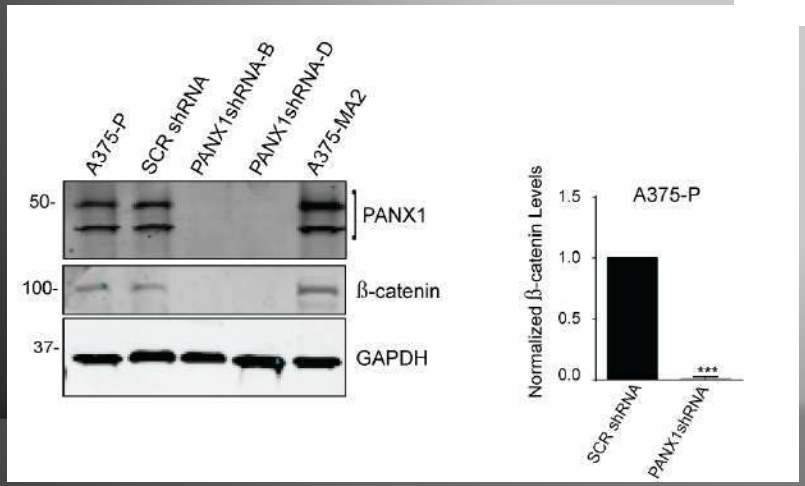


Cell surface and intracellular PANX1 in melanoma cells

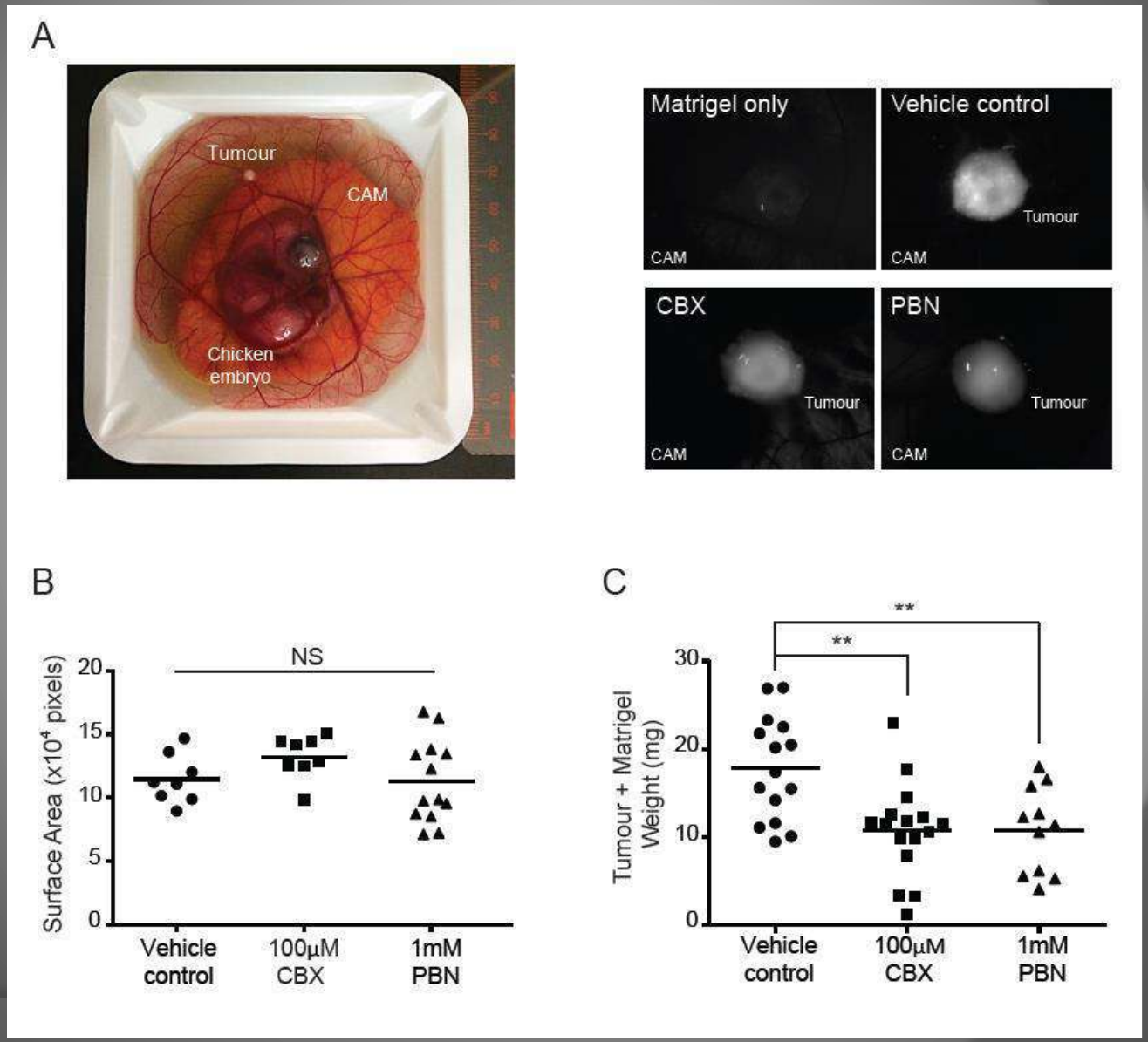




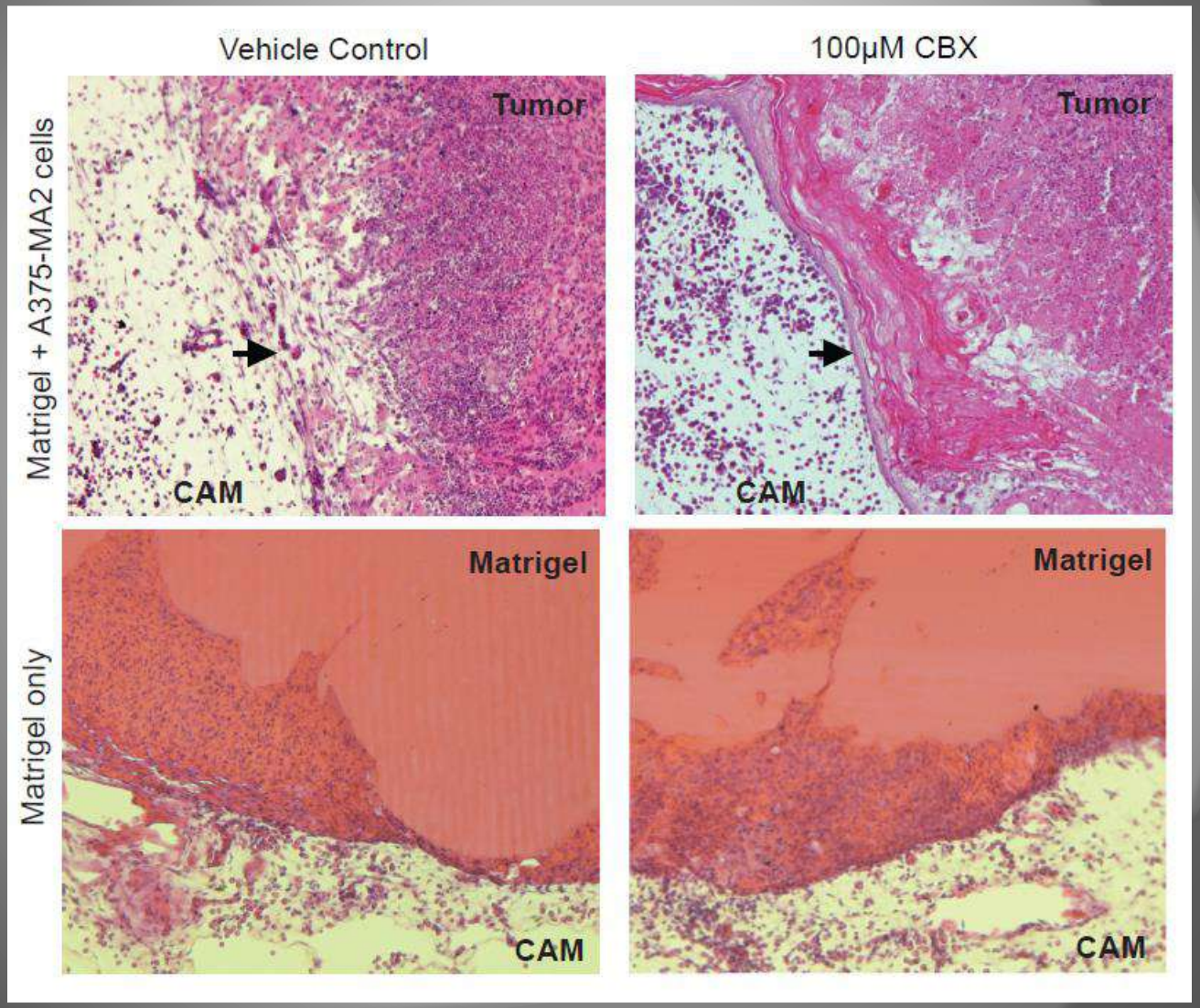




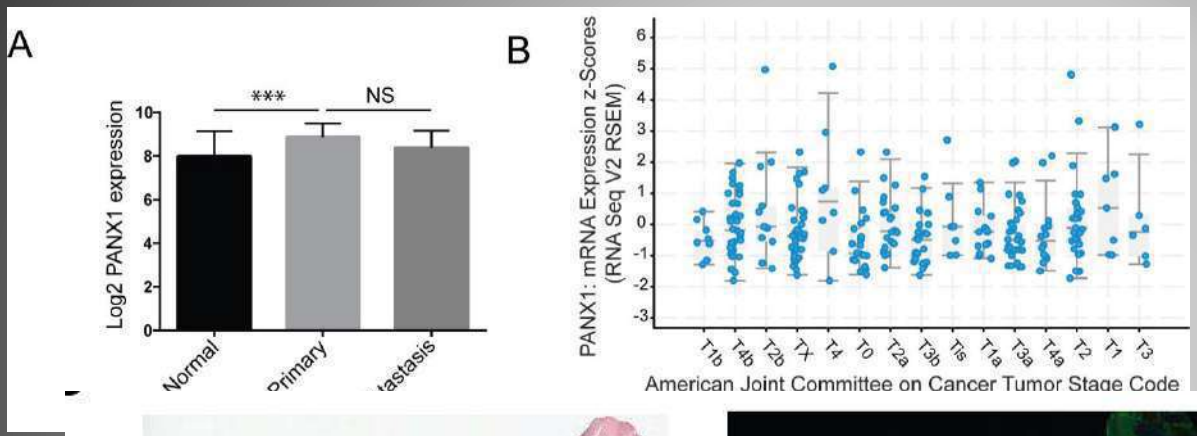
A375-MA2 tumour growth is significantly reduced by applying PANX1 channel blockers

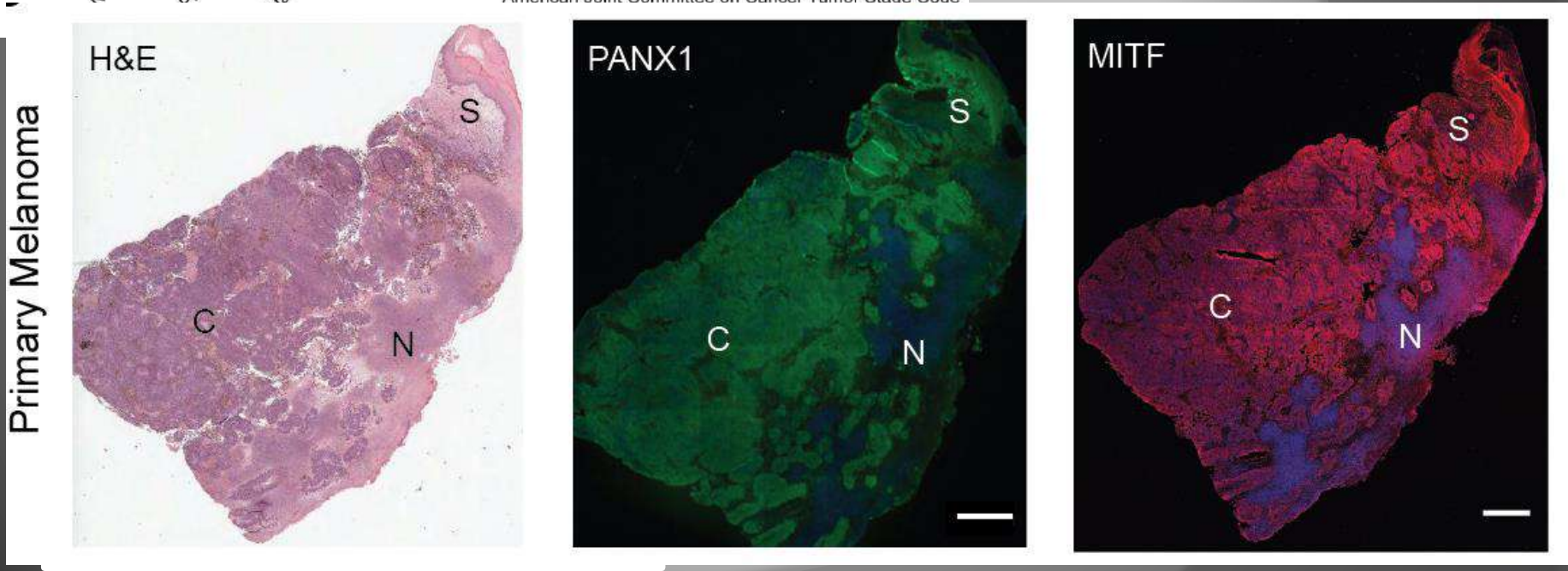


A375-MA2 tumours are less attached and invasive when treated with PANX1 channel blockers

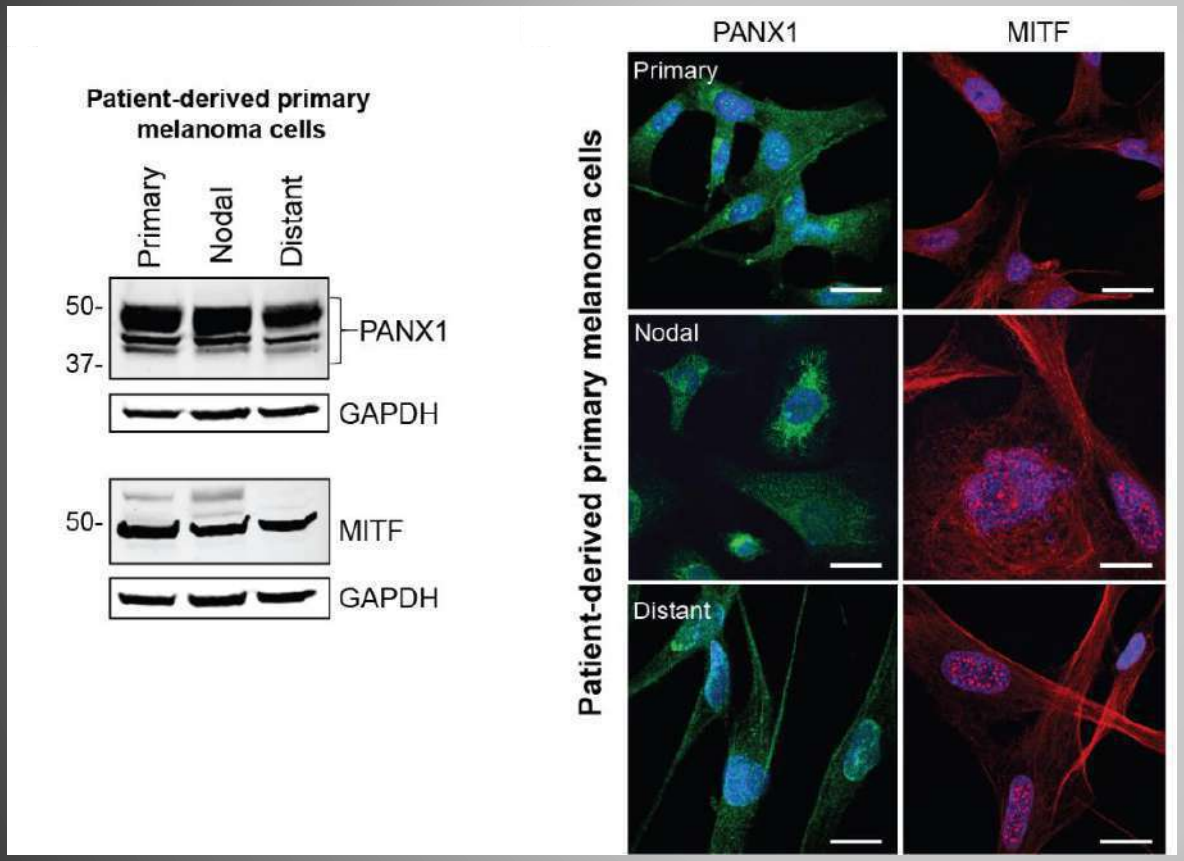


PANX1 is Expressed at All Stages of Melanoma Progression in Patient-Derived Biopsies



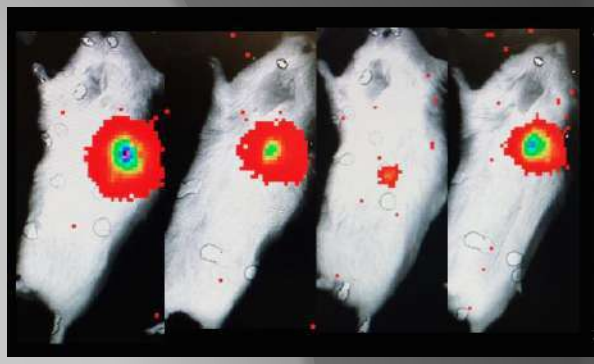


Patient-derived xenografts

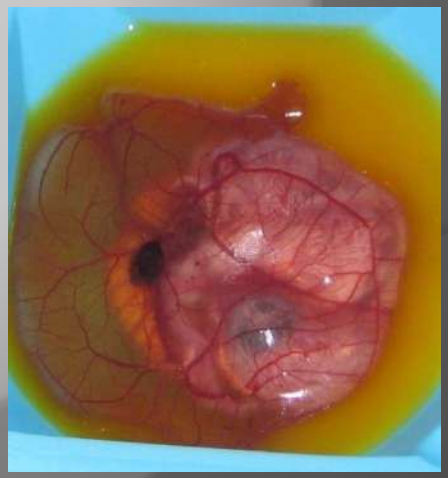


Freeman et al. *Cancers* 2019

Patient-derived xenograft



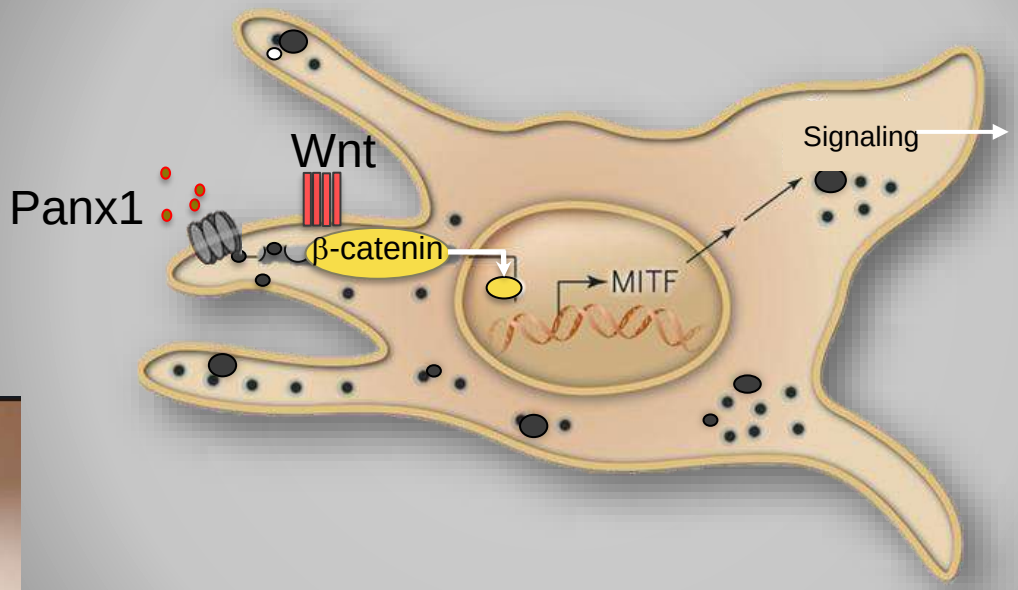
High-throughput blocker testing in chick-CAM



What is the mechanism?

Combination therapies

Wnt signaling pathway in melanoma



- Melanin production
- Proliferation
- Survival

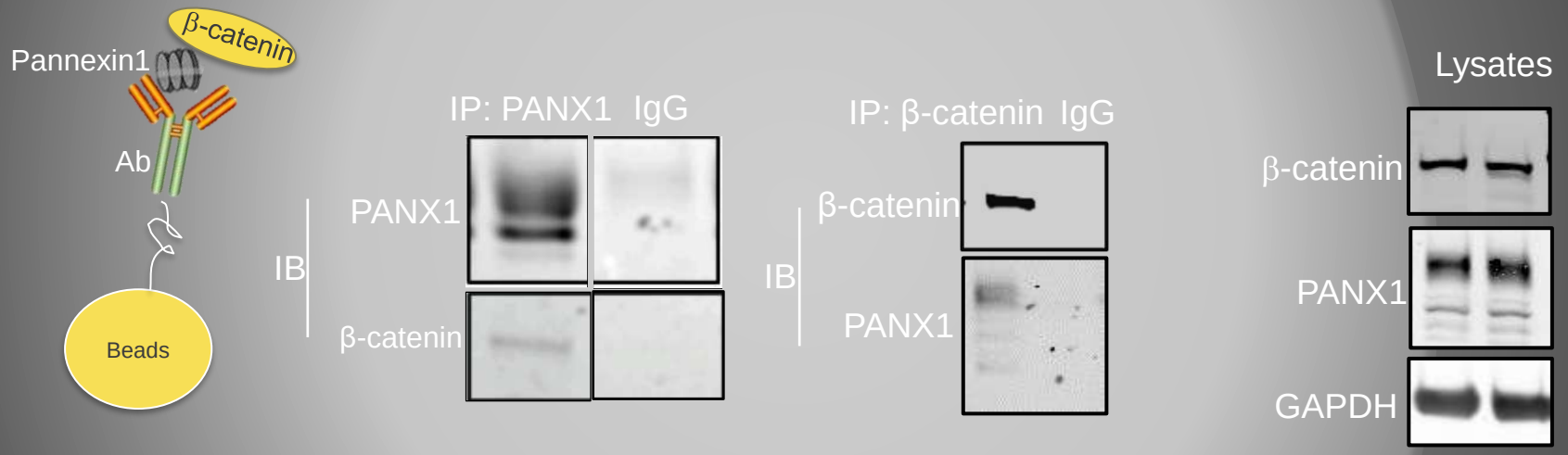
Dr. Samar Sayedyahossein

Modified from https://biology.stackexchange.com

MITF; Microphthalmia-associated Transcription Factor

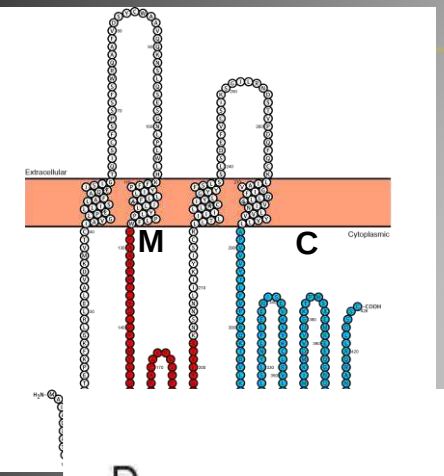
Pannexin1 interacts with β-catenin in human melanoma cells

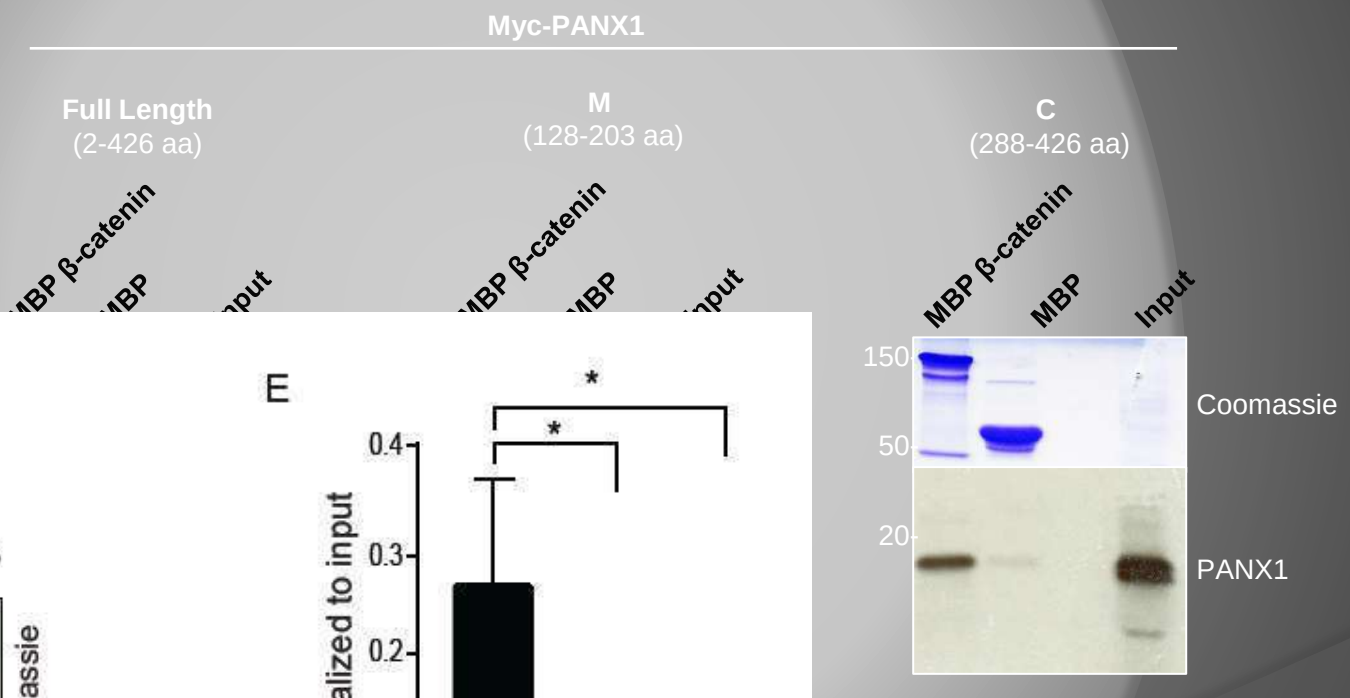
A375-P, A375-MA2, 131/4-5B1

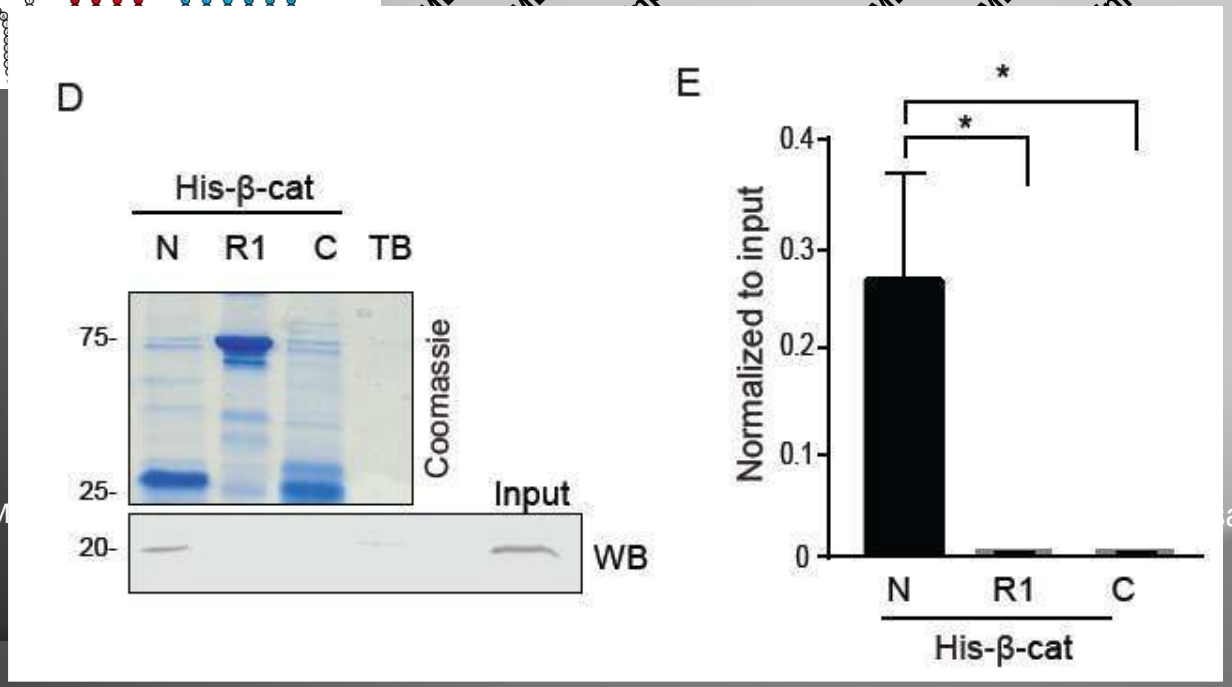


Sayedyahossein *et al.*, JBC 2021

C-terminal region of Pannexin1 directly binds to N-terminus of β-catenin

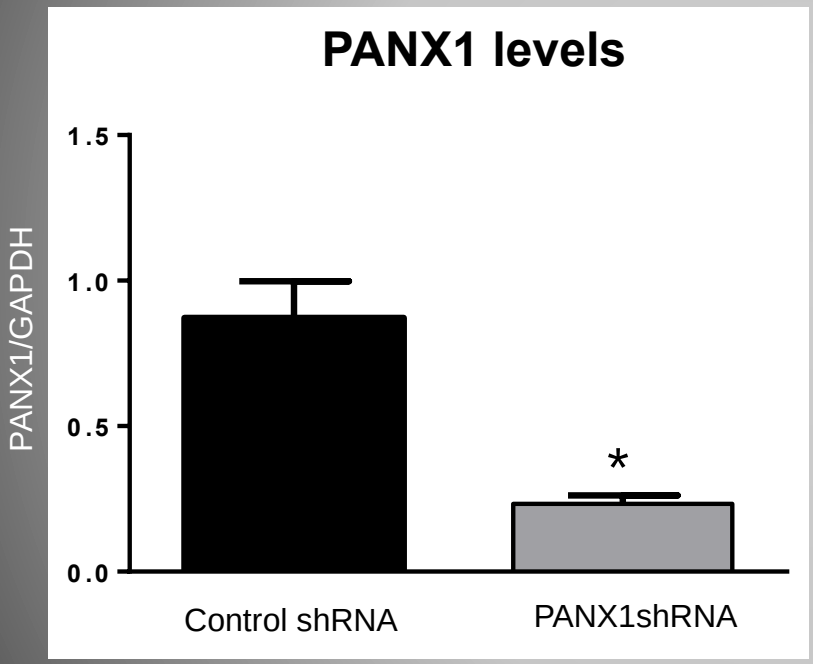


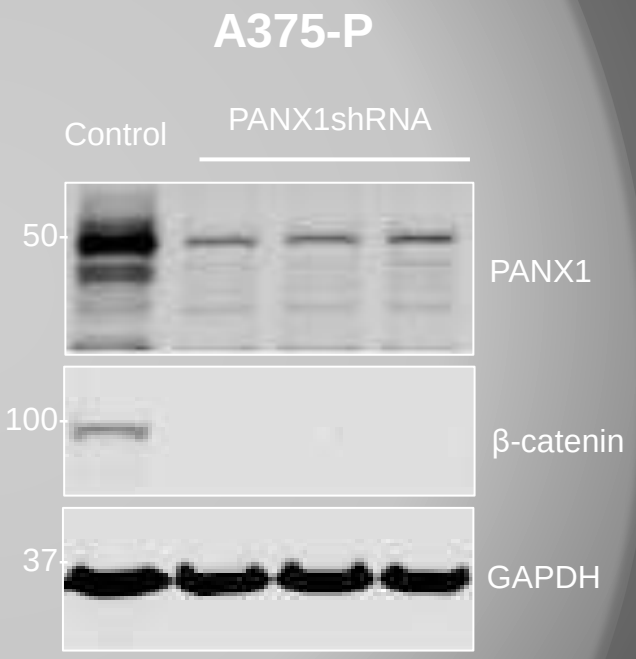




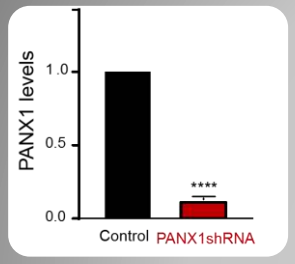
M ay, Zhigang Li (Dr. David Sacks's Lab, NIH)

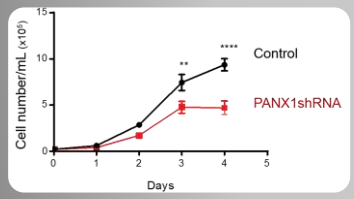
Knocking down Pannexin1 decreases β-catenin levels

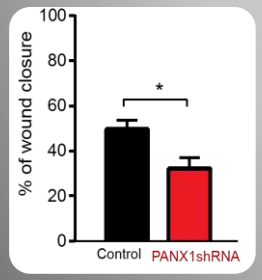


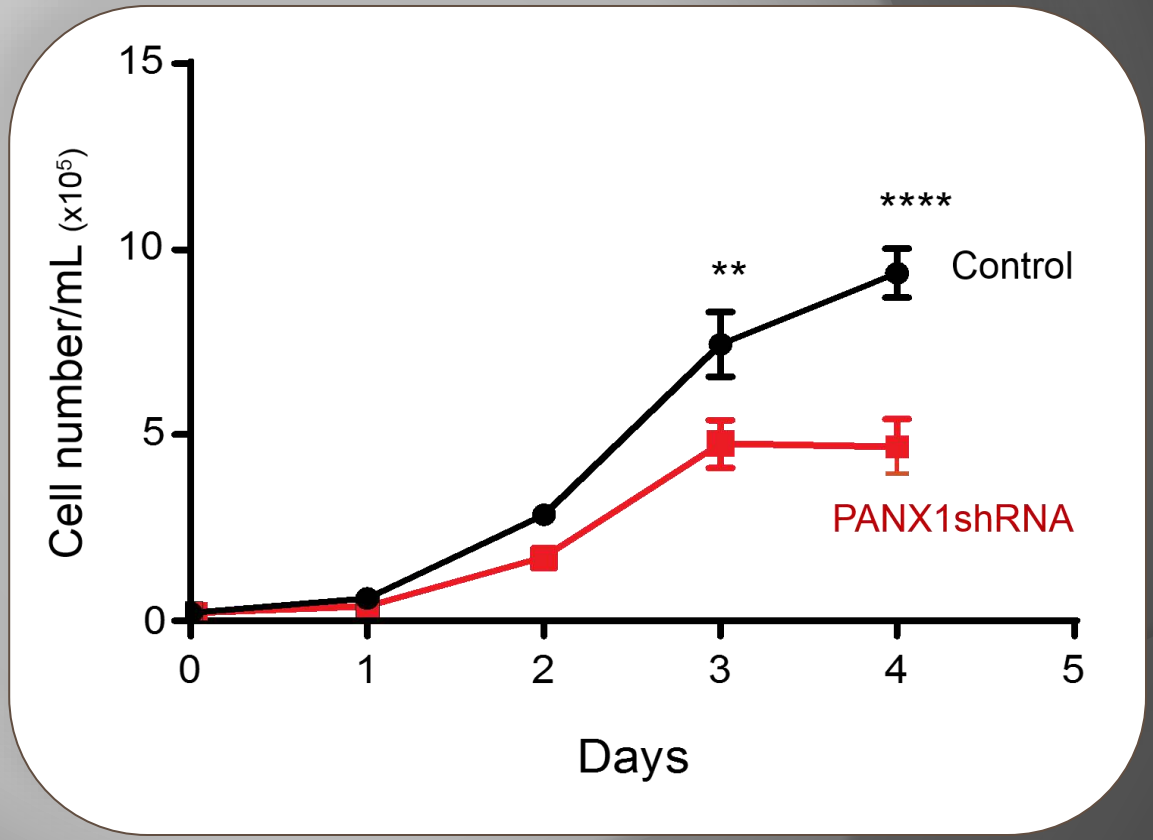


Knocking down Pannexin1 reduces growth and migration of melanoma cells

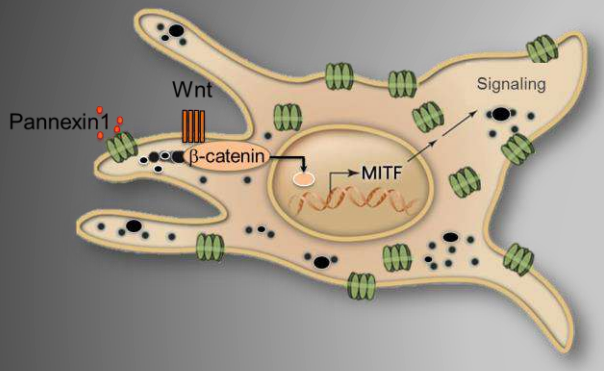


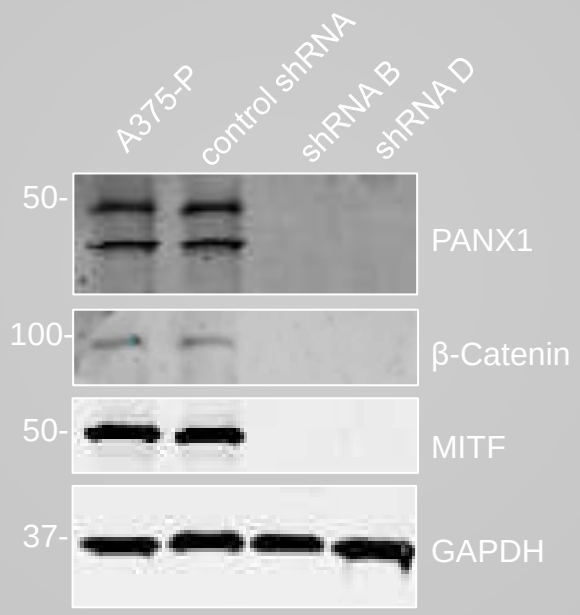


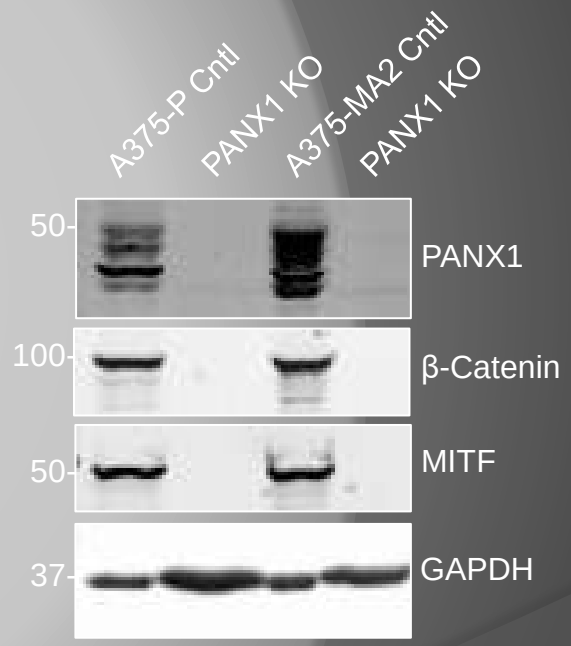




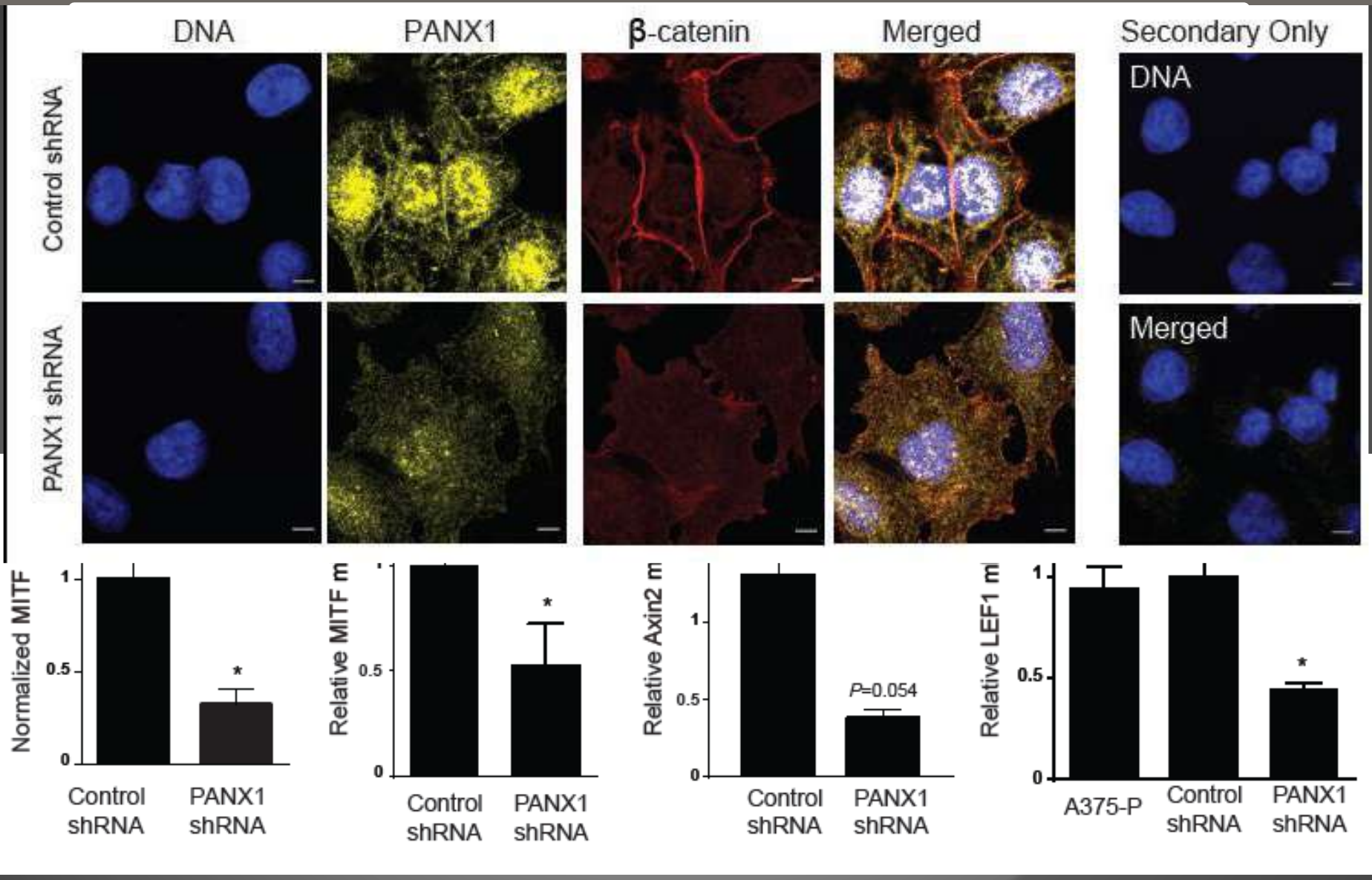
Pannexin1 reduction decreases β-catenin and MITF levels



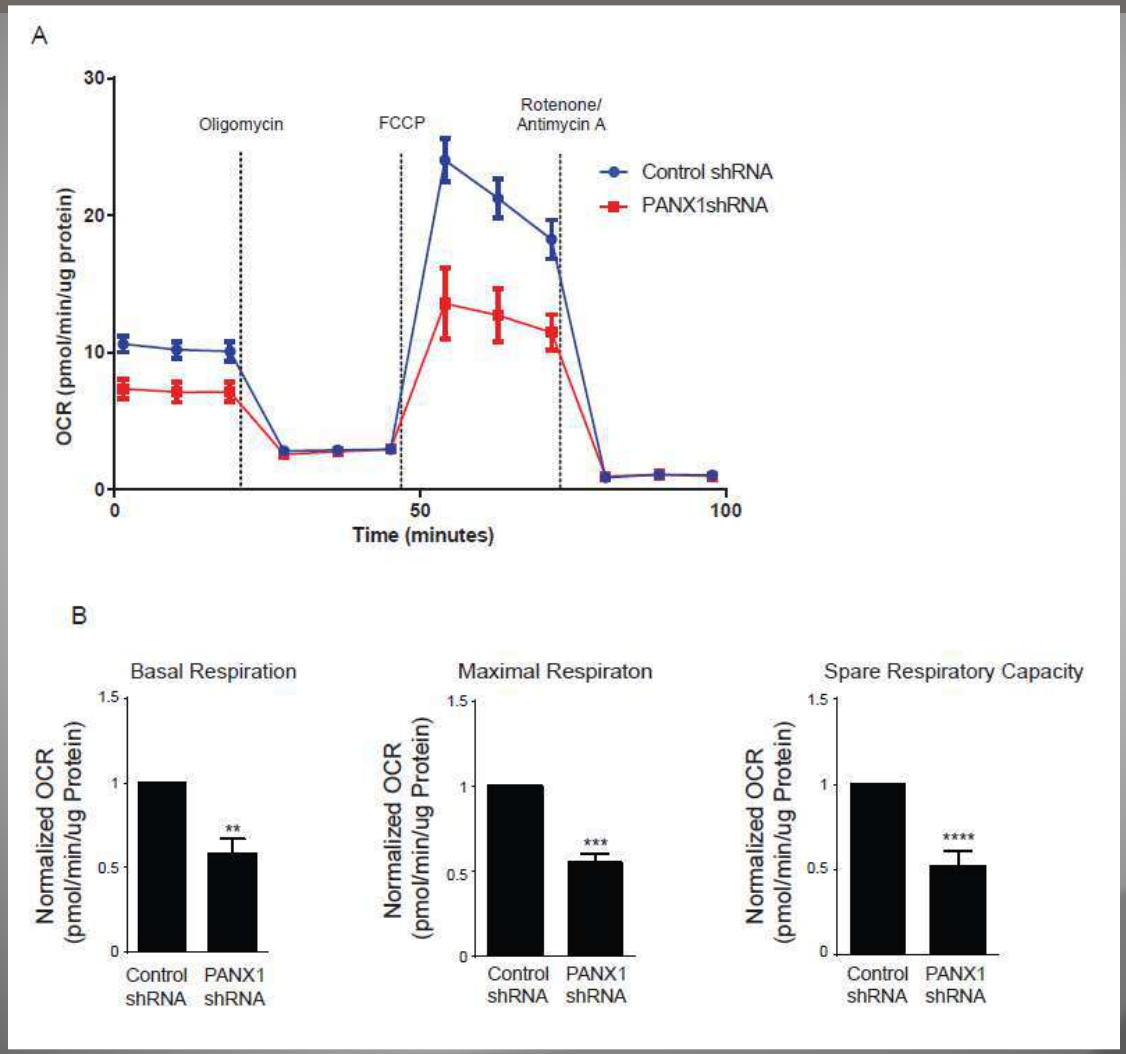




Knocking down Pannexin1 decreases Wnt signalling



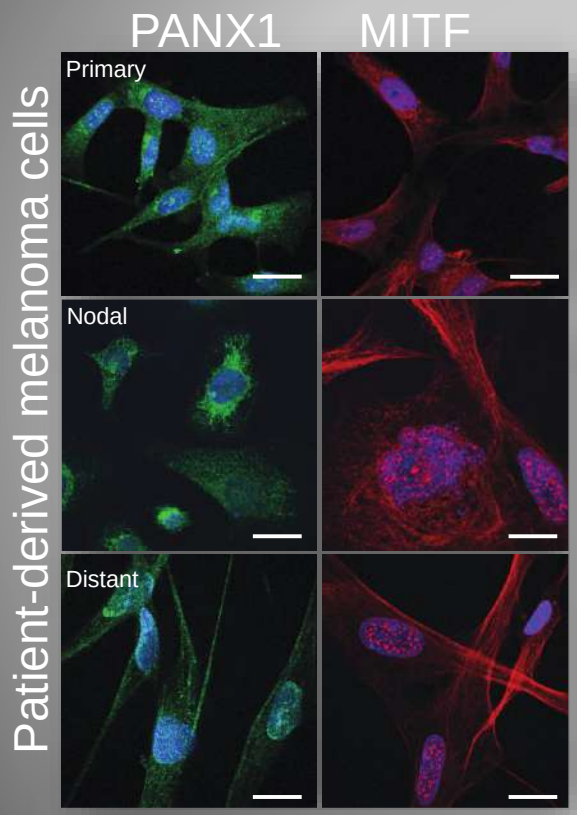
PANX1-deficient melanoma cells have impaired mitochondrial metabolic activity



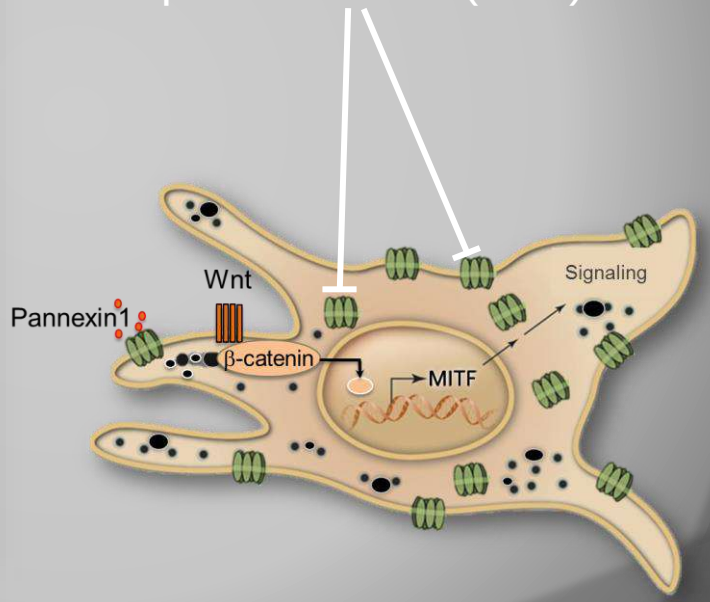
Agilent Seahorse XF^{e}24

Sayedyahossein *et al.*, 2021

Pannexin1 in patient-derived melanoma cells



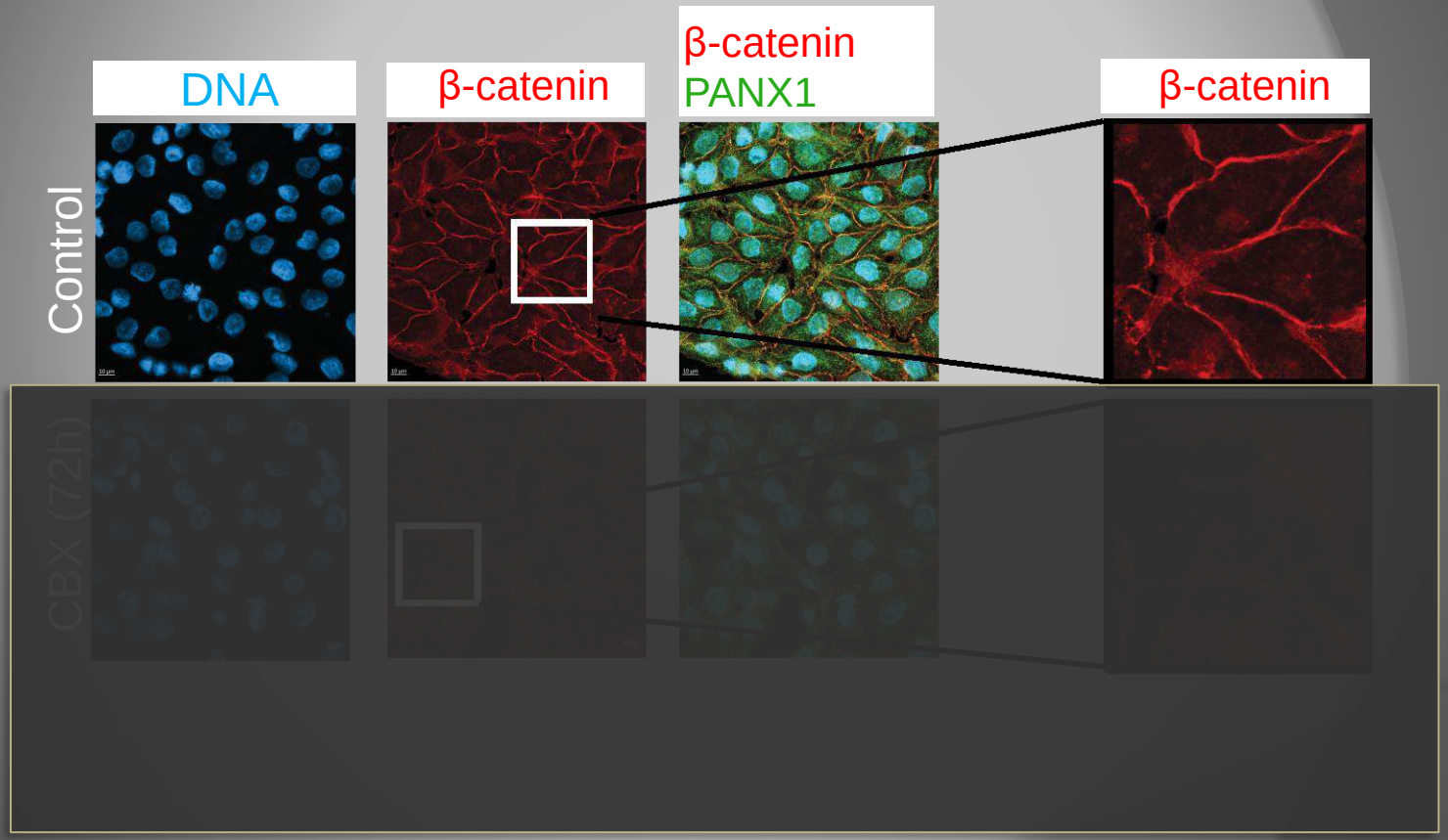
Blockers
Carbenoxolone (CBX)
Probenecid (PBN)
Spironolactone (SPL)



Freeman *et al.*, Cancers, 2019

In collaboration with Dr Steven Latosinsky and Dr Aaron Grant at LHSC

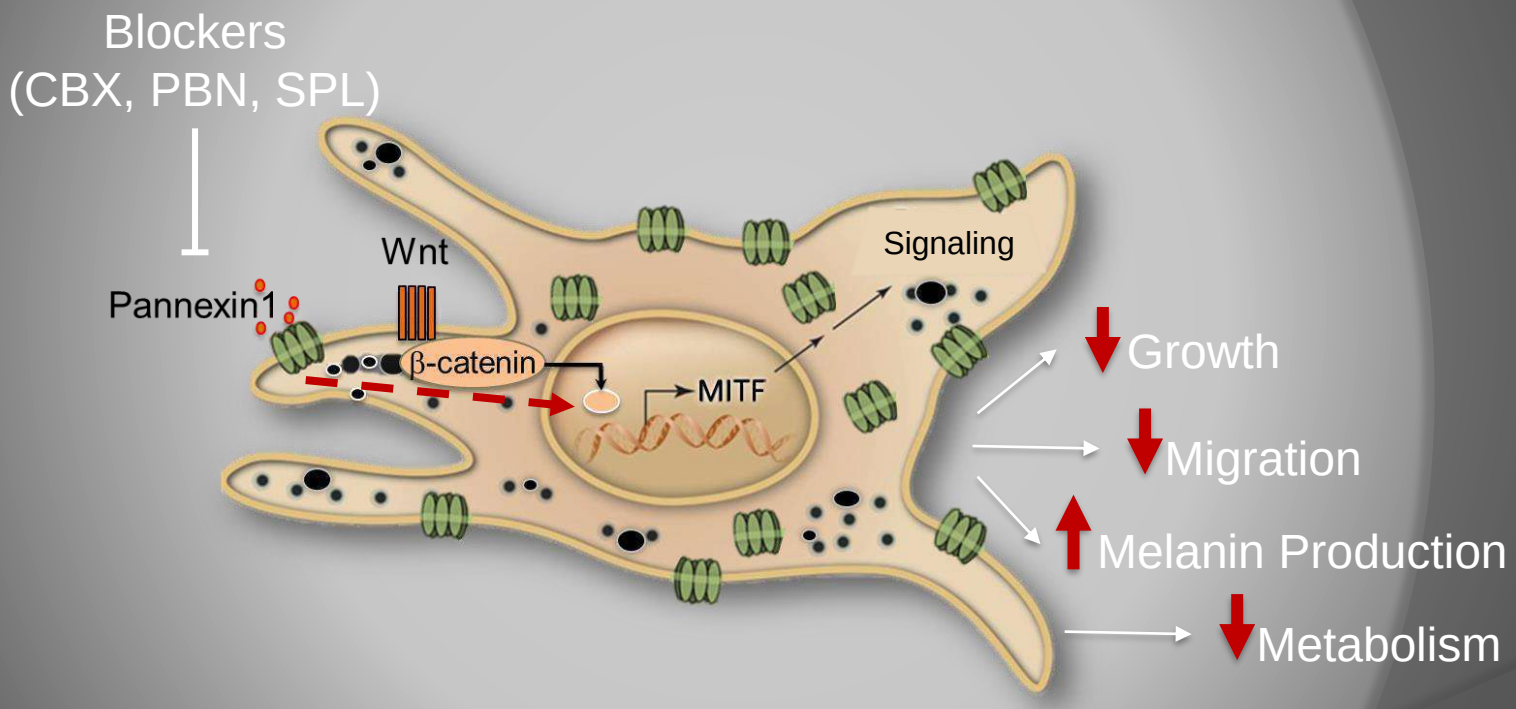
Pannexin1 blockers alter β-catenin subcellular localization



A375-P, A375-MA2, 131/4-5B1

Sayedyahossein *et al.*, 2021

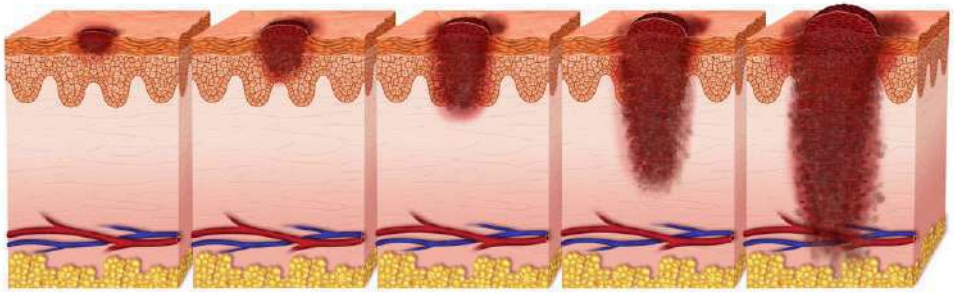
Pannexin1 modulates Wnt/β-catenin pathway



How about in vivo?

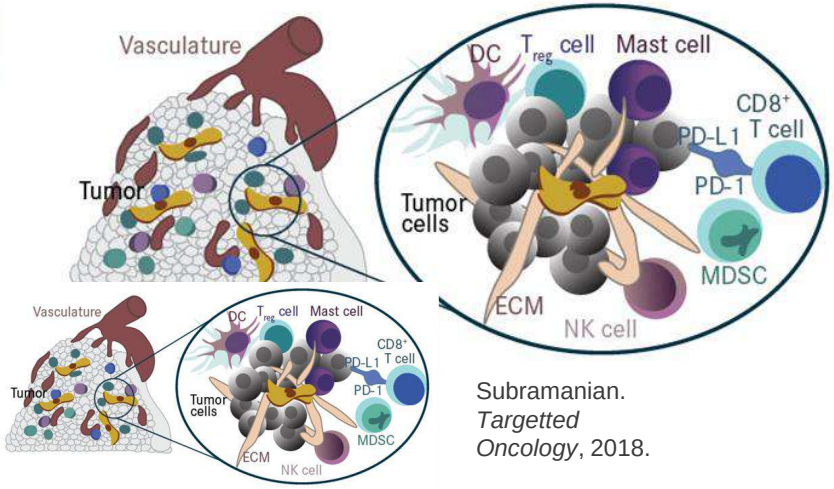
PANX1 in Melanoma and the Tumour Immune Microenvironment

- PANX1 as a tumour promoter *in vitro*:
  - Highly expressed in melanoma tumours
    - Genetic/pharmacological PANX1-KO
      - Reduced proliferation
      - Reduced migration



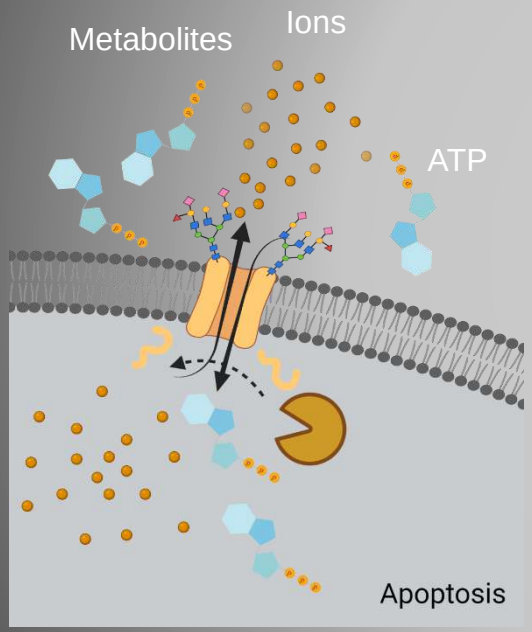
Ramirez. *Alamy stock photo*, 2013.

- PANX1 immunological functions:
  - Inflammasome activation
  - Release of pro-inflammatory cytokines
  - Activation/migration of leukocytes
    - **PANX1 and immune infiltration of tumours?**



Subramanian. *Targetted Oncology*, 2018.

PANX1 channel function mediates inflammation

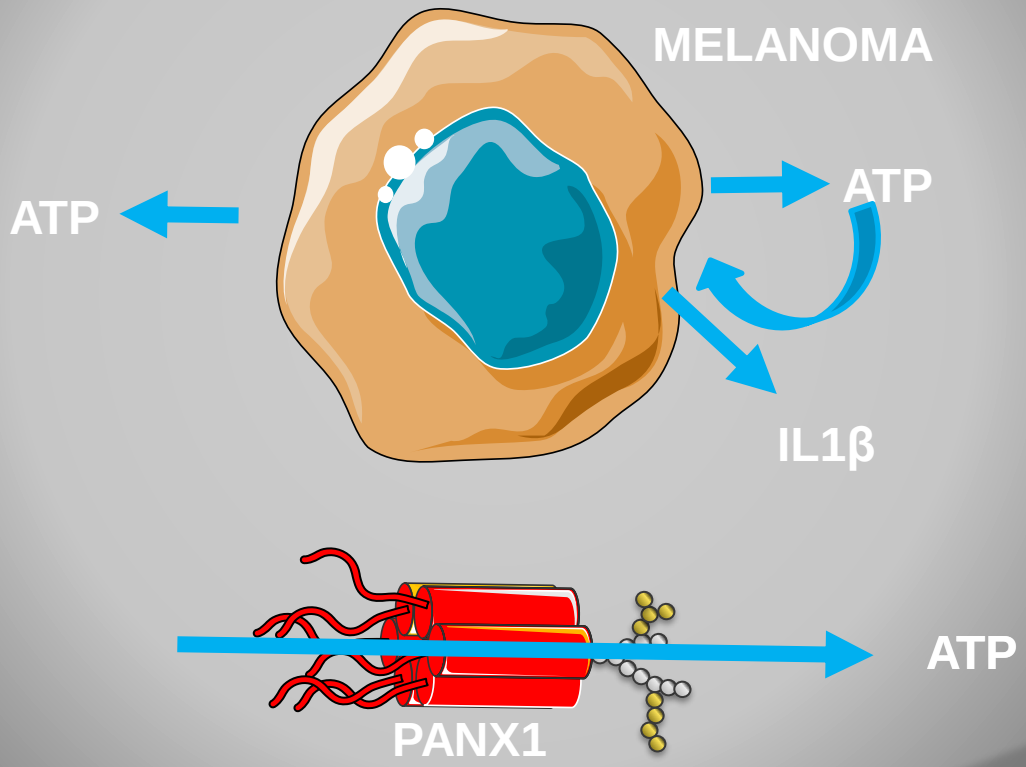


Created with BioRender.com

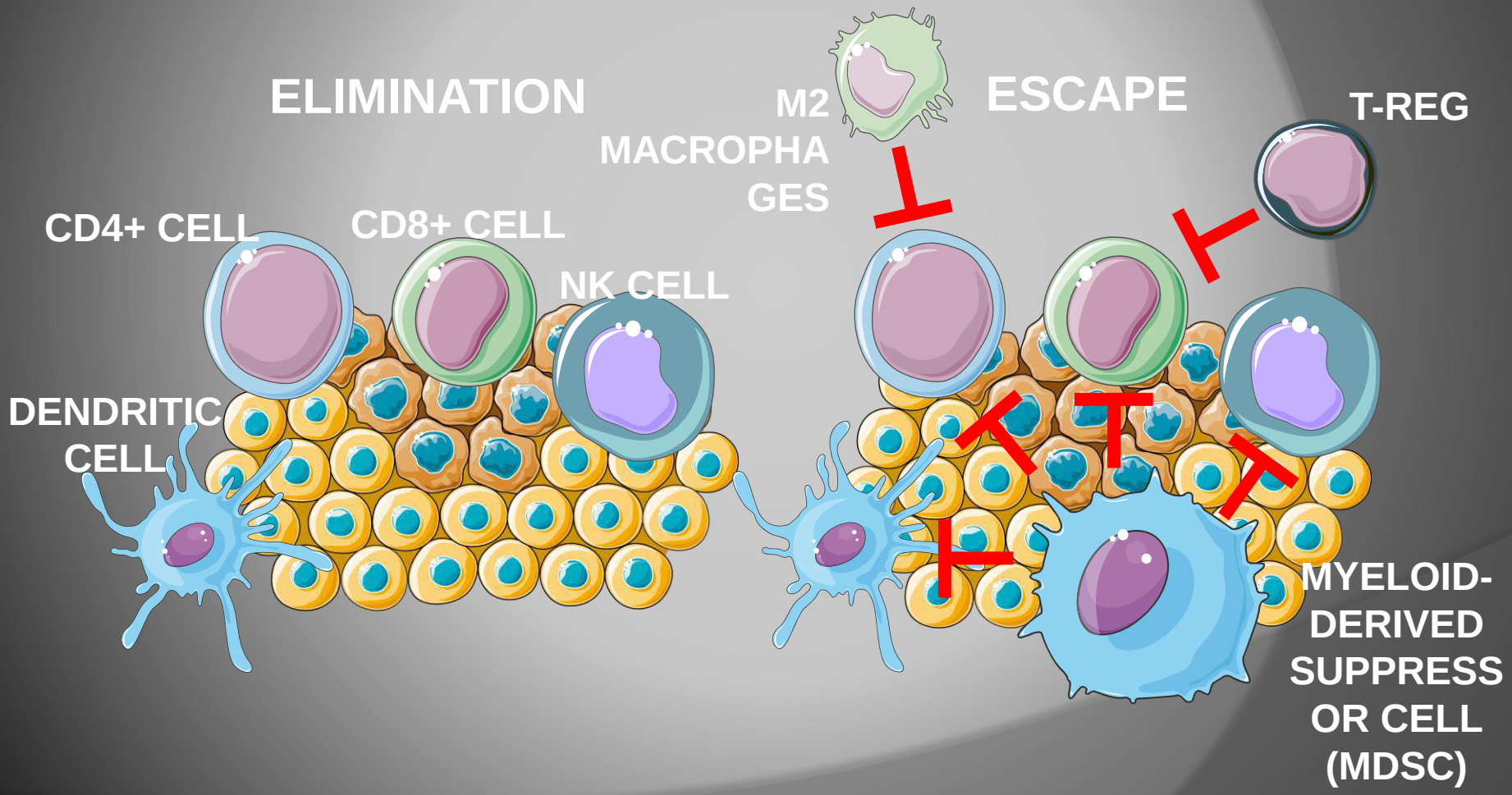
- PANX1-dependent ATP release attracts monocytes and macrophages (Chekeni *et al* 2010)
- PANX1 is required for chemokine-mediated migration of CD4+ T-lymphocytes in experimental autoimmune encephalomyelitis (Velasquez *et al* 2016)
- Myeloid PANX1 channels play a major role in the leukocyte infiltration triggered by traumatic brain injury (Seo *et al* 2020)

- Unknown role during inflammation in the context of cancer

IL1β and purinergic signalling promote immunosuppressive activity

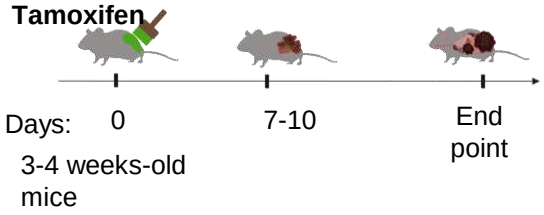


Immune cell infiltration of melanoma



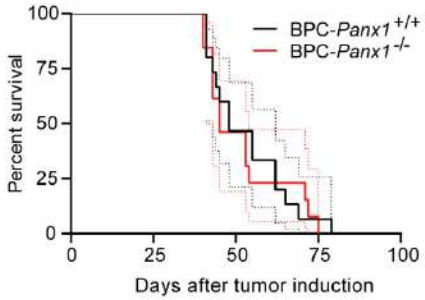
Panx1 global deletion does not hamper tumor progression of the BrafV600E/Pten(del) mouse melanoma model

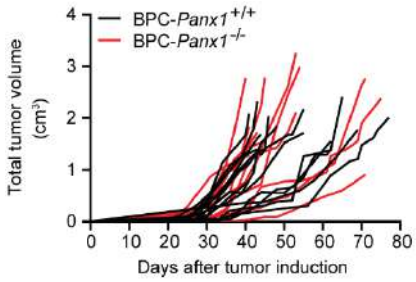
Braf(V600E)/Pten(del)/Cre => BPC

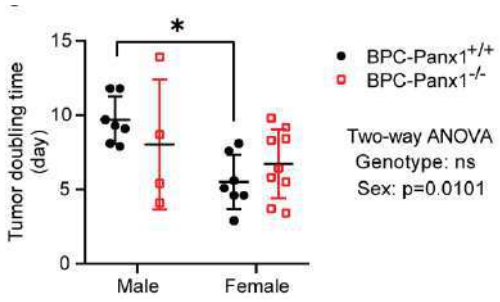


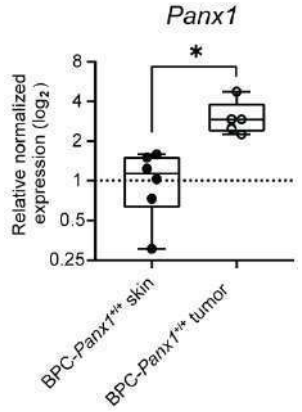
BPC-Panx1$^{+/+}$ **BPC-Panx1$^{-/-}$**

♂ n=8; ♀ n=7 ♂ n=4; ♀ n=9





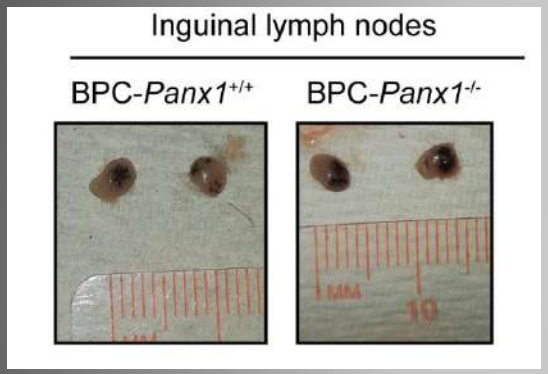


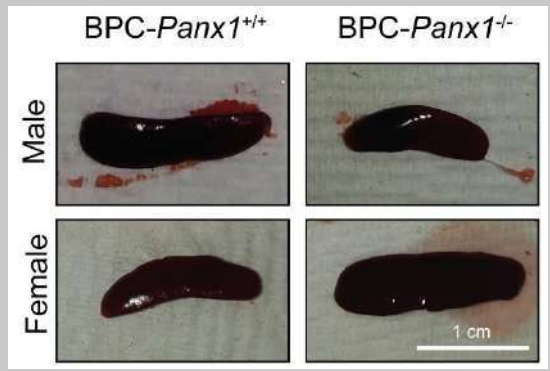


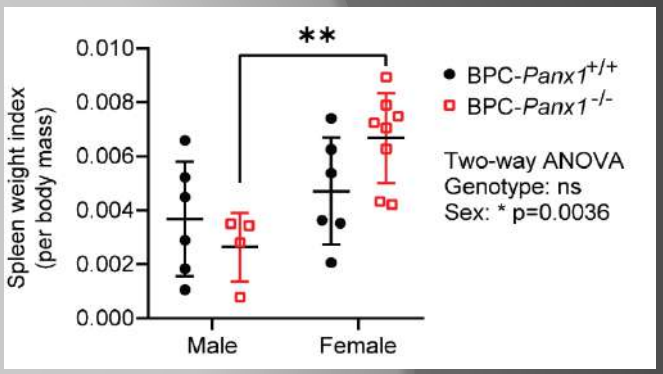
- Tumor growth rate was higher in BPC-Panx1$^{+/+}$ females

Paired t-tests p<0.05

Global deletion of *Panx1* did not prevent primary melanoma spread to lymph nodes and caused splenomegaly in tumor-bearing female BPC-mice



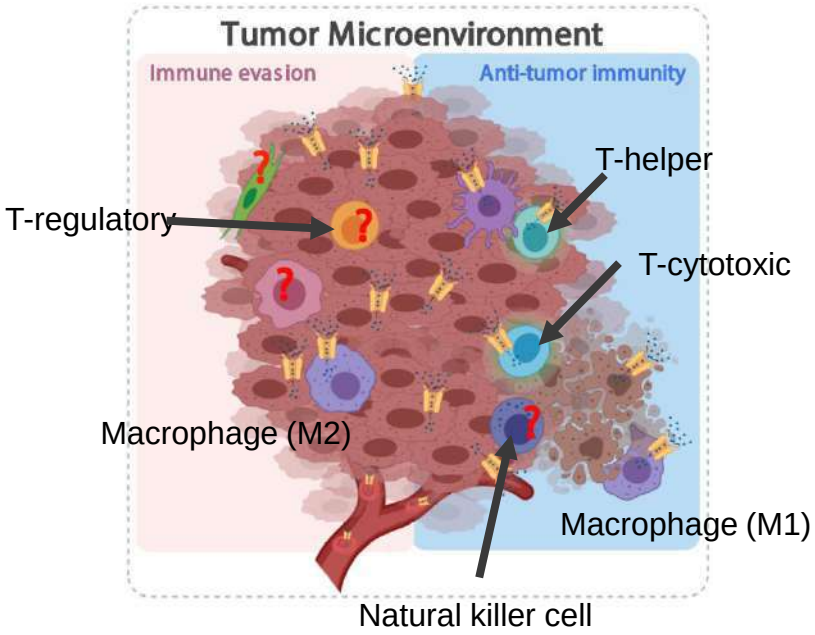




- No evident melanoma metastasis in other organs

- Increased spleen size **only** in tumor-bearing female BPC-*Panx1*$^{-/-}$ mice.
- Unknown implications for the antitumor immune system's response in BPC-*Panx1*$^{-/-}$ mice.

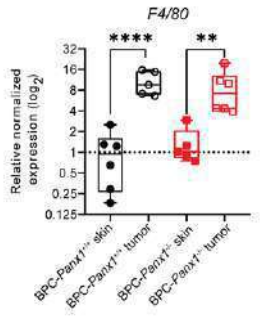
Panx1-deficient mice had a significant increase in *CD8* mRNA transcript expression in skin and tumors



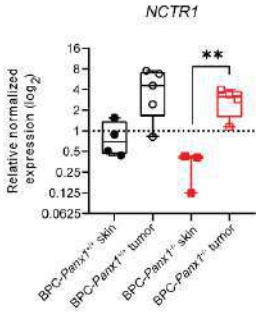
Modified from Laird & Penuela 2021

? Unknown PANX1 expression

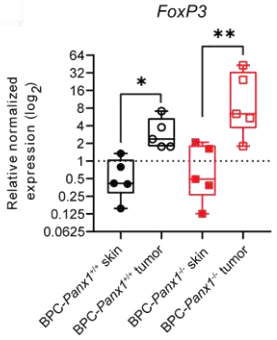
Macrophages



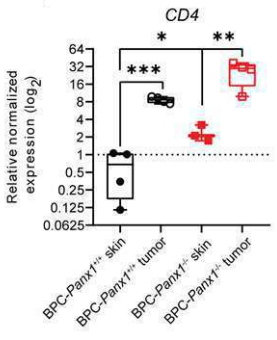
Natural killer cells



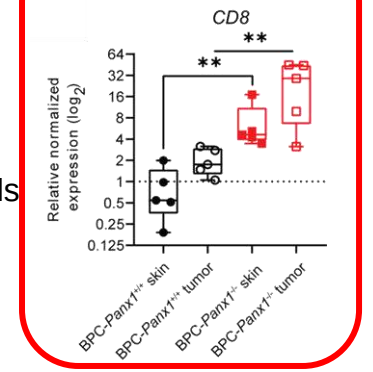
Regulatory T-cells



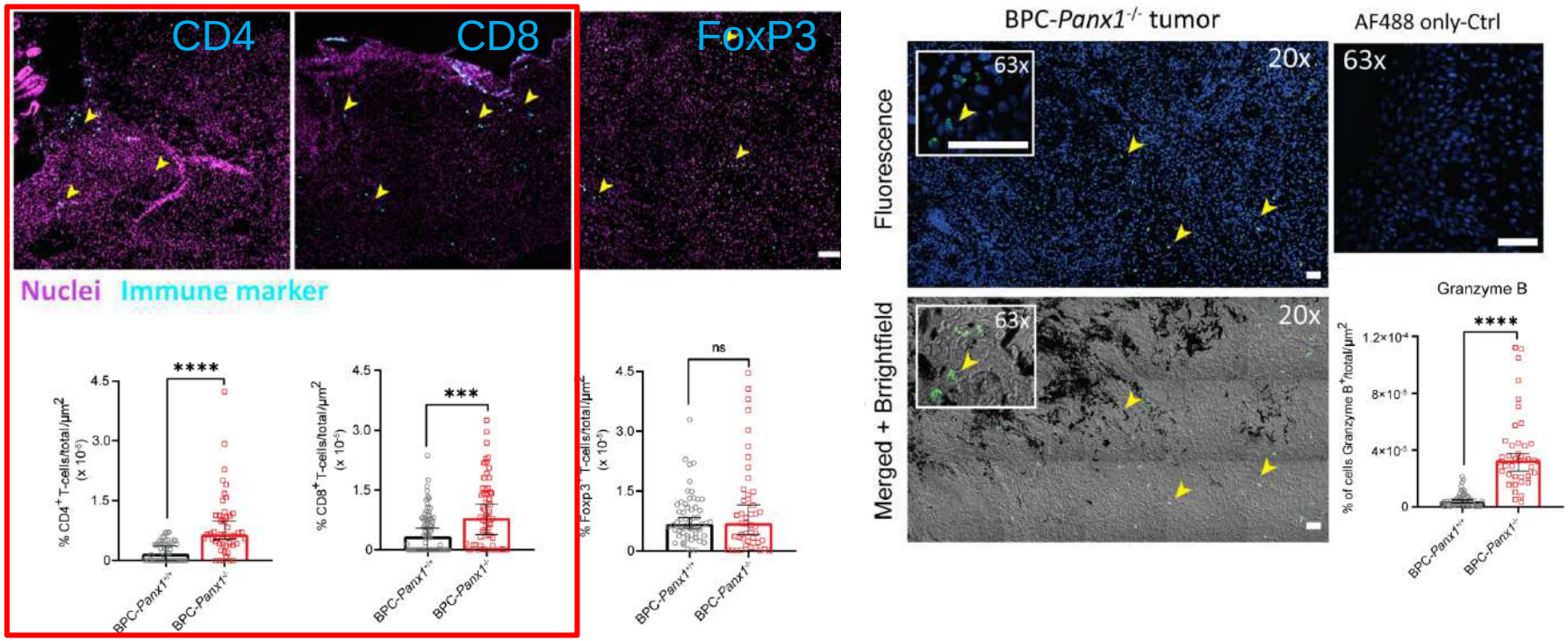
Helper CD4+ T-cells



Cytotoxic CD8+ T-cells

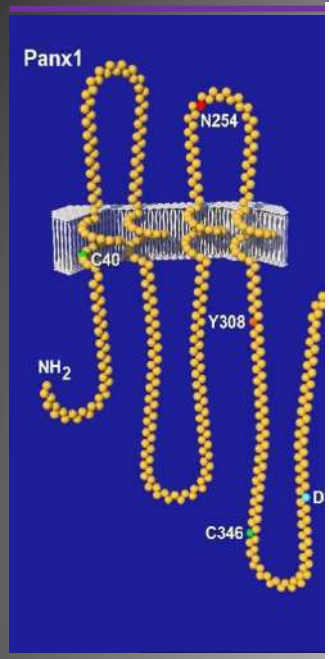


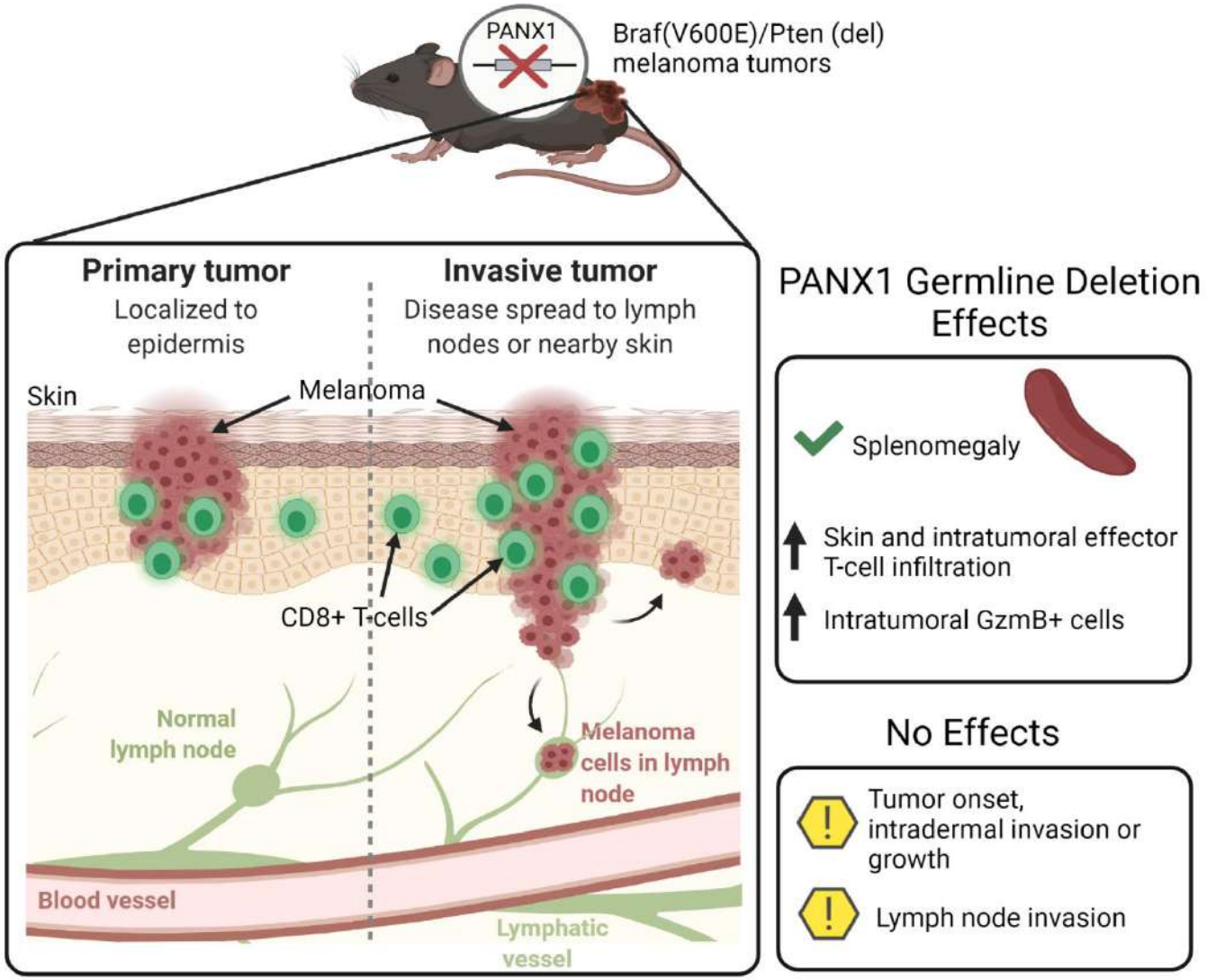
Effector CD4+, CD8+ T-lymphocytes and Granzyme B+ cells are significantly increased in BPC-*Panx1*$^{-/-}$ tumors



Sanchez-Pupo et al *Mol Oncol* 2024

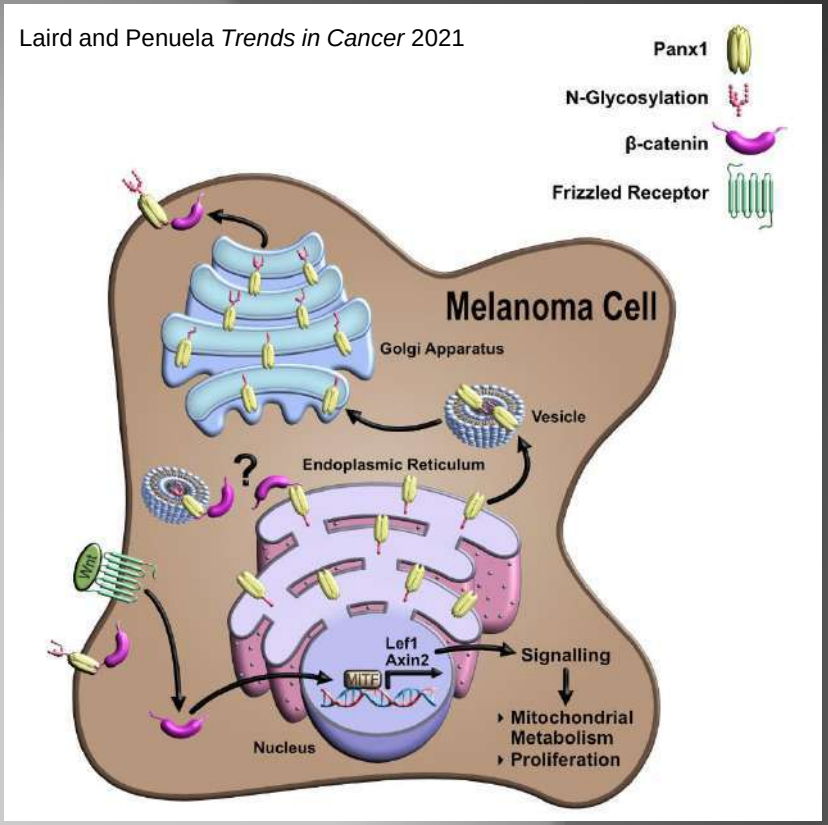
Pannexin1 modulates Wnt/β-catenin pathway in melanoma and can be targeted for cancer treatments

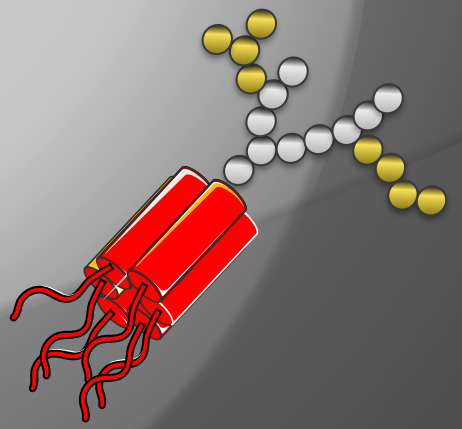




Summary

- PANX1 is highly expressed in melanoma and other cancers and its deletion or inhibition can slow growth, migration, and tumour formation.
- Signaling effects of PANX1 include its direct interaction with β-catenin and its regulation of the Wnt signaling pathway
- Knocking down or inhibiting PANX1 reduces cell growth and mitochondrial metabolism via β-catenin and the Wnt pathway
- PANX1 interacts directly with the actin cytoskeleton and may also have a scaffolding function independent of its channel function
- Pharmacological inhibition of PANX1 may be a tool in combination therapies for melanoma, glioblastoma and other cancers





Thank you!

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https://www.schulich.uwo.ca/penuelalab/

Hiring postdocs
and graduate students!
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of PUBLIC HEALTH

frontiers
in Artificial Intelligence

Thomas Hartung & team

CAAT

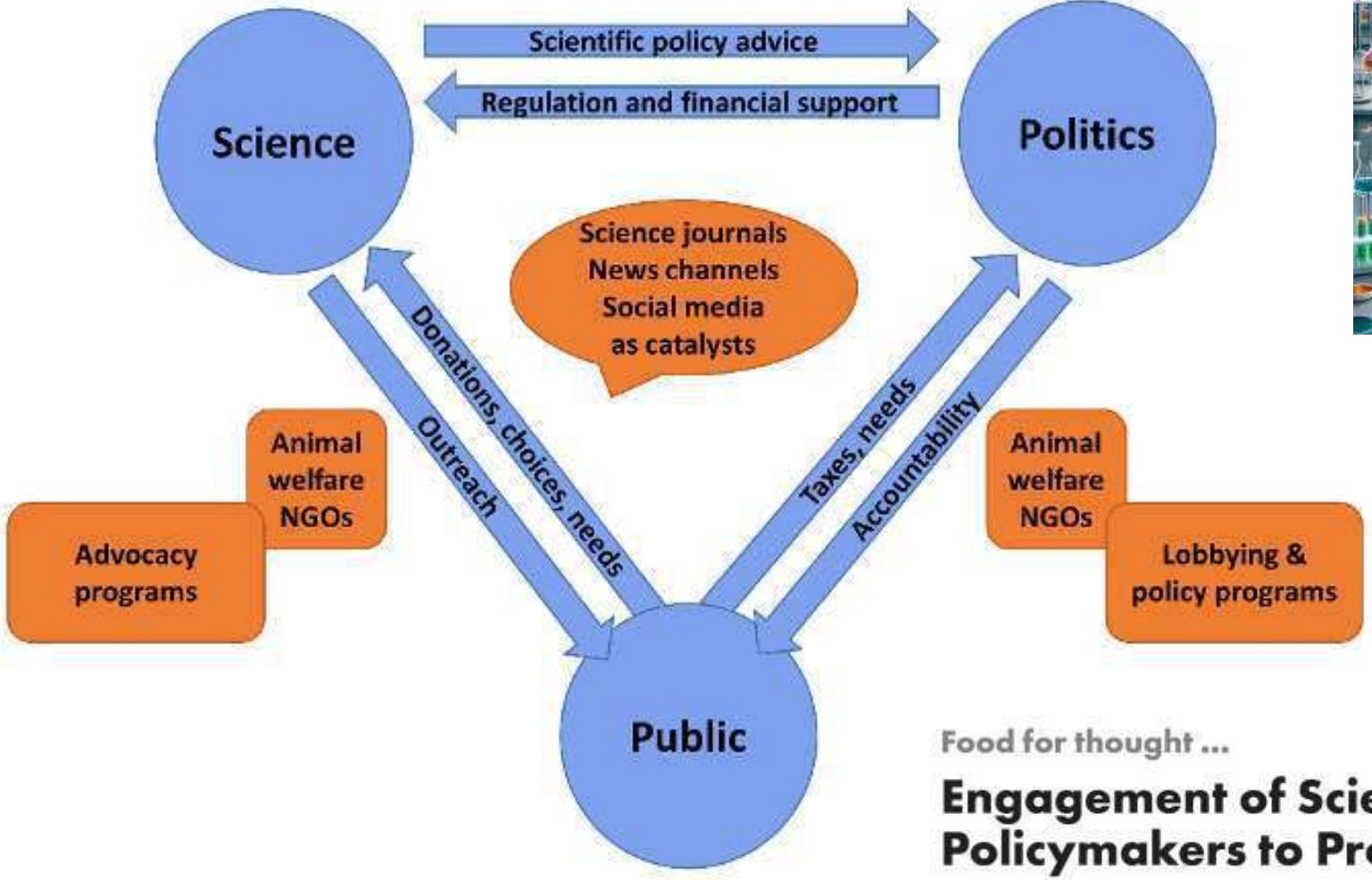
CAAT eu

The scAInce of drug development and toxicology

slides

https://share.zight.com/4guGZq7G

CAAT as ‘lubricant’



Food for thought ...

Engagement of Scientists with the Public and Policymakers to Promote Alternative Methods

Sonja von Aulock[1], Francois Busquet[2,3], Paul Locke[4], Kathrin Herrmann[4,5] and Thomas Hartung[2,4]

2019: 350,000 registered chemicals
~25,000 somewhat tested
US: 1,000 pre-marketing notifications /a with minimal data

US: 6,7 million vaping products submitted
6 Electronic Nicotine Delivery Systems (ENDS) admitted

US: 4,500 food additives
80% inadequate data

Challenges for mixtures, biologicals, cell therapies, medical devices, nanoparticles and micro-plastics

Drugs and pesticides well-assessed but need for front-loading / Green Toxicology

"We thought you'd like to meet Reggie. He's the rat who we experimented on to find a cure for you."

Not different for efficacy models

The “best” animal tests:

OECD guidelines
GLP
high-dose
no disease model

Acute & topical tox

(six tests, 350-750 chemicals, Luechtefeld et al., 2016)

81% reproducible; 69% sensitive

Carcinogenicity

(317 & 121 chemicals, Gray et al. 1995 & Gottmann et al., 2001)

64% inter-species

57% reproducible

Reproductive tox

(105 & 396 chemicals, Hurtt et al., 2003 & Bailey et al., 2005)

60-74% inter-species

Repeat-dose tox

(310 & 37 chemicals, Wang & Gray, 2015)

68-80% inter-species

Human side-effects pred. by rodents

(150 & 182 drug candidates, Olson et al. 2000 & Monticelli et al., 2017)

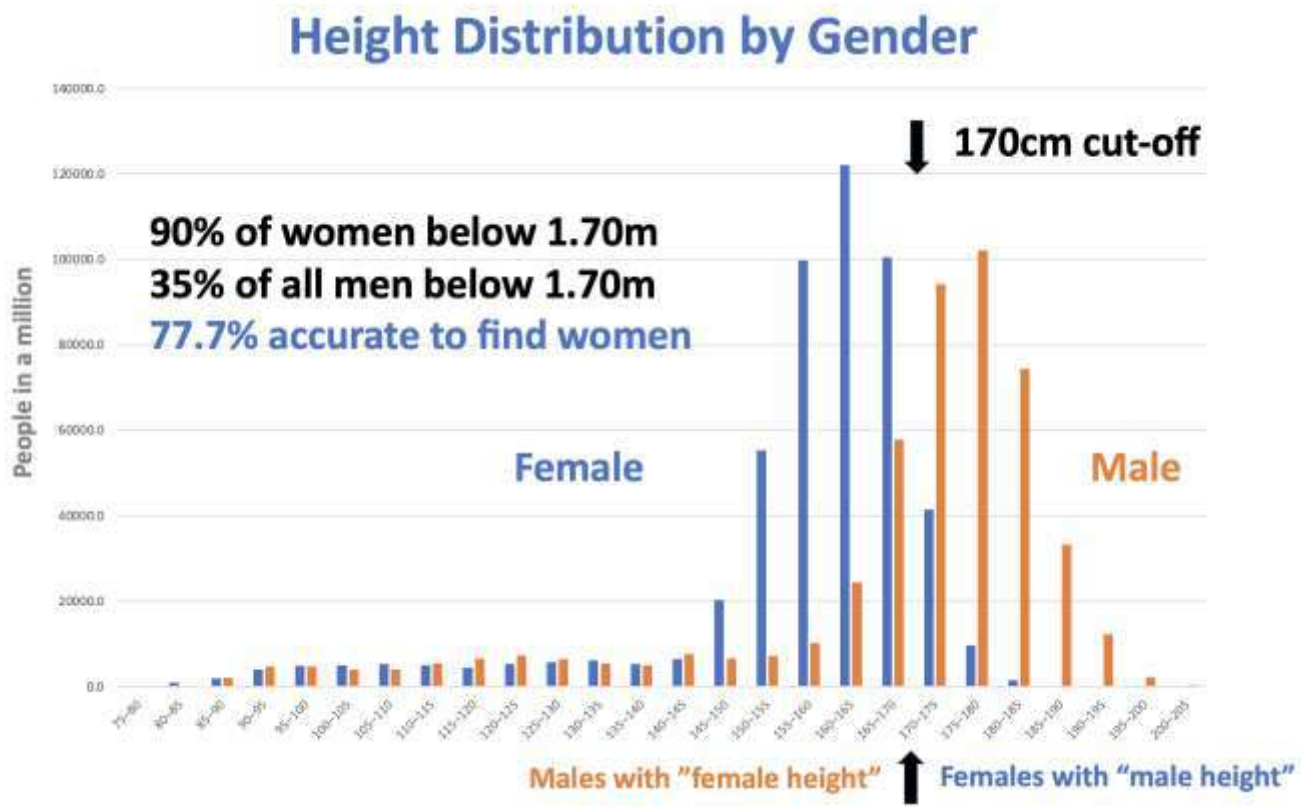
43-48% sensitive

Animals 60-80% predictive

frontiers | Frontiers in Drug Discovery

The (misleading) role of animal models in drug development

Thomas Hartung[1,2]*



Targeting lay audiences

https://doi.org/10.3389/fddsv.2024.1355044

The problem of finding good drugs with limited accuracy of the (animal) tools

Finding 1 in 10,000,
90% accurate tests

Test 1: 90% probability not to lose "The One"
1 good in 1000 remaining.

Test 3: 27% probability that we lost "The One"
11 candidates remaining.

Finding 1 in 10,000,
80% accurate tests

Test 1: 80% probability not to lose "The One"
1 good in 2000 remaining.

Test 4: 59% probability that we lost "The One"
16 candidates remaining.

Finding 1 in 10,000,
70% accurate tests

Test 1: 70% probability not to lose "The One"
~1 good in 3000 remaining.

Test 6: 82% probability that we lost "The One"
7 candidates remaining.

REVIEW article

Front. Drug Discov.
Sec. Technologies and Strategies to Enable Drug Discovery
Volume 4 - 2024 | doi: 10.3389/fddsv.2024.1355044

This article is part of the Research Topic
Drug Discovery and Development Explained: Introductory Notes for the General Public
View all 10 Articles >

The (misleading) role of animal models in drug development

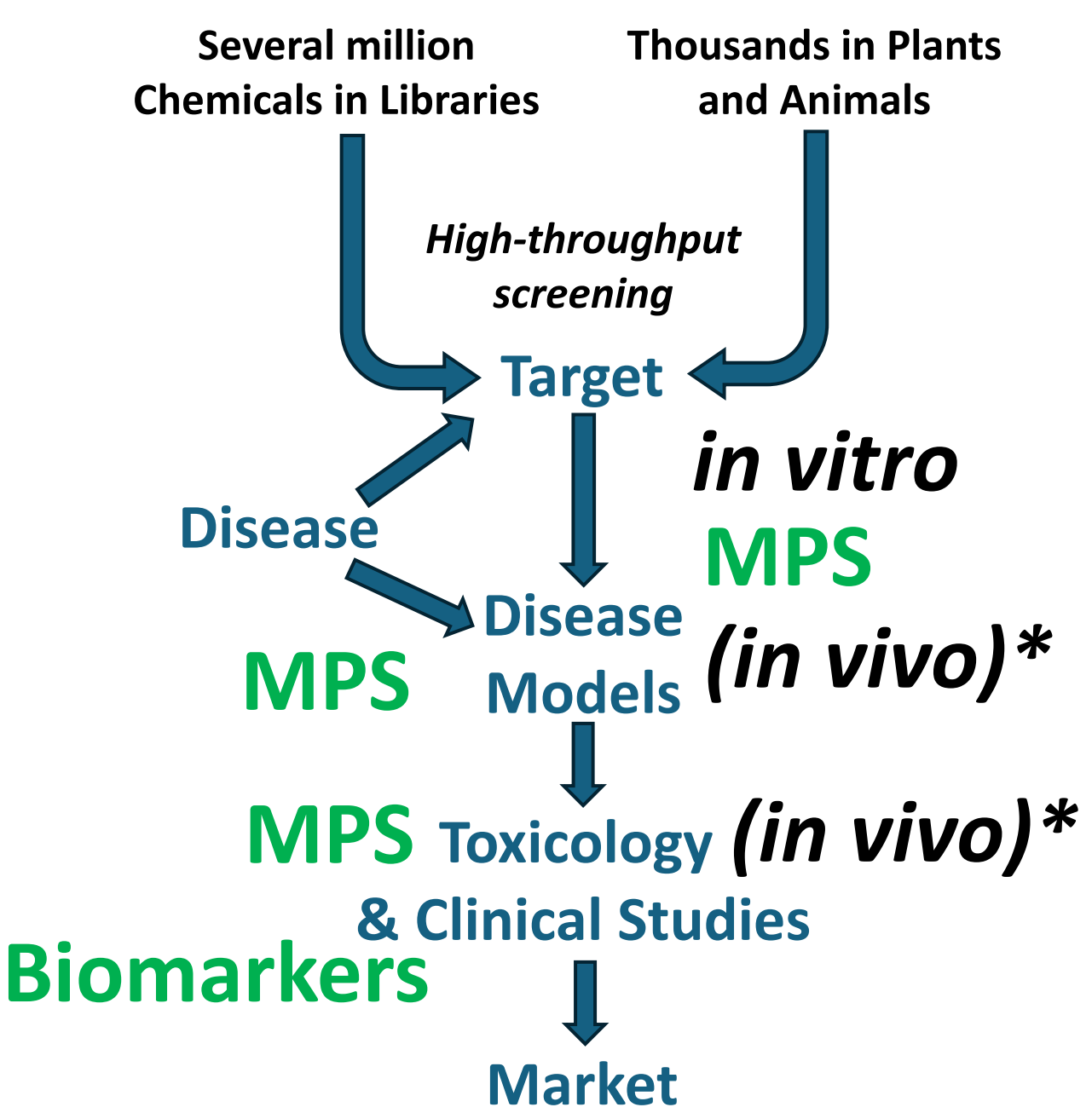
Provisionally Accepted

Thomas Hartung[1*]

“in vivitrosi”

with MPS, AI and biomarkers

as new kids on the block



*not for biologicals, i.e., 50% of new drugs

Data: double every 18month
= 90% in last three years

Computer: double every 24 months (Moore's law)

AI: double every 3 months since 2010

frontiers in Artificial Intelligence

KEYSTONE SYMPOSIA
Accelerating Life Science Discovery

50 YEARS

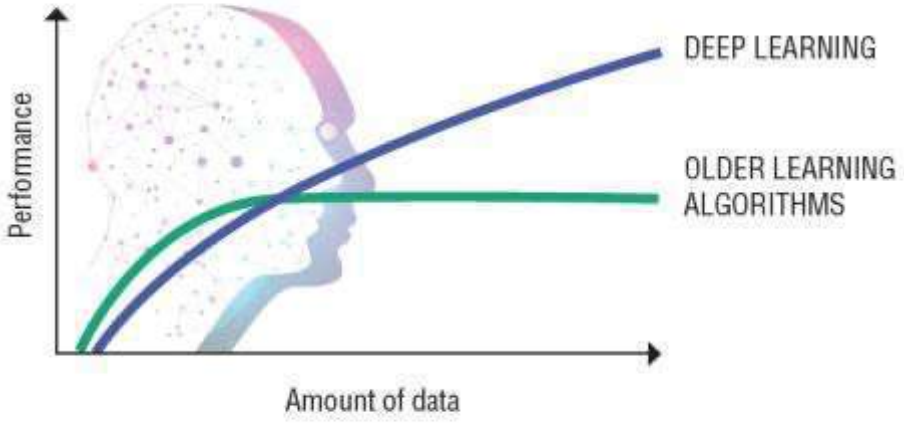
AI in Biomedicine

1-3 May 2024 (virtual)

WORLD ECONOMIC FORUM

2023: AI-facilitated healthcare
2024: LLM in science

Why Deep Learning?

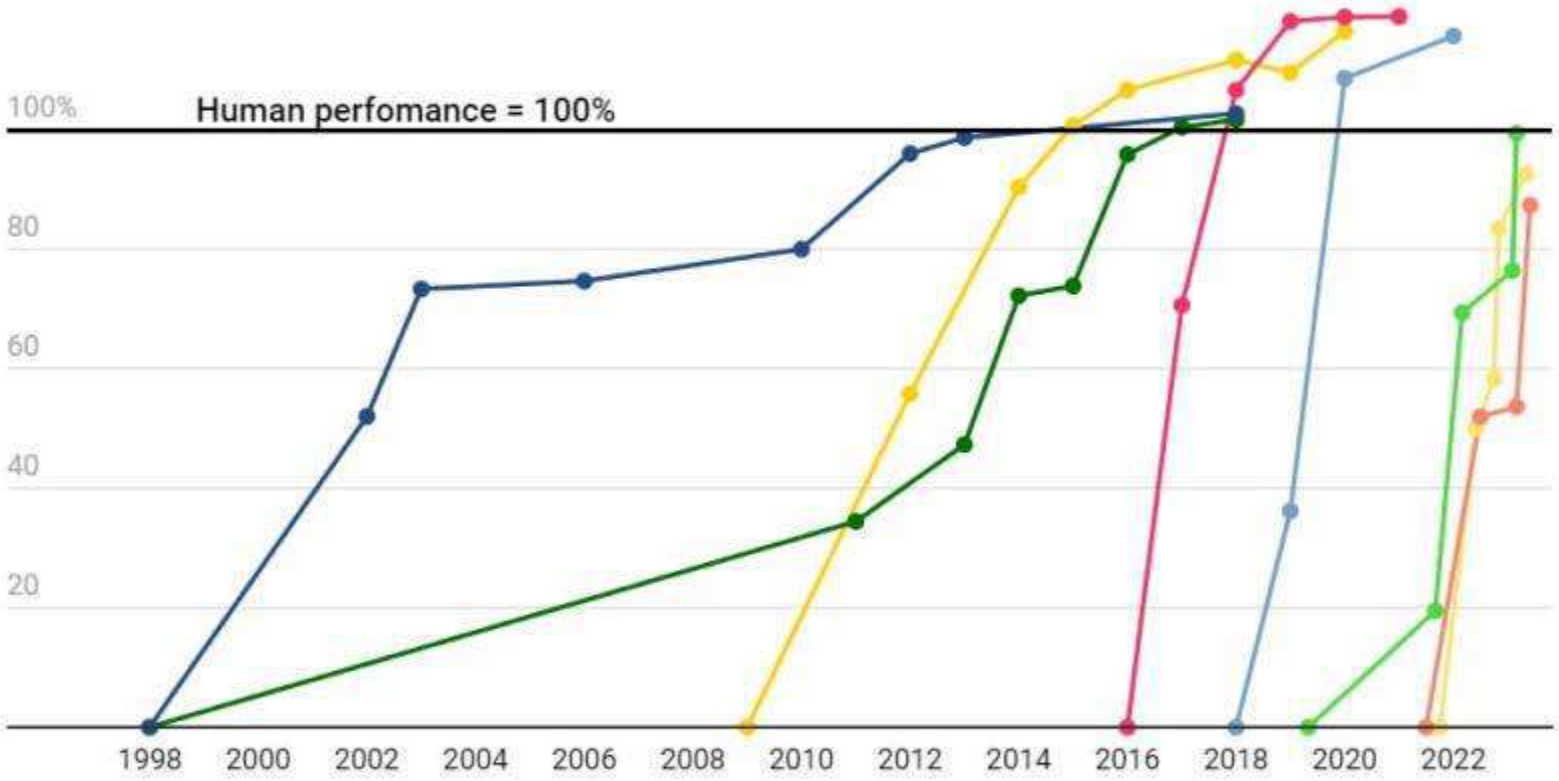


How do data science techniques scale with amount of data?

AI has surpassed humans at a number of tasks and the rate at which humans are being surpassed at new tasks is increasing

State-of-the-art AI performance on benchmarks, relative to human performance

Handwriting recognition · Speech recognition · Image recognition · Reading comprehension · Language understanding · Common sense completion · Grade school math · Code generation

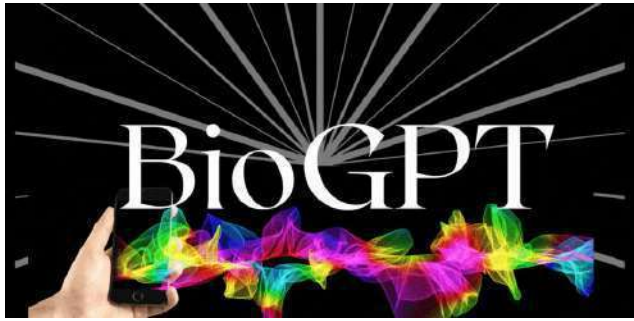


For each benchmark, the maximally performing baseline reported in the benchmark paper is taken as the "starting point", which is set at 0%. Human performance number is set at 100%. Handwriting recognition = MNIST, Language understanding = GLUE, Image recognition = ImageNet, Reading comprehension = SQuAD 1.1, Reading comprehension = SQuAD 2.0, Speech recognition = Switchboard, Grade school math = GSK8k, Common sense completion = HellaSwag, Code generation = HumanEval.

Chart: Will Henshall for TIME • Source: ContextualAI

TIME

AI surpasses human performance, e.g., annotating scientific papers



2023

BioGPT and human annotator have comparable performance in biomedical research test

Selected performances on PubMedQA, which tests biomedical language processing

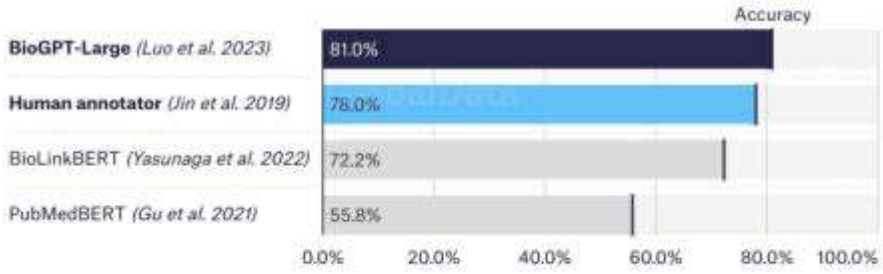
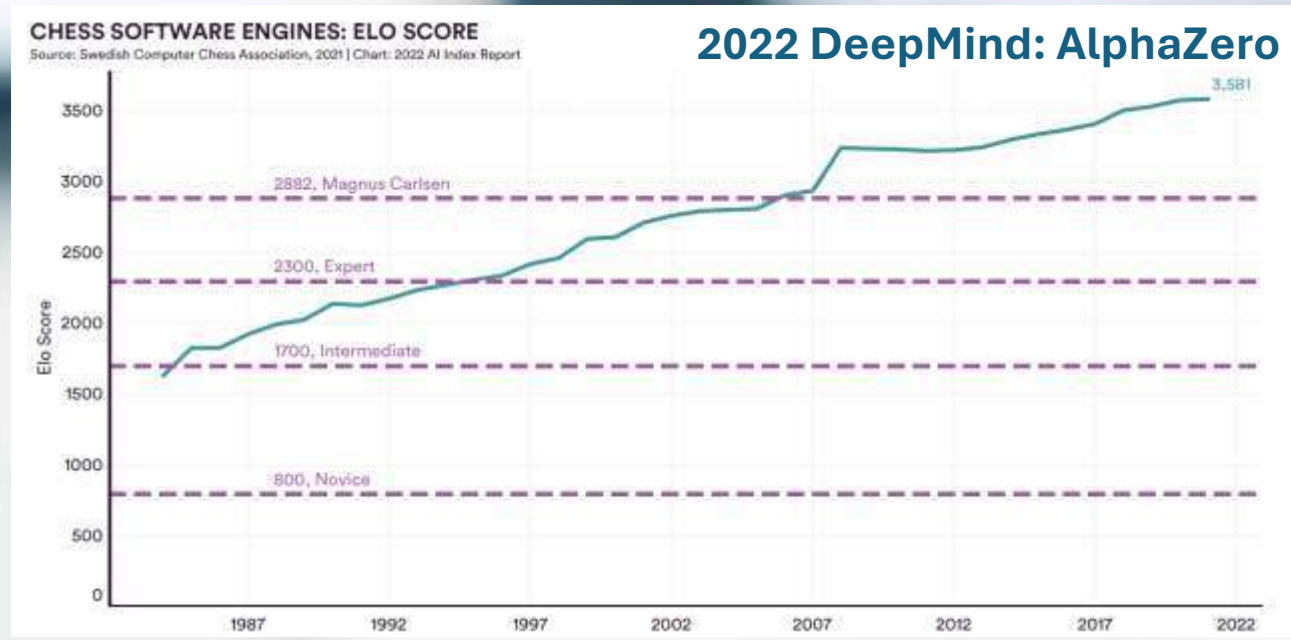


Chart: GlobalData • Source: PubMedQA



AI plays better and different

Plagiarism?

Bias

Data gaps

Black box

Hallucination

Autonomous AI

Productivity

Information retrieval

Evidence integration of Big Data

Multi-modal

Toward xAI

Human-in-loop

ToxAIcology

Big Data

- High-content (~omics & imaging)
- High-throughput (Robotized testing, e.g., Tox21 & ToxCast)
- Sensors
- Literature, Internet
- Legacy studies

Big Computer

AI & Machine Learning

- Natural Language Processing (Large Language Models)

Big Sense

- Data retrieval
- Evidence integration (systematic reviews, risk assessments)
- Predictive toxicology
- Digital pathology
- Reporting

Food for Thought ...

ToxAIcology – The Evolving Role of Artificial Intelligence in Advancing Toxicology and Modernizing Regulatory Science

Thomas Hartung[1,2]

Archives of Toxicology
https://doi.org/10.1007/s00204-023-03666-2

REVIEW ARTICLE

Artificial intelligence (AI)—it's the end of the tox as we know it (and I feel fine)*

Nicole Kleinstreuer[1] · Thomas Hartung[2,3]

Artificial intelligence as the new frontier in chemical risk assessment

Thomas Hartung[1,2]*

frontiers | Frontiers in Artificial Intelligence

Sep 2023

PEER REVIEW

Provisional Peer-Reviewed Toxicity Values for

p-Isopropyltoluene
(CASRN 99-87-6)

Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

EPA United States Environmental Protection Agency

18 months
Costs not disclosed

OpenAI
ChatGPT 3.5

99% of ChatGPT users 👎

GPT-4

1% of ChatGPT users
"40% more factually correct"
👍

+

KEYMATE.AI
SEARCH
CHATGPT
PLUGIN

0.1% of ChatGPT users
👍

> Pharmacol Toxicol. 1996 Nov;79(5):225-30. doi: 10.1111/j.1600-0773.1996.tb00264.x.

Four weeks' inhalation exposure of rats to p-cymene affects regional and synaptosomal neurochemistry

H R Lam [1], O Ladefoged, G Ostergaard, S P Lund, L Simonsen

perplexity

Giv
fou
ssessm
ingredient

ANTHROPIC
CLAUDE 2

👍
👍

https://sfmagazine.com/technotes/february-2019-wipo-u-s-and-china-lead-the-world-in-ai-innovation/

ACCEPTED MANUSCRIPT

Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility

Thomas Luechtefeld, Dan Marsh, Craig Rowlands, Thomas Hartung

Toxicological Sciences, kfy152, https://doi.org/10.1093/toxsci/kfy152

Published: 11 July 2018

2018

10 million structures

600,000 with data

9 most common toxicity tests

190,000 chemical's hazard

cross-validation: 87% correct

AI Animal Replacement

Tom Luechtefeld

2020: Human Skin Sensitization

AI predicted 506 chemicals 80% correctly

Animal 74% correct

2022: Nine most used animal tests predicted by AI

AI predicted 4700+ food chemicals 83% correctly in 1h

= 38,000 animal studies at $250+ million

2023: Systemic toxicities

AI predicted 75% cancer risk of 950 chemicals and 82% reproductive tox of 1152 chemicals correctly

ONTOX

Copilot for safety sciences

Literature

Databases

Internet

biobricks-ai/
bricktools

a set of tools for auditing bricks

3 Contributors · 1 Issue · 1 Star · 0 Forks

LLM

~50 BioBricks constructed to datePublic
release of toxicology BioBricks upcoming

ChemHarmony:
integrates chembl, pubchem, ctdbase etc.:
200 million triplets of
substance/property/result

DATA

Can we make a better similarity metric?

Structural similarity

(e.g., Morgan fingerprints)

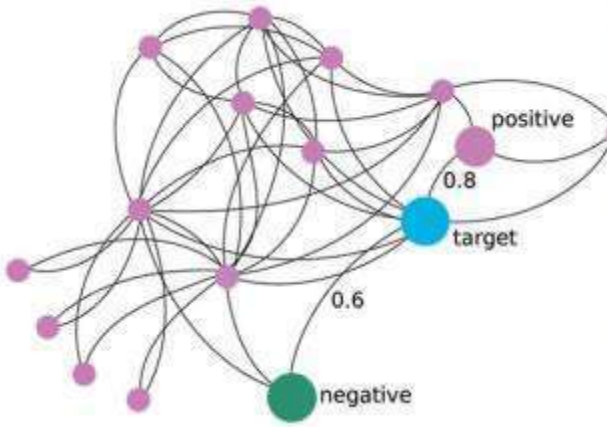
Biological similarity

Test run:

Adding biological similarity increased accuracy by ~10%

DATA

AOP networks
Biological &
physiological maps



From perturbation of physiology

Probability of hazard

RASAR
+ QSAR

From chemical structure and properties

The problem

ALTEX 2022

"Probability is the very guide of life."
Cicero (106 – 43 B.C.)

Food for Thought …

Probabilistic Risk Assessment – the Keystone for the Future of Toxicology

Alexandra Maertens[1], Emily Golden[1], Thomas H. Luechtefeld[1,2], Sebastian Hoffmann[1,3], Katya Tsaioun[1] and Thomas Hartung[1,4]

pdf of The probable future of toxicology – probabilistic risk assessment

The Probable Future of Toxicology – Probabilistic Risk Assessment

Alexandra Maertens[1], Eric Antignac[2], Emilio Benfenati[3], Denise Bloch[4], Ellen Fritsche[5], Sebastian Hoffmann[6], Joanna Jaworska[7], George Loizou[8], Kevin McNally[8], Przemyslaw Piechota[1], Erwin L. Roggen[9], Marc Teunis[10] and Thomas Hartung[1,11]

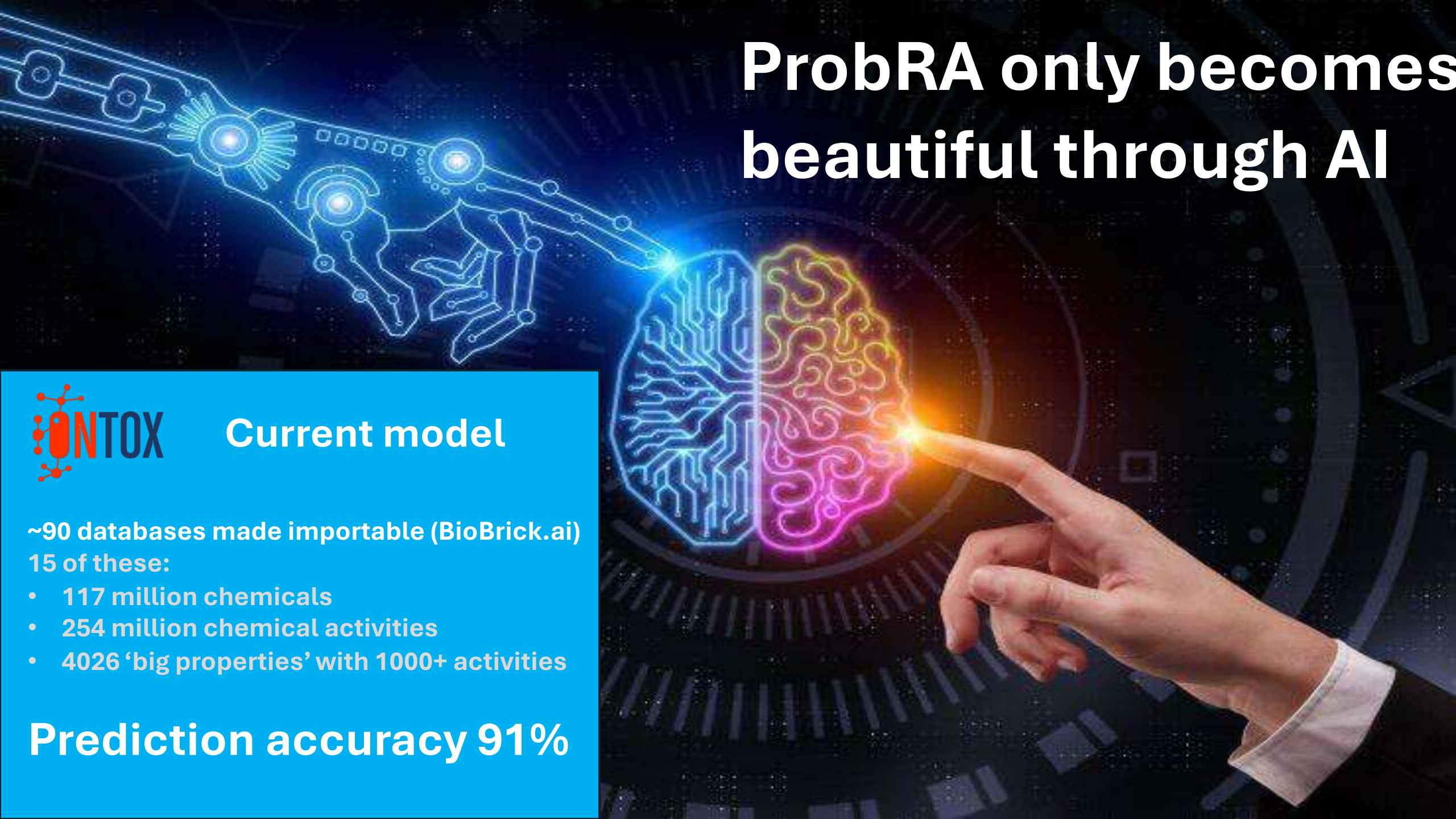
i4 transatlantic think tank for toxicology

AAT

ONTOX

3 Workshops 2022, 2023 & 2024 in Ranco, Italy

ProbRA only becomes beautiful through AI



ONTOX

Current model

~90 databases made importable (BioBrick.ai)
15 of these:

- **117 million chemicals**
- **254 million chemical activities**
- **4026 ‘big properties’ with 1000+ activities**

Prediction accuracy 91%

Call for a Human Exposome Project

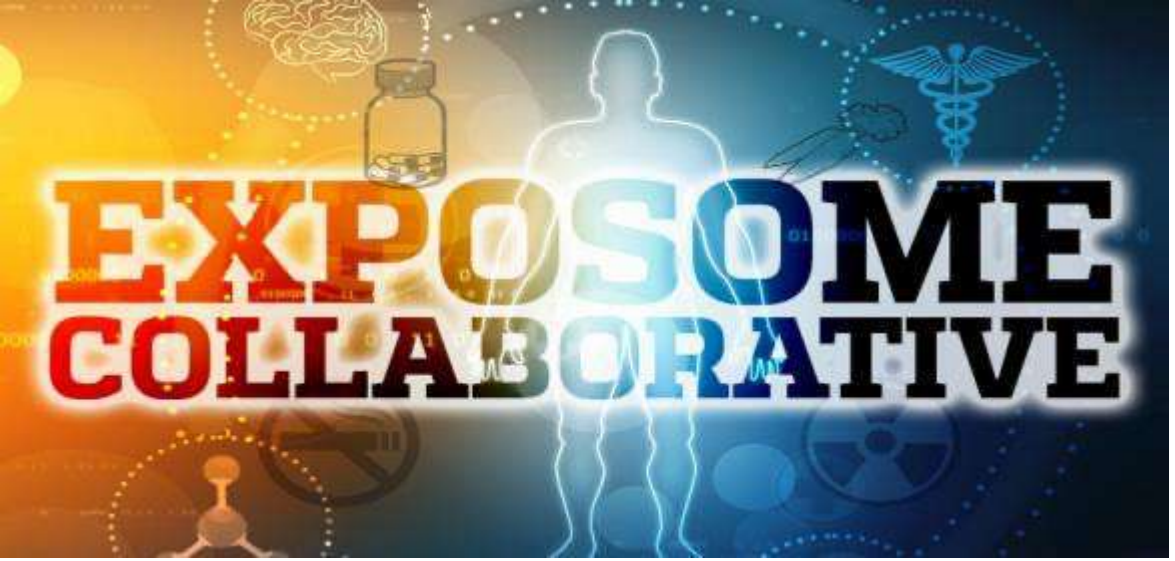
1. Exposure-driven
2. Technology-enabled
3. Evidence-integrated

Food for Thought ...

ALTEX 2023

A Call for a Human Exposome Project

Thomas Hartung[1,2]



Exposome & A.I. = E.I. (Exposome Intelligence)

metabolites

MDPI

Review

Metabolomics in Preclinical Drug Safety Assessment: Current Status and Future Trends

Fenna Sillé [1] and Thomas Hartung [1,2,*]

https://doi.org/10.3390/metabo14020098

Human EXPOSOME Moonshot

Washington, DC
12-16 May 2025

Fenna Sillé

Join the Human Exposome Moonshot!

Food for Thought ...

The Implementation Moonshot Project for Alternative Chemical Testing (IMPACT) toward a Human Exposome Project

Fenna C. M. Sillé[1], Francois Busquet[2], Suzie Fitzpatrick[3], Kathrin Herrmann[1], Lisa Leenhouts-Martin[4], Thomas Luechtefeld[1,5], Alexandra Maertens[1], Gary W. Miller[6], Lena Smirnova[1], Katya Tsaioun[1] and Thomas Hartung[1,7,8]

- **Integrate Disruptive Technologies with Existing Knowledge**
- **Accelerate Product Development**
- **Optimize Prevention and democratize Healthcare Access**

AI, MPS, ~omics, sensors…. Human Exposome

Slides available:

https://share.zight.com/4guGZq7G

Attachment 6

QSAR modeling for the prediction of pharmacokinetics and bioactivities of therapeutic peptides

Presenter: *Carmen Ortiz González*

3rd PANACHE Workshop

7th October 2024

ProtoQSAR

MOLDRUG AI Systems
Machine Learning-Driven Drug Discovery & Optimization

PANACHE

In silico drug discovery

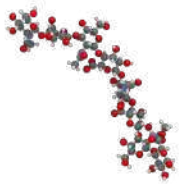
In silico methods for hit identification

ADMET* prediction

Structure determination

Types of candidates

Small molecules



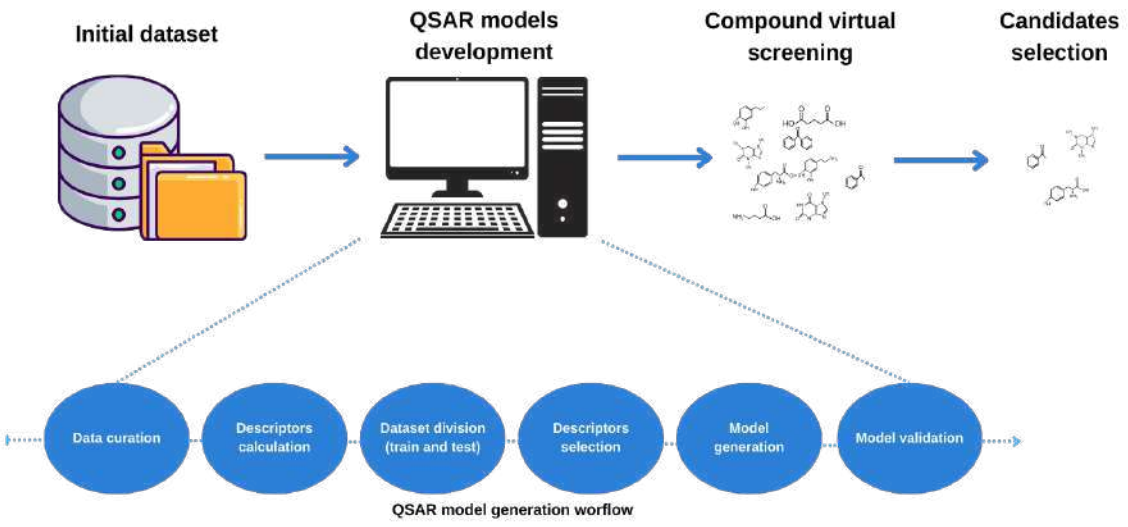
Peptides

*(Adsorption, Distribution, Metabolism, Excretion, Toxicity)

Properties attributed to peptides:

- High specificity and activity
- High degradation rate
- Reduction of toxic metabolites release
- Reused by human body

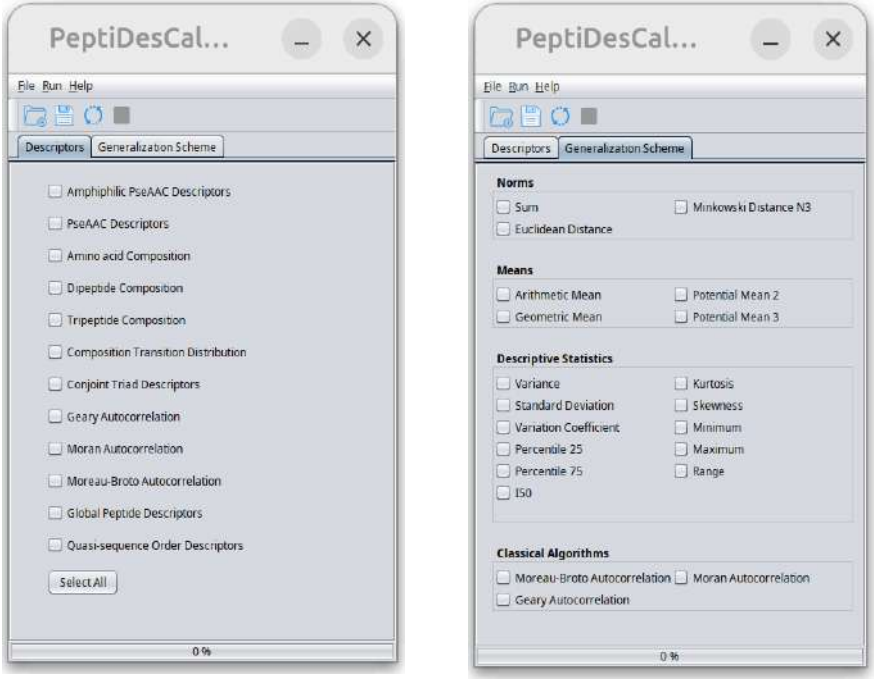
Quantitative Structure-Activity Relationship (QSAR)



- Statistical models for predicting unknown molecular physico-chemical or biological parameter
- Model training with known data
- Virtual screening with datasets of chemical structures
- Selection of best candidates

PeptiDesCalculator

- Java-based software for calculation of descriptors for peptides
- User-friendly Graphical User Interface
- Parallelized processed



Barigye, S. J., Gómez-Ganau, S., Serrano-Candelas, E., & Gozalbes, R. (2021). PeptiDesCalculator: software for computation of peptide descriptors. Definition, implementation and case studies for 9 bioactivity endpoints. Proteins: Structure, Function, and Bioinformatics, 89(2), 174-184.

PeptiDesCalculator

Property	Inhibitors	Non-inhibitors	Accuracy	Sensitivity	Specificity	Precision	Mathew's correlation coefficient
Hepatitis C inhibition	182	225	0.79	0.81	0.78	0.75	0.59
Anti-breast cancer	75	165	0.79	0.65	0.86	0.7	0.52
Anti-skin cancer	39	149	0.86	0.66	0.91	0.66	0.57
Anti-colon cancer	47	180	0.78	0.61	0.85	0.57	0.44
C. albicans activity inhibition	120	661	0.69	0.75	0.59	0.71	0.35
P. aeruginosa activity inhibition	505	385	0.78	0.81	0.75	0.8	0.56
Listeria activity inhibition	39	149	0.82	0.84	0.75	0.88	0.58
HIV inhibition	261	270	0.79	0.77	0.81	0.81	0.58

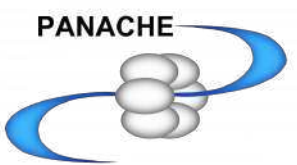
Barigye, S. J., Gómez-Ganau, S., Serrano-Candelas, E., & Gozalbes, R. (2021). PeptiDesCalculator: software for computation of peptide descriptors. Definition, implementation and case studies for 9 bioactivity endpoints. Proteins: Structure, Function, and Bioinformatics, 89(2), 174-184.

Conclusions

- Peptides present properties that makes them optimal therapeutic candidates
- PeptiDesCalculator allows users to calculate specific descriptors for peptides
- QSARs of peptides performed with PeptiDesCalculator have demonstrated their robustness and reliability

THANK YOU!

For more info, contact **info@protoqsar.com**



NANOBODY-BASED PANX1 CHANNEL INHIBITORS REDUCE INFLAMMATION IN ACUTE LIVER INJURY

7/10/2024

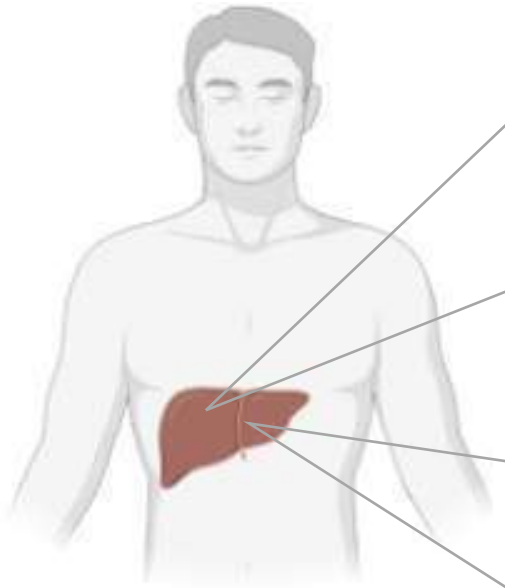
Daan Peeters

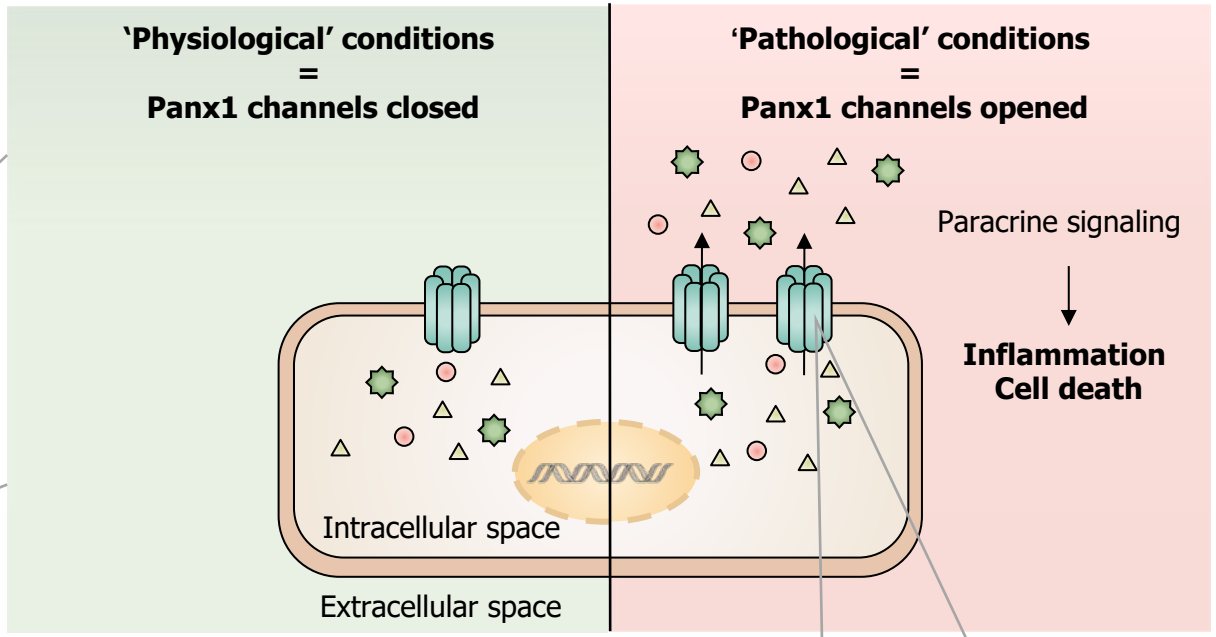
3rd PANACHE workshop

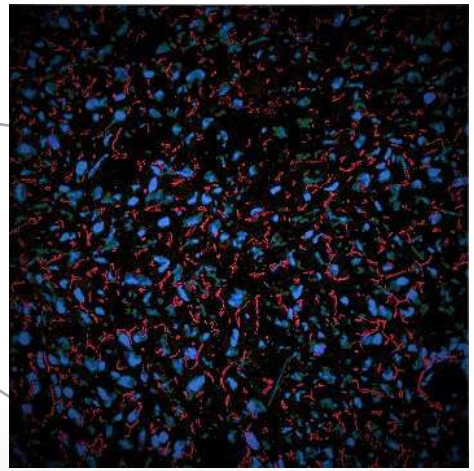
VRIJE
UNIVERSITEIT
BRUSSEL

INTRODUCTION

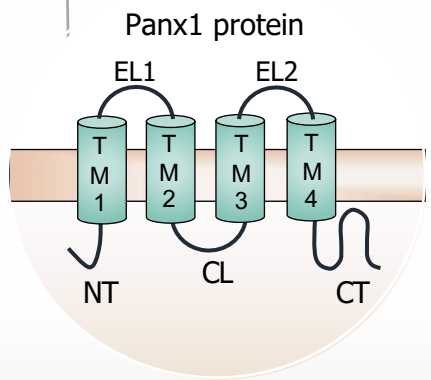
PROJECT RATIONALE







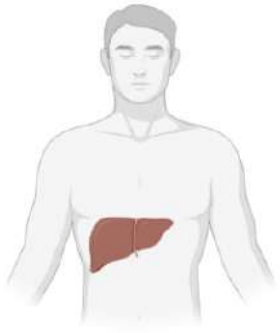
Panx1 protein
Nucleus



Maes M. *et al*. (2017) *Arch Toxicol*. 91:2245-2261 | 2

INTRODUCTION

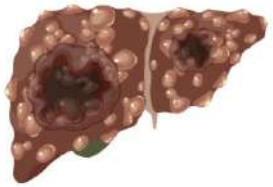
PROJECT RATIONALE



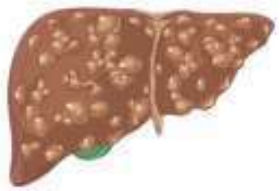
Inflammation + cell death

Liver disease

Acute liver injury

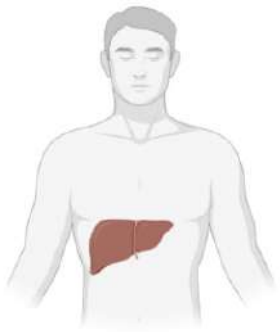


Chronic liver disease



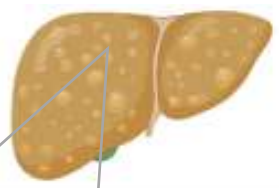
INTRODUCTION

PROJECT RATIONALE

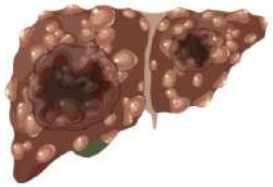


Inflammation + cell death

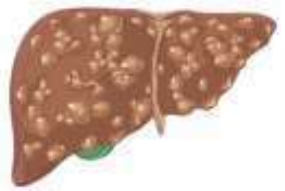
Liver disease

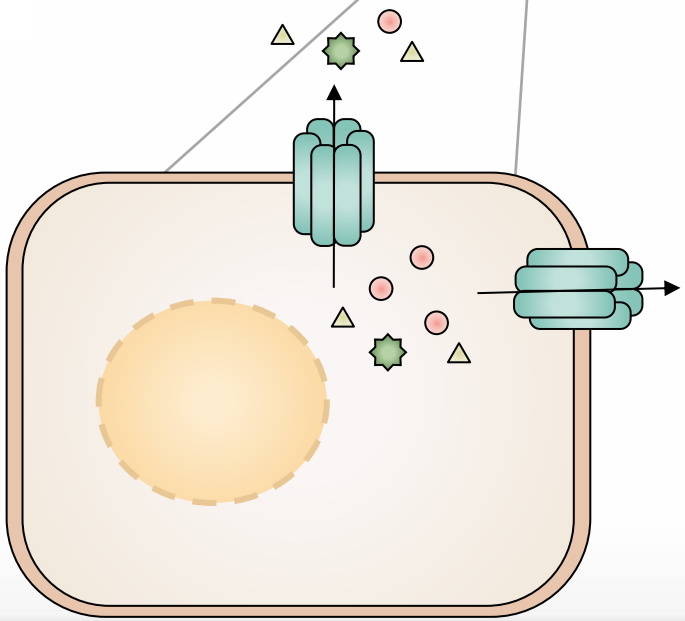


Acute liver injury



Chronic liver disease

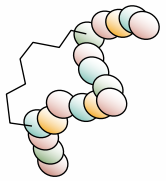




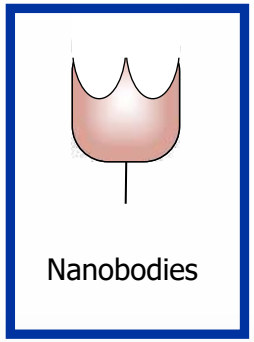
Panx1 channel inhibitors

Lack of specificity
Lack of potency
Lack of stability

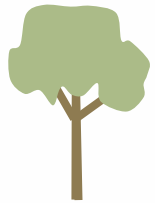
Novel Panx1 channel inhibitors



Peptidomimetics



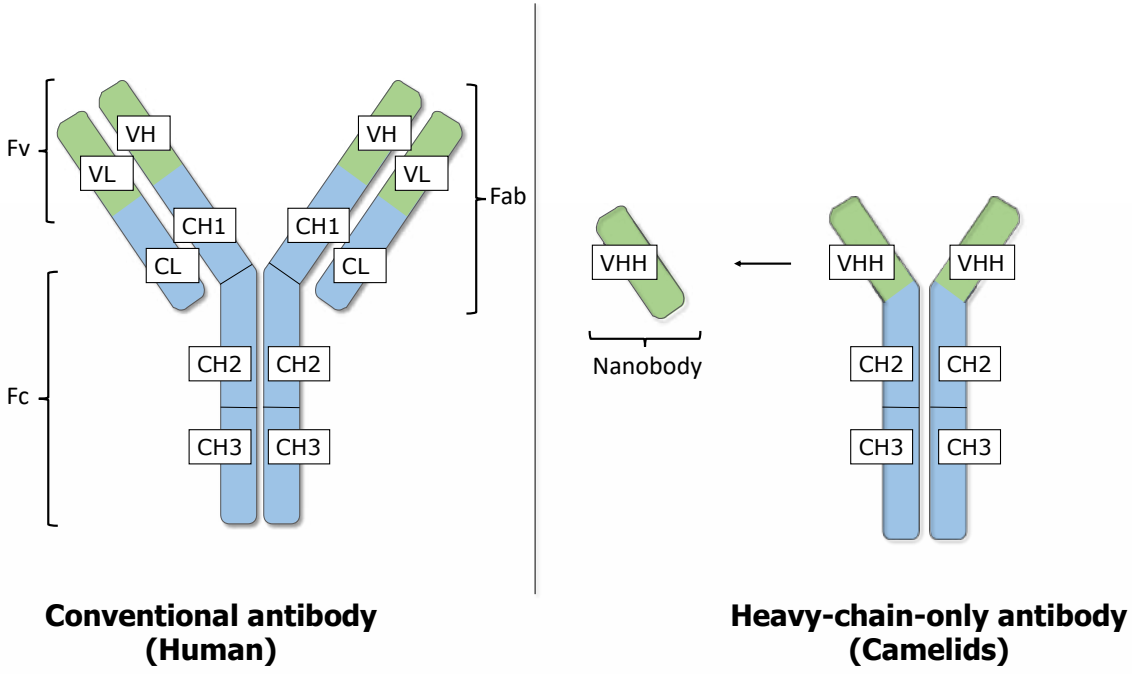
Nanobodies



Natural compounds

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

NANOBODY GENERATION AND *IN VITRO* CHARACTERIZATION

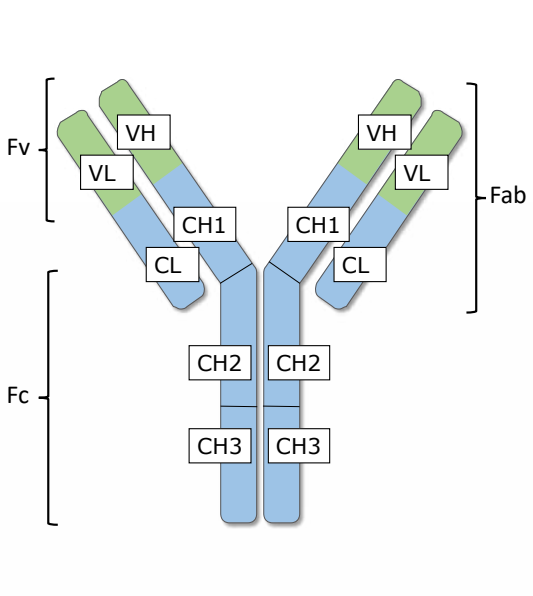


Conventional antibody (Human)

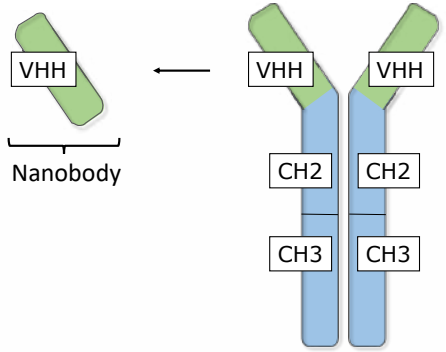
Heavy-chain-only antibody (Camelids)

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

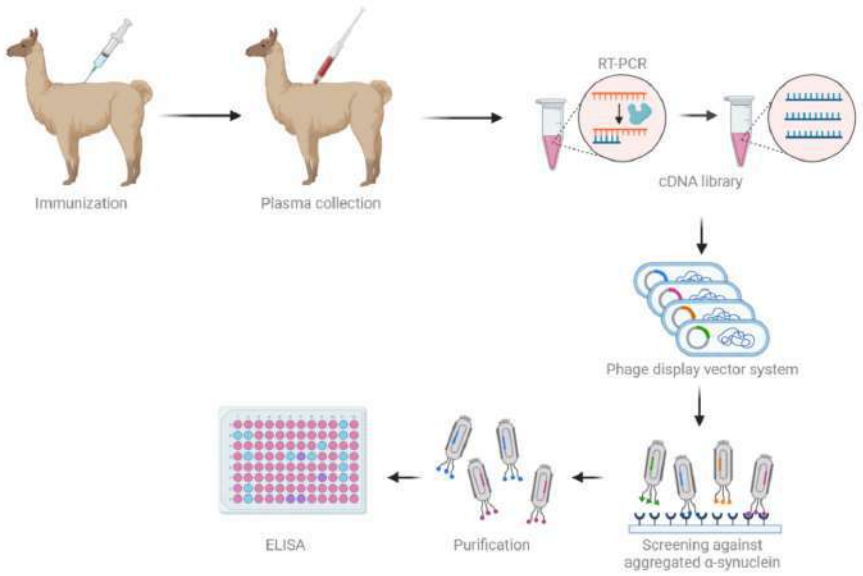
NANOBODY GENERATION AND *IN VITRO* CHARACTERIZATION



Conventional antibody (Human)



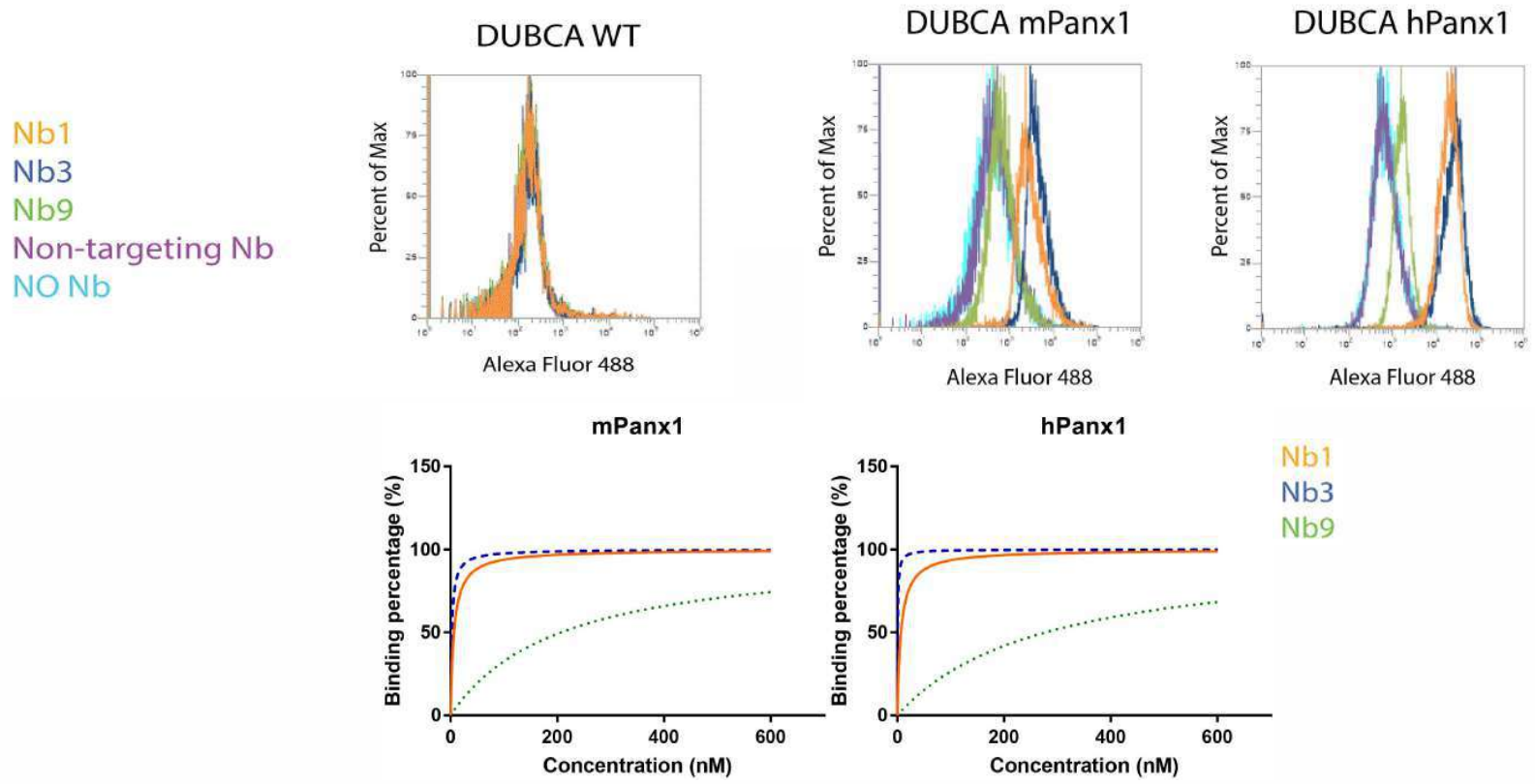
Heavy-chain-only antibody (Camelids)



Van Campenhout R. et al. (2021) Biomolecules. 11:63 | 6
Schiellerup, O. (2024) BioRender.

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

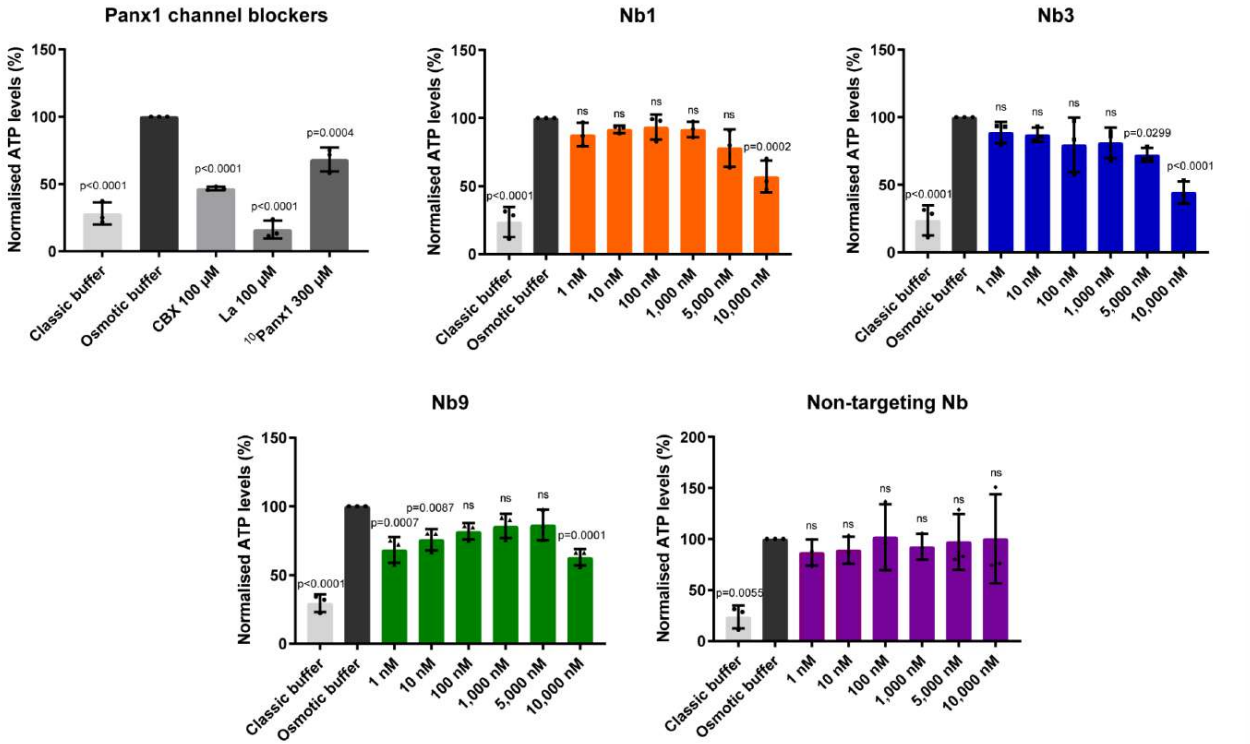
NANOBODY GENERATION AND *IN VITRO* CHARACTERIZATION



Panx1 nanobodies show cross-reactive binding to murine and human Panx1

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

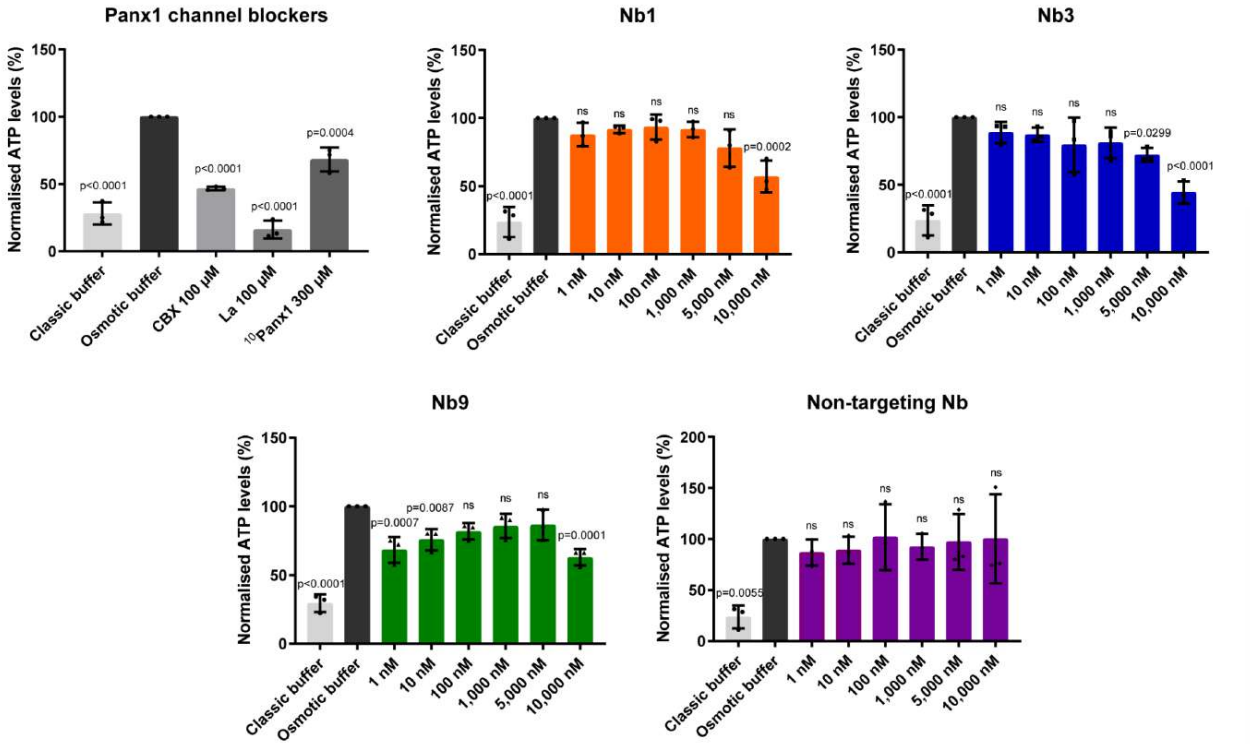
NANOBODY GENERATION AND *IN VITRO* CHARACTERIZATION



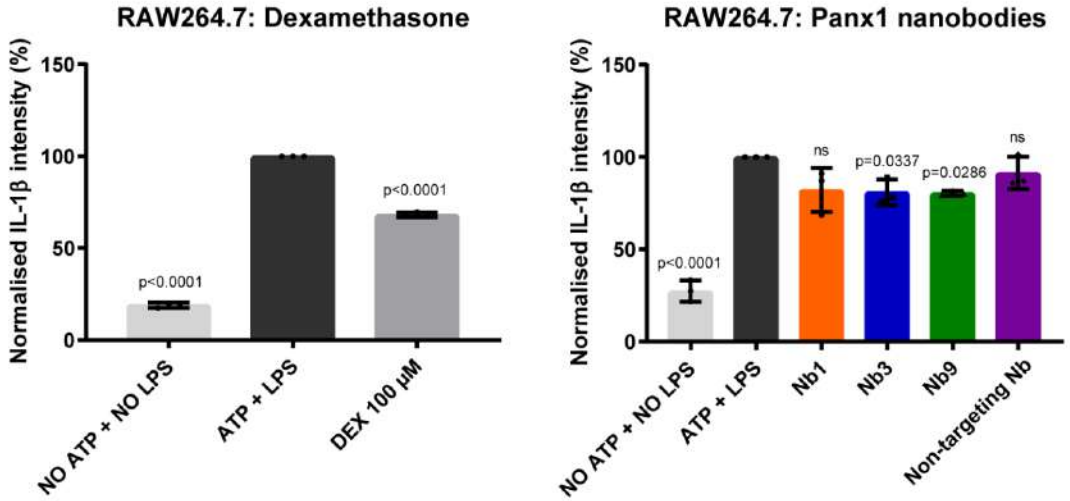
Panx1 nanobodies block Panx1 channel activity *in vitro*

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

NANOBODY GENERATION AND *IN VITRO* CHARACTERIZATION



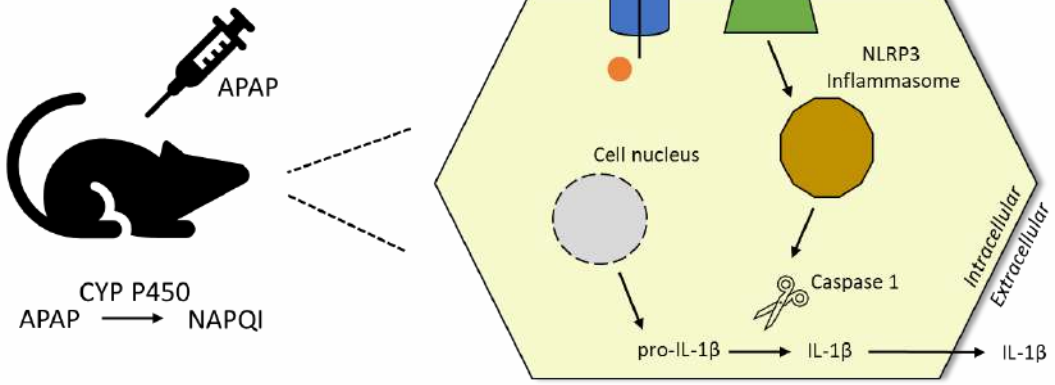
Panx1 nanobodies block Panx1 channel activity *in vitro*

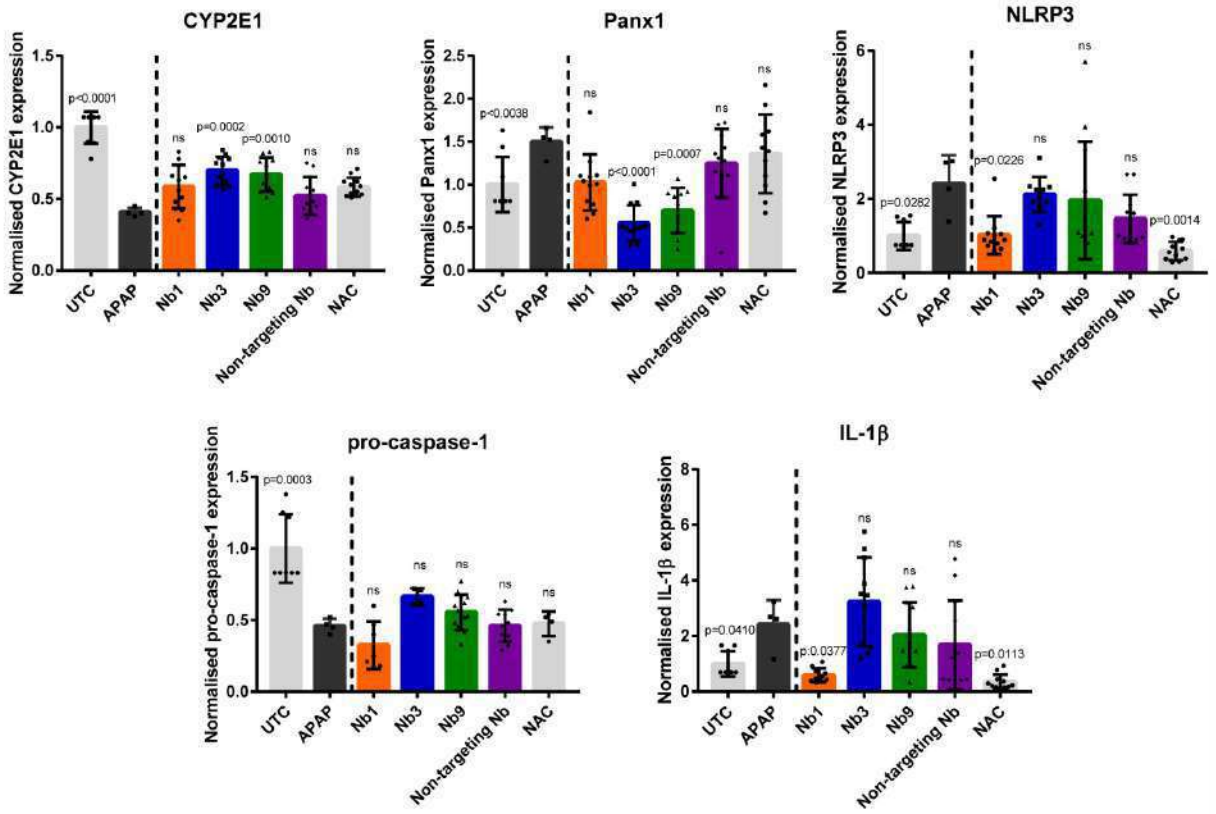


Panx1 nanobodies show anti-inflammatory effects *in vitro*

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

THE *IN VIVO* TESTING OF PANX1 NANOBODIES

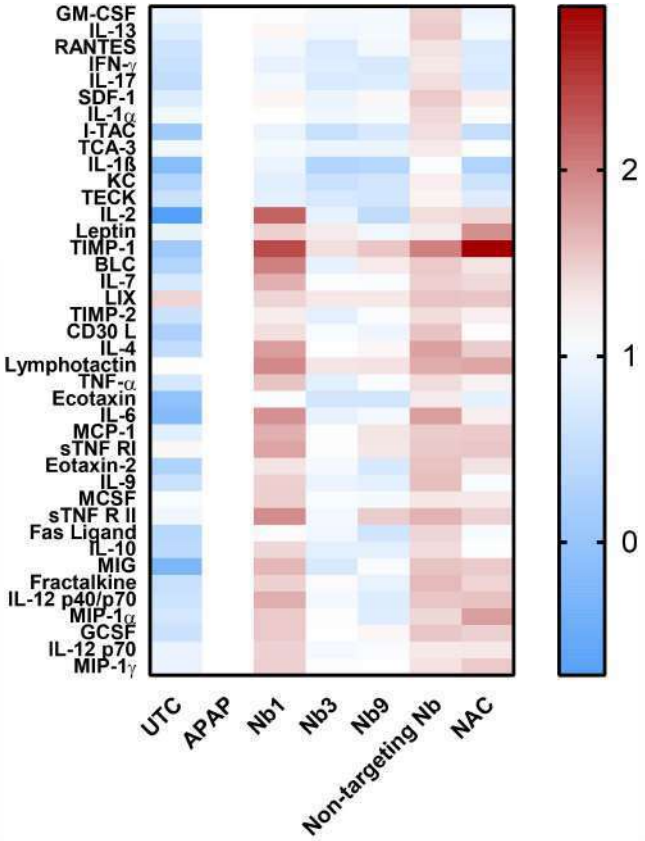


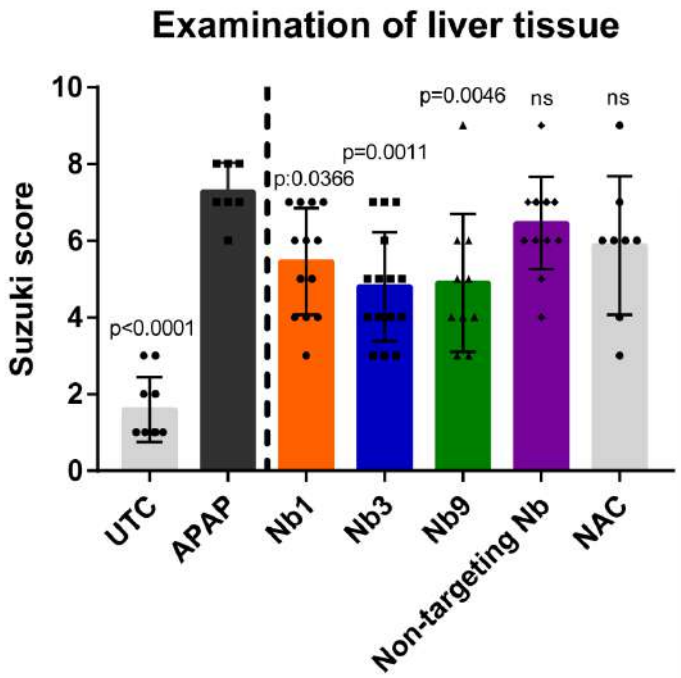


Panx1 nanobodies affect NLRP3 inflammasome components

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

THE *IN VIVO* TESTING OF PANX1 NANOBODIES





Panx1 nanobodies reduced expression levels of serum cytokines and liver damage following APAP overdosing

DEVELOPMENT AND CHARACTERIZATION OF NANOBODIES DIRECTED AGAINST PANX1

CONCLUSION AND FUTURE PERSPECTIVES

- As demonstrated for the case of acute liver injury, the Panx1 nanobodies hold great promise as anti-inflammatory agents
- Explore Panx1 nanobodies as treatment of other acute/chronic inflammatory (hepatic) diseases

UNIVERSITÉ DE GENÈVE

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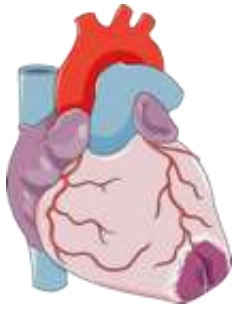
A new stable Panx1 peptidomimetic for the prevention of myocardial ischemia/reperfusion injury

Malaury Tournier

Dept of Pathology and Immmunology

October 7th 2024

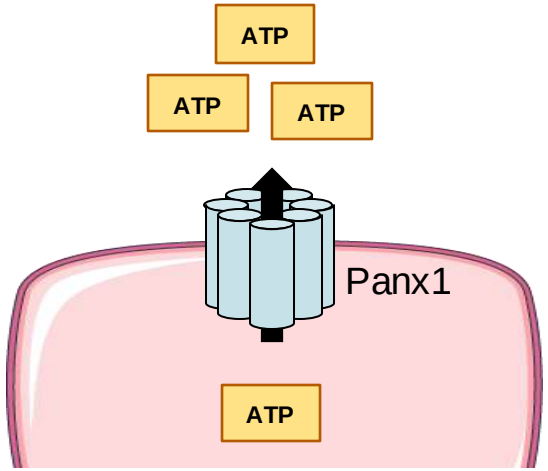
Inhibiting Panx1 channels in ischemic heart disease



Ischemia/Reperfusion
(I/R) injury

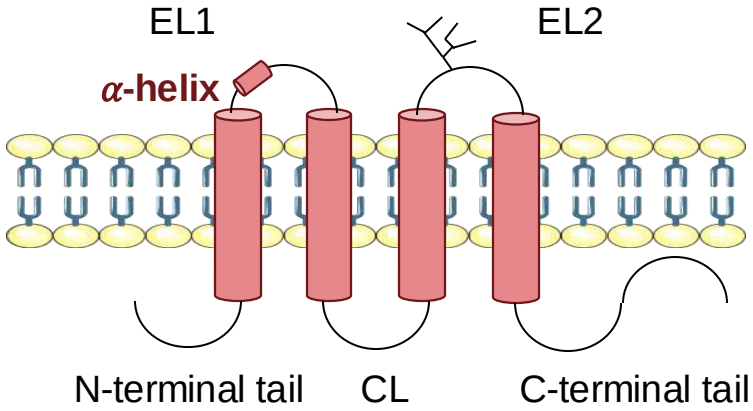
NLRP3 inflammasome activation

Leukocyte adhesion



- **^{10}Panx1: Panx1 mimetic peptide**

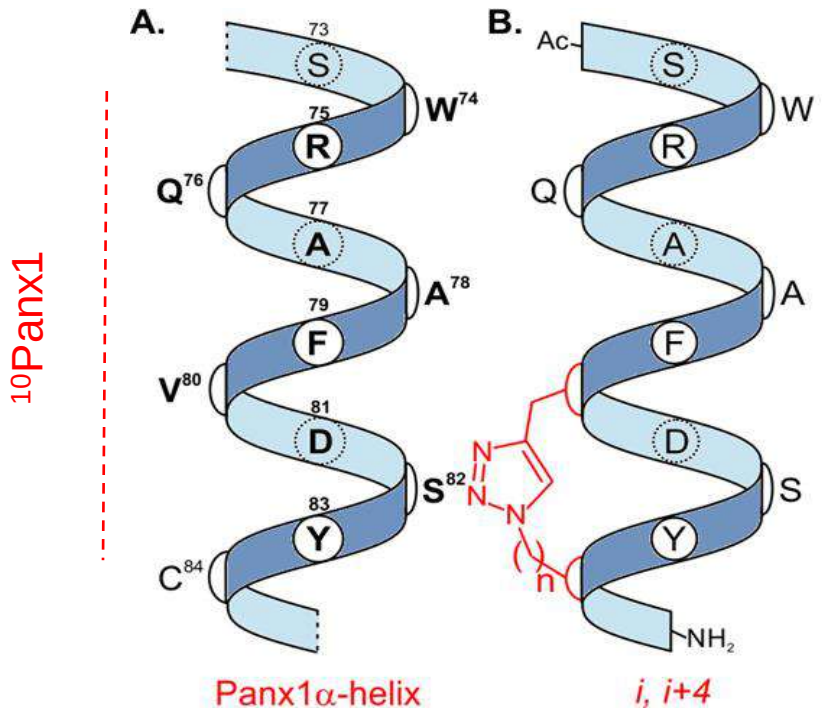
  Helical sequence: WRQAAFVDSYCWA
  ^{10}Panx1 sequence: WRQAAFVDSY



Low stability in plasma

Stapled ^{10}Panx1 analogs for the treatment of ischemic heart disease

- Solid-phase peptide synthesis



n = 3 or 4,
Stapled ^{10}Panx1 analogs

Lamouroux A, unpublished data

SBL-PX1-42

In vitro:

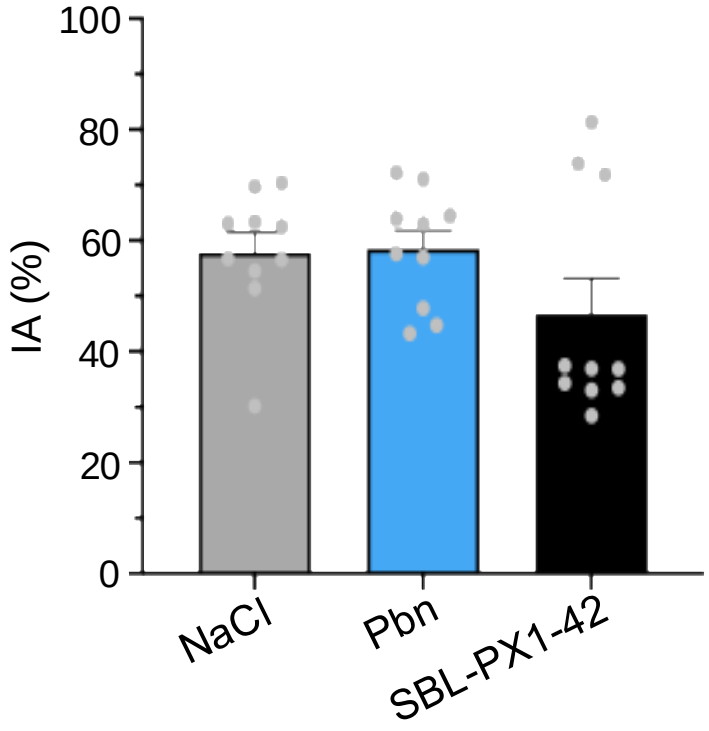
- ✓ Efficient Panx1 channel inhibition in endothelial cells (ECs)
- ✓ Specific (no inhibition in Panx1-deficient cardiomyocyte-like cells)
- ✓ Reduced monocyte adhesion to ECs
- ✓ Not cytotoxic
- ✓ **>30 fold more stable ($t_{1/2}$= 66.13 ± 0.52 min)**

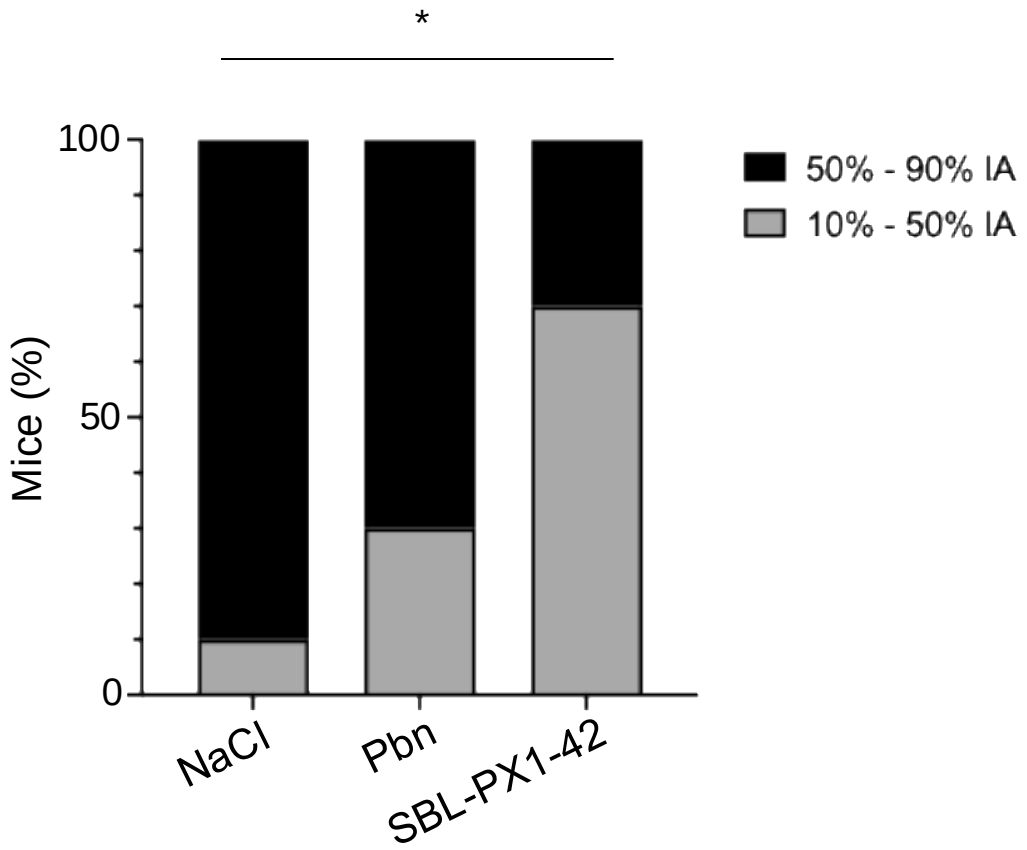
Lamouroux A, Tournier M *et al. J. Med. Chem.* 2023

Ex vivo:

- ✓ No effects on cardiac function = **no cardiotoxicity**

SBL-PX1-42 decreases infarct area 24h after myocardial I/R in WT mice

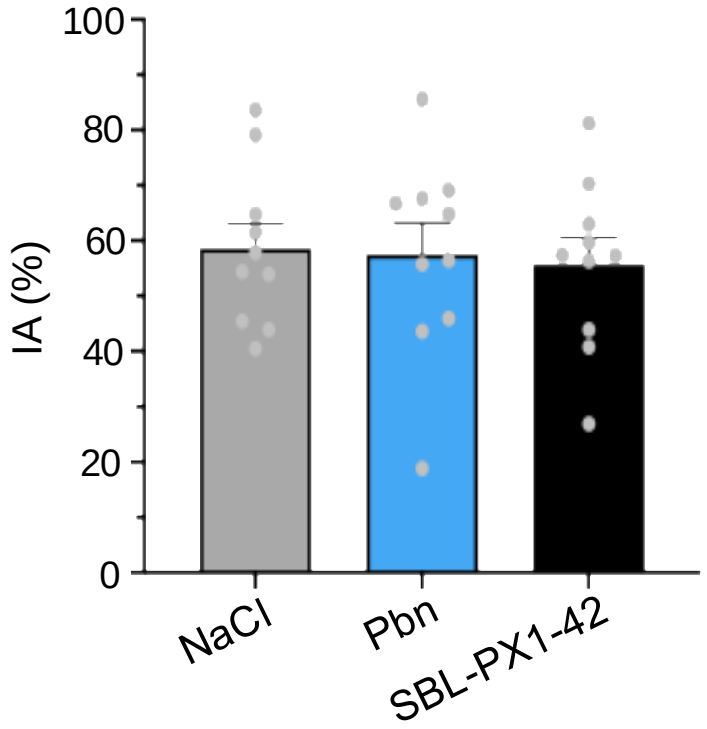




IA: infarct area

Pbn: 2.5mM
SBL-PX1-42: 90μM

SBL-PX1-42 does not affect infarct area 24h after myocardial I/R in *Panx1*$^{-/-}$ mice



IA: infarct area

Pbn: 2.5mM
SBL-PX1-42: 90μM

Conclusions

- **SBL-PX1-42 is a promising stable Panx1 peptidomimetic to protect against myocardial I/R injury.**

- SBL-PX1-42 displays **cardioprotective effects** 24h after myocardial I/R.
  (reduction of infarct size in 70% of the WT mice)

- The cardioprotective effects of SBL-PX1-42 are **dependent of Panx1 channels.**
  (absence of effects in *Panx1*$^{-/-}$ mice)

- The cardioprotective effects of SBL-PX1-42 could be linked to a decrease of leukocyte infiltration to the injured area.

Acknowledgements

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Silymarin and its major components as a potential naturally occurring Panx1 channel inhibitors

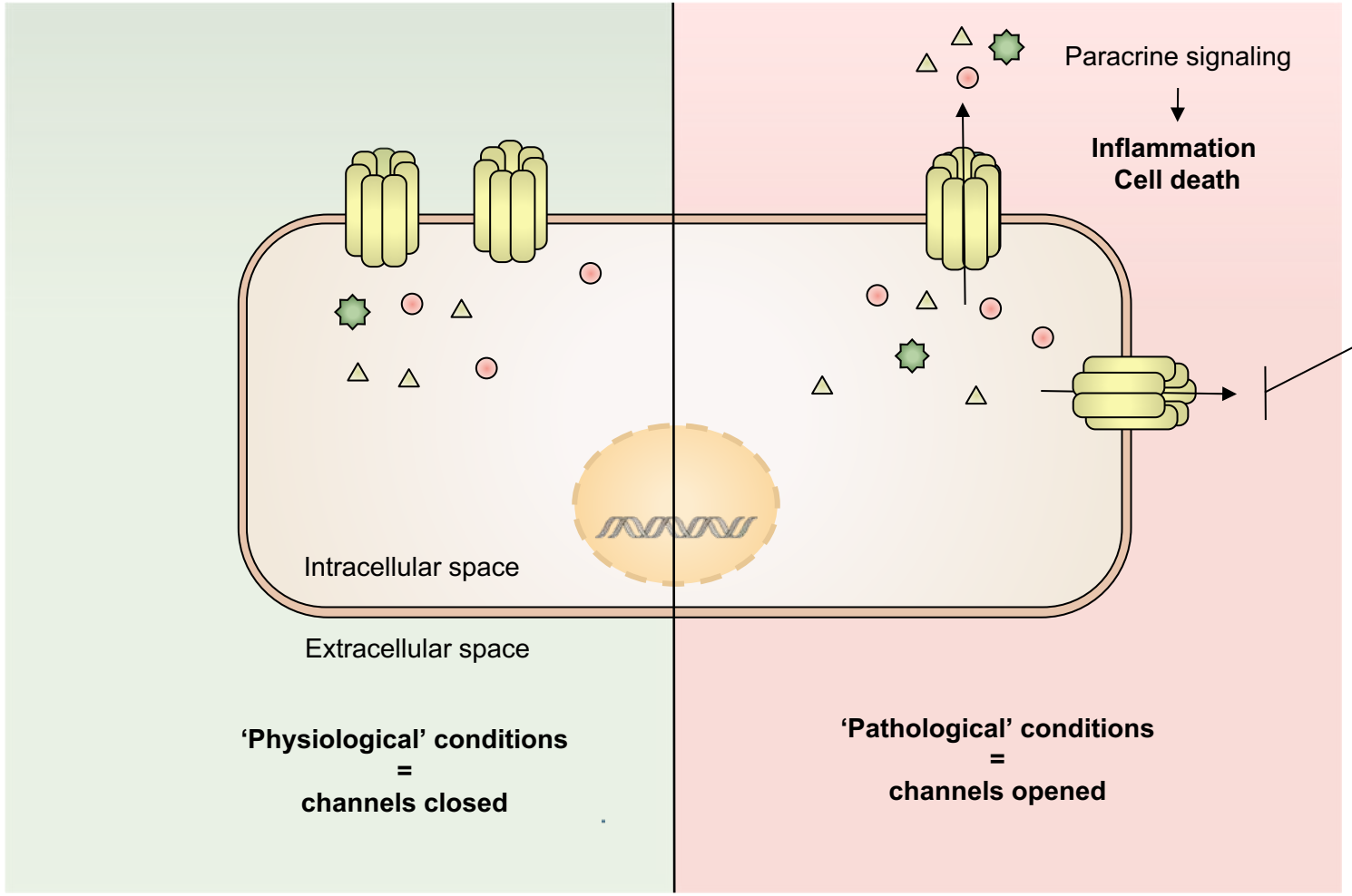
Batuhan YILDIZ, PhD candidate

3rd PANACHE Workshop
7th October 2024

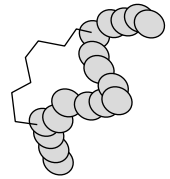
Funded by the Republic of Türkiye Ministry of National Education

YLSY

Introduction: Herbal ingredients as potential Panx1 channel inhibitors

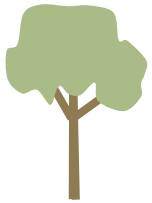


Novel Panx1 channel inhibitors



Peptidomimetics

Nanobodies



Herbal ingredients

- Structural diversity
- Intrinsic bioactivity
- Anti-inflammatory properties

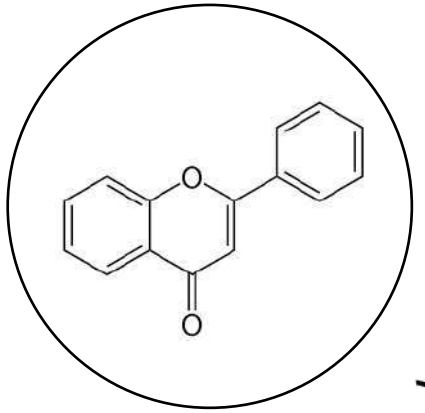
Panx1: Pannexin 1

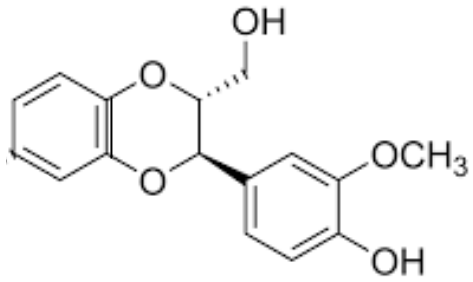
Introduction: The Silymarin complex and its major components

Silymarin complex

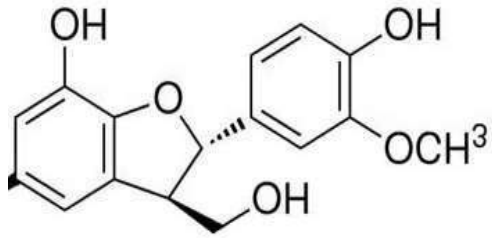
Anti-inflammatory properties
- NF-kβ
- Nrf2
- NRLP3 inflammasome
- …

Flavonoids

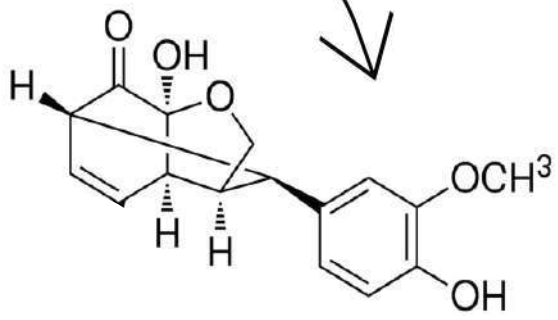




Silibinin (60% to 70%)



Silychristin (20%)

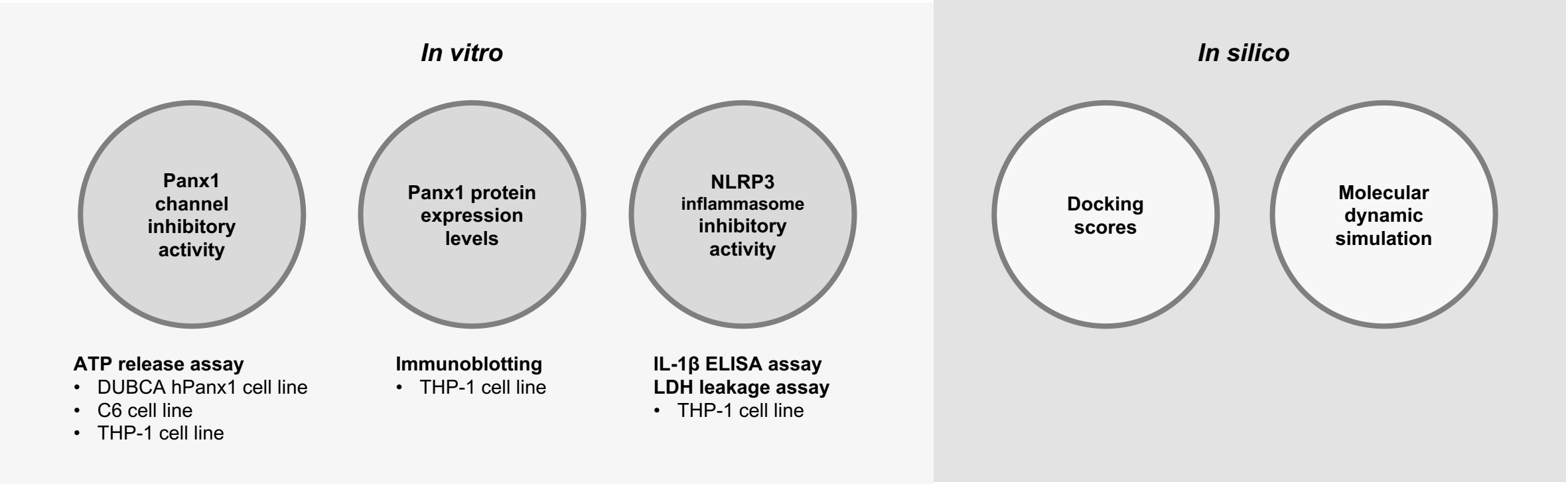


Silydianin (10%)

NF-kβ; Nuclear factor kappa, Nrf2: nuclear factor erythroid 2–related factor 2, NRLP3: Nod-like receptor pyrin domain-containing 3

Objective and experimental setup

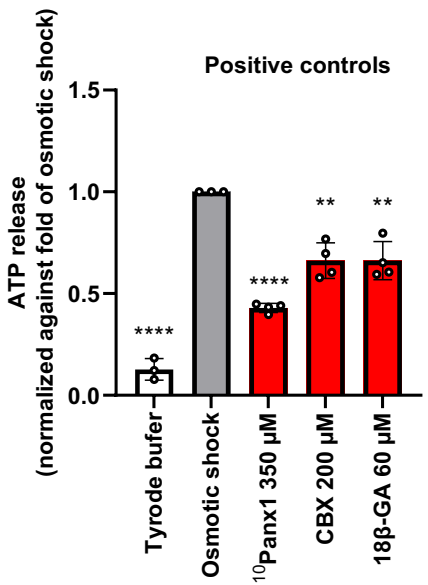
To *in vitro* and *in silico* evaluate the potential Panx1 channel inhibitory activity of the Silymarin complex and its major components (silibinin, silychristin and silydianin)

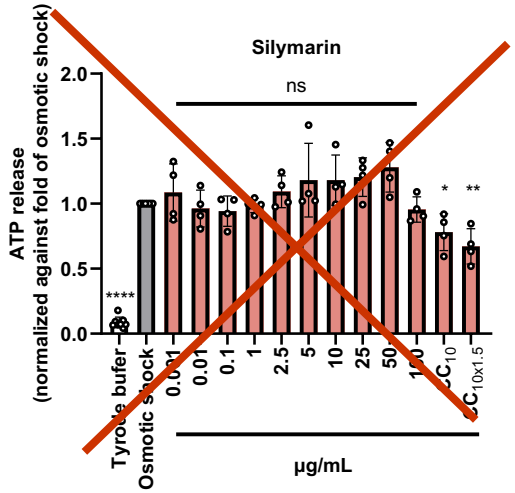


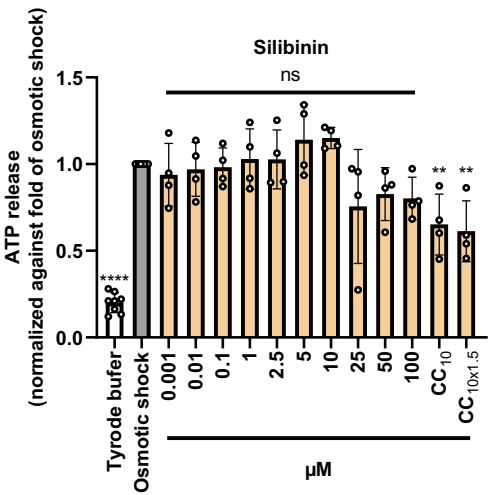
ATP: Adenosine triphosphate, ELISA: Enzyme linked immunosorbent assay, IL-1β: Interleukin- β, LDH: Lactate dehydrogenase, NRLP3: Nod-like receptor pyrin domain-containing 3, Panx1: Pannexin 1

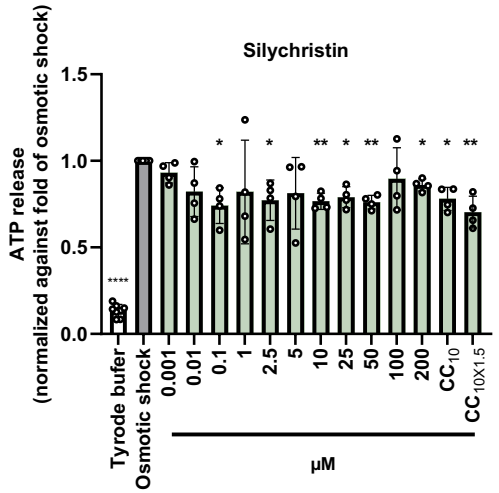
Panx1 channel activity: ATP release assay

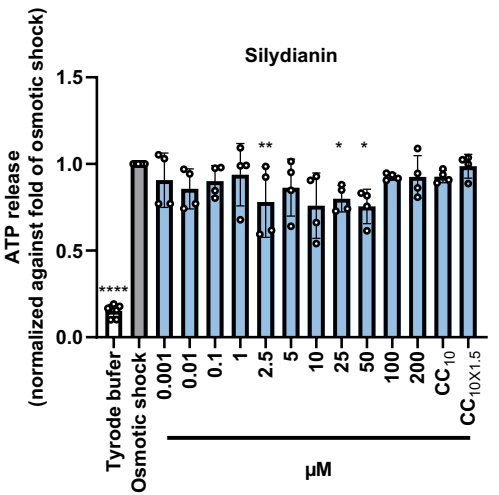
Dubca hPanx1 (high exogenous human Panx1 expression levels)









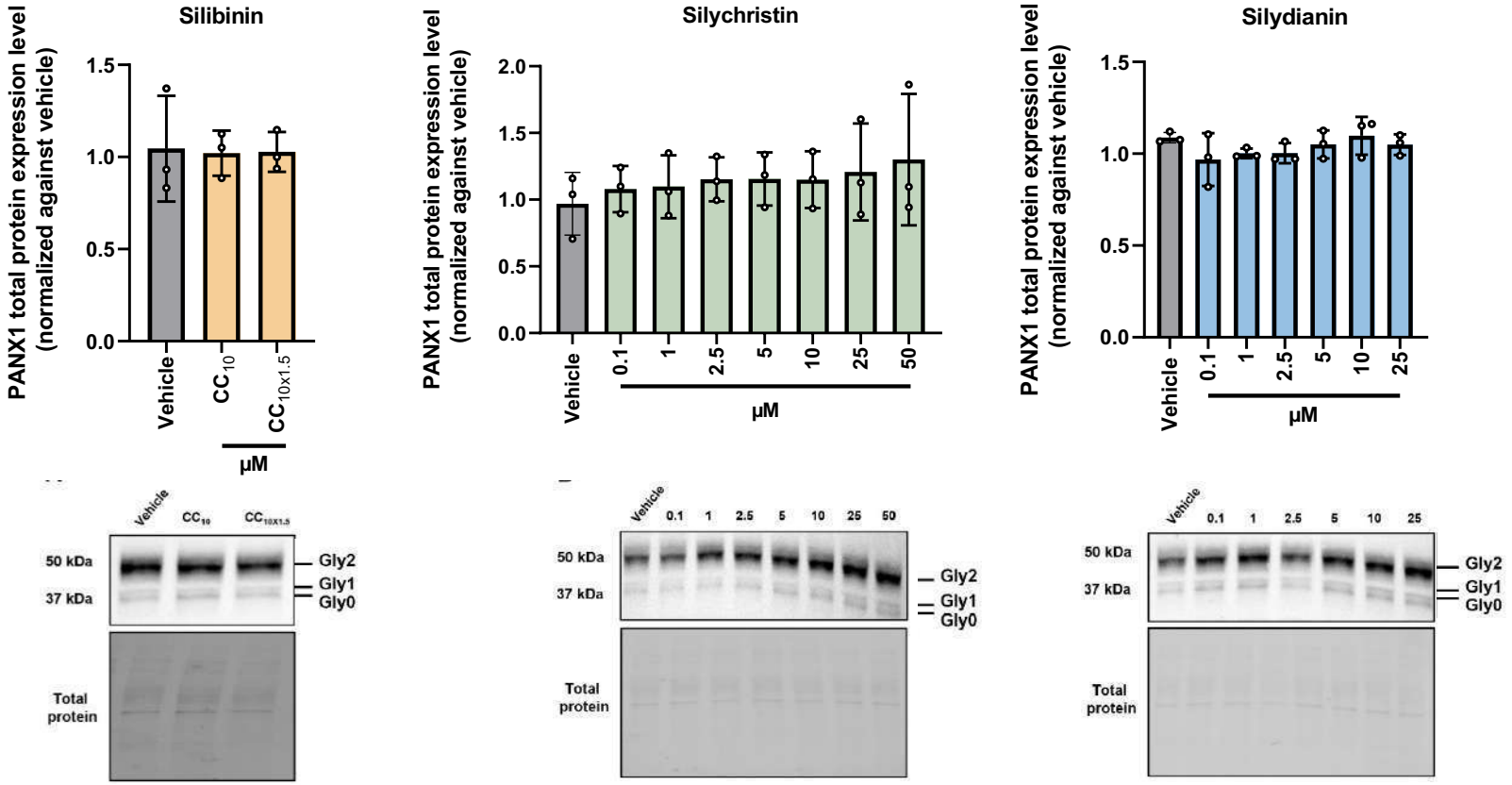


Inhibitory concentrations were validated and confirmed in 2 additional cells lines C6 and THP-1 monocyte-derived macrophages

Data are expressed as mean±SD (N=4, n=4)); **** p-value<0.0001 ; *** p-value<0.001; ** p-value<0.01; * p-value<0.05; ns: not significant 18β-GA: 18β-glycyrrhetinic acid, ATP: Adenosine triphosphate, CBX: Carbenoxolone, Panx1: Pannexin1
All conditions contain 1% DMSO

Panx1 protein expression levels: Immunoblotting

THP-1 monocytes-derived macrophages: 2 hours exposure to Panx1 channel inhibitory concentrations of silibinin, silychristin, and silydianin

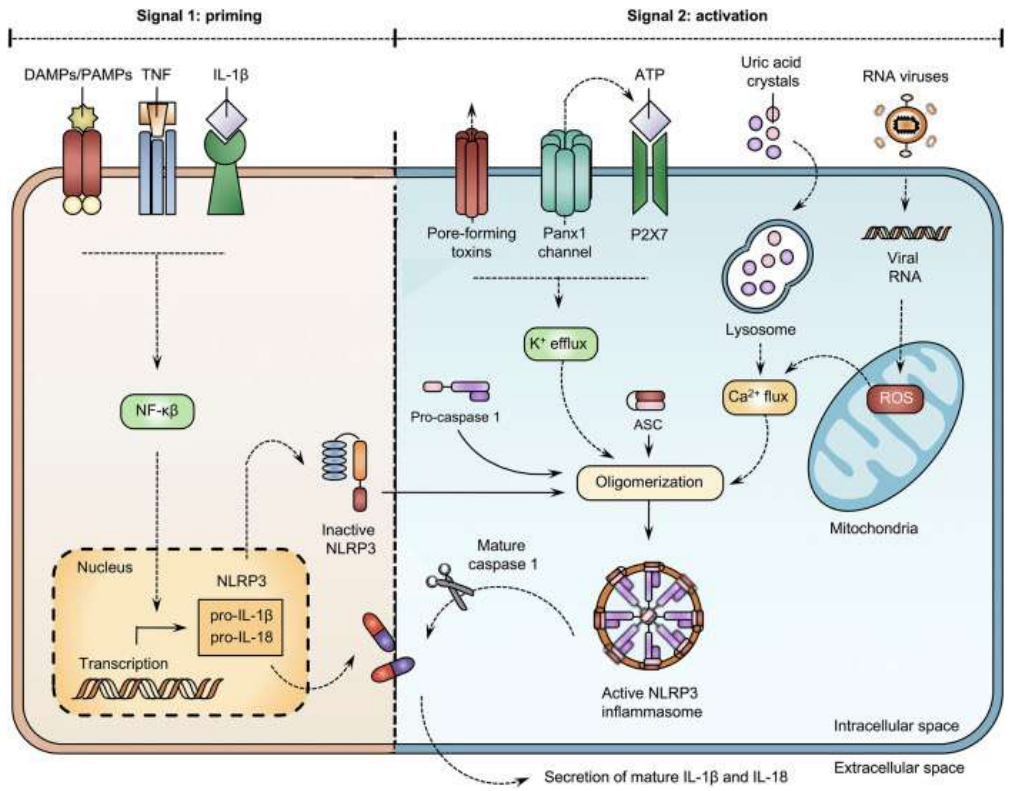


Data are expressed as mean±SD (N=3, n=1)
All conditions contain 0.1% DMSO

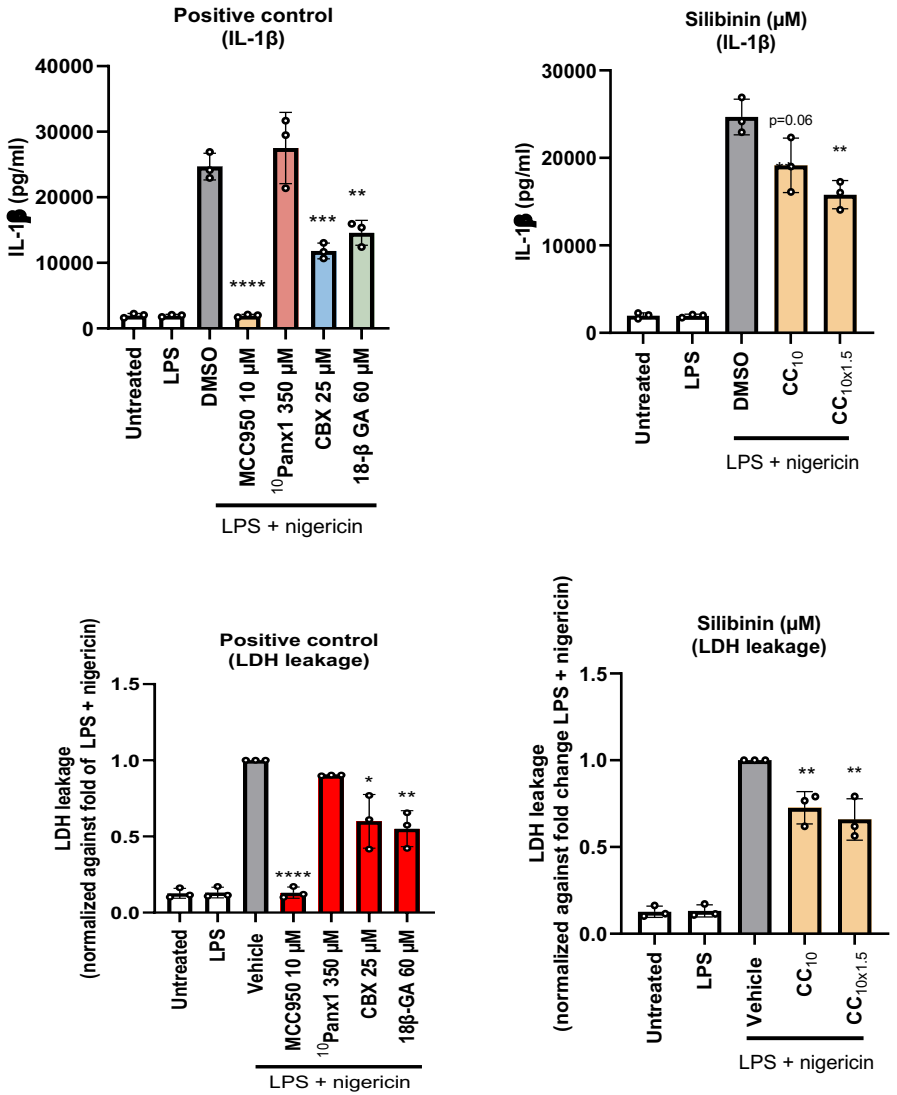
CC_{10}: 10% cytotoxic concentration, Gly: Glycolisation, kDA: Kilodalton, Panx1: Pannexin1

NLRP3 inflammasome inhibitory activity

THP-1 monocytes-derived macrophages:
LPS as priming signal and nigericin as activation signal



Van Campenhout, et al. Frontiers in cell and developmental biology (2023)



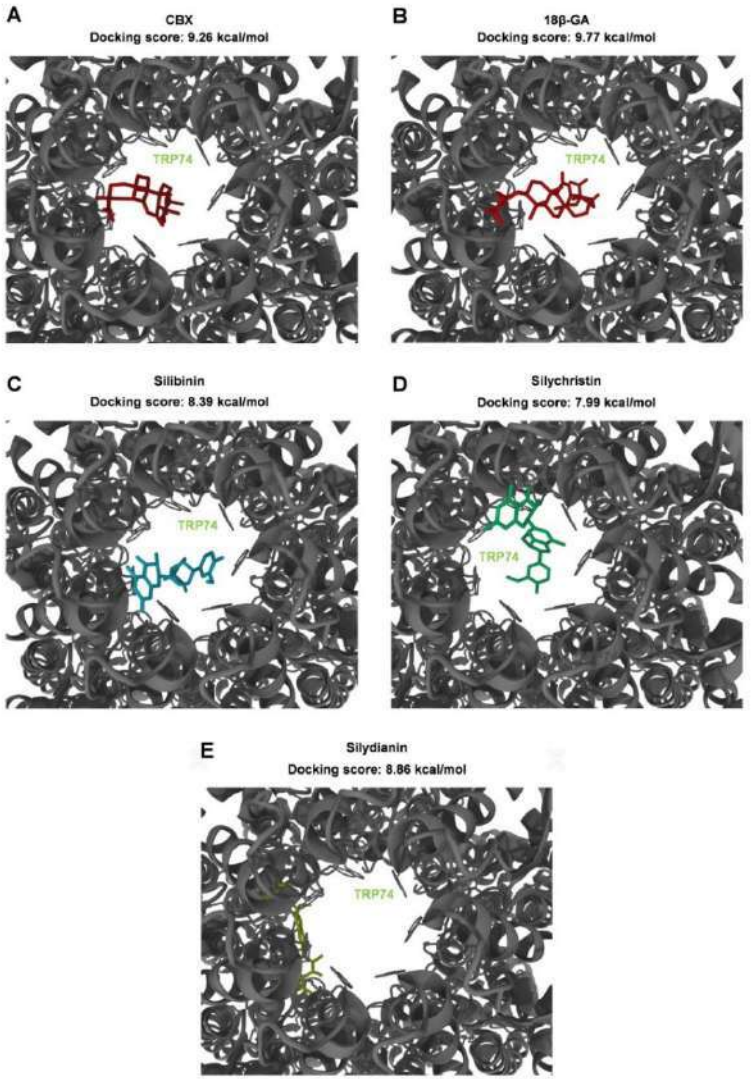
Data are expressed as mean±SD (N=3, n=4)); **** p-value<0.0001 ; *** p-value<0.001; ** p-value<0.01; * p-value<0.05; ns: not significant
All conditions contain 0.1% DMSO

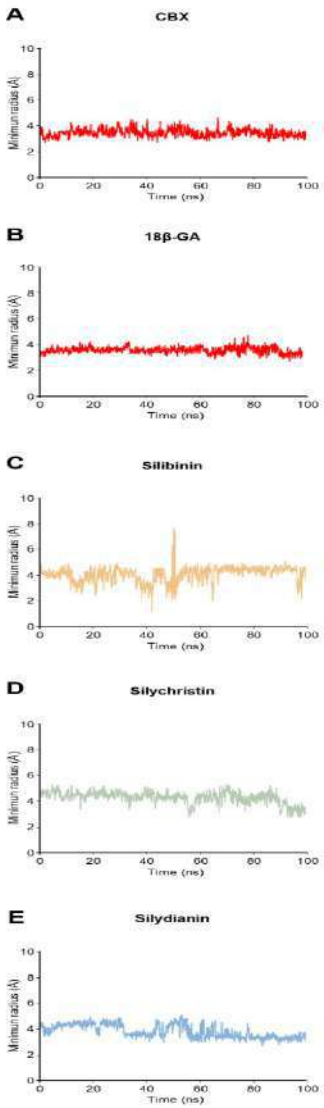
18β-GA: 18β-glycyrrhetinic acid, CBX: Carbenoxolone, CC_{10}: 10% cytotoxic concentration, DMSO: Dimethyl sulfoxide, IL-1β: Interleukin- β, LDH: Lactate dehydrogenase LPS: Lipopolysaccharides

In silico studies: Docking scores and molecular dynamic simulation

	Docking score (Kcal/mol)	Main contact residues
CBX	9.26	TRP 74
18β-GA	9.77	TRP 74
Silibinin	8.39	GLN 56 and ILE 60
Silychrisin	7.99	TRP 74
Silydianin	8.86	TRP 74

Higher docking score = higher binding affinity





18β-GA: 18β-glycyrrhetinic acid, CBX: Carbenoxolone, GLN 56: Glycine 56, ILE 60: Isoleucine 60, TRP 74: Tryptophan 74

Conclusions

The current *in vitro* and *in silico* studies provide substantial evidence for the Panx1 channel inhibitory properties of silibinin, silychristin, and silydianin and suggest a novel alternative anti-inflammatory mechanism of these compounds based on the inhibition of Panx1 channel activity.

Silymarin and its major components as a potential naturally occurring Panx1 channel inhibitors

Batuhan YILDIZ, PhD candidate

3rd PANACHE Workshop
7th October 2024

Funded by the Republic of Türkiye Ministry of National Education

YLSY

ProtoQSAR

PANACHE

3rd PANACHE workshop

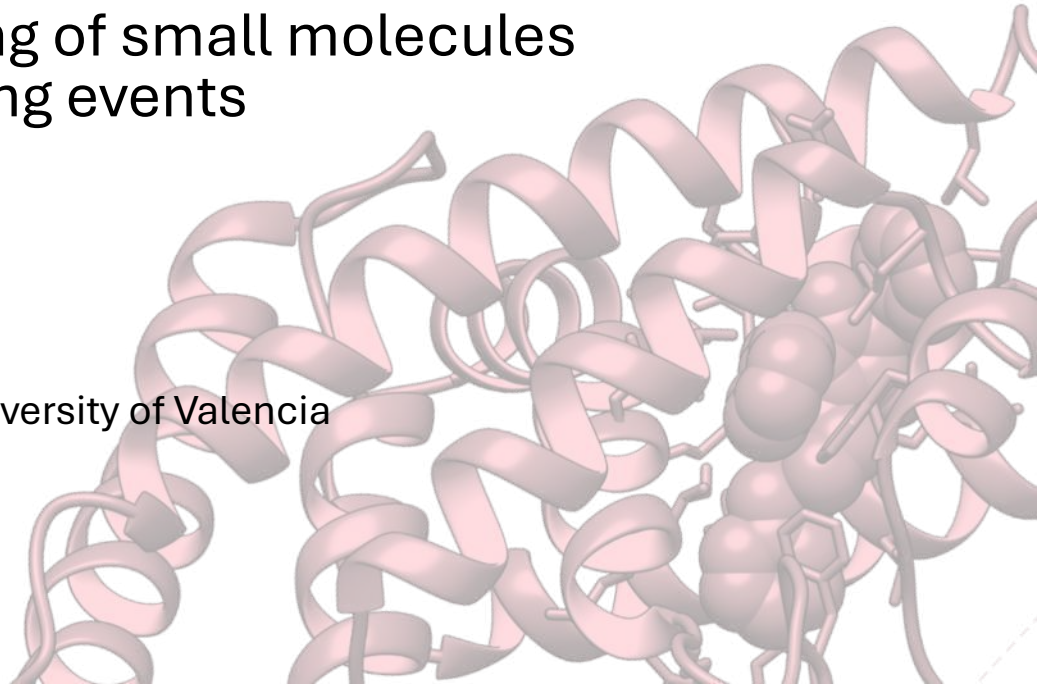
DOCKTOX

Molecular docking for *in silico* screening of small molecules targeting molecular initiating events

Rita Ortega Vallbona

PhD Student – ProtoQSAR

Chemistry PhD Programme - Polytechnic University of Valencia



ONTOX

This work was performed in the context of the ONTOX project (https://ontox-project.eu/) that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 963845. ONTOX is part of the ASPIS project cluster (https://aspis-cluster.eu/).

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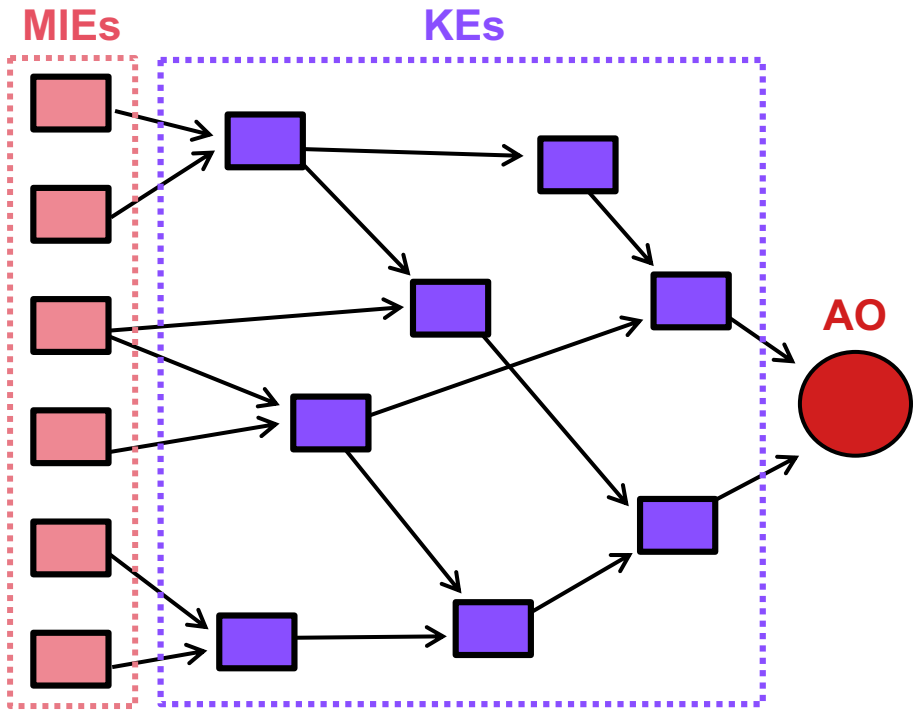
Fons Europeu de Desenvolupament Regional

Una manera de fer Europa

Adverse Outcome Pathway (AOP)



- Molecular Initiating Events (MIEs)
- Key Events (KEs)
- Adverse Outcome (AO)

Manuscript in preparation: Ortega-Vallbona, R., *et al*. "*DockTox: Targeting Molecular Initiating Events in Organ Toxicity through Molecular Docking*."

Protein preparation workflow

1. Align structures
- Superpose protein structures
- Use protein chains as reference

2. Prepare aligned structures
- Separate protein and ligand
- Docking box of individual structure
- Save information of reference structures

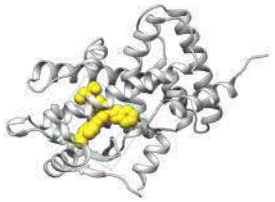
3. Add Hs to protein
- Select protein structure with the best resolution
- Clean structure and add Hs
- Energy minimization

4. Calculate interactions
- Add Hs to ligands
- Merge each ligand with selected protein structure
- Calculate interactions profile

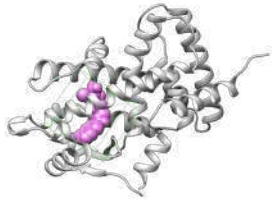
5. Create consensus set
- Consensus docking grid
- Consensus list of interactions
- Prepared reference protein structure

Individual reference structures

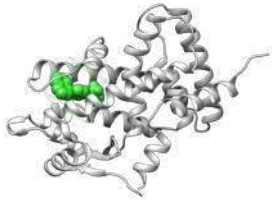
PPARα: 6KB4



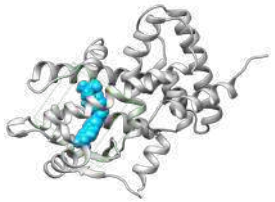
PPARα2: 3VI8



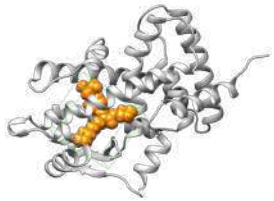
PPARα3: 5HYK



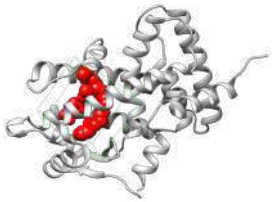
PPARα4: 2P54

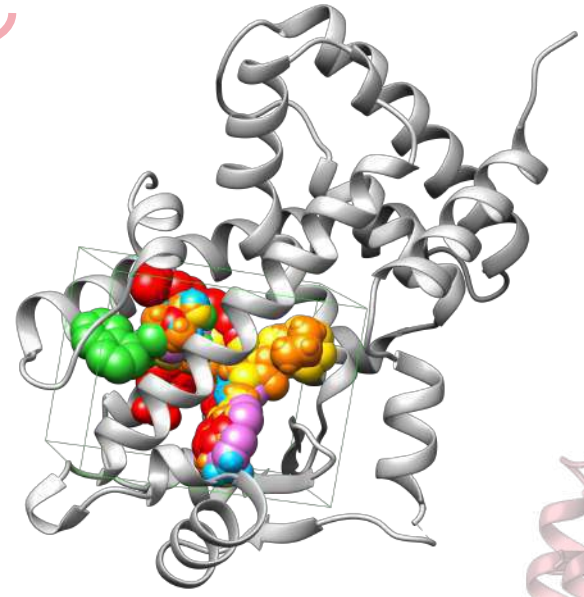


PPARα5: 6KAY



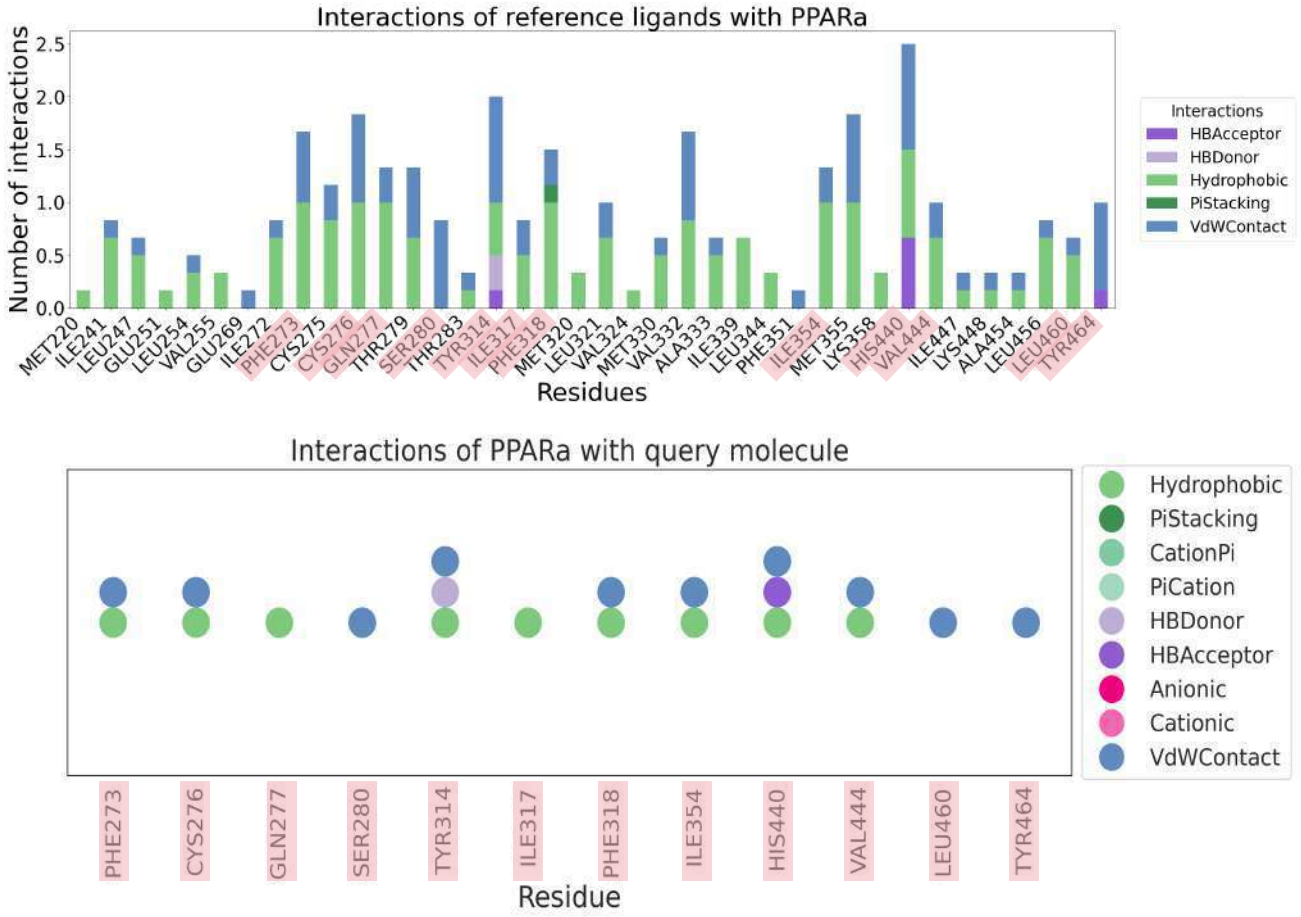
PPARα6: 1KKQ





Manuscript in preparation: Ortega-Vallbona, R., *et al*. "*DockTox: Targeting Molecular Initiating Events in Organ Toxicity through Molecular Docking*."

Interaction fraction



$$Interaction\ Fraction = \frac{I_q \cap I_r}{I_r}$$

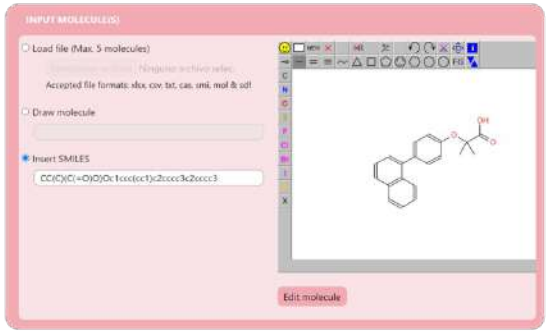
- I_q = interactions of query molecule
- I_r = interactions in reference list
- $I_q \cap I_r$ = number of interactions of query molecule that coincide with the reference list

Manuscript in preparation: Ortega-Vallbona, R., *et al*. "*DockTox: Targeting Molecular Initiating Events in Organ Toxicity through Molecular Docking*."

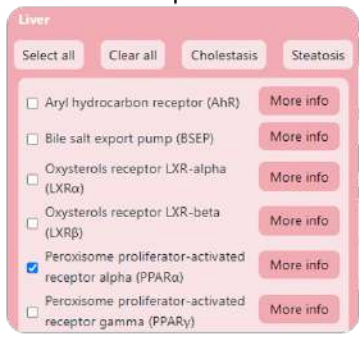
DockTox workflow

Input

Query small molecule



Select protein



Docking

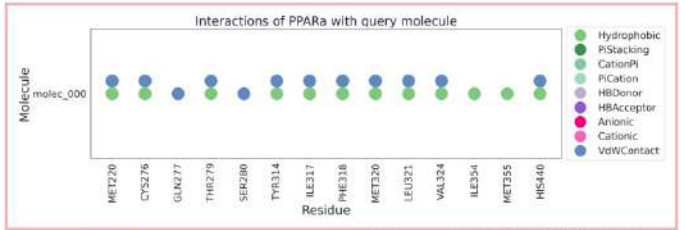
- Conformer generation
- Docking on prepared protein structure complexes
- Calculation of interactions

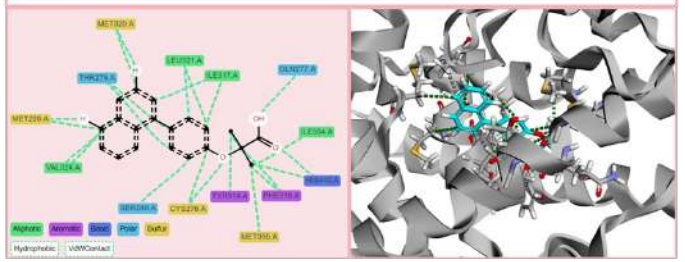
Output

- Binding energy
- Protein interactions
- Comparison to reference interactions
- Interaction fraction

DOCKTOX

Visualization of interactions





ID	SMILES	Binding affinity	Hydrophobic (of 34)	HBDonor (of 1)	HBAcceptor (of 3)	PiStacking (of 1)	Anionic (of 0)	Cationic (of 0)	CationPi (of 0)	PiCation (of 0)	VdWContact (of 30)	Total (of 69)	interaction fraction	Reference PDB	Resolution PDB	Conformer embedding method	Conformer optimization method
molec_000	CC(C)(C(=O)O)Oc1ccc(cc1)c2cccc3c2cccc3	-9.3	12	0	0	0	0	0	0	0	9	21	0.3	6KB4	1.42	ETKDGv3	MMFF94

Manuscript in preparation: Ortega-Vallbona, R., *et al*. "*DockTox: Targeting Molecular Initiating Events in Organ Toxicity through Molecular Docking*."

Proteins in DockTox

Liver	
AhR	Aryl hydrocarbon receptor
PXR	Pregnane X receptor
LXRa	Liver X receptor α
LXRb	Liver X receptor β
PPARa	Peroxisome proliferator activated receptor α
PPARg	Peroxisome proliferator activated receptor γ
BSEP	Bile salt export pump
OATP1B1	Organic anion transporting polypeptide 1B1
PgP	P-glycoprotein

Brain	
ACHE	Acetylcholinesterase
TTR	Transthyretin
THRa	Thyroid receptor α
THRb	Thyroid receptor β
HDAC2	Histone Deacetylases 2
HDAC4	Histone Deacetylases 4
HDAC6	Histone Deacetylases 6
HDAC7	Histone Deacetylases 7
HDAC8	Histone Deacetylases 8
BMP	Bone morphogenetic protein
TXNRD1	Thioredoxin reductase 1

Kidney	
COX1	Cyclooxigenase 1
POLG1	Mitochondrial DNA polymerase γ
ACE	Angiotensin converting enzyme

Try it here!

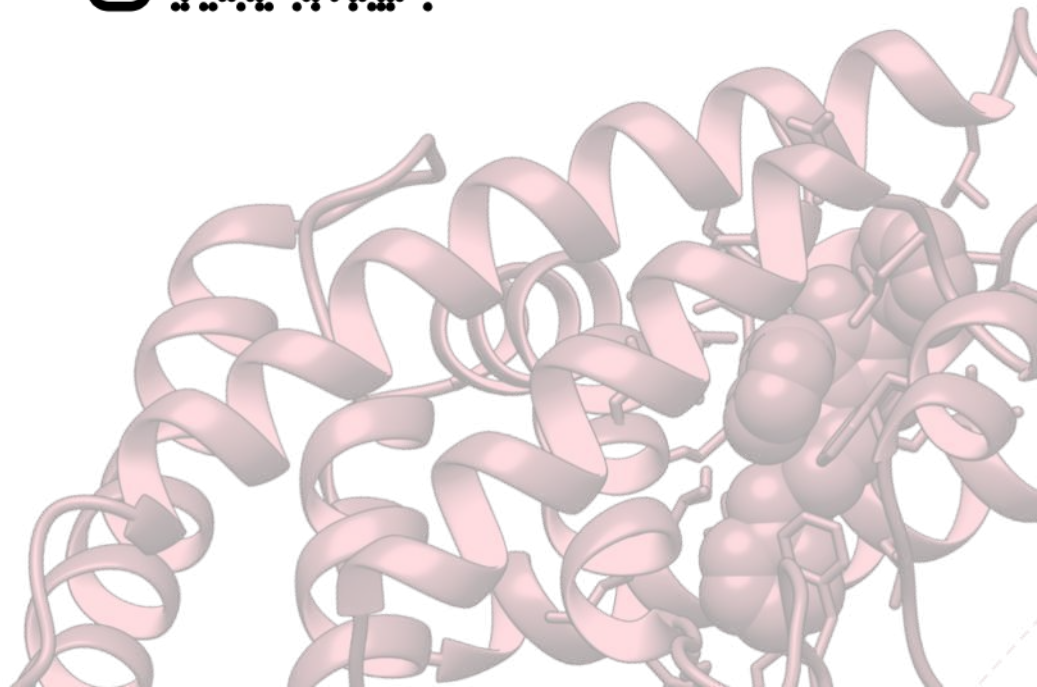
Manuscript in preparation: Ortega-Vallbona, R., *et al*. "*DockTox: Targeting Molecular Initiating Events in Organ Toxicity through Molecular Docking*."

ProtoQSAR

DOCKTOX

Thank you!

Try it here!



This work was performed in the context of the ONTOX project (https://ontox-project.eu/) that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 963845. ONTOX is part of the ASPIS project cluster (https://aspis-cluster.eu/).

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Cold exposure rejuvenates the metabolic phenotype of *Panx1*$^{-/-}$ mice

Filippo Molica, PhD

PANACHE workshop, October 7th, 2024

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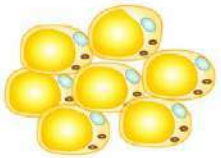
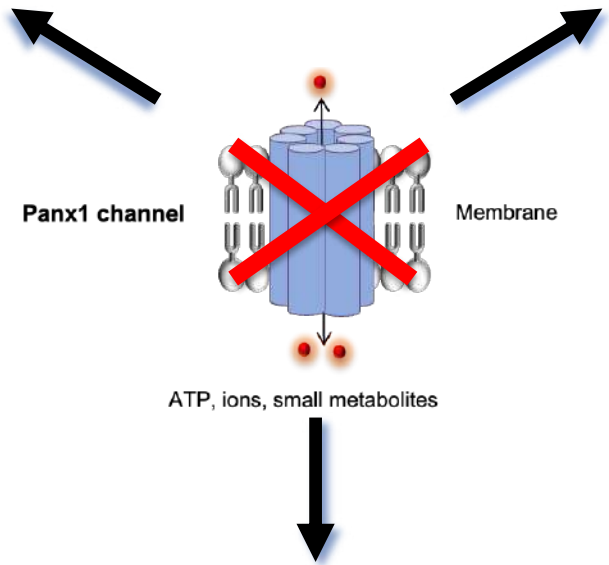
Panx1 and metabolic regulation

ASC differentiation and fat accumulation



(Adamson SE *et al. Mol. Metab.* 2015)
(Lee VR *et al. Sci. Rep.* 2018)



Increased fat mass

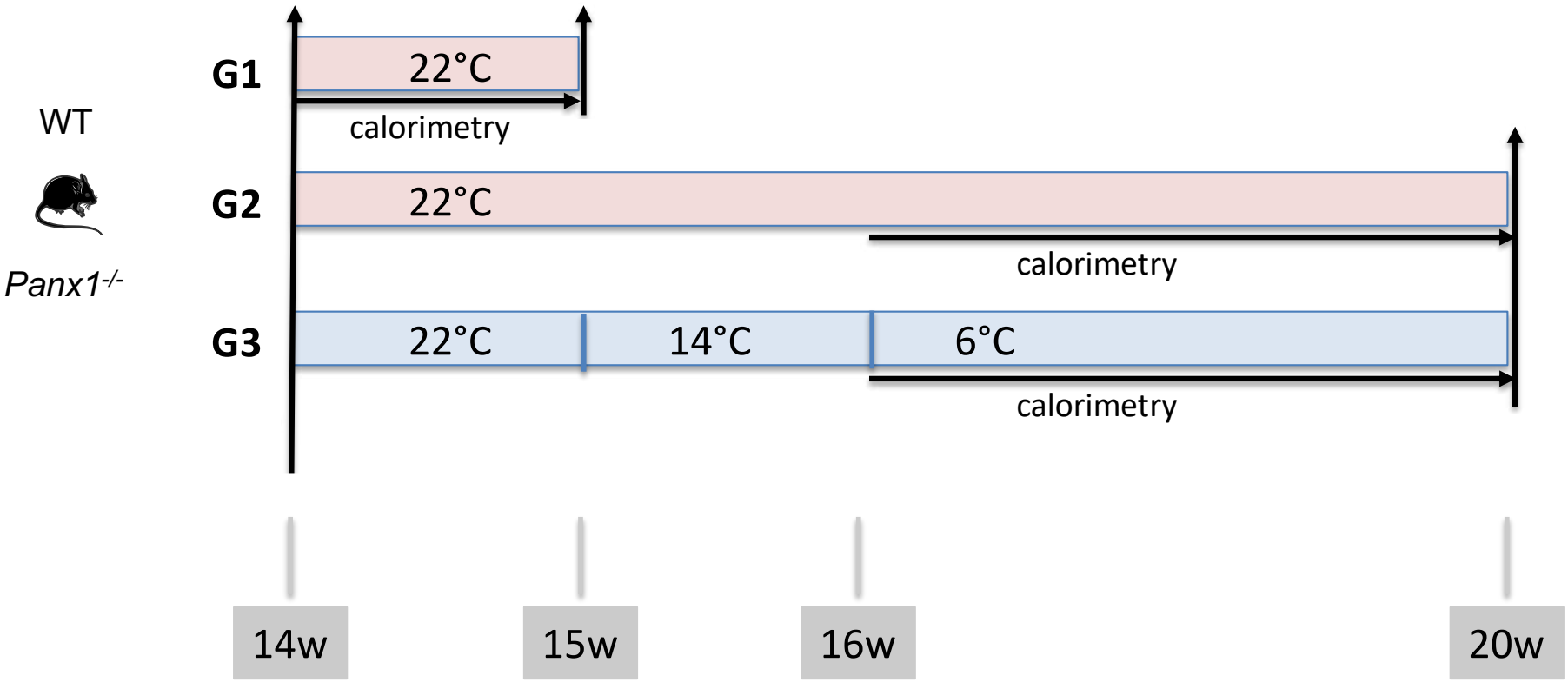
(Lee VR *et al. Sci. Rep.* 2018)

β3-dependent thermogenic response

(Senthivinayagam S *et al. Mol. Metab.* 2021)

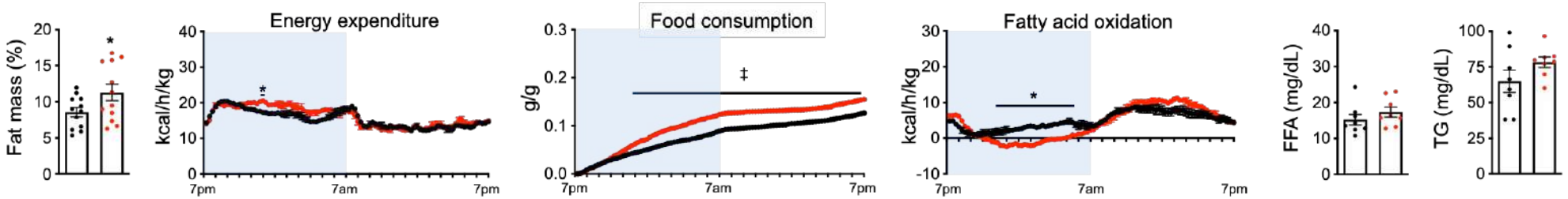
Is Panx1 involved in the beneficial metabolic effects of cold exposure?

Methods:

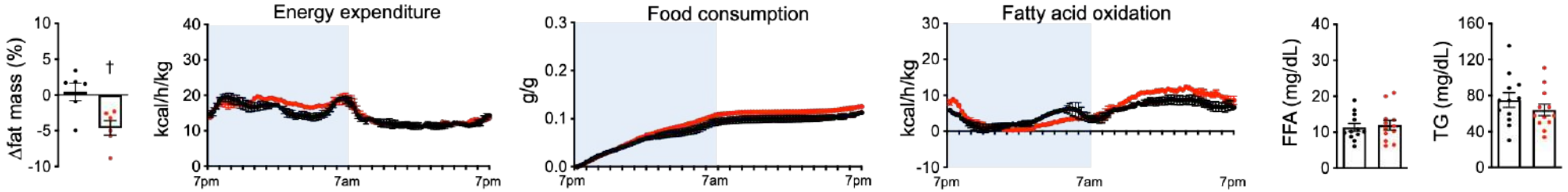


Cold exposure preserves the metabolic phenotype of *Panx1-/-* mice

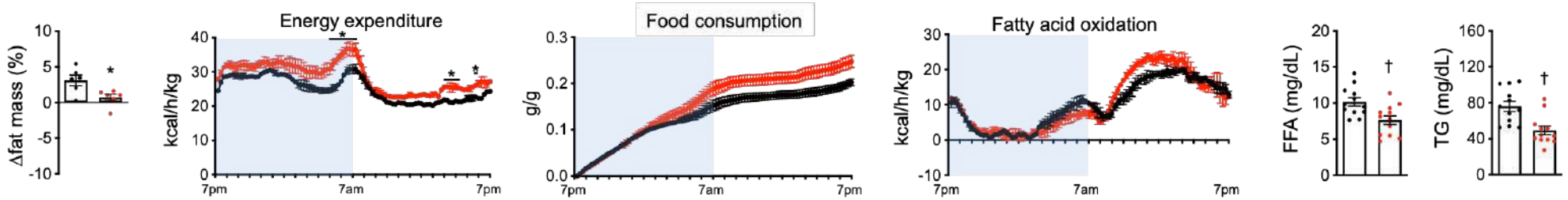
15wo; housing 22°C



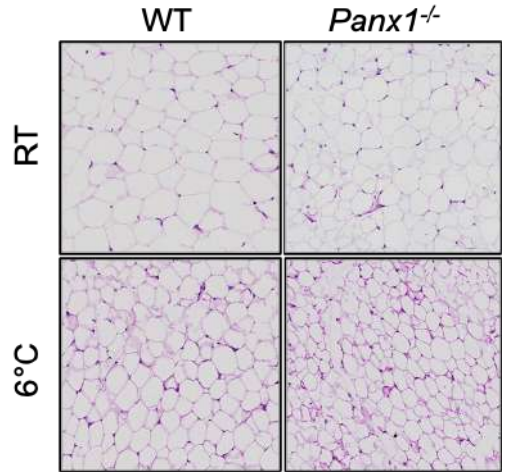
20wo; housing 22°C

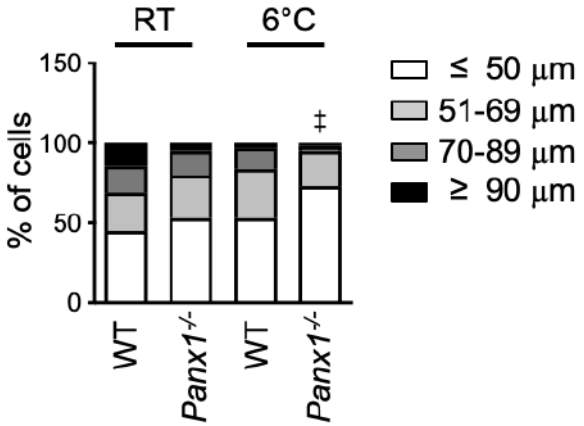


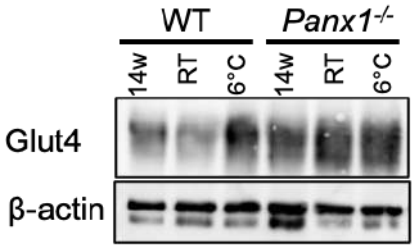
20wo; housing 6°C

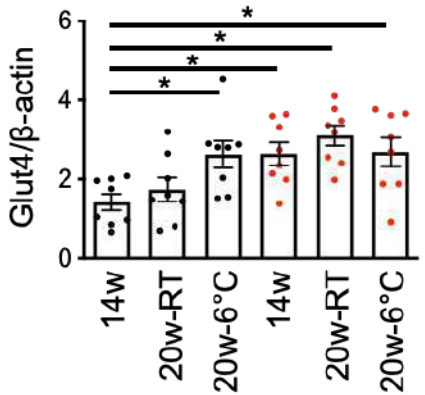


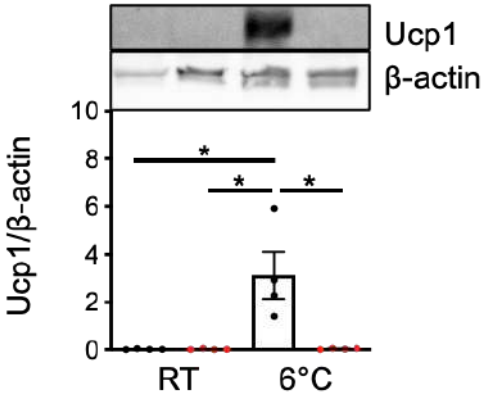
Cold-induced adipose tissue browning is independent of Ucp1











Conclusions

Panx1 plays a role in the regulation of adipose tissue and energy metabolism.

The white adipose tissue of cold-exposed *Panx1*$^{-/-}$ mice displays alterations in adipocyte morphology and function.

Panx1$^{-/-}$ mice fail to express the key thermogenic marker Ucp1, indicating Ucp1-independent thermogenesis.

Acknowledgements

biomolecules

MDPI

Article

Cold Exposure Rejuvenates the Metabolic Phenotype of *Panx1*$^{-/-}$ Mice

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