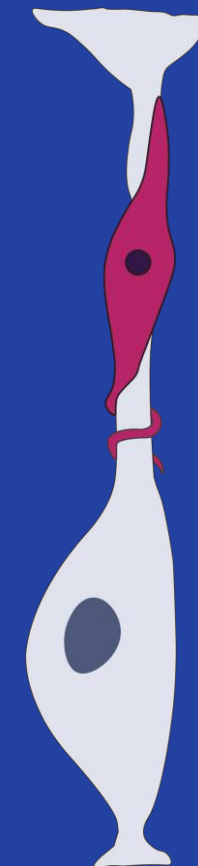


# Ontology-based AI-driven innovative approach using developmental neurotoxicity New Approach Methodologies for Next Generation Risk Assessment



ESTIV



<https://ontox-project.eu/>

ASCCT-ESTIV Award Winners Webinar Series

Japanese Society of Alternatives to Animal Experiments (JSAAE) best oral presentation award



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 963845



Dr. Eliska Kuchovska

K. Bartmann, L.Ladeira, A. Donmez, L. Saborowski, N. Gorts, D. Polozij, F. Bendt, M. Schade, G. Raad, R. Lesage, A. Gamba, B. Staumont, M. Lislien, O. Myhre, H. Dirven, L. Geris, E. Fritsche

22.11.2024

# DNT safety assessment of chemicals: Urgency & Purpose

<200 tested  
chemicals

untested  
chemicals



→ **WHY?**

# DNT safety assessment of chemicals: Urgency & Purpose

<200 tested chemicals

untested chemicals



→ WHY?



OECD/OCDE

426

Adopted:  
16 October 2007

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Developmental Neurotoxicity Study





# DNT safety assessment of chemicals: Urgency & Purpose

<200 tested chemicals

untested chemicals



→ WHY?



OECD/OCDE 426  
Adopted: 16 October 2007

**OECD GUIDELINE FOR THE TESTING OF CHEMICALS**

Developmental Neurotoxicity Study



→ PARADIGM SHIFT NEEDED



New Approach Methodologies

# Ontology: from the data collection to the regulatory application



## Goal:

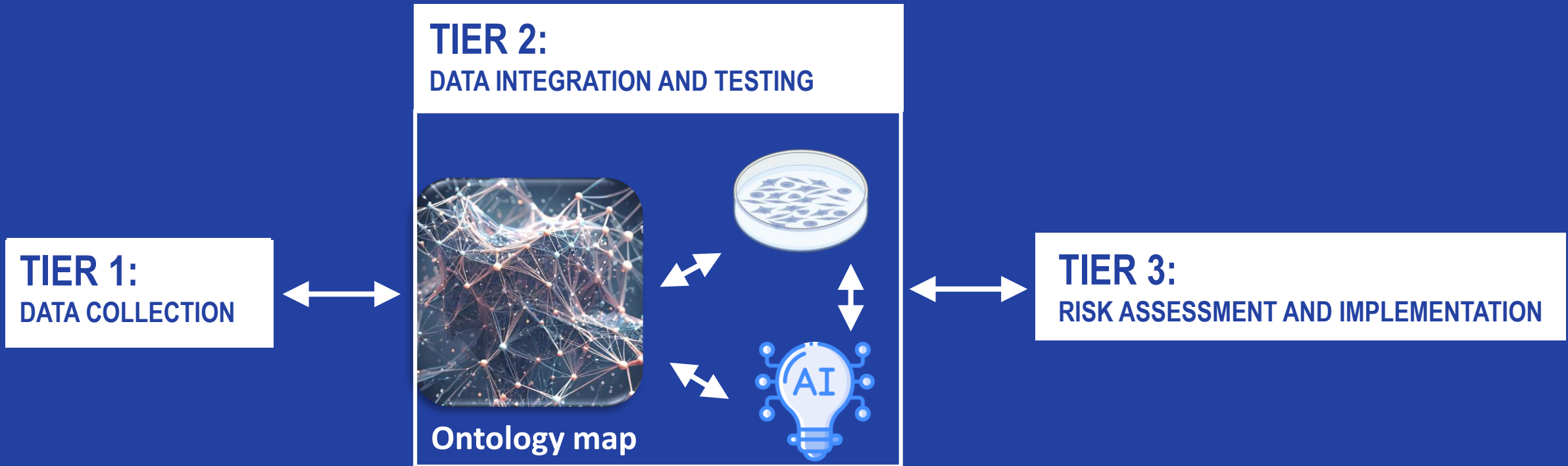
to create New Approach Methodologies that will provide a functional and sustainable solution for advancing human risk assessment of chemicals without the use of animal testing

# Ontology: from the data collection to the regulatory application



Goal:

to create New Approach Methodologies that will provide a functional and sustainable solution for advancing human risk assessment of chemicals without the use of animal testing



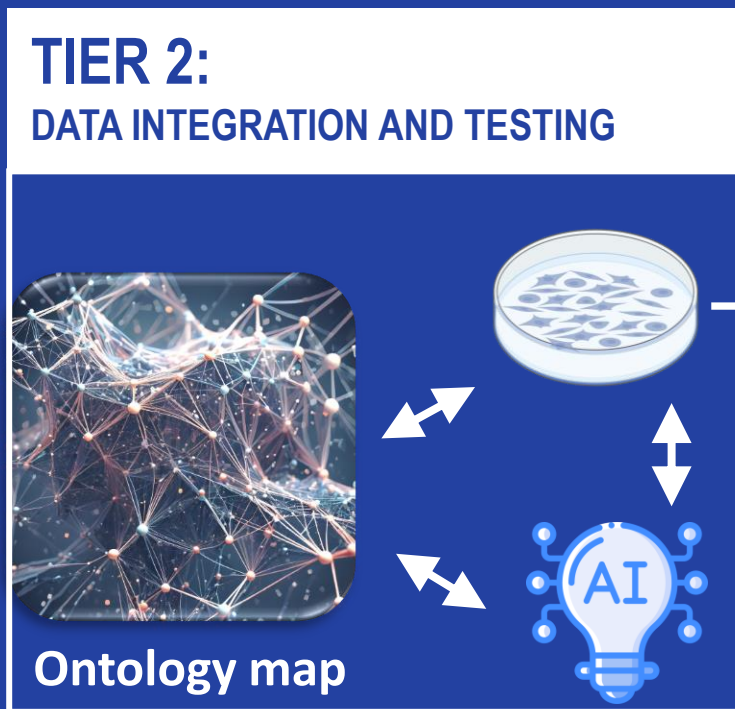
# Ontology: from the data collection to the regulatory application



Goal:

to create New Approach Methodologies that will provide a functional and sustainable solution for advancing human risk assessment of chemicals without the use of animal testing

**TIER 1:**  
DATA COLLECTION



Ontology map

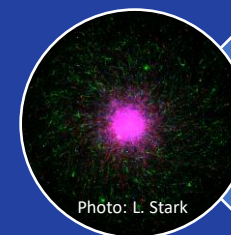
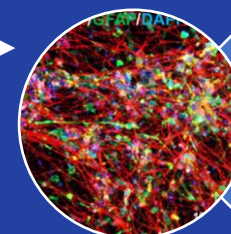
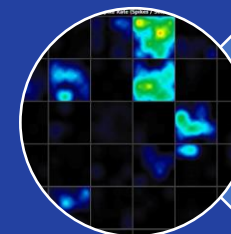


Photo: L. Stark

**Neurosphere Assay (DNT IVB)**  
(Koch et al. 2022)



**Synaptogenesis Assay**  
(Davidsen et al. 2021)



**Neural Network Formation Assay**  
(Bartmann et al. 2023)

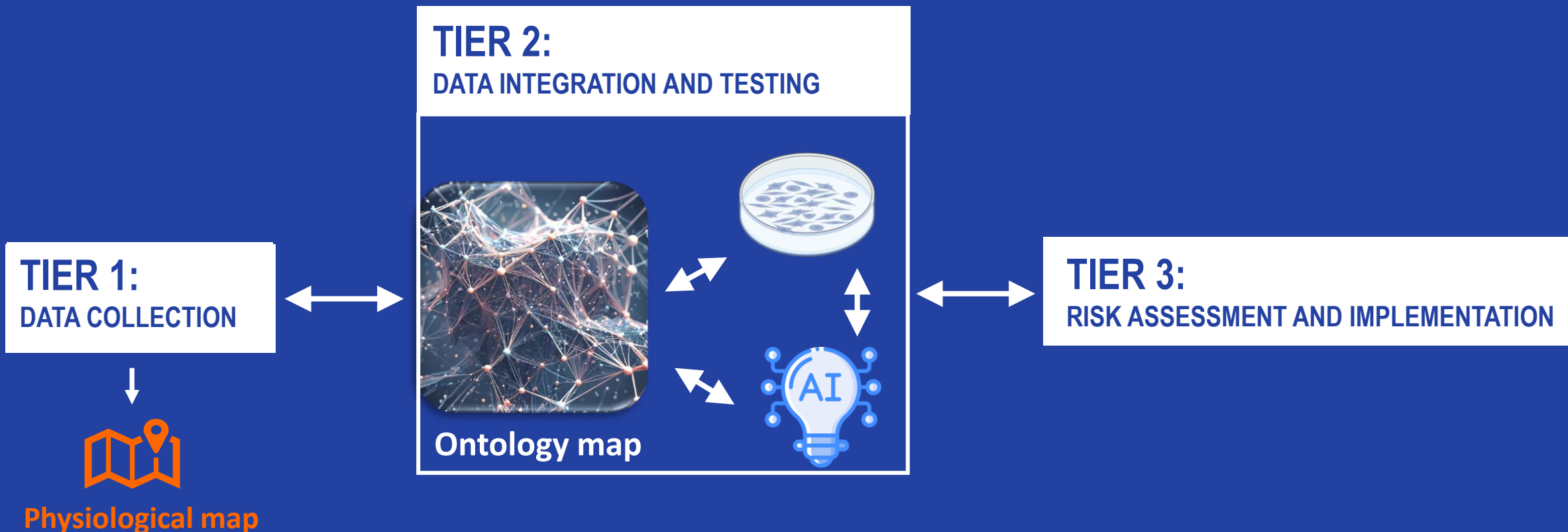


# Ontology: from the data collection to the regulatory application



Goal:

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# Physiological map: analytical strategy

## Physiological map (PM)

Vinken et al. 2021

---

# Physiological map: analytical strategy

## Physiological map (PM)

Vinken et al. 2021



Non-systematic literature review



Standardized documentation following PM guidelines



Staumont et al. unpublished

CellDesigner™



Map design using SBGN

Le Novère et al. 2009



Visualization in Minerva

Gawron et al. 2016

SBGN = Systems Biology Graphical Notation<sup>3</sup>

# Physiological map: analytical strategy

## Physiological map (PM)

Vinken et al. 2021

## Multilayered PM of the developing brain

**IUF**  
LEIBNIZ RESEARCH  
INSTITUTE FOR  
ENVIRONMENTAL  
MEDICINE

**NIPH**  
Norwegian Institute of Public Health

**LIÈGE**  
université



Non-systematic  
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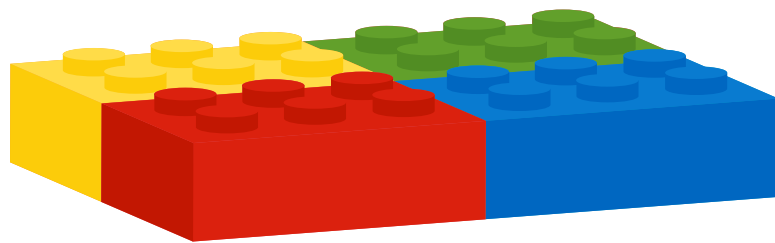
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# Physiological map: analytical strategy

## Physiological map (PM)

Vinken et al. 2021



Submaps

Multilayered PM of the developing brain

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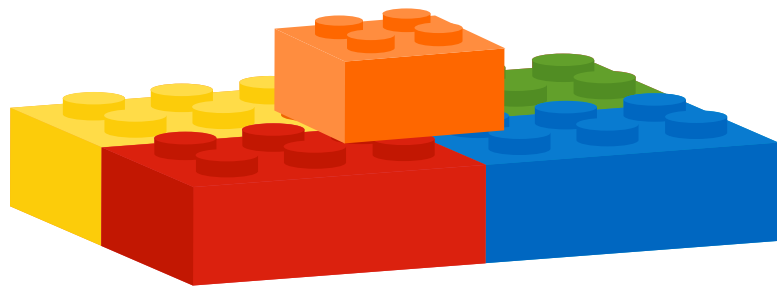
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# Physiological map: analytical strategy

## Physiological map (PM)

Vinken et al. 2021



Cell-cell interactions

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Multilayered PM of the developing brain

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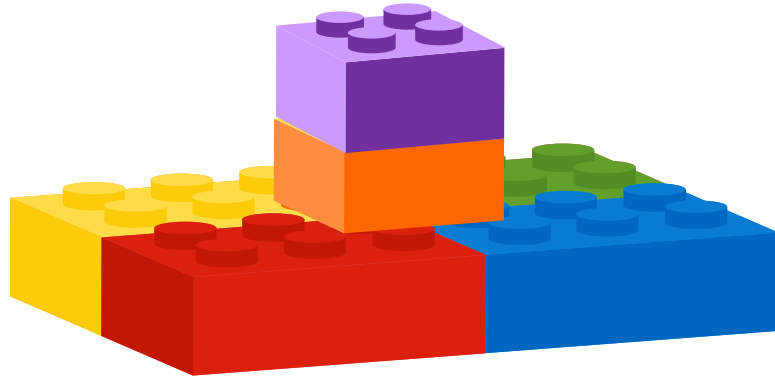
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Overview

Cell-cell interactions

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Multilayered PM of the developing brain

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LIÈGE  
université

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# Physiological map: analytical strategy

## Physiological map (PM)

Vinken et al. 2021

100%  
human  
-based



Overview

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Non-systematic  
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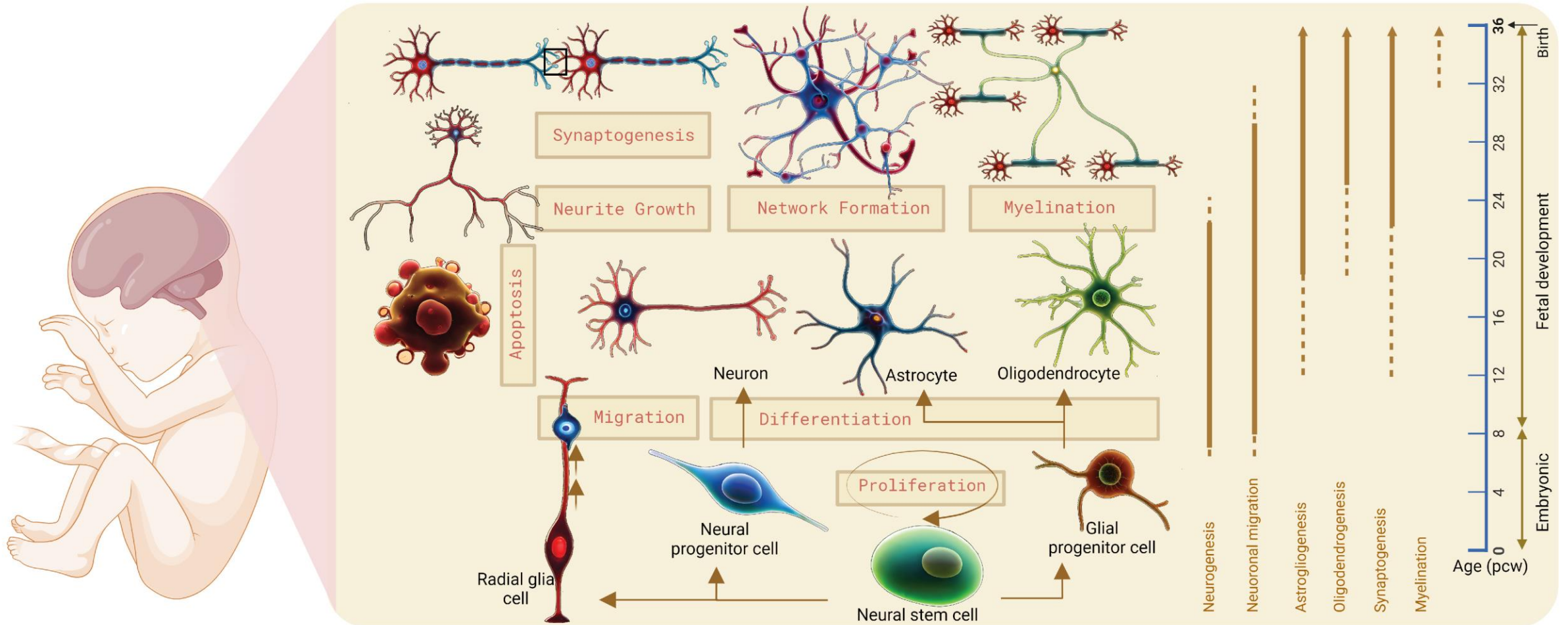
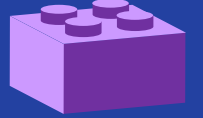


Visualization in  
Minerva

Gawron et al. 2016



# Physiological map: overview

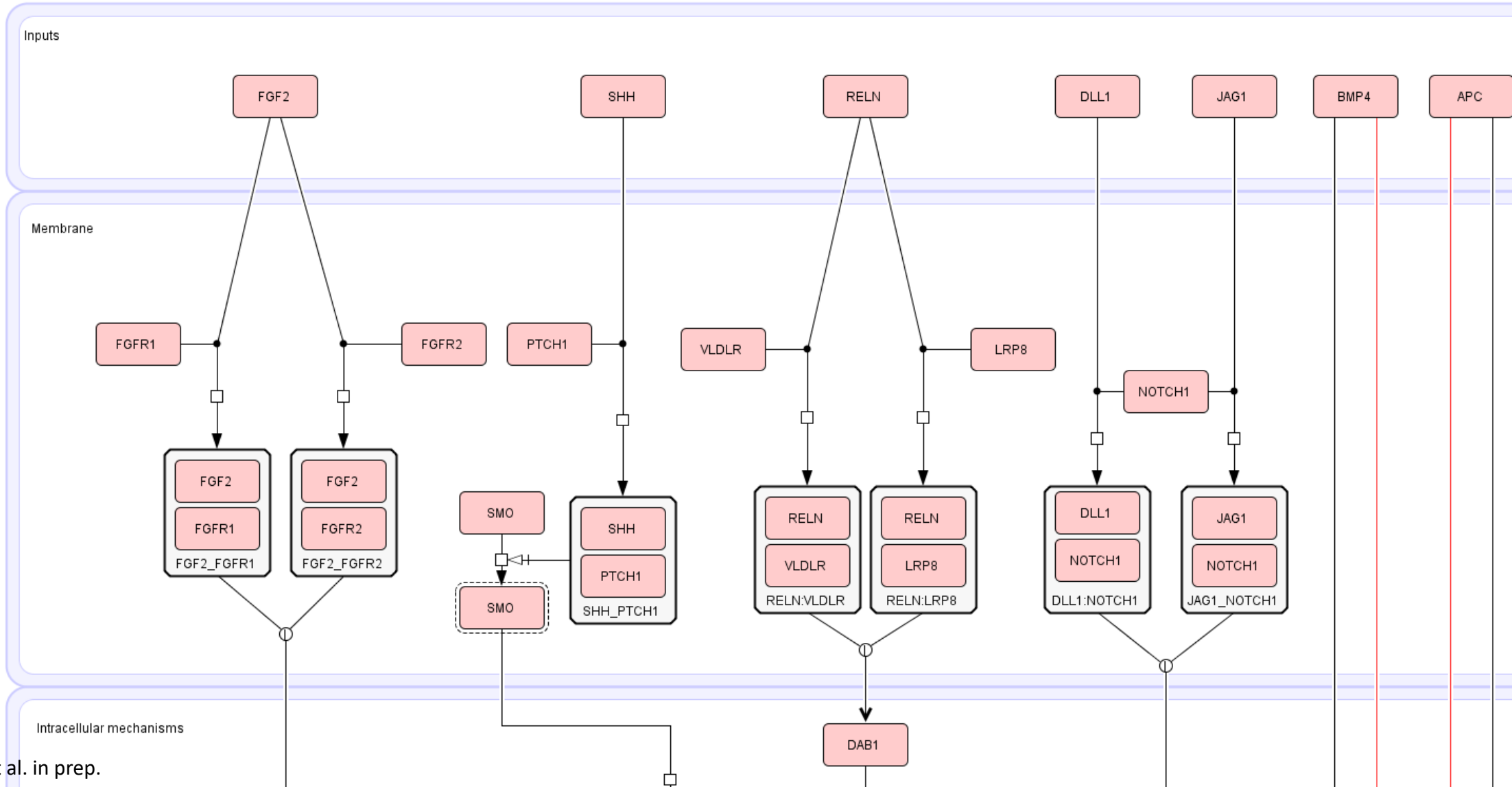
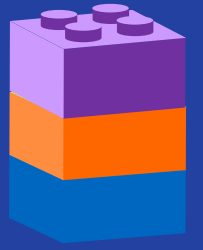


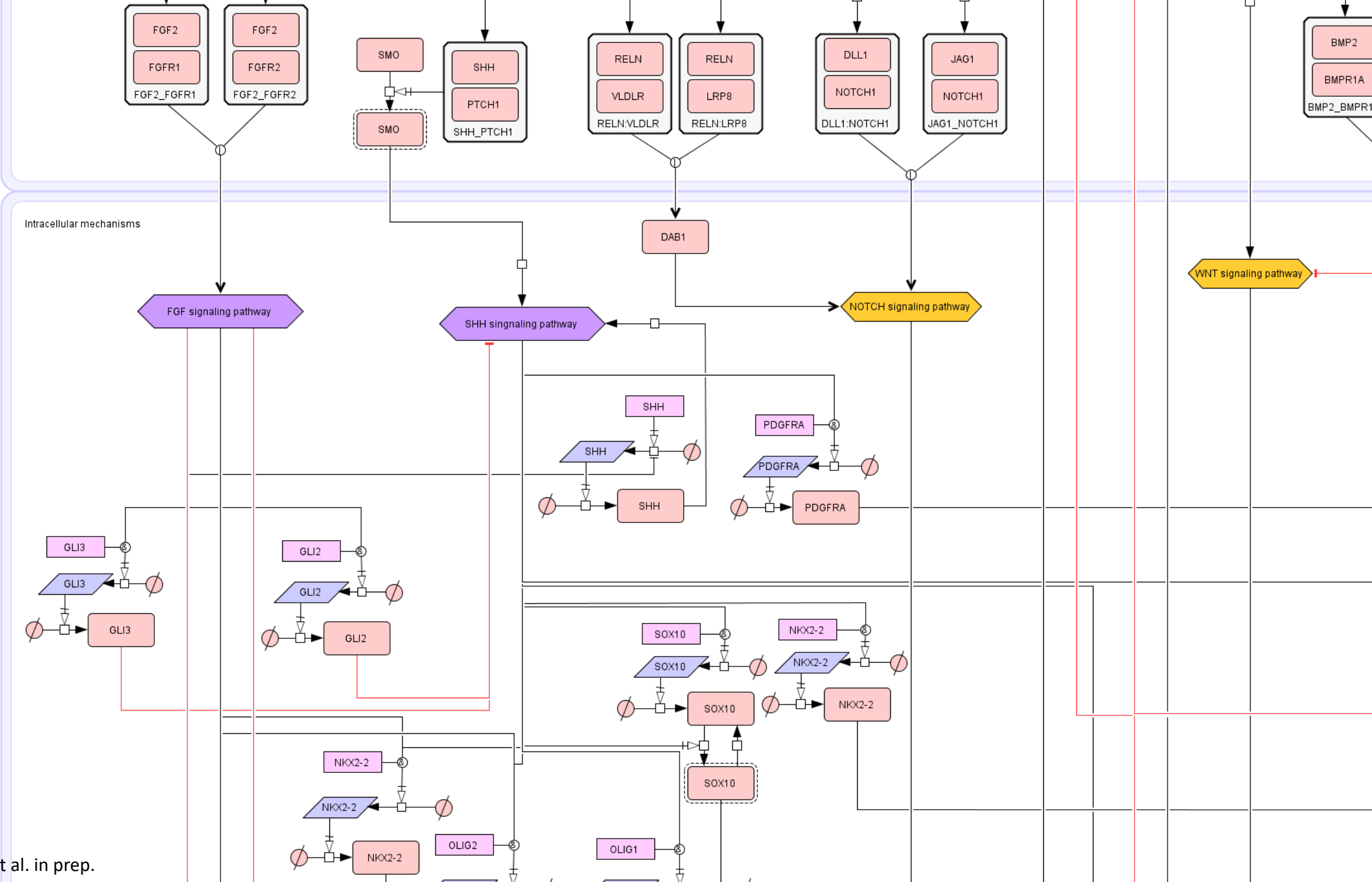






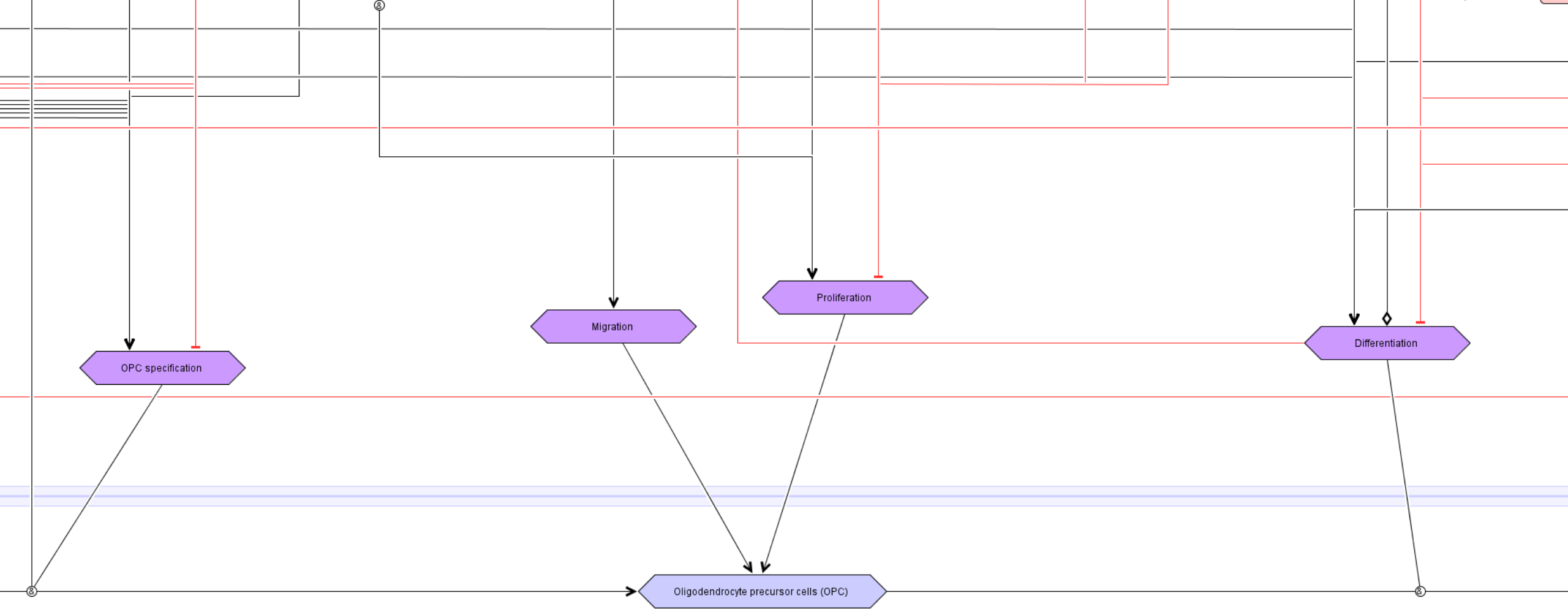
# Physiological map: oligodendrocyte submap

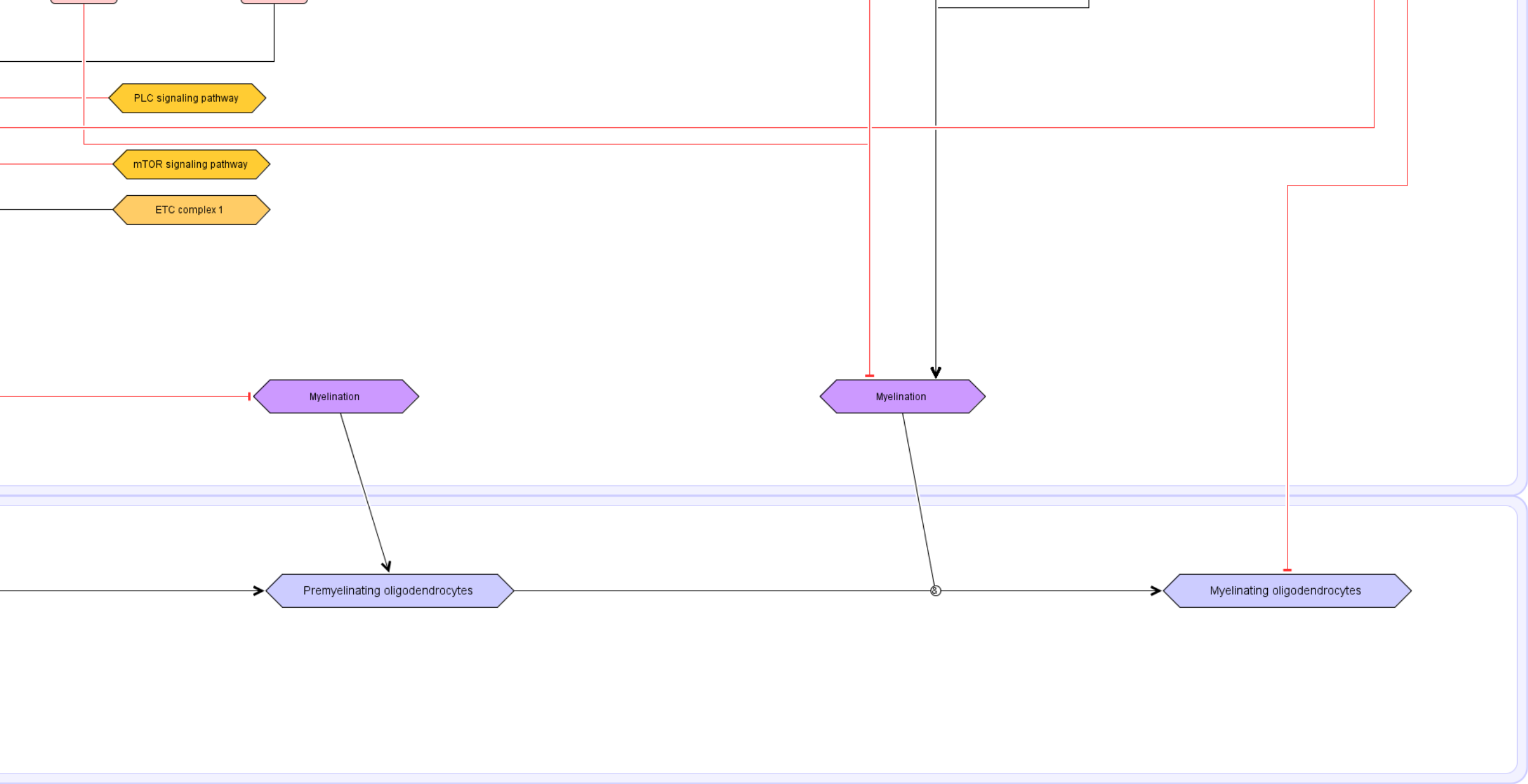




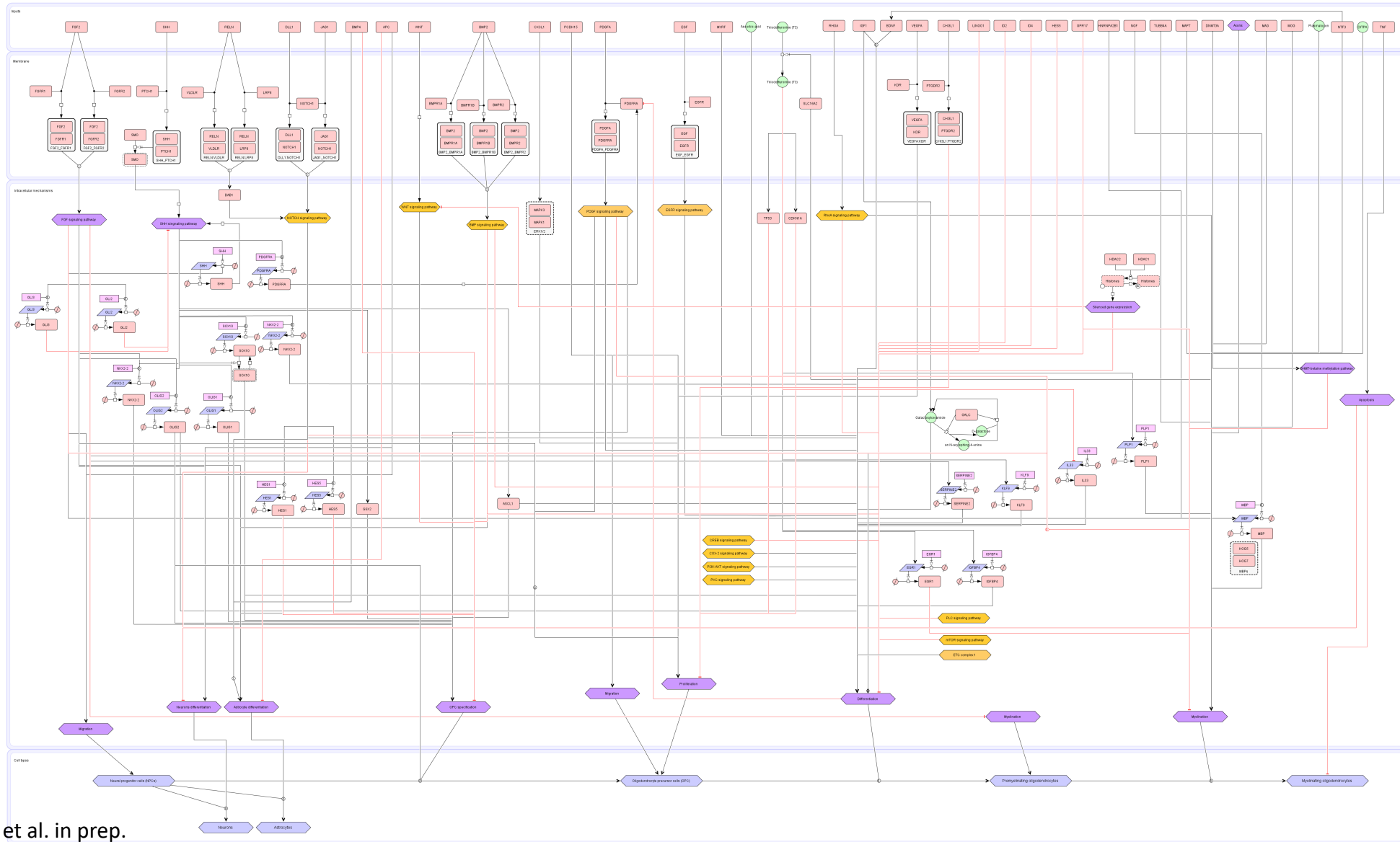
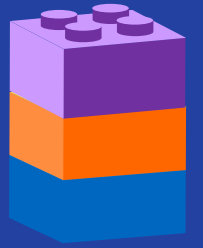








# Physiological map: oligodendrocyte submap



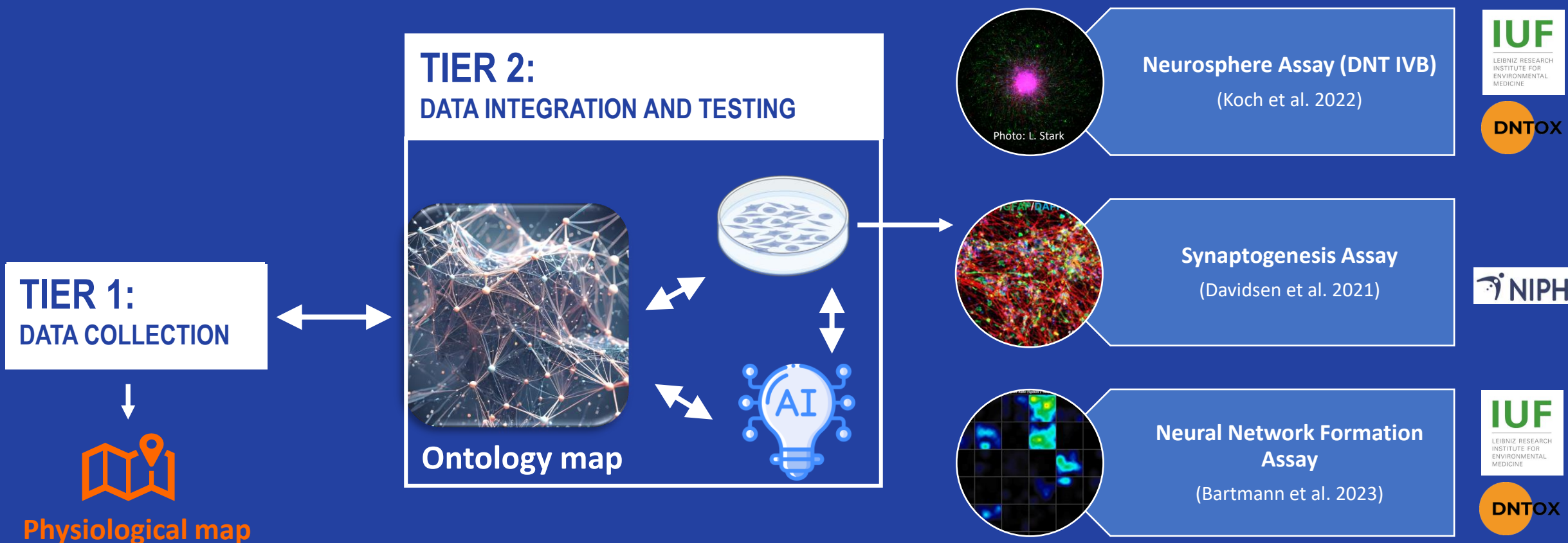


# Ontology: from the data collection to the regulatory application

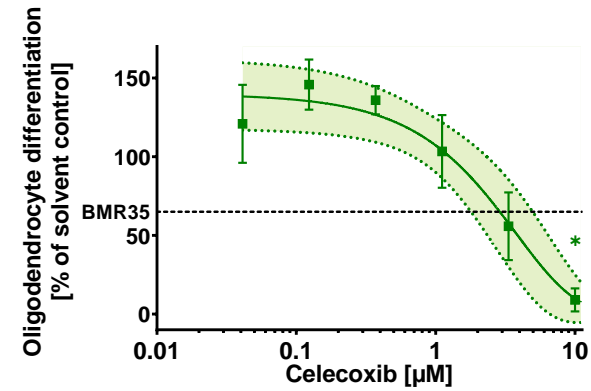


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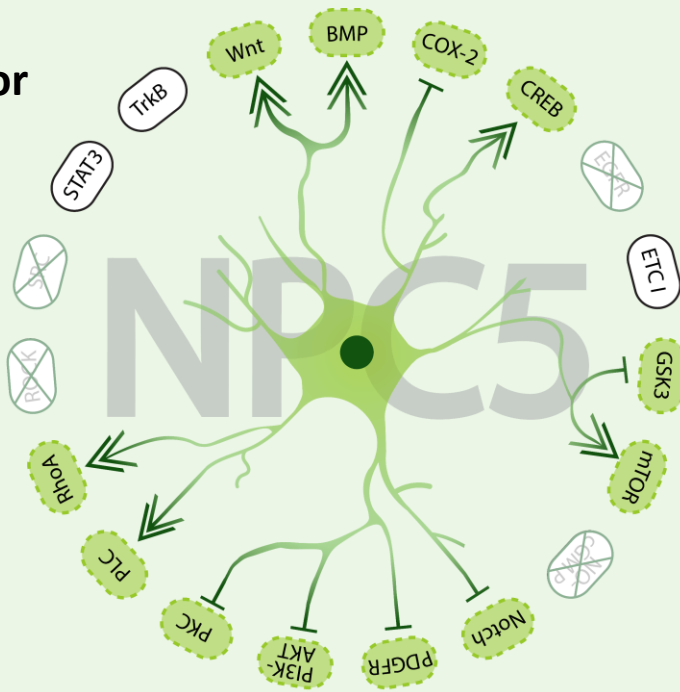


# Oligodendrocyte differentiation (NPC5 assay\*)



**Celecoxib – COX-2 inhibitor**  
 BMC = 1.61 (µM)

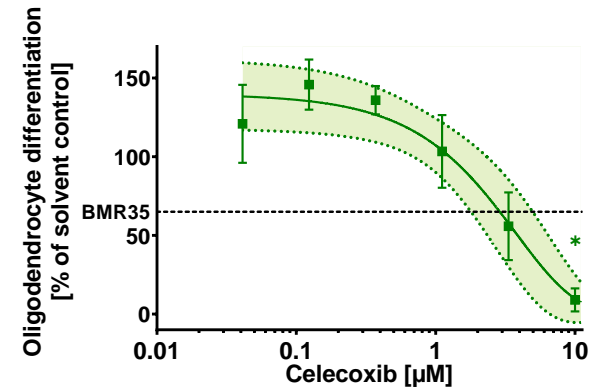
- ↓ OL differentiation
- ↑ OL differentiation
- pathway inhibition
- pathway activation
- no effect
- pathway not tested



Kristina Bartmann

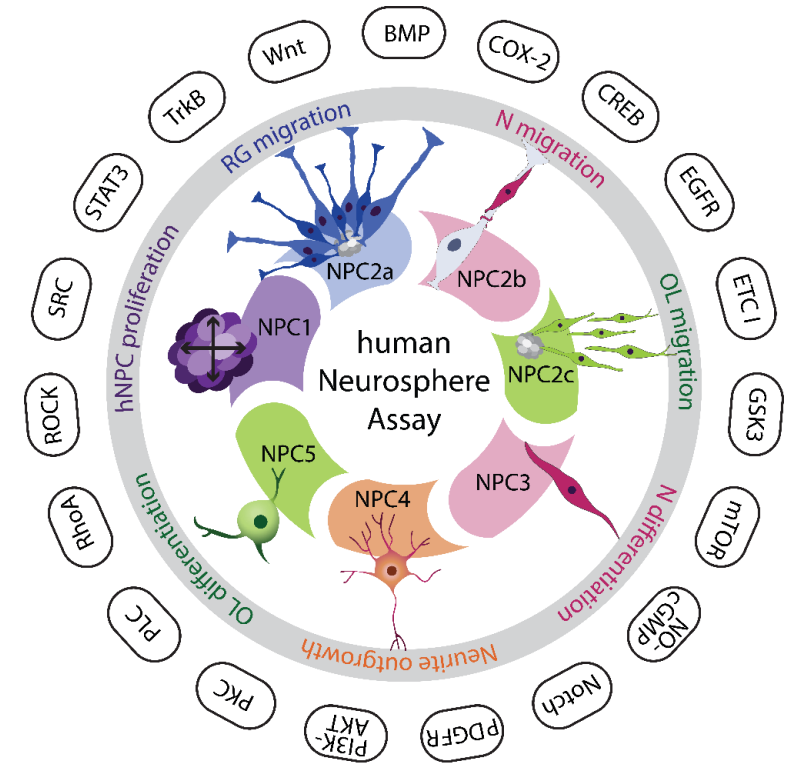
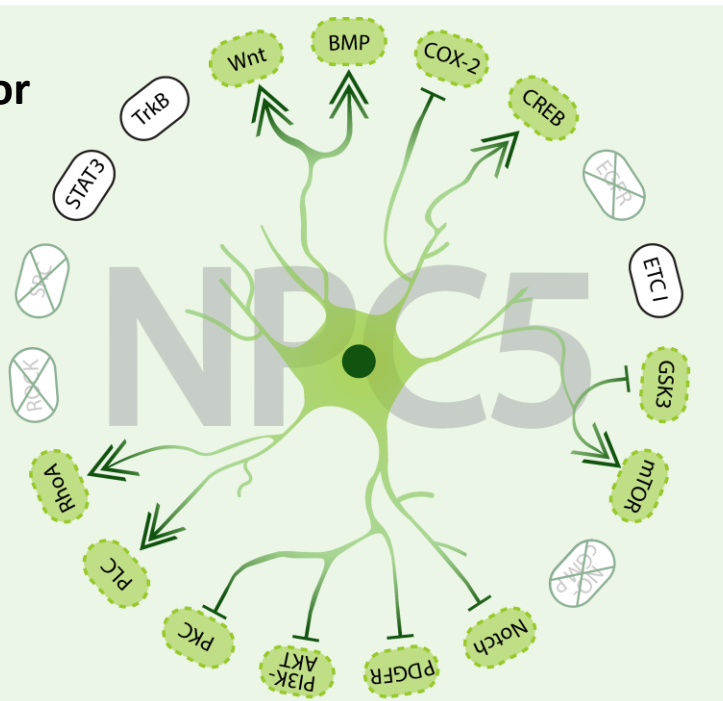
\*part of the DNT IVB (Koch et al. 2022; Crofton Mundy 2021) <sup>7</sup>

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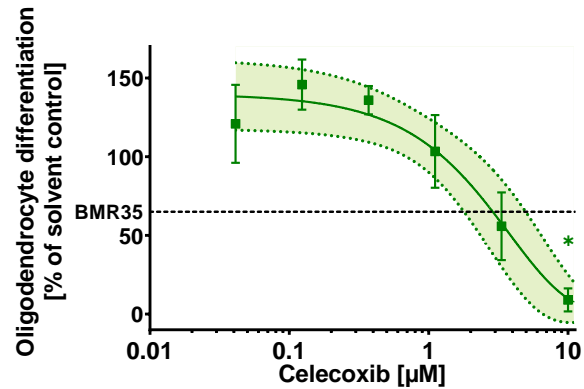


**Biological applicability domain  
of the Neurosphere Assay**

↓ uncertainty      ↑ human relevance

\*part of the DNT IVB (Koch et al. 2022; Crofton Mundy 2021) <sup>7</sup>

# Oligodendrocyte differentiation (NPC5 assay\*)



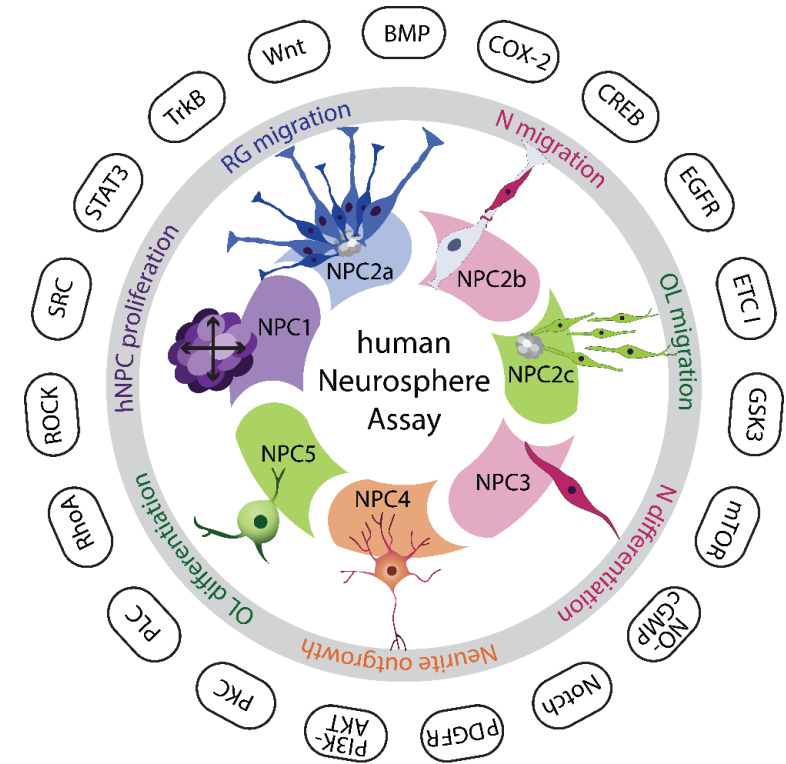
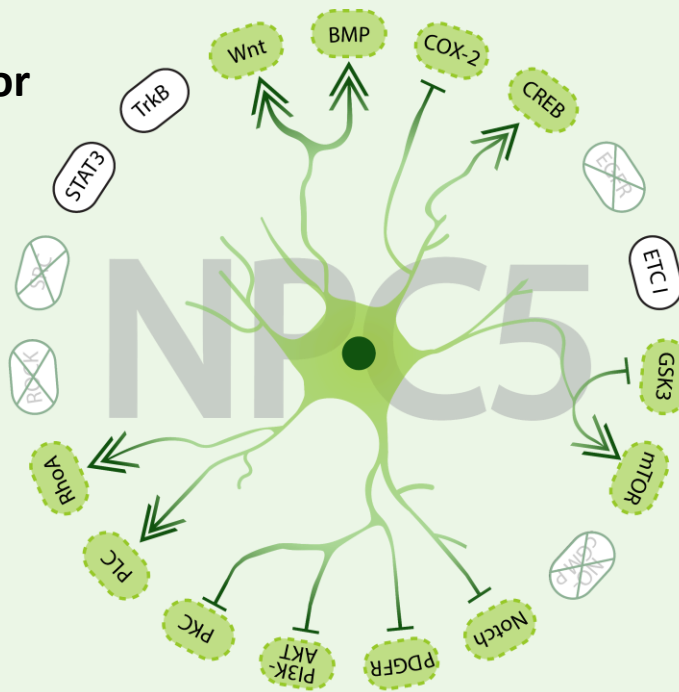
Related human neurodevelopmental disorder:

## Cyclooxygenase-2 (COX-2)

- Neonatal white matter injury (Shiow et al. 2017)

**Celecoxib – COX-2 inhibitor**  
BMC = 1.61 (µM)

- ↓ OL differentiation
- ↑ OL differentiation
- pathway inhibition
- pathway activation
- no effect
- pathway not tested



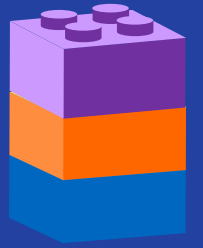
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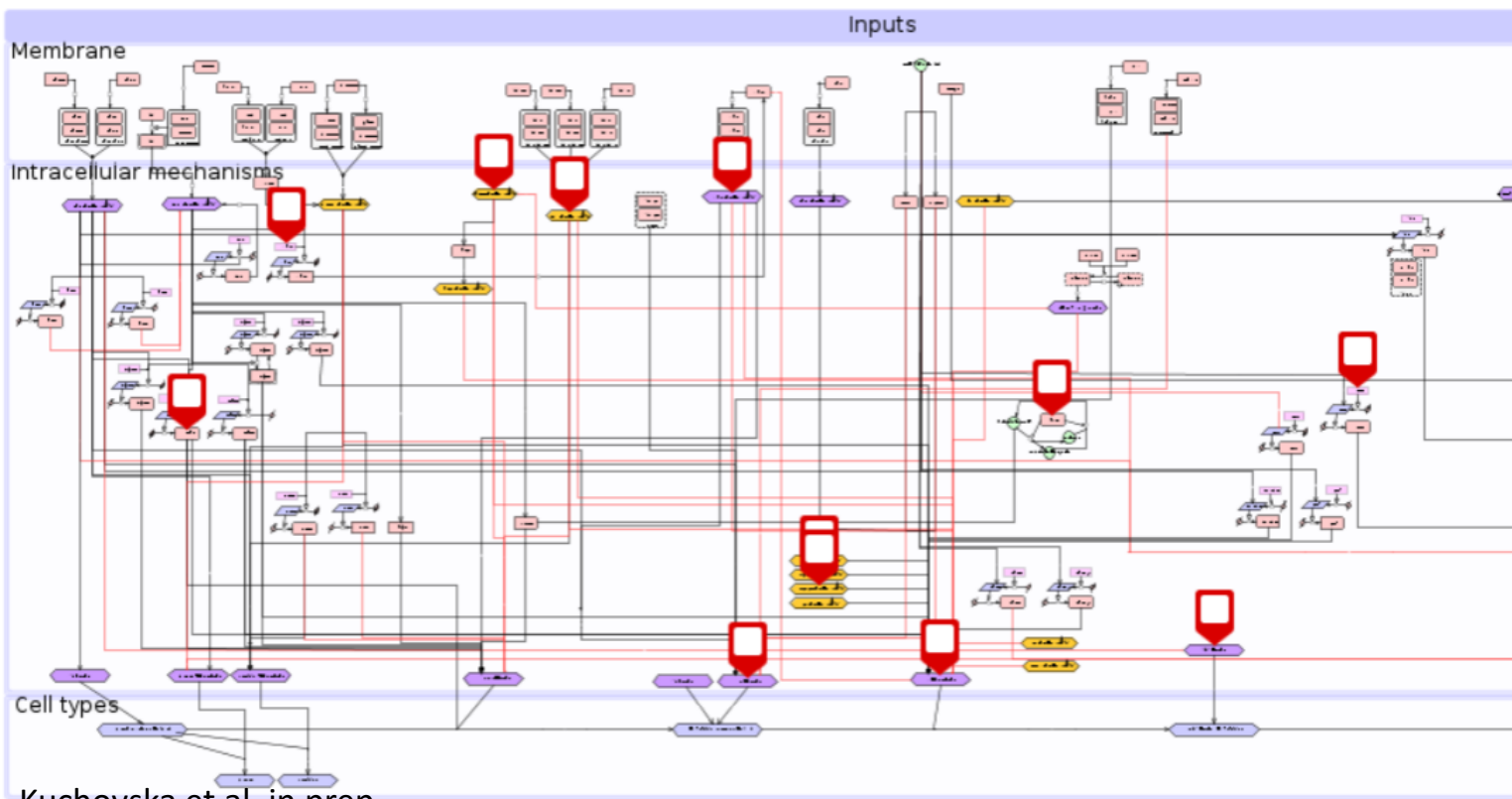
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# Physiological map: oligodendrocyte submap



## Plugin: neurodevelopmental disorders

In cooperation with Hesam Korki, Valentin Groues, and Marek Ostaszewski (MINERVA, ELIXIR-LU)

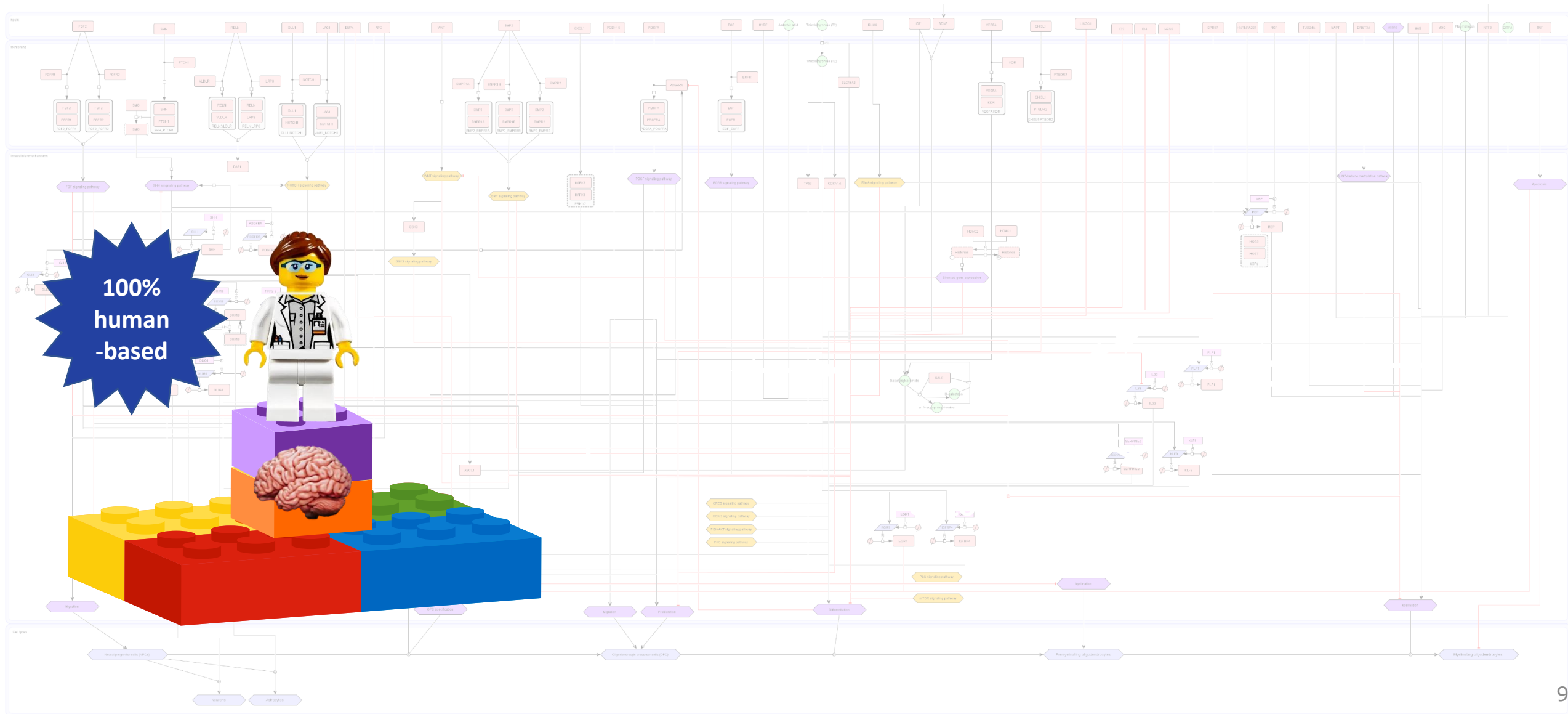


Disease	Entity	Relevance	Reference
Down syndrome	PDGFRA	Oligodendrocyte differentiation	<a href="https://doi.org/10.1093/hmg/dds052">10.1093/hmg/dds052</a>
Neonatal white matter injury	PGE2	Oligodendrocyte differentiation	<a href="https://doi.org/10.1002/glia.23212">10.1002/glia.23212</a>
Alexander disease	PI3K-AKT signaling pathway	Oligodendrocyte differentiation	<a href="https://doi.org/10.1016/j.stem.2018.07.009">10.1016/j.stem.2018.07.009</a>
Fragile X-associated tremor/ataxia syndrome (FXTAS)	PI3K-AKT signaling pathway	Oligodendrocyte differentiation	<a href="https://doi.org/10.1073/pnas.2300052120">10.1073/pnas.2300052120</a>
Pelizaeus-Merzbacher disease	PLP1	Oligodendrocyte differentiation	<a href="https://doi.org/10.1016/j.stem.2019.09.003">10.1016/j.stem.2019.09.003</a>





# Physiological maps: Purpose in



100%  
human-  
based





# Physiological maps: Purpose in



100%  
human-  
based



Knowledgebase

1.



*In vitro* assays

2.



Adverse  
outcome  
pathways

3.



Predictive *in silico* models

4.



Ontology  
map

5.

# Physiological map → Ontology map → NAM

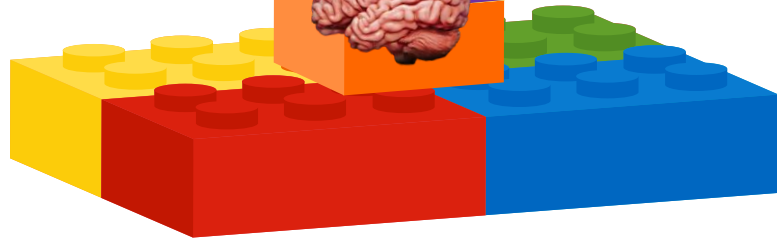
NAM = New Approach Methodology

**TIER 1:**  
DATA COLLECTION



**TIER 2:**  
DATA INTEGRATION AND TESTING

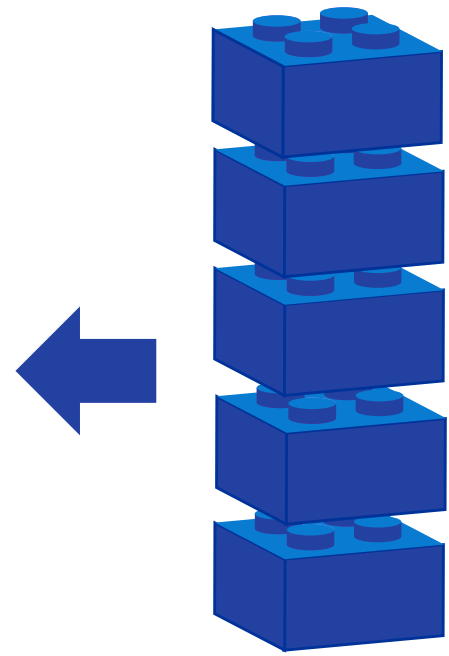
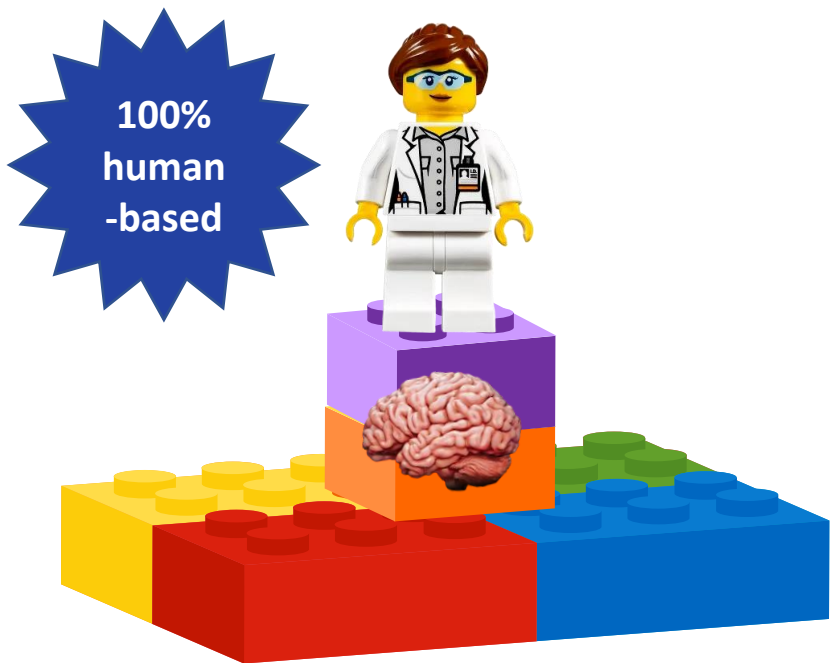
100%  
human  
-based



Ontology map

# Physiological map → Ontology map → NAM

NAM = New Approach Methodology



# Physiological map → Ontology map → NAM

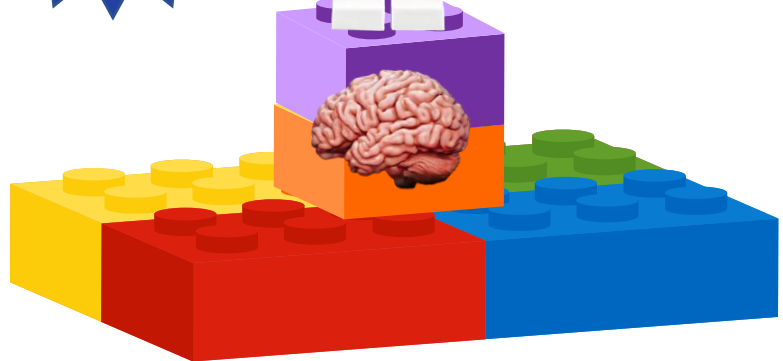
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DATA COLLECTION

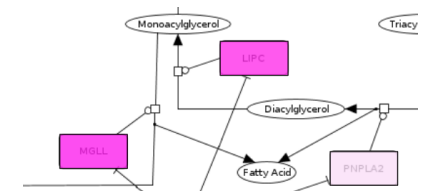
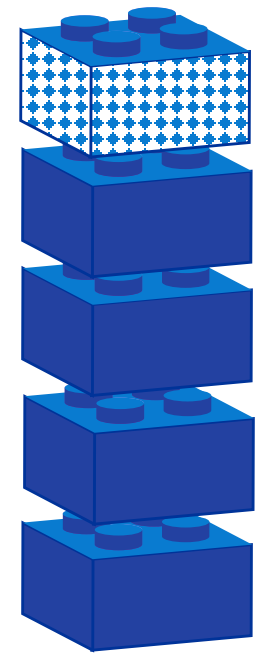
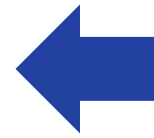


**TIER 2:**  
DATA INTEGRATION AND TESTING

100%  
human  
-based



Ontology map



Healthy transcriptomics  
BrainSpan atlas  
(Allen Institute for Brain Science)

# Physiological map → Ontology map → NAM

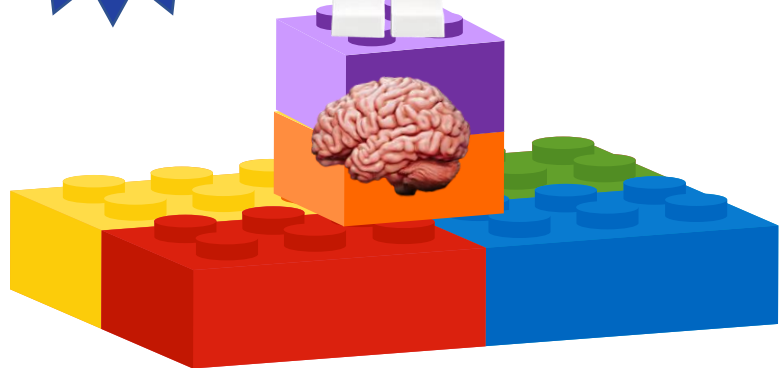
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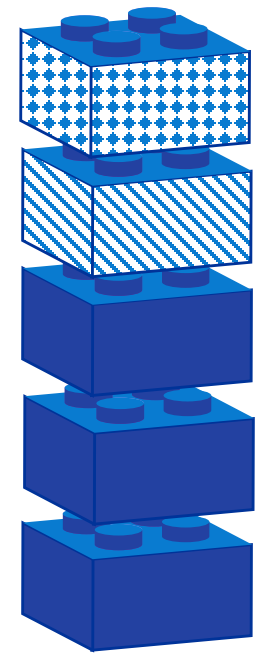
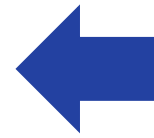


**TIER 2:**  
DATA INTEGRATION AND TESTING

100%  
human  
-based



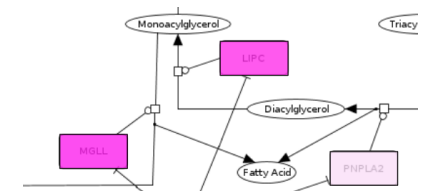
Ontology map



Healthy transcriptomics

Disease transcriptomics

Based on a literature search of relevant neurodevelopmental disorders





# Physiological map → Ontology map → NAM

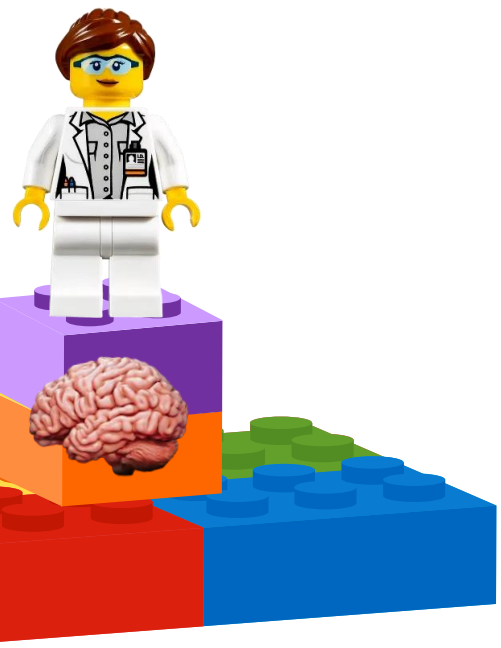
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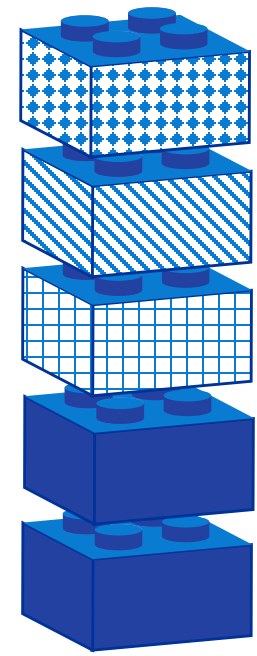
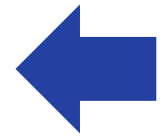


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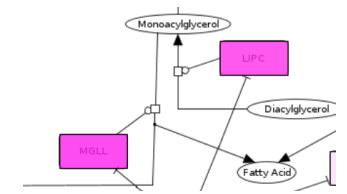
100%  
human  
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Ontology map

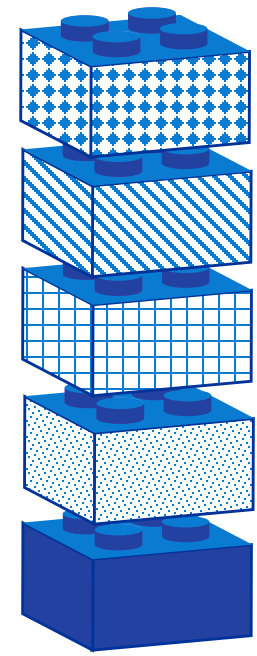
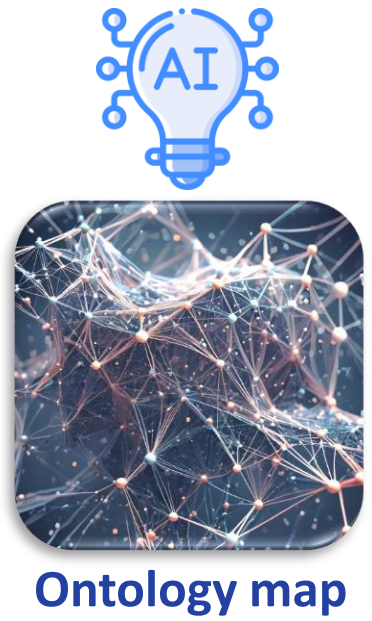
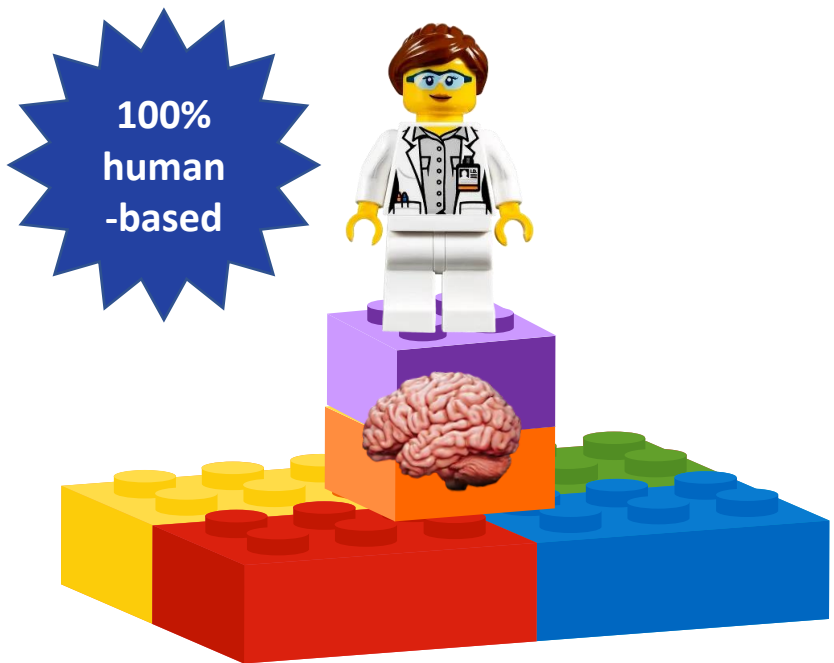


- Healthy transcriptomics
  - Disease transcriptomics
  - In vitro* model transcriptomics
- Klose et al. 2021  
Lislien, Kuchovska, Kapr et al. 2024



# Physiological map → Ontology map → NAM

NAM = New Approach Methodology



- Healthy transcriptomics
- Disease transcriptomics
- In vitro* model transcriptomics
- Physico-chemical data



Lislien, Kuchovska, Kapr et al. 2024



# Physiological map → Ontology map → NAM

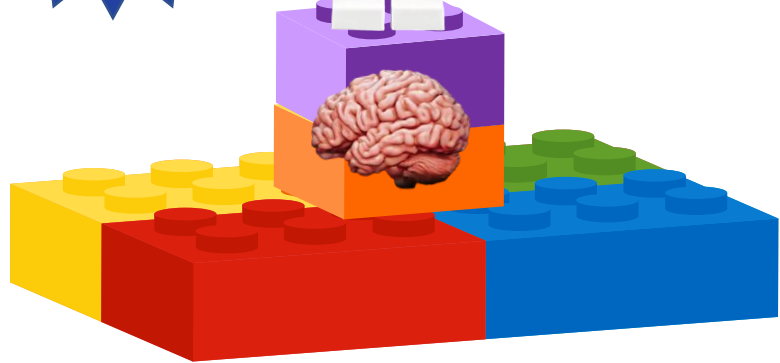
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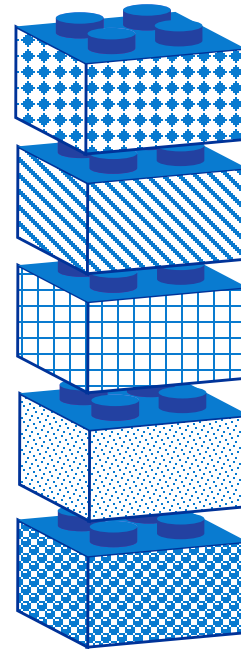
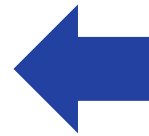


**TIER 2:**  
DATA INTEGRATION AND TESTING

100%  
human  
-based



Ontology map



Healthy transcriptomics

Disease transcriptomics

*In vitro* model transcriptomics

Physico-chemical data

AOP network    AOP = adverse outcome pathway



Lislien, Kuchovska,  
Kapr et al. 2024

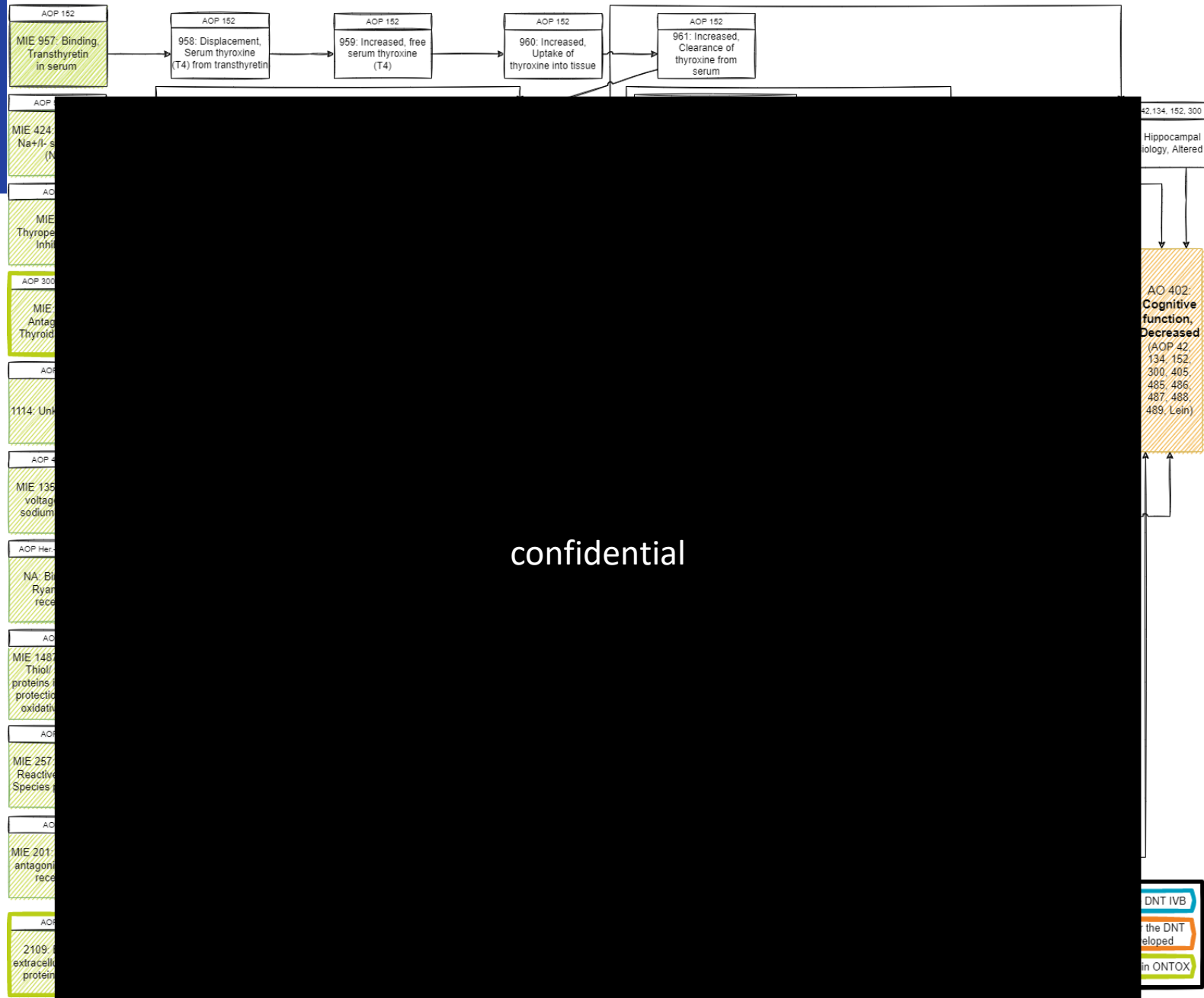


ISTITUTO DI RICERCHE  
FARMACOLOGICHE  
MARIO NEGRI · IRCCS

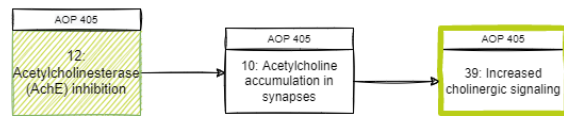
# AOP network

- 16 AOPs
- 2 adverse outcomes of interest

AOP wiki: AOPs 13, 17, 42, 54, 134, 152, 300, 442  
 Bal-Price et al. 2015  
 Hernández-Jerez et al. 2021  
 Klose et al. 2021  
 Klose et al. 2022



confidential



AOP = adverse outcome pathway; KE = key event  
 ICD-11 = 11th International Classification of Diseases system

# AOP network

- 16 AOPs
- 2 adverse outcomes of interest

AOP wiki: AOPs 13, 17, 42, 54, 134, 152, 300, 442  
 Bal-Price et al. 2015  
 Hernández-Jerez et al. 2021  
 Klose et al. 2021  
 Klose et al. 2022

- KEs links to neurodevelopmental disorders (ICD-11)
- KE & disease gaps in the AOP wiki

[Front Toxicol](#), 2024; 6: 1285768.

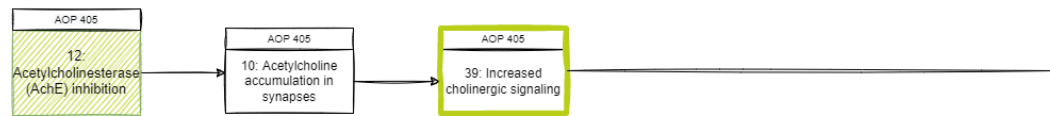
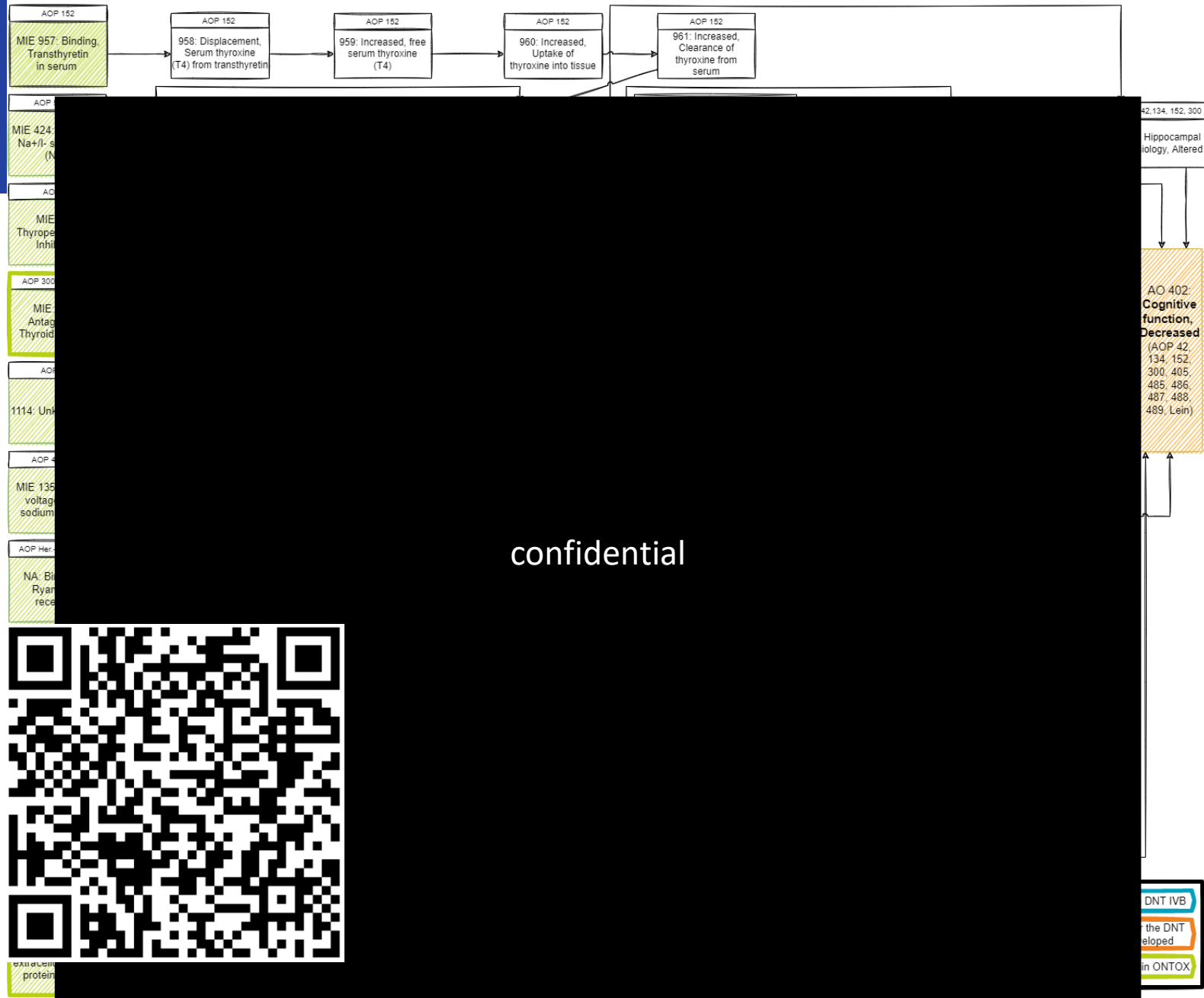
Published online 2024 Mar 8. doi: [10.3389/tox.2024.1285768](https://doi.org/10.3389/tox.2024.1285768)

PMCID: PMC10958381

PMID: [38523647](https://pubmed.ncbi.nlm.nih.gov/38523647/)

Comprehensive mapping of the AOP-Wiki database: identifying biological and disease gaps

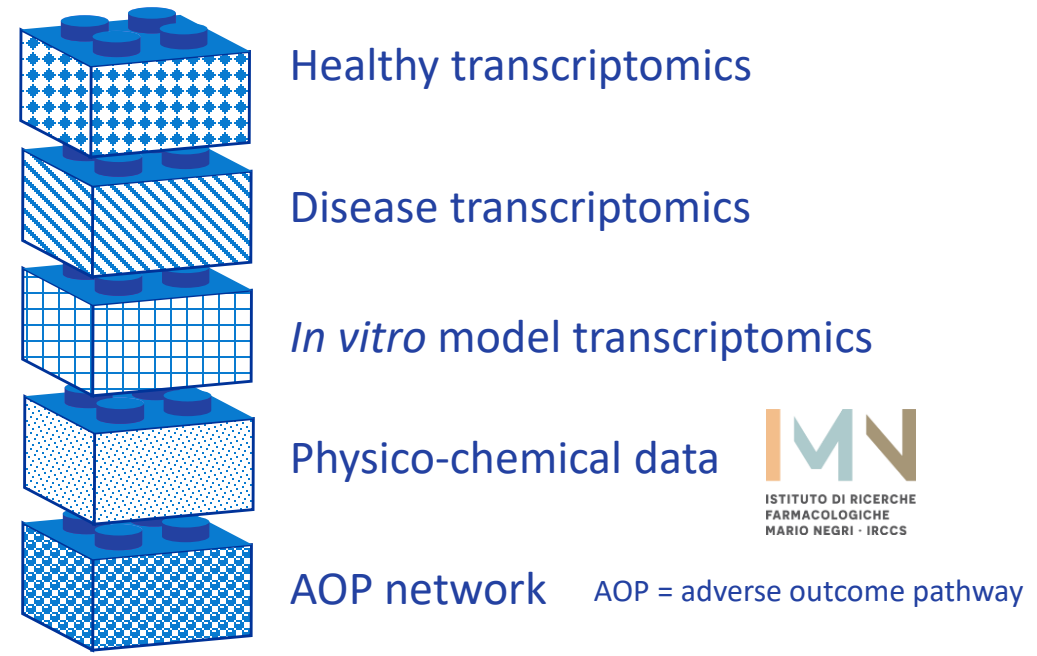
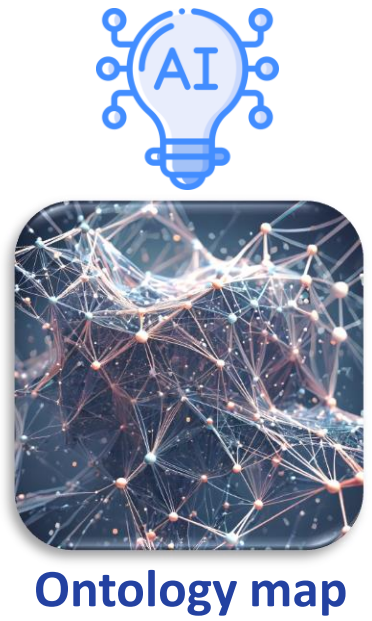
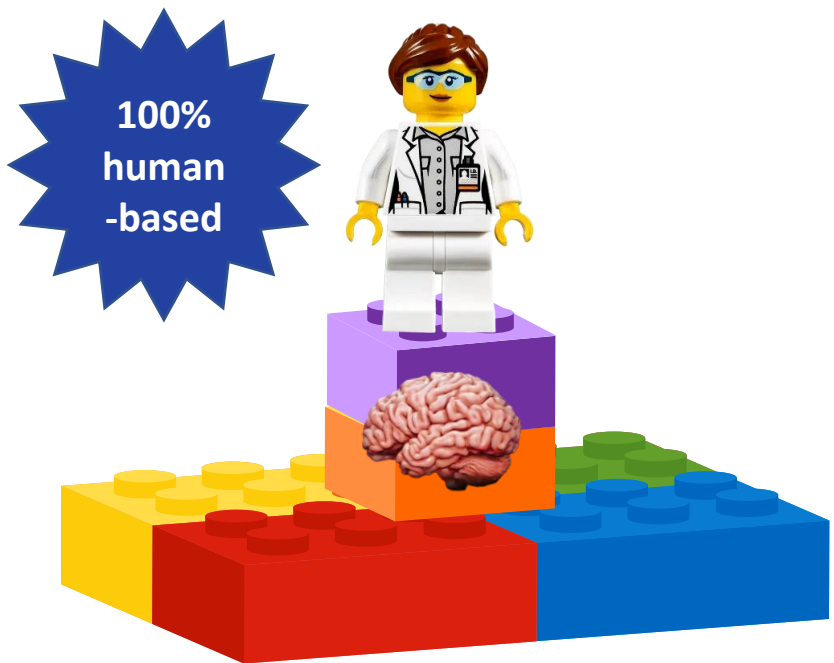
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DNT IVB  
 the DNT  
 in ONTOX

# Physiological map → Ontology map → NAM

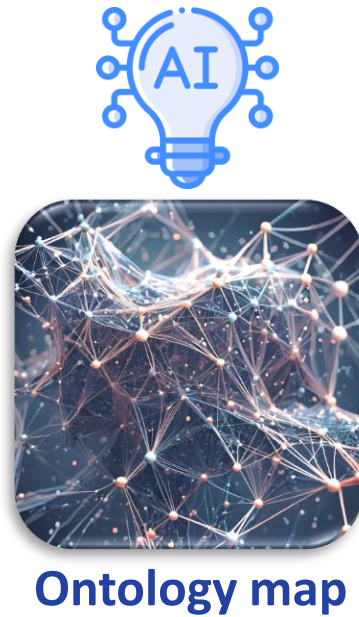
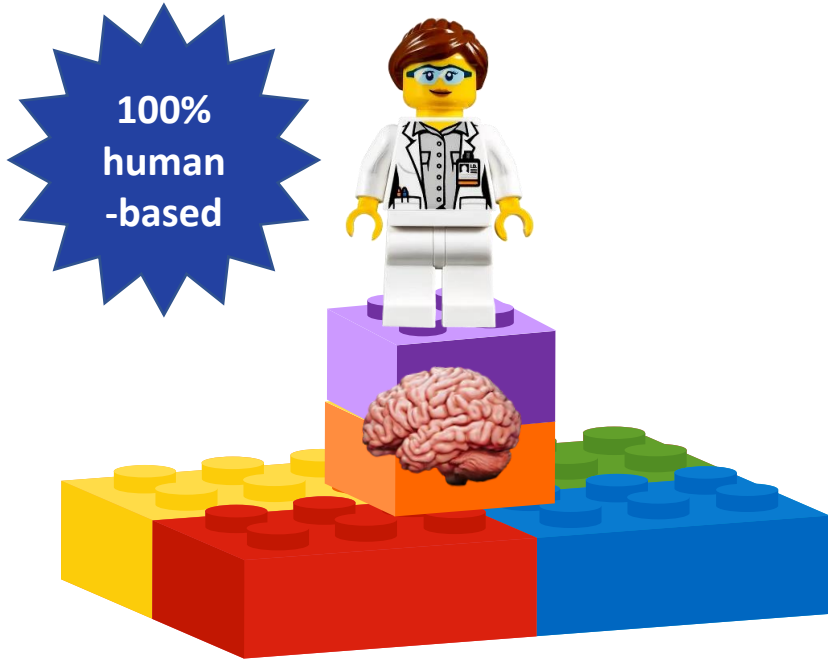
NAM = New Approach Methodology





# Physiological map → Ontology map → NAM

NAM = New Approach Methodology



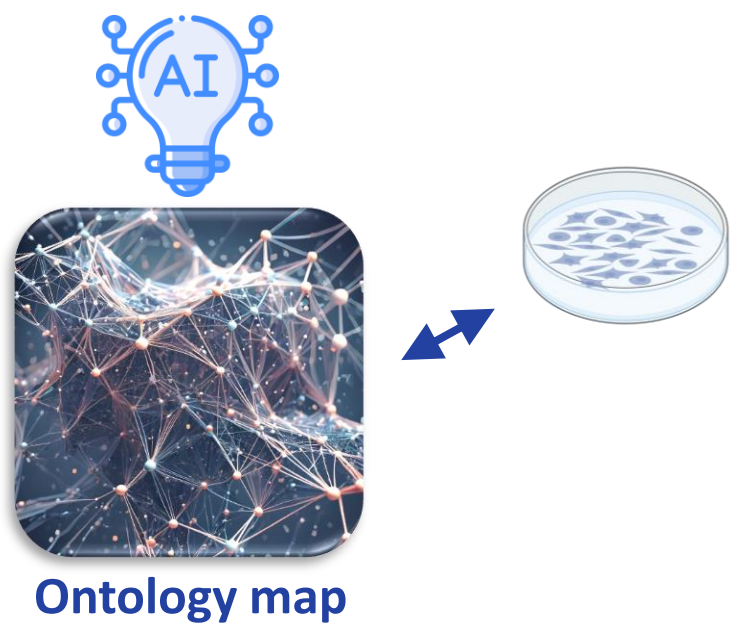
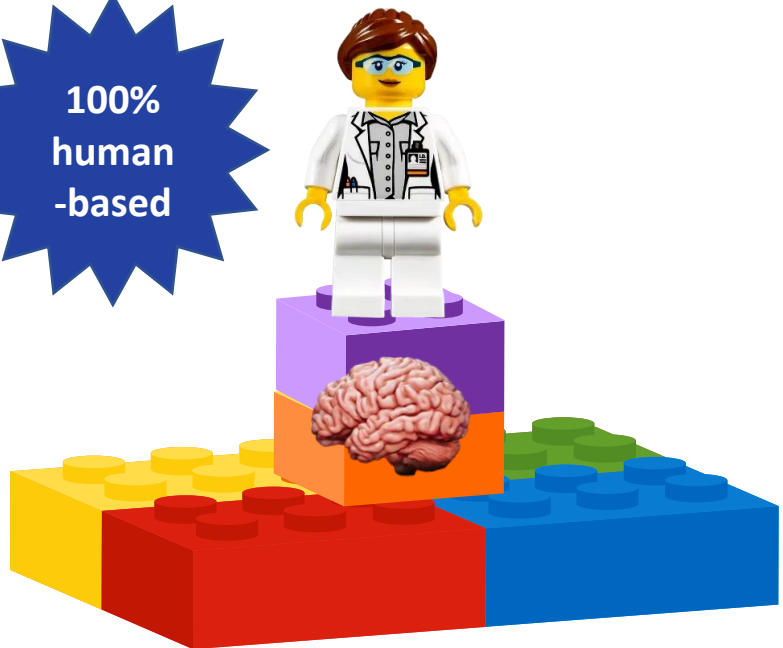


# Physiological map → Ontology map → NAM


NAM = New Approach Methodology



100%  
human  
-based



Ontology map

- QIVIVE
  - PBPK
  - QSAR
  - RASAR
  - qAOP network
  - Exposure assessment
  - Communication with stakeholders
  - ...
- 

Ongoing case study of probabilistic risk assessment:  
**PFOA**

**STAY TUNED!**

PFOA: Perfluorooctanoic acid

QIVIVE = quantitative in vitro to in vivo extrapolation; PBPK = Physiologically based pharmacokinetic modelling; QSAR = Quantitative structure-activity relationship; RASAR = Read-across structure-activity relationship; qAOP = quantitative adverse outcome pathway

# Solution for a safer human brain development



Physiological map → Ontology map → New Approach Methodology

**INNOVATIVE**

→ Paradigm shift

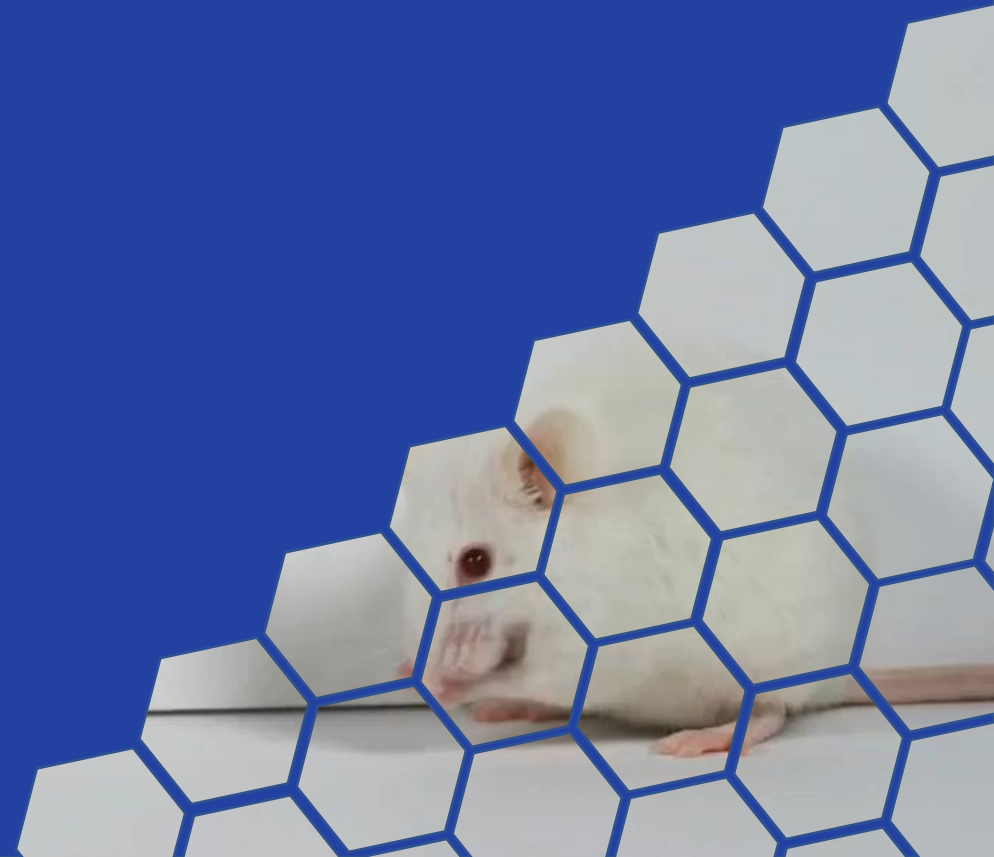
**ETHICAL**



→ Risk assessment of chemicals

**SUSTAINABLE**

**RELIABLE**



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**Thank you for  
your attention!**



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