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"Establishment of interdisciplinary teams of young scientists in the field of fundamental and applied research relevant to medical practice"
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EUROPEAN UNION

EUROPEAN SOCIAL FUND

PPARγ-related hepatotoxic mode-of-action: quantitative characterization and *in silico* study of the molecular initiating event involving receptor activation

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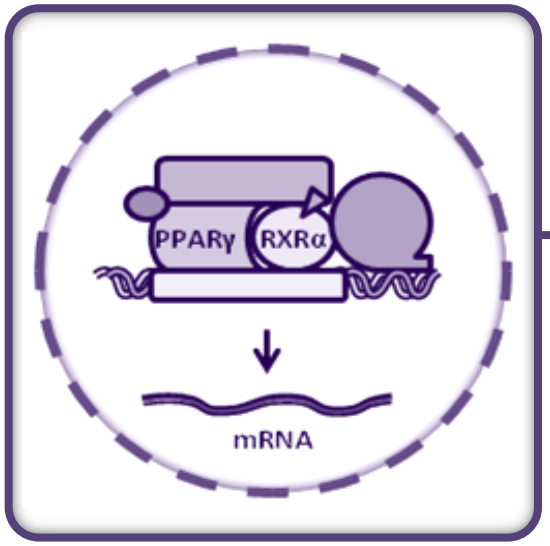
CONTENT

1. Introduction
2. Workflow
3. Evaluation of key events
4. PPARγ ligand database
5. 3D QSAR
6. Perspectives

INTRODUCTION



NAFLD

- NAFL = simple steatosis
- NASH = NAFL + inflammation

Source	Exposure	Molecular Initiating Events	Organelle Level Effects	Cellular Effects	Tissue Effects	Organ Effects	Organism Effects	Population Effects	Community Effects

Toxicity Pathway

Mode of Action

Adverse Outcome Pathway

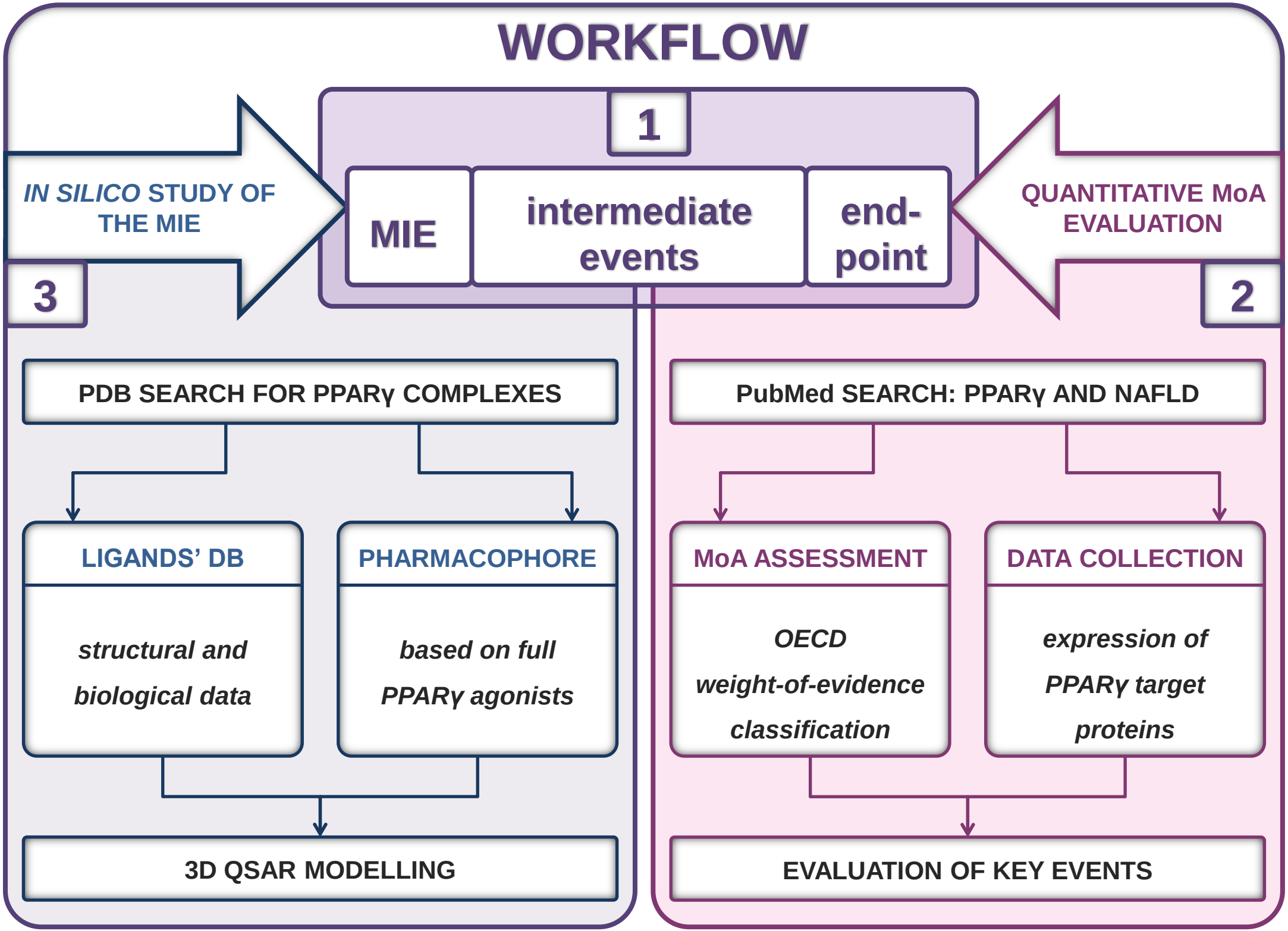
Source to Outcome Pathway

MoA RELATED TASKS

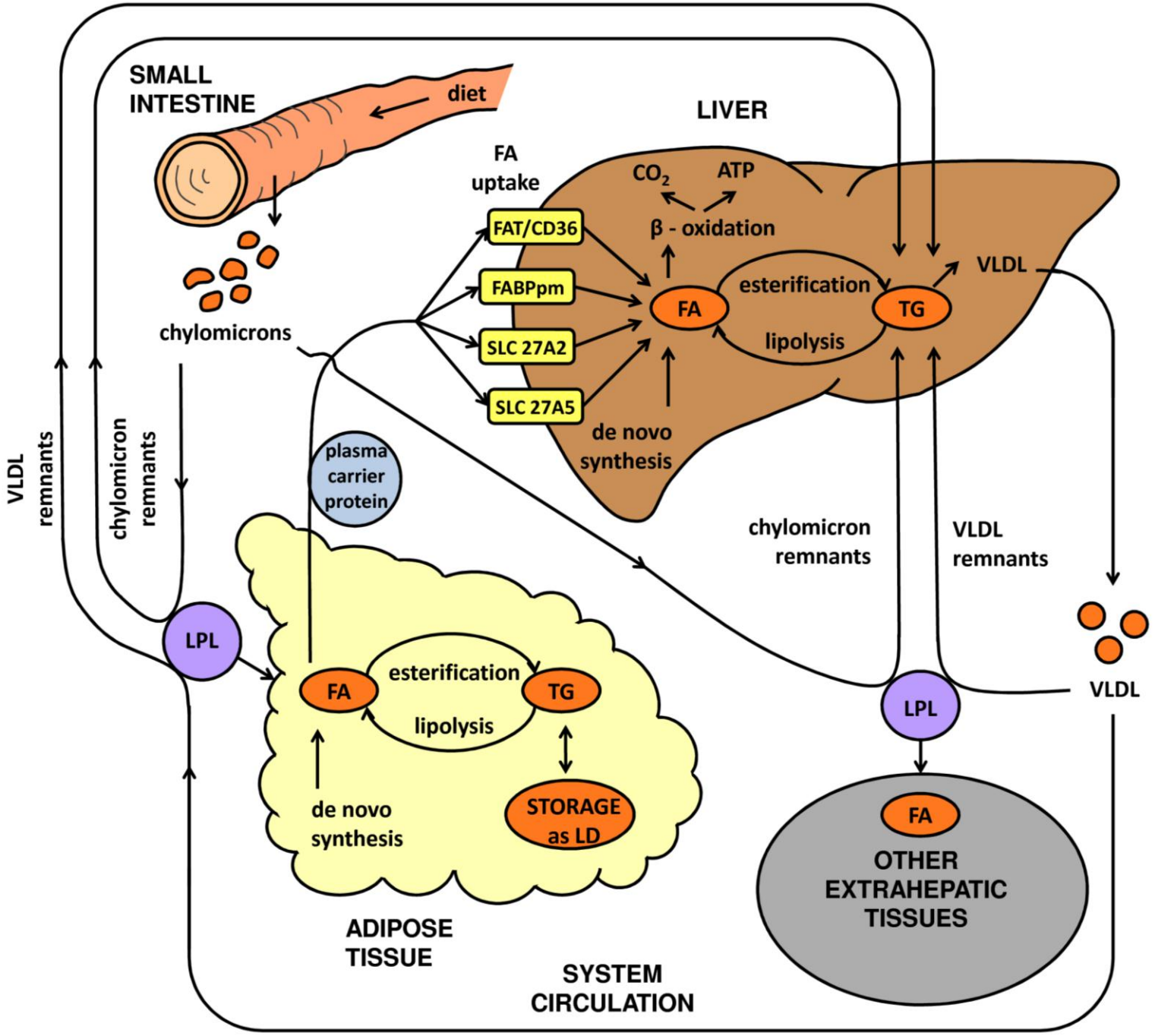
1. **Meta study of the existing experimental evidence linking the MIE and the adverse effect**

2. **Evaluation of the outlined key events**

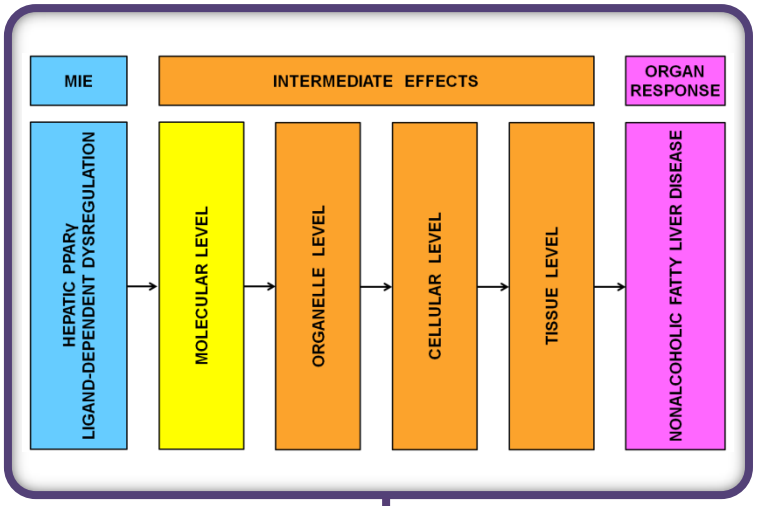
3. **Development of explanatory and predictive *in silico* models of the MIE**


WORKFLOW
1
IN SILICO STUDY OF THE MIE
MIE
intermediate events
end-point
QUANTITATIVE MoA EVALUATION
3
2
PDB SEARCH FOR PPARγ COMPLEXES
PubMed SEARCH: PPARγ AND NAFLD
LIGANDS' DB
structural and biological data
PHARMACOPHORE
based on full PPARγ agonists
MoA ASSESSMENT
OECD weight-of-evidence classification
DATA COLLECTION
expression of PPARγ target proteins
3D QSAR MODELLING
EVALUATION OF KEY EVENTS

BODY LIPID EXCHANGE

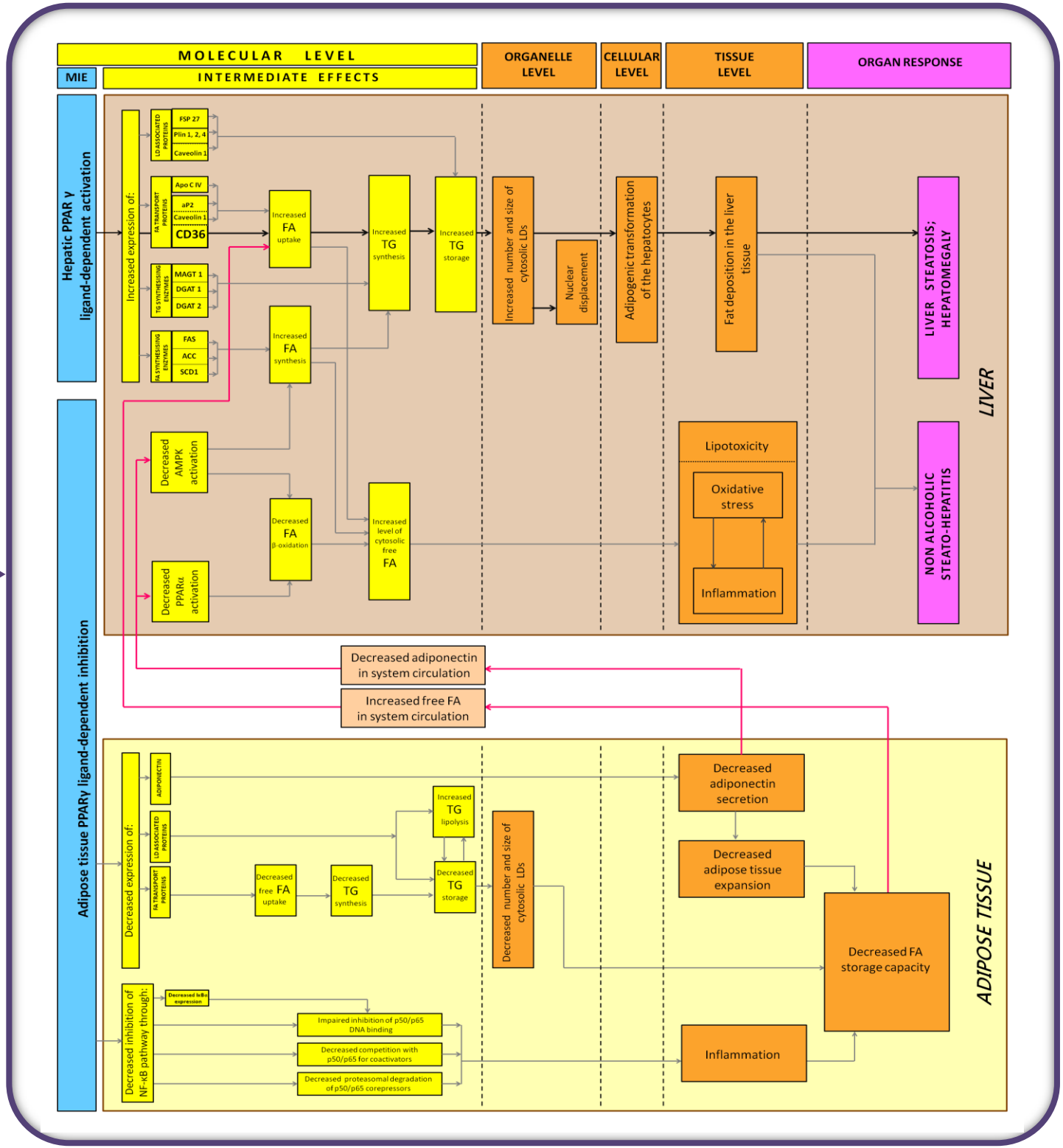


MoAs

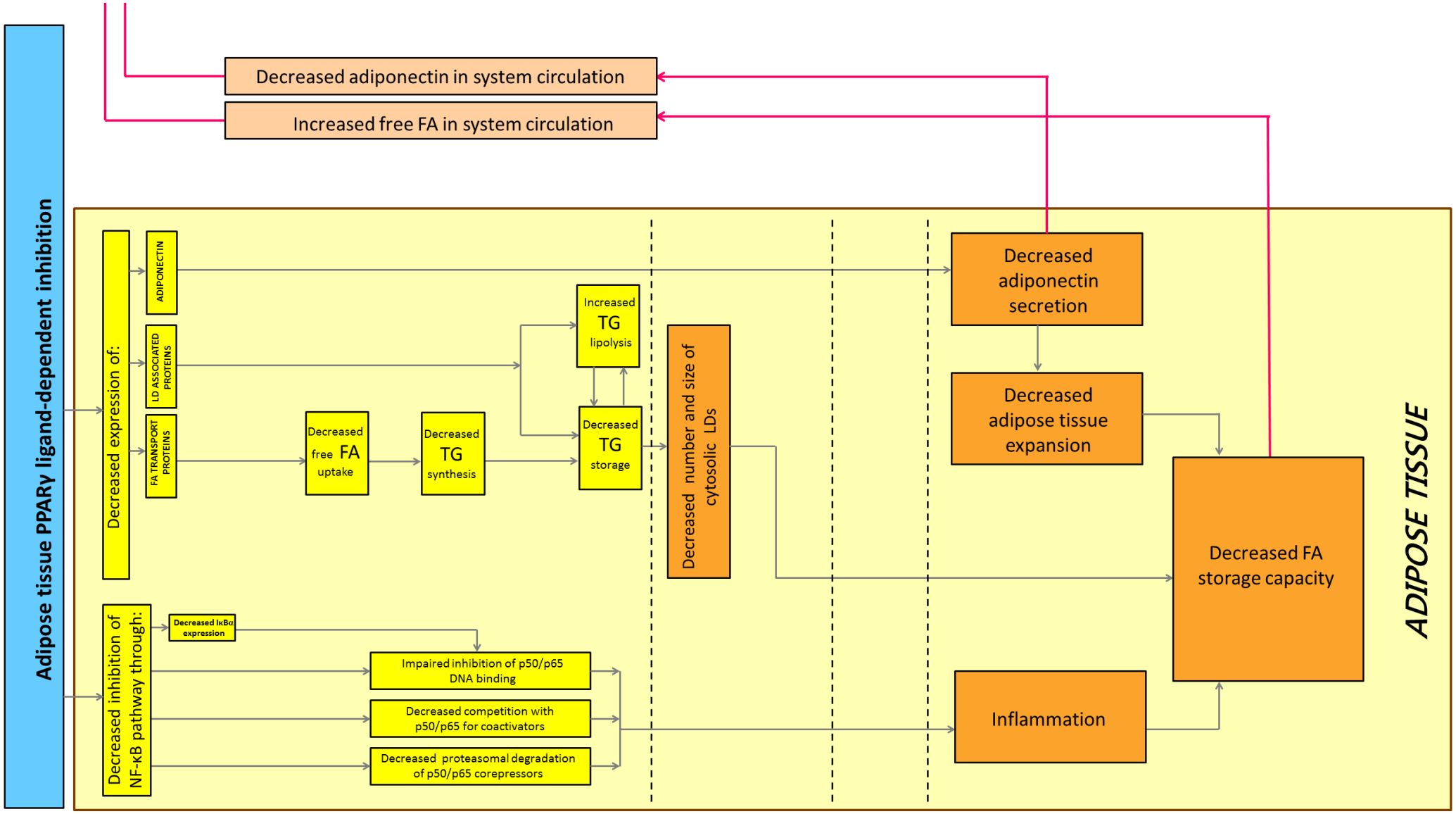


Two specific features of our MoAs

- **MIEs**
  - *PPARγ Inhibition*
  - *PPARγ Activation*
- **Sites of action**
  - *Tissue specificity*
  - *Ligand binding site*

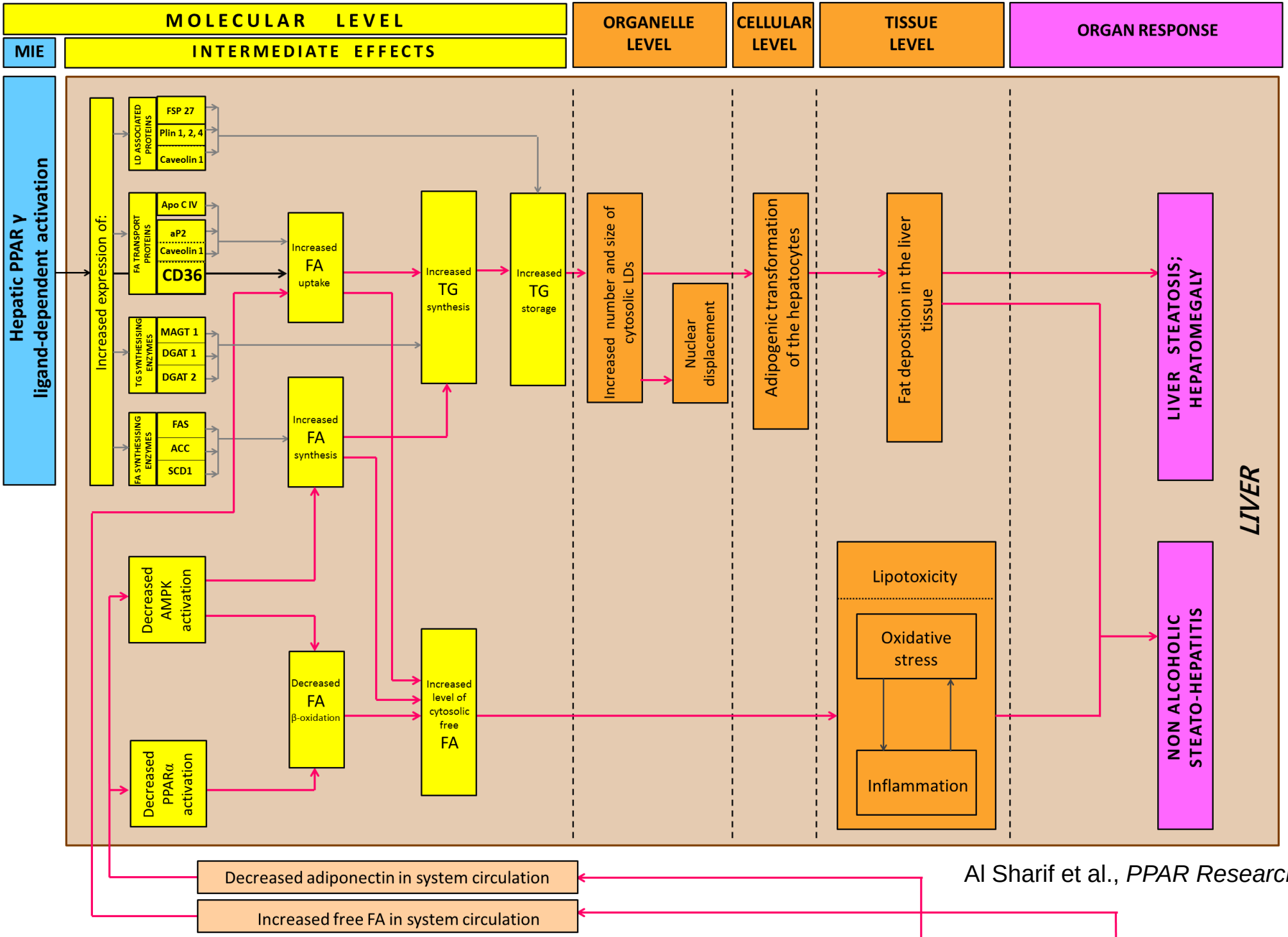


MoA with MIE in adipose tissue



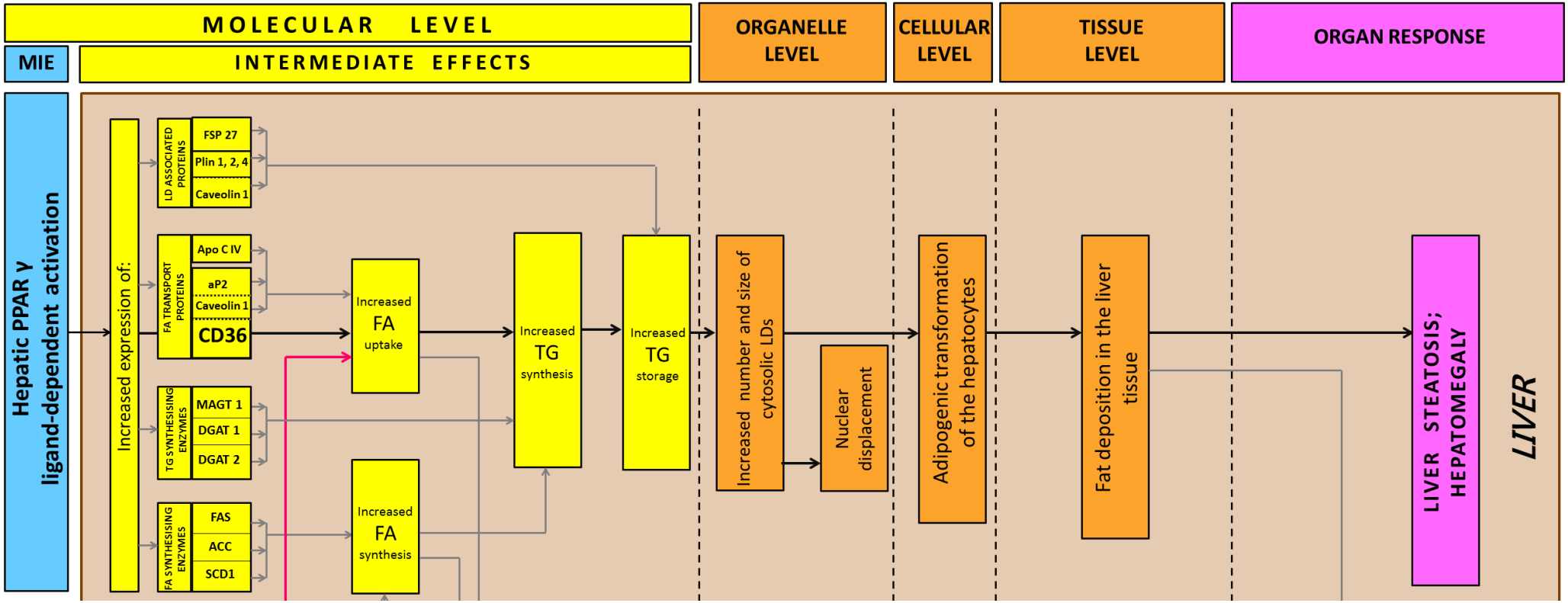
Al Sharif et al., *PPAR Research*, 2014

MoA with MIE in adipose tissue



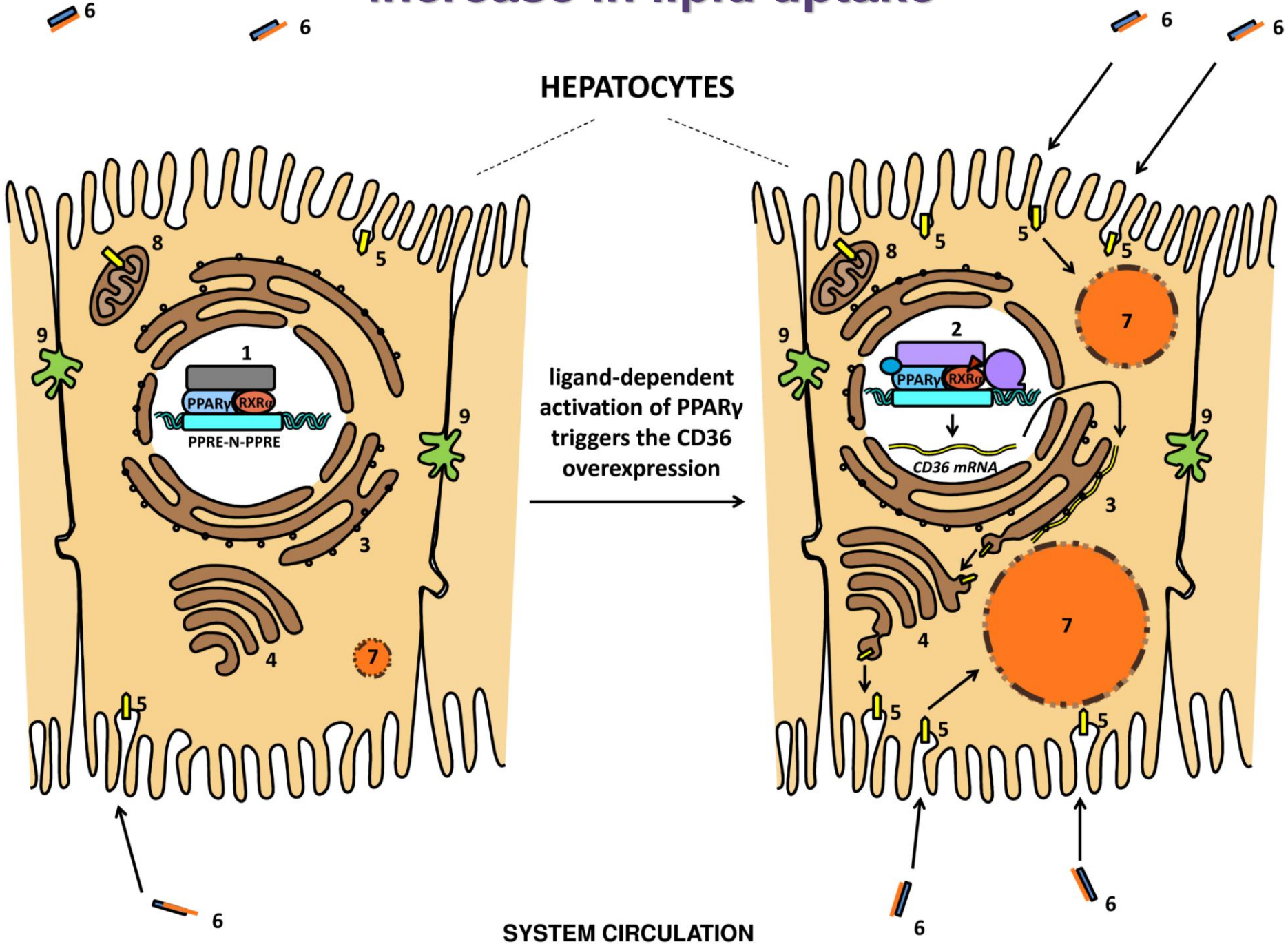
Al Sharif et al., *PPAR Research*, 2014

MoA with MIE in liver



Al Sharif et al., *PPAR Research*, 2014

PPARγ triggered and CD36 protein mediated increase in lipid uptake



WORKFLOW

IN SILICO STUDY OF THE MIE

MIE

intermediate events

end-point

QUANTITATIVE MoA EVALUATION

2

PDB SEARCH FOR PPARγ COMPLEXES

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structural and biological data

PHARMACOPHORE

based on full PPARγ agonists

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OECD weight-of-evidence classification

DATA COLLECTION

expression of PPARγ target proteins

EVALUATION OF KEY EVENTS

WORKFLOW

MIE	intermediate events	end-point

OECD WEIGHT-OF-EVIDENCE CLASSIFICATION

2

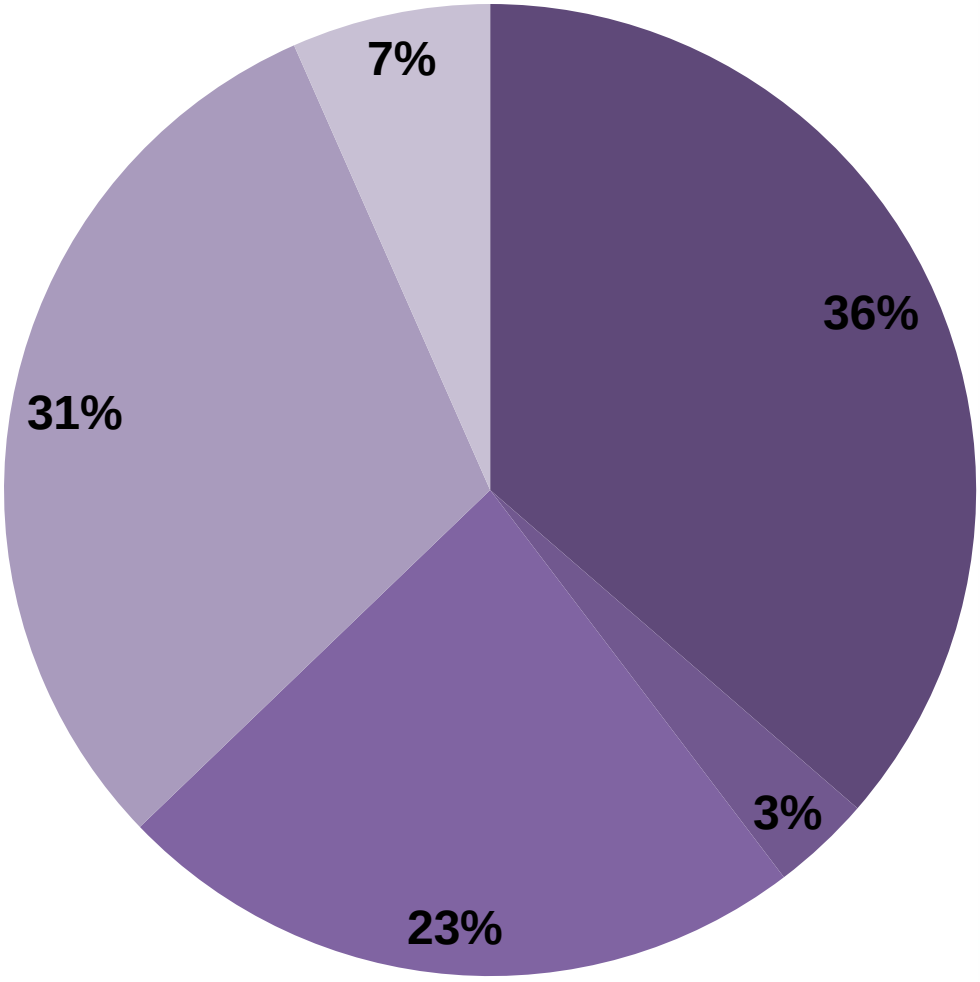
- ***Extent of development of the assay***
- ***Relationship between key event and apical endpoint***

- ***very strong***
- ***strong***
- ***moderate***
- ***weak***
- ***very weak***

MoA EVALUATION

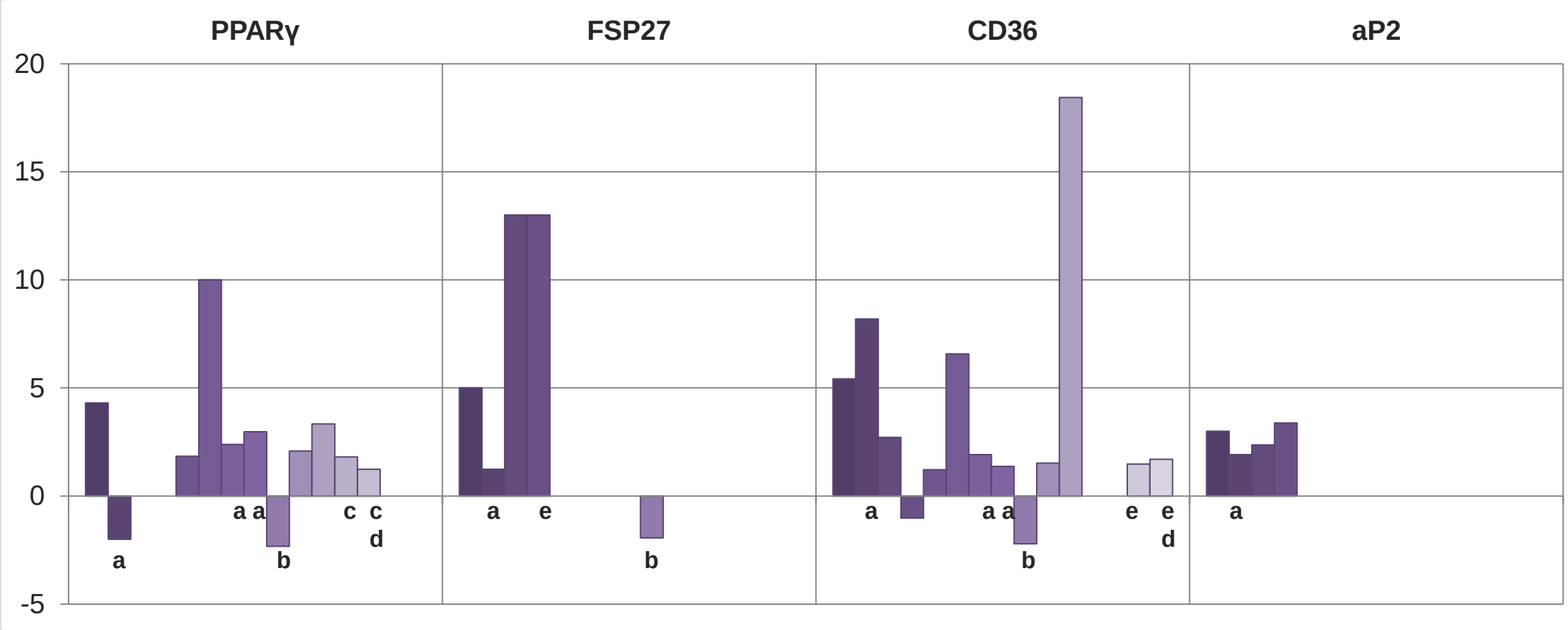
ANALYSED EVENTS

- MIE
- LD ASSOCIATED PROTEINS
- FA TRANSPORT PROTEINS
- INCREASED FA UPTAKE
- INCREASED TG STORAGE
- INCREASED NUMBER OR SIZE OF LD
- NAFLD AT TISSUE AND ORGAN LEVEL



Distribution of the assays by type

QUANTITATIVE DATA FOR KEY EVENTS - 1



Effect of natural ligands (mainly from diet) on the mRNA levels of PPARγ and some of its targets

General experiment type: WT + HFD (variants) + quantitative RT-PCR analysis

Aditional:

a PPARγ deficient line

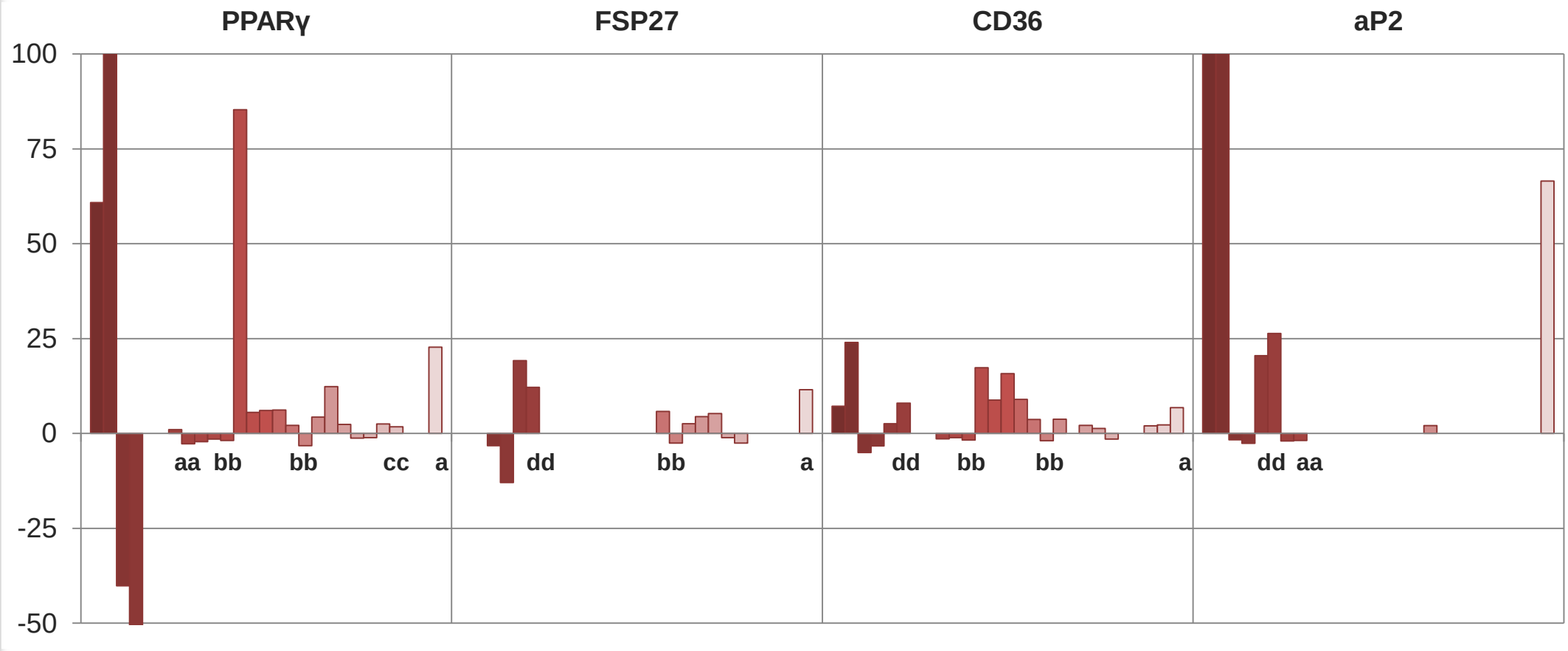
b *In vitro* treatment with ceramide (endogenous suppressor)

c Semiquantitative RT-PCR analysis

d Obese, hypercholesterolemic, diabetic line

e Microarray analysis

QUANTITATIVE DATA FOR KEY EVENTS - 2



Effect of genetic manipulation and genetic background on the mRNA and protein levels of PPARγ and some of its targets

General experiment type: PPARγ up- or downregulation + CD + quantitative RT-PCR analysis

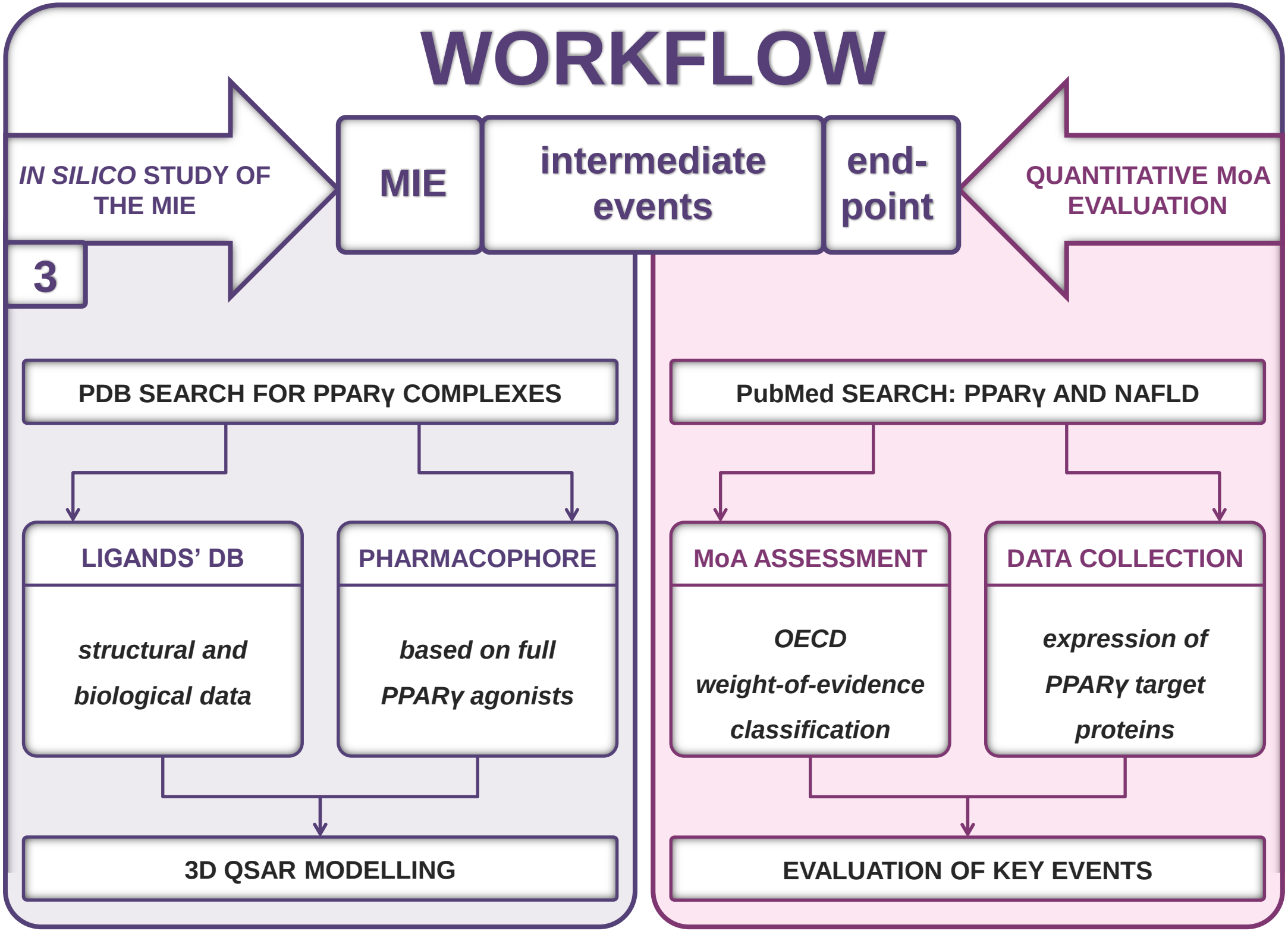
Aditional:

a Western blot analysis

b HFD

c Semiquantitative RT-PCR analysis

d Microarray analysis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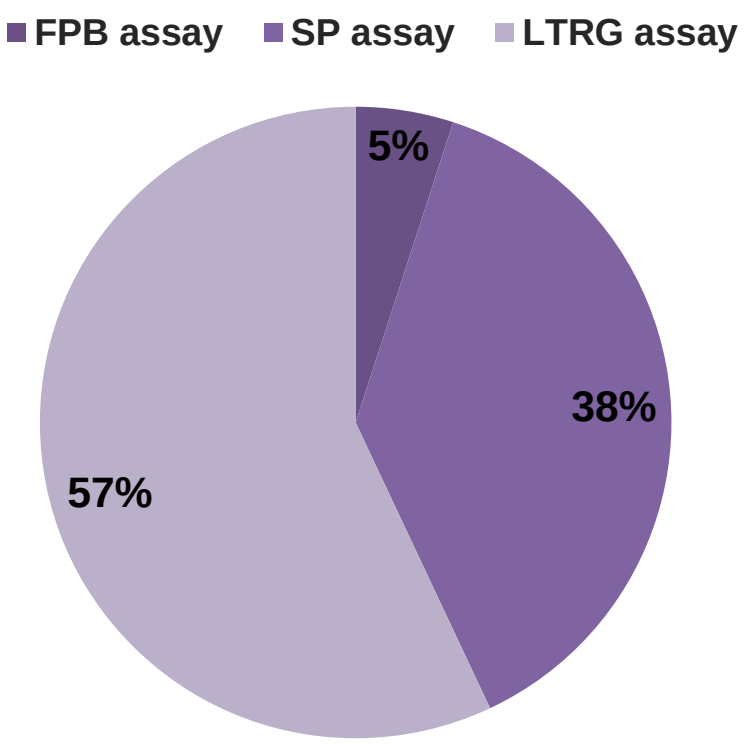
EVALUATION OF KEY EVENTS

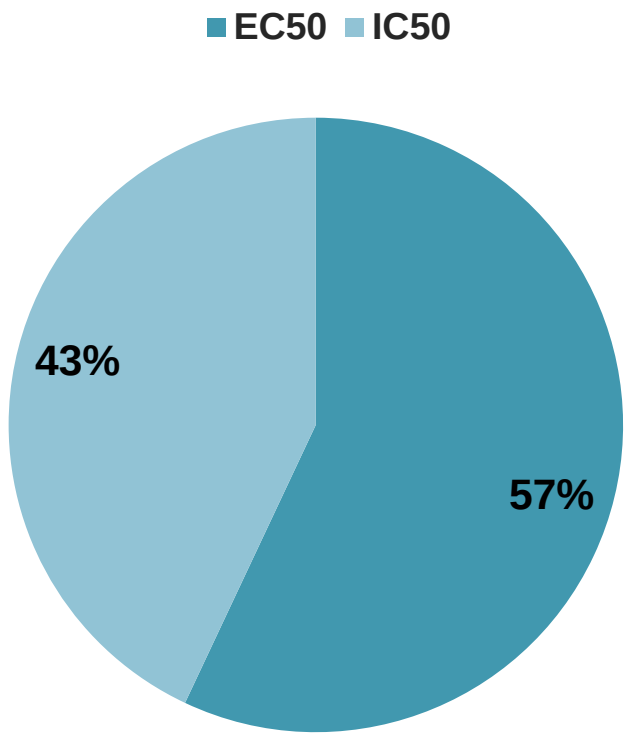
PPARγ LIGANDS' DATABASE

- Activity data for 250 compounds extracted so far;
- Distribution of data according to:

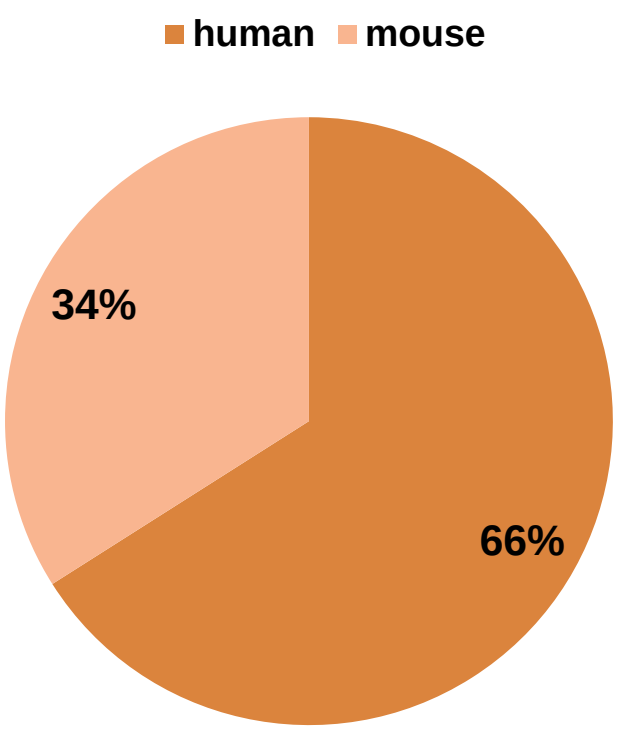
A) Type of the assay



B) Reported endpoint



C) Experimental subject



In silico modelling: 3D QSAR

PDB SEARCH FOR PPARγ COMPLEXES

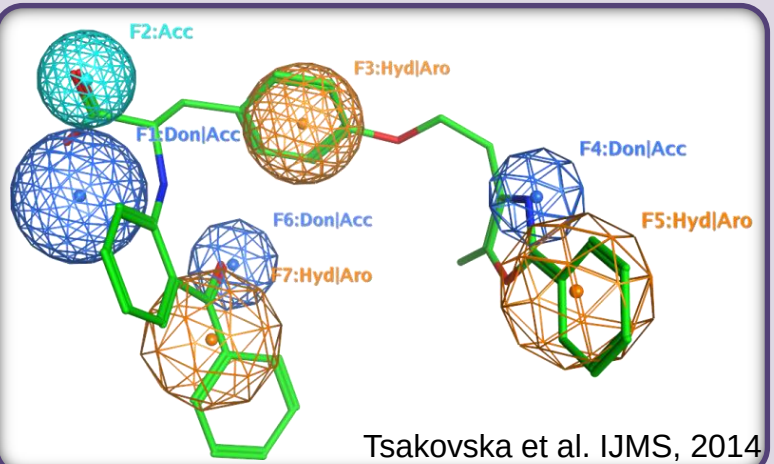
TRAINING SET FROM OUR DATABASE OF 250 PPARγ LIGANDS

- Full agonists
- Human & Hepatic
- Same assay
- EC50 & IC50 (2 log units activity span)
- structural variability

PHARMACOPHORE GENERATION AND REFINEMENT

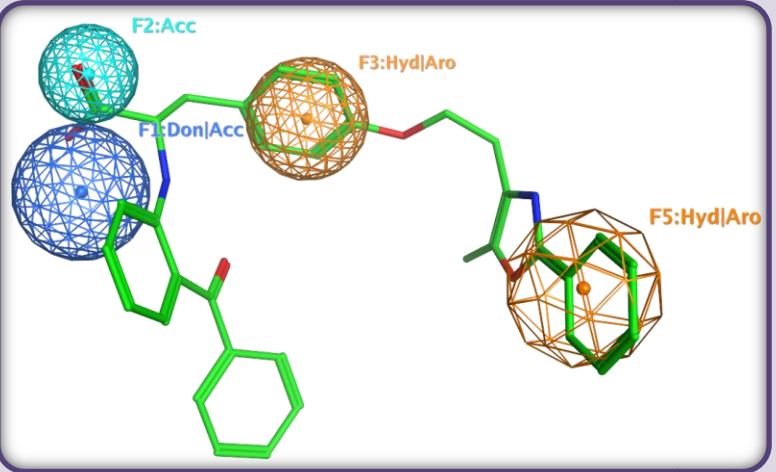
- F1: Don|Acc
- F2: Acc
- F3: Hyd|Aro
- F4: Hyd|Aro

7 feature pharmacophore



Tsakovska et al. IJMS, 2014

4 feature pharmacophore



N = 21 COMPOUNDS

Docking + pharmacophore

N = 18 (BEST MODEL)

CoMFA/CoMSIA

N = 21 (BEST POSES)

In silico modelling: CoMSIA

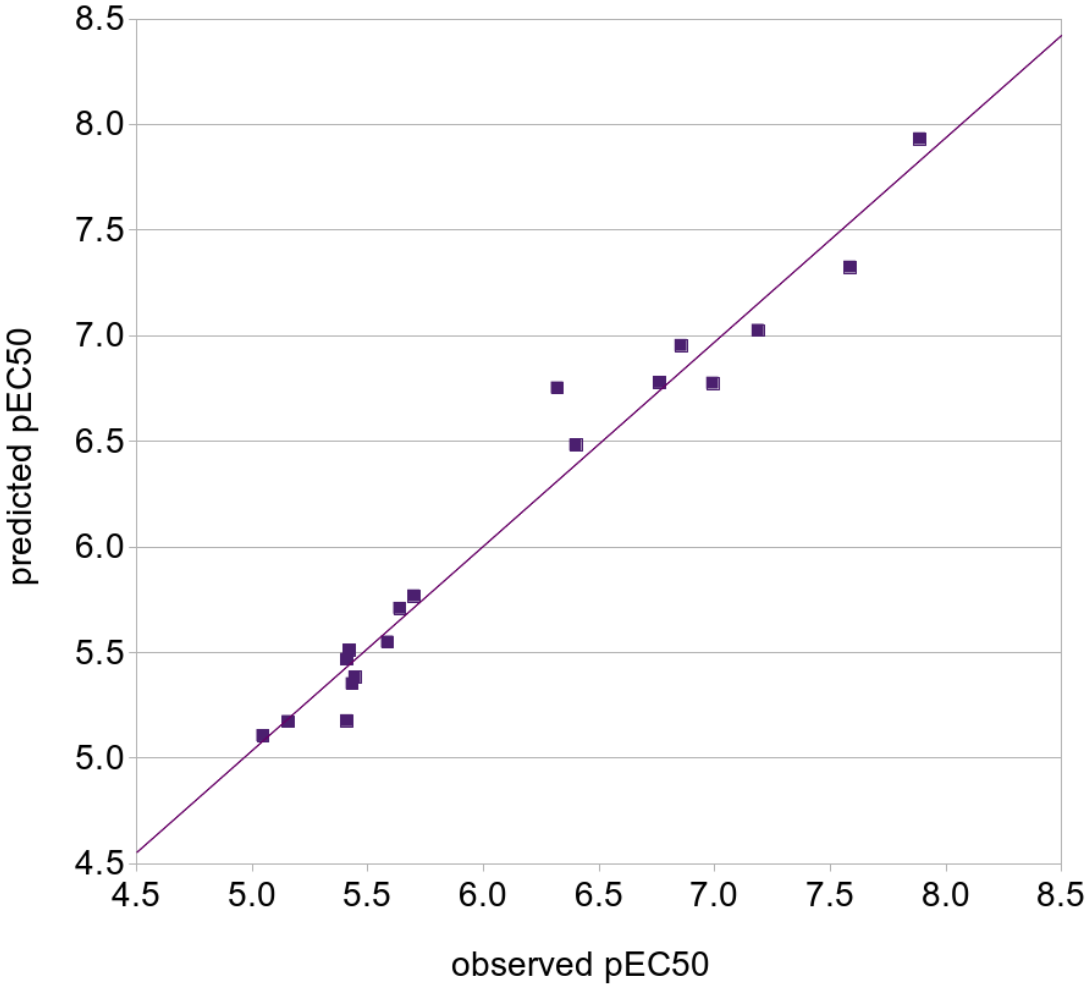
PREDICTION

- $q_{CV}^2 = 0.716$
- $C_{opt} = 5$
- SEP = 0.564

FIT

- $r^2 = 0.965$
- SEE = 0.198
- $p << 0.05$

Training set
Predicted vs. Observed pEC50

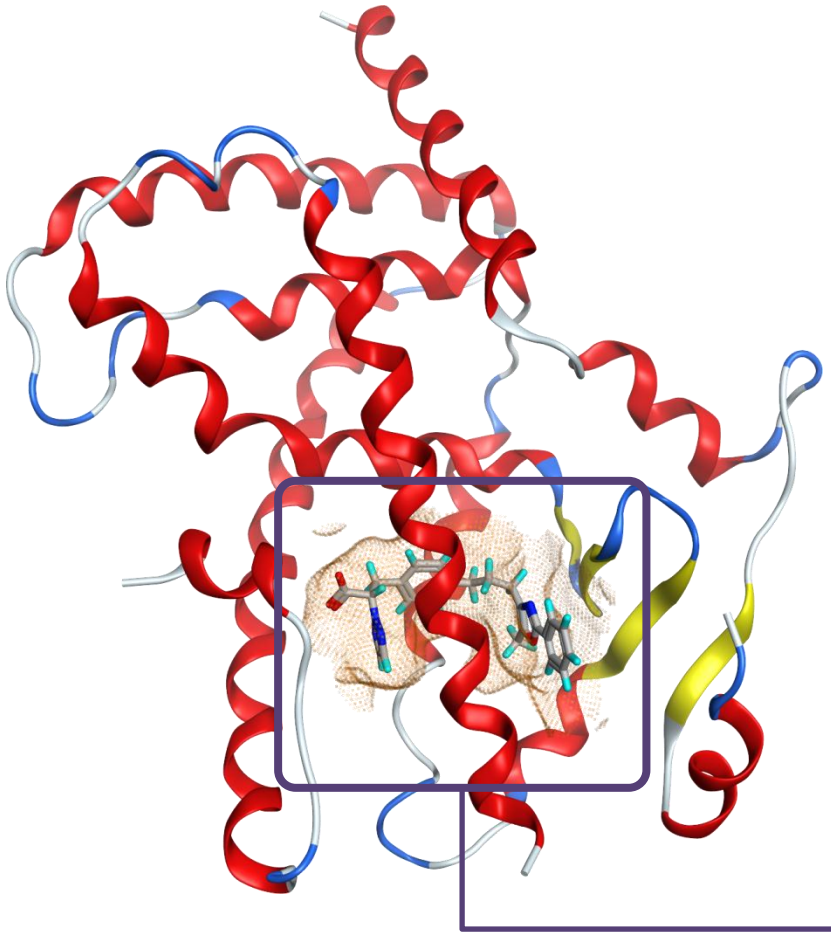


$pEC50_{pred} = 0.97\ pEC50_{obs} + 0.20$

$R^2 = 0.967$

CONTOUR MAP OF PPARγ AGONISTS

PPARγ ligand binding domain with the template structure

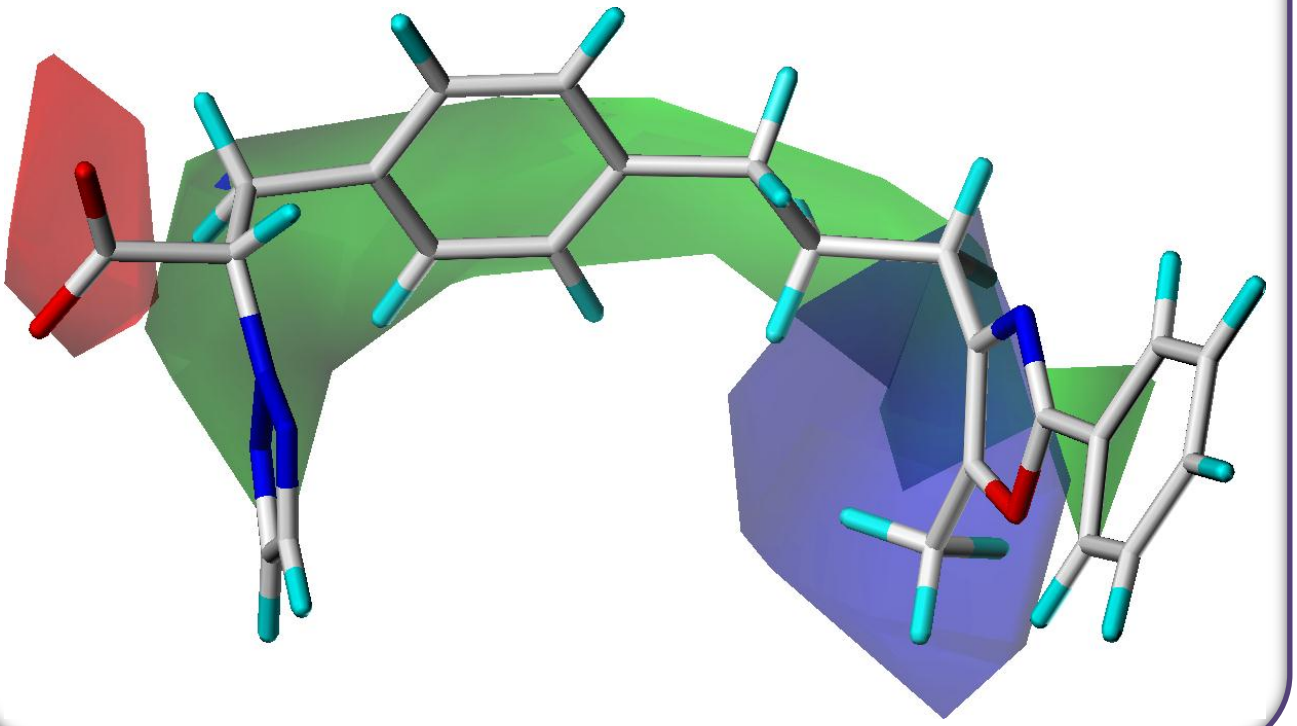


Favorable for activity molecular fields

electronegative

bulky

electropositive



3D QSAR – EXTERNAL VALIDATION

PDB SEARCH FOR PPARγ COMPLEXES

PHARMACOPHORE

TRAINING SET

TEST SET SELECTION

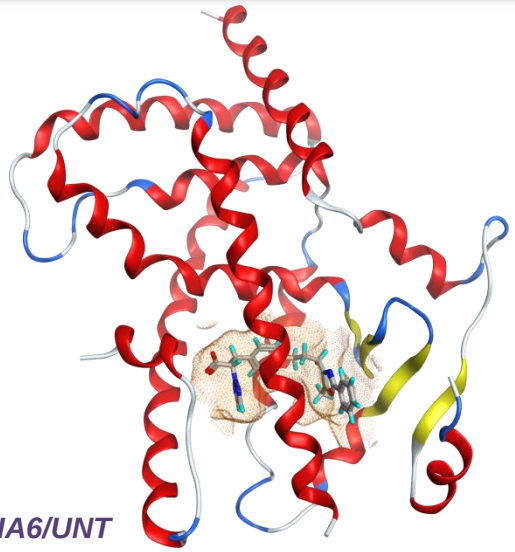
- Full agonists
- Structure similarity
- BA data inspection

BA PREDICTION BY THE BEST MODEL (N = 18)

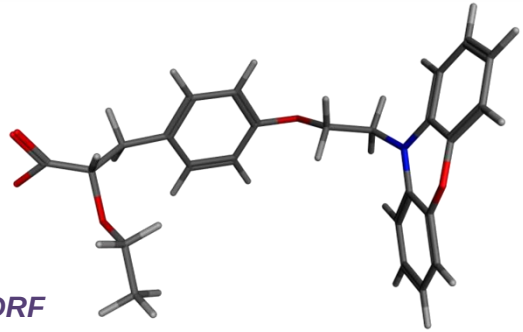
EXTERNAL VALIDATION

$$R^2_{pred} = 1 - \frac{\sum \left(Y_{pred(test)} - Y_{exp(test)} \right)^2}{\sum \left(Y_{exp(test)} - \overline{Y}_{exp(training)} \right)^2}$$

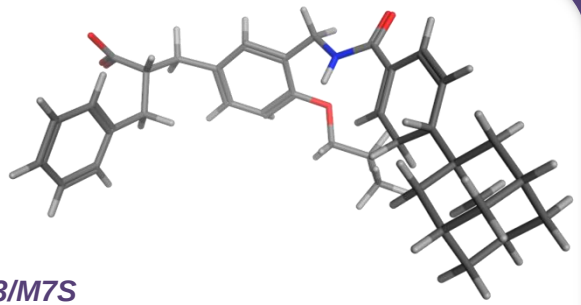
TEST SET: PREDICTIVE $R^2 = 0.628$



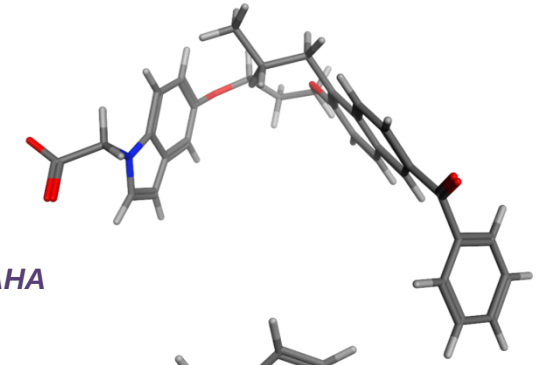
3IA6/UNT



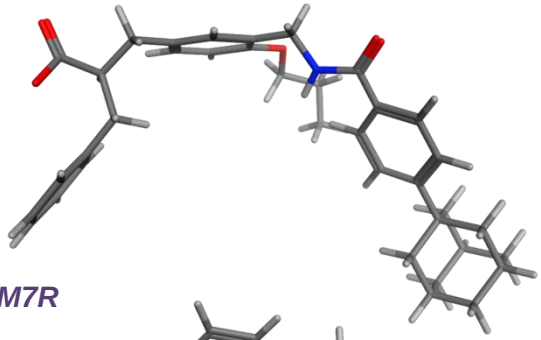
1NYX/DRF



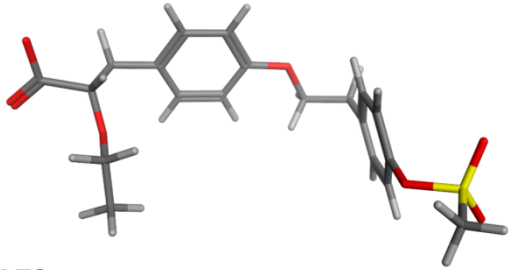
3AN3/M7S



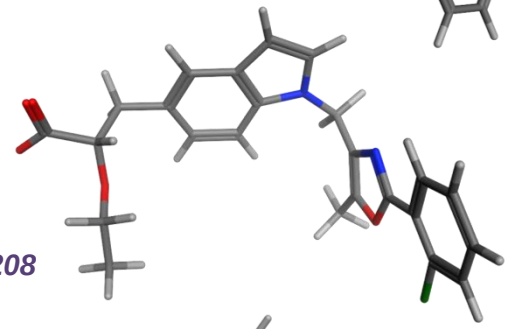
2F4B/AHA



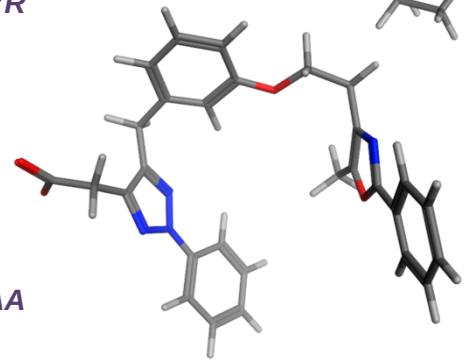
3AN4/M7R



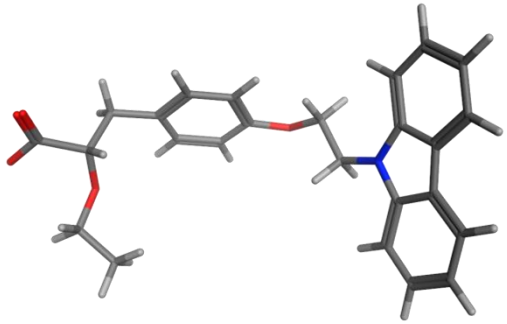
1I7I /AZ2



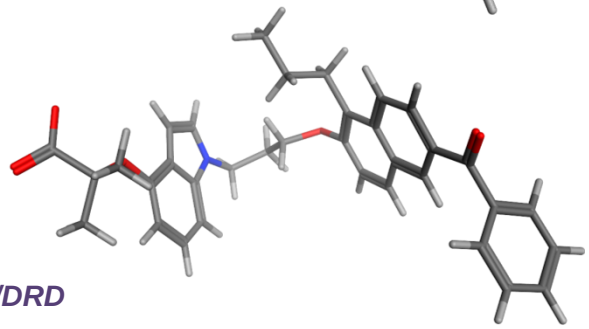
2GTK/208



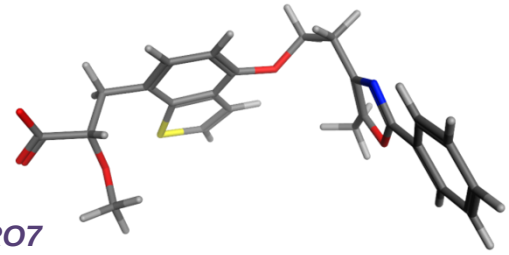
3BC5/ZAA



1KNU/YPA



2HWR/DRD



3G9E/RO7

SUMMARY

MoA key events outlined and evaluated

PPARγ ligands' database created

Predictive and explanatory *in silico* models derived

PERSPECTIVES

Refinement, update and further evaluation of MoAs

Further development of the *in silico* model (activity and structural data variability)

ACKNOWLEDGEMENTS

BULGARIAN ACADEMY of SCIENCES 1869

Department QSAR & Molecular modelling, IBPhBME – BAS

Ilza Pajeva **Petko Alov**

Ivanka Tsakovska **Vessela Vitcheva**

Cosmetics Europe
the personal care association

SEURAT-1

COSMOS

SEVENTH FRAMEWORK PROGRAMME

EUROPEAN UNION

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ESF Bulgaria
EUROPEAN SOCIAL FUND

SEURAT-1 Research Initiative

Towards the replacement of in vivo repeated dose systemic toxicity testing

- Major European research initiative addressing the global long-term strategic target SEURAT - *Safety Evaluation Ultimately Replacing Animal Testing*.

- Jointly funded by the European Commission and Cosmetics Europe for 5 years starting from Jan 2011.

- Aim: Provide a blueprint for future implementation of mechanism-based, integrated toxicity testing strategies into modern safety assessment approaches.

SEURAT-1 at WC9

The SEURAT-1 corner is hosted on the JRC ECVAM booth

Meet SEURAT-1, get information about SEURAT-1 presence at WC9 and gather contacts

Cosmetics Europe
the personal care association

European Commission

More information on www.seurat-1.eu

Thank you

for your

attention!