AOPs, biological networks, and data analysis

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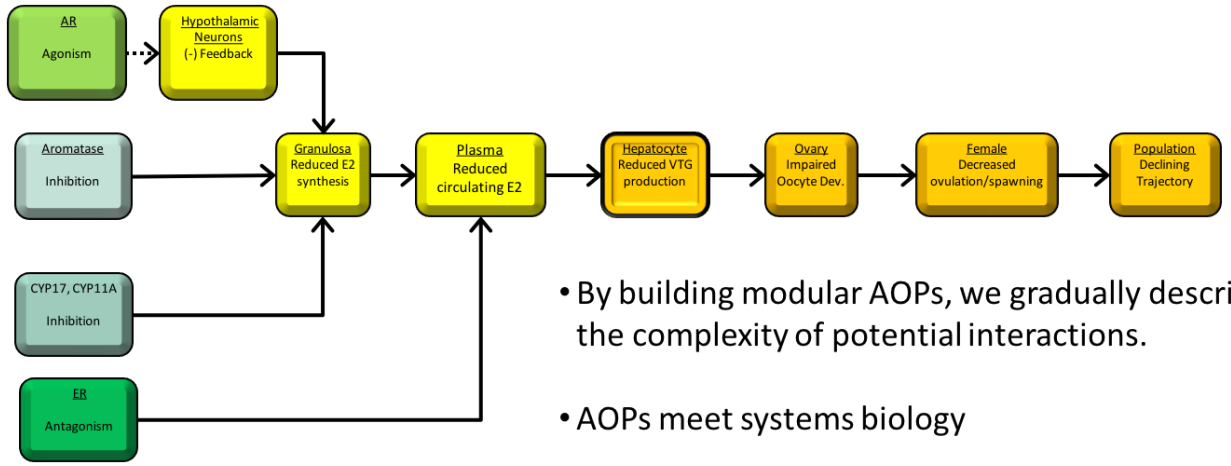
- Bridging biological networks and AOPs
- Using Omics data with AOPs

Adverse Outcome Pathways are:

-Pragmatic and simple representations of essential events

-Composed of measurable Events generally representing in vitro or in vivo assays

- Linear but can be integrated to form networks



- By building modular AOPs, we gradually describe the complexity of potential interactions.
- AOPs meet systems biology

But..

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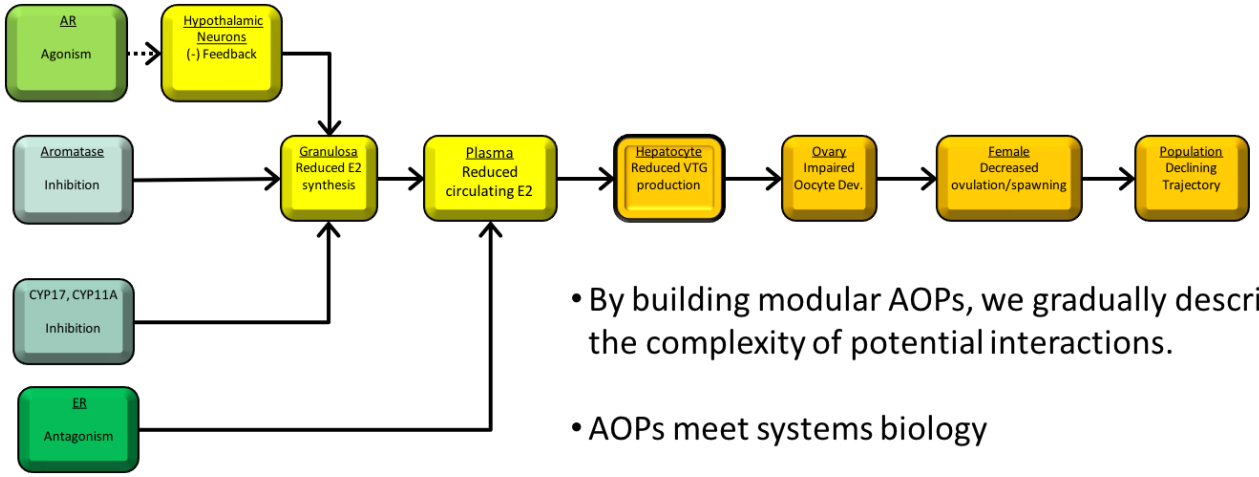
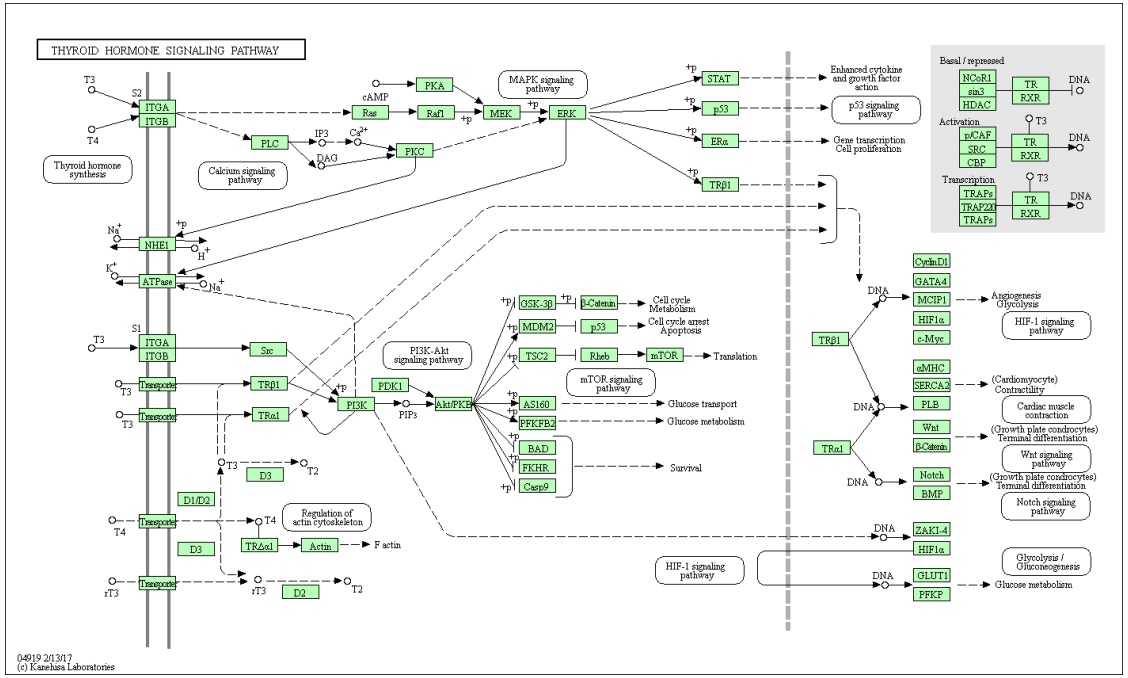


- By building modular AOPs, we gradually describe the complexity of potential interactions.
- AOPs meet systems biology

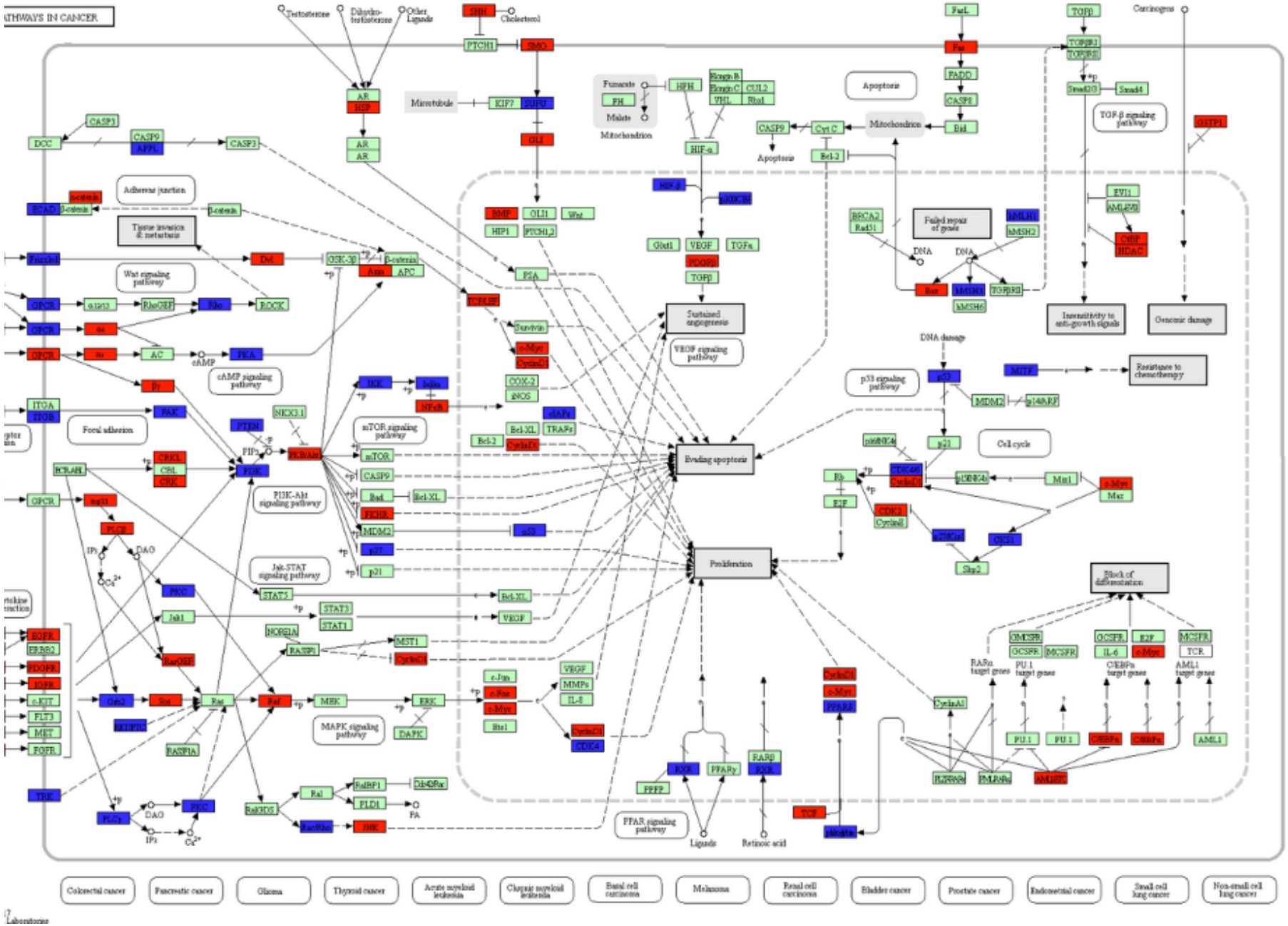
Life is complicated



KEGG thyroid signaling pathway

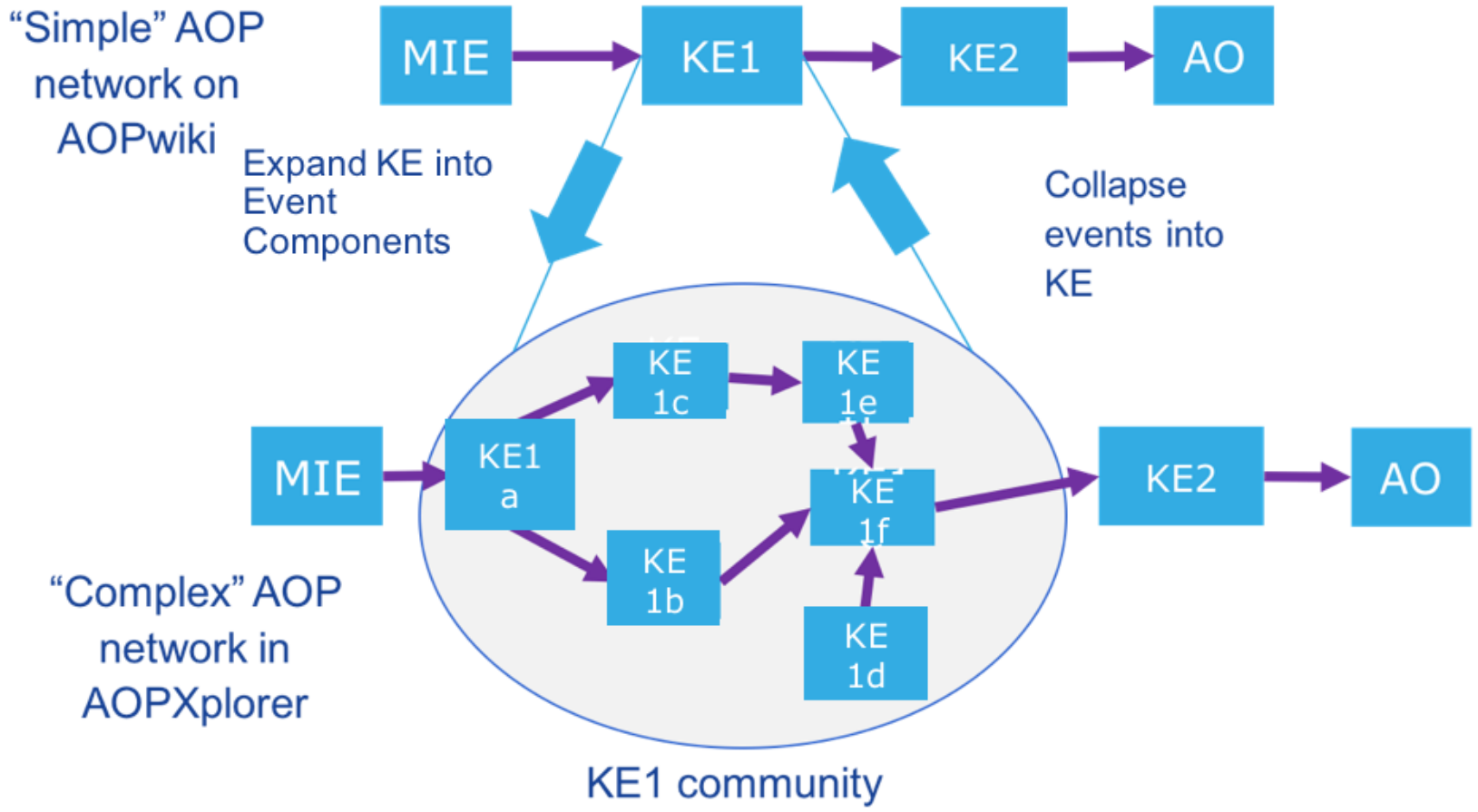
Omics can tell us a lot about what's happening in tissue

But this doesn't fit well into the simple AOP concept



AOPXplorer – Simple and Complex networks

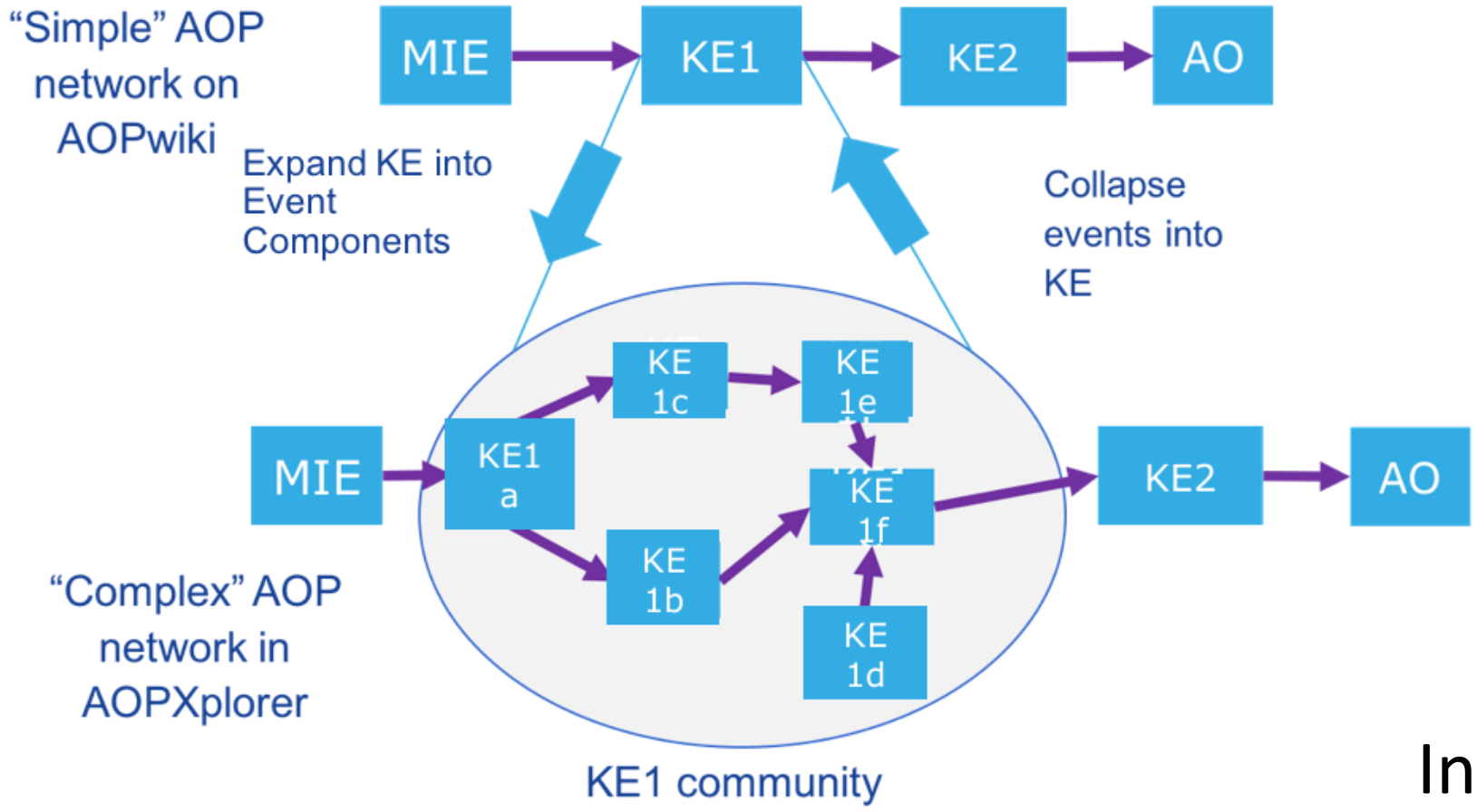
Networks will bridge AOPs and biological pathways using KE communities



Multiple components per Key Event

AOPXplorer – Simple and Complex networks

Networks will bridge AOPs and biological pathways using KE communities

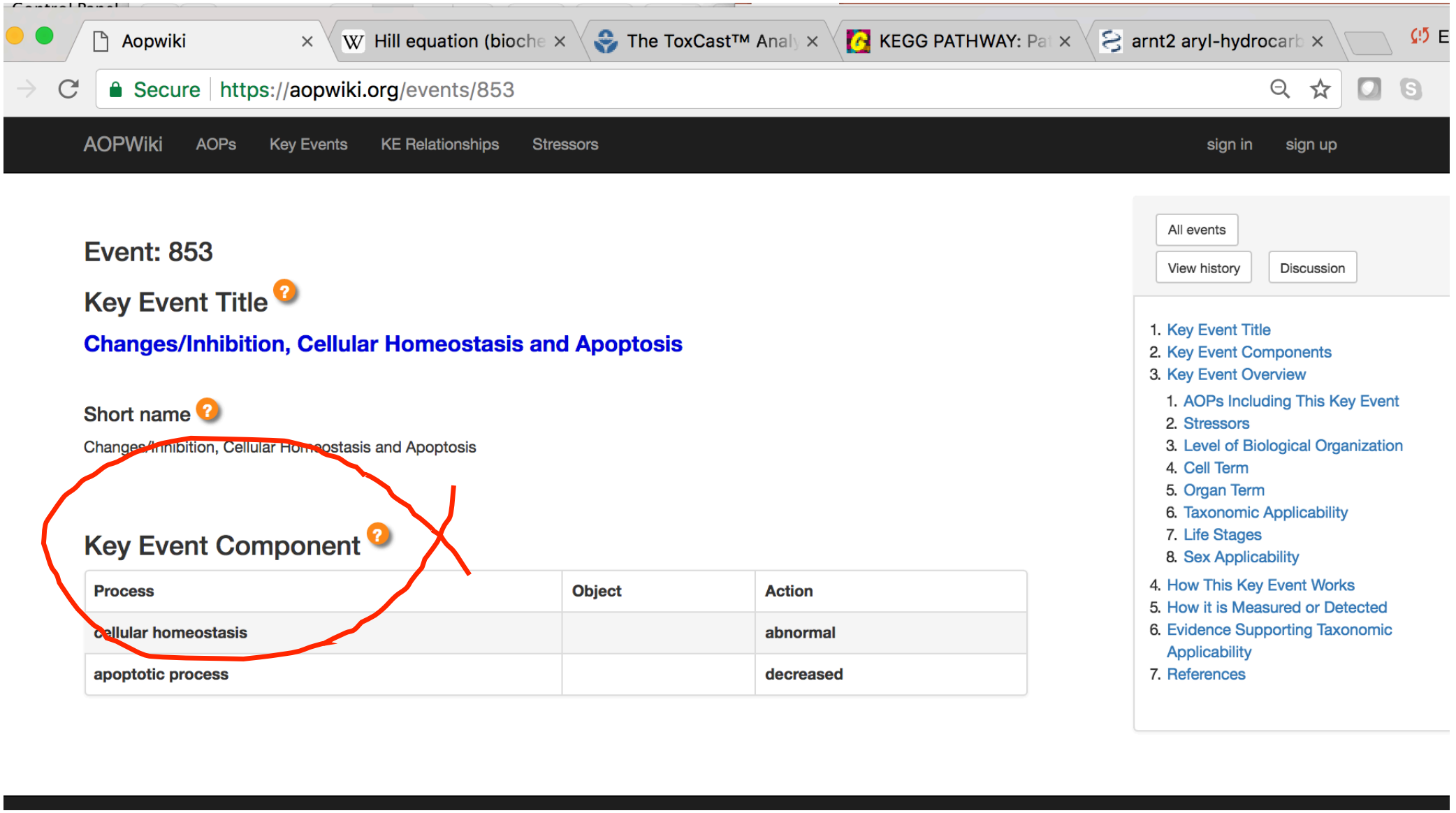


AOP

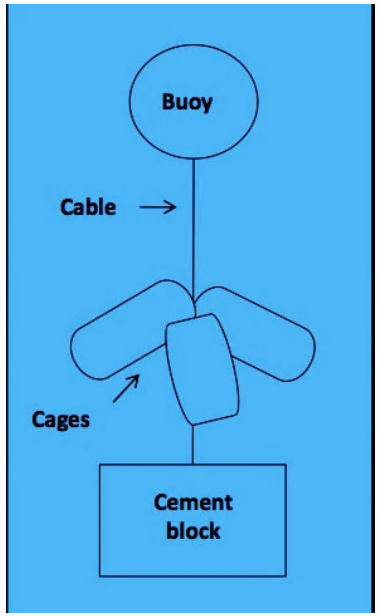
Biological networks

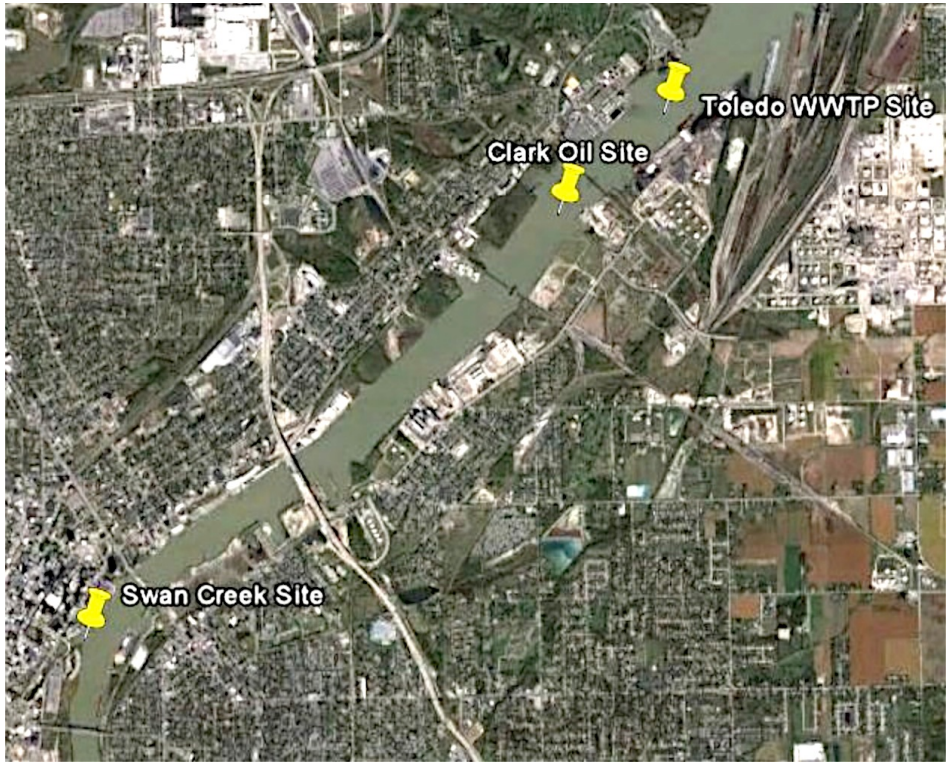
Multiple components per Key Event

Interface with systems biology and biological networks


Aopwiki
Hill equation (bioche
The ToxCast™ Analy
KEGG PATHWAY: Pat
arnt2 aryl-hydrocarb
Secure
https://aopwiki.org/events/853
AOPWiki
AOPs
Key Events
KE Relationships
Stressors
sign in
sign up
Event: 853
Key Event Title
Changes/Inhibition, Cellular Homeostasis and Apoptosis
Short name
Changes/Inhibition, Cellular Homeostasis and Apoptosis
Key Event Component
Process
Object
Action
cellular homeostasis
abnormal
apoptotic process
decreased
All events
View history
Discussion
1. Key Event Title
2. Key Event Components
3. Key Event Overview
1. AOPs Including This Key Event
2. Stressors
3. Level of Biological Organization
4. Cell Term
5. Organ Term
6. Taxonomic Applicability
7. Life Stages
8. Sex Applicability
4. How This Key Event Works
5. How it is Measured or Detected
6. Evidence Supporting Taxonomic Applicability
7. References

Example: Monitoring of effects of chemicals in rivers on caged fathead minnows using transcriptomics

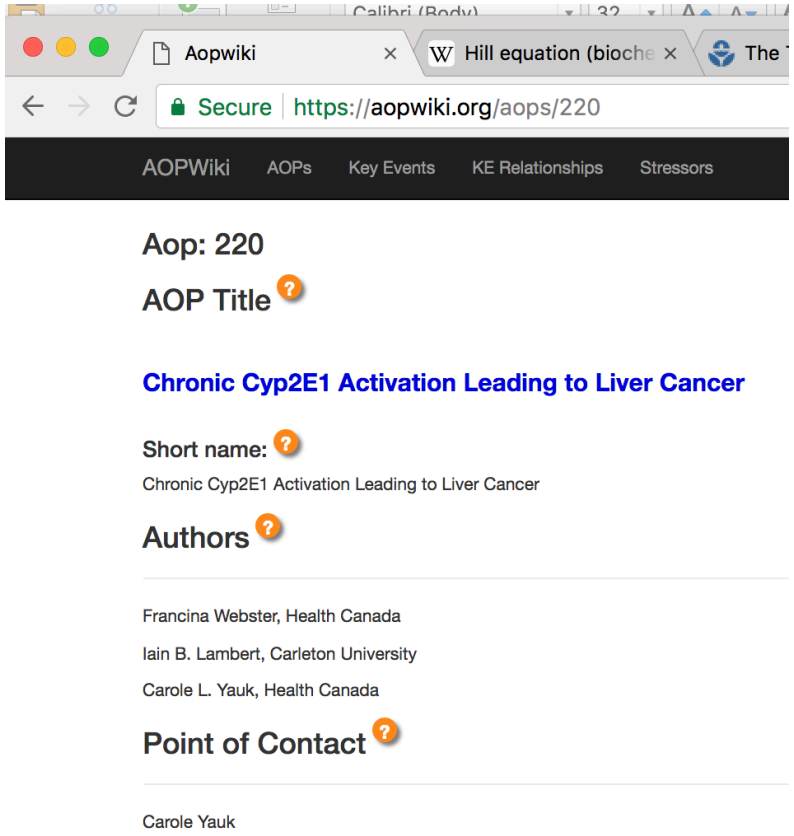


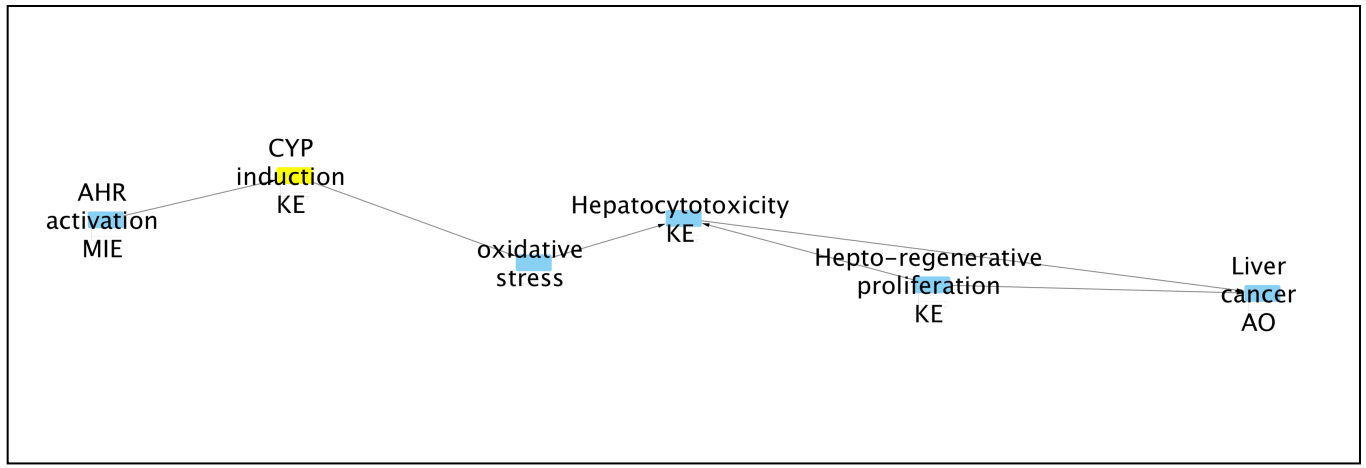


Maumee river and Detroit river

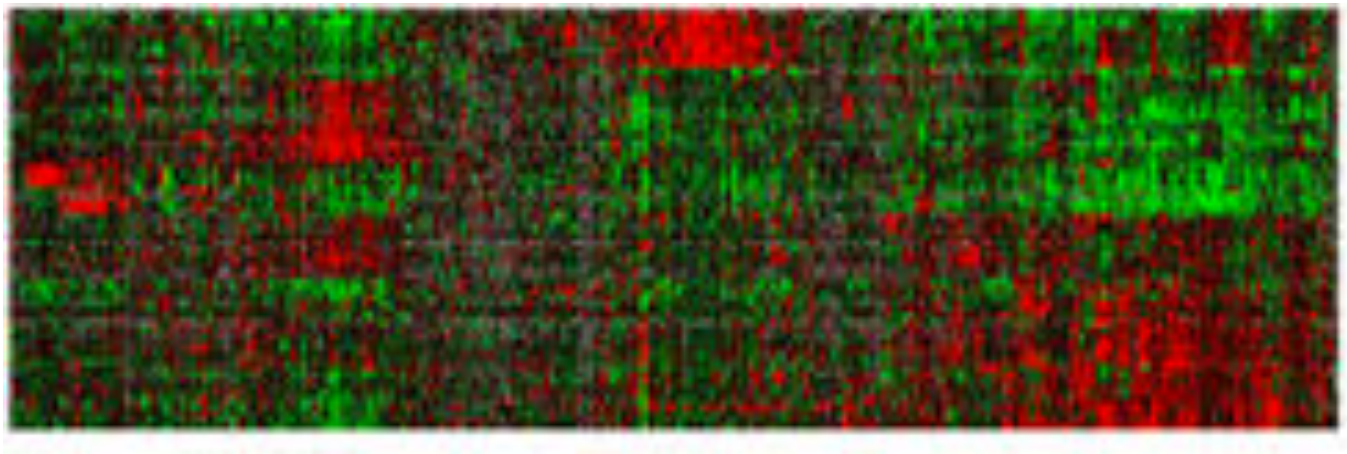
Sampling of the rivers has indicated a high incidence of tumor in native fish
Adult males exposed 4 days in rivers. Gene expression in liver analyzed.

How do we use AOPs to link observed effects to outcome of concern?



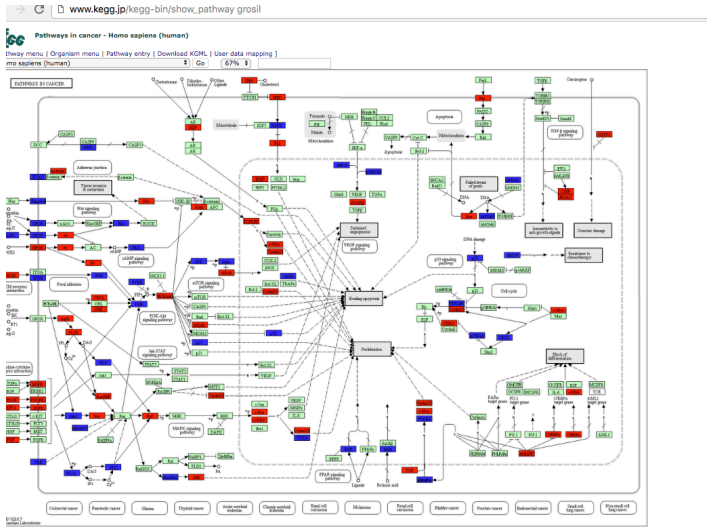


Cancer AOP

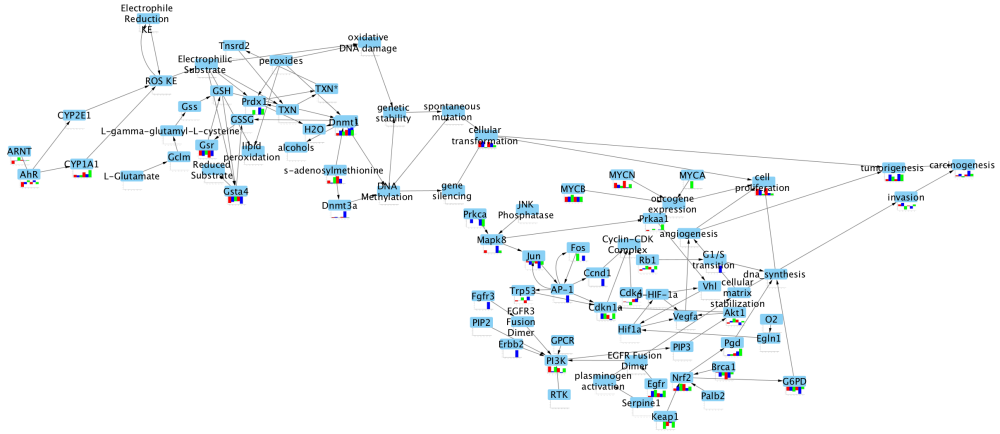


Liver gene expression

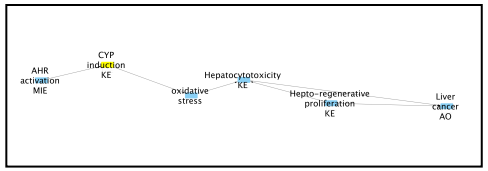
Use Key event components and subnetworks to relate to AOP



KEGG pathways in Cancer

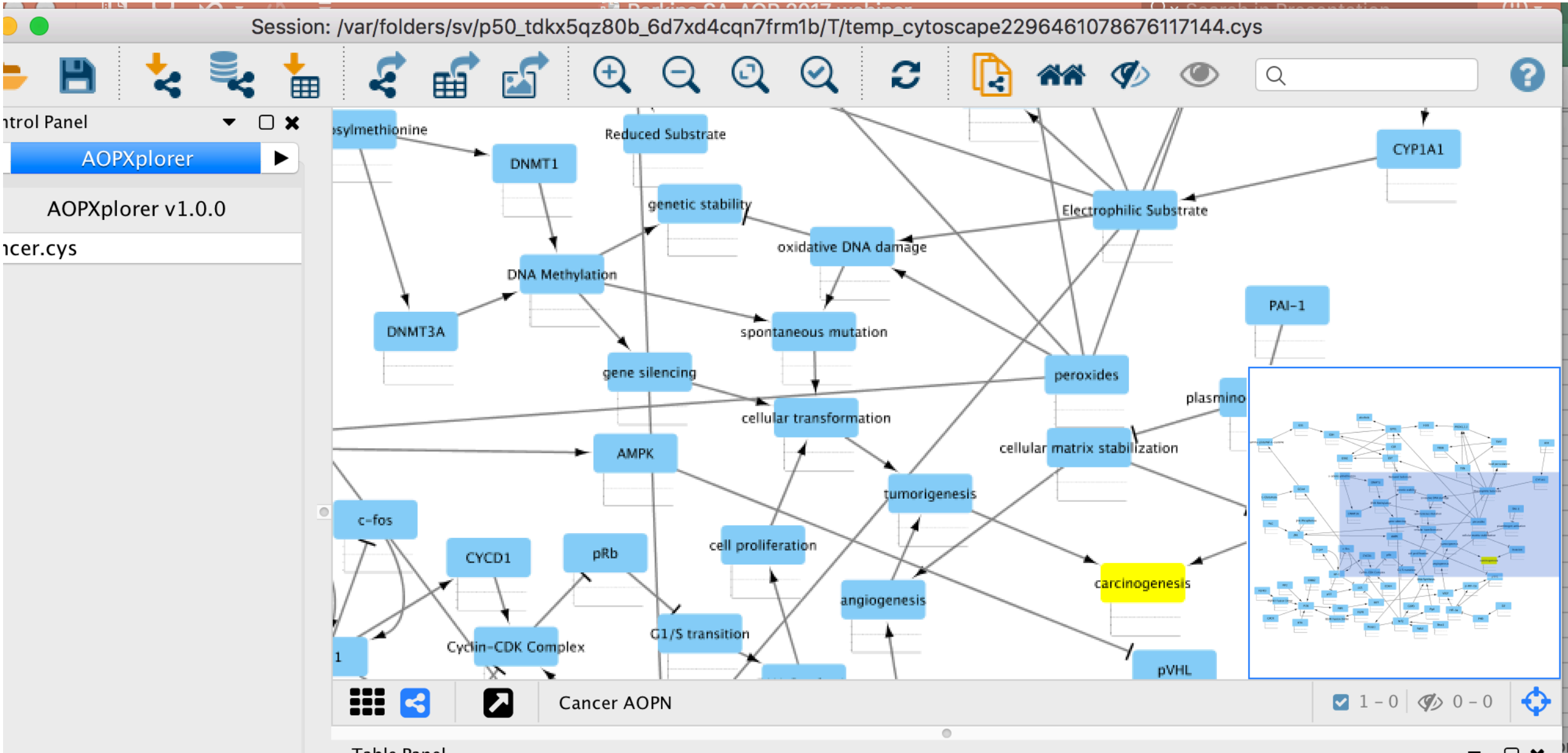


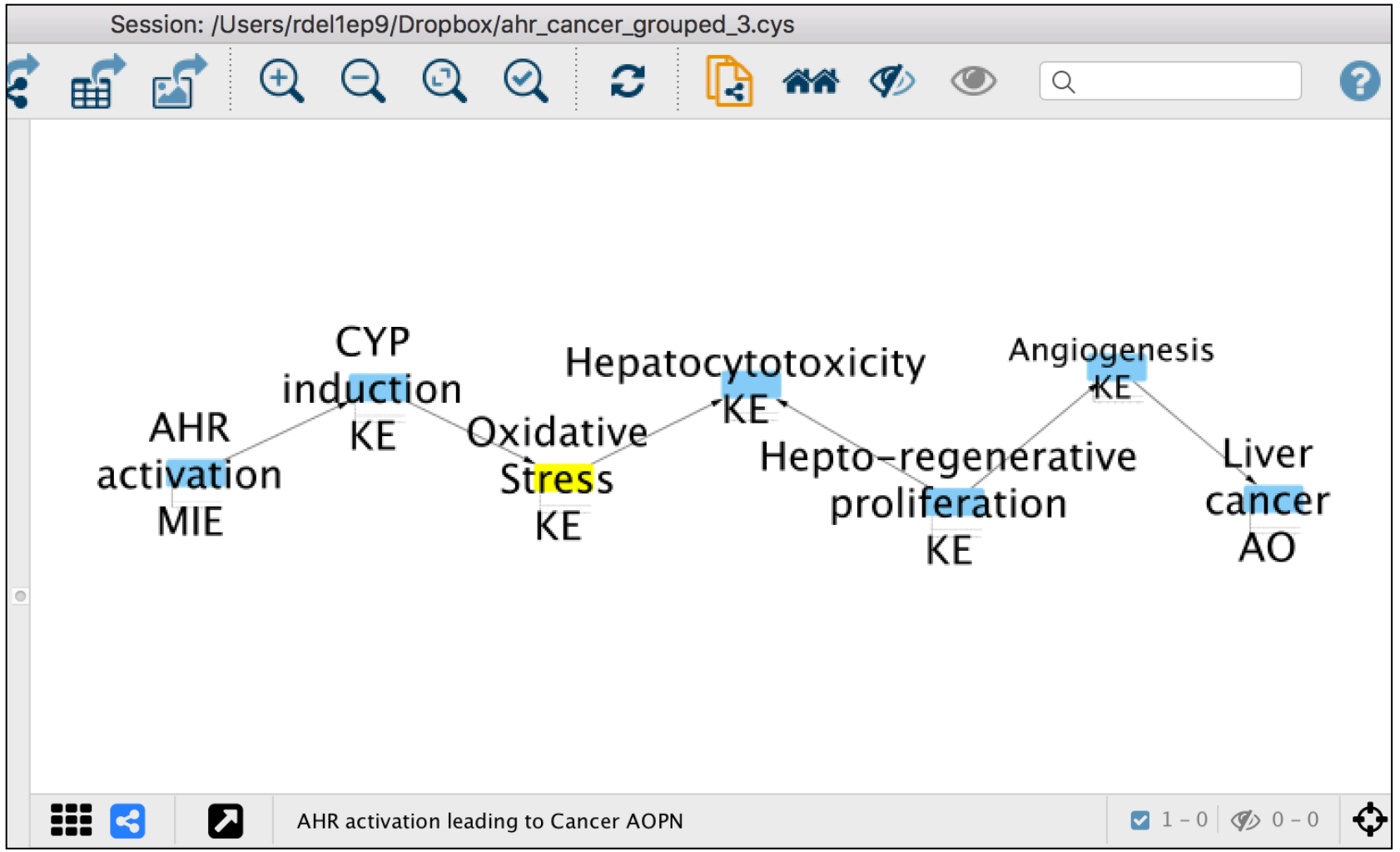
AhR activation leading to liver cancer network



Liver Cancer AOP

We first developed a detailed AhR cancer network

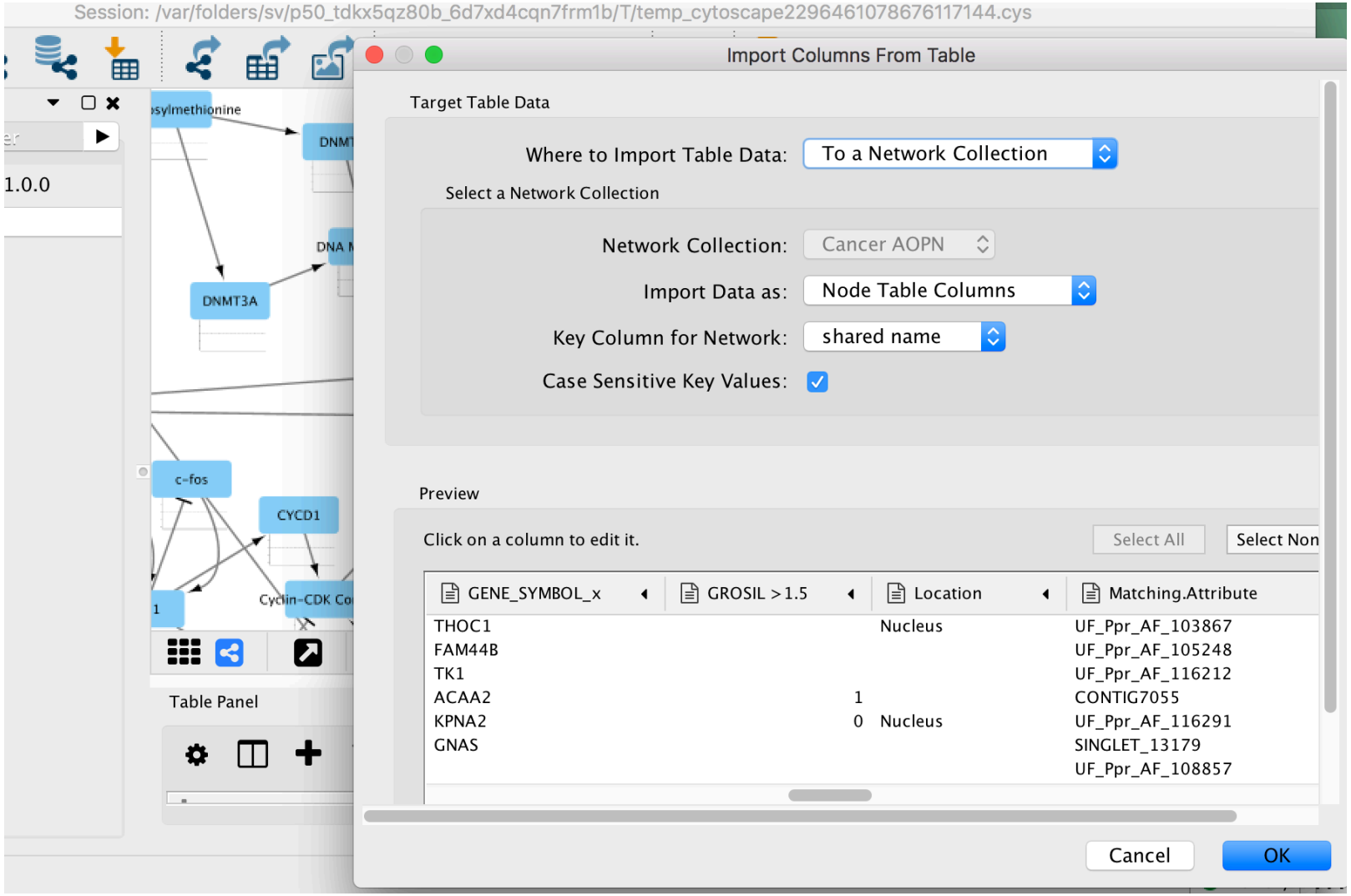


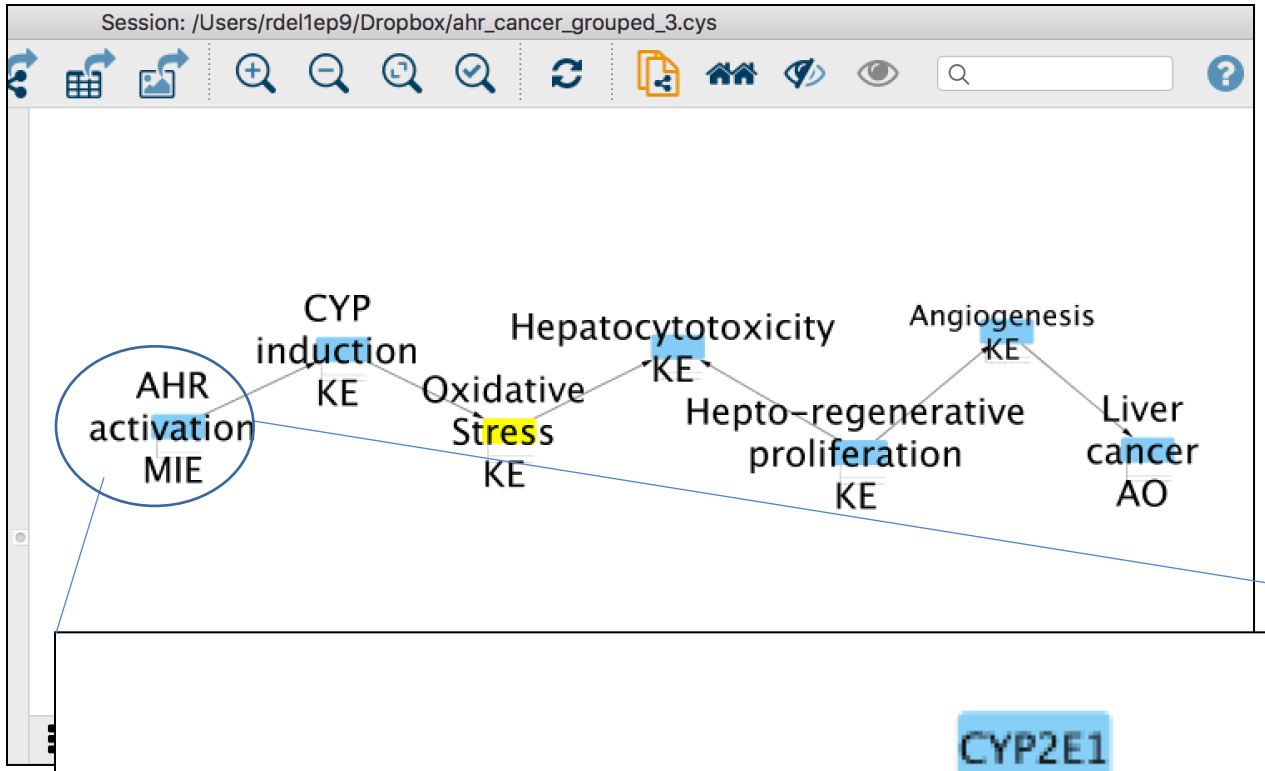


We fit the subnetworks of genes and pathways to relevant events in the AOP

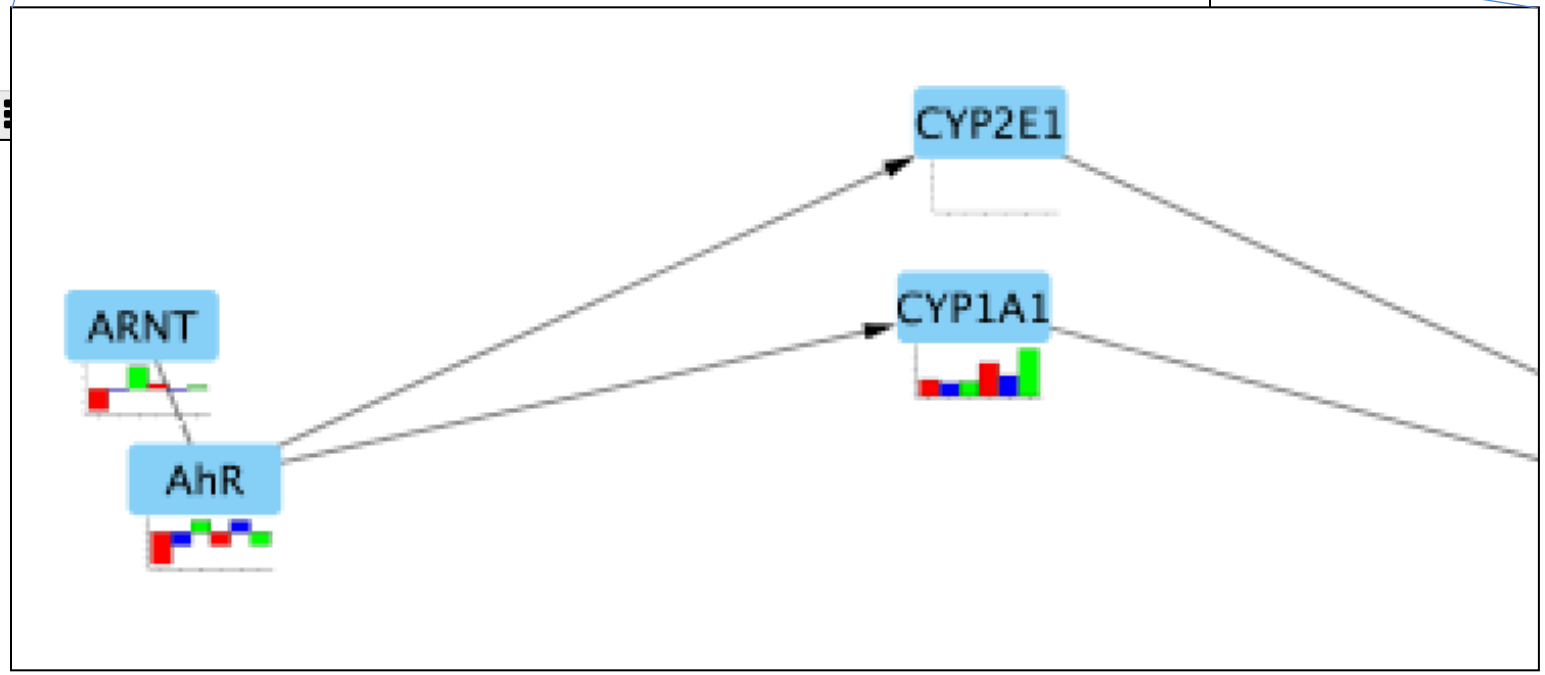
AOP for AhR activation leading to liver cancer

We imported transcriptomic, gene enrichment, PCR, and inferred values for genes in network

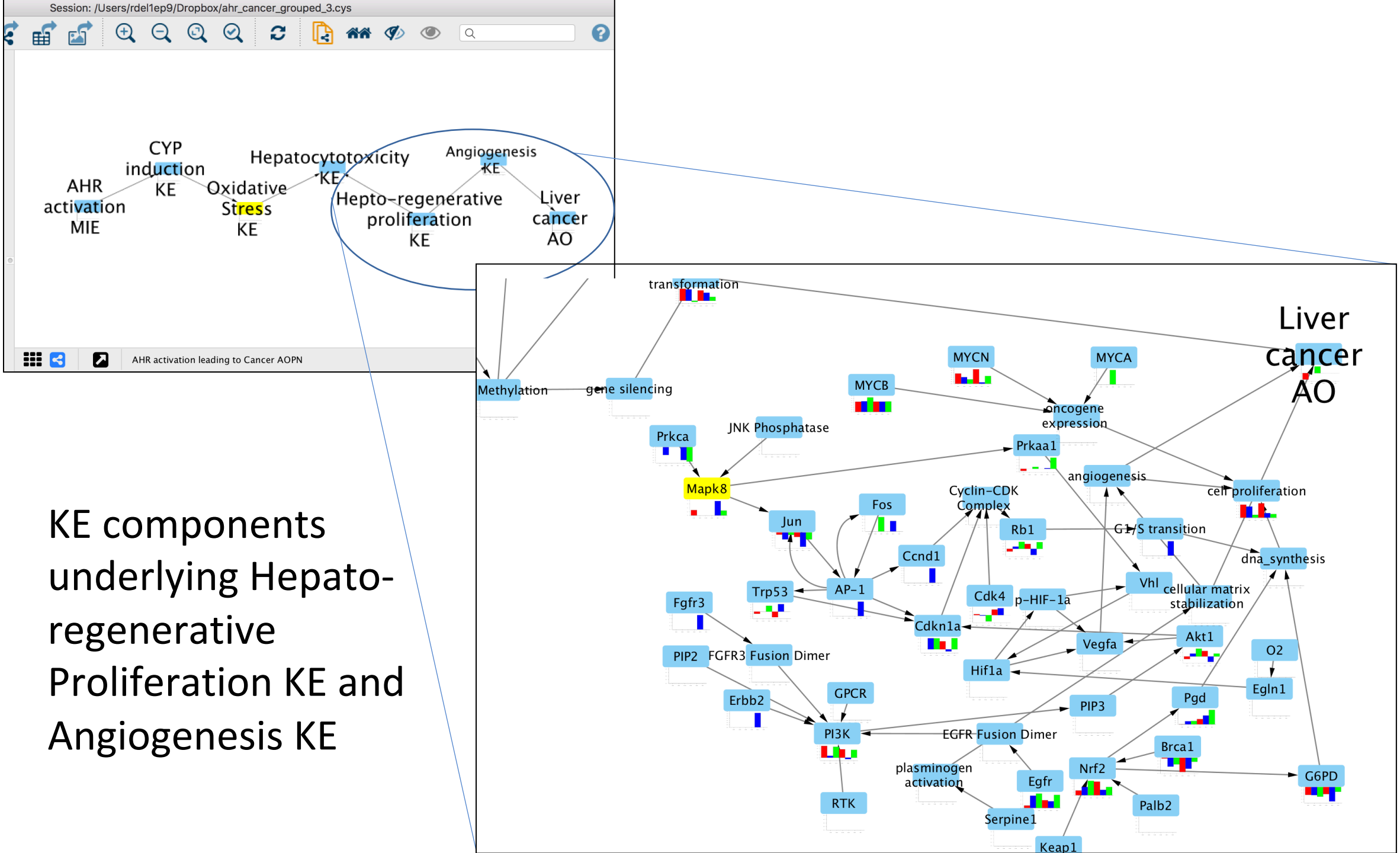




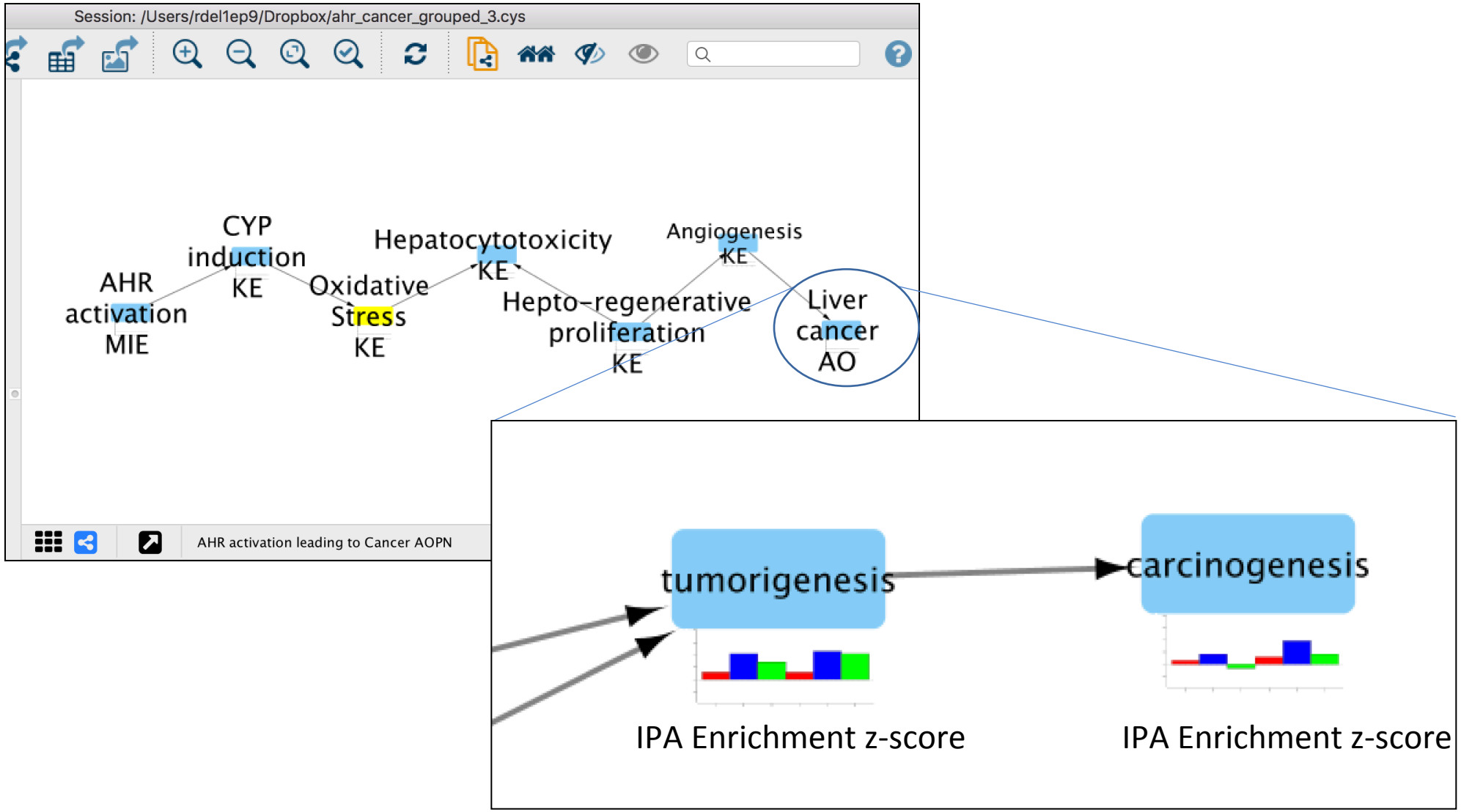
Can now examine genes and subnetworks as event components



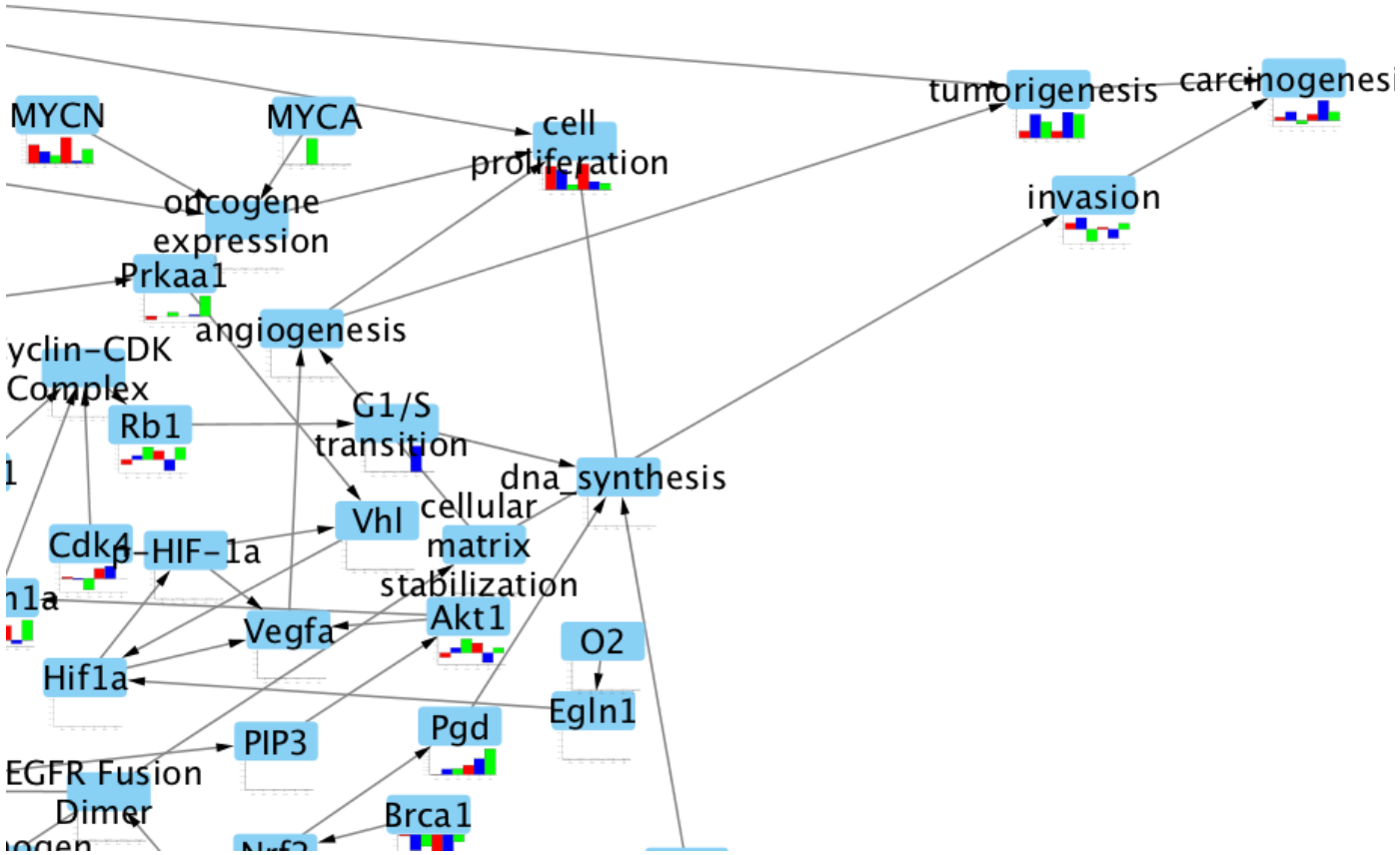
KE components underlying Ahr activation and Cyp induction KE



KE components underlying Hepato-regenerative Proliferation KE and Angiogenesis KE



KE components underlying Liver Cancer AO



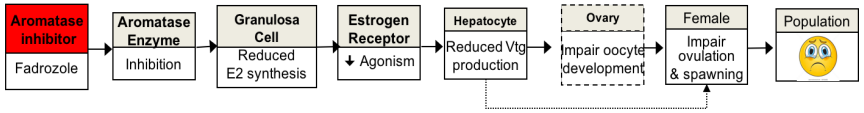
The AOP network with gene expression or other data may be useful in a weight of evidence for assessing and communicating potential for Cancer in exposed animals

Table of KE and KE components for AhR leading to cancer

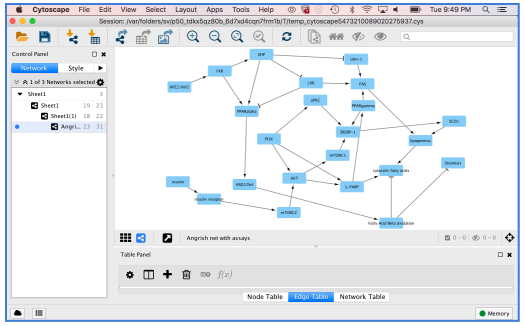
Activation of AHR in the liver	**SWANC**	**CLARKO**	**TOLEDO**	**WYAND**	**GROSIL**	**TRENT**
AHR upstream activation						
CYP1A1						
Oxidative Stress						
NFE2L2 upstream regulator						
NRF2-mediated Oxidative Stress Response						
DNA damage/alkylation						
Cell Cycle: G2/M DNA Damage Checkpoint Regulation						
DNA Double-Strand Break Repair by Homologous Recombination zscore						
DNA Double-Strand Break Repair by Non-Homologous End Joining						
Activation of DNA repair genes ALKBH genes, XRCC1 and 5, and DNA methylation genes						
Apoptosis	**SWANC**	**CLARKO**	**TOLEDO**	**WYAND**	**GROSIL**	**TRENT**
p53 Signaling z-score						
Cdkn1a/p21 expression						
Proliferation						
VEGFAB						
Vegf activation						
MYCA expression						
MYCB expression	2.27	2.3631	3.212	2.286	2.317	2.277
cell proliferation of tumor cell lines p value	1.43E-15	2.73E-12	3.41E-18	1.69E-17	8.30E-25	1.95E-21
cell proliferation of tumor cell lines z score	2.16	1.84	0.464	2.381	0.743	0.596
Tumor						
liver tumor (p-value)	4.66E-16	2.27E-14	4.53E-09	9.75E-14	6.48E-39	2.27E-14
liver tumor (z score)	0.599	2.085	1.419	0.588	2.273	2.085

AOPXplorer + High throughput transcriptomics = AOP-based hazard assessment

Identify hazard threshold or safe exposure limit based on change in pathways known to lead to Adverse Outcome



Adverse Outcome Pathway based point of departure provides a meaningful toxicological context

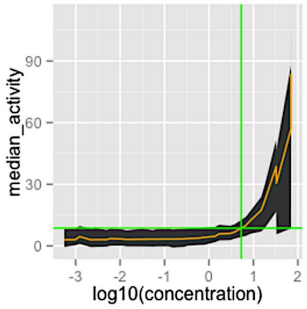


Identify DEG with monotonic concentration response

Species	TNT RfD mg/ kg-day
C. elegans	0.273
Daphnia magna	0.062
Zebrafish embryo	0.015
Human iPSC hepatocytes	0.335
IRIS RfD (Dog)	0.0005

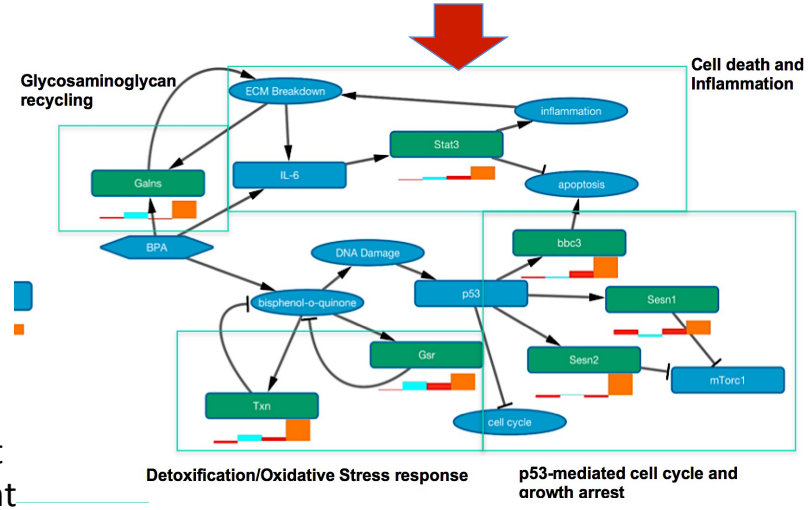
Use of AOP t-POD for Oral reference dose

Note; Human cells had 10x uncertainty factor. EPA IRIS RfD has1000x uncertainty factor.



Determine POD of genes/event most proximal to apical endpoint (aop R package)

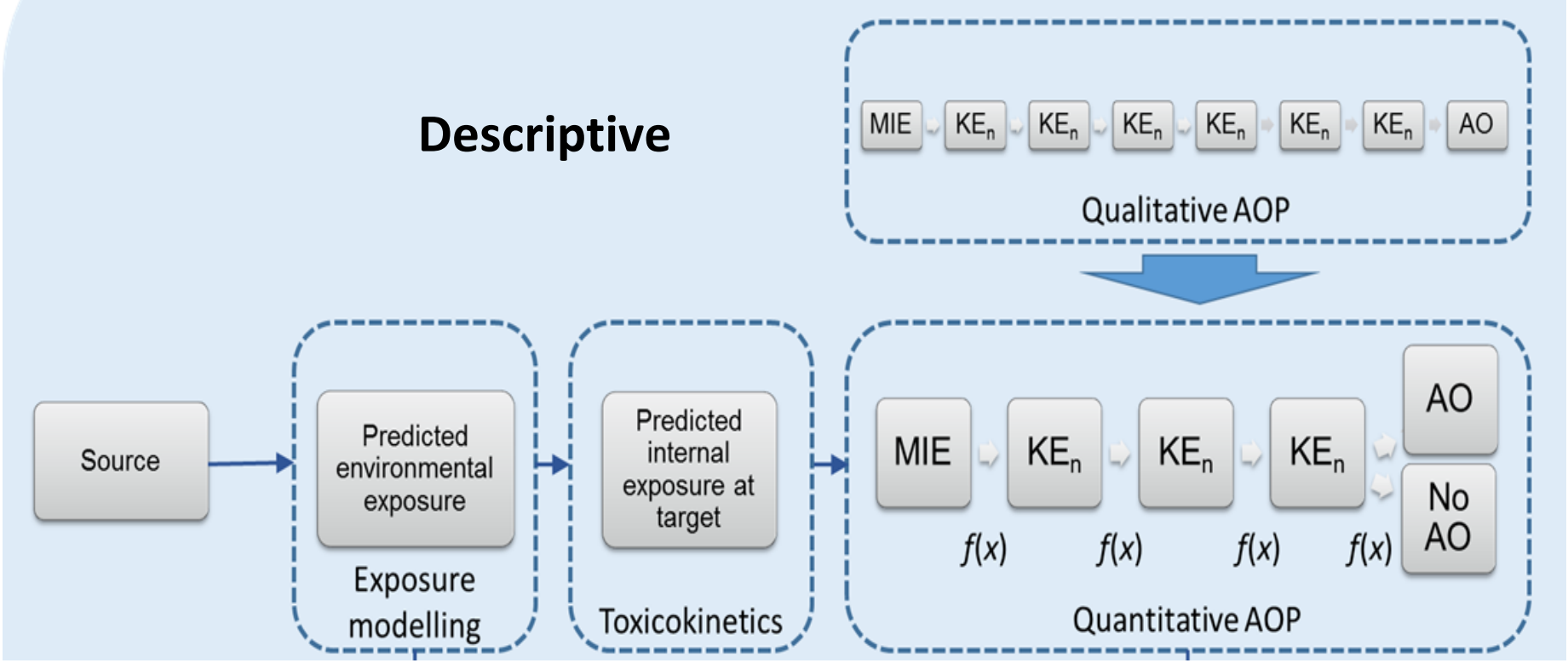
Bootstrap natural Spline Metaregression (Burgoon et al 2016)



Identify plausible AOPs via AOP networks from AOPwiki or computationally through Reactome, KEGG, BioCyc and literature

Quantitative approaches for AOPs

Translation of an AOP into a quantitative and computational AOP model



A qAOP captures response-response relationships between Key Events

qAOP model is dependent upon the question being asked

Simple models for

- **Screening level questions**
- **Prioritization**

Complex models for

- **Quantifying impacts on populations**
- **High Biological fidelity and lower uncertainty**

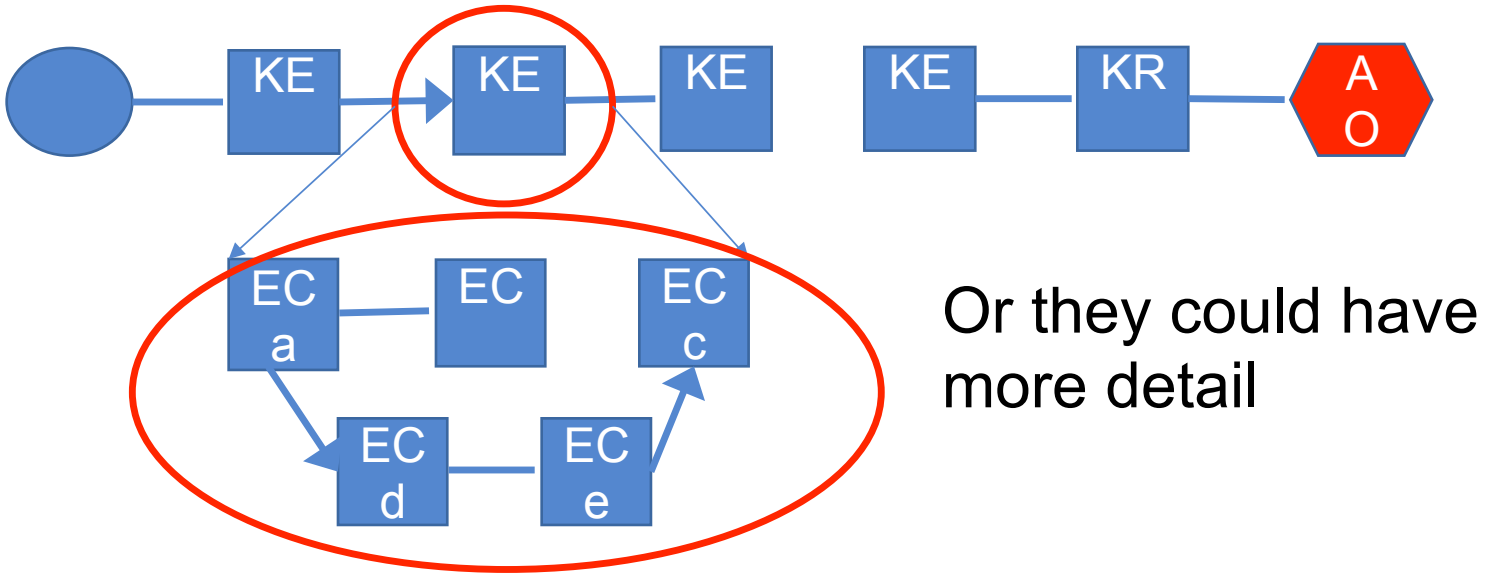
AOPs are conceptual models for qAOPs

AOP

Must incorporate the AOP, but …

qAOP

May not model all details of the AOP



Or they could have more detail

The TRACE levels of documenting qAOPs

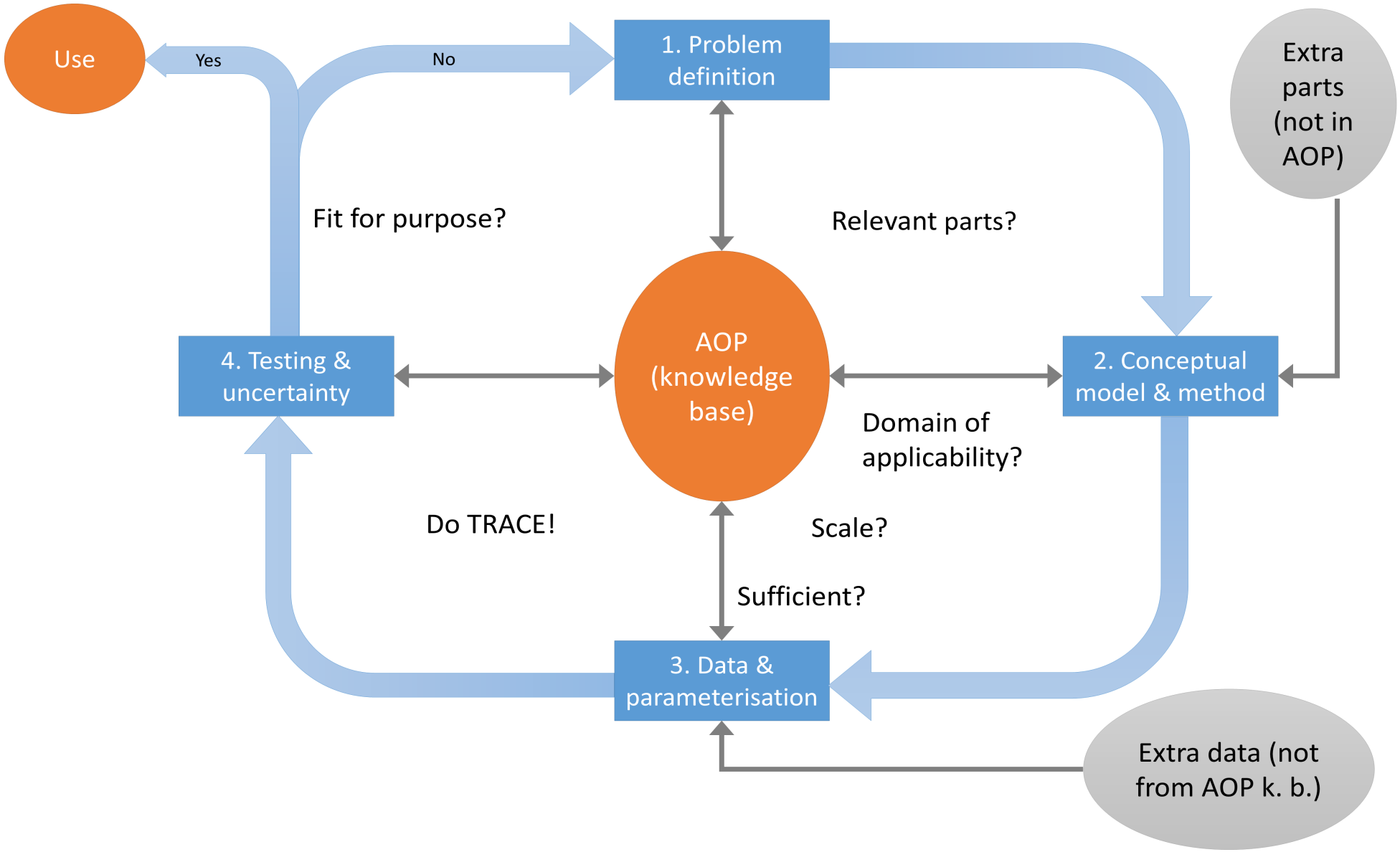
Level	Step	Description and examples
Development	Problem formation	- Predict an endpoint of regulatory relevance in chemical hazard and risk assessment - Estimate which combination of MIE/KE is required to trigger an AO
	Model design and formulation (≠ programming)	Decide whether TKTD, statistical or correlative models may best describe the quantitative relations required in the anticipated decision making context.
	Implementation	Implement the model. A combination of different models targeting the need to describe different KERs by different approaches may be considered.
	Parametrization and calibration	Obtain parameters for the different AOP levels from literature, the AOP-KB, or by conducting additional experiments. Thresholds that trigger KEs or differential equations describing relationships –are parametrization.
Analysis	Verification and sensitivity analysis	Test whether the quantitative model adequately describe the relation of MIE, KE and AO and identify parameters that would have the strongest impact on the AO prediction.
	Validation	Validate the model using different chemicals
Application	Quantification of uncertainties	Compare to experimental data and estimate the deviation, identify data gaps
	Results	Decide whether the confidence is sufficient, the problem can be addressed.
Repeat	Rerun steps to optimize the model or adopt the problem formulation (increase feasibility)	Revise and repeat the modeling chain if performance deviates from the expected results.

Transparent and comprehensive **model** evaluation and documentation.

Types of models and needs

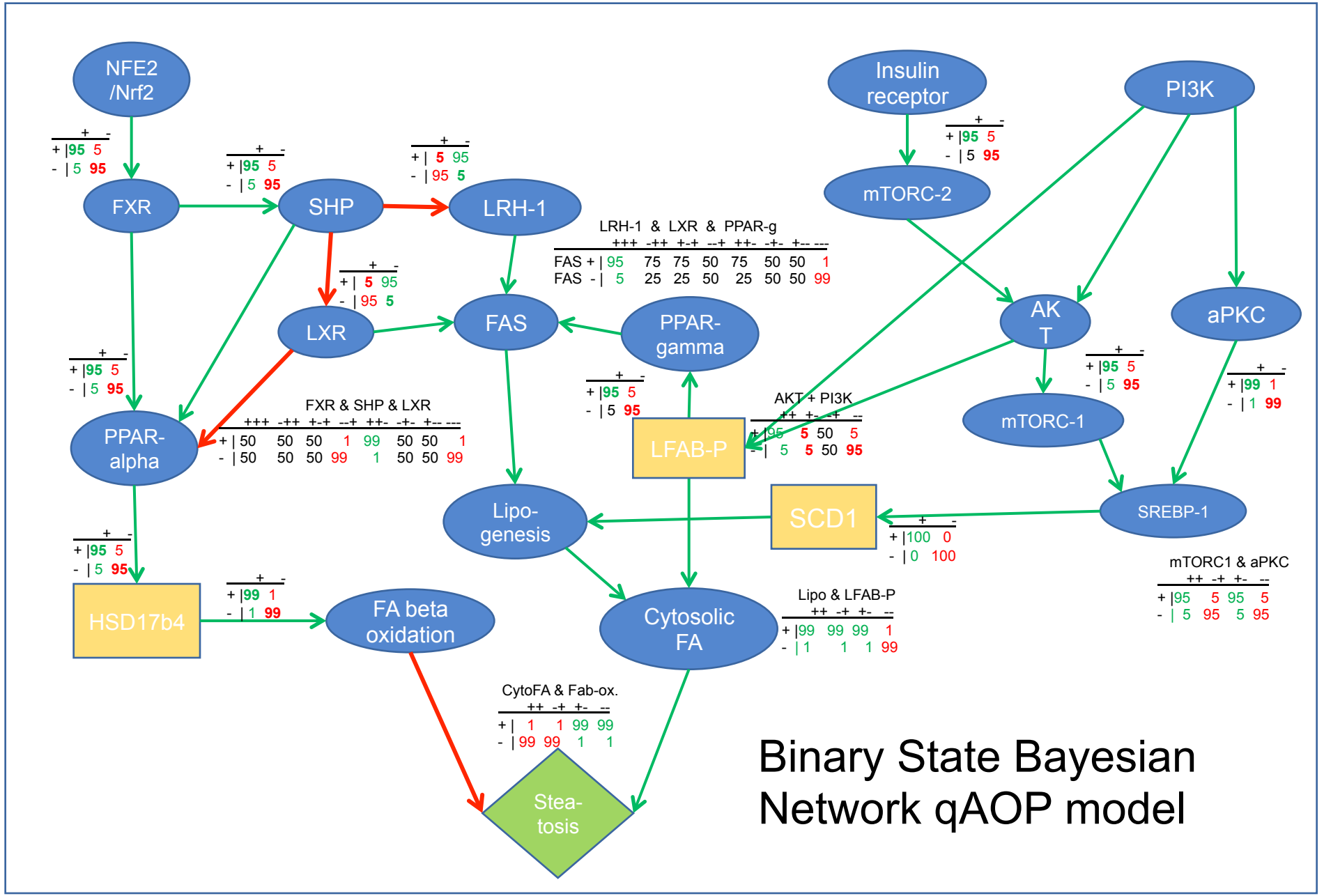
	Description of Key Event Relationship	Relevant Models & Analyses	Typical Data Needs	Case Studies and Applications
AOP	Directed: $KE_A \rightarrow K_B$ e.g. all KERs in AOP represent a causal linkage	Network/graph analyses techniques	Graph structure providing the connectivity with KEs	
	Directed and signed relationship: $KE_A \xrightarrow{\pm} KE_B$ e.g. increasing and decreasing, i.e. $\uparrow KE_A \Rightarrow \downarrow KE_B$	[a]		
	Direction and scalar-weighted relationship: $KE_A \xrightarrow{\pm w_{A,B}} KE_B$ e.g. simple weights	[a]	Expert judged weights	Semi-quantitative weight of evidence analysis
qAOP	Direction and functional relationship: $KE_A \xrightarrow{f} KE_B$			
	Probabilistic, e.g. probability of KE activation	Bayesian Networks	Expert judged probabilities, Experimental data	predicting MoA, predicting states of KE
	Non-linear, e.g. saturable response	Regression modelling	Experimental data on KEs under different levels of perturbation	
	Time-dependent, e.g. acute vs chronic KE activation	Ordinary differential equation (ODE) Individual based models, Leslie projection matrix	Independent parameter measurement temporal response data	Predicting temporal response on HPG axis {Conolly, 2017 #8177} Dynamic Energy Budget Modelling (case study?) (relevant ABM example)

Making AOP models

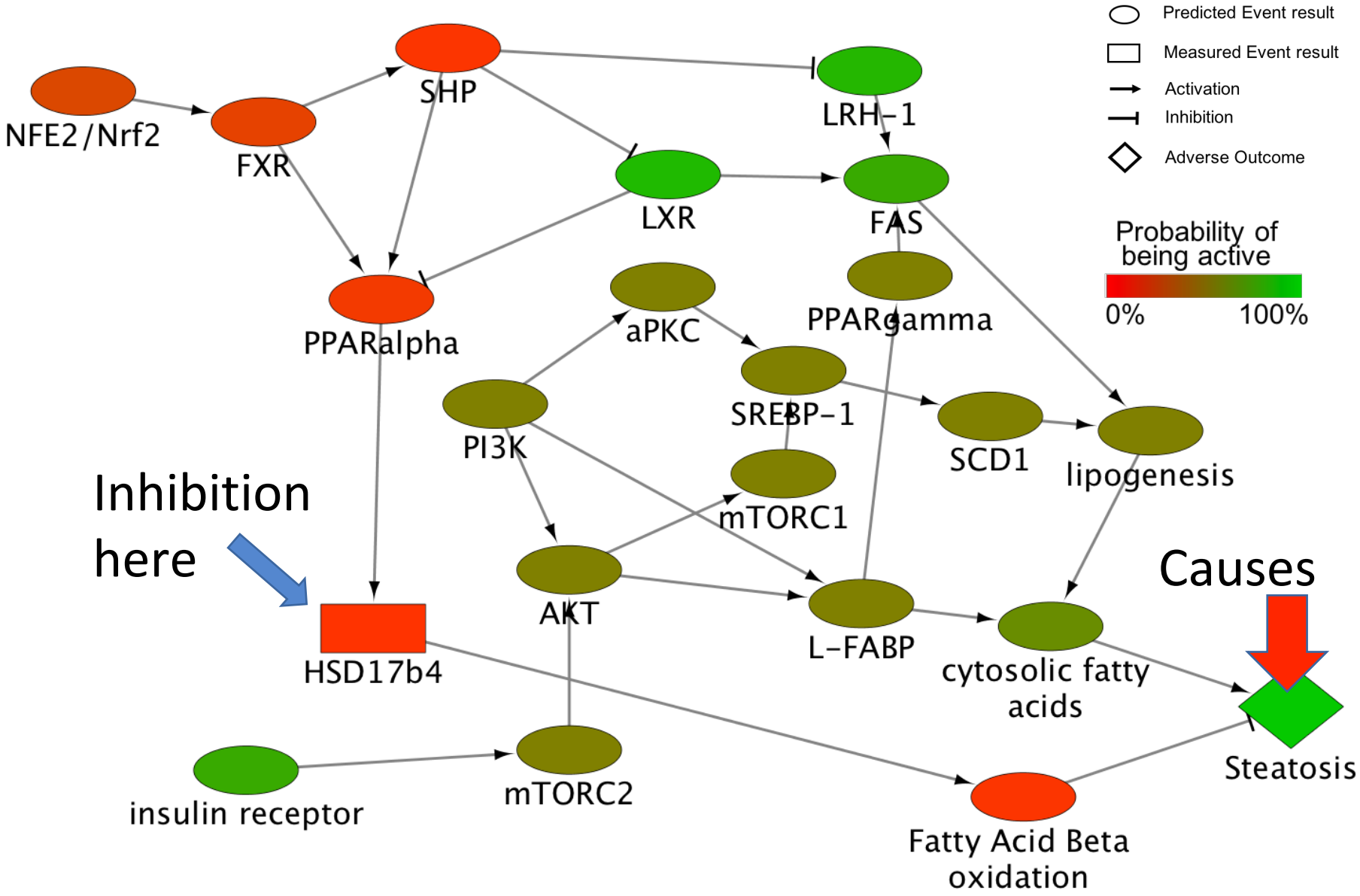


Application of qAOP models

Predicting effect of assay measurements of events in an AOP network



Steatosis causal AOP network



BISCT: Bayesian Bayesian Inference for Substance and Chemical Toxicity

BISCT: Bayesian Inference for Substance and Chemical Toxicity

Get your predictions in 3 easy steps!

1) Choose your input data file

Data File wyeth-14643.txt

2) Choose the bayes net

Steatosis

3) Press Go!

Go!

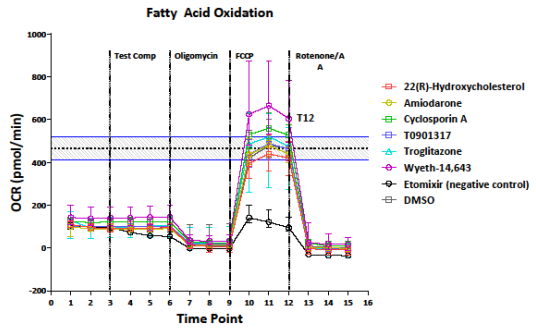
Node Name	Probability Node Active	Probability Node Inactive
fxr	0.9146166942269095	0.08538330577309054
shp	0.9600049377330028	0.039995062266997235
lxr	0.03820189573315146	0.9617981042668485
ppara	0.9825216509386533	0.01747834906134661
hsd17b4	0.9966636270441364	0.0033363729558637274
fatty_acid_beta_oxid...	1.0	0.0
lrh1	0.062317604948029265	0.9376823950519708
mtorc2	0.31216955755495784	0.6878304424450421
akt	0.2912995083943976	0.7087004916056024
lfabp	0.3077300343196365	0.6922699656803635
influx	0.0	1.0
pparg	1.0	0.0
fas	0.06431722805348873	0.9356827719465112
mtorc1	0.3121695575549579	0.6878304424450421
apkc	0.9111399684630369	0.08886003153696316
srebp1	1.0	0.0
scd1	1.0	0.0
lipogenesis	0.0	1.0
steatosis	0.01	0.99

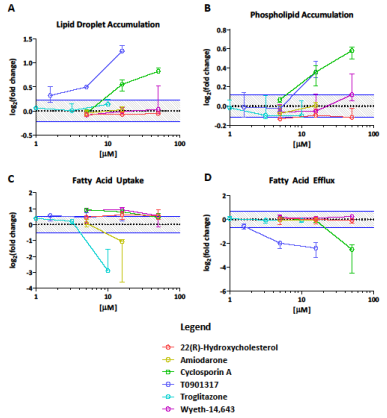
Software Version 1.0.0
Content Pack 1.0.0

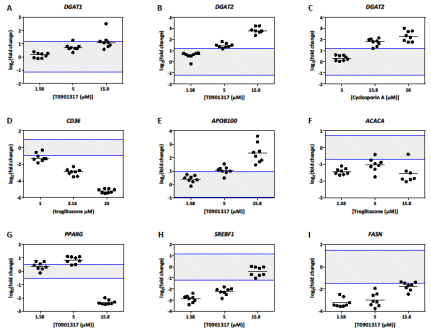
Developed by: Lyle D. Burgoon, Ph.D.
US Army Corps of Engineers, Engineer Research and Development Center
Environmental Laboratory
Bioinformatics and Computational Toxicology Group

Funded by the US Army Environmental Quality and Installations Program

STEATOSIS AOP Bayes Net v1.1 with real data





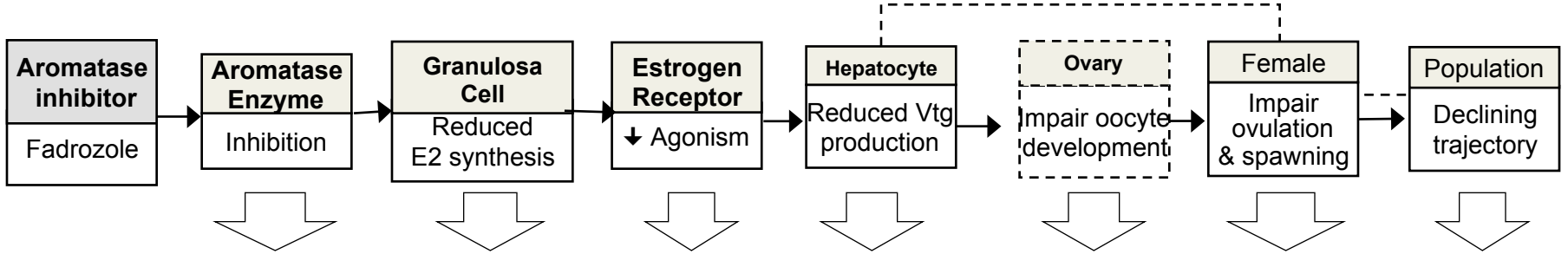


Data was taken from the Angrish, et al (2017, Mechanistic Toxicity Tests Based on an Adverse Outcome Pathway Network for Steatosis, https://doi.org/10.1093/toxsci/kfx121 (https://doi.org/10.1093/toxsci/kfx121)).
We did not reanalyze the data – we took the data directly from the paper.
We aligned the assay data from Angrish, et al to our Steatosis AOP Bayes Nets, and calculated predictions.
Our results concur with those presented by Angrish, et al.:

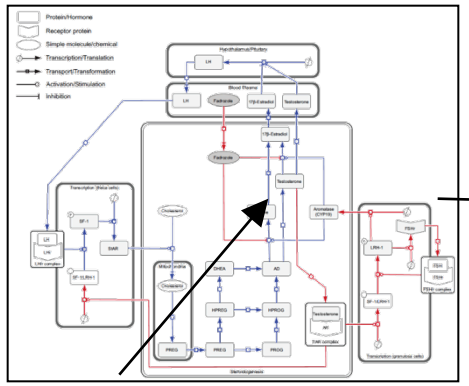
Chemical Steatosis

22(R)-hydroxycholesterol	No	(99% certain)
amiodarone	No	(99% certain)
cyclosporin A	Yes	(99% certain)
T0901317	Yes	(99% certain)
Troglitazone	No	(99% certain)
Wyeth-14,643	No	(99% certain)

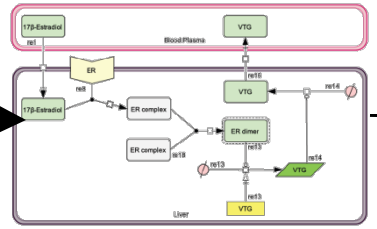
Quantitative Prediction of Reproductive/Population Effects in Fish: Linking Relevant Models Across an AOP



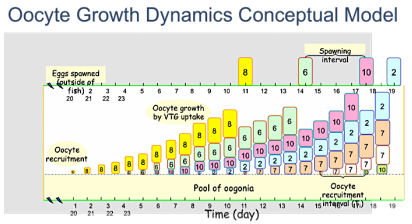
VTG/fecundity correlation



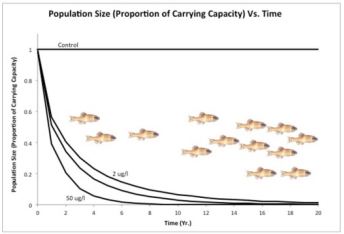
Aromatase inhibition



Vtg production



Oocyte development, ovulation and spawning

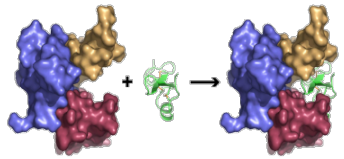


Population declining trajectory

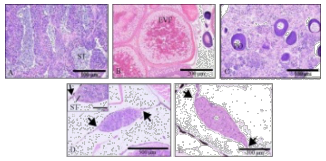
HPG axis model

Oocyte Growth Dynamics model

Population Dynamics model

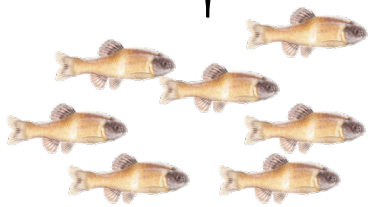


Molecular level



Organ/tissue level

Animal level



Population level

Conolly et al. 2017. Quantitative adverse outcome pathways and their application to predictive toxicology.

Summary

- Biological networks can be integrated into AOPs
  - Useful for hypothesis driven analysis of mixture effects
- Transcriptomics can be useful for examining AOPs with integration of KE components and subnetworks
- Descriptive AOPs can form the basis of quantitative AOP models
- qAOP models vary widely in type and application- but can be very simple of complex

Thanks!

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Caged fish studies

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AOP modeling

Lyle Burgoon, Stefan Scholz, Roman Ashauer, Rory Conolly, Brigitte Landesmann, Cameron Mackay, Cheryl Murphy, Nathan Pollesch, James R. Wheeler, and Anze Zupanic

AOPXplorer and networks are available as a Cytoscape app from with in Cytoscape. See Lyle Burgoon (Lyle.D.Burgoon@usace.army.mil)