NAMs for Estrogenicity

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CompTox Communities of Practice

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Key Points

- Community Needs
  - People are exposed to thousands of chemicals
  - EPA is required to test certain classes of chemicals for potential endocrine disruption, including through interaction with the estrogen receptor (ER)
  - *In vitro* NAMs can be used to rapidly test many chemicals – the ER Pathway Model
- Accounting for Variability
  - There is variability across *in vitro* methods, and within *in vivo* methods (lab-to-lab)
  - *In vitro* to *in vivo* concordance is approximately the same as lab-to-lab *in vivo* concordance
- *In Vivo* vs. *In Vitro* Adversity
  - *In vitro* assays are good predictors of the *in vivo* uterotrophic response
  - Metabolic activation / deactivation is challenging, but NAM methods to address this are being developed (Simmons and Deisenroth)
- Data for Benchmarking New Approaches
  - A large collection of reference chemicals are available
  - 1800+ chemicals have been evaluated *in vitro*, although few have corresponding *in vivo* data
  - Very little direct human data

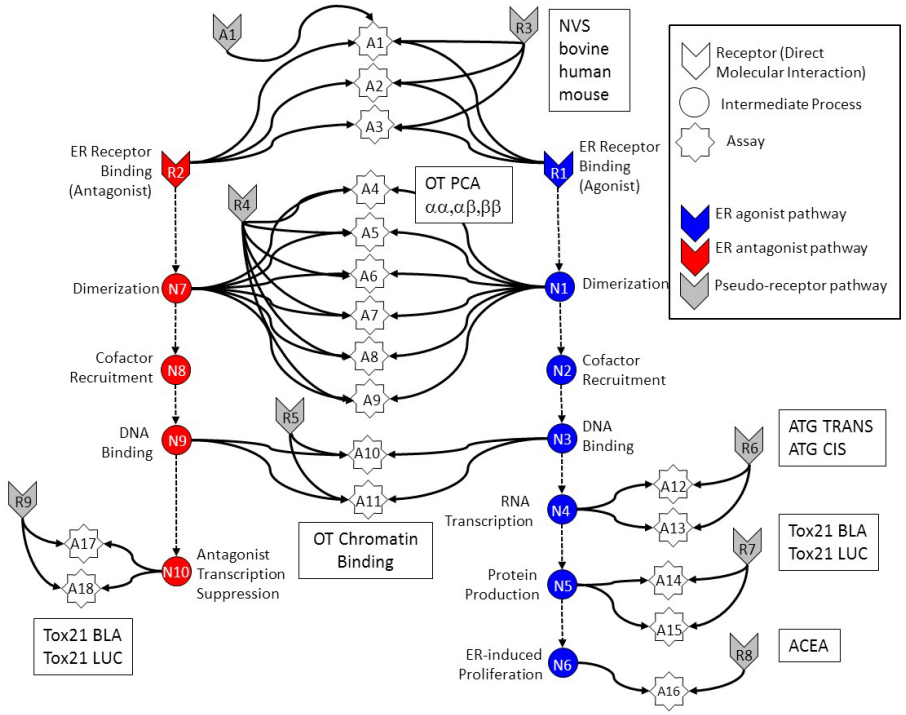
Community Needs

- Over a lifetime, people can be exposed to many thousands of chemicals
  - A minority of these have been thoroughly tested for toxicity, including estrogenicity
- EPA is required to test for potential endocrine disrupting chemicals through the Food Quality Protection Act (FQPA) and other statutes
  - Origin of the Endocrine Disruptor Screening Program (EDSP)
  - There are ~10,000 chemicals on the EDSP queue (pesticides, chemicals potentially found in drinking water)
- *In vitro* NAMs have been used to test 1,800 chemicals and more are in queue
- *In silico* NAMs (QSAR models) have tested all defined chemicals in the EDSP Universe (Mansouri et al.)

https://www.epa.gov/endocrine-disruption

In Vitro Estrogen Receptor Pathway Model

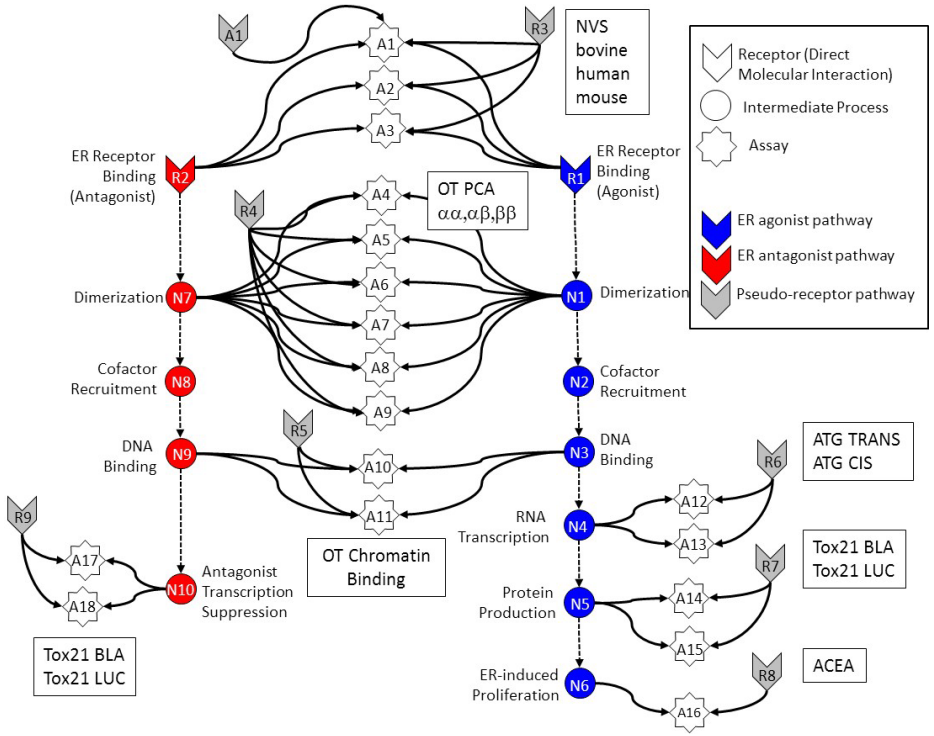
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals

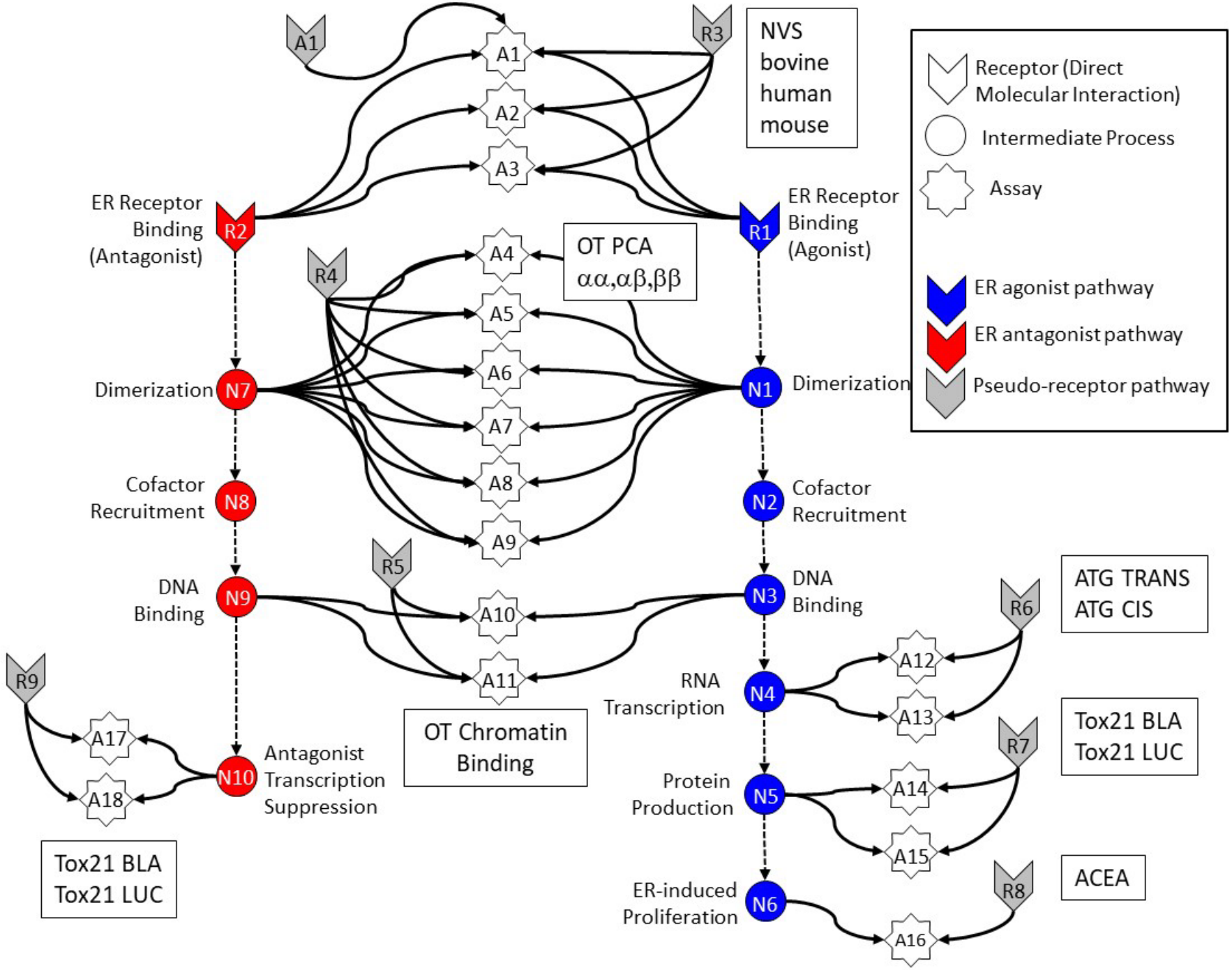


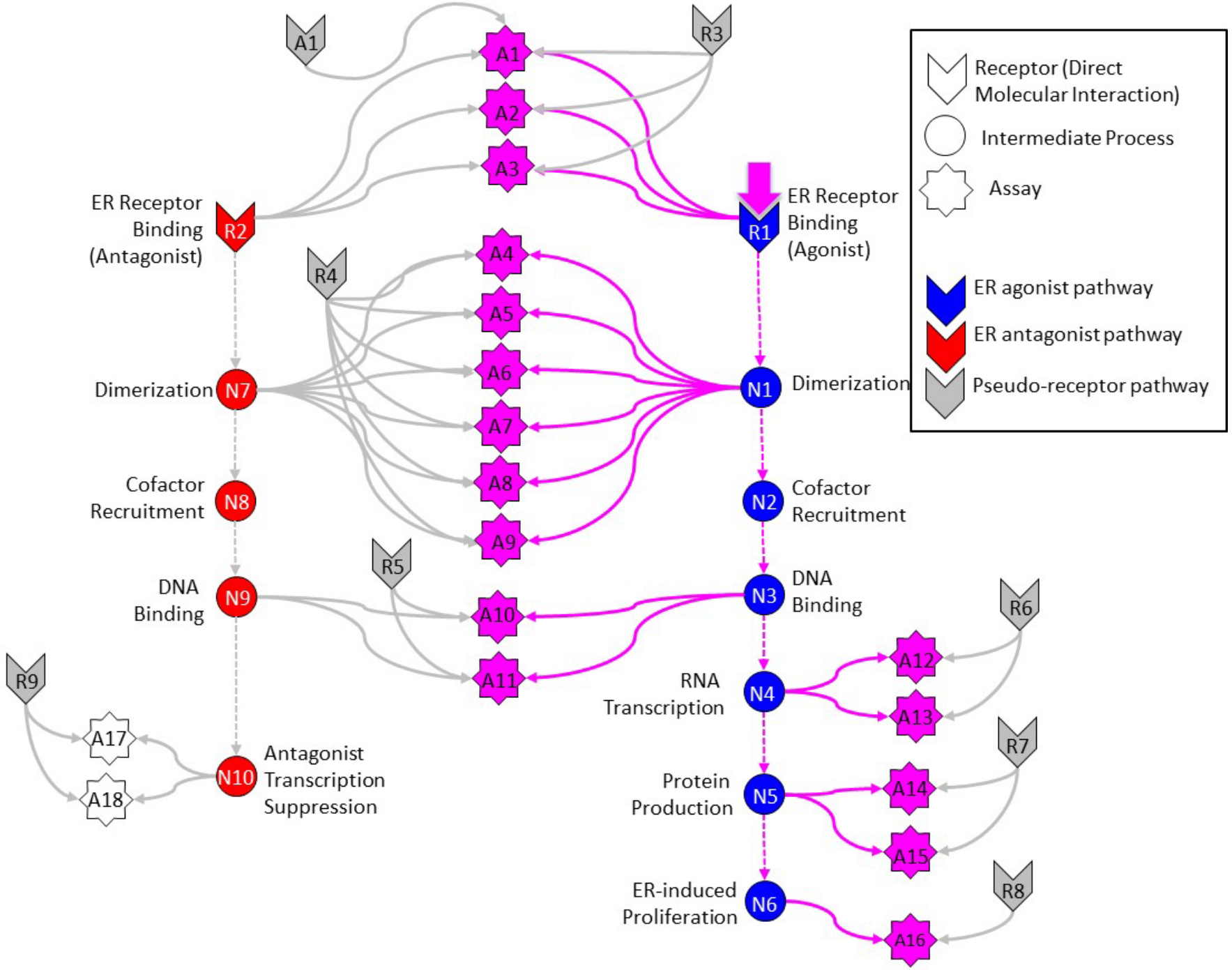
Judson et al: "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor" (EHP 2015)

What Does the Model Do?

- For every concentration, look at the pattern of activity across the assays
  - If pattern is consistent with agonist activity, classify the chemical as an agonist
  - If pattern is consistent with antagonist activity, classify the chemical as an antagonist
  - Else, classify the chemical as acting through some technology or cell-type specific interference process




A1
R3
NVS
bovine
human
mouse
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
A1
A2
A3
ER Receptor Binding (Antagonist)
R2
R1
ER Receptor Binding (Agonist)
A4
OT PCA
αα,αβ,ββ
R4
A5
A6
A7
A8
A9
Dimerization
N7
N1
Dimerization
Cofactor Recruitment
N8
N2
Cofactor Recruitment
DNA Binding
N9
R5
N3
DNA Binding
ATG TRANS
ATG CIS
R6
A10
A11
A12
A13
R9
A17
A18
N10
Antagonist Transcription Suppression
OT Chromatin Binding
RNA Transcription
N4
Tox21 BLA
Tox21 LUC
R7
Protein Production
N5
A14
A15
ER-induced Proliferation
N6
R8
ACEA
A16
Tox21 BLA
Tox21 LUC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Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
ER Receptor Binding (Antagonist)
ER Receptor Binding (Agonist)
Dimerization
Dimerization
Cofactor Recruitment
Cofactor Recruitment
DNA Binding
DNA Binding
RNA Transcription
Protein Production
ER-induced Proliferation
Antagonist Transcription Suppression
R1
R2
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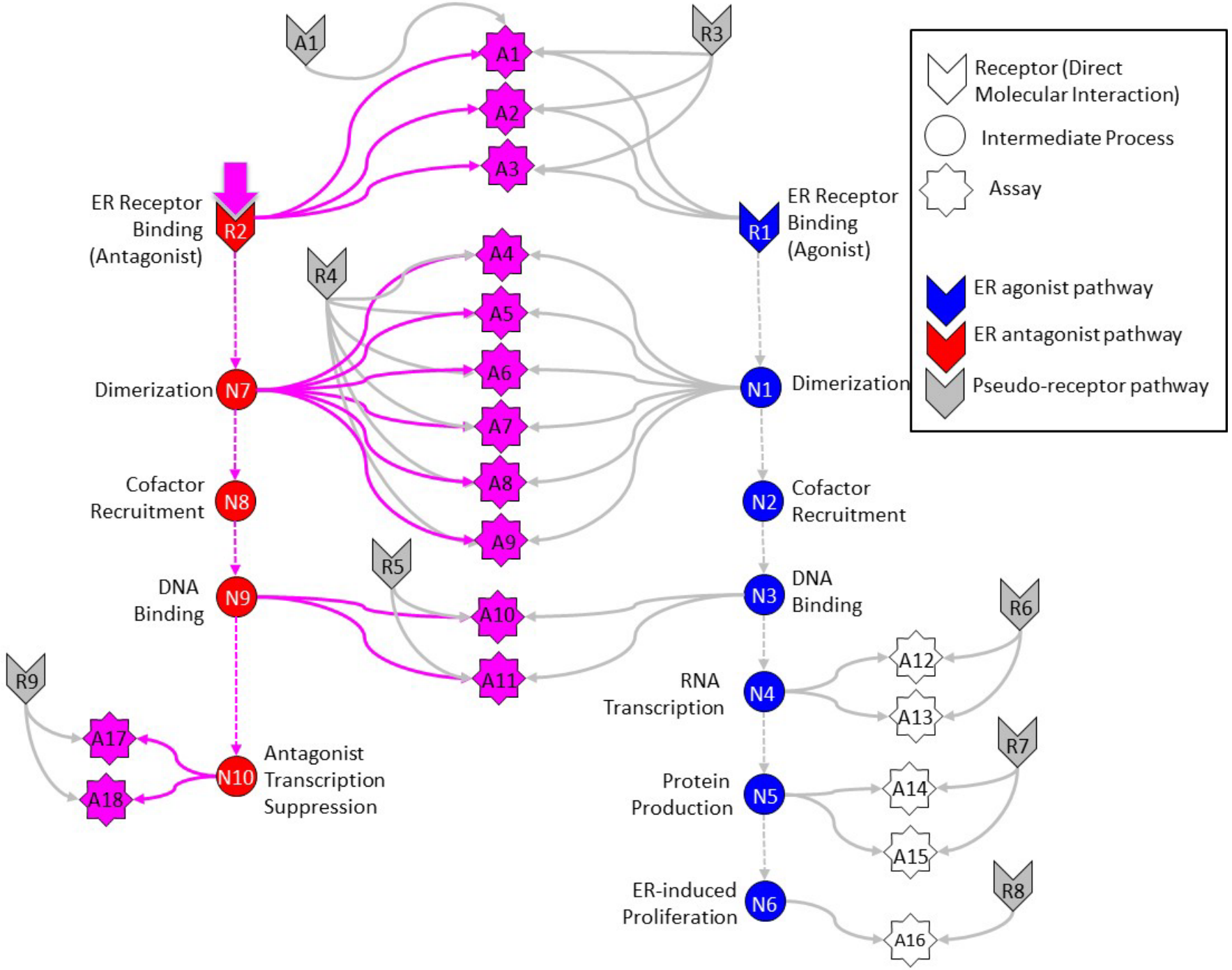
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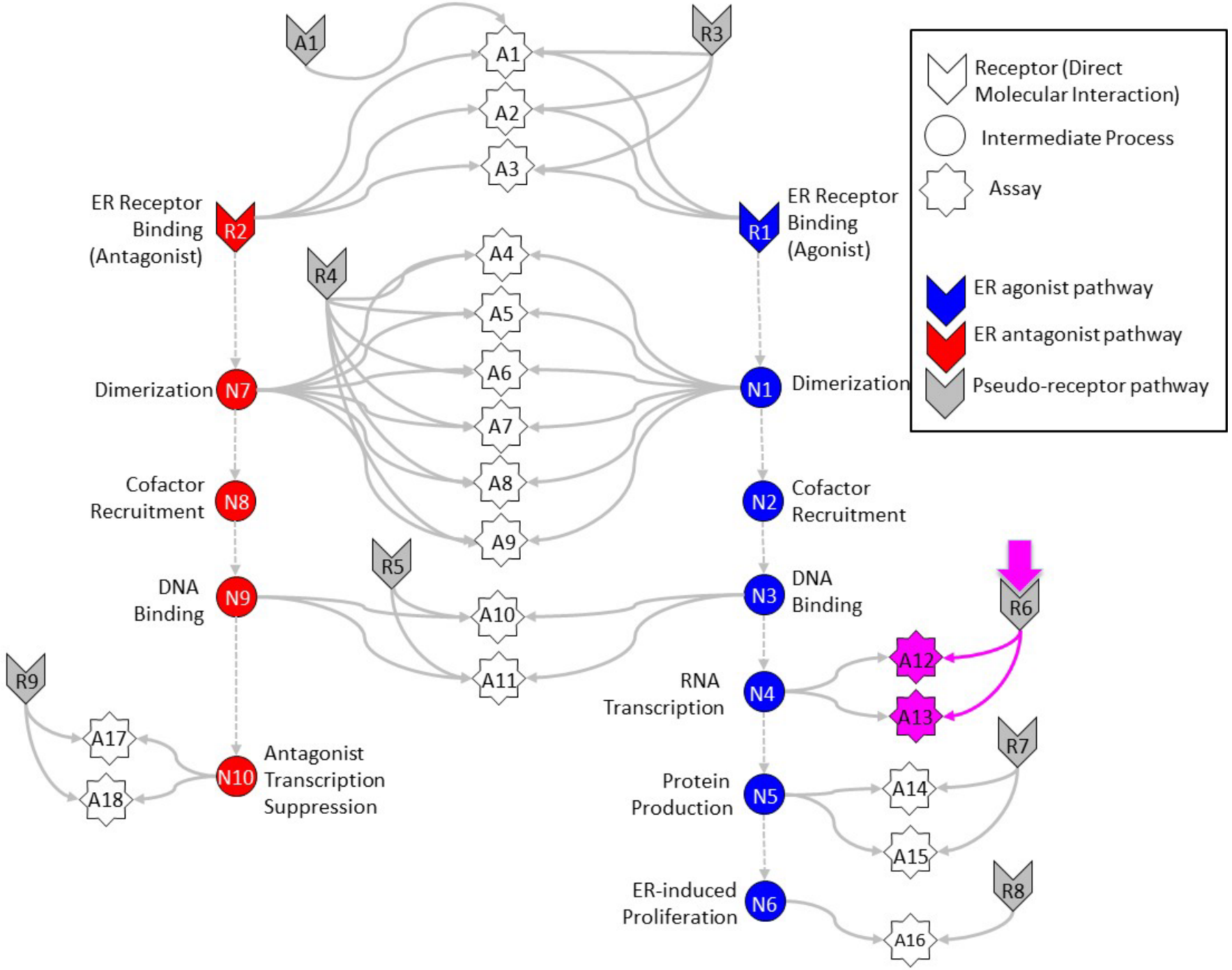
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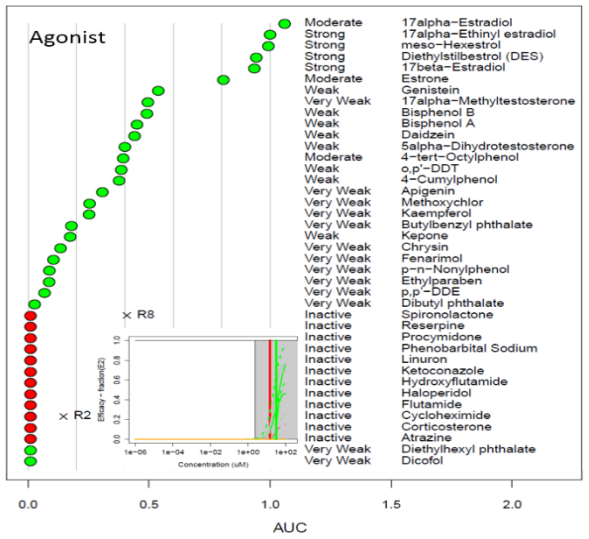
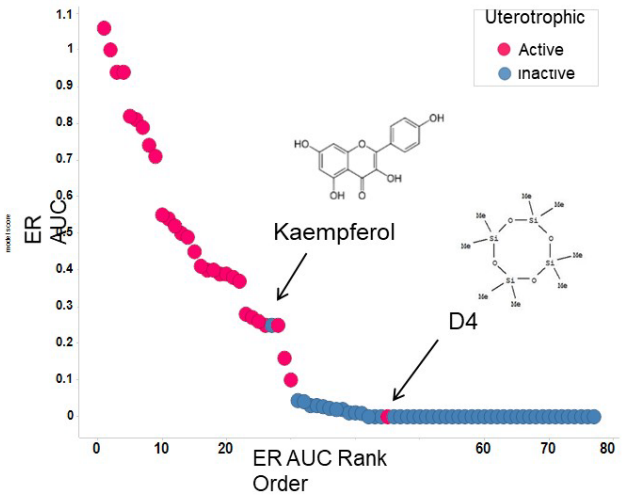
Demonstrate that the full model replicates reference chemical activity



True Positive	26 (25)
True Negative	11 (11)
False Positive	1 (0)
False Negative	2 (2)
Accuracy	**0.93 (0.95)**
Sensitivity	**0.93 (0.93)**
Specificity	**0.92 (1.0)**

In Vitro



True Positive	29
True Negative	46
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.97**

In Vivo

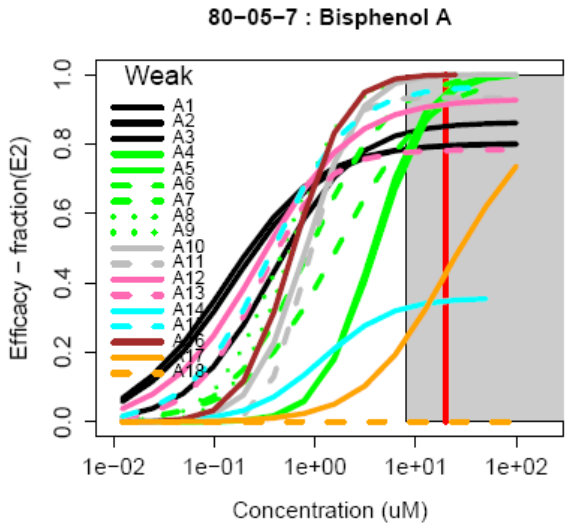
Variability and Concordance

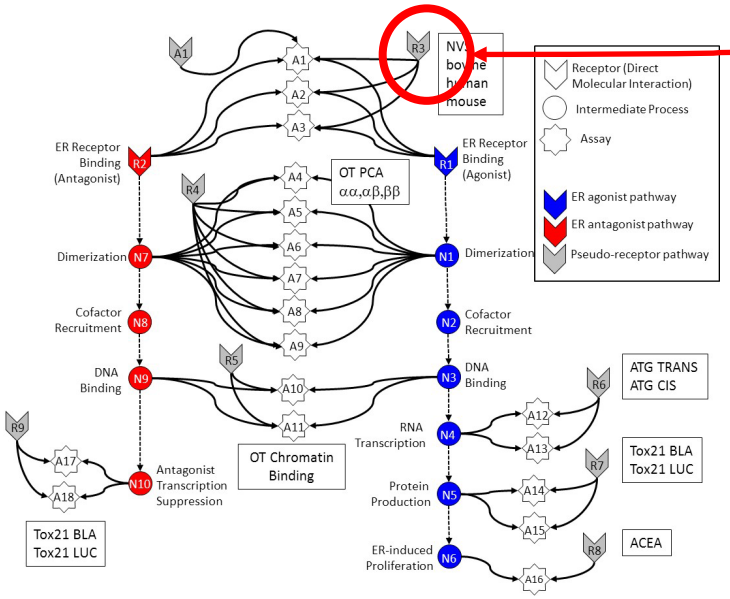
- We have tested 1800 chemicals for estrogenicity in 18 separate *in vitro* assays
  - Different points on the biological pathway
  - Different cell types
  - Different readout technologies
- Key learnings:
  - All *in vitro* (and probably all *in vivo*) assays can show false positive results through "assay interference" – the assay is positive for reasons unrelated to the target the assay is supposed to assess
  - Best to use "orthogonal" assays – if multiple different technologies give the same results, confidence in result (positive or negative) is increased.
  - Quantitative results (potency) may still be variable

Judson et al., Tox.Sci. doi: 10.1093/toxsci/kfw092 (2016)
Judson, et al., ToxSci 148 (1) pp 137-154 (2015)
Browne et al., Environ. Sci. Technol. 2015, 49, 8804−8814, DOI: 10.1021/acs.est.5b02641

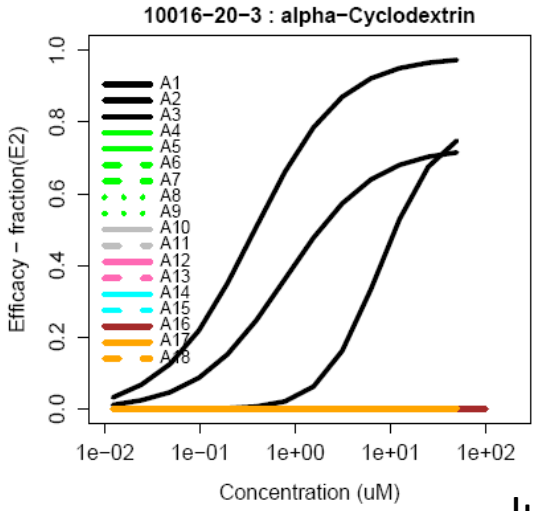
Example of Quantitative Uncertainty

True Agonist





Assay Interference Example “R3”



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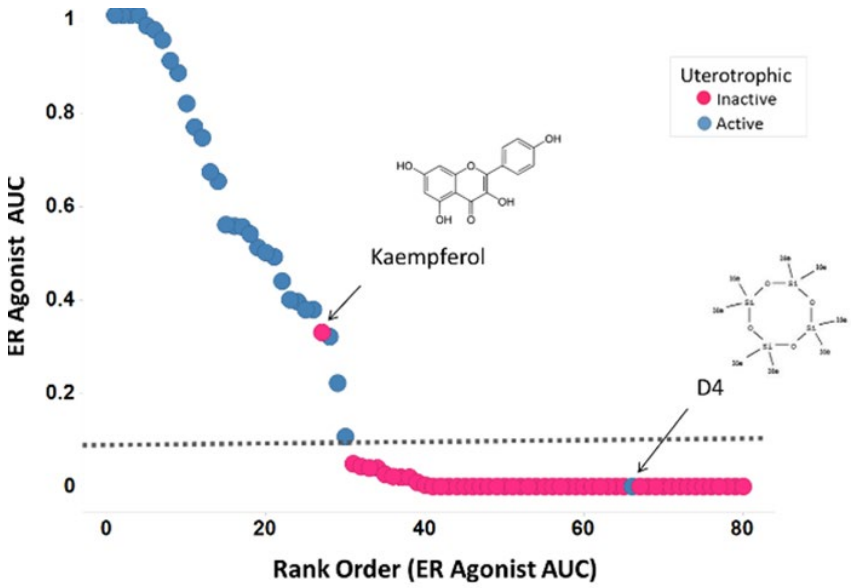
In Vitro to *In Vivo* Concordance



42 Chemicals with *in vitro* and *in vivo* data
1 false negative – chemical was volatile *in vitro*
1 false positive – chemical is metabolically deactivated *in vivo*

Variability in published *in vivo* uterotrophic data for Bisphenol A
Potency spans ~3 orders of magnitude

Browne et al. Environ. Sci. Technol. 2015, 49, 8804−8814, DOI: 10.1021/acs.est.5b02641

In Vivo and *In Vitro* Adversity

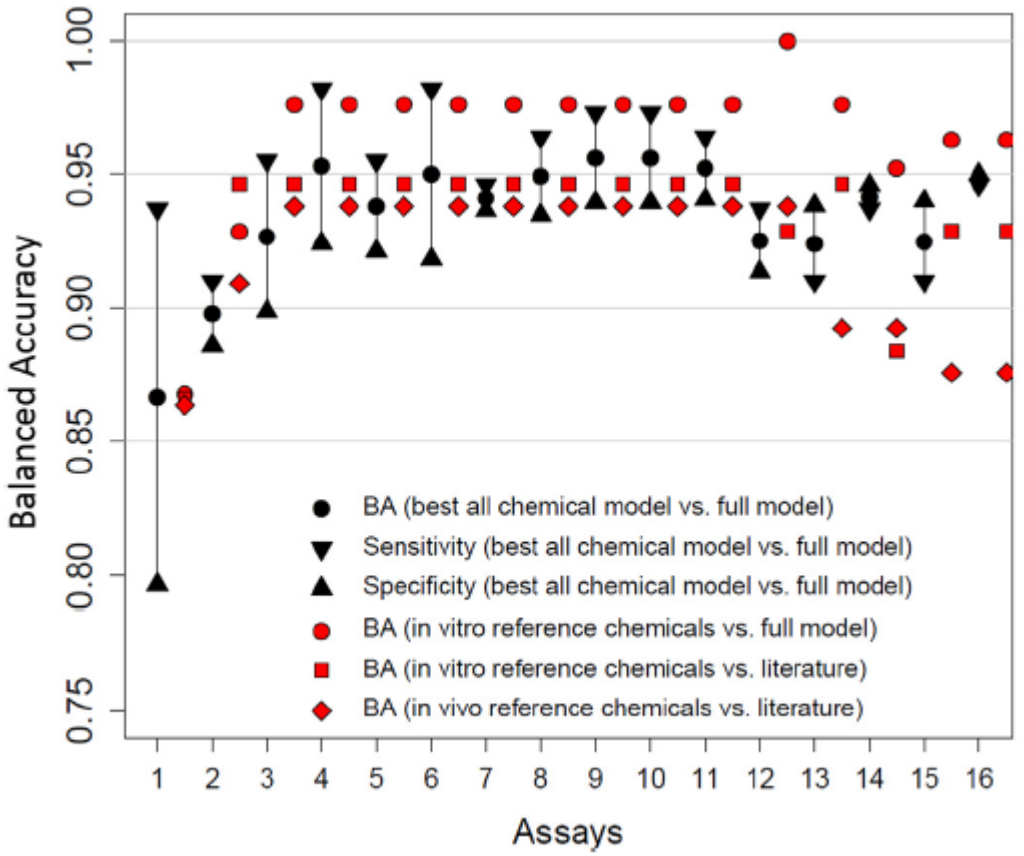
- NAMs can model some of the *in vivo* effects of estrogenic chemicals
- Estrogen receptor (ER) *in vitro* assays answer the following questions
  - Does the chemical interact with the ER?
  - Is the interaction in an agonist or antagonist mode?
  - Does the chemical cause ER-dependent cell proliferation?
  - What blood concentration is required to have an ER-related effect?
  - Assays test (mostly) the human ER
- In vitro toxicokinetics assays and models answer the following question
  - What human oral dose is required to reach the blood level that is required to have an ER-related effect?
- Exposure NAM models answer the following question
  - Are humans likely to be exposed at levels that cause ER-related effects?
- Cannot directly predict what the adverse phenotype will be seen

Data for Benchmarking New Methods

- Does a chemical interact with ER?
  - Good reference chemicals and published assays to compare against (see Browne et al. (2016))
- Is the chemical estrogenic through a non-genomic mechanism (GPR30)?
  - Reference chemicals and methods are sparse
  - Not clear how important this is for environmental chemicals
- Does the chemical affect estrogen signaling through the steroidogenesis pathway?
  - Chemicals can block the production of estrogen
  - Reference chemicals and methods exist (e.g. see Haggard et al. (2017))

Practical Application of the ER Model

- The original model uses 18 assay
  - Expensive to run
  - Some are no longer commercially available
  - Many are redundant
- To address this, “subset” models have been developed
  - Using as few as 4 assays, results can match the full model, within its uncertainty
- ORD is developing in-house subset models for testing new chemicals for ER (and AR) to meet Program Office needs



Regulatory Toxicology and Pharmacology 91 (2017)

What's Special About the ER Model?

- One of few *in vitro* models to be considered for use in a regulatory context
  - Multiple peer-reviewed publications
  - 3 Scientific Advisory Panels
  - 5+ years of internal review
  - Review by OECD (IATA)
  - Actual use by EFSA
- Highest level of assay-to-assay concordance testing for a single MIE (18 ER) assays that will probably ever be carried out at EPA
- Formed basis for comparable analysis for AR activity
- Data was used to develop a large battery of QSAR models (CERAPP)

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