Mathematical modelling of skin sensitization:
Guiding *in vitro* assay development for use in novel risk assessment methods

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Unilever, Safety & Environmental Assurance Centre

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Overview

- Background:
  - Why apply mathematical modelling to consumer safety risk assessments?
- The model:
  - An *in silico* model of skin sensitization induction
- Application within consumer safety risk assessment:
  - Focussing *in vitro* assay research / development and guiding data integration

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EU Cosmetics Directive

7th Amendment – March 2003

If the cosmetic product is to be marketed in the EU:

- alternative, non-animal tests must be used once validated
- animal testing and marketing bans on finished products
- animal testing and marketing bans on ingredients:
    - from March 2009: tests for acute (local) effects
    - from March 2013: more complex tests (including LLNA)

- threat to innovation and a major business risk
- challenge: market safe products without animal testing
- opportunity: apply new technologies in risk assessment

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Unilever's R&D Activities: 2004 →

"Mulberry / ASAT Programme"
(Assuring Safety without Animal Testing)

Objective: deliver safe new products without animal testing

- Developed and published "conceptual approach" – Fentem *et al*. ATLA, 2004

- Assessing feasibility of "conceptual approach" in practice
  - Invested in developing new capabilities
  - Evaluating applicability of new technologies and models for risk assessment: case study – skin allergy (sensitization)

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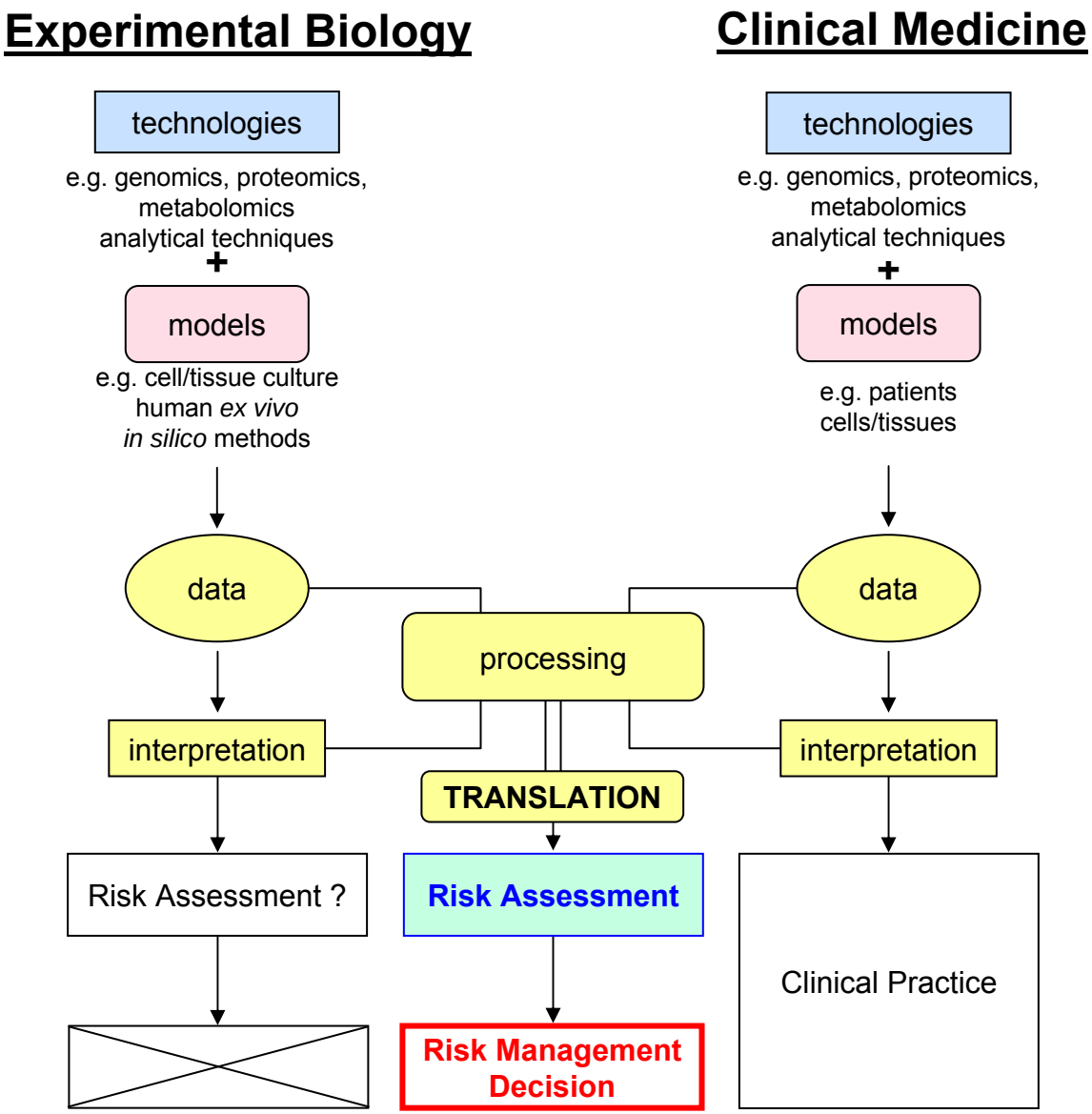
Conceptual Approach



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Skin Allergy: ‘building blocks’ of non-animal approach

- Risk Assessment
  - model development and experimental data generation driven by RA needs
- Models
  - experimental work on developing cell-based assays, peptide binding assays, and integrating dermal kinetics & metabolism
- Technologies
  - feasibility of omics and new informatics platforms explored through study of human skin inflammation
- Data Integration
  - tools developed to construct and analyse biological networks

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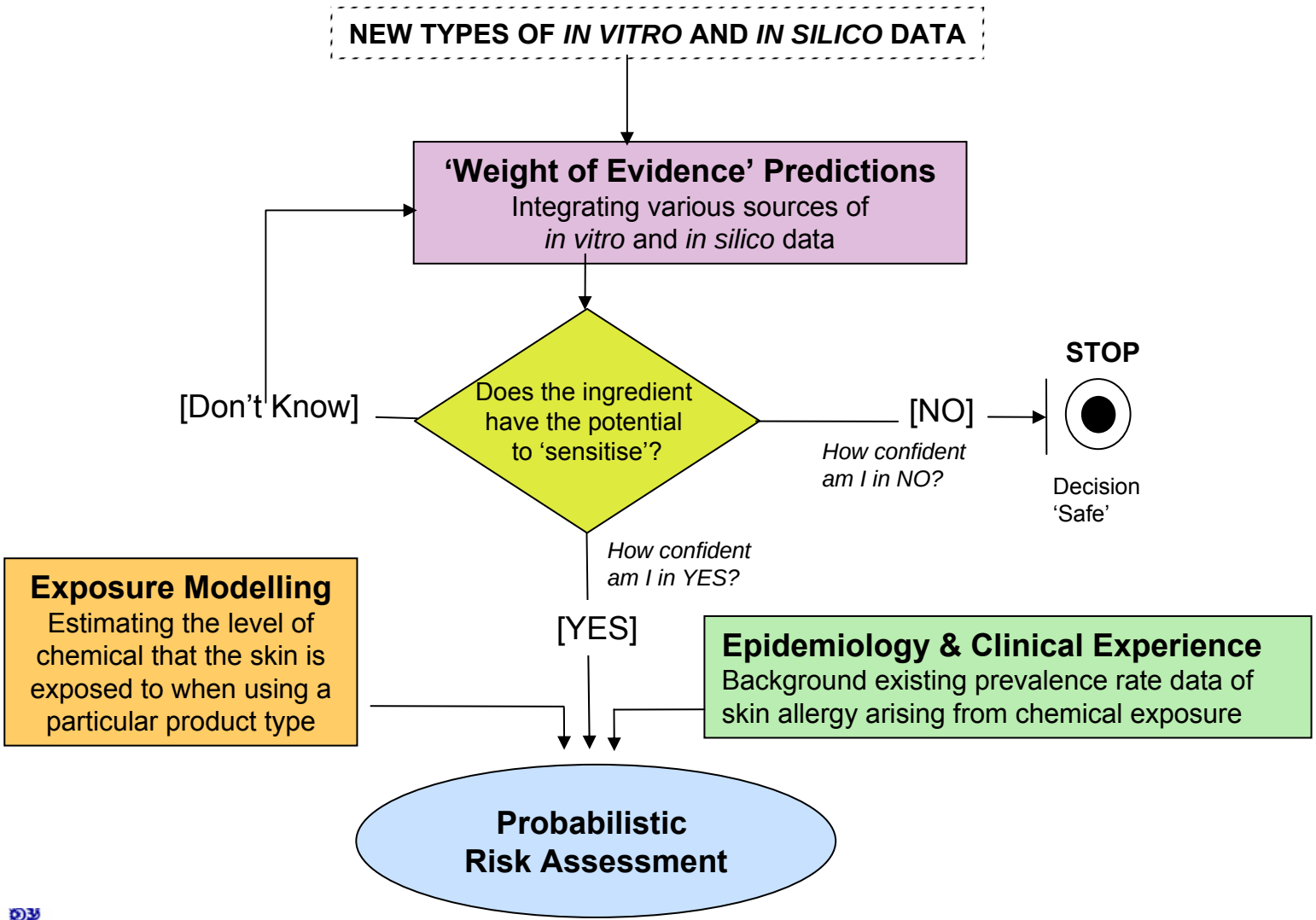
New Risk Assessment Framework



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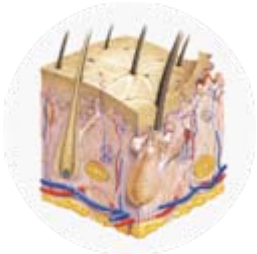
Why apply mathematical modelling to consumer safety risk assessment?



To focus research

- Creating a ‘snap shot’ of what we know and don’t know about chemical-induced skin sensitization will allow effective targeting of investigative research

To guide assay development

- Evaluating the relative contribution of each biological pathway to skin sensitization induction will allow effective model and biomarker selection

To inform new risk assessment approaches

- The model represents a tool for guiding the integration and weighting of different forms of non-animal data

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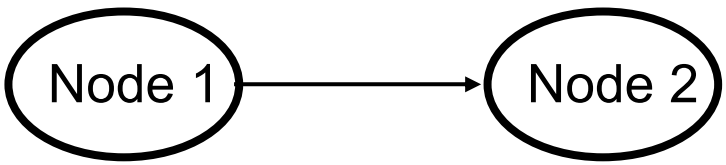
What is an mathematical model of skin sensitization?

- Computer-based representation of biology of sensitization induction described using mathematical equations
- Model limited in scope to cells/mediators/events known to have a role in skin sensitization (mouse/human data)
- Impact of 8 well-characterised sensitizers/non-sensitizers captured through effect on biological system
- Entelos Physiolab software used to visualise model
  - Nodes - Things (i.e. cells, mediators etc.)
  - Arrows - Link nodes, characterise effect of one thing (node) on another



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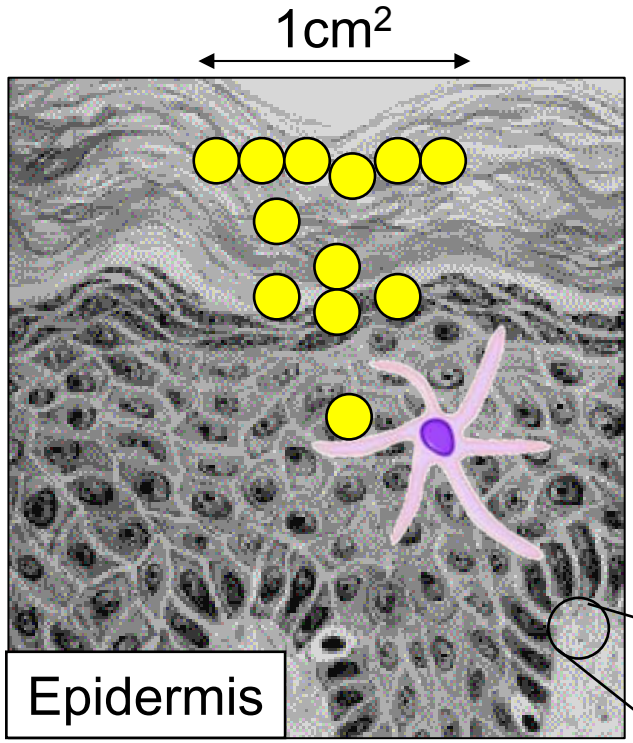
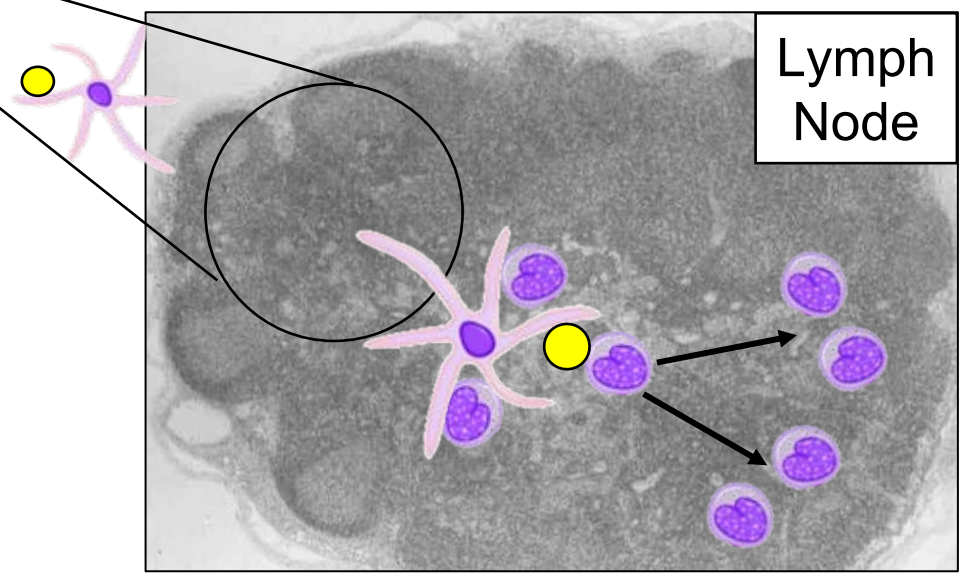
Biological scope of *in silico* model

- Model covers biological pathways required for sensitization induction
- Two main biological compartments





= test chemical

= Langerhans' cell

= T cell

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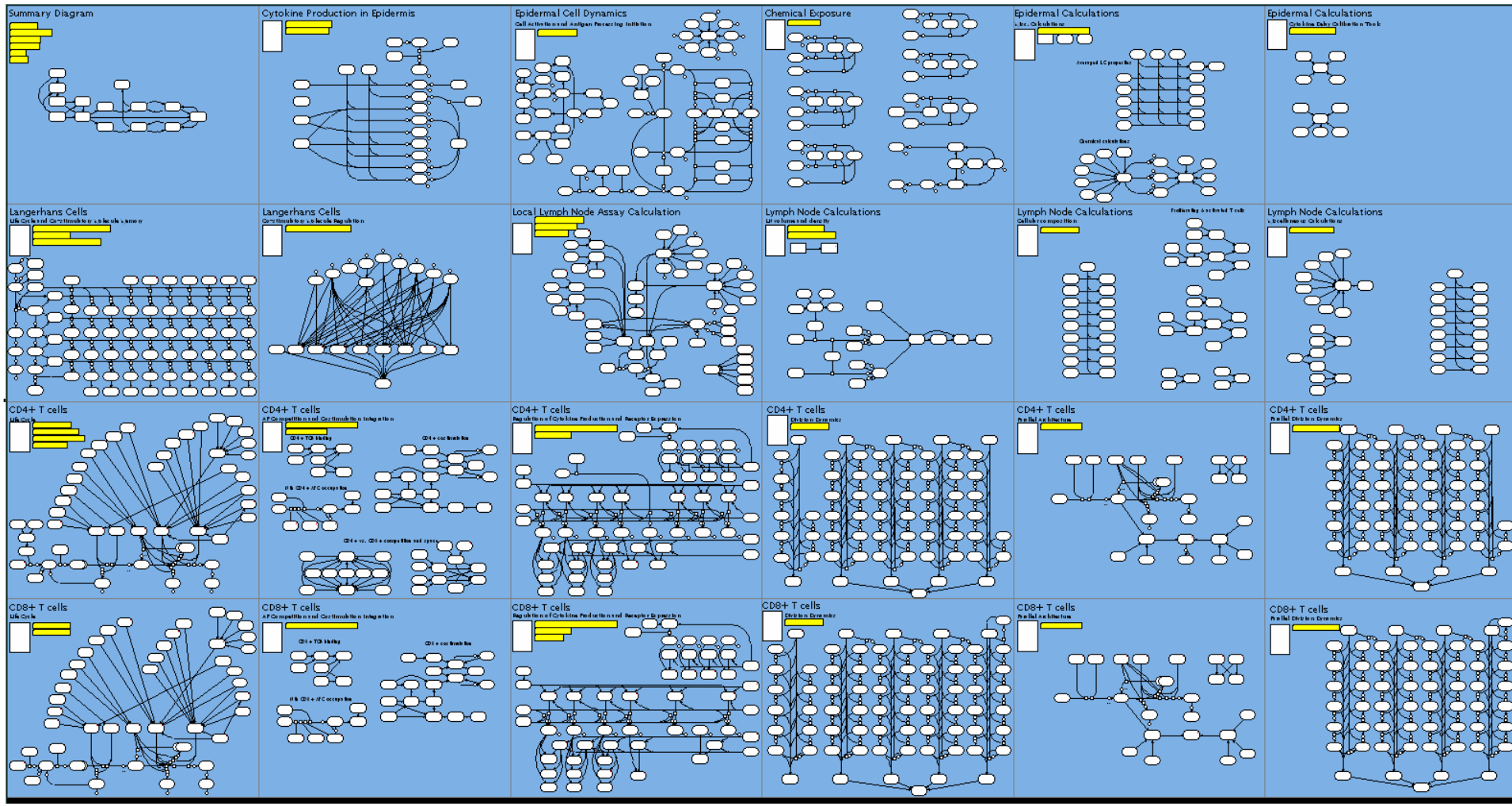
In Silico Model Overview



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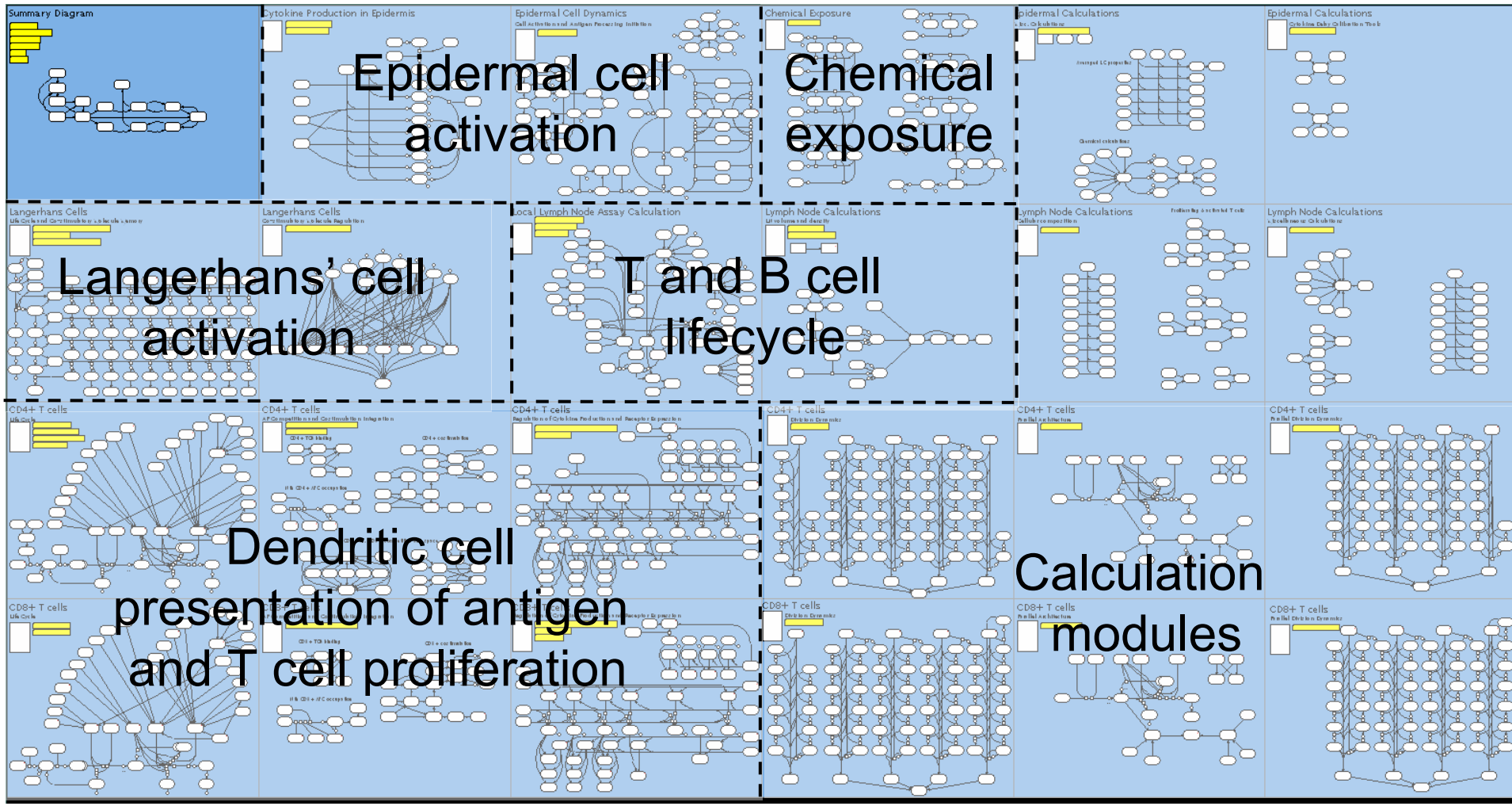
In Silico Model Overview



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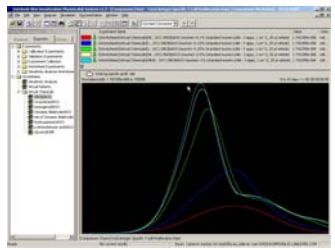
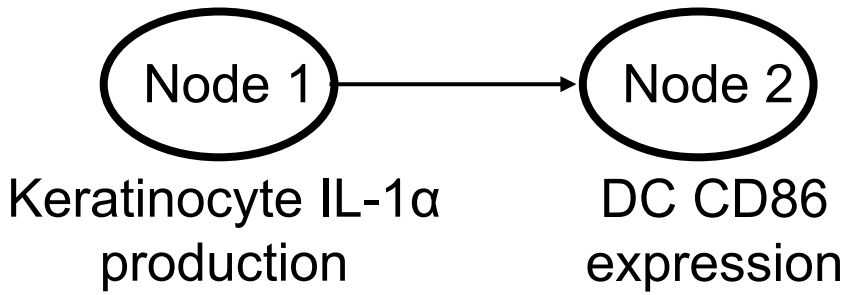
Model Development and Sensitivity Analysis I

- **Qualitative Modelling**
  - Information from contemporary literature used to define cellular/molecular interactions

- **Quantitative Modelling**
  - Dynamic interactions of the biological system represented using mathematical equations & published experimental data





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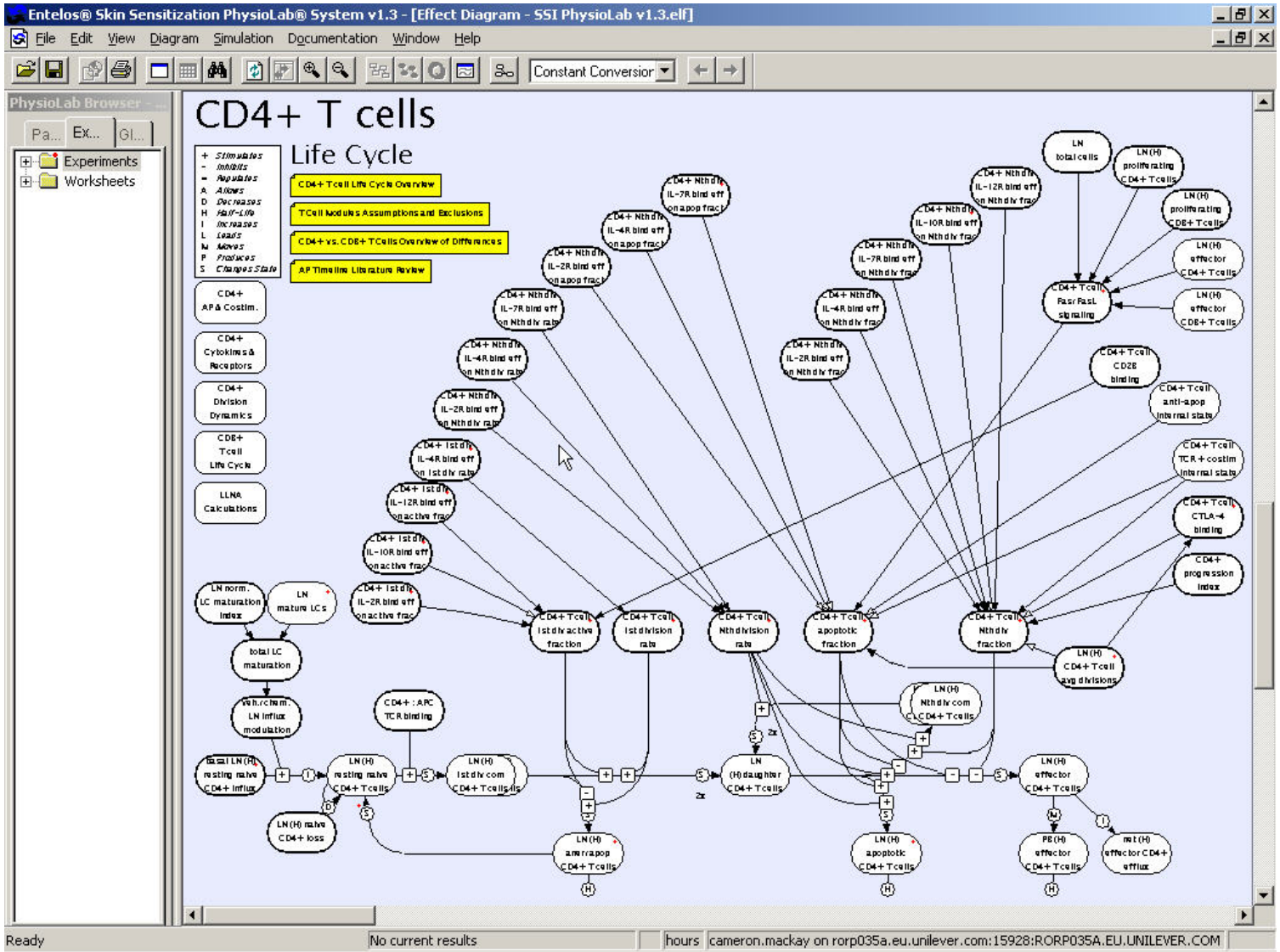
In Silico Model – Example



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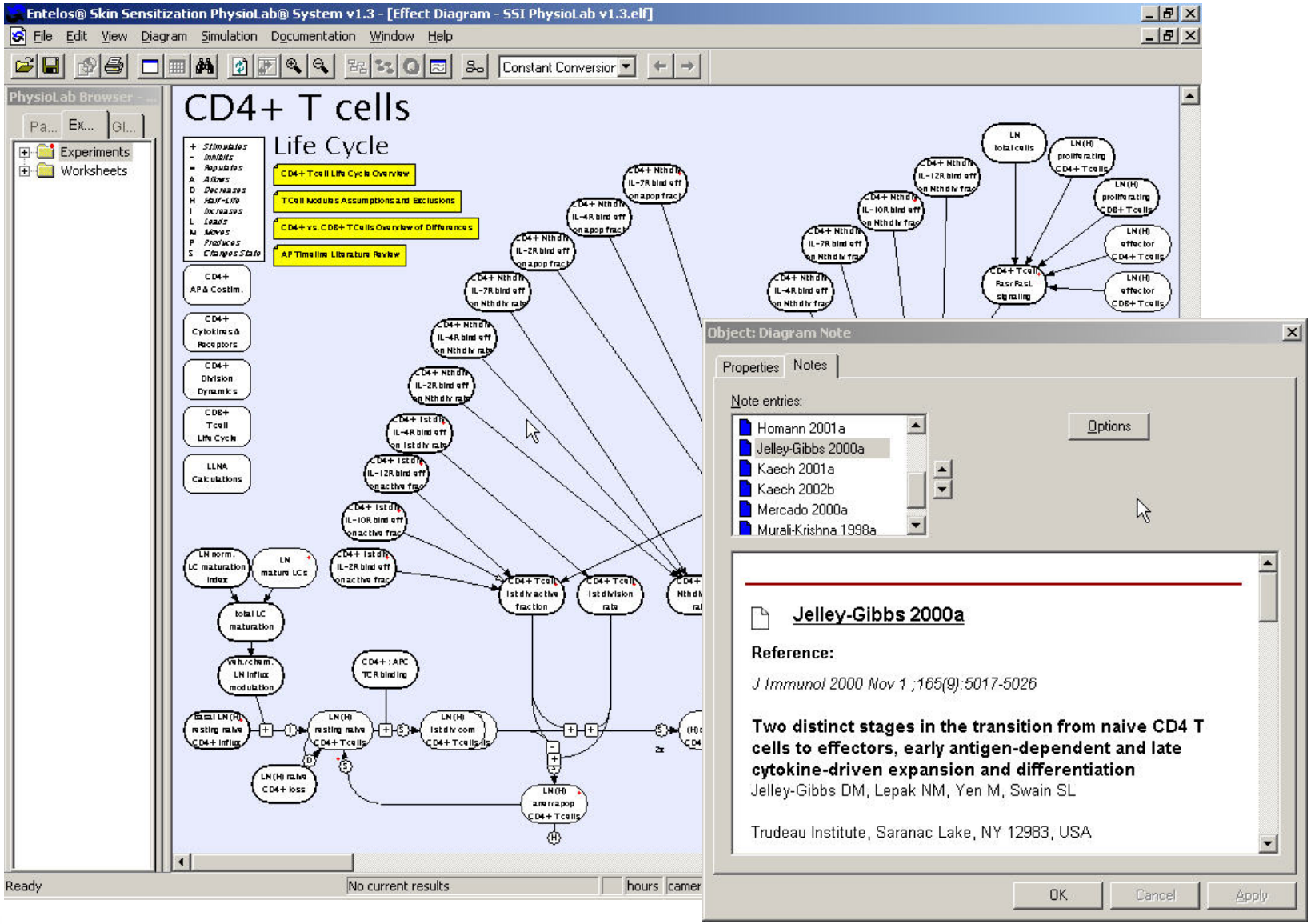
In Silico Model – Example



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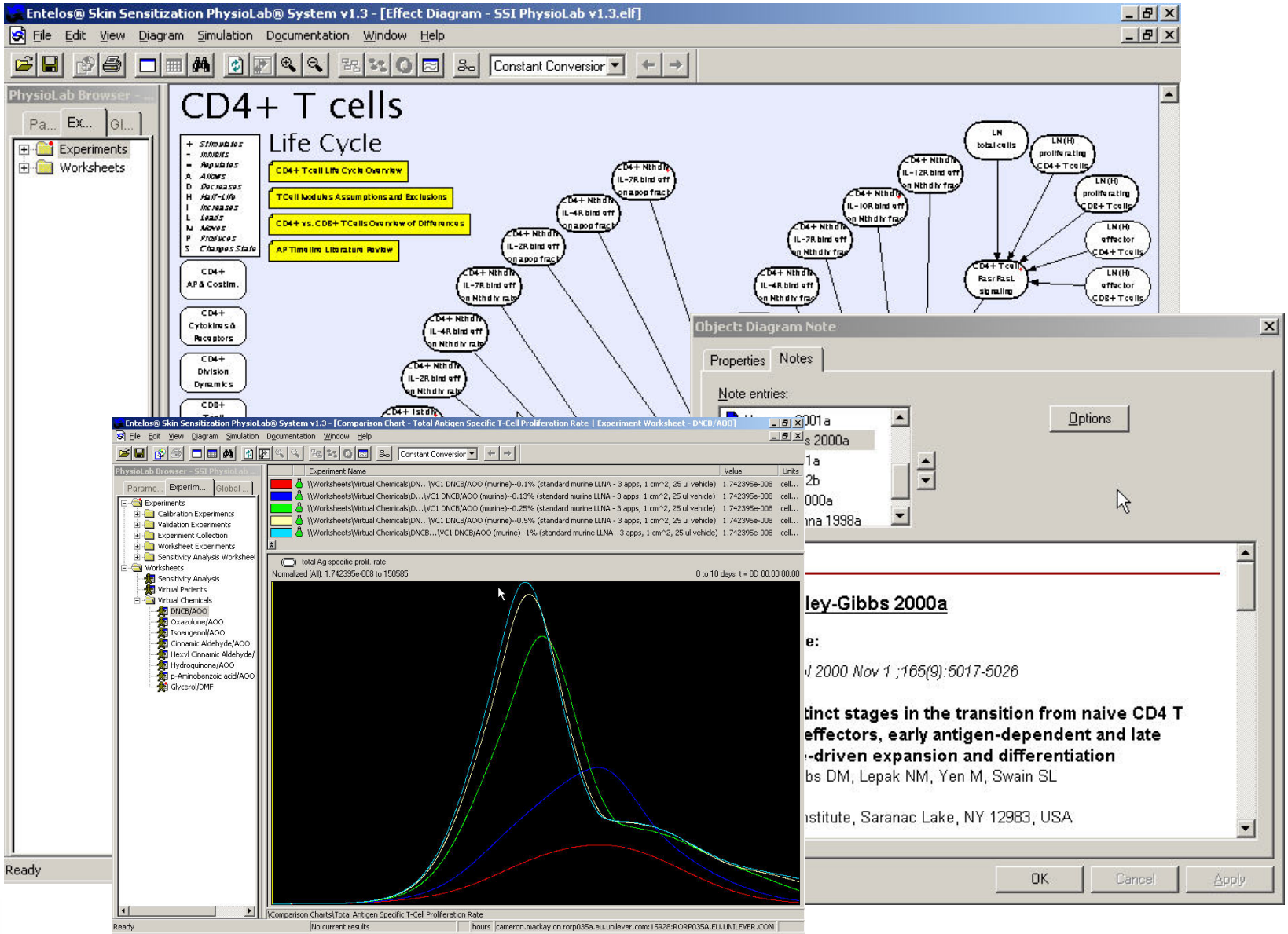
In Silico Model – Example



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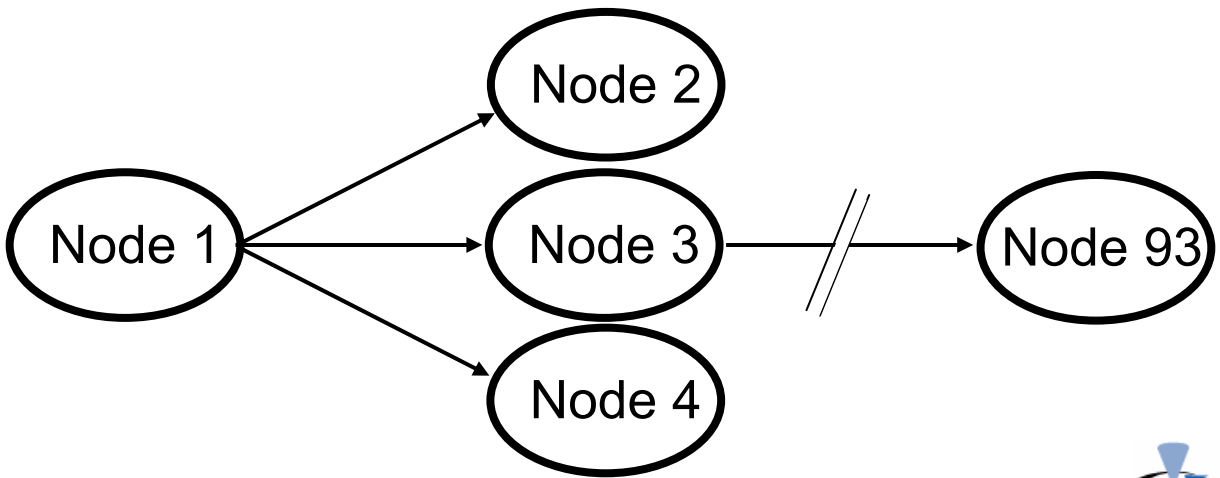
Model Development and Sensitivity Analysis II

- **Model Calibration**
  - Replication of published results from 35 key experiments within the model (e.g. Keratinocyte mediator release)
- **Model Validation**
  - Reproduction of system-level biological response (e.g. LLNA experiment)
- **Sensitivity analysis**
  - Identification of pathways with largest influence on biological response (e.g. max Ag-specific T cell proliferation)



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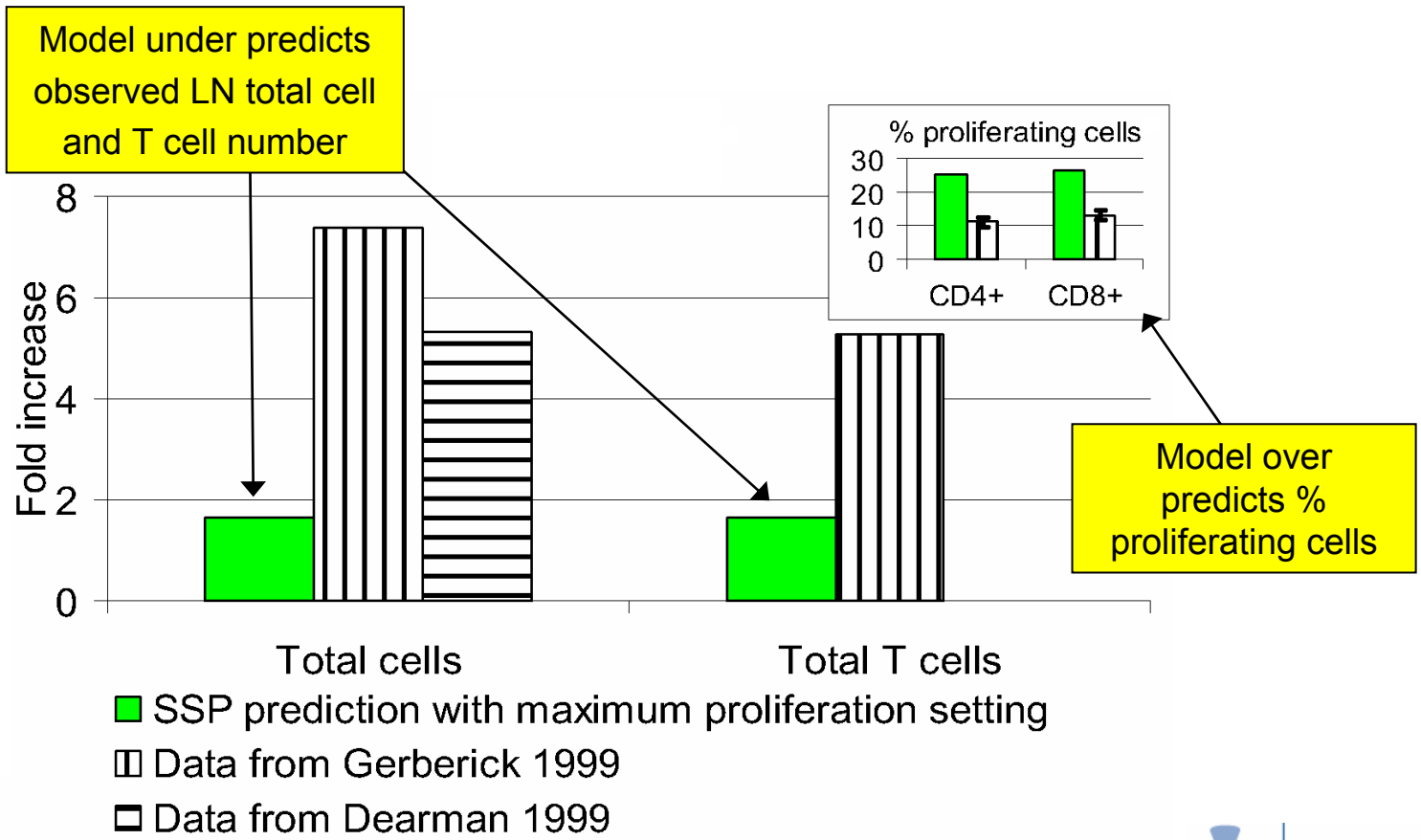
Model Insights

- During calibration phase, model was unable to reproduce published lymph node cell number data (example: 0.25% DNCB exposure in LLNA shown)



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Modelling reveals new biological insights

- **Hypothesis 1**: T cells must undergo > 7 proliferations in sensitizer-induced responses.
- Model required > 20 proliferations to match data
  - Still over predicted % of proliferating cells
  - No experimental evidence to support this hypothesis and runs against infection data (approx. 5-6 proliferations)
- **Hypothesis 2**: Increased recruitment of lymphocytes to the lymph node supplements the total cell population
- Does experimental evidence support this hypothesis?

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Modelling reveals new biological insights

- Tedla *et al*. 1998. *J. Immunol.* **161**. 5663-5672
  - DNFB (sensitizer) exposure on skin induces mouse LN chemokine production (MIP-1α/β)
  - Peripheral leukocyte numbers depleted by 50% at 30mins after exposure

- Soderberg *et al*. 2005. *PNAS*. **45**. 16315-16320
  - TLR agonist intradermal exposure and Herpes Simplex virus infection caused massive recruitment of naïve lymphocytes to LN.
  - Most LN cells are non-proliferating (95%)
  - Proposed mechanism is via vessel re-modelling: greatly increases LN cell turnover.

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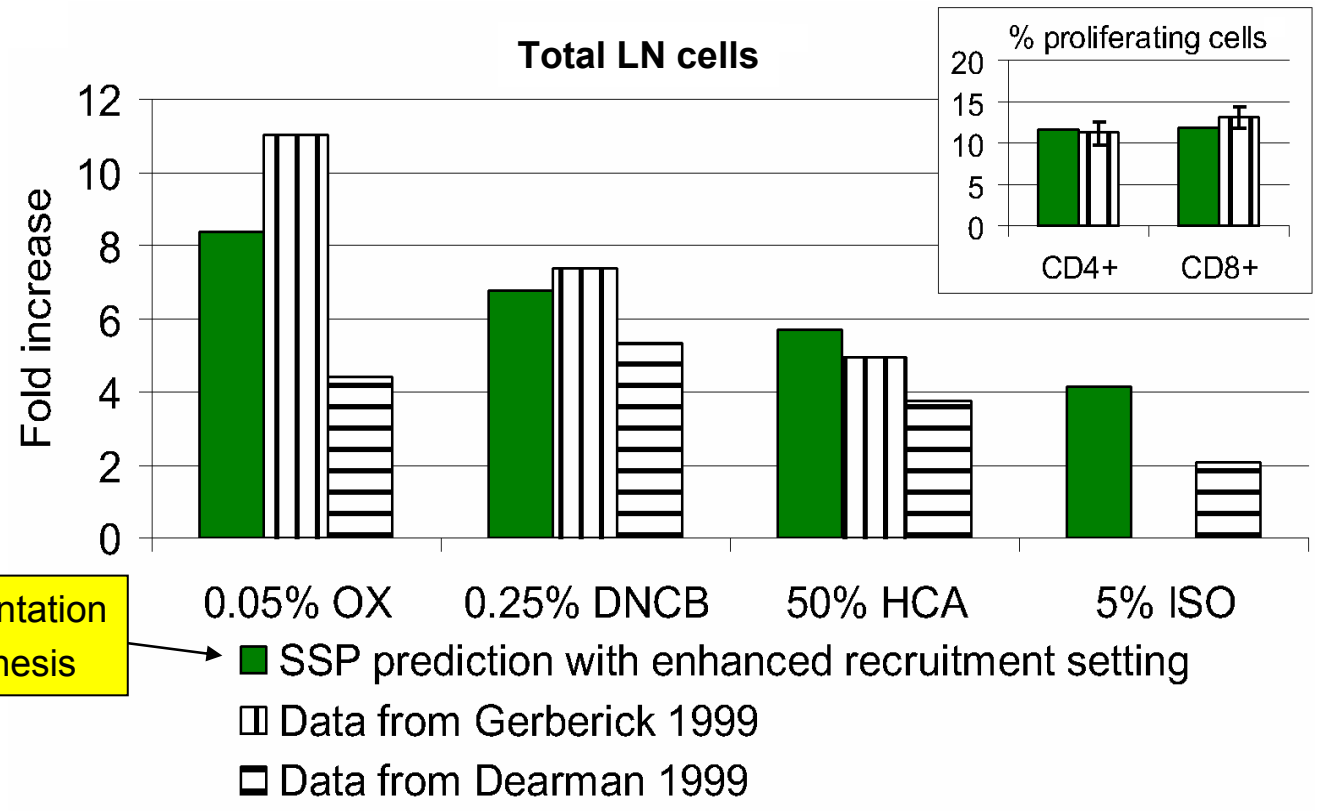
New Insight: Cell recruitment to the lymph node

Hypothesis 2 implemented



Model implementation with new hypothesis

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Model Sensitivity Analysis

- Aim:
  - To evaluate relative contribution of individual pathways to overall biological response (e.g. Max Ag-specific T cell proliferation)
- Method:
  - Controls – assigned control dose for prototypic weak/moderate/strong sensitizers
  - Experiments – vary model parameters to up/down-regulate biological pathways
- Results:
  - Record model predicted outcomes under control and perturbed conditions – approx. 30,000 simulations performed
  - Calculate fold change in outcome relative to control
  - High fold change = high influence of pathway on response

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Measure outcomes and modulated pathways

Modulated pathways:

Category •Subcategory	•Parameter/Location
Chemical exposure	•haptenated protein half-life •binding efficiency
Epidermal inflammation	•epidermal LC mat/mig induction •epidermal IL-1a production •epidermal IL-1b production •epidermal TNF-a production •epidermal IL-8 production •epidermal IL-10 production •epidermal GM-CSF production •epidermal cytokine production (all together)
Non-specific response	•veh/chem LN influx modulation •LN mature LCs
Antigen presentation	•space/LC •LN norm. mature LC MHC I •LN norm. mature LC MHC II •LN norm. mature MHCI and MHCII together •LN norm. mature LC B7-1 •LN norm. mature LC B7-2 •LN norm. mature LC anti-apop •LN norm. mature LC IL-12 prod •total LN LC phenotype (all markers together)

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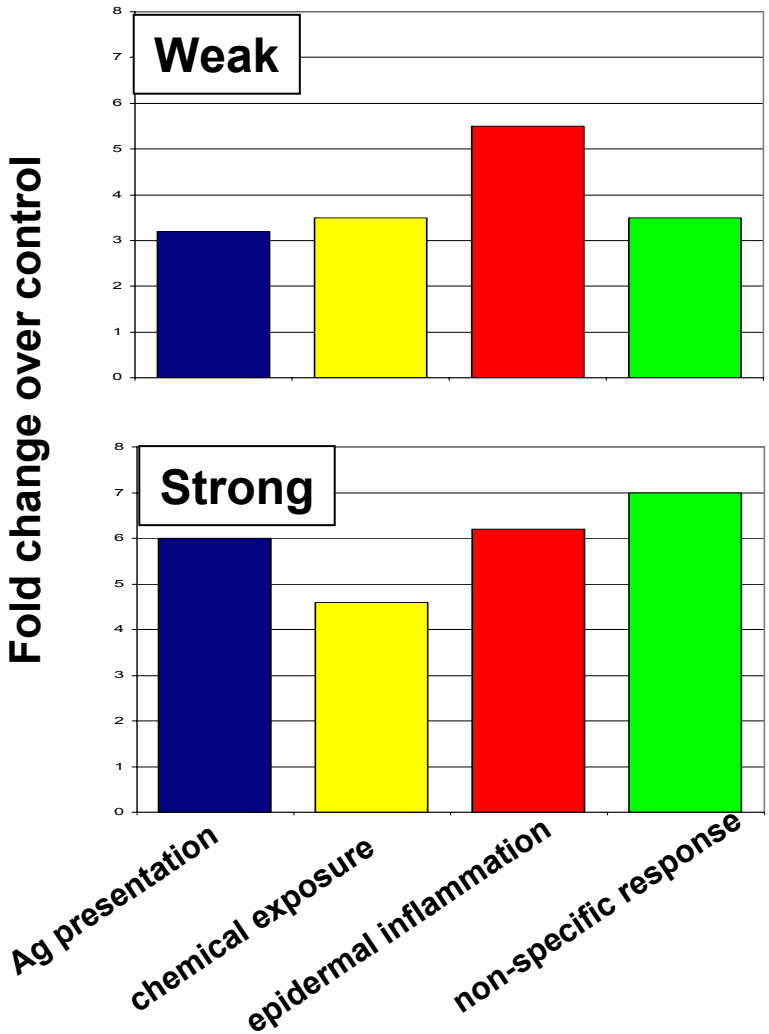
Relative pathway contribution: Maximum Antigen-specific T cell proliferation



- Max Ag-specific T cell proliferation selected as ideal measure of skin sensitization induction
- Epidermal inflammation and (Ag) non-specific effects have a significant influence over Ag-specific T cell proliferation
- All categories are significantly influential across sensitizer strength

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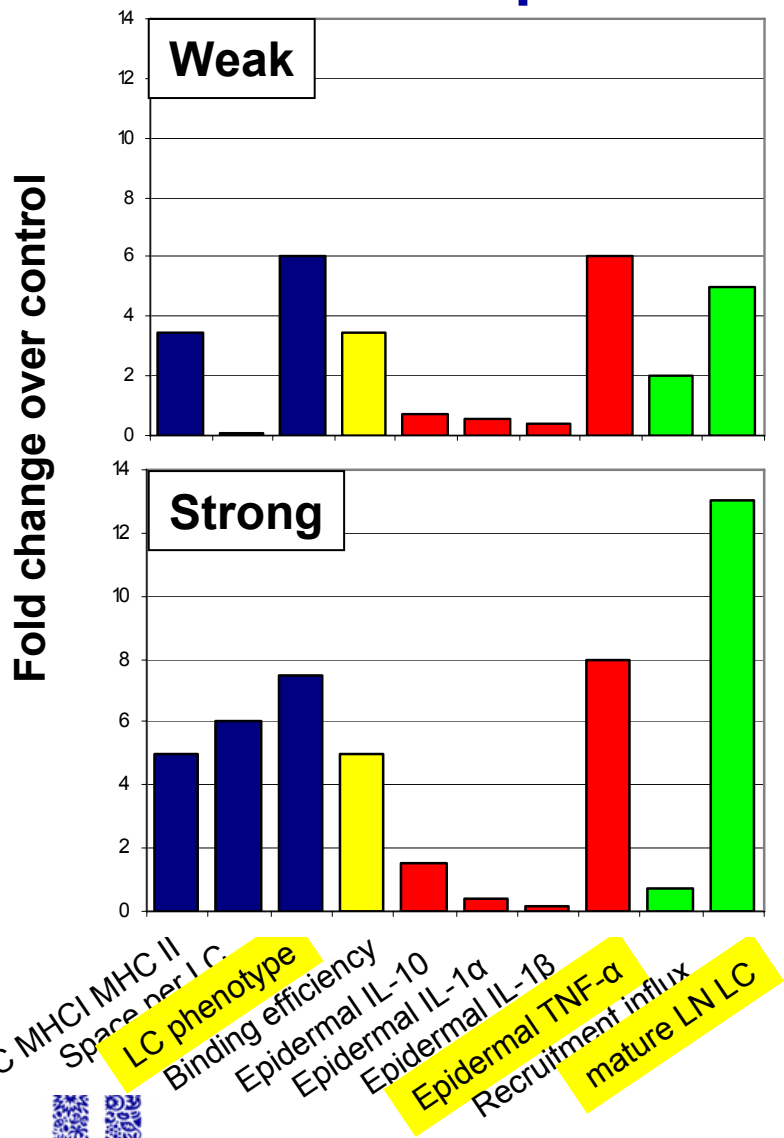
Relative pathway contribution: Maximum Antigen-specific T cell proliferation



- Epidermal inflammation (e.g. TNFα release) has most significant effect over Ag-specific T cell proliferation
  - Due to role in induction of LC migration to LN
- Number of mature LN LCs and LC phenotype also have a significant effect on Ag-specific T cell proliferation

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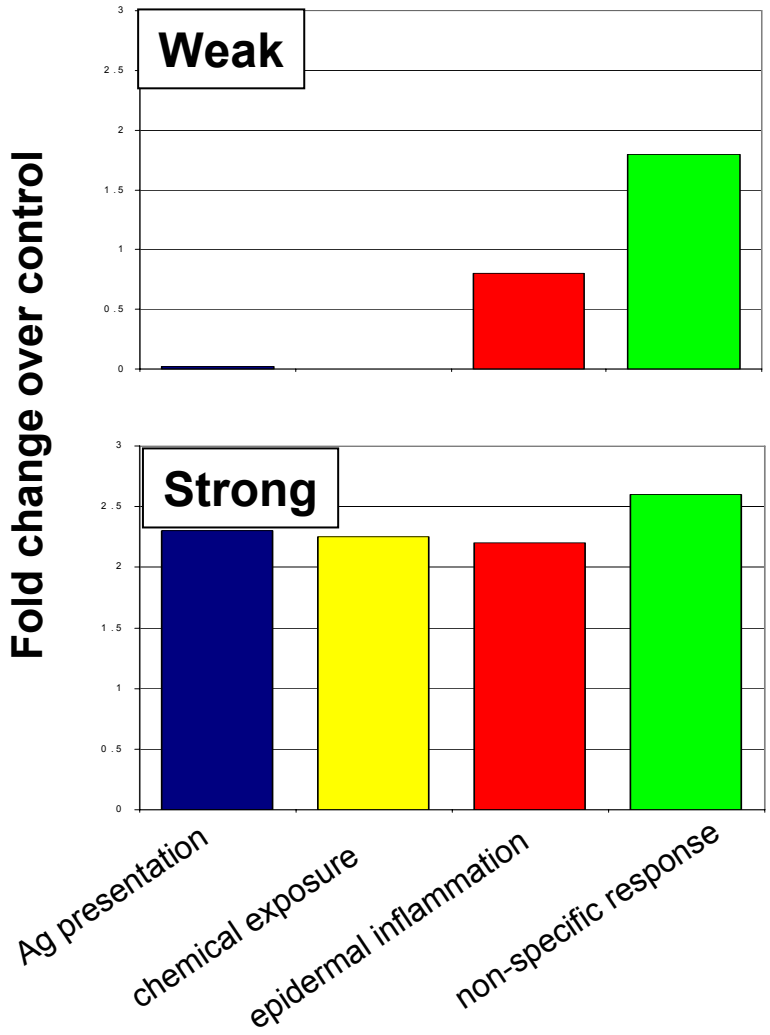
Relative pathway contribution: LLNA Stimulation Index

- LLNA SI measures both Ag- and Ag-non-specific T cell proliferation
- Weak Sensitizers
  - Epidermal inflammation and (Ag) non-specific responses dominate the LLNA SI
- Strong sensitizers
  - Chemical exposure and antigen presentation pathways become more important



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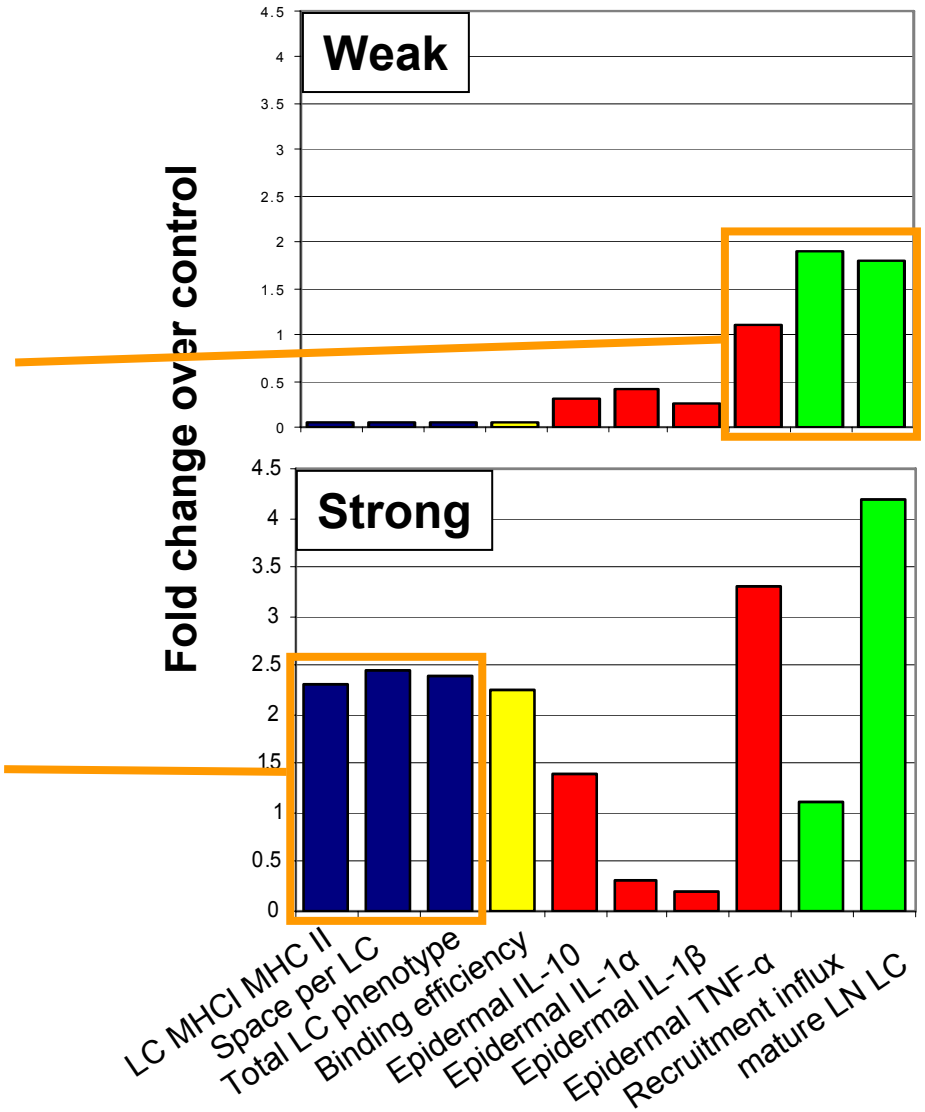
Relative pathway contribution: LLNA Stimulation index

- Weak sensitizers
  - LLNA SI is dominated by Ag-non-specific T cell proliferation (e.g. epidermal inflammation pathways)
- Strong Sensitizers
  - LLNA SI includes a stronger contribution from Ag-specific T cell proliferation (i.e. Ag presentation pathways have strong influence on SI)



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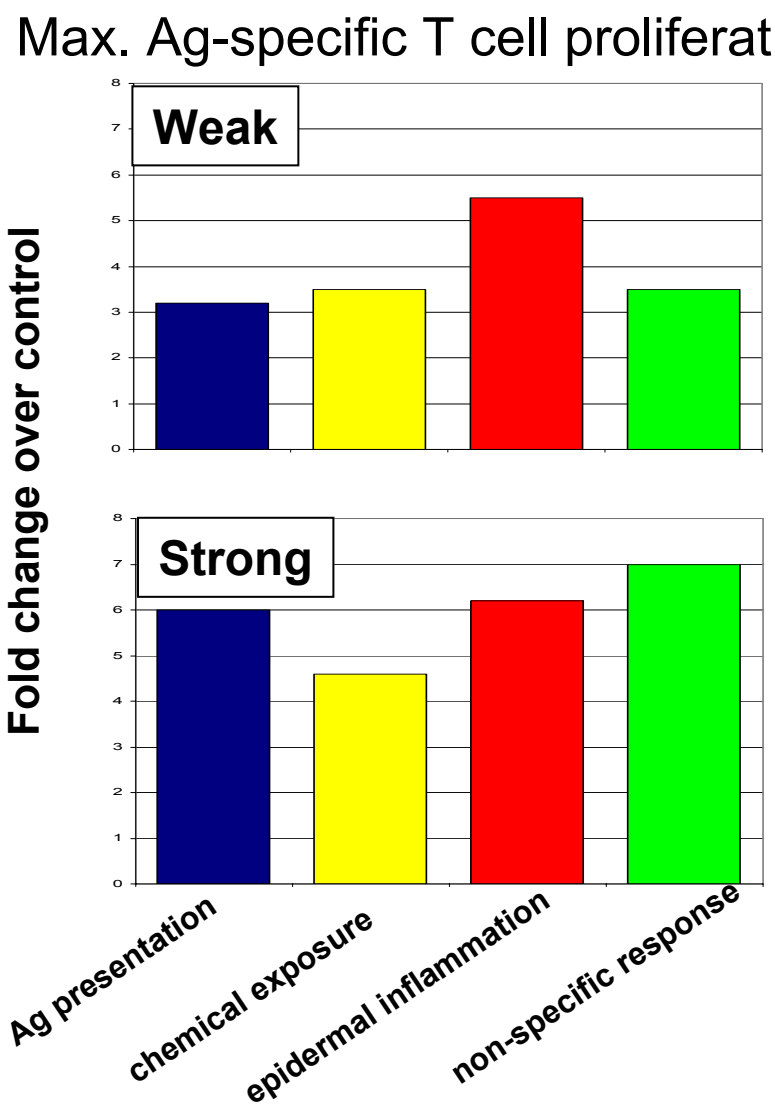
Insights for *in vitro* assay development



- An array of predictive assays that cover all key categories should allow Ag-specific T cell proliferation to be confidently predicted
- Several model systems in development should be capable of generating these key pieces of data:
  - Chemical Exposure – Peptide Binding
  - Epidermal Inflammation – 3D Skin models
  - Ag presentation – DC activation or *in vitro* T cell proliferation
- (Ag) non-specific response may require *in silico* prediction or new assay type

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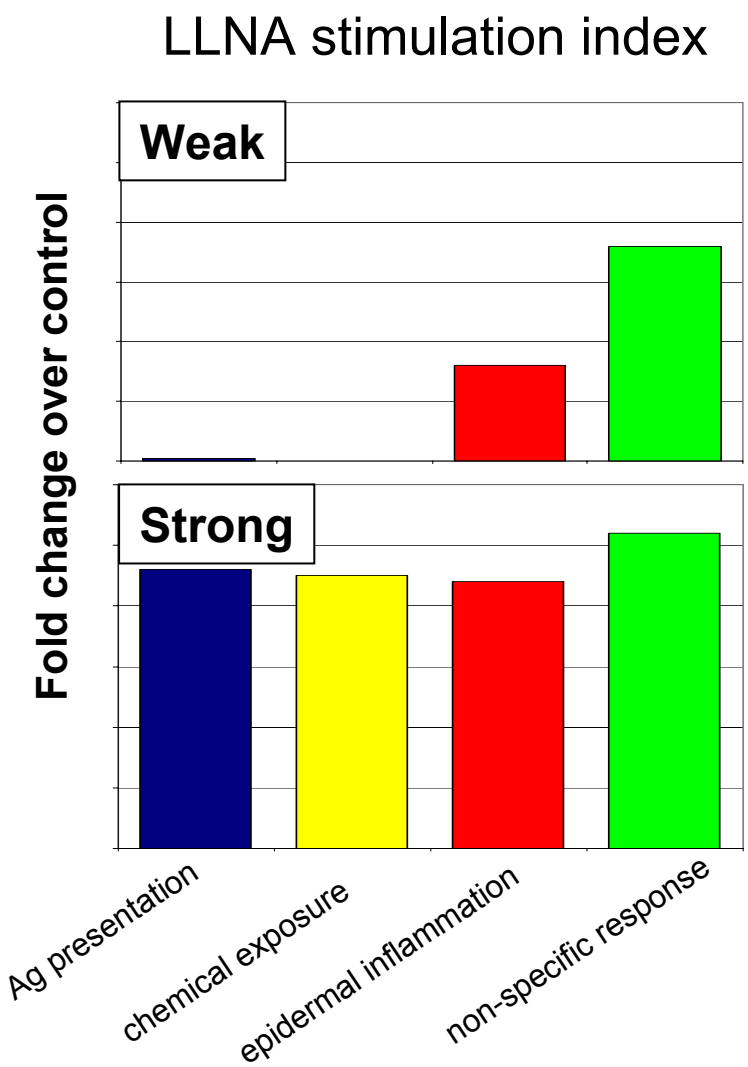
Insights for non-animal assay development



- Traditional *in vitro* assay validation (i.e. through direct correlation *in vivo* animal data) will not be possible
- Relative influence of different biological pathways on LLNA SI will vary across different chemicals
- Integration of data from multiple assays, delivering different types of hazard information, will be required

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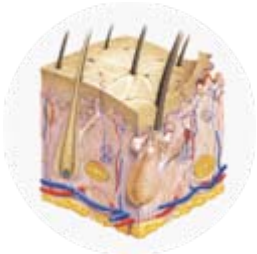
Next Steps



To focus research

- Skin allergy research programme realigned to address key knowledge gaps
  - Future research data will be used to inform the model where possible

To guide assay development

- *In vitro* assay development research verified as broadly relevant by sensitivity analysis
  - *In silico* pathway analysis used to guide selection of experimental parameters

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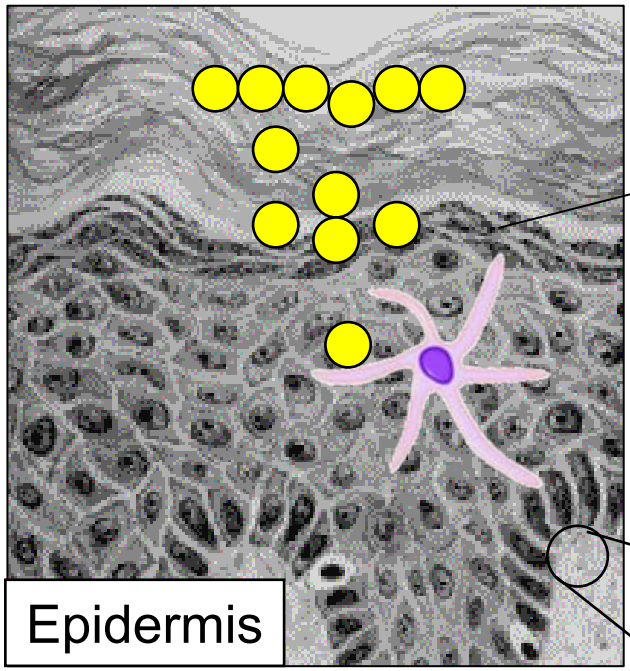
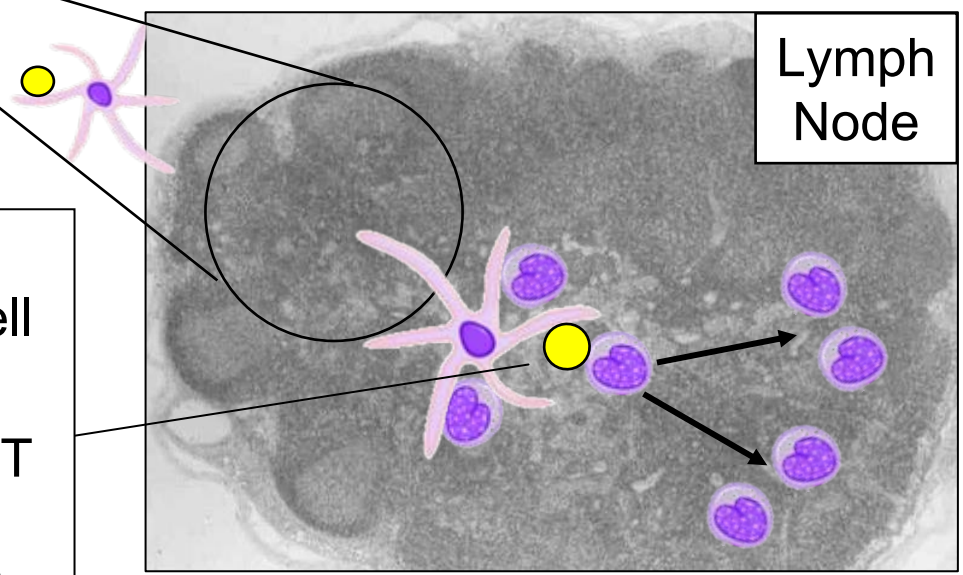
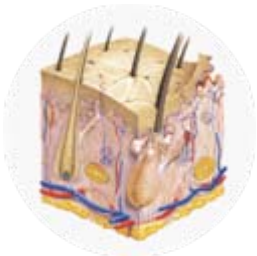


- How does the frequency and/or specificity of hapten: protein binding relate to sensitizer potency?
- Do sensitizers activate DC solely via indirect mechanisms (e.g. inflammatory signal release) or are direct mechanisms (e.g. receptor-mediated) also involved?



- How does sensitizer potency correlate to naïve, specific T cell clone frequency?
- What is the role of regulatory T cells and other lymphocyte subsets (i.e. B cells, NK cells)?

Next Steps



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To inform new Risk Assessment approaches

- Model provides biological rationale for guiding integration of different forms of animal data
  - e.g. what is value of epidermal inflammation data?

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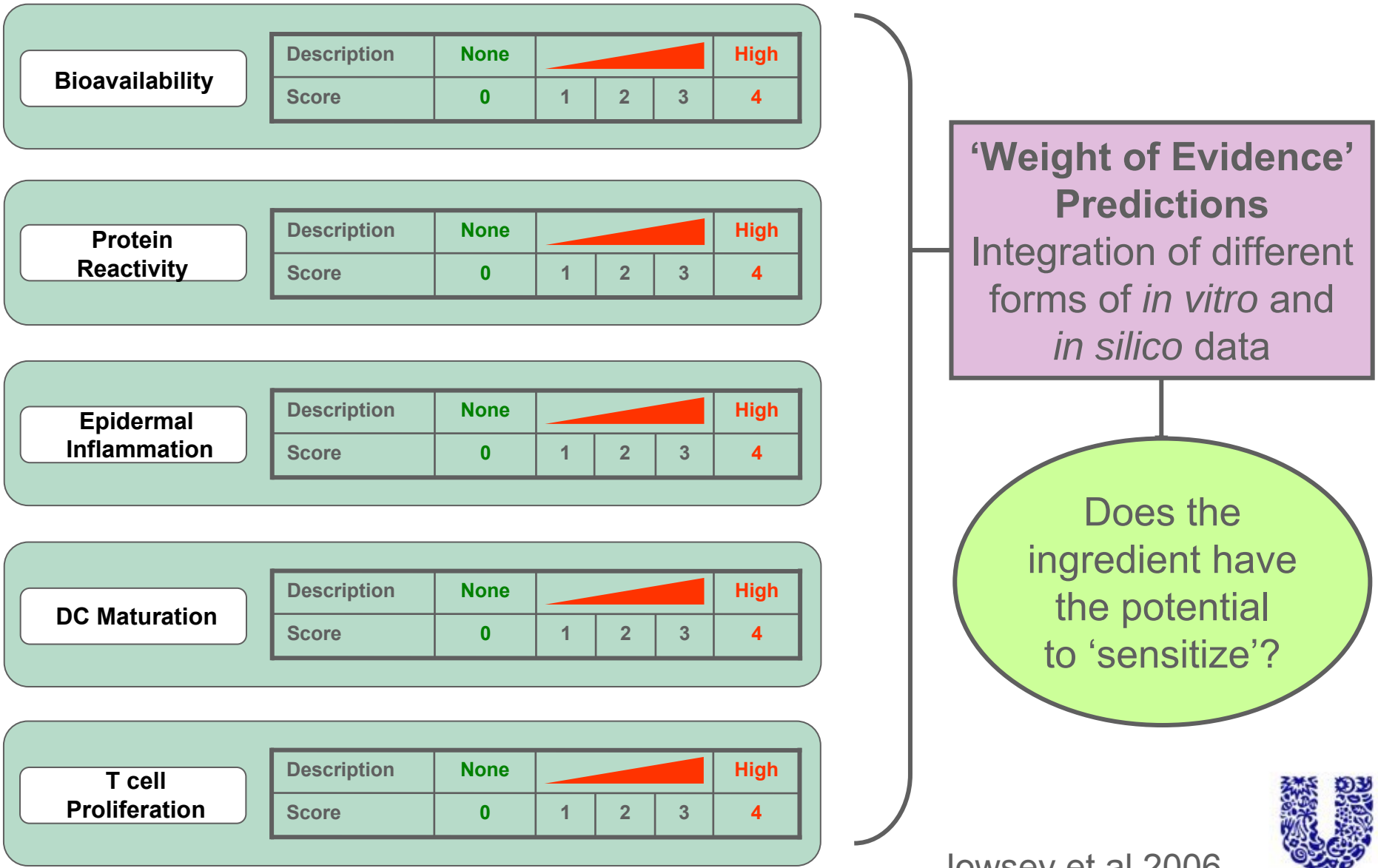
Integration of different forms of non-animal data



Jowsey et al 2006

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Acknowledgements

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  - Catherine Clapp
  - Ian Jowsey
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  - Cameron MacKay
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