Mathematical modelling of skin sensitization: Practicalities of the modelling process

Dr Cameron MacKay

Safety & Environmental Assurance Centre

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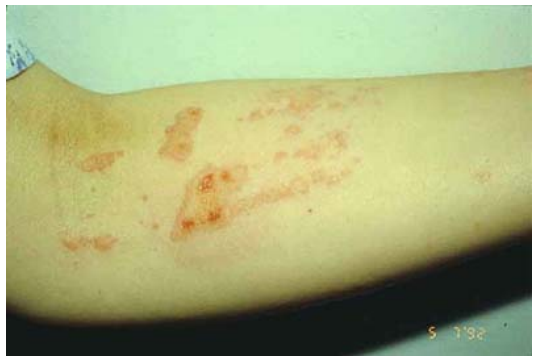
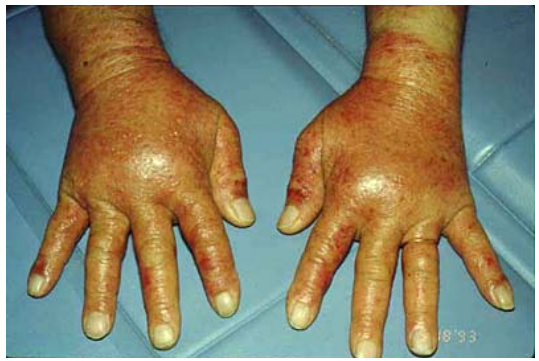
Skin Sensitization





- Allergic Contact Dermatitis:
  - occurs due to the presence of allergen-specific T cells in the circulation (sensitisation)
  - allergen re-exposure induces recruitment of allergen-specific T cells into the skin where they mediate an inflammatory response (elicitation)
- To assure consumer safety, animal data (e.g. mouse local lymph node assay) is currently used in risk assessment of skin sensitisation

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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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Project Objectives

- Use data in the published literature to construct a computer-based mathematical model of the induction of skin sensitization
- Use this model to interrogate the biology and determine the biological pathways having the greatest influence on the endpoint (T-cell proliferation)
- Use this information to build a strategy for obtaining *in vitro* assays that are predictive of the induction of skin sensitization

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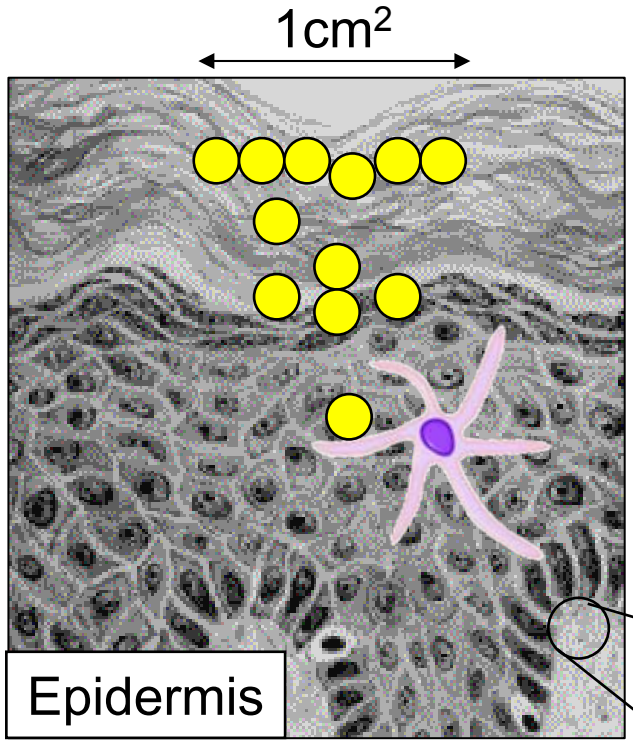
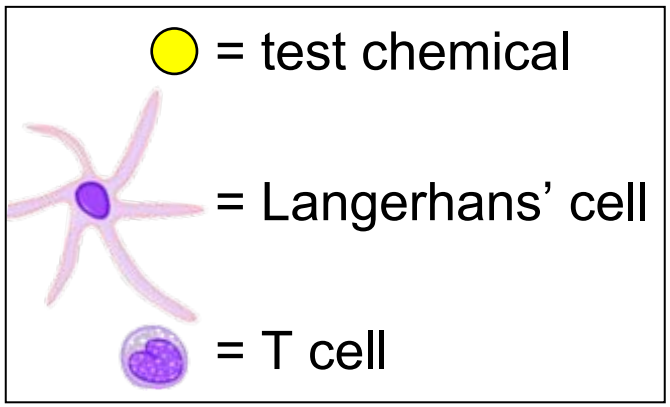
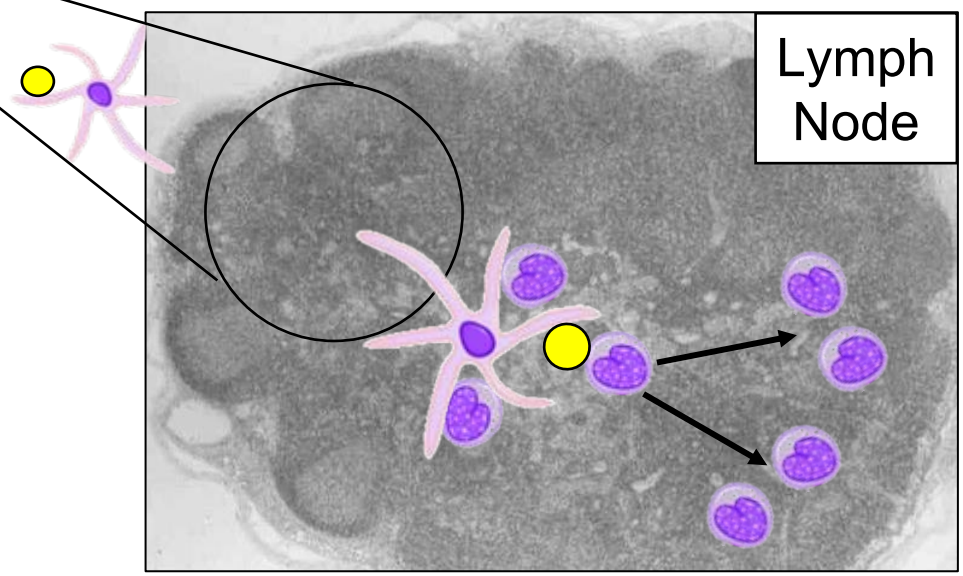
Biological scope of *in silico* model

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







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Approach Overview

- Stage I: Qualitative platform design
  - Documentation of pathways
  - Knowledge gaps
- Stage II: Quantitative platform development
  - Calibration experiments
  - Virtual patients
- Stage III: Platform validation & exploration
  - Validation experiments
  - Sensitivity analysis
  - Assay recommendations
  - Areas for additional platform development identified

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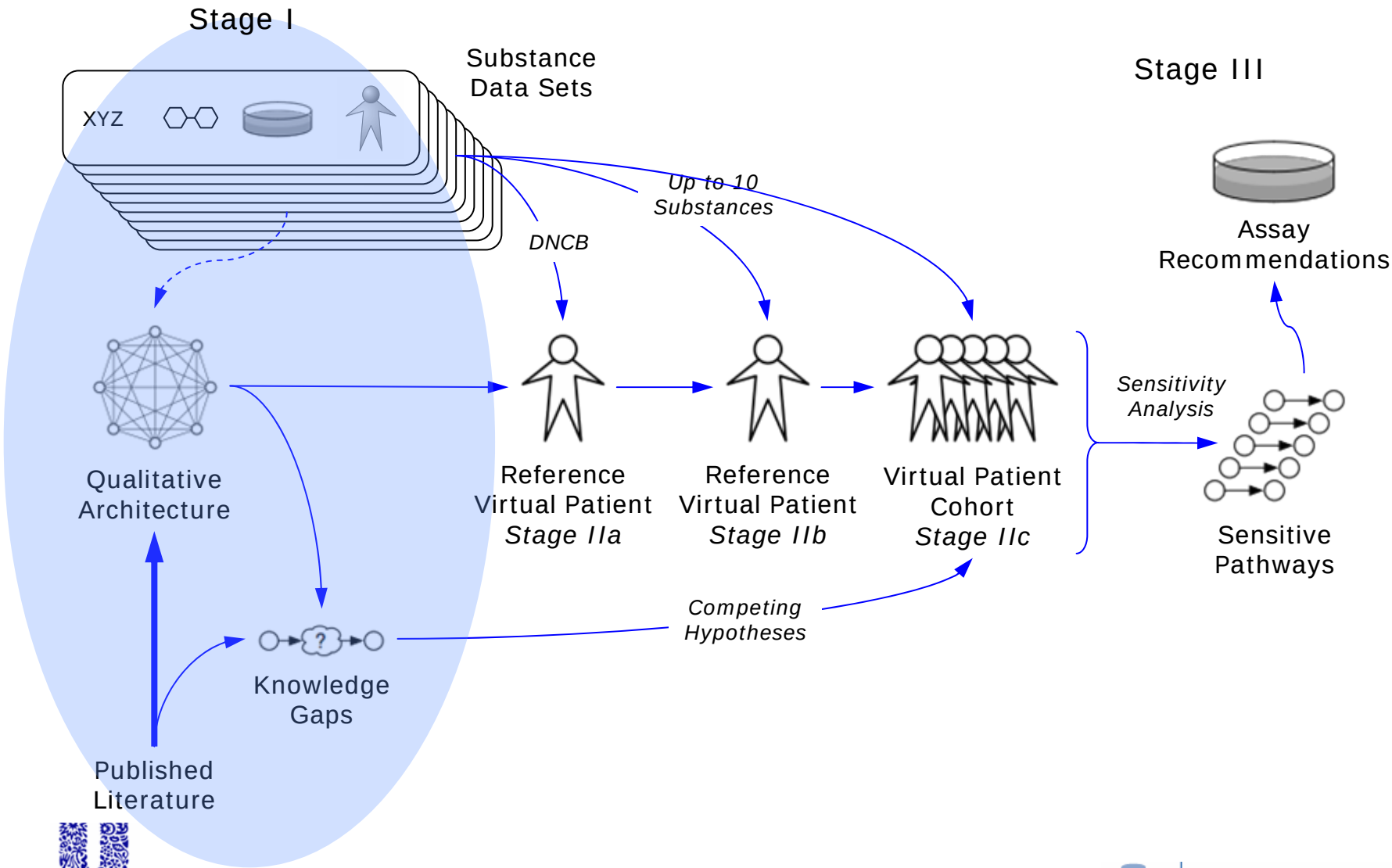
Skin Sensitization Induction PhysioLab Platform (SSIPP)



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Model development – stage I

- Qualitative Modelling
  - 496 papers used in mapping biological processes
- Types of information required
  - Released cell mediators
  - Regulated surface markers
  - Regulators of T-cell proliferation
- Identify knowledge gaps

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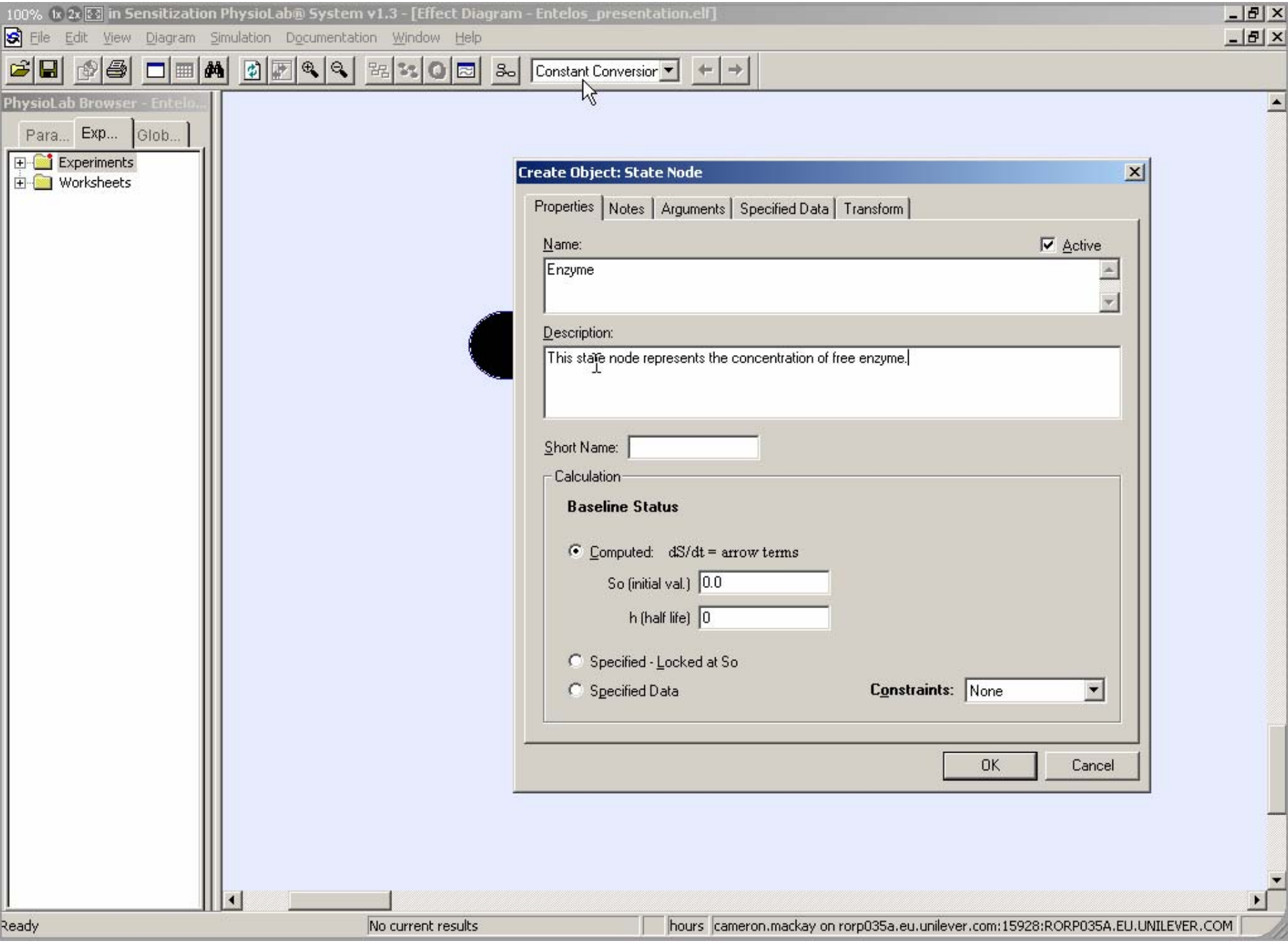
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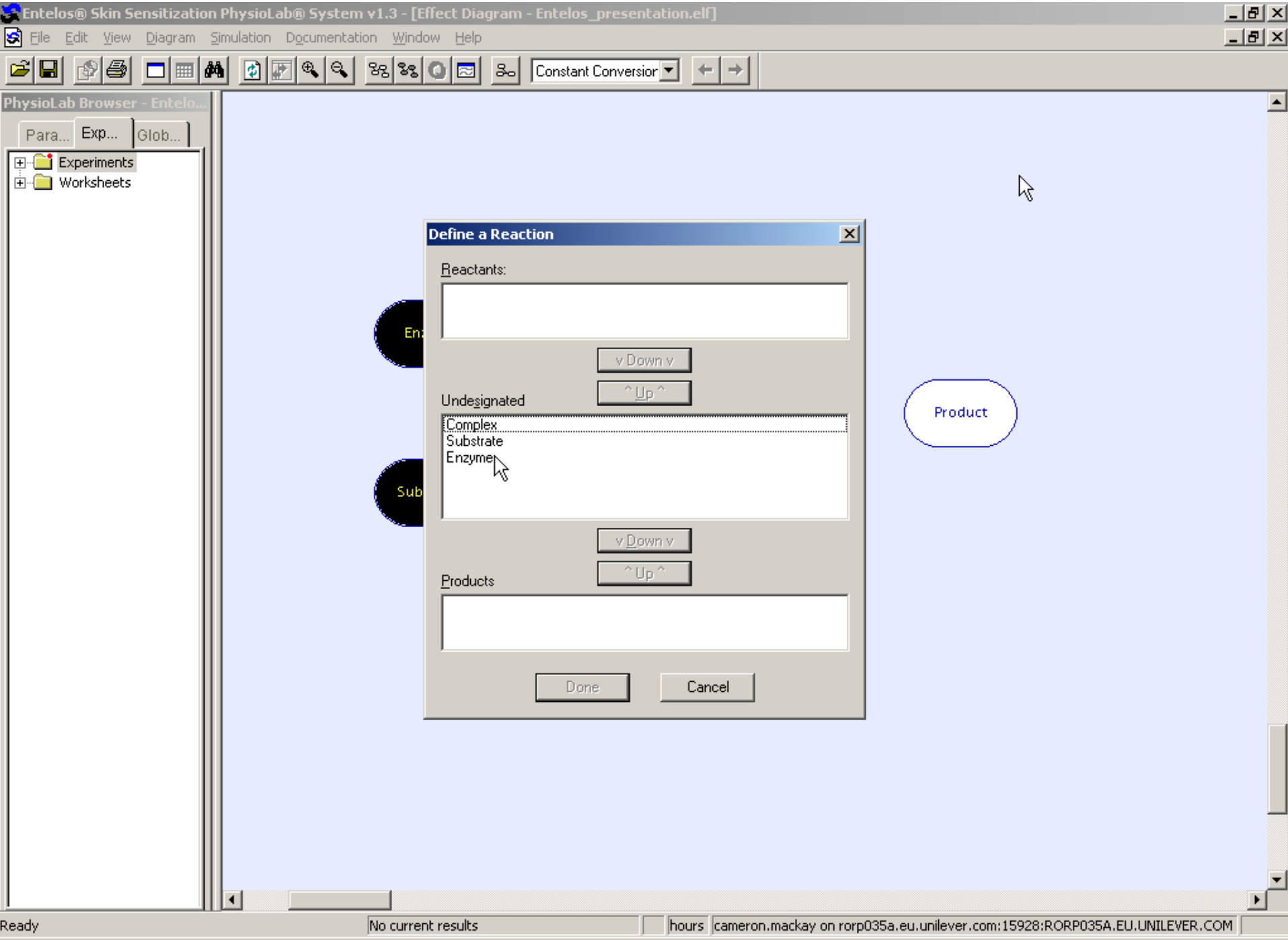
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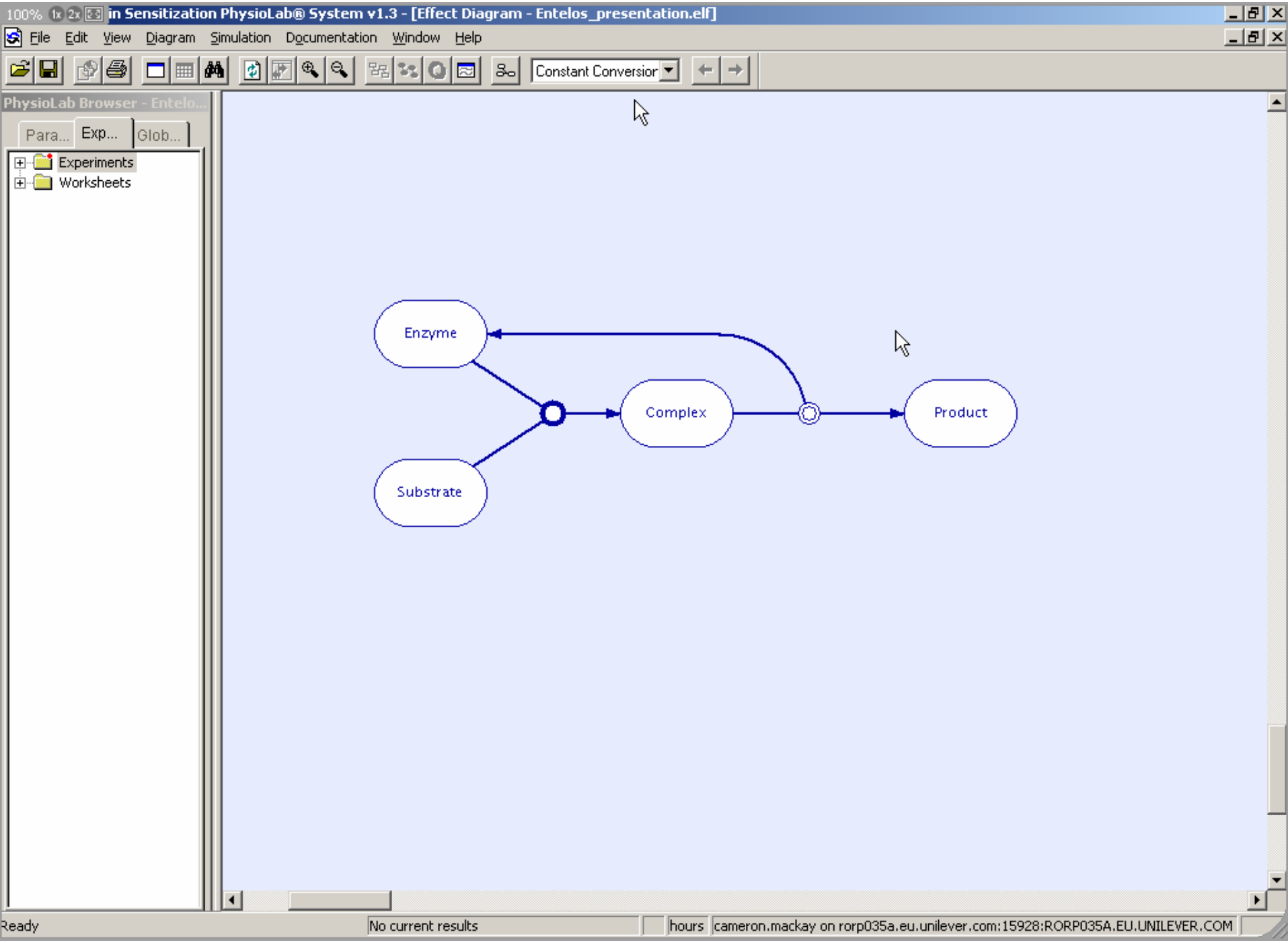
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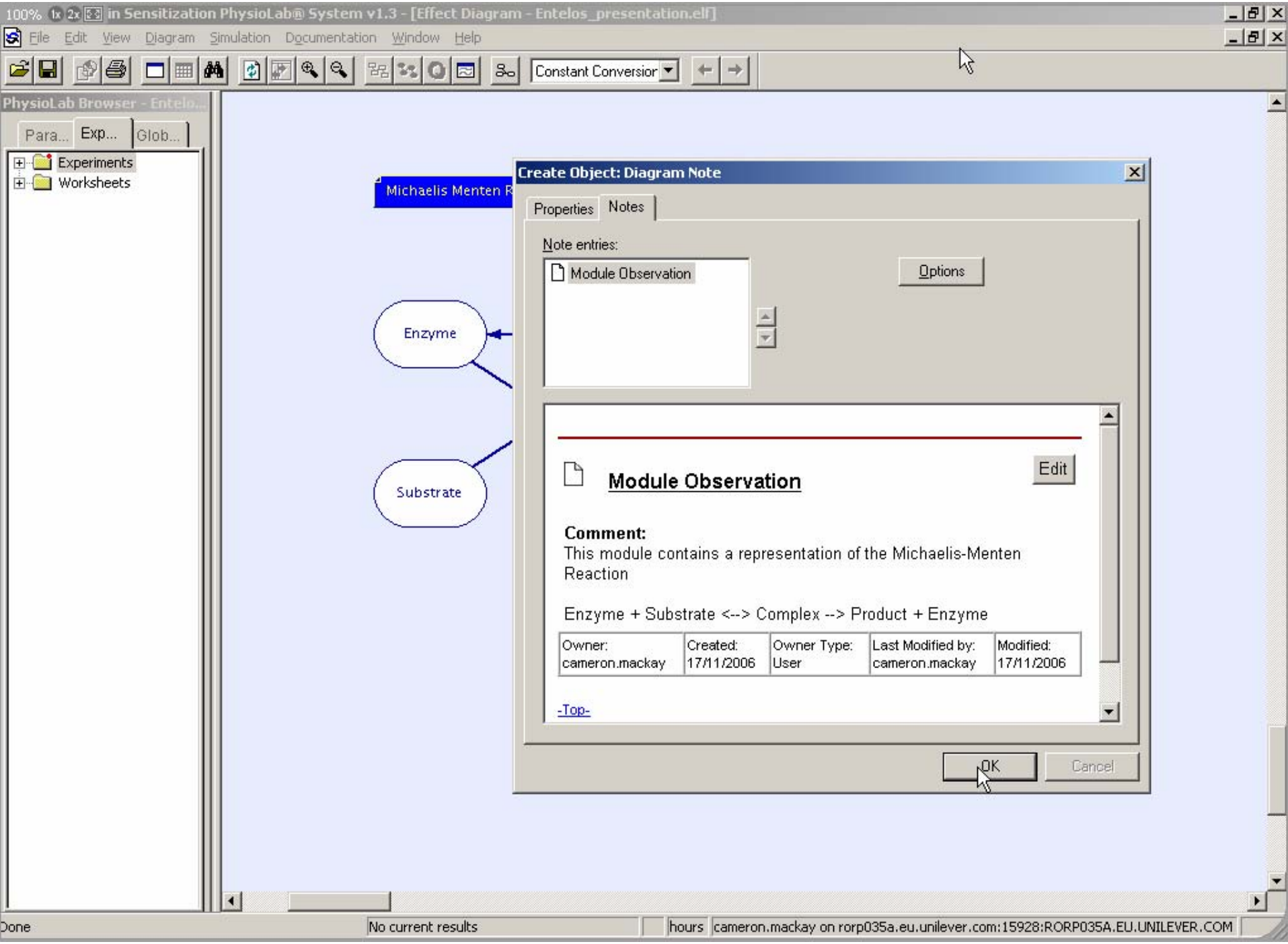
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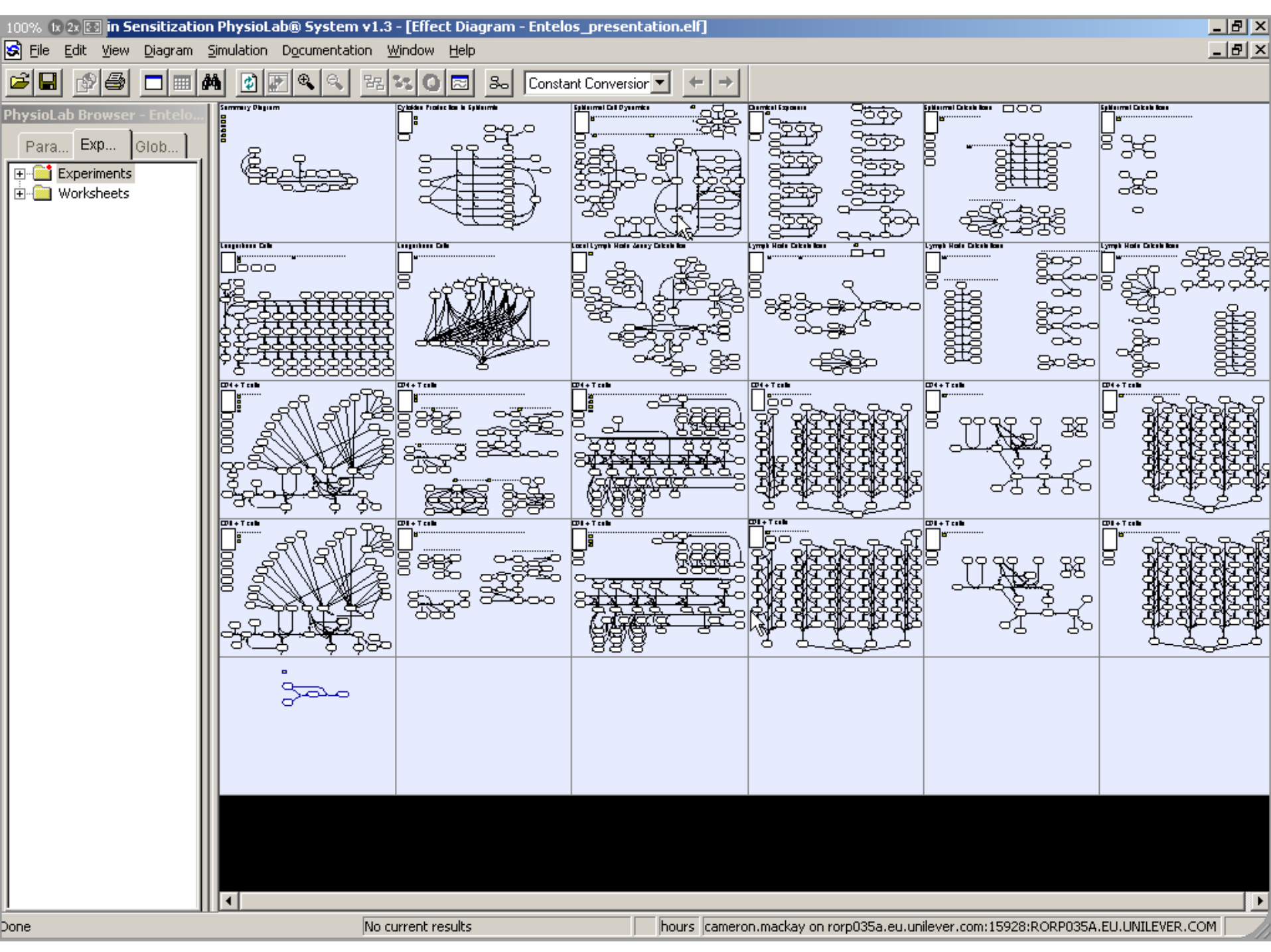
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Competing Hypothesis: programmed vs. progressive T-cell expansion

- Two competing theories of T-cell expansion
- Programmed hypothesis
  - Single APC encounter triggers T-cell division *(Foulds 2002)*
  - Quality of encounter determines the time spent in cell division
- Progressive hypothesis
  - Repeated stimulation required to maintain cell division *(Gett 2003, Lanzavecchia 2002)*
  - Number of available APCs and the quality of the stimulation determines the time spent in cell division
- Approach
  - Implement and evaluate both mechanisms

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CD4+ T cells

AP Competition and Costimulation Integration

+ *Stimulates*
- *Inhibits*
= *Regulates*
A *Allows*
D *Decreases*
H *Half-Life*
I *Increases*
L *Leads*
M *Moves*
P *Produces*
S *Changes State*

CD8+ AP & Costim.

CD4+ T cell Life Cycle

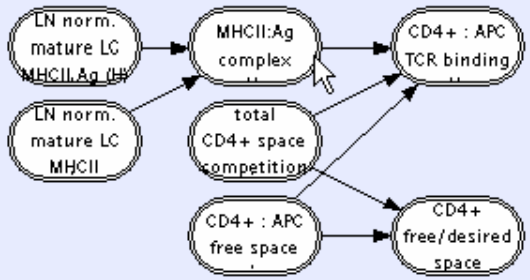
LC Life Cycle & Memory

CD4+ T cells AP Competition and Costimulation Integration Overview

Programmed vs. Progressive Hypothesis

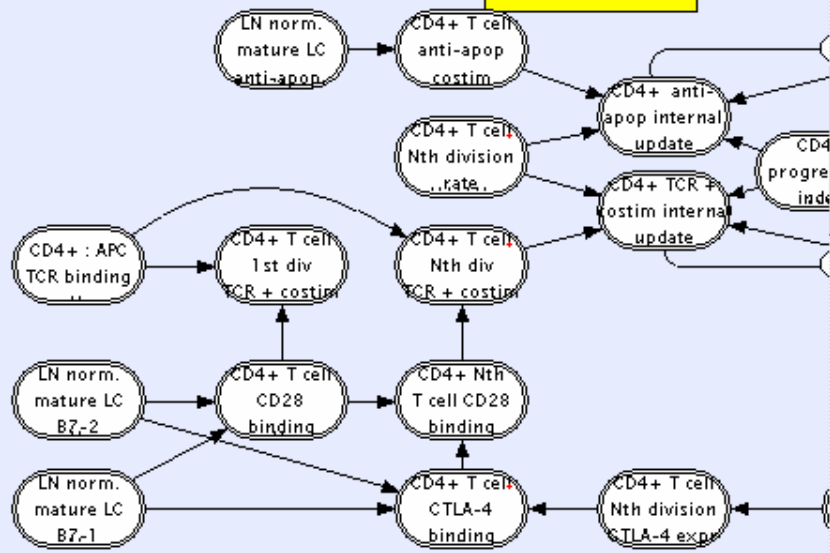
CD4+ TCR binding

TCR Binding



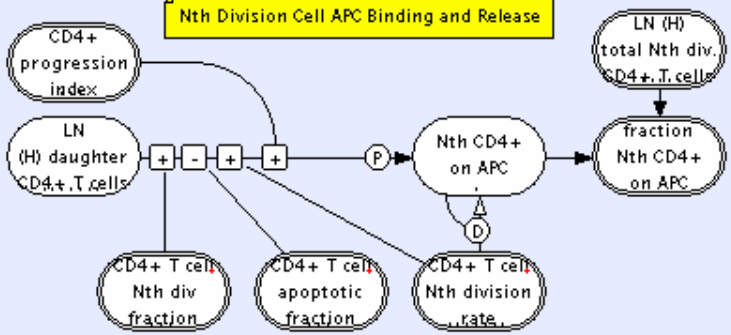
CD4+ costimulation

Costimulation Effects

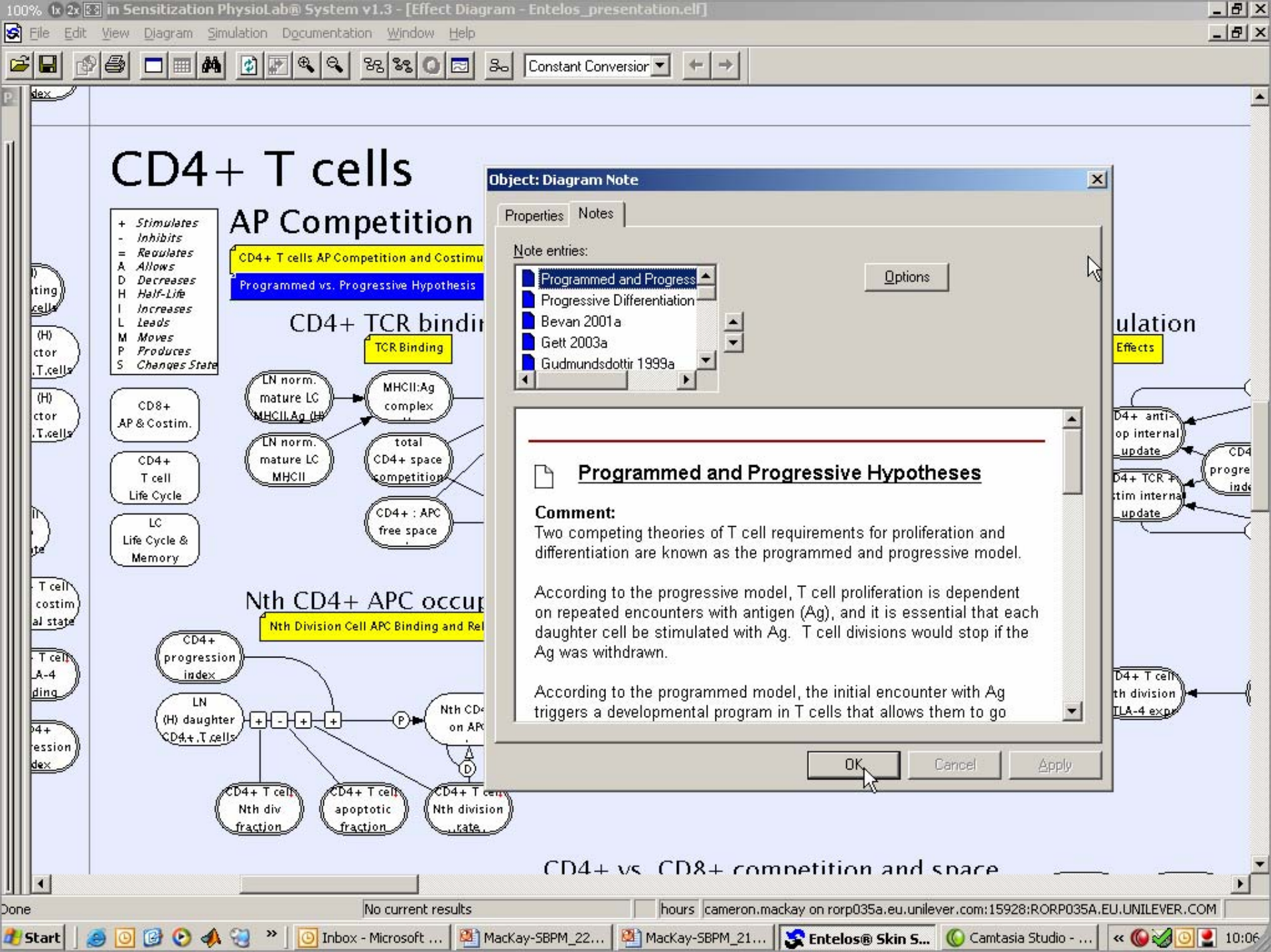


Nth CD4+ APC occupation

Nth Division Cell APC Binding and Release



CD4+ vs. CD8+ competition and space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Two competing theories of T cell requirements for proliferation and differentiation are known as the programmed and progressive model.
According to the progressive model, T cell proliferation is dependent on repeated encounters with antigen (Ag), and it is essential that each daughter cell be stimulated with Ag. T cell divisions would stop if the Ag was withdrawn.
According to the programmed model, the initial encounter with Ag triggers a developmental program in T cells that allows them to go
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J Immunol. 2002 Feb 15;168(4):1528-32.

Cutting edge: CD4 and CD8 T cells are intrinsically different in their proliferative responses.

Foulds KE, Zenewicz LA, Shedlock DJ, Jiang J, Troy AE, Shen H

Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

In this study, we compared the proliferation and differentiation of Ag-specific CD4 and CD8 T cells following Listeria infection. Our results show that CD4 T cells responding to infection divide a limited number of times, with progeny exhibiting proliferative arrest in early divisions. Even with increased infectious doses, CD4 T cells display this restricted proliferative pattern and are not driven to undergo extensive clonal expansion. This is in striking contrast to CD8 T cells, which undergo extensive proliferation in response to infection. These differences are also evident when CD4 and CD8 T cells receive uniform anti-CD3 stimulation in vitro. Together, these results suggest that CD4 and CD8 T cells are programmed to undergo limited and extensive proliferation, respectively, to suit their function as regulator and effector cells.

MeSH

- Adoptive Transfer
- Animals
- CD4-Positive T-Lymphocytes/*immunology
- CD8-Positive T-Lymphocytes/*immunology
- Cells, Cultured
- Comparative Study
- Flow Cytometry
- Kinetics
- Listeria Infections/*immunology
- *Lymphocyte Activation
- Mice
- Mice, Inbred BALB C
- Mice, Inbred C57BL
- Mice, Transgenic
- Ovalbumin/genetics/immunology
- Recombinant Proteins/immunology

Cutting Edge: CD4 and CD8 T Cells Are Intrinsically Different in Their Proliferative Responses[1]

Kathryn E. Foulds, Lauren A. Zenewicz, Devon J. Shedlock, Jiu Jiang, Amy E. Troy, and Hao Shen[2]

In this study, we compared the proliferation and differentiation of Ag-specific CD4 and CD8 T cells following *Listeria* infection. Our results show that CD4 T cells responding to infection divide a limited number of times, with progeny exhibiting proliferative arrest in early divisions. Even with increased infectious doses, CD4 T cells display this restricted proliferative pattern and are not driven to undergo extensive clonal expansion. This is in striking contrast to CD8 T cells, which undergo extensive proliferation in response to infection. These differences are also evident when CD4 and CD8 T cells receive uniform anti-CD3 stimulation in vitro. Together, these results suggest that CD4 and CD8 T cells are programmed to undergo limited and extensive proliferation, respectively, to suit their function as regulator and effector cells. *The Journal of Immunology*, 2002, 168: 1528–1532.

Murine listeriosis has been a useful model for investigating T cell responses to infection (reviewed in Ref. 1). The infectious agent, *Listeria monocytogenes* (LM),[3] is a Gram-positive bacterium that invades host cells, escapes from the endosome, and replicates within the host cell cytosol (2). LM proteins are presented by both MHC class I and class II pathways and stimulate strong CD8 and CD4 T cell responses (1, 3). The in vivo dynamics of the CD8 T cell response to infection has been studied extensively through the analysis of specific responses to native LM epitopes as well as foreign epitopes expressed by recombinant LM (3–5). Two recent studies have shown that the extent of CD8 T cell proliferation is not determined by the amount or duration of Ag presentation (6, 7), leading to the hypothesis that CD8 T cells undergo autonomous clonal expansion in an Ag-independent fashion following in vivo priming. Further studies both in vitro and in vivo have demonstrated that stimulation of CD8 T cells triggers a developmental program that instructs daughter cells to continue to divide and differentiate into effector and memory T cells (7–10).

Much less is known about the in vivo dynamics of CD4 T cell responses to infection. While several MHC class II-restricted LM epitopes have been defined through the in vitro analysis of T cell clones (1, 11), the frequencies of CD4 T cells responding to these known epitopes are too low to allow the direct measurement of Ag-specific CD4 T cell responses. This limitation has thus far hindered our ability to assess the in vivo dynamics of CD4 T cell responses in most infections. In this study, we developed a system to quantitate Ag-specific CD4 T cell responses in vivo, using an adoptive transfer model (12) that couples the use of a recombinant LM expressing OVA with OVA-specific transgenic cells. Our results show that the extent of CD4 T cell proliferation and differentiation is strikingly different from that of CD8 T cells.

Materials and Methods

Mice, Abs, and bacterial strains

BALB/c-Tg(NSE/DO11.10)10Lob, C57BL/6-P14, OT-I, and OT-II mice were previously described (13–16). OT-I and OT-II mice were bred onto the B6/PL-Thy1.1 background. B6.PL-Thy1.1 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and BALB/c-Thy1.1 mice were obtained from C. Surh at The Scripps Institute (La Jolla, CA). All mAbs were from BD PharMingen (San Diego, CA), except the KJ1-26 from Caltag Laboratories (Burlingame, CA). Construction and Western blot analysis of rLM-OVA and rLM-gp33 strains were performed as described (17). Both strains were derived from the wild-type strain 10403s and described previously (18, 19). The LD_{10} of rLM-OVA in BALB/c mice is ~5×10^5 CFU and the LD_{50} of rLM-OVA and rLM-gp33 in C57BL/6 mice is ~5×10^6 CFU.

Analysis of T cell proliferation following LM infection in vivo

Splenocytes from DO11.10, P14, OT-I, or OT-II transgenic mice were labeled with CFSE as described (20). A total of 2×10^7 CFSE-labeled splenocytes (2×10^6 specific cells) were transferred into Thy1.1 or Thy1.2 congenic mice (21), which were then infected with the indicated doses of rLM-OVA or rLM-gp33. For the OT-I/OT-II combined transfer, T cells were enriched by depleting splenocytes with B220 and MHC II MicroBeads by MACS (Miltenyi Biotec, Auburn, CA) and CFSE labeled, and 2×10^6 of each cell type were cotransferred per mouse. Proliferation of transferred cells was visualized by FACS analysis of their CFSE profile. Transferred DO11.10 cells were identified by staining with mAb to Thy1.2, CD4, and the KJ1-26 clonotypic mAb, P14 cells were identified by staining with mAb to Thy1.2, CD8, and/or the D^b/gp33 tetramer (22, 23), and OT-I and OT-II cells were identified by staining with mAb to Thy1.1, Vα2, and CD8 or CD4, respectively. Intracellular IFN-γ staining was performed as described following 5 h of in vitro stimulation with 5 μM $OVA_{257-264}$ or 1 μM gp33–41 particles (23).

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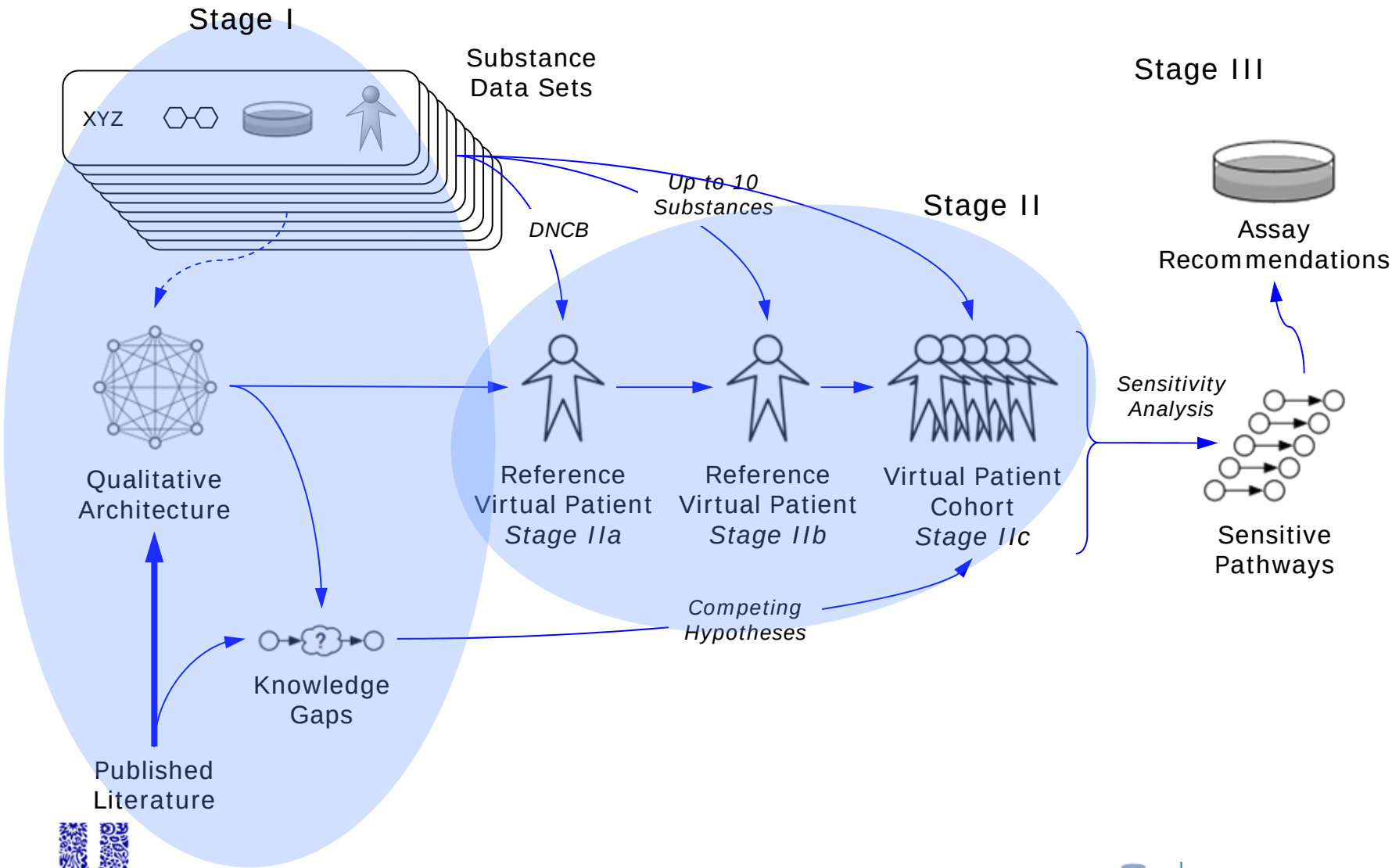
[1] This work was supported by National Institutes of Health Grants AI-49023 and AI-46304 (to H.S.).

[2] Address correspondence and reprint requests to Dr. Hao Shen, Department of Microbiology, School of Medicine, University of Pennsylvania, 3610 Hamilton Walk, Philadelphia, PA 19104. E-mail address: hshen@mail.med.upenn.edu

[3] Abbreviations used in this paper: LM, *Listeria monocytogenes*; LCMV, lymphocytic choriomeningitis virus.

Skin Sensitization Induction PhysioLab Platform (SSIPP)



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Model development – stage II

- Quantitative Modelling
  - The dynamic interactions of the biological system were represented using mathematical equations (ordinary differential equations)

- Model Calibration: published results from 35 key experiments were replicated by the model including:
  - Epidermal cytokine production
  - Langerhans cell and T cell surface marker expression
  - Langerhans cell migration
  - Lymph node cytokine production

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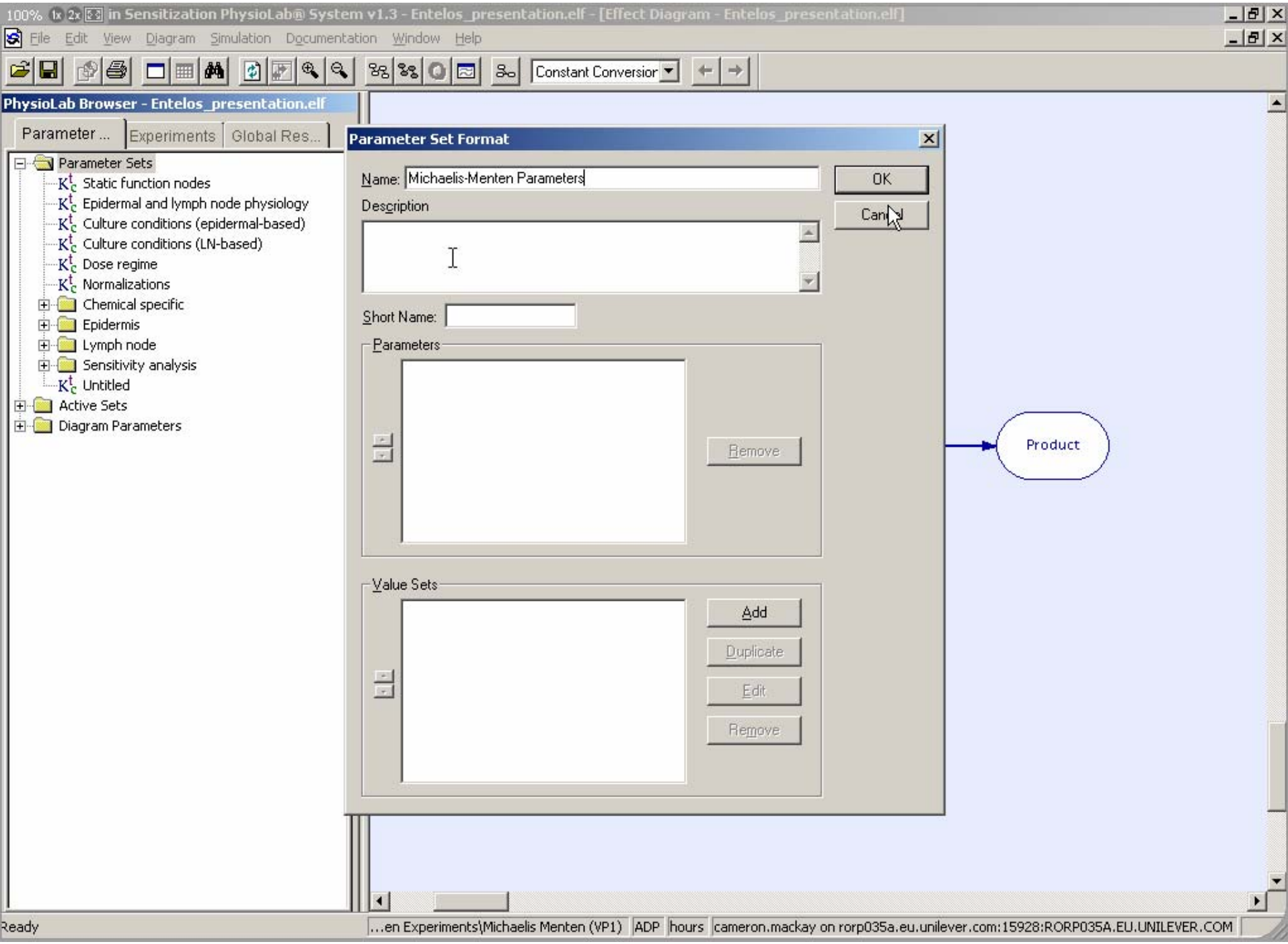
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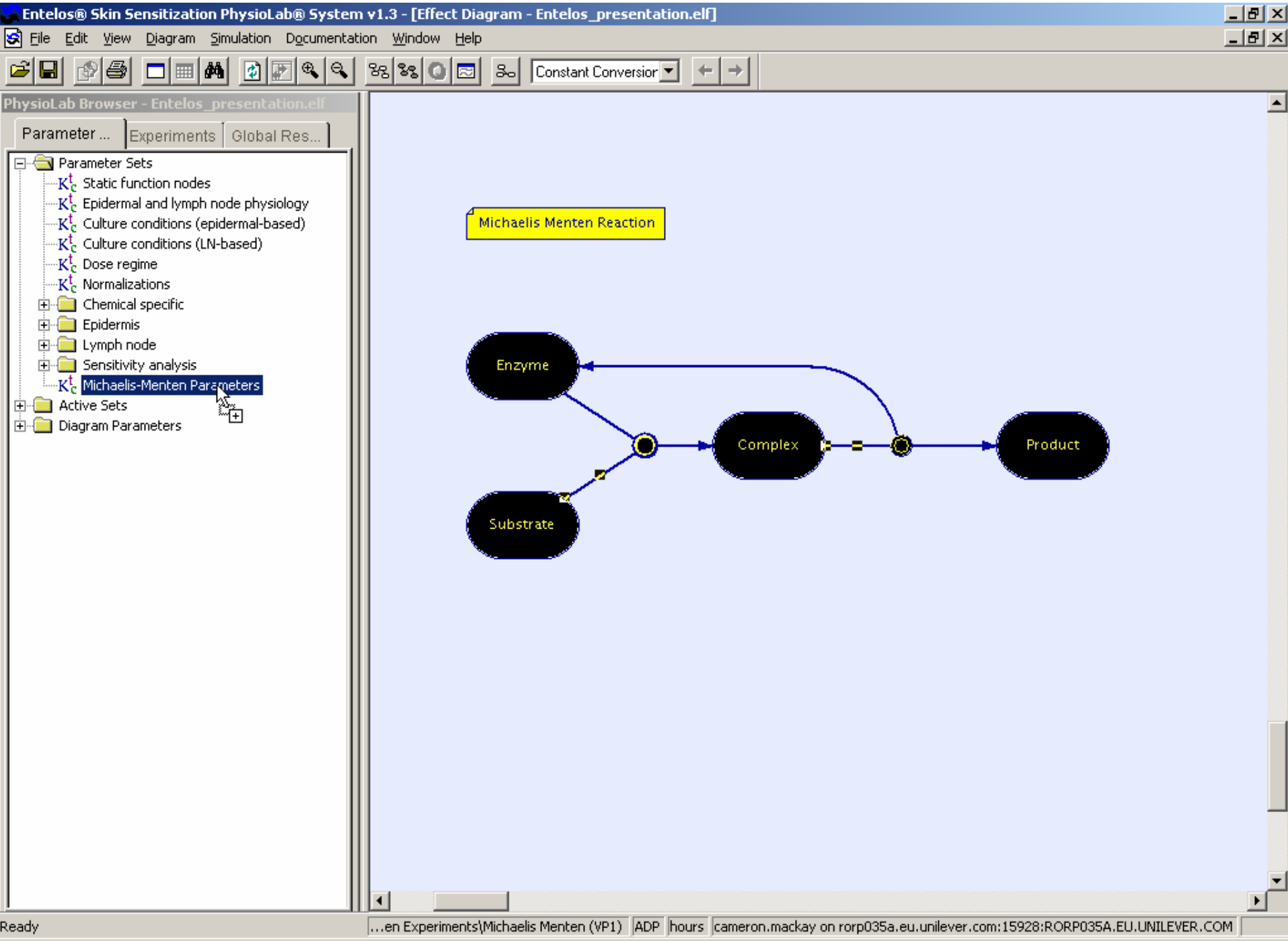
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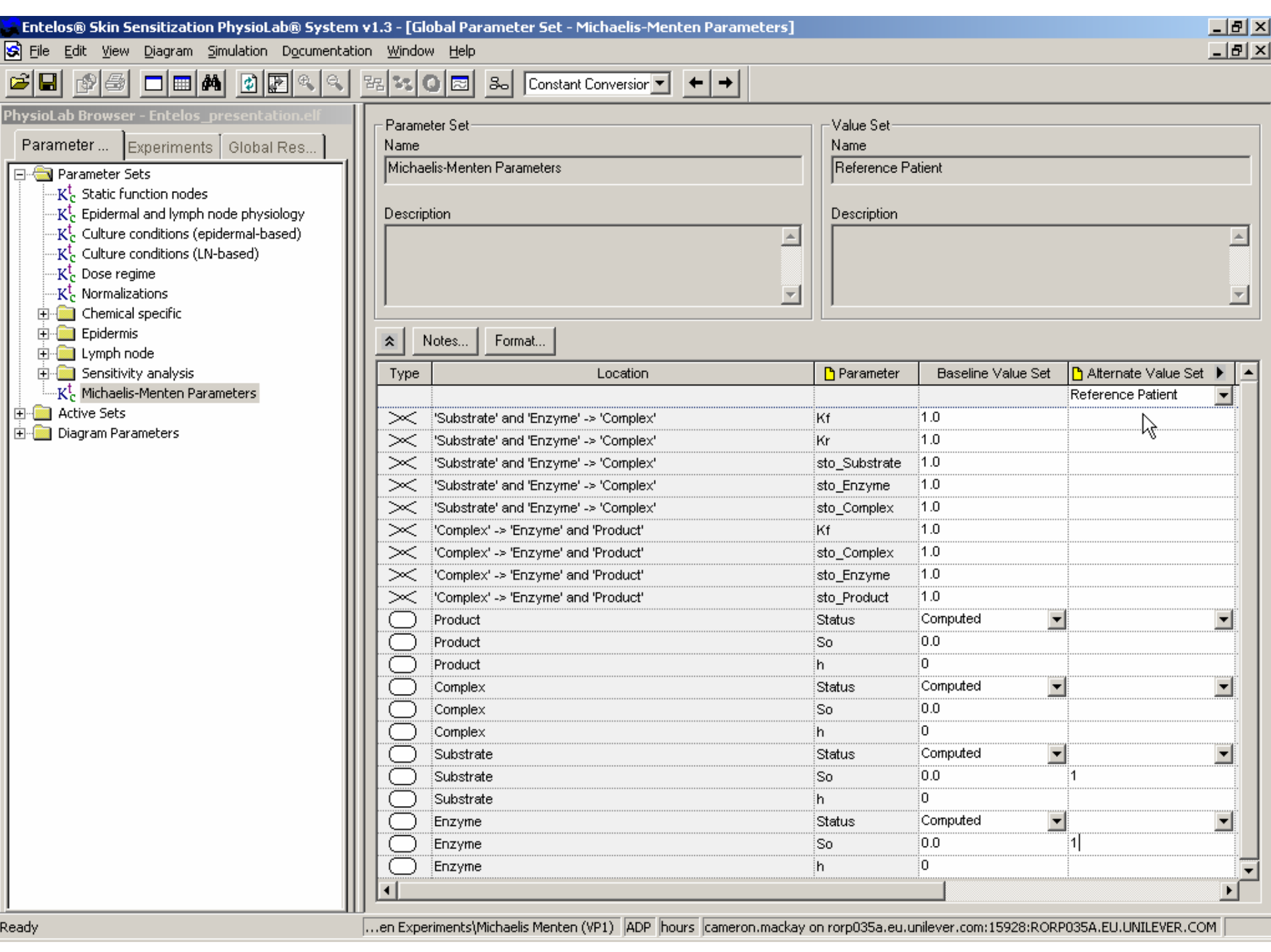
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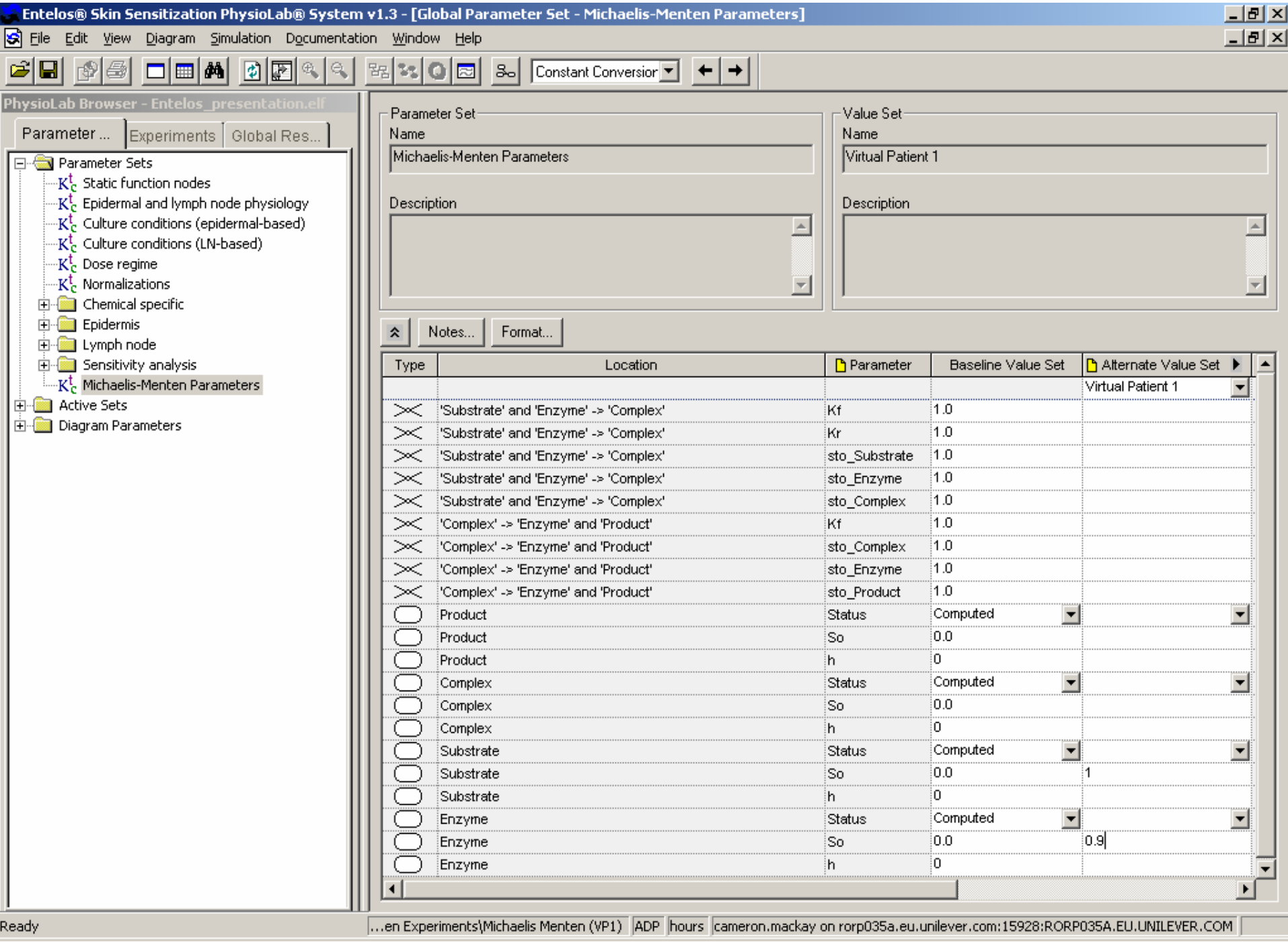
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Global Parameter Set - Michaelis-Menten Parameters]
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Parameter ... Experiments Global Res...
Parameter Sets
Static function nodes
Epidermal and lymph node physiology
Culture conditions (epidermal-based)
Culture conditions (LN-based)
Dose regime
Normalizations
Chemical specific
Epidermis
Lymph node
Sensitivity analysis
Michaelis-Menten Parameters
Active Sets
Diagram Parameters
Parameter Set
Name
Michaelis-Menten Parameters
Description
Value Set
Name
Virtual Patient 1
Description
Notes... Format...
Type Location Parameter Baseline Value Set Alternate Value Set
Virtual Patient 1
'Substrate' and 'Enzyme' -> 'Complex' Kf 1.0
'Substrate' and 'Enzyme' -> 'Complex' Kr 1.0
'Substrate' and 'Enzyme' -> 'Complex' sto_Substrate 1.0
'Substrate' and 'Enzyme' -> 'Complex' sto_Enzyme 1.0
'Substrate' and 'Enzyme' -> 'Complex' sto_Complex 1.0
'Complex' -> 'Enzyme' and 'Product' Kf 1.0
'Complex' -> 'Enzyme' and 'Product' sto_Complex 1.0
'Complex' -> 'Enzyme' and 'Product' sto_Enzyme 1.0
'Complex' -> 'Enzyme' and 'Product' sto_Product 1.0
Product Status Computed
Product So 0.0
Product h 0
Complex Status Computed
Complex So 0.0
Complex h 0
Substrate Status Computed
Substrate So 0.0 1
Substrate h 0
Enzyme Status Computed
Enzyme So 0.0 0.9
Enzyme h 0
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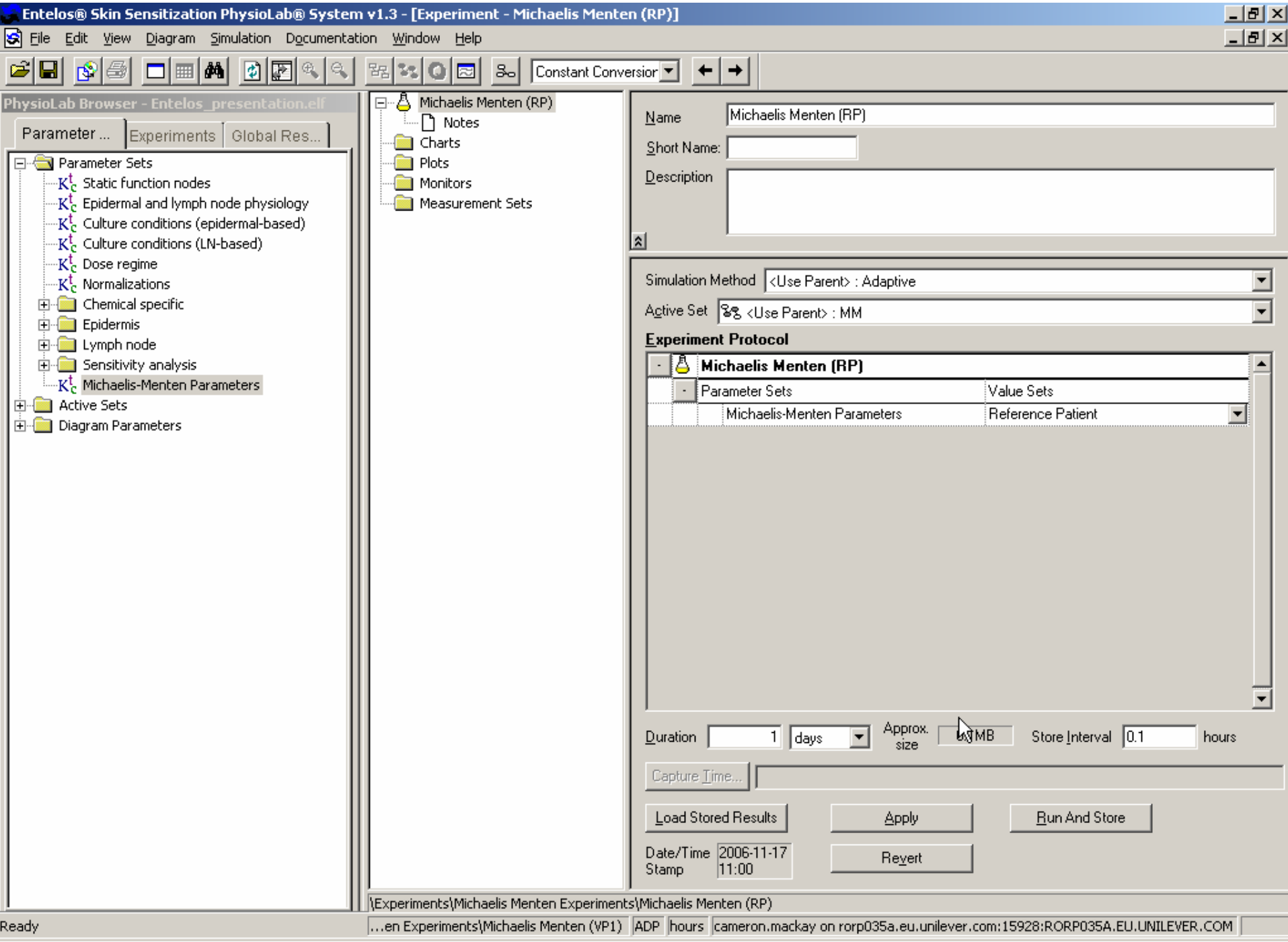
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Michaelis Menten (RP)]
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Parameter Sets
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Epidermal and lymph node physiology
Culture conditions (epidermal-based)
Culture conditions (LN-based)
Dose regime
Normalizations
Chemical specific
Epidermis
Lymph node
Sensitivity analysis
Michaelis-Menten Parameters
Active Sets
Diagram Parameters
Michaelis Menten (RP)
Notes
Charts
Plots
Monitors
Measurement Sets
Name Michaelis Menten (RP)
Short Name:
Description
Simulation Method <Use Parent> : Adaptive
Active Set <Use Parent> : MM
Experiment Protocol
Michaelis Menten (RP)
Parameter Sets
Value Sets
Michaelis-Menten Parameters
Reference Patient
Duration 1 days
Approx. size 0 MB
Store Interval 0.1 hours
Capture Time...
Load Stored Results
Apply
Run And Store
Date/Time Stamp 2006-11-17 11:00
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\Experiments\Michaelis Menten Experiments\Michaelis Menten (RP)
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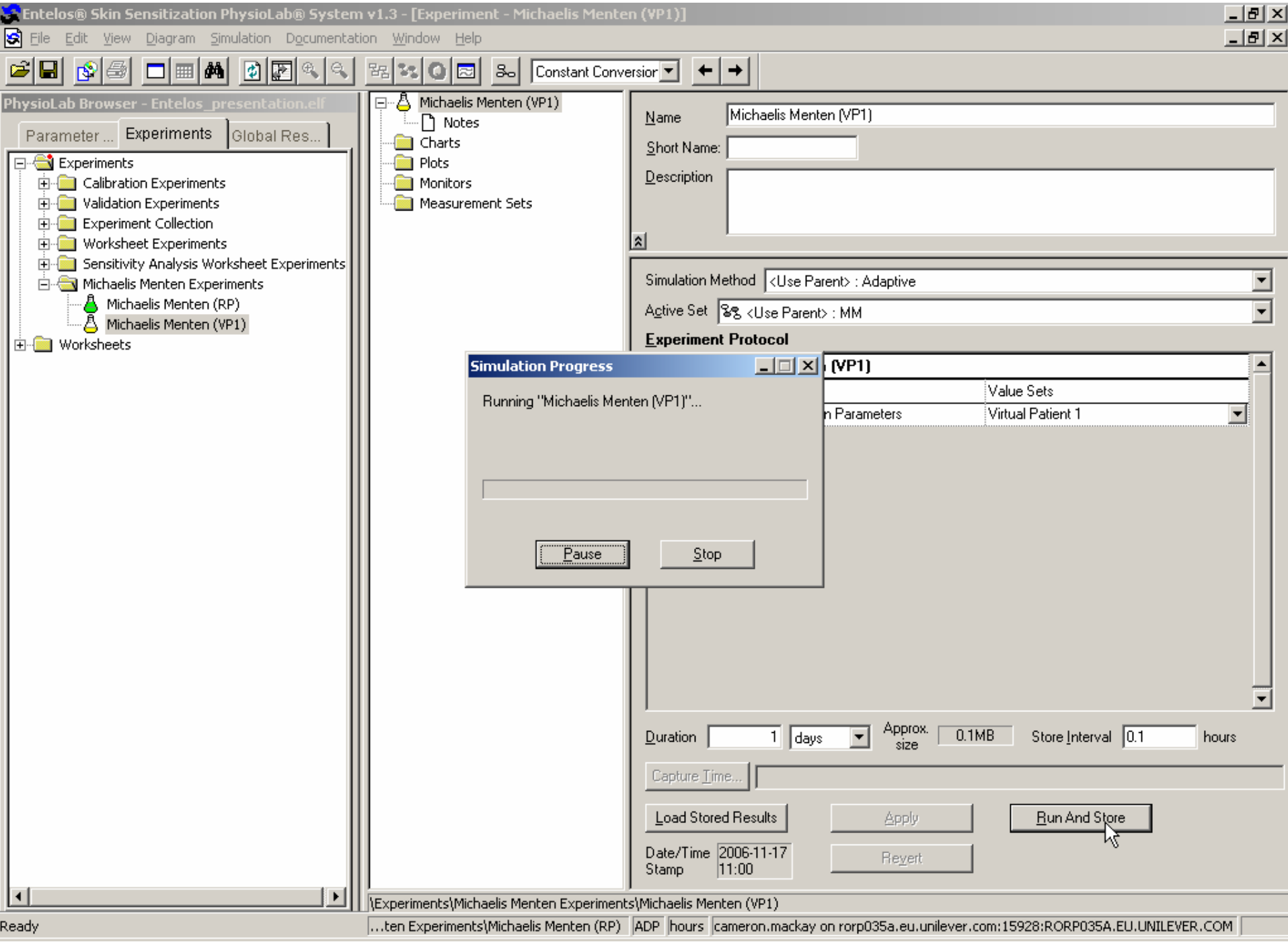
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Michaelis Menten (VP1)]
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Experiments
Calibration Experiments
Validation Experiments
Experiment Collection
Worksheet Experiments
Sensitivity Analysis Worksheet Experiments
Michaelis Menten Experiments
Michaelis Menten (RP)
Michaelis Menten (VP1)
Worksheets
Michaelis Menten (VP1)
Notes
Charts
Plots
Monitors
Measurement Sets
Name Michaelis Menten (VP1)
Short Name:
Description
Simulation Method <Use Parent> : Adaptive
Active Set <Use Parent> : MM
Experiment Protocol
(VP1)
Value Sets
n Parameters
Virtual Patient 1
Simulation Progress
Running "Michaelis Menten (VP1)"...
Pause
Stop
Duration 1 days
Approx. size 0.1MB
Store Interval 0.1 hours
Capture Time...
Load Stored Results
Apply
Run And Store
Date/Time Stamp 2006-11-17 11:00
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Entelos® Skin Sensitization PhysioLab® System v1.3 - [Global Comparison Chart - Michaelis-Menten Graphs]

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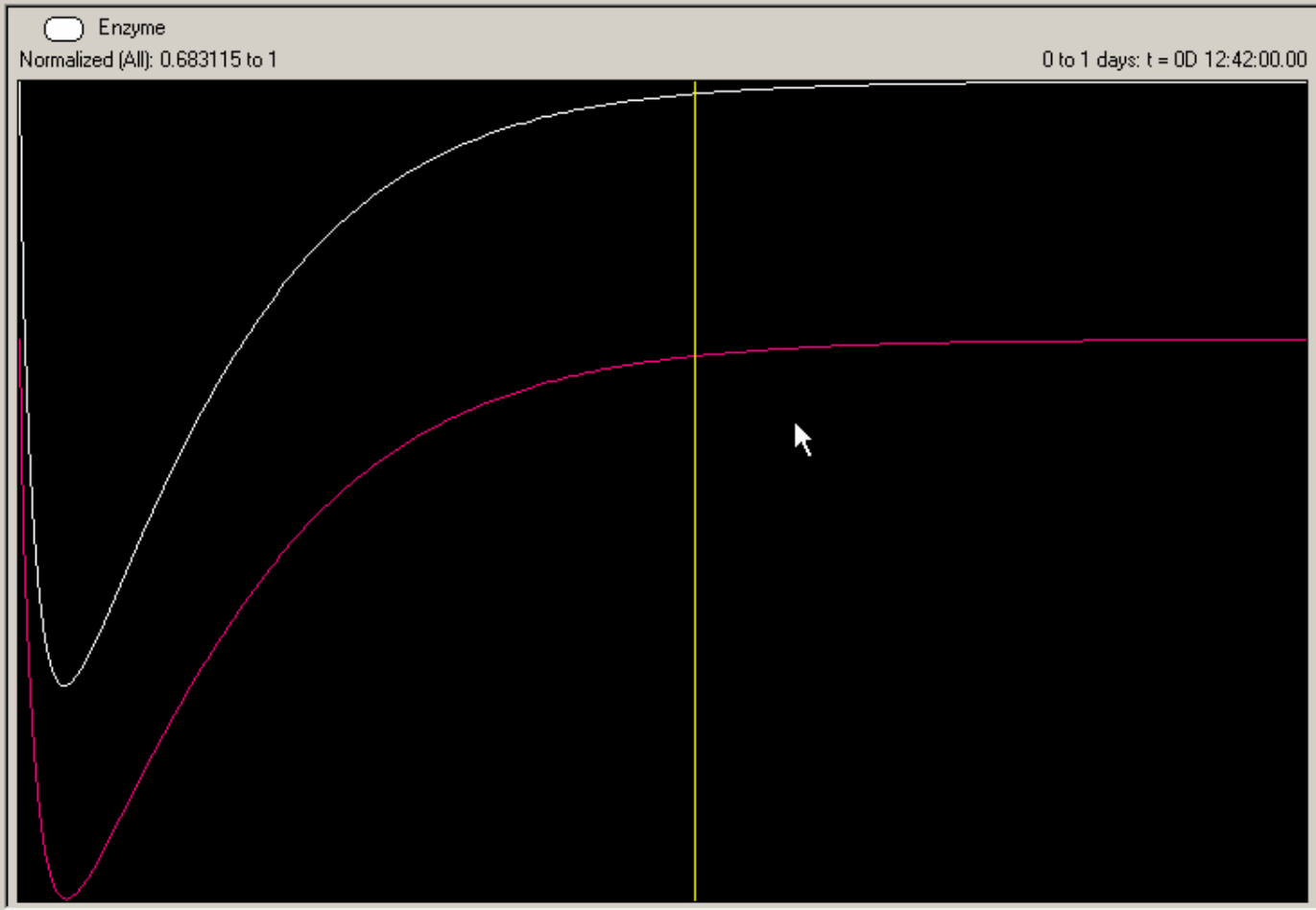
Parameter ... | Experiments | Global Res...

- Experiments
  - Calibration Experiments
  - Validation Experiments
  - Experiment Collection
  - Worksheet Experiments
  - Sensitivity Analysis Worksheet Experiments
  - Michaelis Menten Experiments
    - Michaelis Menten (RP)
    - Michaelis Menten (VP1)
- Worksheets

Experiment Name	Value	Units
\Experiments\Michaelis Menten Experiments\Michaelis Menten (RP)	0.995154	
\Experiments\Michaelis Menten Experiments\Michaelis Menten (VP1)	0.893545	



\Comparison Charts\Michaelis-Menten Graphs

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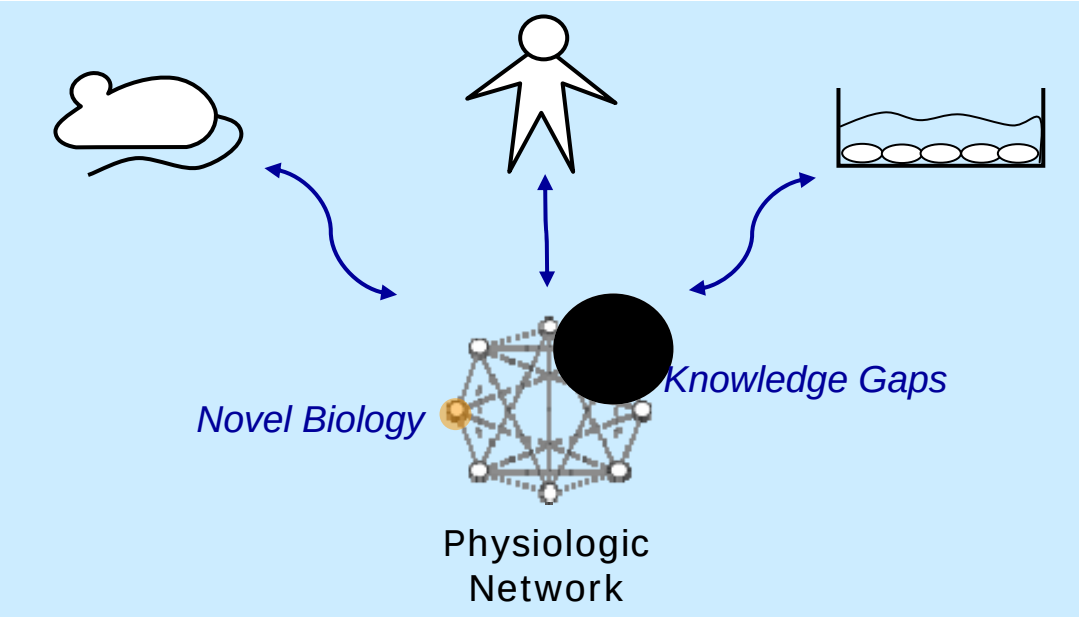
Calibration process ensures proper subsystem behavior



- Implemented > 35 *in vitro, in vivo, ex vivo* experiments from 31 references

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Model development – stage II

- Reference Patient (RP)
  - The calibration of the model thought to be most like the underlying biology
- Identification of knowledge gaps in Stage I leads to the formulation of alternative hypotheses
- Virtual Patients (VPs)
  - Alternative biological hypotheses to the RP represented by a number of distinct Virtual Patients (VPs)
  - The model can be interrogated for each VP
  - Conclusions robust to the knowledge gaps are obtained

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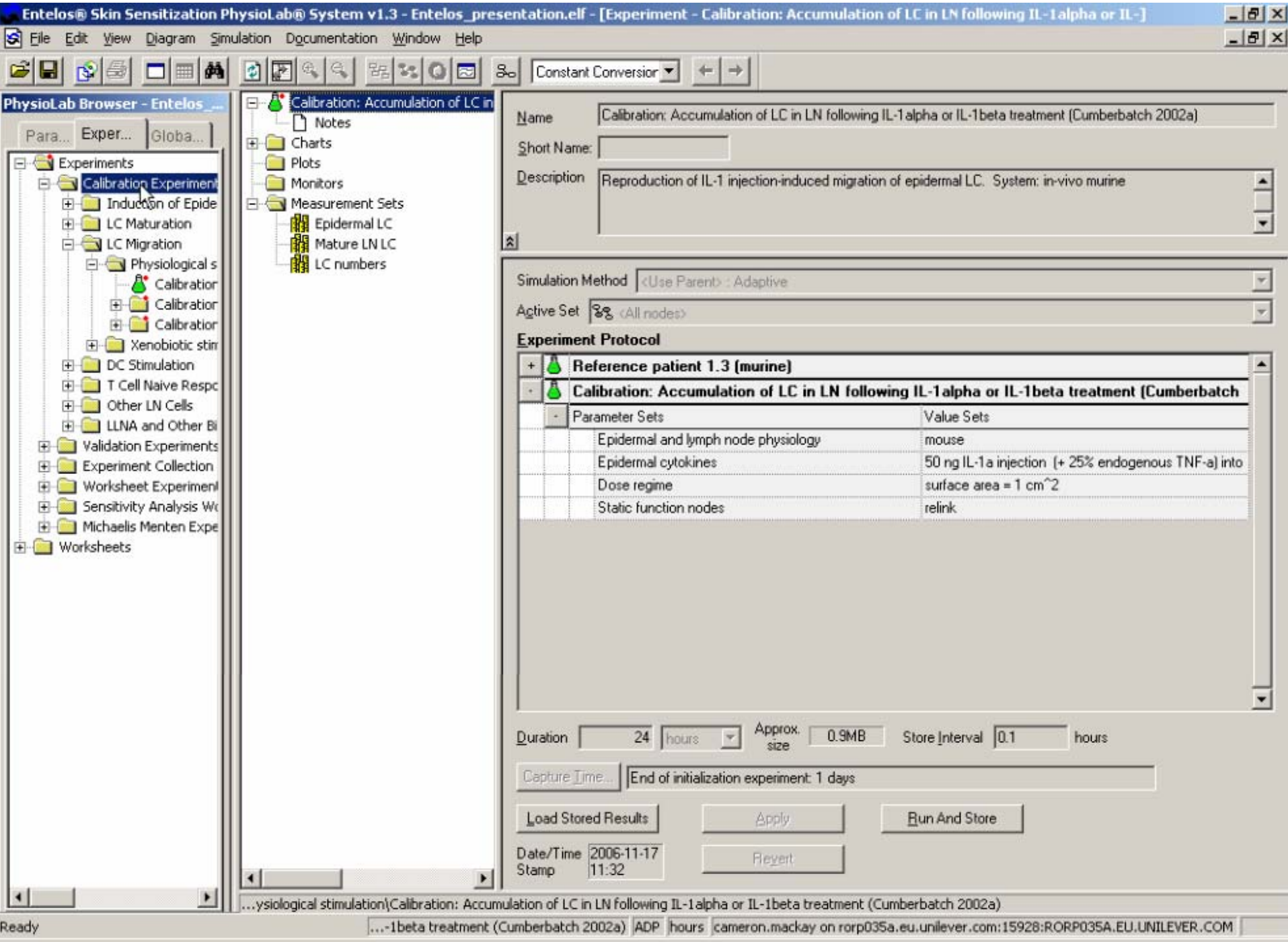
Entelos® Skin Sensitization PhysioLab® System v1.3 - Entelos_presentation.elf - [Experiment - Calibration: Accumulation of LC in LN following IL-1alpha or IL-]
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LC Migration
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Worksheets
Calibration: Accumulation of LC in
Notes
Charts
Plots
Monitors
Measurement Sets
Epidermal LC
Mature LN LC
LC numbers
Name
Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatch 2002a)
Short Name:
Description
Reproduction of IL-1 injection-induced migration of epidermal LC. System: in-vivo murine
Simulation Method
<Use Parent> : Adaptive
Active Set
<All nodes>
Experiment Protocol
Reference patient 1.3 (murine)
Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatch
Parameter Sets
Value Sets
Epidermal and lymph node physiology
mouse
Epidermal cytokines
50 ng IL-1a injection (+ 25% endogenous TNF-a) into
Dose regime
surface area = 1 cm^2
Static function nodes
relink
Duration
24
hours
Approx. size
0.9MB
Store Interval
0.1
hours
Capture Time...
End of initialization experiment: 1 days
Load Stored Results
Apply
Run And Store
Date/Time Stamp
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...ysiological stimulation\Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatch 2002a)
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Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatc]

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    - T Cell Naive Respc
    - Other LN Cells
    - LLNA and Other Bi
  - Validation Experiments
  - Experiment Collection
  - Worksheet Experimenl
  - Sensitivity Analysis Wc
  - Michaelis Menten Expe
- Worksheets

- Calibration: Accumulation of LC in
  - Notes
  - Charts
  - Plots
  - Monitors
  - Measurement Sets
    - Epidermal LC
    - Mature LN LC
    - LC numbers

- Calibration: Accumulation of LC in LN following I
  - Charts
  - Plots
  - Monitors

- Experimental Protocol
- Interpretation of Results
- Cumberbatch 2002a

Experimental Protocol

Comment:
Epidermal emigration and subsequent lymph node accumulation of LC following subcutaneous injection of IL-1alpha or IL-1beta was measured in BALB/c mice.

To approximate the IL-1-induced TNF-alpha production by keratinocytes, the 50 ng IL-1 injection is accompanied by an endogenous TNF-alpha concentration of 25% that of IL-1.

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Entelos	06/04/2006	Entelos	Entelos	05/04/2006

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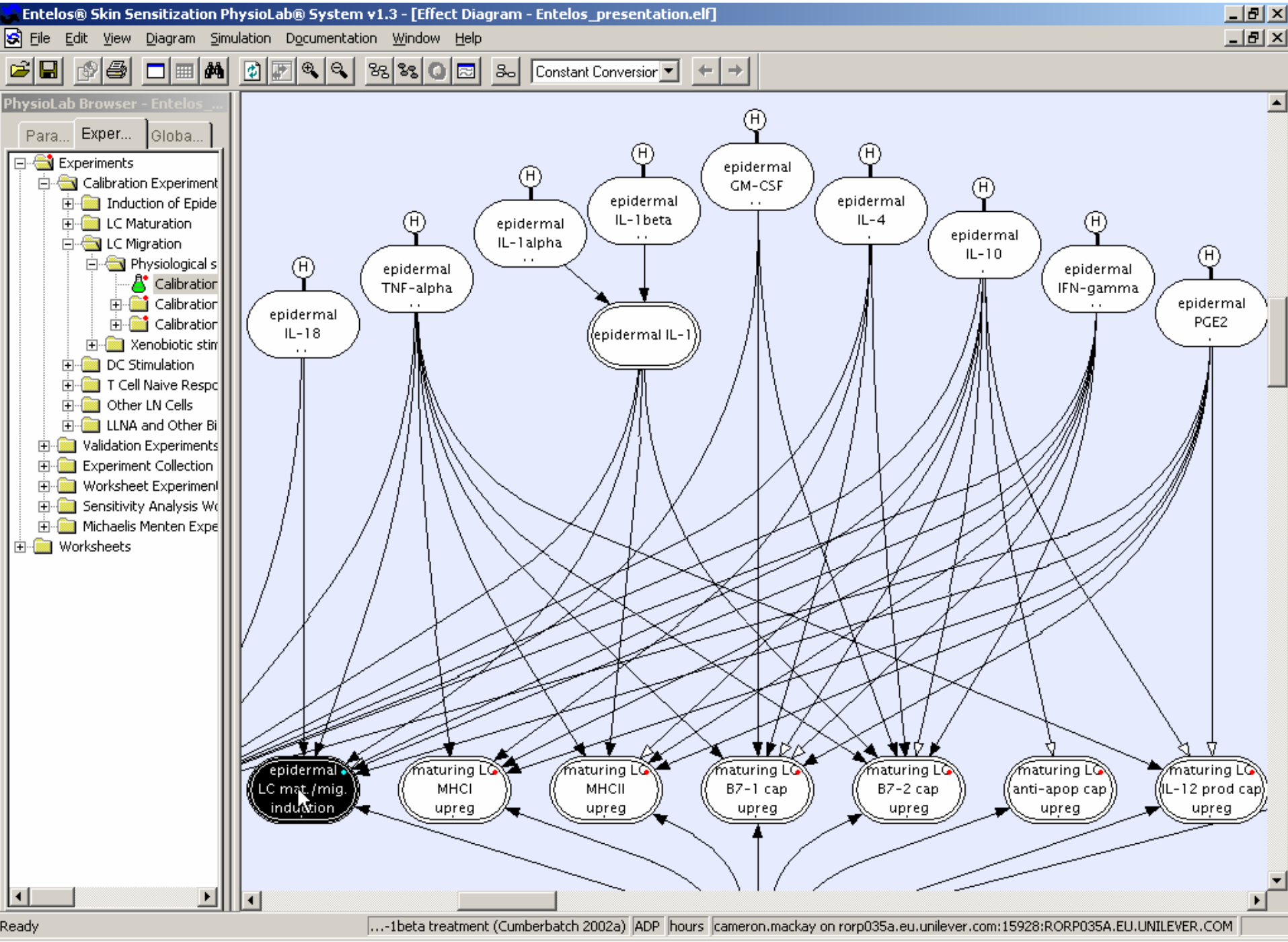
Interpretation of Results

Comment:
As shown below, epidermal LC density was assessed at 2, 4 and 17 hours after injection (Figure 1 in paper):

time after injection [hours]	epidermal LC density, approximate [LC/mm^2]
0	850

...ysiological stimulation\Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatch 2002a)

Done ...-1beta treatment (Cumberbatch 2002a) ADP hours cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM


Entelos® Skin Sensitization PhysioLab® System v1.3 - [Effect Diagram - Entelos_presentation.elf]
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epidermal IL-18
epidermal TNF-alpha
epidermal IL-1alpha
epidermal IL-1beta
epidermal GM-CSF
epidermal IL-4
epidermal IL-10
epidermal IFN-gamma
epidermal PGE2
epidermal IL-1
epidermal LC mat./mig. induction
maturing LC MHCI upreg
maturing LC MHCII upreg
maturing LC B7-1 cap upreg
maturing LC B7-2 cap upreg
maturing LC anti-apop cap upreg
maturing LC IL-12 prod cap upreg
Ready
...-1beta treatment (Cumberbatch 2002a)
ADP
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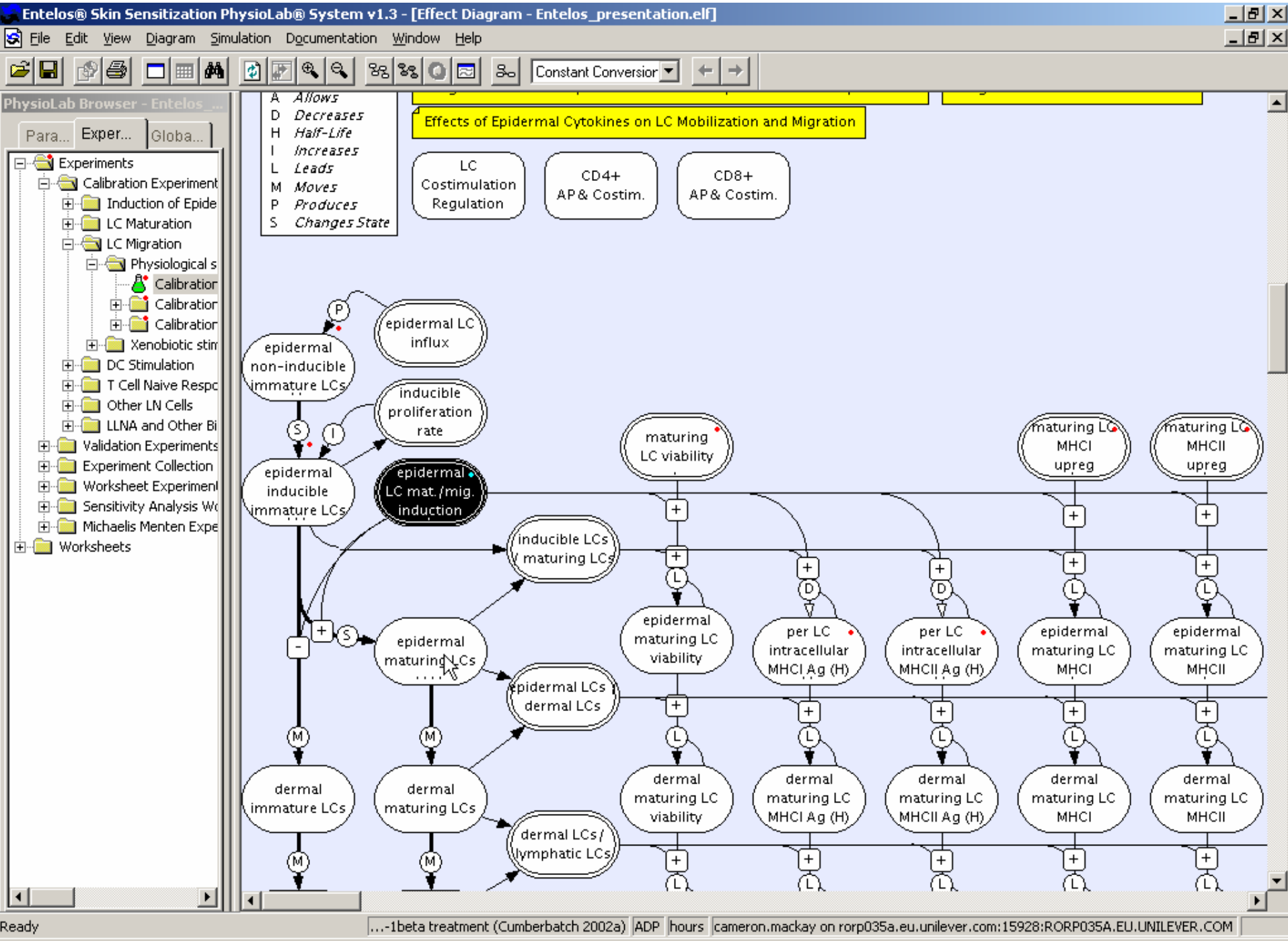
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Effect Diagram - Entelos_presentation.elf]
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DC Stimulation
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Other LN Cells
LLNA and Other Bi
Validation Experiments
Experiment Collection
Worksheet Experimenl
Sensitivity Analysis Wc
Michaelis Menten Expe
Worksheets
A Allows
D Decreases
H Half-Life
I Increases
L Leads
M Moves
P Produces
S Changes State
Effects of Epidermal Cytokines on LC Mobilization and Migration
LC Costimulation Regulation
CD4+ AP& Costim.
CD8+ AP& Costim.
epidermal non-inducible immature LCs
epidermal LC influx
inducible proliferation rate
epidermal inducible immature LCs
epidermal LC mat./mig. induction
maturing LC viability
maturing LC MHCI upreg
maturing LC MHCII upreg
inducible LCs / maturing LCs
epidermal maturing LCs
epidermal LCs / dermal LCs
epidermal maturing LC viability
per LC intracellular MHCI Ag (H)
per LC intracellular MHCII Ag (H)
epidermal maturing LC MHCI
epidermal maturing LC MHCII
dermal immature LCs
dermal maturing LCs
dermal LCs / lymphatic LCs
dermal maturing LC viability
dermal maturing LC MHCI Ag (H)
dermal maturing LC MHCII Ag (H)
dermal maturing LC MHCI
dermal maturing LC MHCII
Ready
...-1beta treatment (Cumberbatch 2002a)
ADP
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Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatc]

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    - DC Stimulation
    - T Cell Naive Respc
    - Other LN Cells
    - LLNA and Other Bi
  - Validation Experiments
  - Experiment Collection
  - Worksheet Experimenl
  - Sensitivity Analysis Wc
  - Michaelis Menten Expe
- Worksheets

- Calibration: Accumulation of LC in
  - Notes
- Charts
- Plots
- Monitors
- Measurement Sets
  - Epidermal LC
  - Mature LN LC
  - LC numbers

- Calibration: Accumulation of LC in LN following I
  - Charts
  - Plots
  - Monitors

- Experimental Protocol
- Interpretation of Results
- Cumberbatch 2002a

Interpretation of Results

Comment:
As shown below, epidermal LC density was assessed at 2, 4 and 17 hours after injection (Figure 1 in paper):

time after injection [hours]	epidermal LC density, approximate [LC/mm^2]
0	850
2	730
4	640
17	520

Lymph node accumulation was measured at 17 hour mark (Figure 3 in paper),~5,000 DC above baseline.

Model-predicted values (shown in Charts & Measurement Sets folders) for epidermal LC density are in good agreement with Cumberbatch-provided data. Correlation for lymph node LC accumulation at the 17 hour mark is fair.

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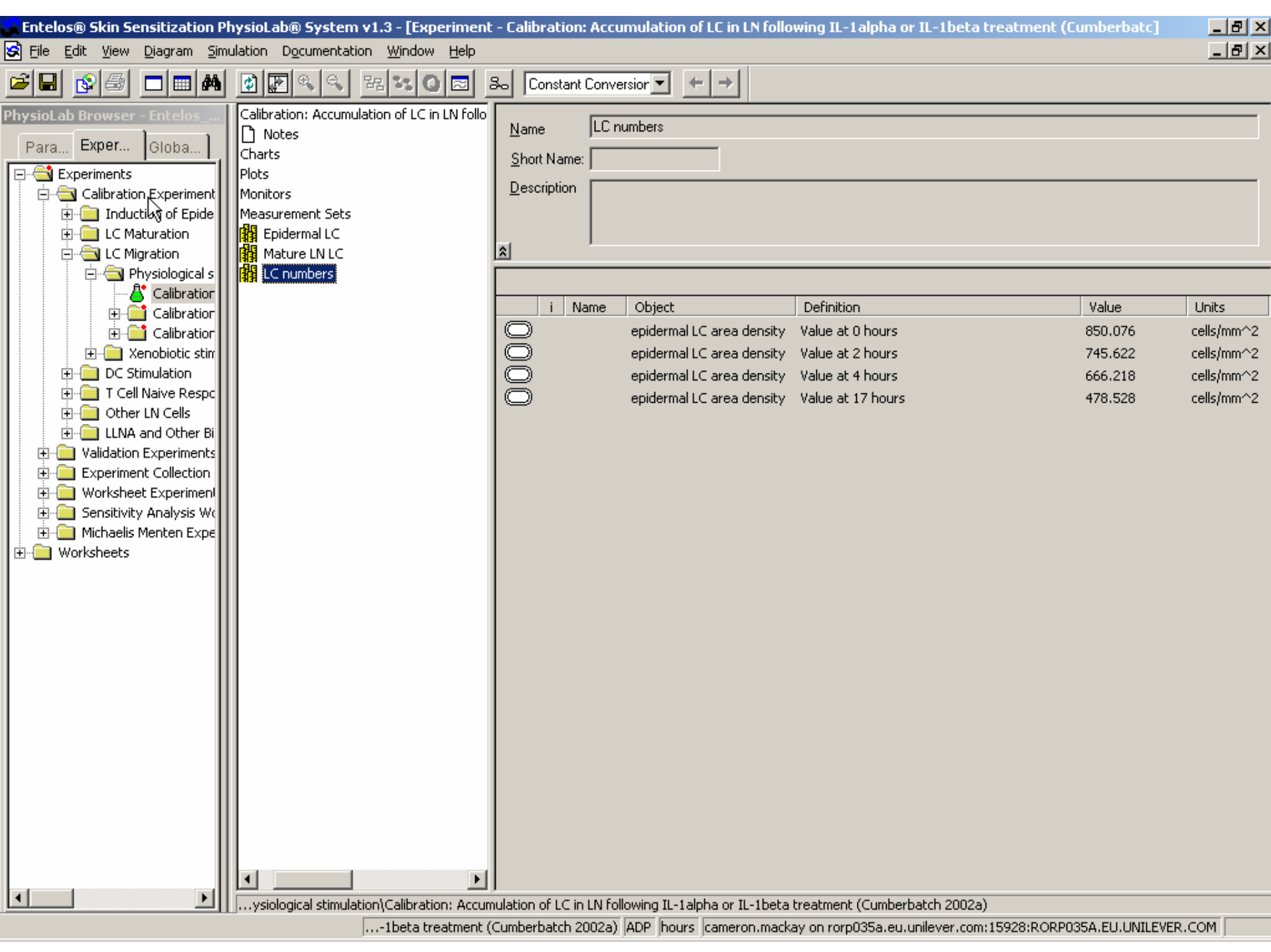
...ysiological stimulation\Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatch 2002a)

Done ...-1beta treatment (Cumberbatch 2002a) ADP hours cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM


Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatc]
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Notes
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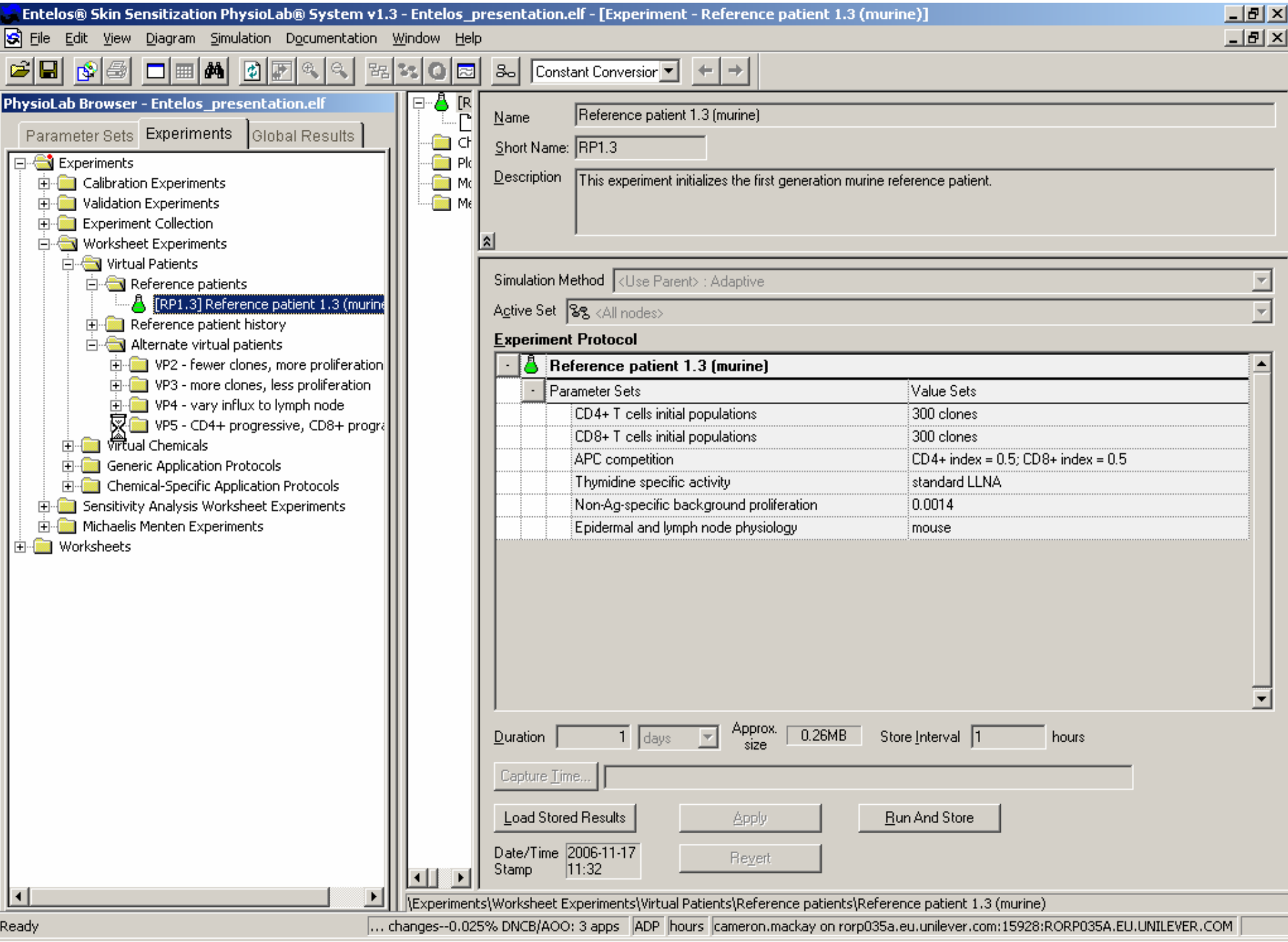
Plots
Monitors
Measurement Sets
Epidermal LC
Mature LN LC
LC numbers
Name LC numbers
Short Name:
Description
i Name Object Definition Value Units
epidermal LC area density Value at 0 hours 850.076 cells/mm^2
epidermal LC area density Value at 2 hours 745.622 cells/mm^2
epidermal LC area density Value at 4 hours 666.218 cells/mm^2
epidermal LC area density Value at 17 hours 478.528 cells/mm^2
...ysiological stimulation\Calibration: Accumulation of LC in LN following IL-1alpha or IL-1beta treatment (Cumberbatch 2002a)
...-1beta treatment (Cumberbatch 2002a) ADP hours cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM


Entelos® Skin Sensitization PhysioLab® System v1.3 - Entelos_presentation.elf - [Experiment - Reference patient 1.3 (murine)]
File Edit View Diagram Simulation Documentation Window Help
Constant Conversion
PhysioLab Browser - Entelos_presentation.elf
Parameter Sets
Experiments
Global Results
Experiments
Calibration Experiments
Validation Experiments
Experiment Collection
Worksheet Experiments
Virtual Patients
Reference patients
[RP1.3] Reference patient 1.3 (murine
Reference patient history
Alternate virtual patients
VP2 - fewer clones, more proliferation
VP3 - more clones, less proliferation
VP4 - vary influx to lymph node
VP5 - CD4+ progressive, CD8+ progra
Virtual Chemicals
Generic Application Protocols
Chemical-Specific Application Protocols
Sensitivity Analysis Worksheet Experiments
Michaelis Menten Experiments
Worksheets
Name Reference patient 1.3 (murine)
Short Name: RP1.3
Description This experiment initializes the first generation murine reference patient.
Simulation Method <Use Parent> : Adaptive
Active Set <All nodes>
Experiment Protocol
Reference patient 1.3 (murine)
Parameter Sets
Value Sets
CD4+ T cells initial populations
300 clones
CD8+ T cells initial populations
300 clones
APC competition
CD4+ index = 0.5; CD8+ index = 0.5
Thymidine specific activity
standard LLNA
Non-Ag-specific background proliferation
0.0014
Epidermal and lymph node physiology
mouse
Duration 1 days
Approx. size 0.26MB
Store Interval 1 hours
Capture Time...
Load Stored Results
Apply
Run And Store
Date/Time Stamp 2006-11-17 11:32
Revert
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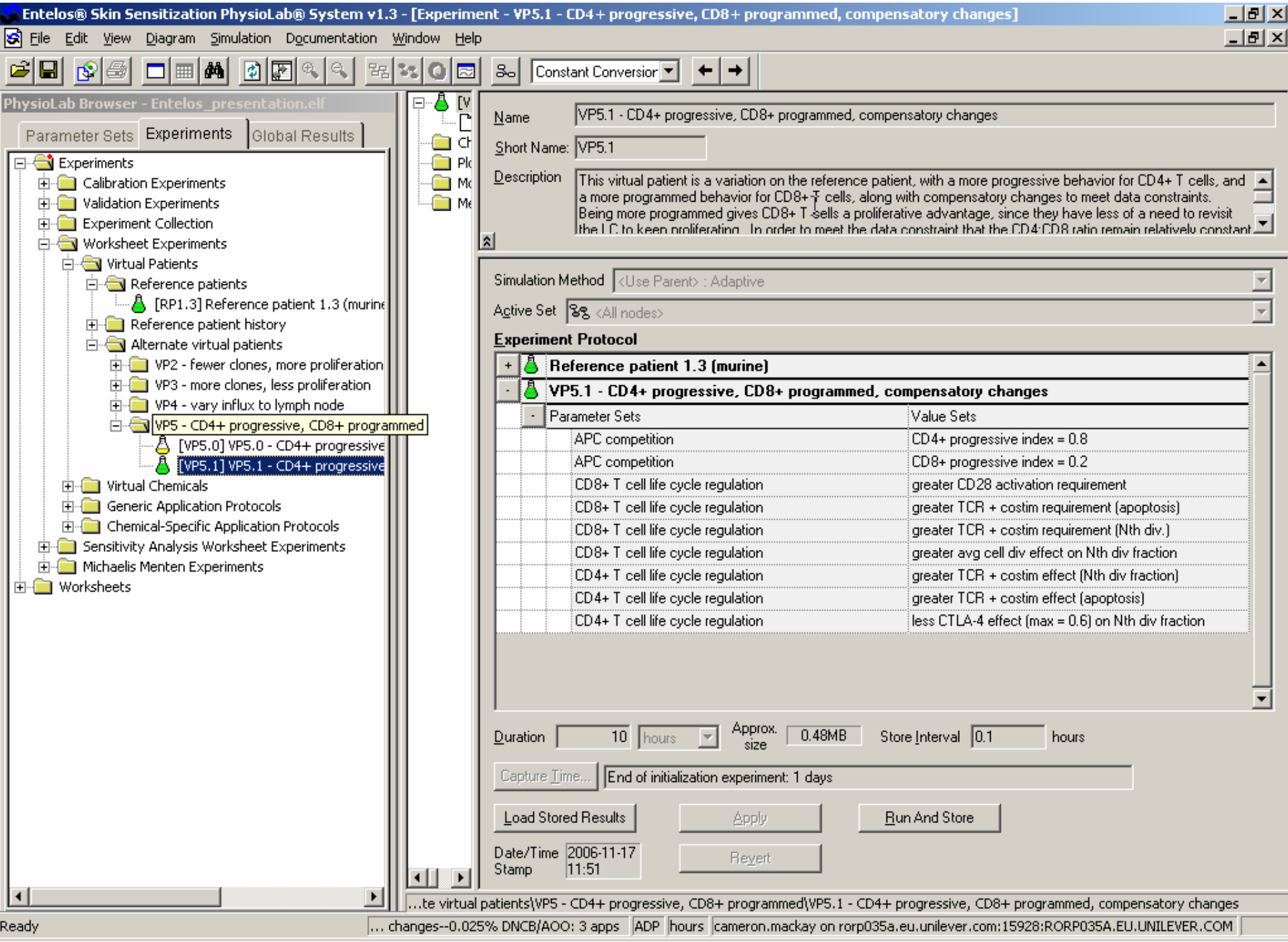
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes]
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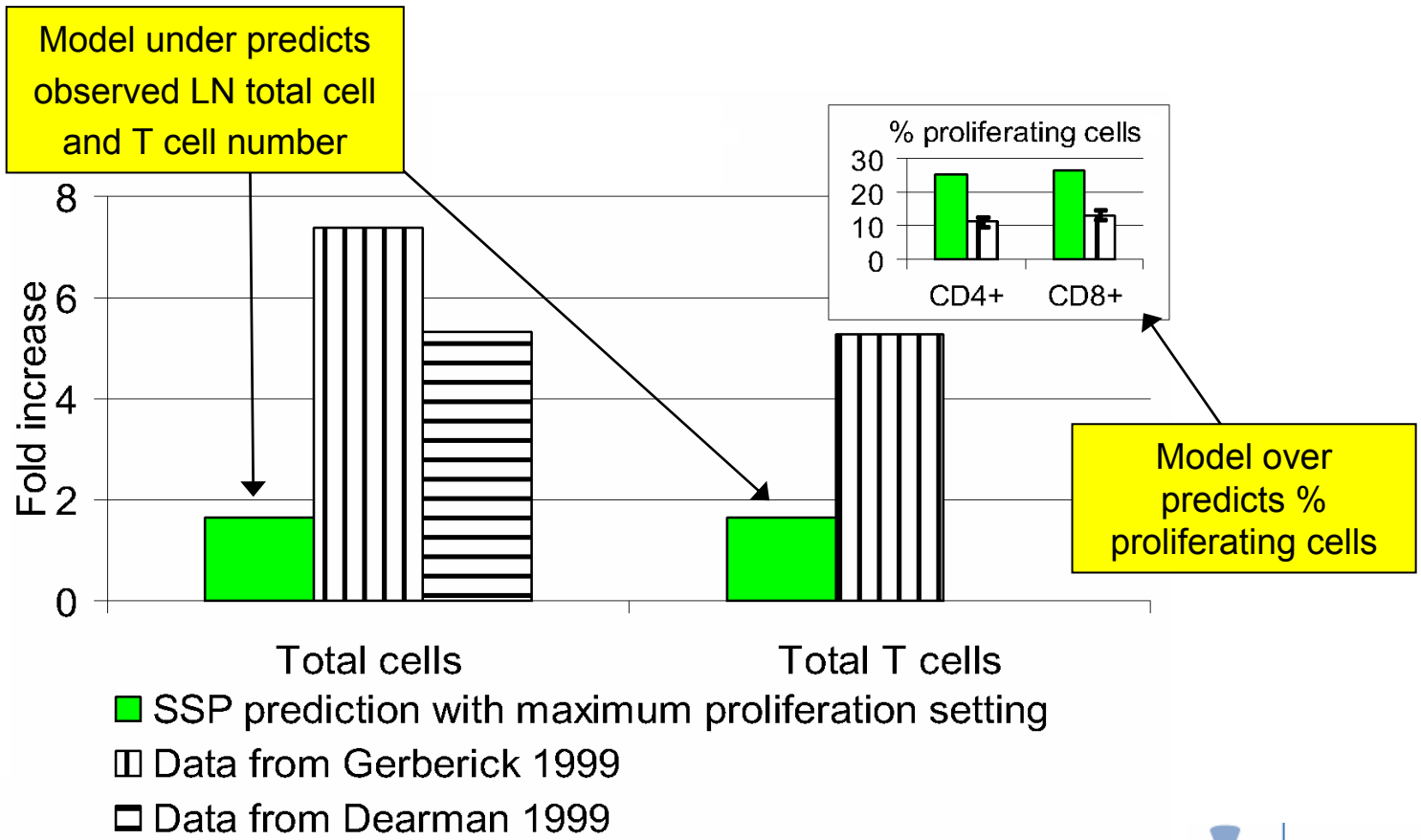
Experiments
Global Results
Experiments
Calibration Experiments
Validation Experiments
Experiment Collection
Worksheet Experiments
Virtual Patients
Reference patients
[RP1.3] Reference patient 1.3 (murine
Reference patient history
Alternate virtual patients
VP2 - fewer clones, more proliferation
VP3 - more clones, less proliferation
VP4 - vary influx to lymph node
VP5 - CD4+ progressive, CD8+ programmed
[VP5.0] VP5.0 - CD4+ progressive
[VP5.1] VP5.1 - CD4+ progressive
Virtual Chemicals
Generic Application Protocols
Chemical-Specific Application Protocols
Sensitivity Analysis Worksheet Experiments
Michaelis Menten Experiments
Worksheets
Name
VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes
Short Name:
VP5.1
Description
This virtual patient is a variation on the reference patient, with a more progressive behavior for CD4+ T cells, and a more programmed behavior for CD8+ T cells, along with compensatory changes to meet data constraints. Being more programmed gives CD8+ T cells a proliferative advantage, since they have less of a need to revisit
Simulation Method
<Use Parent> : Adaptive
Active Set
<All nodes>
Experiment Protocol
Reference patient 1.3 (murine)
VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes
Parameter Sets
Value Sets
APC competition
CD4+ progressive index = 0.8
APC competition
CD8+ progressive index = 0.2
CD8+ T cell life cycle regulation
greater CD28 activation requirement
CD8+ T cell life cycle regulation
greater TCR + costim requirement (apoptosis)
CD8+ T cell life cycle regulation
greater TCR + costim requirement (Nth div.)
CD8+ T cell life cycle regulation
greater avg cell div effect on Nth div fraction
CD4+ T cell life cycle regulation
greater TCR + costim effect (Nth div fraction)
CD4+ T cell life cycle regulation
greater TCR + costim effect (apoptosis)
CD4+ T cell life cycle regulation
less CTLA-4 effect (max = 0.6) on Nth div fraction
Duration
10
hours
Approx. size
0.48MB
Store Interval
0.1
hours
Capture Time...
End of initialization experiment: 1 days
Load Stored Results
Apply
Run And Store
Date/Time Stamp
2006-11-17 11:51
Revert
...te virtual patients\VP5 - CD4+ progressive, CD8+ programmed\VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes
Ready
... changes--0.025% DNCB/AOO: 3 apps
ADP
hours
cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM

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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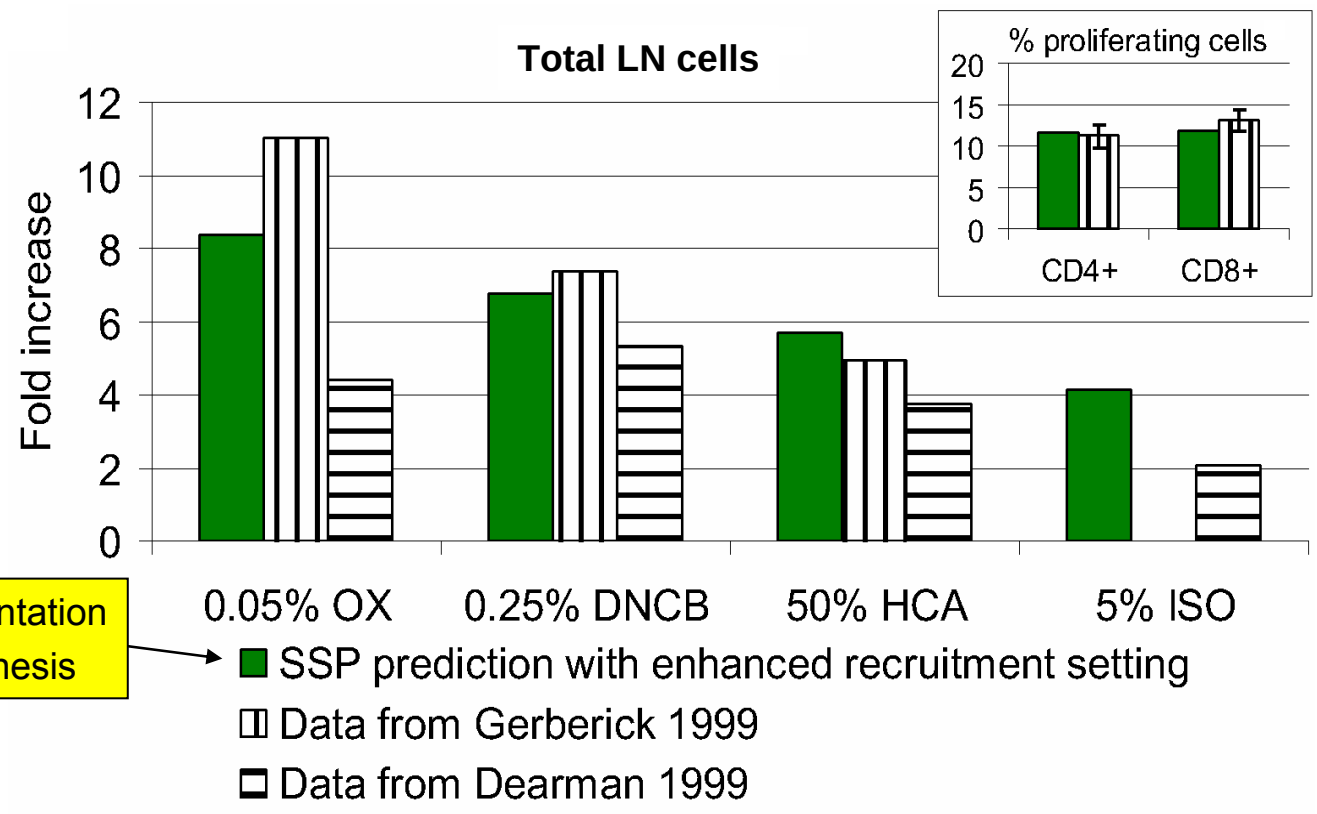
New Insight: Cell recruitment to the lymph node

Hypothesis 2 implemented



Model implementation with new hypothesis

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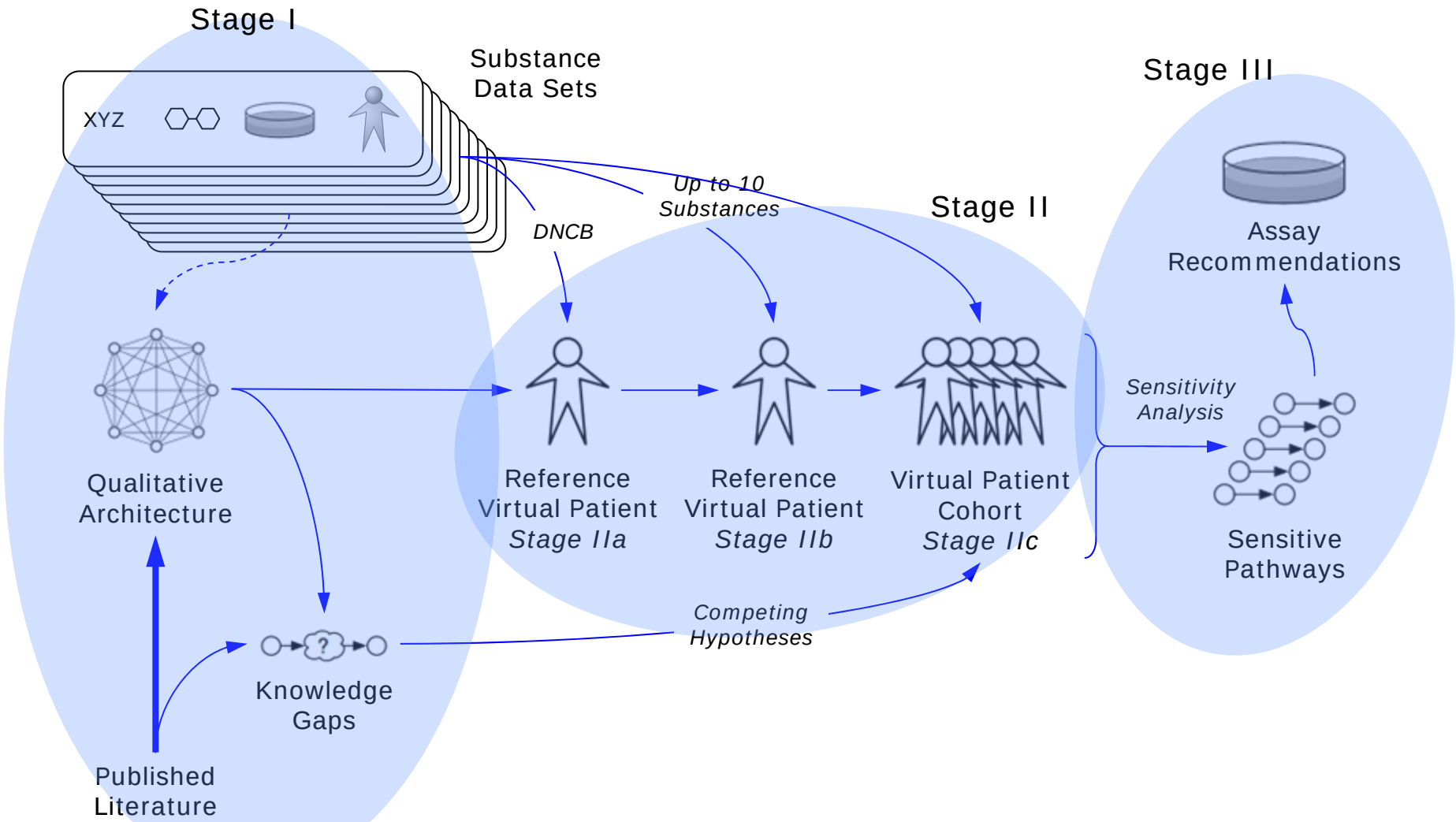
Skin Sensitization Induction PhysioLab Platform (SSIPP)



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Model development – stage III: validation process

- Goal: Ensure that the calibrated mechanisms together reproduce the system-level physiologic behaviors associated with chemical sensitization
  - Demonstrate accurate prediction of T cell count and proliferation rate
  - Demonstrate the ability to capture chemical properties and behaviors
- Approach: Implementation of 30 validation experiments from 15 papers
- Considerations:
  - Protocol: standard & modified LLNA
  - Outputs: SI, relative LN composition, absolute cell numbers
  - Other literature: supporting or inconsistent data
  - Relevance: quantitative or qualitative match expected
- Outcome:
  - Complete agreement with 28/30 experiments
  - Partial agreement with 2/30 experiments

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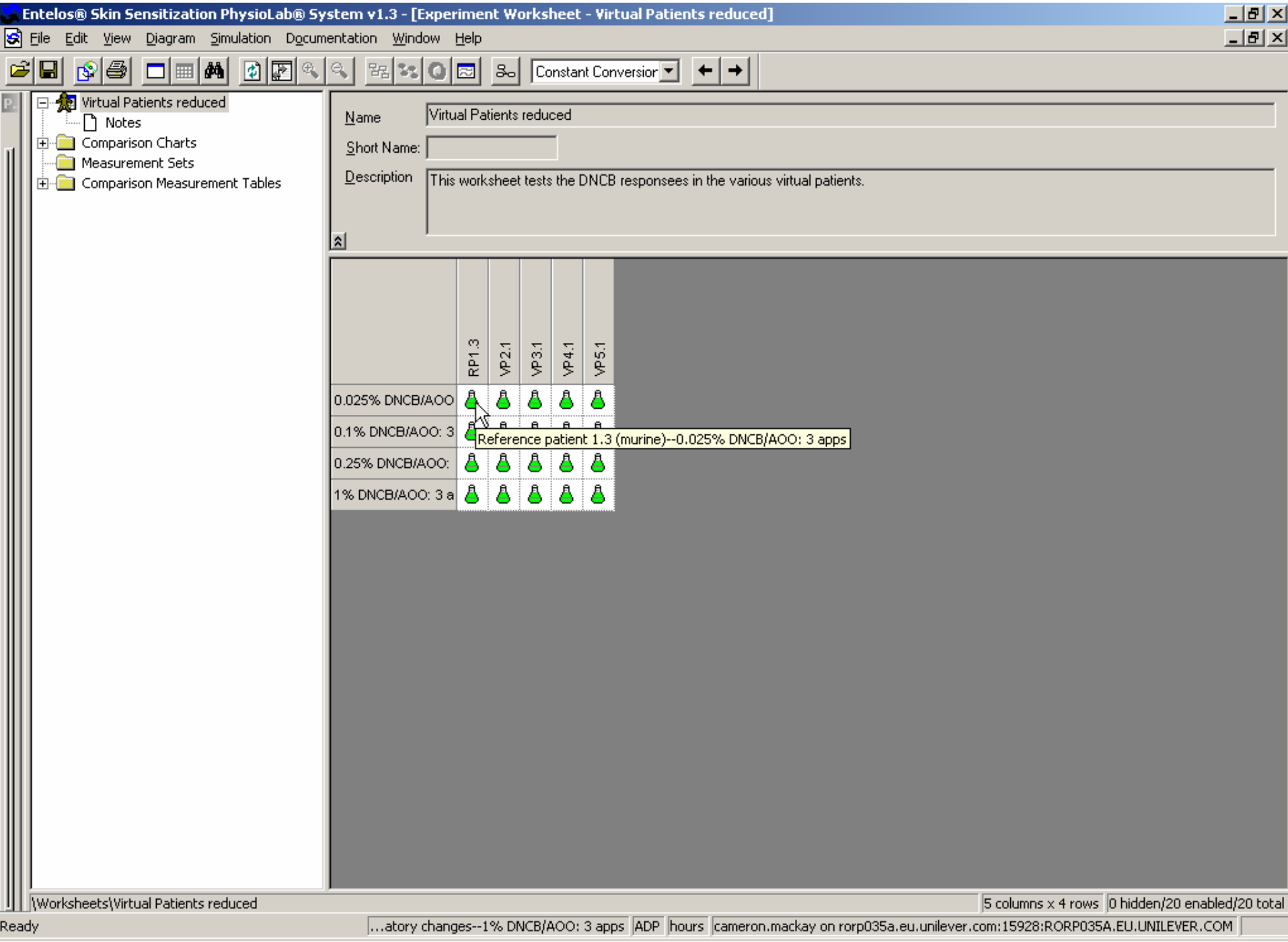
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment Worksheet - Virtual Patients reduced]
File Edit View Diagram Simulation Documentation Window Help
Constant Conversior
Virtual Patients reduced
Notes
Comparison Charts
Measurement Sets
Comparison Measurement Tables
Name
Virtual Patients reduced
Short Name:
Description
This worksheet tests the DNCB responsees in the various virtual patients.
RP1.3
VP2.1
VP3.1
VP4.1
VP5.1
0.025% DNCB/AOO
0.1% DNCB/AOO: 3
0.25% DNCB/AOO:
1% DNCB/AOO: 3 a
Reference patient 1.3 (murine)--0.025% DNCB/AOO: 3 apps
\Worksheets\Virtual Patients reduced
5 columns x 4 rows
0 hidden/20 enabled/20 total
Ready
...atory changes--1% DNCB/AOO: 3 apps
ADP
hours
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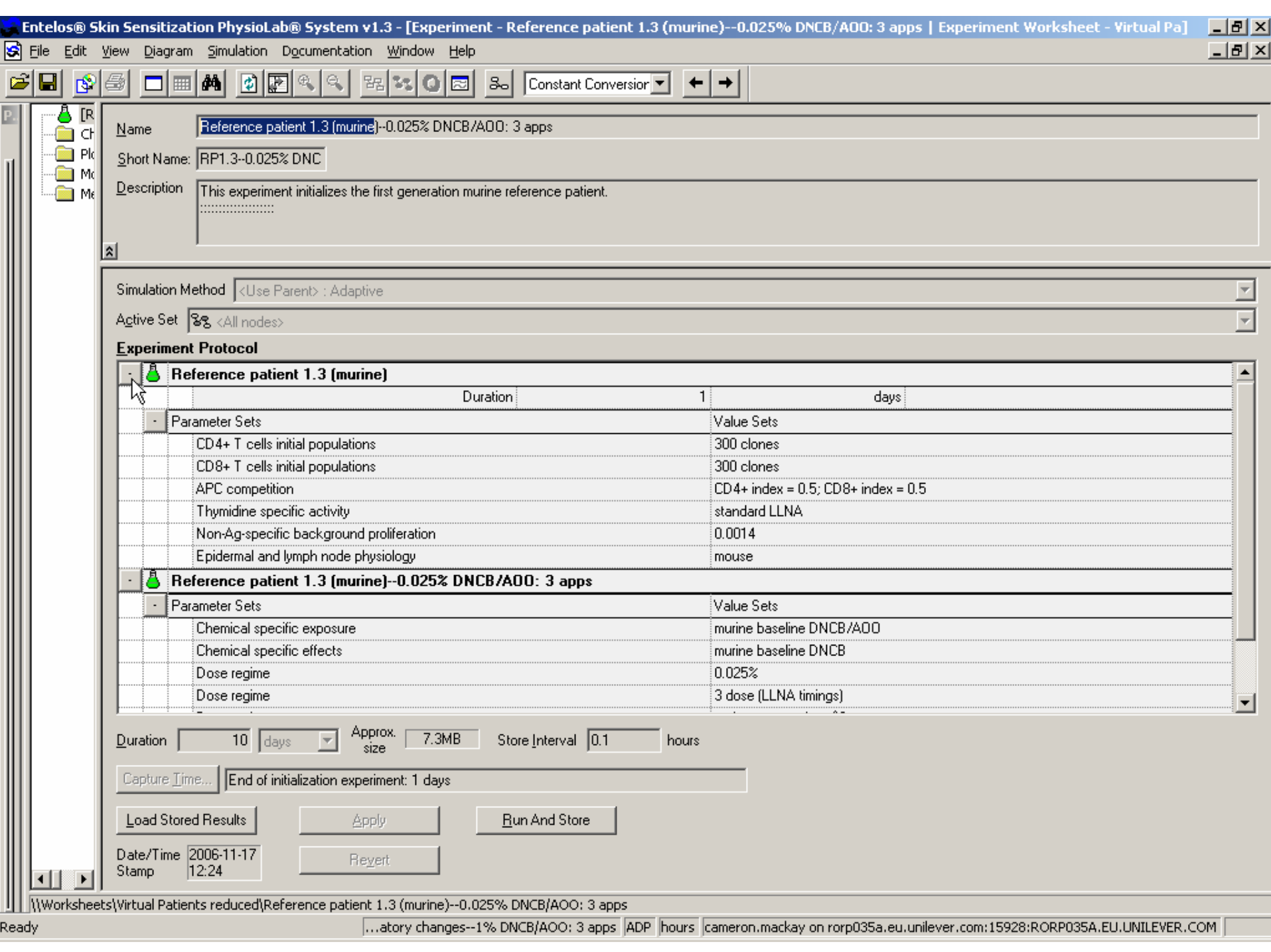
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Reference patient 1.3 (murine)--0.025% DNCB/AOO: 3 apps | Experiment Worksheet - Virtual Pa]
File Edit View Diagram Simulation Documentation Window Help
Constant Conversior
Name
Reference patient 1.3 (murine)--0.025% DNCB/AOO: 3 apps
Short Name:
RP1.3--0.025% DNC
Description
This experiment initializes the first generation murine reference patient.
Simulation Method
<Use Parent> : Adaptive
Active Set
<All nodes>
Experiment Protocol
Reference patient 1.3 (murine)
Duration 1 days
Parameter Sets Value Sets
CD4+ T cells initial populations 300 clones
CD8+ T cells initial populations 300 clones
APC competition CD4+ index = 0.5; CD8+ index = 0.5
Thymidine specific activity standard LLNA
Non-Ag-specific background proliferation 0.0014
Epidermal and lymph node physiology mouse
Reference patient 1.3 (murine)--0.025% DNCB/AOO: 3 apps
Parameter Sets Value Sets
Chemical specific exposure murine baseline DNCB/AOO
Chemical specific effects murine baseline DNCB
Dose regime 0.025%
Dose regime 3 dose (LLNA timings)
Duration 10 days
Approx. size 7.3MB
Store Interval 0.1 hours
Capture Time...
End of initialization experiment: 1 days
Load Stored Results
Apply
Run And Store
Date/Time Stamp 2006-11-17 12:24
Revert
\\Worksheets\Virtual Patients reduced\Reference patient 1.3 (murine)--0.025% DNCB/AOO: 3 apps
Ready
...atory changes--1% DNCB/AOO: 3 apps
ADP
hours
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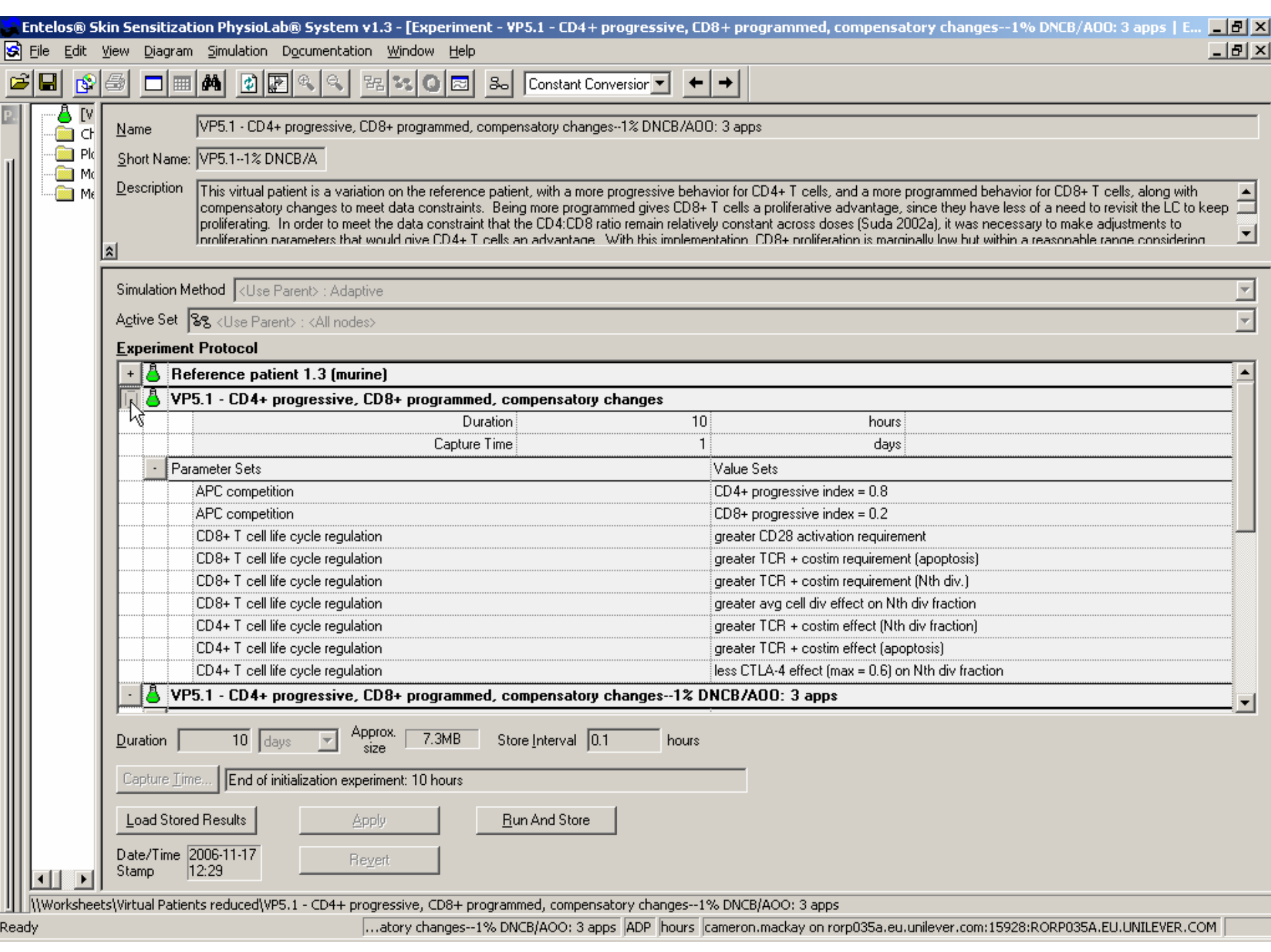
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes--1% DNCB/AOO: 3 apps | E...
File Edit View Diagram Simulation Documentation Window Help
Constant Conversior
Name
VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes--1% DNCB/AOO: 3 apps
Short Name:
VP5.1--1% DNCB/A
Description
This virtual patient is a variation on the reference patient, with a more progressive behavior for CD4+ T cells, and a more programmed behavior for CD8+ T cells, along with compensatory changes to meet data constraints. Being more programmed gives CD8+ T cells a proliferative advantage, since they have less of a need to revisit the LC to keep proliferating. In order to meet the data constraint that the CD4:CD8 ratio remain relatively constant across doses (Suda 2002a), it was necessary to make adjustments to
Simulation Method
<Use Parent> : Adaptive
Active Set
<Use Parent> : <All nodes>
Experiment Protocol
Reference patient 1.3 (murine)
VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes
Duration 10 hours
Capture Time 1 days
Parameter Sets
Value Sets
APC competition
CD4+ progressive index = 0.8
APC competition
CD8+ progressive index = 0.2
CD8+ T cell life cycle regulation
greater CD28 activation requirement
CD8+ T cell life cycle regulation
greater TCR + costim requirement (apoptosis)
CD8+ T cell life cycle regulation
greater TCR + costim requirement (Nth div.)
CD8+ T cell life cycle regulation
greater avg cell div effect on Nth div fraction
CD4+ T cell life cycle regulation
greater TCR + costim effect (Nth div fraction)
CD4+ T cell life cycle regulation
greater TCR + costim effect (apoptosis)
CD4+ T cell life cycle regulation
less CTLA-4 effect (max = 0.6) on Nth div fraction
VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes--1% DNCB/AOO: 3 apps
Duration 10 days
Approx. size 7.3MB
Store Interval 0.1 hours
Capture Time...
End of initialization experiment: 10 hours
Load Stored Results
Apply
Run And Store
Date/Time Stamp 2006-11-17 12:29
Revert
\\Worksheets\Virtual Patients reduced\VP5.1 - CD4+ progressive, CD8+ programmed, compensatory changes--1% DNCB/AOO: 3 apps
Ready
...atory changes--1% DNCB/AOO: 3 apps
ADP
hours
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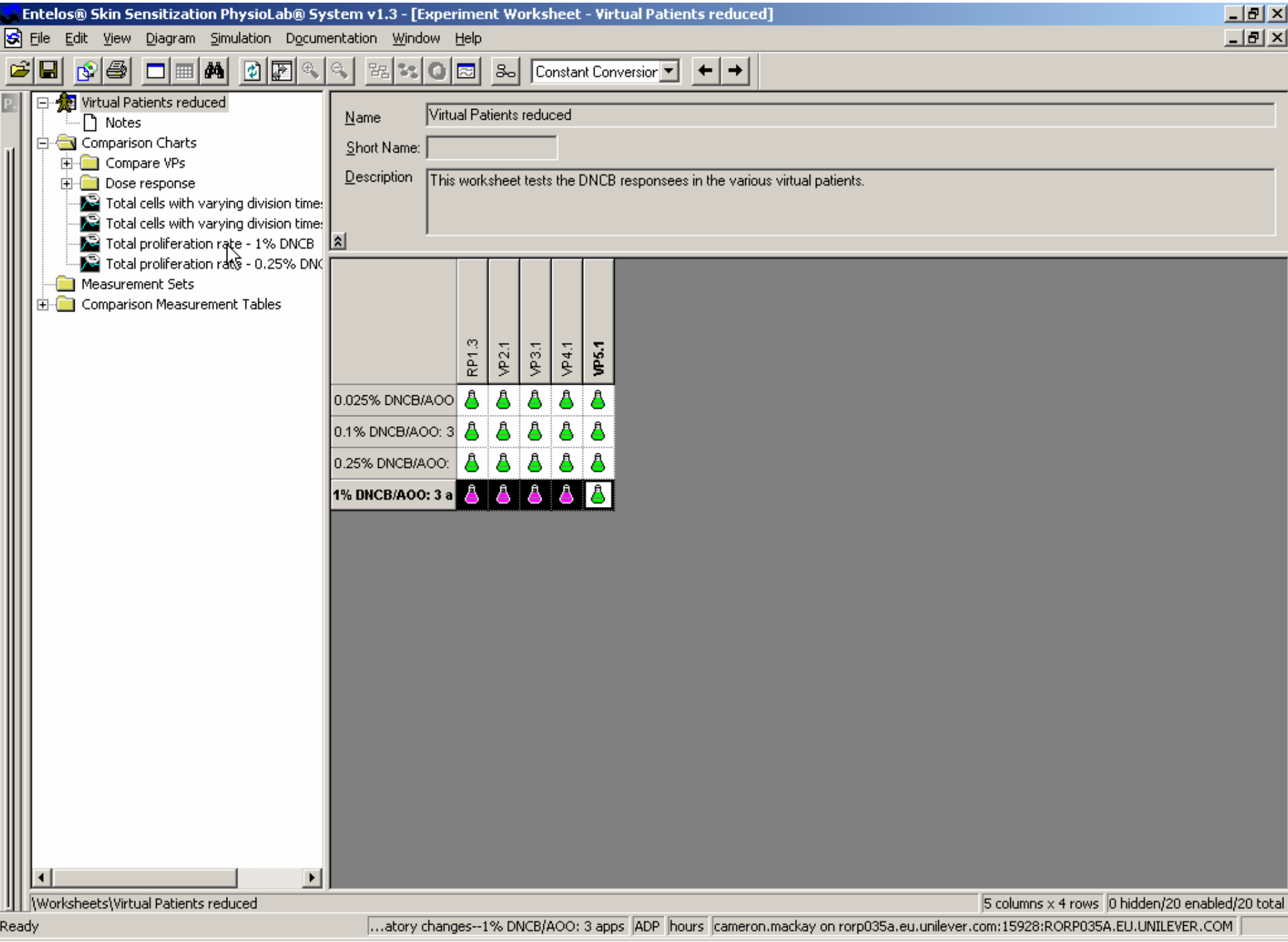
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment Worksheet - Virtual Patients reduced]
File Edit View Diagram Simulation Documentation Window Help
Constant Conversion
Virtual Patients reduced
Notes
Comparison Charts
Compare VPs
Dose response
Total cells with varying division time
Total cells with varying division time
Total proliferation rate - 1% DNCB
Total proliferation rate - 0.25% DNC
Measurement Sets
Comparison Measurement Tables
Name
Virtual Patients reduced
Short Name:
Description
This worksheet tests the DNCB responsees in the various virtual patients.
RP1.3
VP2.1
VP3.1
VP4.1
VP5.1
0.025% DNCB/AOO
0.1% DNCB/AOO: 3
0.25% DNCB/AOO:
1% DNCB/AOO: 3 a
\Worksheets\Virtual Patients reduced
5 columns x 4 rows
0 hidden/20 enabled/20 total
Ready
...atory changes--1% DNCB/AOO: 3 apps
ADP
hours
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Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment Worksheet - Virtual Patients reduced | Comparison Chart - Total proliferation rate - 1% DNCB]

File Edit View Diagram Simulation Documentation Window Help

Constant Conversior

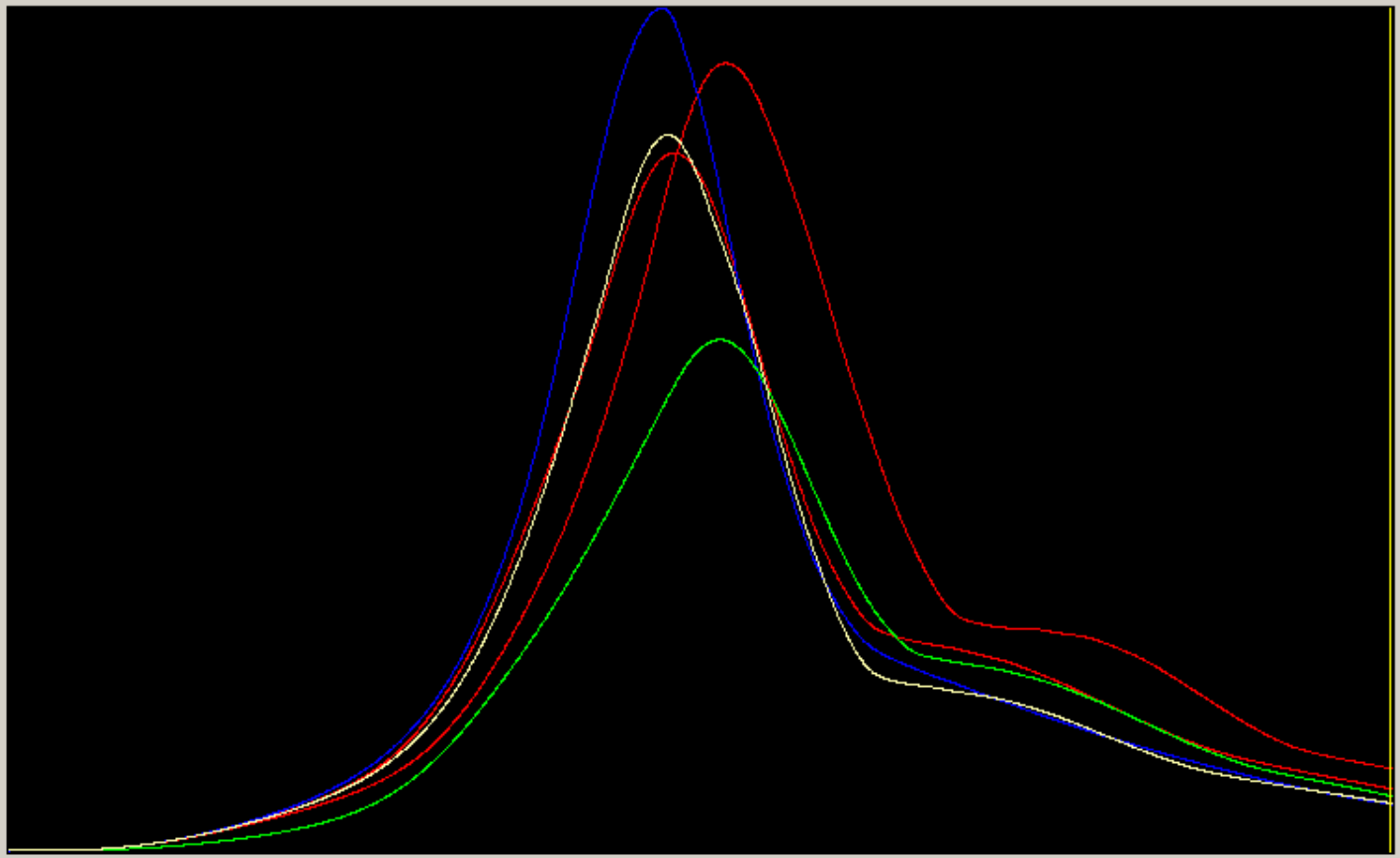
- Virtual Patients reduced
  - Notes
  - Comparison Charts
  - Measurement Sets
  - Comparison Measurement Tables
    - 0.25% DNCB response type compar
    - 0.25% DNCB - % activated and pro
    - 1% DNCB response type comparison

Experiment Name	Value	Units
\\Worksheets\Virtual Patients reduced\Reference patient 1.3 (murine)--1% DNCB/AOO: 3 apps	17623.6	cell...
\\Worksheets\Virtual Patients reduced\VP2.1 - fewer clones, more proliferation, compensatory changes--1% ...: 3 apps	22480.7	cell...
\\Worksheets\Virtual Patients reduced\VP3.1 - more clones, less proliferation, compensatory changes--1% D...: 3 apps	13927.1	cell...
\\Worksheets\Virtual Patients reduced\VP4.1 - reduced influx modulation--1% DNCB/AOO: 3 apps	15953.6	cell...
\\Worksheets\Virtual Patients reduced\VP5.1 - CD4+ progressive, CD8+ programmed, compensatory change...: 3 apps	14203	cell...

total proliferation rate

Normalized (All): 2718.5 to 204943

0 to 10 days: t = 9D 23:24:00.00



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Ready

...atory changes--1% DNCB/AOO: 3 apps | ADP | hours | cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM

Entelos® Skin Sensitization PhysioLab® System v1.3 - [Comparison Measurement Table - 1% DNCB response type comparison | Experiment Worksheet - Virtual Patie...

File Edit View Diagram Simulation Documentation Window Help

Constant Conversion

Name	Object	RP1.3--1% D...	VP2.1--1% D...	VP3.1--1% D...	VP4.1--1% D...	VP5.1--1% D...	Units
	LN total cells (Value at 0 days)	1.950000e+006	1.950000e+006	1.950000e+006	1.950000e+006	1.950000e+006	cells
	LN total cells (Value at 5.2083 days)	1.565468e+007	1.579994e+007	1.627434e+007	8.218700e+006	1.568219e+007	cells
	LN total cells (Value at 5.2083 days - delta%)	702.804	710.253	734.581	321.472	704.215	%
	LN total cells (Time at max)	4.91426	5.27235	4.85272	5.1923	4.90061	
	LN total T cells (Value at 0 days)	1.521000e+006	1.521000e+006	1.521000e+006	1.521000e+006	1.521000e+006	cells
	LN total T cells (Value at 5.2083 days)	1.003534e+007	1.049808e+007	1.012458e+007	5.809979e+006	1.003230e+007	cells
	LN total T cells (Value at 5.2083 days - delta%)	559.786	590.209	565.653	281.984	559.586	%
	LN total T cells (Time at max)	4.65636	5.0071	4.62372	5.04461	4.66082	
	LN total CD4+ T cells (Value at 5.2083 days)	6.555542e+006	6.611684e+006	6.404027e+006	3.691862e+006	6.629299e+006	cells
	LN total CD8+ T cells (Value at 5.2083 days)	3.479799e+006	3.886397e+006	3.720550e+006	2.118117e+006	3.403002e+006	cells
	LN (H) proliferating CD4+ T cells (Value at 5.2083 days)	705562	920695	609260	617832	772923	cells
	LN (H) proliferating CD8+ T cells (Value at 5.2083 days)	301108	587441	341710	372041	234303	cells
	LN (H) proliferating CD4+ T cells (Time at max)	4.89765	5.47684	4.88169	5.15308	4.98204	
	LN (H) proliferating CD8+ T cells (Time at max)	4.4835	4.84626	4.48651	4.85518	4.47072	
	LN CD4+ T cell % prolif. (Value at 5.2083 days)	10.7628	13.9253	9.51369	16.735	11.6592	%
	LN CD8+ T cell prolif. % (Value at 5.2083 days)	8.65304	15.1153	9.18438	17.5647	6.8852	%
	LN B cell fraction (Value at 0 days)	0.11	0.11	0.11	0.11	0.11	fraction
	LN B cell fraction (Value at 5.2083 days)	0.285234	0.262516	0.306965	0.220636	0.28668	fraction
	LN T cell fraction (Value at 0 days)	0.78	0.78	0.78	0.78	0.78	fraction
	LN T cell fraction (Value at 5.2083 days)	0.641044	0.664438	0.622119	0.706922	0.639726	fraction

\Comparison Measurement Tables\1% DNCB response type comparison

Ready ...atory changes--1% DNCB/AOO: 3 apps ADP hours cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM

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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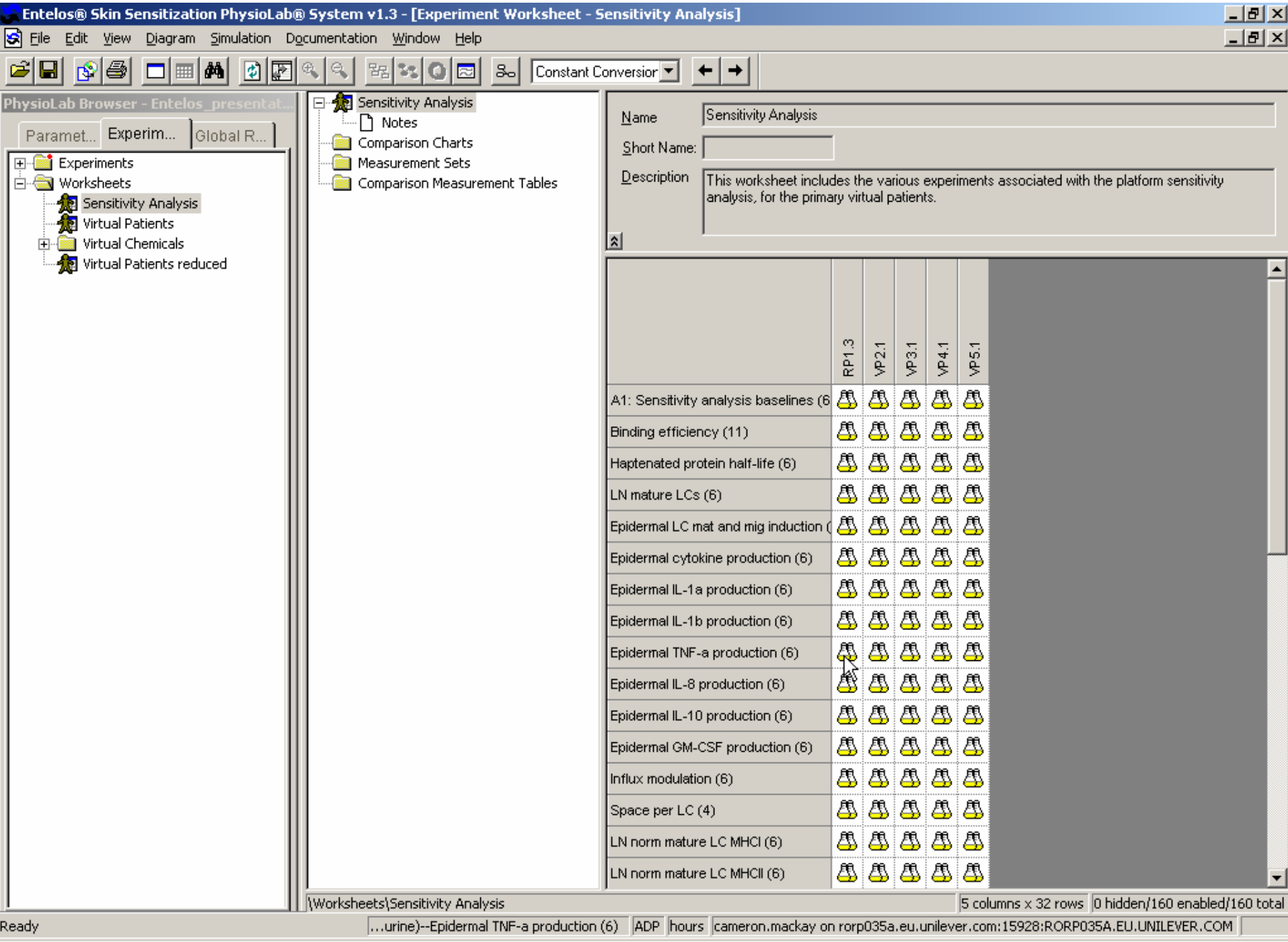
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment Worksheet - Sensitivity Analysis]
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Experiments
Worksheets
Sensitivity Analysis
Virtual Patients
Virtual Chemicals
Virtual Patients reduced
Sensitivity Analysis
Notes
Comparison Charts
Measurement Sets
Comparison Measurement Tables
Name Sensitivity Analysis
Short Name:
Description This worksheet includes the various experiments associated with the platform sensitivity analysis, for the primary virtual patients.
RP1.3
VP2.1
VP3.1
VP4.1
VP5.1
A1: Sensitivity analysis baselines (6
Binding efficiency (11)
Haptenated protein half-life (6)
LN mature LCs (6)
Epidermal LC mat and mig induction (
Epidermal cytokine production (6)
Epidermal IL-1a production (6)
Epidermal IL-1b production (6)
Epidermal TNF-a production (6)
Epidermal IL-8 production (6)
Epidermal IL-10 production (6)
Epidermal GM-CSF production (6)
Influx modulation (6)
Space per LC (4)
LN norm mature LC MHCI (6)
LN norm mature LC MHCII (6)
\Worksheets\Sensitivity Analysis
5 columns x 32 rows
0 hidden/160 enabled/160 total
Ready
...urine)--Epidermal TNF-a production (6)
ADP
hours
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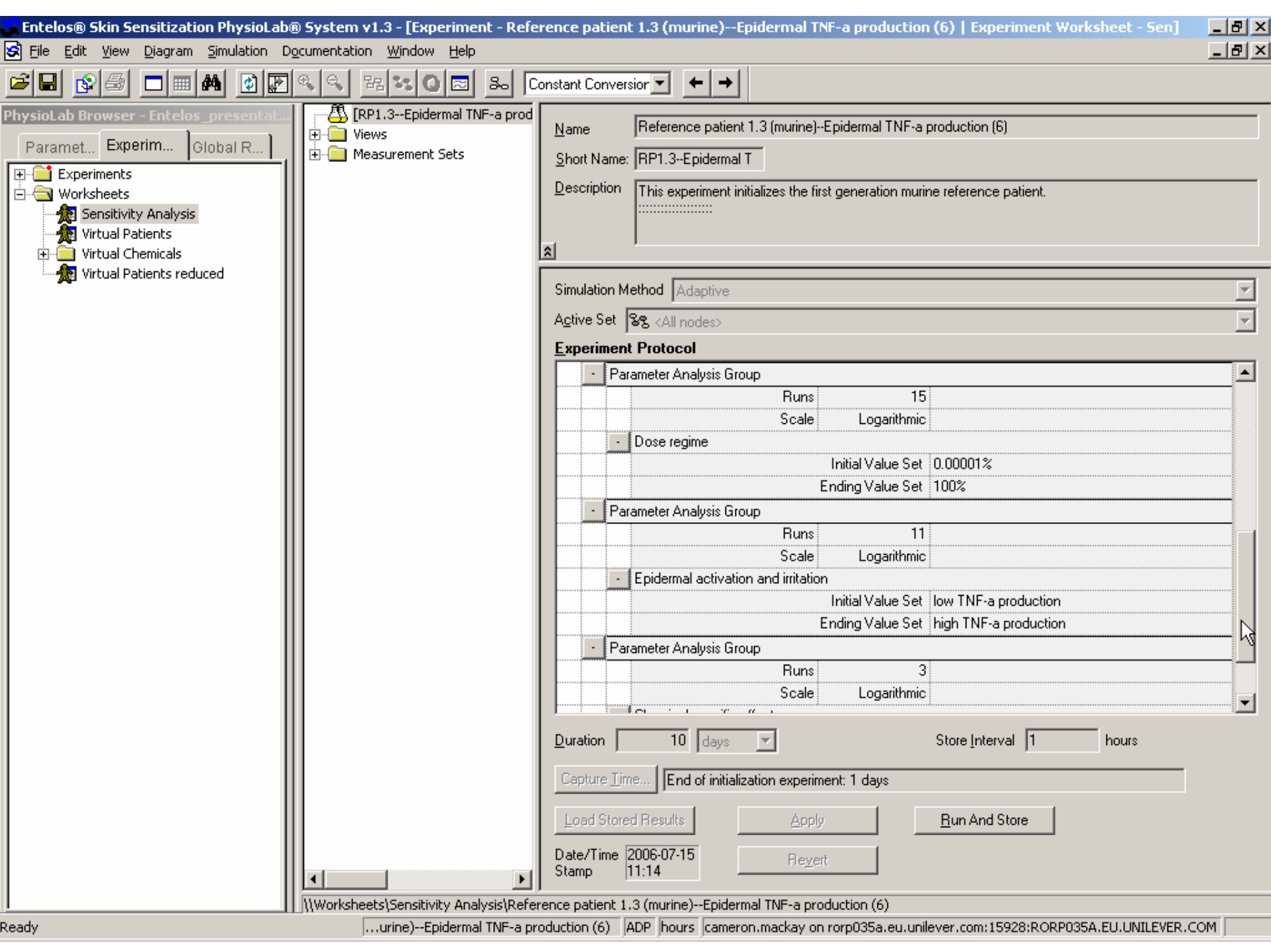
Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Reference patient 1.3 (murine)--Epidermal TNF-a production (6) | Experiment Worksheet - Sen]
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Paramet... Experim... Global R...
Experiments
Worksheets
Sensitivity Analysis
Virtual Patients
Virtual Chemicals
Virtual Patients reduced
[RP1.3--Epidermal TNF-a prod
Views
Measurement Sets
Name Reference patient 1.3 (murine)--Epidermal TNF-a production (6)
Short Name: RP1.3--Epidermal T
Description This experiment initializes the first generation murine reference patient.
Simulation Method Adaptive
Active Set <All nodes>
Experiment Protocol
Parameter Analysis Group
Runs 15
Scale Logarithmic
Dose regime
Initial Value Set 0.00001%
Ending Value Set 100%
Parameter Analysis Group
Runs 11
Scale Logarithmic
Epidermal activation and irritation
Initial Value Set low TNF-a production
Ending Value Set high TNF-a production
Parameter Analysis Group
Runs 3
Scale Logarithmic
Duration 10 days
Store Interval 1 hours
Capture Time...
End of initialization experiment: 1 days
Load Stored Results
Apply
Run And Store
Date/Time Stamp 2006-07-15 11:14
Revert
\\Worksheets\Sensitivity Analysis\Reference patient 1.3 (murine)--Epidermal TNF-a production (6)
Ready
...urine)--Epidermal TNF-a production (6)
ADP
hours
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Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment - Reference patient 1.3 (murine)--Epidermal TNF-a production (6) | Parametric Analysis View -]

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Constant Conversion

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Paramet... Experim... Global R...

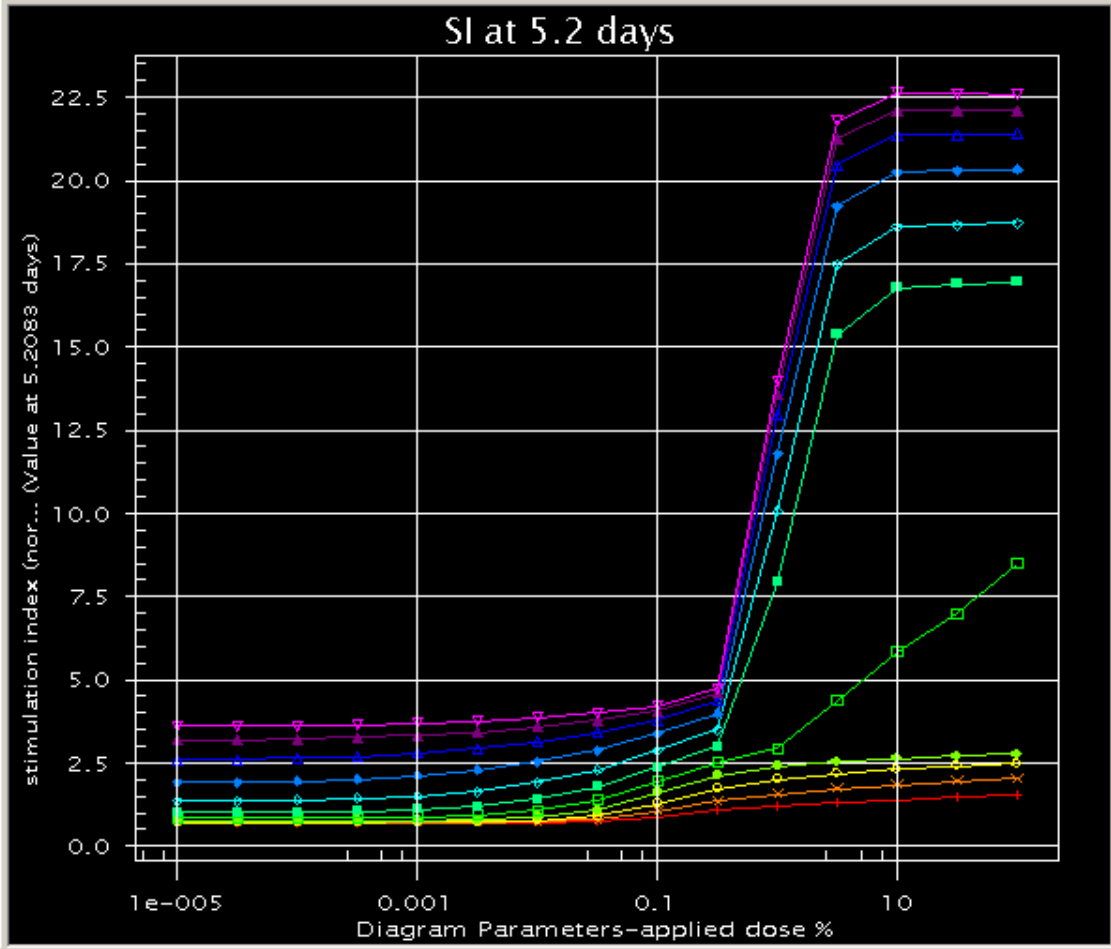
- Experiments
- Worksheets
  - Sensitivity Analysis
  - Virtual Patients
  - Virtual Chemicals
  - Virtual Patients reduced

- [RP1.3--Epidermal TNF-a prod
- Views
  - SI at 5.2 days
  - Radioactivity at 5.2 days
- Measurement Sets

Name: SI at 5.2 days

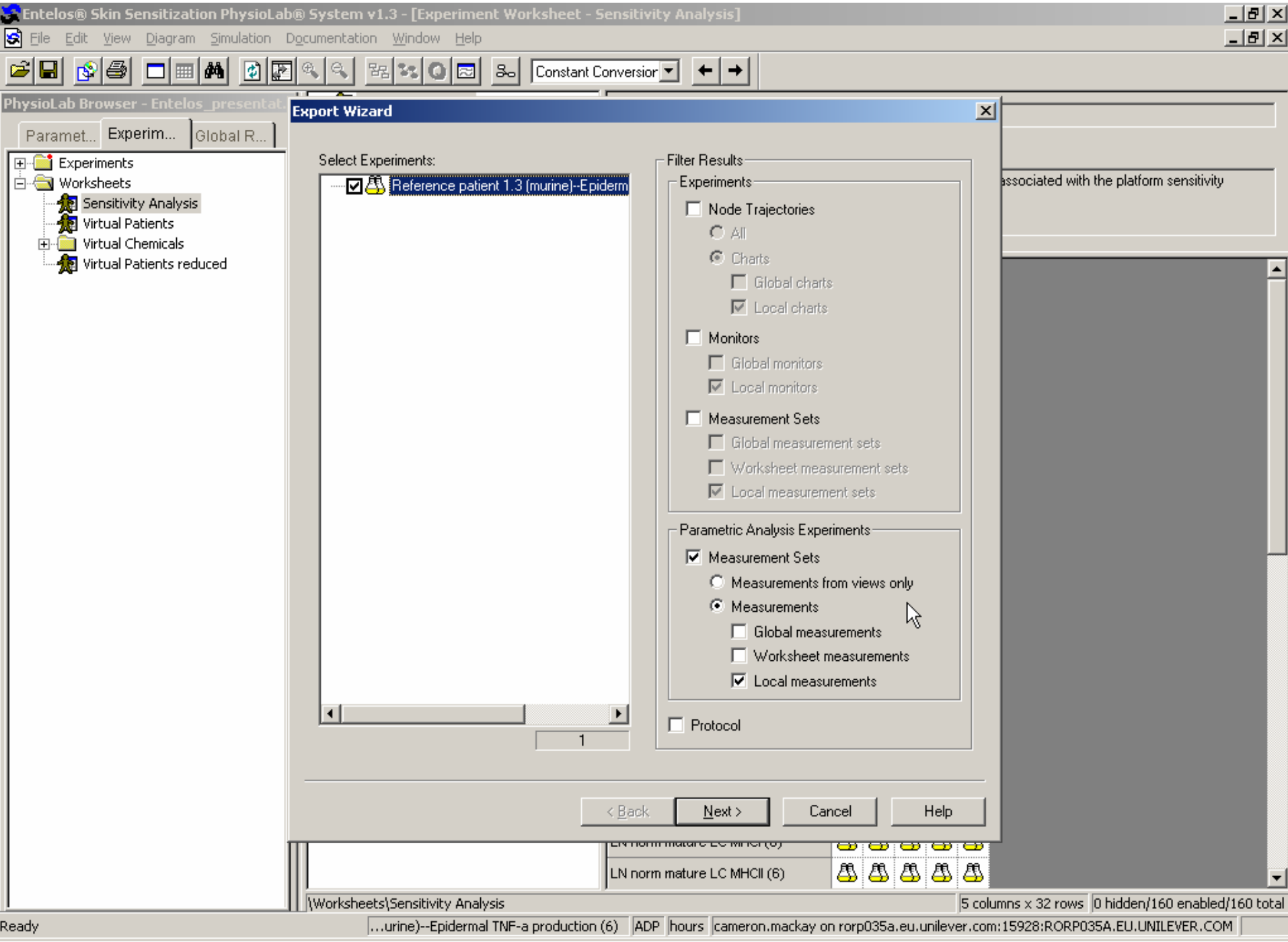
Short Name:

Description:



\\Worksheets\Sensitivity Analysis\Reference patient 1.3 (murine)--Epidermal TNF-a production (6)

Ready ...urine)--Epidermal TNF-a production (6) ADP hours cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM


Entelos® Skin Sensitization PhysioLab® System v1.3 - [Experiment Worksheet - Sensitivity Analysis]
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Constant Conversion
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Paramet... Experim... Global R...
Experiments
Worksheets
Sensitivity Analysis
Virtual Patients
Virtual Chemicals
Virtual Patients reduced
Export Wizard
Select Experiments:
Reference patient 1.3 (murine)--Epiderm
1
Filter Results
Experiments
Node Trajectories
All
Charts
Global charts
Local charts
Monitors
Global monitors
Local monitors
Measurement Sets
Global measurement sets
Worksheet measurement sets
Local measurement sets
Parametric Analysis Experiments
Measurement Sets
Measurements from views only
Measurements
Global measurements
Worksheet measurements
Local measurements
Protocol
< Back
Next >
Cancel
Help
associated with the platform sensitivity
LN norm mature LC MHCII (6)
\Worksheets\Sensitivity Analysis
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0 hidden/160 enabled/160 total
Ready
...urine)--Epidermal TNF-a production (6)
ADP
hours
cameron.mackay on rorp035a.eu.unilever.com:15928:RORP035A.EU.UNILEVER.COM

Microsoft Excel - RP1.3--LLNA# 1 dose (1#day) (PMeas).xls

File Edit View Insert Format Tools Data Window Help

Type a question for help

SnagIt Window

B28 fx 20

	K	L	M	N	O
1	User name on DB				
2	cameron.mackay				
3					
4					
5					
6					
7					
8					
9					
10					
11					
12	SA: Primary outputs	SA: Key outputs	SA: Key outputs	SA: Key outputs	SA: Key outputs
13					
14					
15	total Ag specific prolif. rate	LN CD4+ T cell % prolif.	LN CD8+ T cell prolif. %	CD4+ T cell TCR + costim internal state	CD8+ T cell TCR + costim internal state
16	Time at max	Value at 5.2 days	Value at 5.2 days	Value at 5.2 days	Value at 5.2 days
17	17.19595047	4.82E-14	8.11E-14	1.56E-11	4.46E-11
18	17.15483495	4.93E-14	8.16E-14	1.61E-11	4.51E-11
19	17.14196564	1.70E-13	2.56E-13	4.80E-11	1.19E-10
20	73.19260172	1.90E-12	2.75E-12	5.60E-10	1.30E-09
21	77.49140239	2.97E-11	4.64E-11	7.53E-09	1.61E-08
22	85.07462546	8.42E-10	1.47E-09	1.27E-07	2.42E-07
23	102.7949656	2.78E-08	4.68E-08	2.75E-06	4.51E-06
24	117.4150049	7.32E-07	6.24E-07	6.41E-05	8.75E-05
25	119.6859492	1.14E-05	8.41E-06	0.001137851	0.001340279
26	114.6299825	0.000173063	0.000123746	0.014026579	0.015494343
27	104.6320234	0.003034383	0.002033218	0.139458569	0.143765668
28	145.3592711	0.177602645	0.22863282	0.635312663	0.589084166
29	195.7560905	1.434695045	1.693532041	0.848223081	0.775397154
30	184.0506442	1.861931787	2.526689322	0.876246879	0.813223898
31	183.1347231	1.878842556	2.564813968	0.877464828	0.814849304
32	68.31804773	3.01E-13	4.73E-13	2.12E-11	5.53E-11

Ag specific proliferation | LN Cellularity | TRM | RP1.3--LLNA# 2 dose (2#day) (PM | RP1.3--LLN

Ready NUM

Virtual patient exploration revealed the sensitivity analysis to be robust

- The same 14 pathways came up as most sensitive across all virtual patients (ranking did change slightly)
- Biological knowledge gaps/variability explored in the virtual patients does not materially affect the importance of the sensitive pathways

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Areas for model development

Computational/Analysis

- Enhanced sensitivity analysis (global)

Data generation

- Epidermal cytokine profiles
- Exposure parameters
- Activated LC phenotypes
- Number of naïve reactive clones

Expansion of scope

- Increased KC/LC cross-regulation in epidermis
- Expanded LC phenotype
- Mechanisms of LN lymphocyte recruitment regulation
- Memory T cell generation

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Acknowledgements

- Unilever
  - Catherine Clapp
  - Ian Jowsey
  - David Lockley
  - Gavin Maxwell
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  - Christina Friedrich
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