University of Connecticut Health Center

The VCell Database

– Sharing, Publishing, Reusing VCell Models –

http://vcell.org

National Institute of General Medical Sciences

NIH Roadmap

National Center for Research Resources

Richard D. Berlin

Center for Cell Analysis and Modeling

National Technology Center for Networks and Pathways

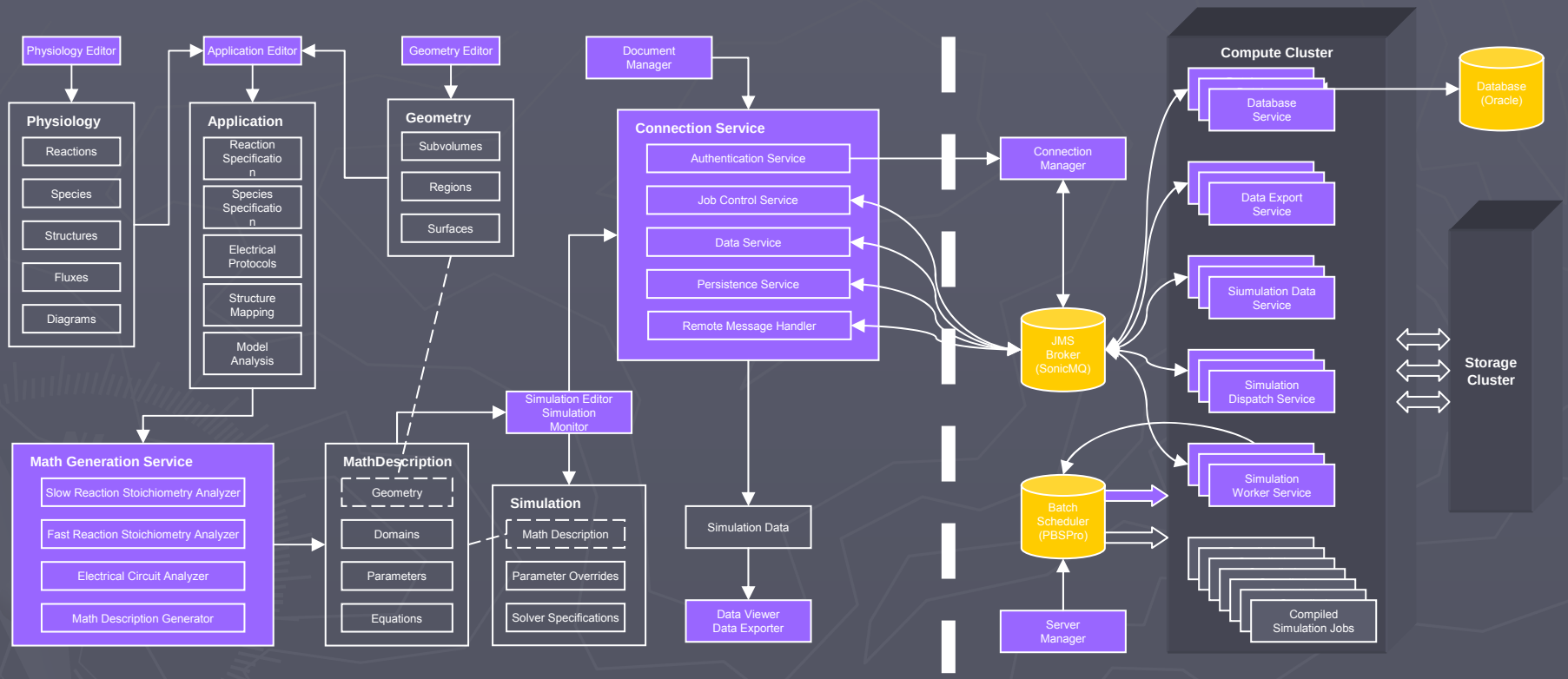
Design Requirements

- Resources
  - Compilers, libraries, add-ons, HPC hardware – NO!
- Portability
  - Run on Windows, Mac, Unix
- Availability
  - Some simulations run for weeks...
- Sharing and Persistence
  - Collaboration; immutable and reproducible public models
- Maintenance and Interoperability
  - Continuous feature updates; backwards compatibility; links to other data/services; interchange with other tools

Minimal Usage Requirements

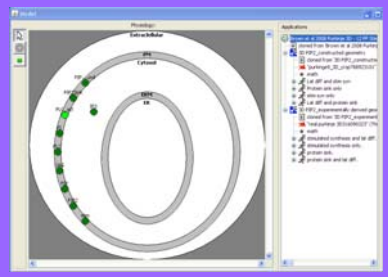
- Registration
  - Free; open source
- Java
  - Version 1.5 or later (except Mac – 1.4 required)
- Internet connection
  - Required for:
    - Database access
    - Running simulations
    - Viewing results
  - But also standalone versions...
- A large monitor... !

Distributed Architecture

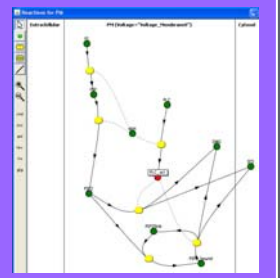


Physiology

Molecules
Structures
(topology)

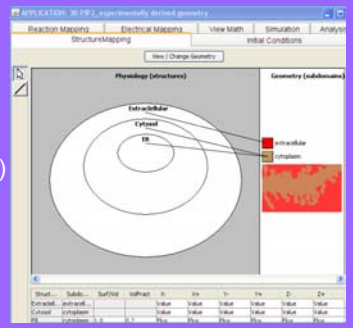


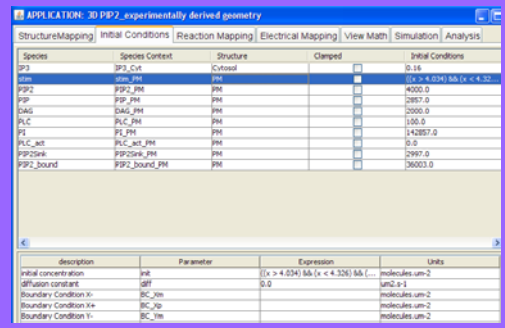
Reactions
Fluxes



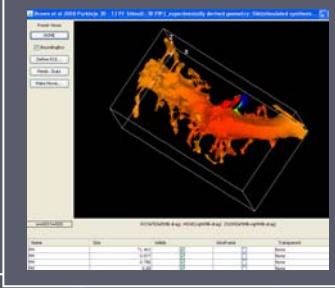
Applications

Structure mapping
(topology to geometry)
Initial Conditions
Boundary conditions
Diffusion constants (if spatial)
Electrophysiology protocols
Enable/disable reactions
Fast reactions
Model analysis
Stochastic rate conversion





Results

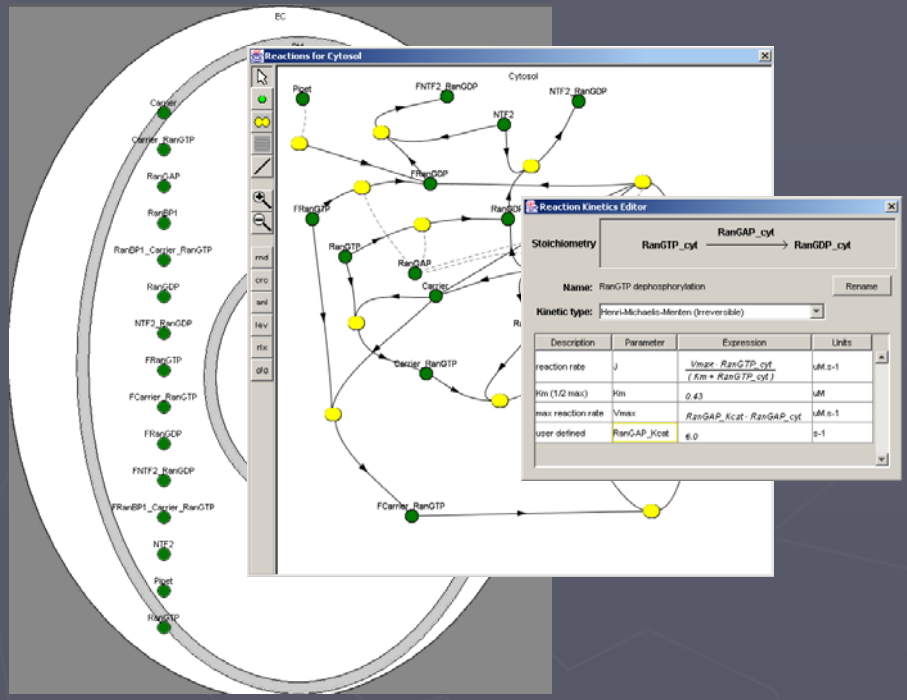


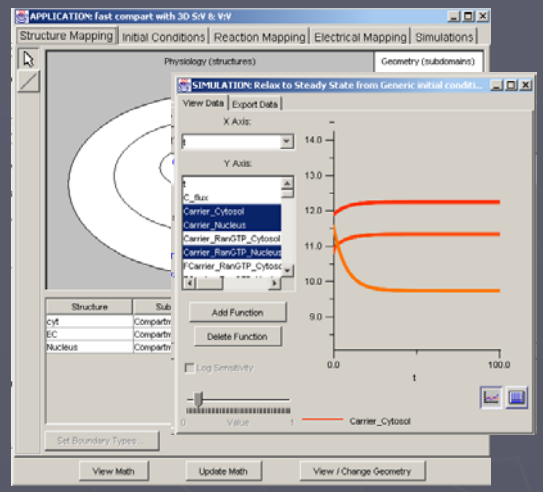
Simulations

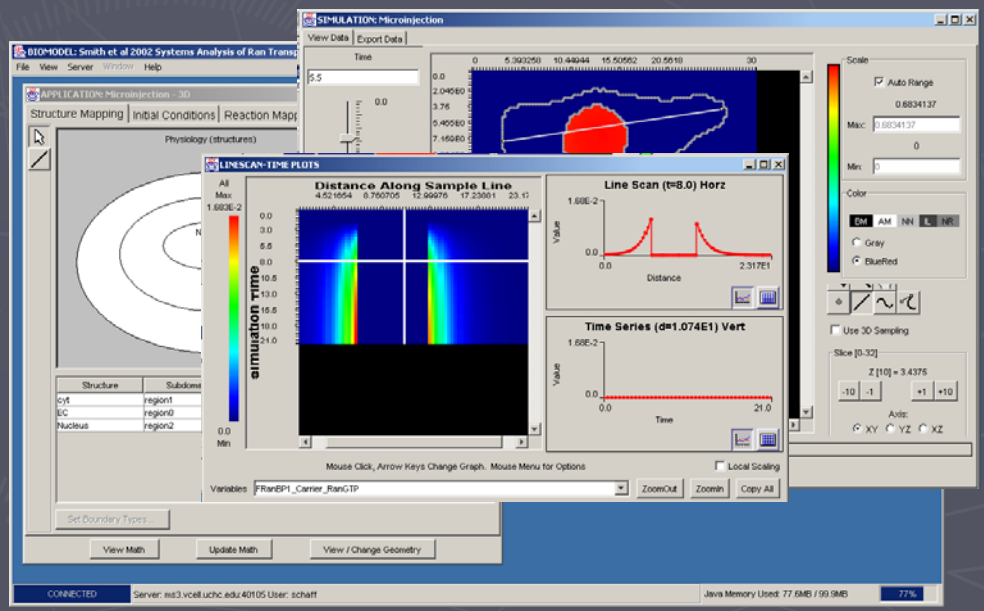
Timecourse
Timestep
Mesh size
Solver type
Solver settings
Parameter changes
Parameter scans
Parameter sensitivity

Mathematical Description

(view-only, automatically generated)







single model

locations/molecules/mechanisms

non-spatial apps

ODEs, sensitivity analysis

multiple simulations

spatial apps

1D,2D,3D PDEs

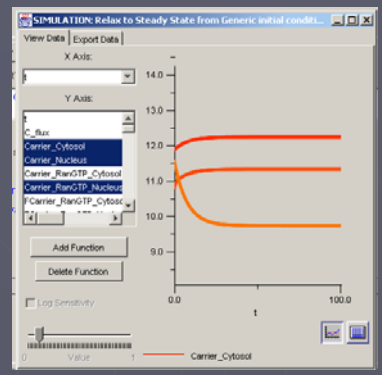
reaction/diffusion/advection

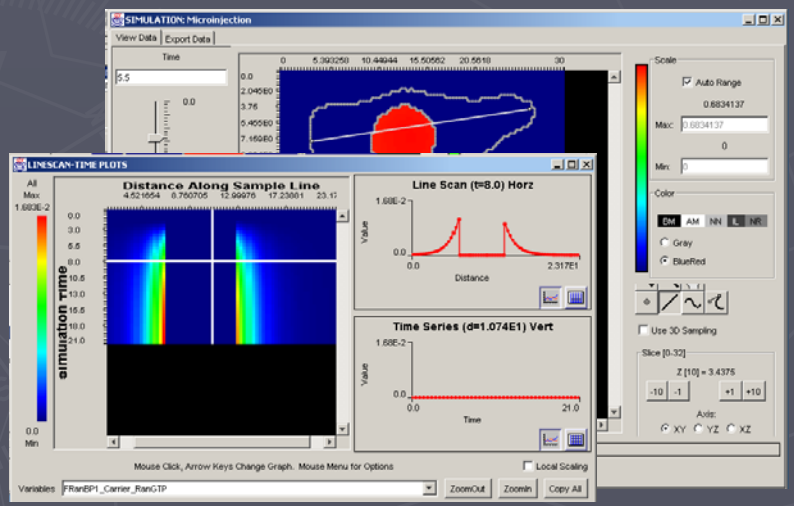
multiple simulations

Math Models

non-spatial “Math Model”

ODEs, sensitivity analysis
multiple simulations



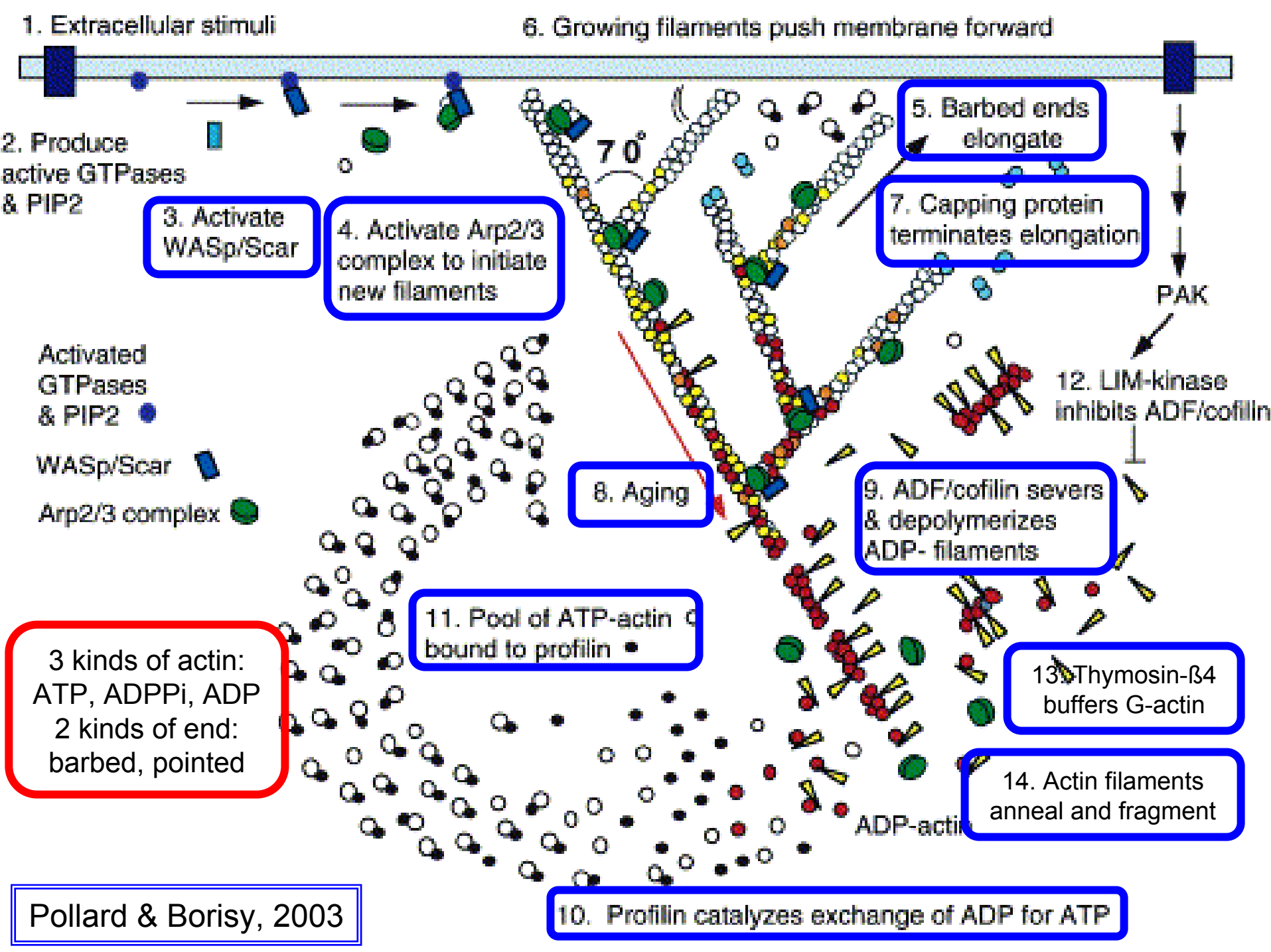


spatial “Math Model”

1D,2D,3D PDEs
reaction/diffusion/advection
multiple simulations

Current Scope and Future Plans

- Intended Users
  - Biologists
  - Biophysicists/Mathematicians
- Modeling Domain
  - Compartmental (0D) or Spatial (1D, 2D, 3D)
  - Reaction/Diffusion/Membrane Transport
  - Electric Potential and Currents
  - Advection & Directed Transport
  - Membrane Diffusion
- Algorithms and Solvers
  - Deterministic – ODE and PDE
  - Stochastic and Hybrid
  - Parameter Scans
  - Parameter Estimation
- Under development
  - Complexes and Rules
  - Stand-alone, Grid-Enabled & Customized Versions
  - Protocols & Virtual Experiments
  - Plug-ins, Modules, Web Services
  - Constraint Handling
  - Mechanical Forces
  - Cell motility


1. Extracellular stimuli
6. Growing filaments push membrane forward
2. Produce active GTPases & PIP2
3. Activate WASp/Scar
4. Activate Arp2/3 complex to initiate new filaments
70°
5. Barbed ends elongate
7. Capping protein terminates elongation
PAK
12. LIM-kinase inhibits ADF/cofilin
Activated GTPases & PIP2
WASp/Scar
Arp2/3 complex
8. Aging
9. ADF/cofilin severs & depolymerizes ADP- filaments
11. Pool of ATP-actin bound to profilin
13. Thymosin-ß4 buffers G-actin
3 kinds of actin: ATP, ADPPi, ADP
2 kinds of end: barbed, pointed
14. Actin filaments anneal and fragment
ADP-actin
10. Profilin catalyzes exchange of ADP for ATP
Pollard & Borisy, 2003

Biophysical Journal Volume 91 November 2006 3519–3528

Kinetics of the Formation and Dissociation of Actin Filament Branches Mediated by Arp2/3 Complex

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ABSTRACT The actin filament network at the leading edge of motile cells relies on localized branching by Arp2/3 complex to generate "mother" filaments that push the plasma membrane forward. The nucleotide bound to the mother filaments (ATP, ADP, and phosphate, or ADP) may influence the branch dynamics. To determine the effect of the nucleotide bound to the subunits of the mother filament on the formation and stability of branches, we compared the time courses of actin polymerization in bulk samples measured using the fluorescence of pyrene actin with observations of single filaments by total internal reflection fluorescence microscopy. Although the fluorescence of pyrene actin was nearly the same for ADP- and ADP-P$_i$-bound filaments in bulk, the observation of branches in the microscope depends on their binding to the slide. Since the probability that a branch binds to the slide is directly related to its lifetime, we used counts of branches by microscopy on ADP- and ADP-P$_i$-bound filaments to conclude that the nucleotide in the mother filament does not affect the initial branching event but that branches are an order of magnitude more stable on the sides of new ATP- or ADP-P$_i$ filaments than on ADP-actin filaments.

INTRODUCTION

At the leading edge of motile cells, a host of molecules participates in the continuous assembly and disassembly of a cytoskeleton capable of producing propulsive force. Actin filaments form the scaffold on which all of the other molecules act. By attaching to the sides of these filaments, Arp2/3 complex contributes to the formation of a dense network of short branched filaments (4) capable of providing the traction to push the forces necessary for global cell motility (5).

Timing is important in the assembly and disassembly of these filaments to maintain distinct filament networks within the dynamic cytoskeleton. Actin filaments contain their own internal timer with the subunits hydrolyzing bound ATP within minutes. Proteins utilizing the filament's nucleotide bound state could aid in timing the disassembly or rearrangement of the network. For example, ADF/cofilin increases the rate of phosphate dissociation from ADP-P$_i$ filaments and severs ADP-actin filaments to bind Arp2/3 complex and then binds with higher affinity, promotes dissociation of branches, and severs ADP-actin filaments (6,7).

ATP hydrolysis and phosphate dissociation might also influence the ability of filaments to bind Arp2/3 complex and support the formation of branches, but the published evidence on this matter is inconsistent. Experiments on branch formation in bulk samples using the fluorescence of pyrene actin to measure filament nucleation implicated the nucleotide bound to the Arp2/3 complex and not nucleotide bound to the mother filament (8,9). Microscopic observations of filaments stabilized with rhodamine-phalloidin of filaments on glass slides showed more branches on newly polymerized segments of filaments than older filaments on which the ATP hydrolysis or phosphate release influence observed these studies lacked quantitative information on whether barbed end is available for branching (12), which seems unlikely given microscopic observations of branching from the sides of filaments (10,13).

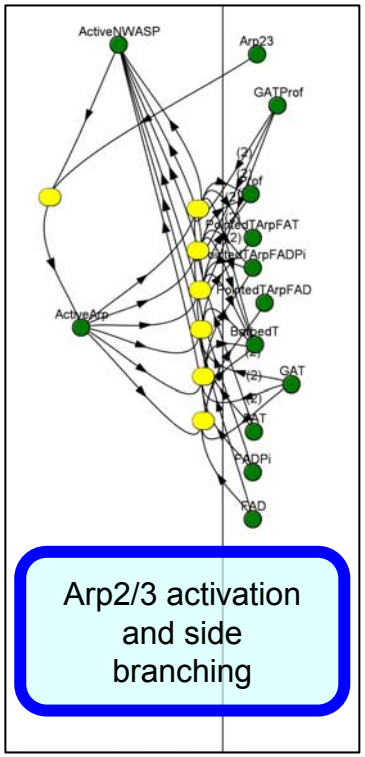
Here we take a new approach to investigate how the nucleotide bound to mother filaments influences their ability to support branching. We compared the number of branches observed by total internal reflection fluorescence (TIRF) microscopy with the number of filament ends formed in bulk samples where polymerization was measured by the fluorescence of pyrene-labeled actin. Branches formed at similar rates in bulk samples whether they contained aging actin filaments or exclusively ADP-P$_i$-bound actin filaments. On the other hand, we observed fewer branches microscopically on older filaments with bound ADP than on new ATP-actin filaments or ADP-actin filaments saturated with phosphate. The number of branches observed on filaments assembled from ATP-actin declined with time, with two exponential rates on highly adhesive surfaces and one exponential rate on less adhesive surfaces. We conclude from our experiments on branching in bulk samples that the nucleotide bound to the mother filament does not influence branch formation, but our microscopic observations show that ATP hydrolysis and γ-phosphate dissociation increase the rate of branch dissociation ~10-fold as mother filaments age.

*Submitted January 6, 2006; and accepted for publication July 28, 2006.
Address reprint requests to Thomas D. Pollard, Tel.: 203-432-5565; Fax: 203-432-6161; E-mail: thomas.pollard@yale.edu.

0006-3495/06/11/3519/10 $2.00
doi: 10.1529/biophysj.106.080937

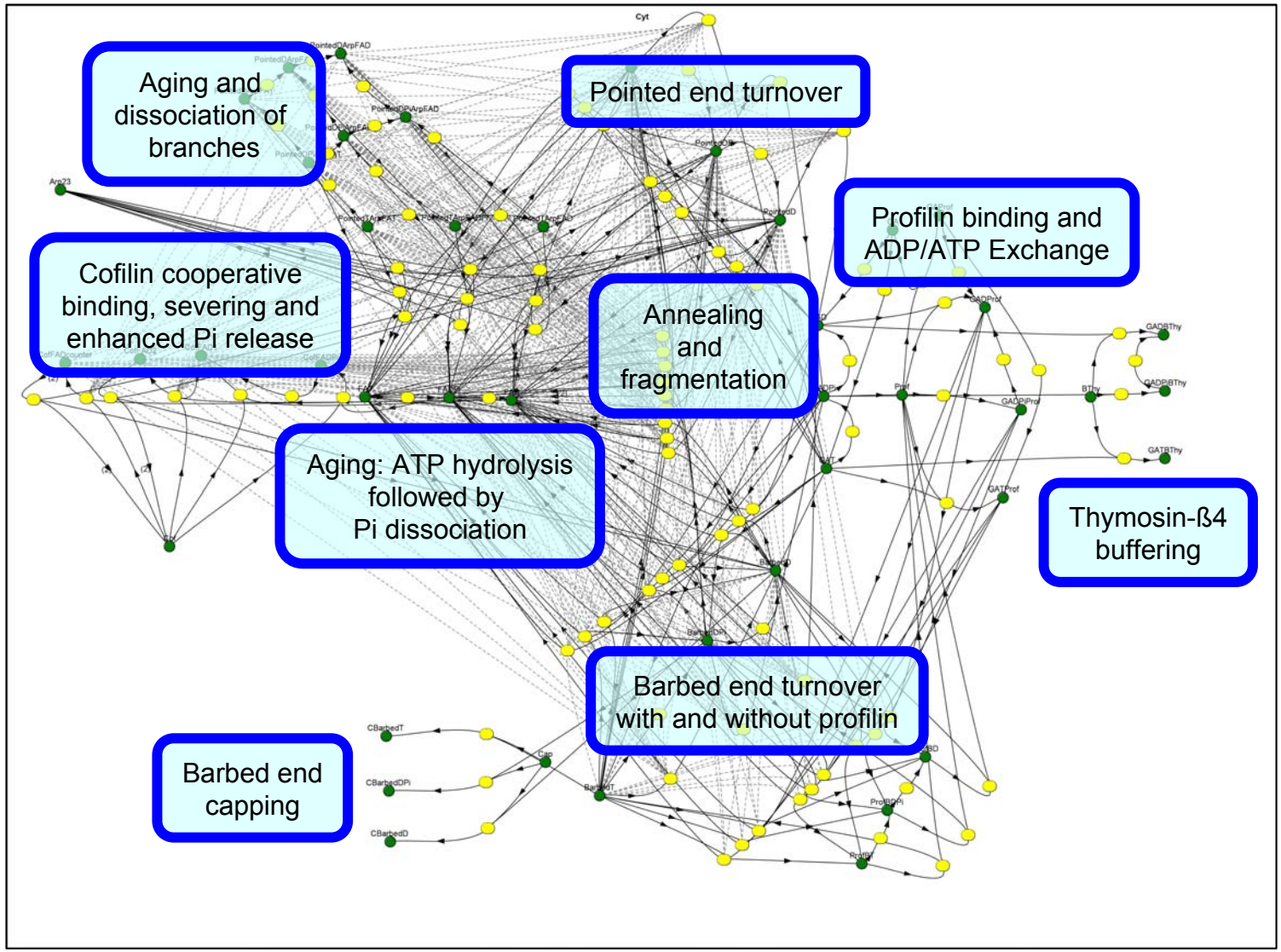
Reaction Network in Virtual Cell

At the Membrane



Arp2/3 activation and side branching

In the Cytosol



Aging and dissociation of branches

Pointed end turnover

Profilin binding and ADP/ATP Exchange

Cofilin cooperative binding, severing and enhanced Pi release

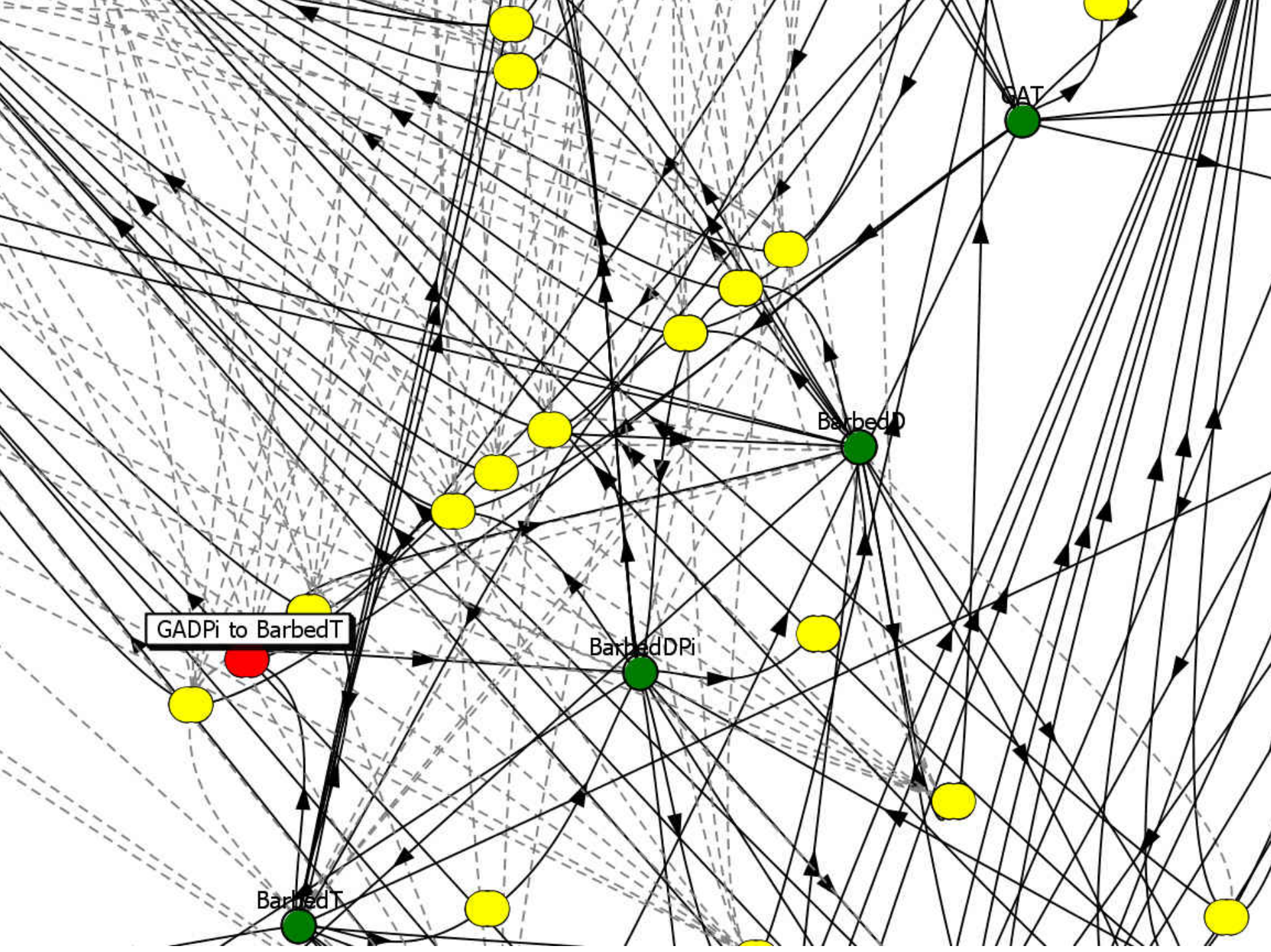
Annealing and fragmentation

Aging: ATP hydrolysis followed by Pi dissociation

Thymosin-ß4 buffering

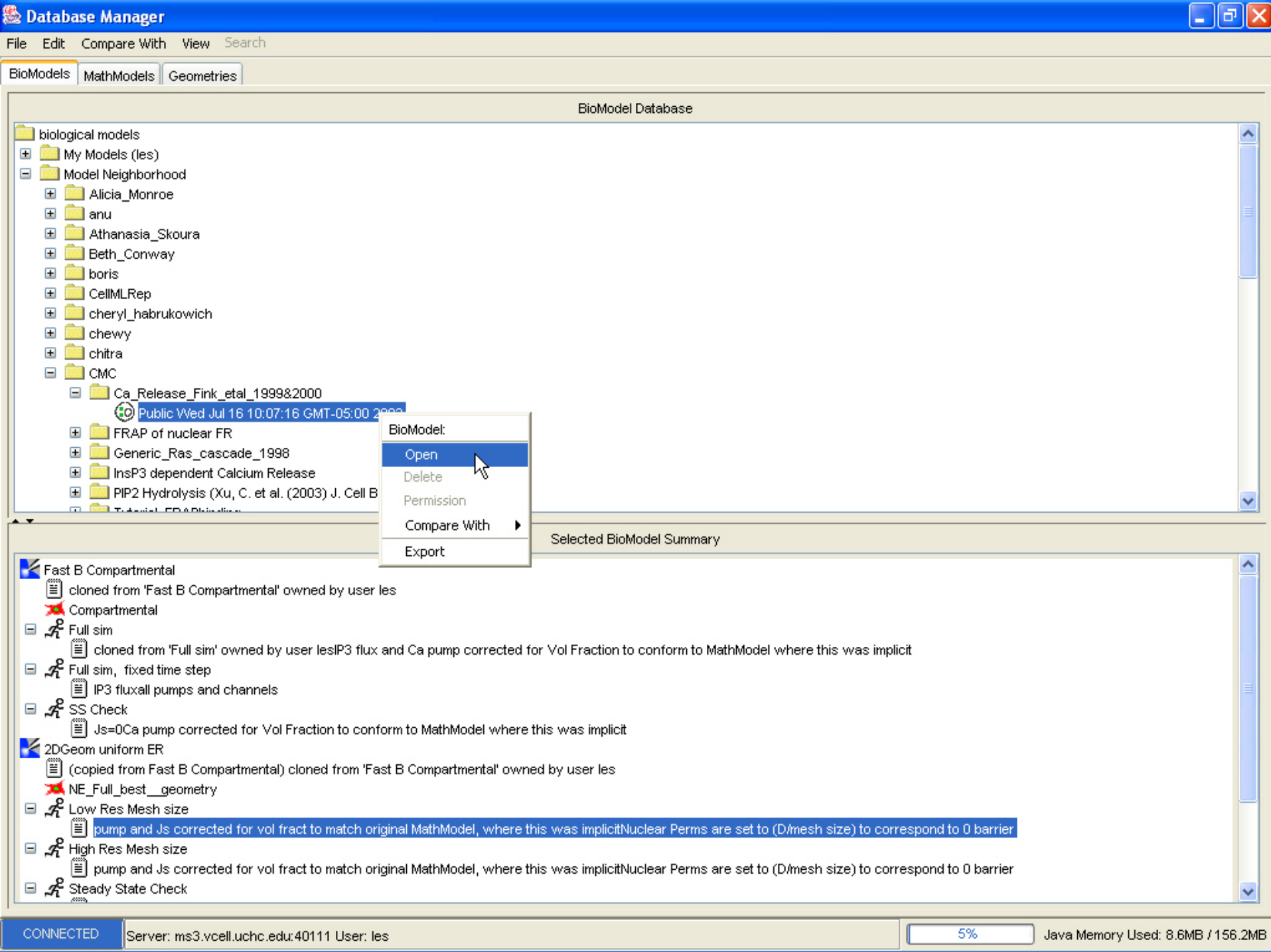
Barbed end turnover with and without profilin

Barbed end capping

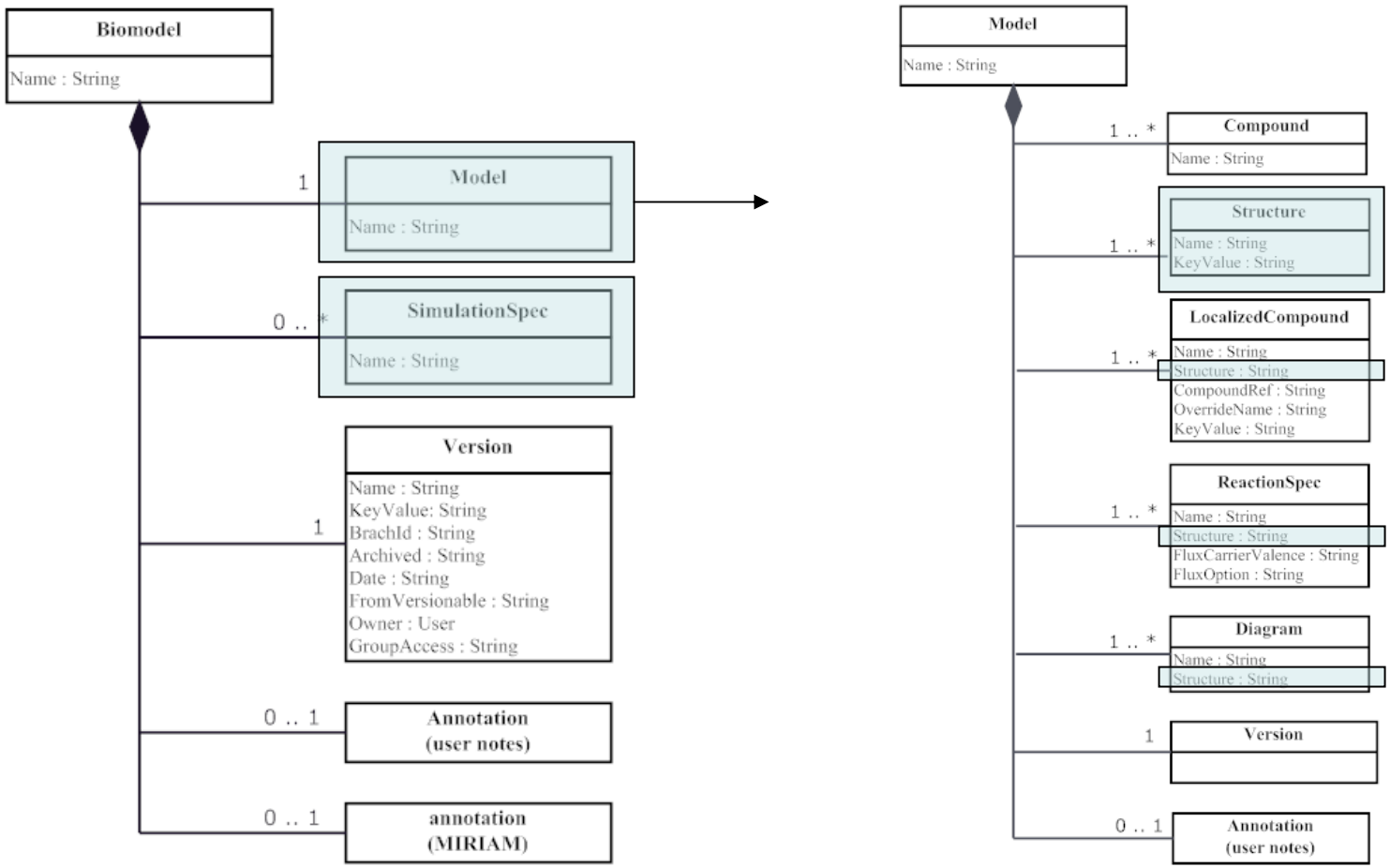

GADPi to BarbedT
BarbedDPi
BarbedT

VCell Top-Level Documents

- Database object containers
  - BioModel
  - MathModel
  - Geometry
- Referential objects
  - ResultSet
  - FieldData

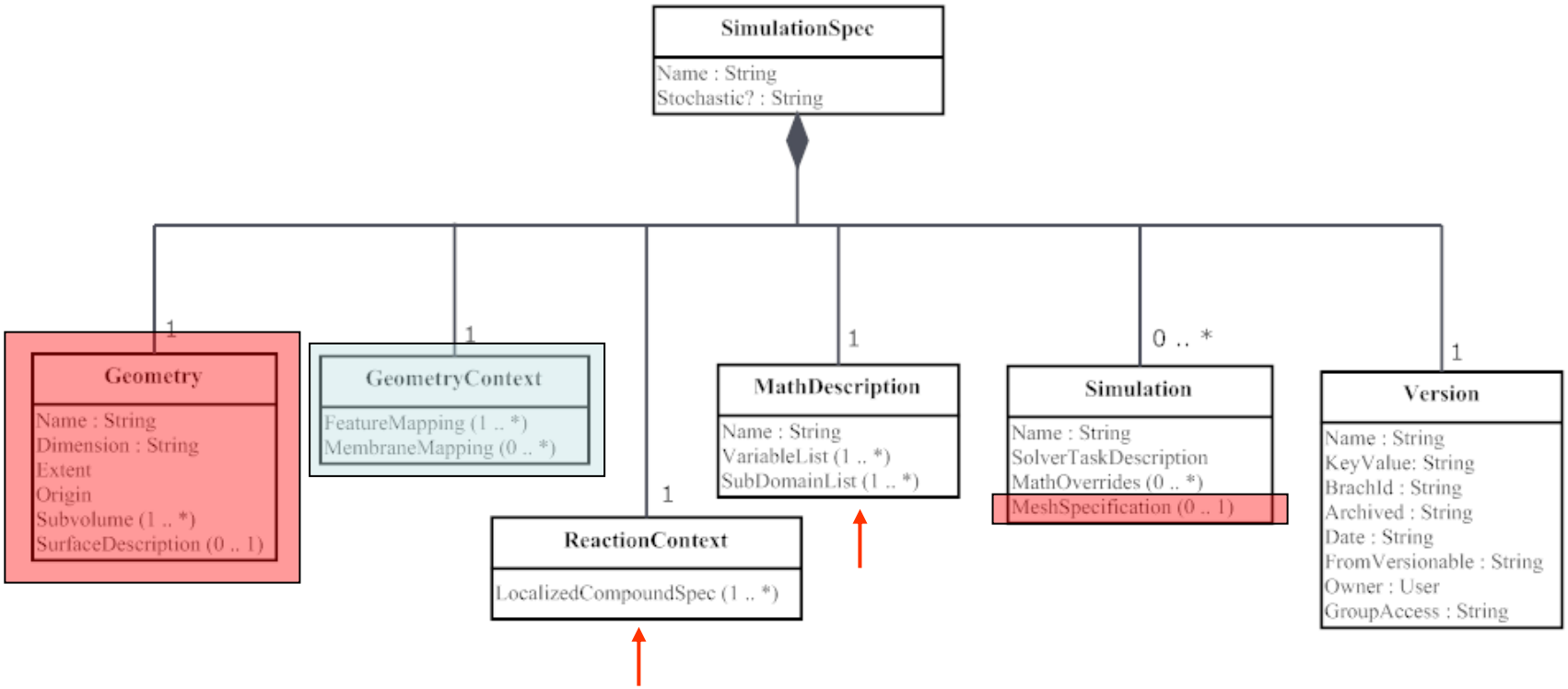

Database Manager
File
Edit
Compare With
View
Search
BioModels
MathModels
Geometries
BioModel Database
biological models
My Models (les)
Model Neighborhood
Alicia_Monroe
anu
Athanasia_Skoura
Beth_Conway
boris
CellMLRep
cheryl_habrukowich
chewy
chitra
CMC
Ca_Release_Fink_etal_1999&2000
Public Wed Jul 16 10:07:16 GMT-05:00
FRAP of nuclear FR
Generic_Ras_cascade_1998
InsP3 dependent Calcium Release
PIP2 Hydrolysis (Xu, C. et al. (2003) J. Cell B
BioModel:
Open
Delete
Permission
Compare With
Export
Selected BioModel Summary
Fast B Compartmental
cloned from 'Fast B Compartmental' owned by user les
Compartmental
Full sim
cloned from 'Full sim' owned by user lesIP3 flux and Ca pump corrected for Vol Fraction to conform to MathModel where this was implicit
Full sim, fixed time step
IP3 fluxall pumps and channels
SS Check
Js=0Ca pump corrected for Vol Fraction to conform to MathModel where this was implicit
2DGeom uniform ER
(copied from Fast B Compartmental) cloned from 'Fast B Compartmental' owned by user les
NE_Full_best__geometry
Low Res Mesh size
pump and Js corrected for vol fract to match original MathModel, where this was implicitNuclear Perms are set to (D/mesh size) to correspond to 0 barrier
High Res Mesh size
pump and Js corrected for vol fract to match original MathModel, where this was implicitNuclear Perms are set to (D/mesh size) to correspond to 0 barrier
Steady State Check
CONNECTED
Server: ms3.vcell.uchc.edu:40111 User: les
5%
Java Memory Used: 8.6MB / 156.2MB

BioModel Object Hierarchy

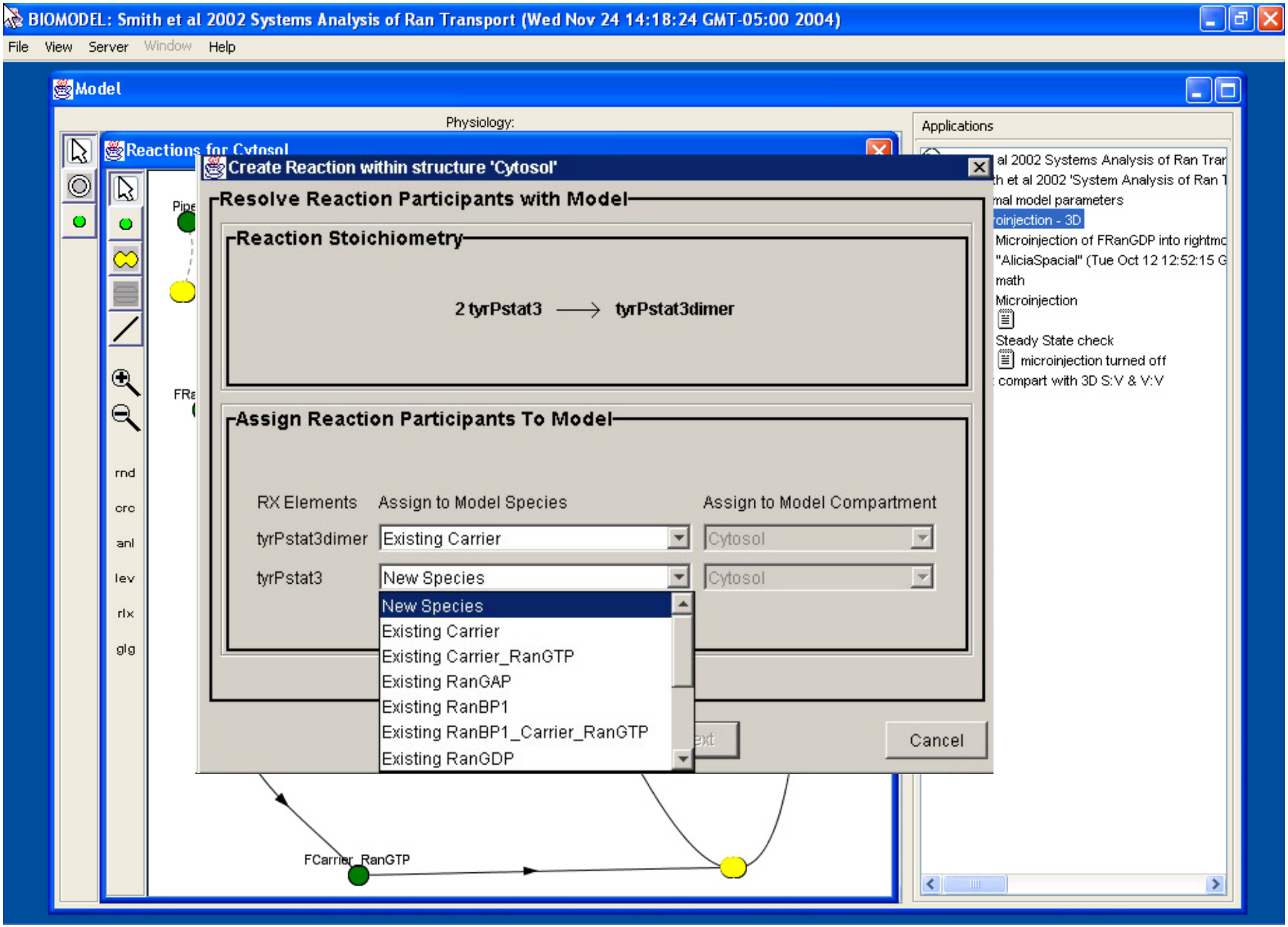


SimulationSpec Element

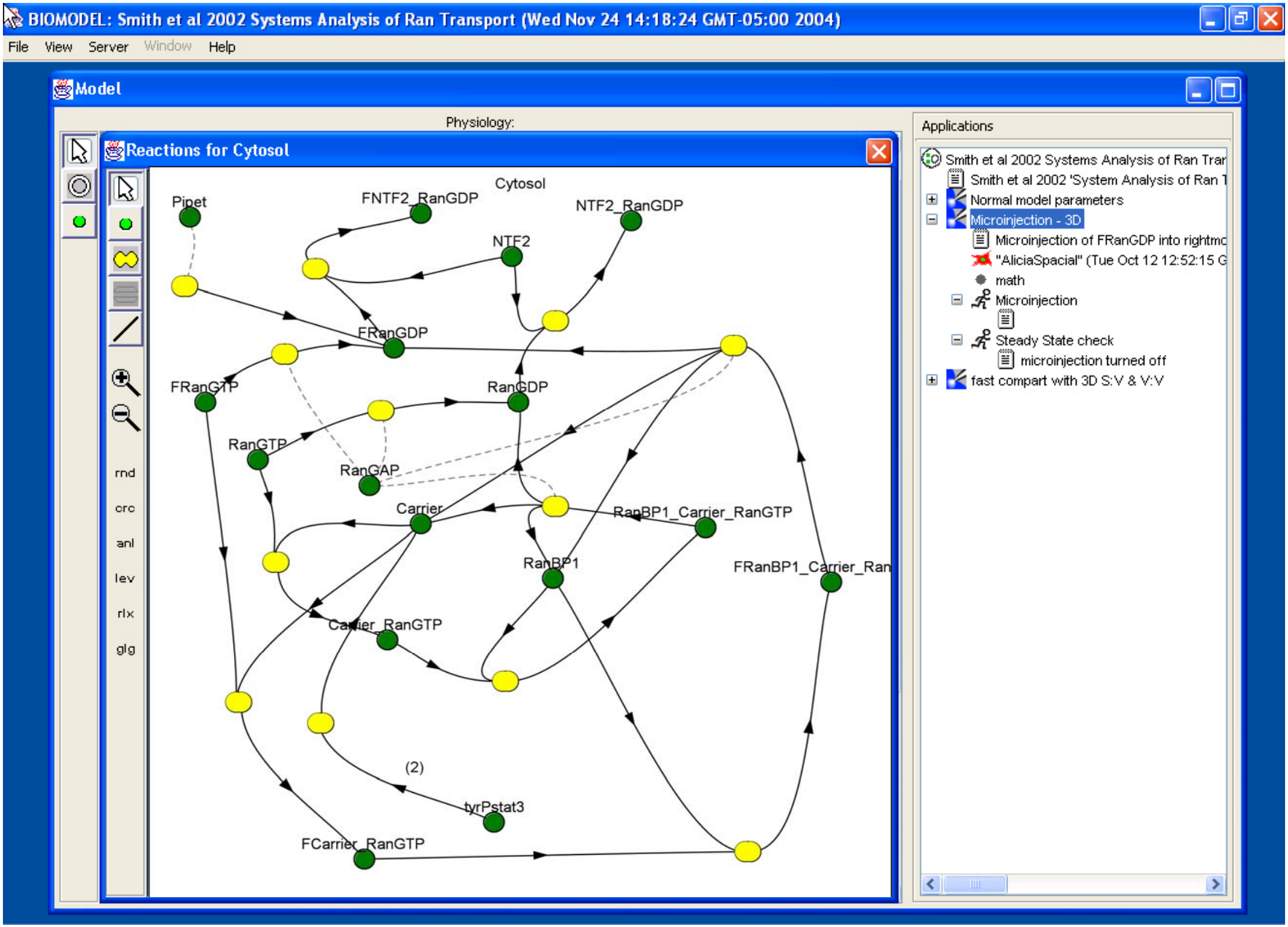
– a.k.a. “Application” wizard, a.k.a. SimulationContext object –



Reactions Database Retrieval



Reactions Database Retrieval



Controlled Vocabulary

KEGG LIGAND database

– **COMPOUND: small molecules**

– **ENZYME: enzyme classifications**

– **REACTION: enzymatic reactions**

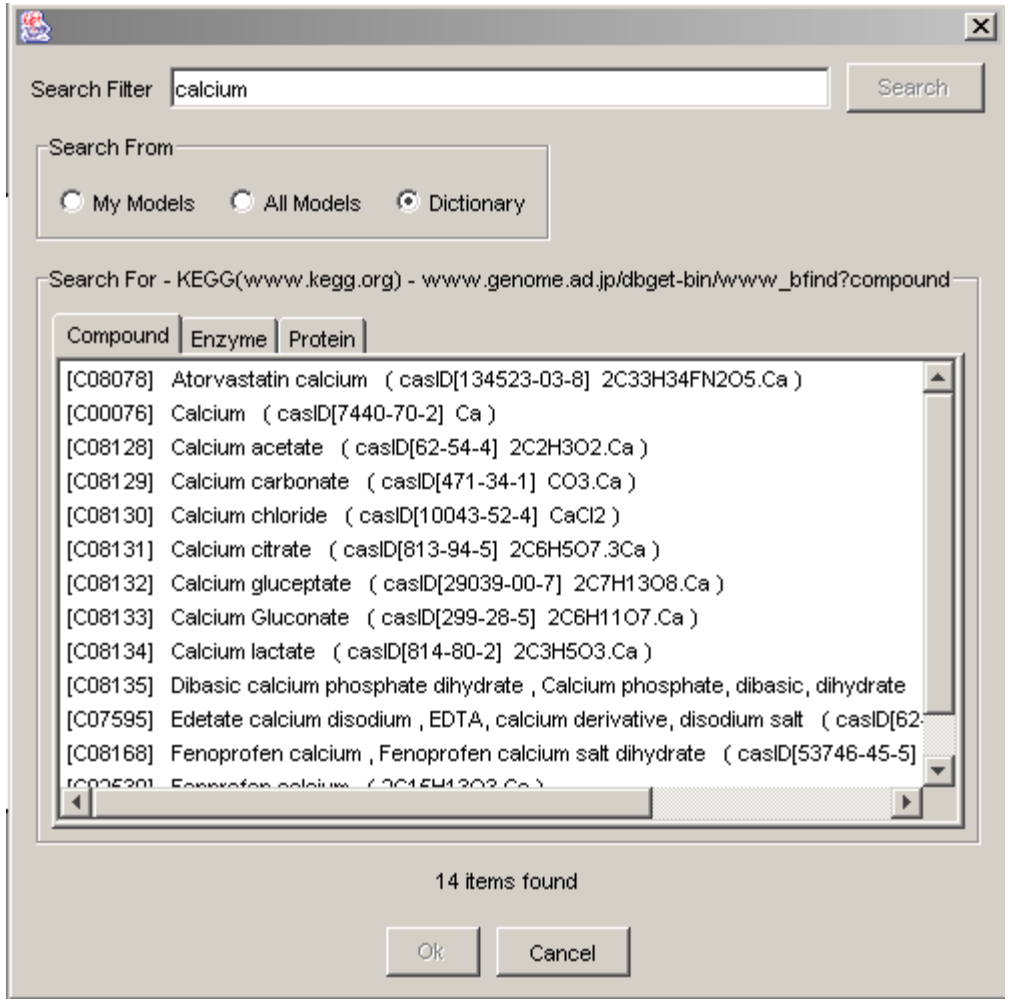
– **GLYCAN: glycolipids, glycoproteins**

SwissProt database

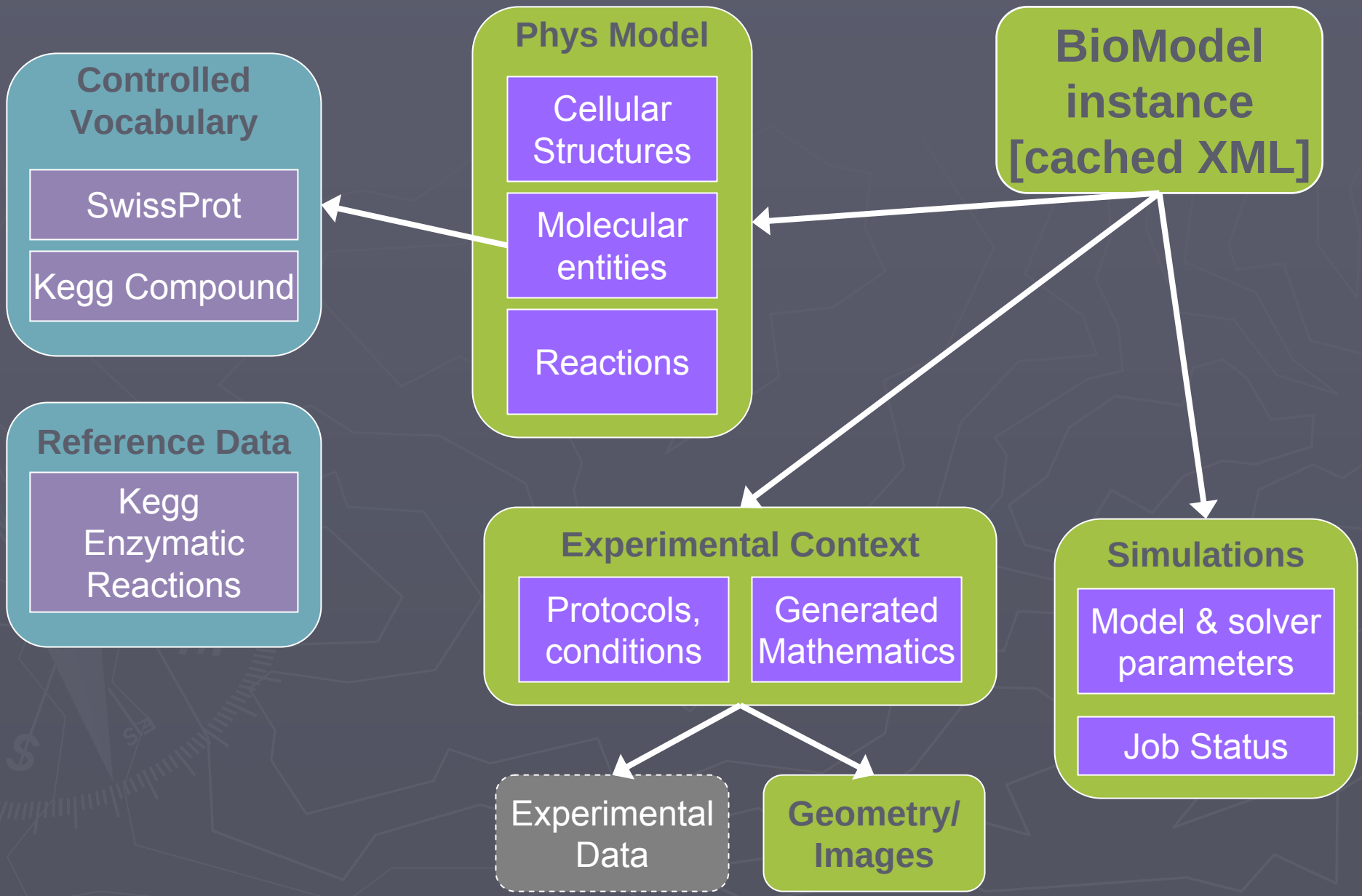
– **proteins**

Species binding

Import Enzymatic Reactions



VCell Database (~60 tables)



Database design features

- Storage of intermediate models.
  - Manage multiple editions of same model.
  - Client allows compare/merge
- Records are immutable
  - Entire models cached in XML (fast loads)
  - Incremental saves (fast saves).
- Access Control
  - Users have private storage
  - supports collaboration (with list of users)
- Scalable:
  - multiple stateless servers
- Reliable:
  - Distributed transactions, persistent messaging middleware
- Searchable (as permitted by access control).
  - Search for reactions from users models or Kegg
  - Bind molecular species to controlled vocabulary (Kegg, SwissProt.)

VCell Database Contents

- Top-level object containers
  - BioModels, MathModels, Geometries
  - All their elements (fine granularity)
  - Link tables and references
- References to external objects
  - ResultSets, FieldData (file-based storage)
- Ancillary data
  - User data (registration info, preferences)
  - Access control lists
- Controlled vocabulary data
  - Kegg, Swissprot bindings

VCell Usage

	Feb-09	May-08	diff	Increase
Users Who Ran Simulations	2224	1885	339	18%
Currently Stored Models	29117	25204	3913	16%
Currently Stored Simulations	160539	118403	42136	36%
Publicly Available Models	687	626	61	10%
Publicly Available Simulations	2377	1945	432	22%

Standards and Resources

- Languages and Ontologies
  - SBML, CellML
    - VCell imports/exports SBML, CellML… ←→ VCML
  - BNGL
  - BioPAX
  - SBO
  - KiSAO…
  - SBGN
  - MIRIAM
  - MIASE – SEDML
- Repositories
  - BioModels, DoQCS, JWS Online
  - CellML model repository
  - Reactome, PID, BioCyc
  - PSLID

Model or Pathway Representations?

- Pathways
  - Qualitative
  - Static
  - No kinetics
  - Often lack spatial context
  - Minimal merging
  - Minimal experimental context
- Models
  - Quantitative
  - Dynamic
  - Kinetics
  - Usually encode some spatial context
  - Frequent merging and other approximations
  - Often encode/depend on experimental context

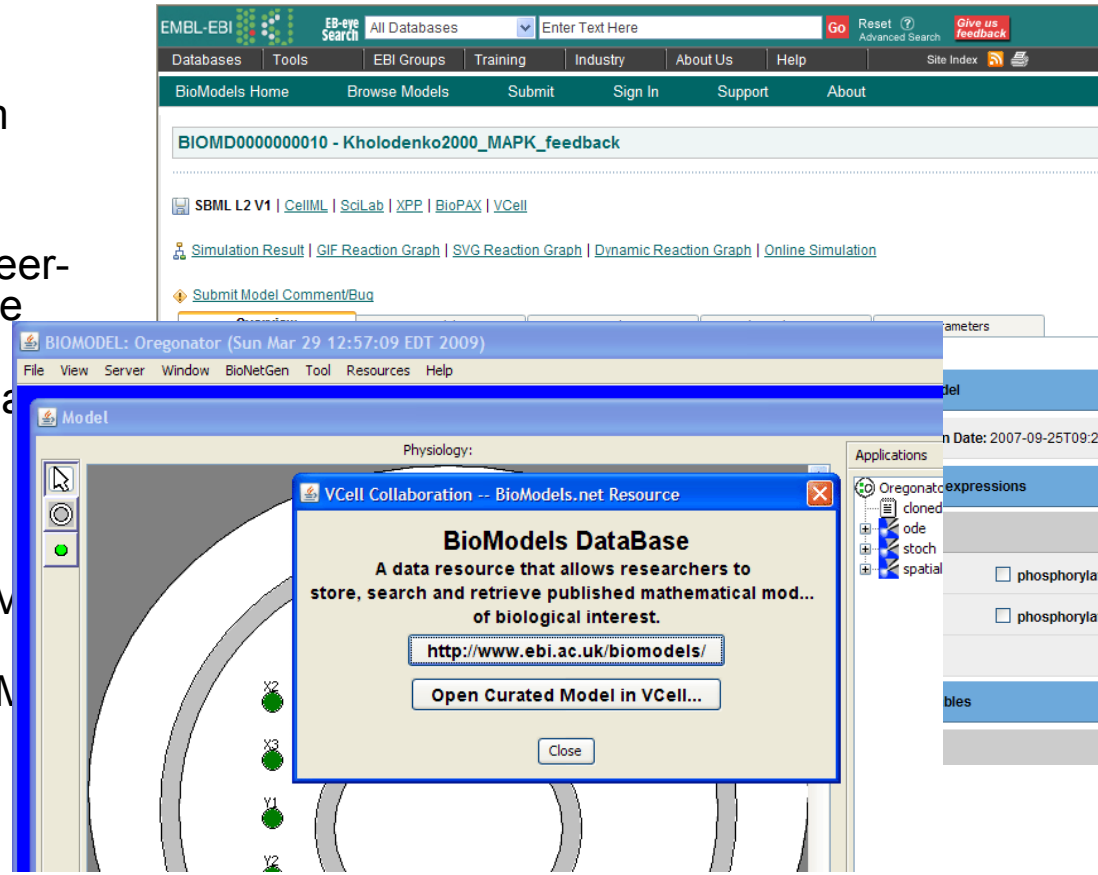
VCML and Model Exchange

Where are the problems?

- VCML is not the DOM for VCell
- VCell separates models from equations (imposes math restrictions)
  - The math is always derived!
  - No support for Rate Rules, Algebraic Rules
  - Units…
- VCell automates compartmental to spatial porting of models (imposes physical “realizability” restrictions)
  - Reactions must have location
  - Molecules can’t cross double boundaries
- VCell supports spatial information
- VCell has hierarchical structure
  - Multiple “Applications”
  - Multiple “Simulations”, includes simulator specification
- VCell includes database and ontology information
  - External bindings, native “roles”

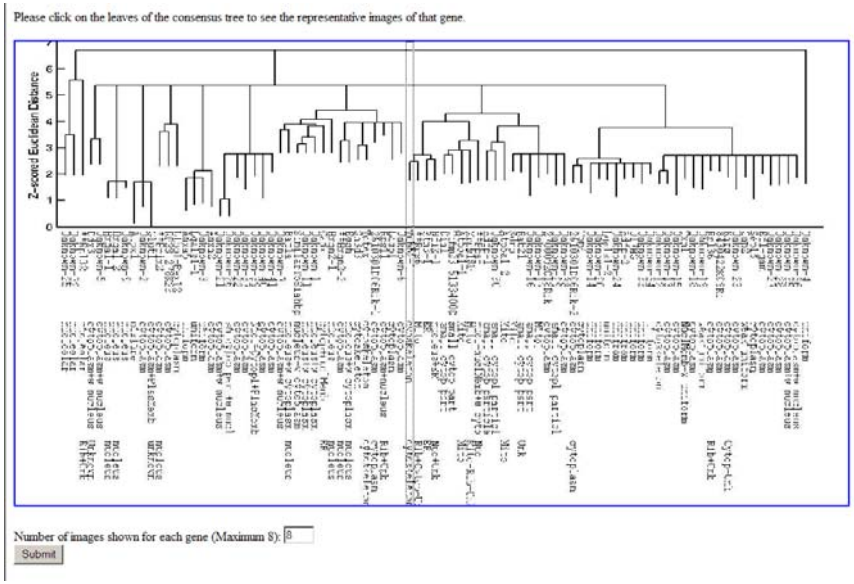
EBI collaboration

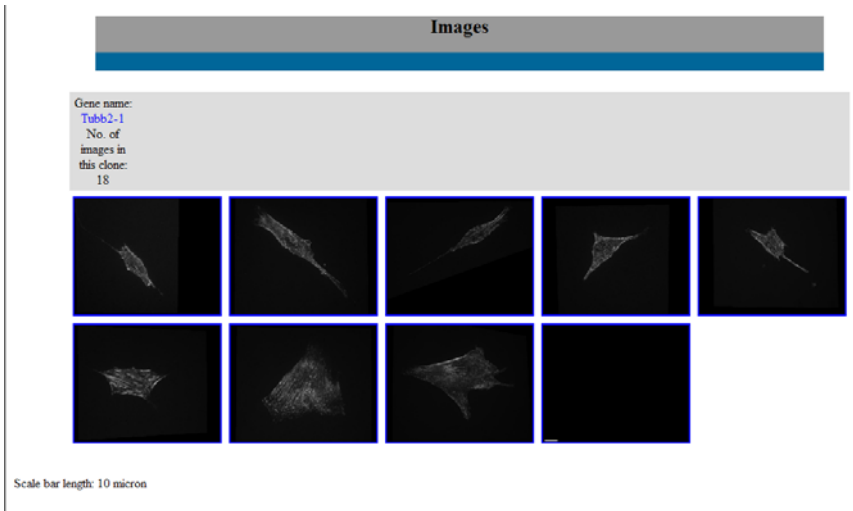
1. What?
   - Connect VCell to BMDB
   - Simulate BMDB models with VCell and search/import from BMDB
2. Why?
   - Provide a source of public, peer-reviewed, curated quantitative models to VCell users
   - Provide support for modules a model aggregation
3. How?
   - Publish VCML specification
   - Create standalone batch SBM <–> VCML converter
   - Create automated links on BM web interface
   - Customize granular query/retrieval API



CMU collaboration

1. What?
   - Connect VCell to PSLID and SLIF
   - Use generative models for “virtual” geometries and “virtual” molecular distributions
2. Why?
   - Provide a large source of public image-based geometries and quantitative data to VCell users
   - Provide realistic “artificial” data to complement/supplant real data
3. How?
   - Search/import interface for CMU databases
   - XML repository of generative models
   - Server-side Matlab libraries
   - Use field data for conversion





PSLID Modules for VCell

1. Asynchronous Communication Layer
   - XML parser for PSLID script queries and downloads
2. Generalized Data Structure
   - Combined geometry regions and protein distributions
3. Custom User Interface
   - Experimental images and generated images

Field Data Structure