CRP-Santé

Wageningen University, Belarusian State University

SIMULATION-BASED ANALYSIS IN THE STUDY OF COMPLEX BIOMOLECULAR SYSTEMS

Membrane Proteins and Actin Polymerization Assays

Petr V. Nazarov

Microarray Center,
CRP-Santé,
Luxembourg

petr.nazarov@crp-sante.healthnet.lu

OUTLINE

I. Modeling

- Types of models
- Stages of model building
- General application of models

II. Membrane Proteins

- Problem definition
- Experimental methods and object: M13 major coat protein
- Simulation-based data analysis
- Results and conclusions

III. Actin Polymerization

- Basic information
- Experimental methods
- Models: simulation and analytical
- Some preliminary results
- Summary

PART I

$$\frac{d[A]}{dt} = \frac{V_a}{1+\frac{[BD]}{K_{I_{ba}}}} - k_a[A] + k_2[AE]$$

$$\frac{d[B]}{dt} = \frac{V_b}{1+\frac{[C]}{K_{I_{cb}}}} - k_b[B] + k_5[BD]$$

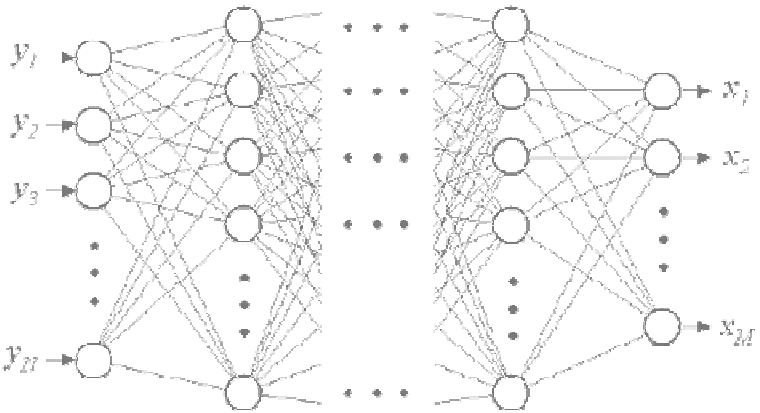
$$\frac{d[C]}{dt} = \frac{V_c}{1+\frac{[AE]}{K_{I_{ac}}}} - k_c[C]$$

$$\frac{d[D]}{dt} = \frac{V_d}{1+\frac{K_{I_{bd}}}{[B]}} - k_d[D] + k_5[BD]$$

$$\frac{d[E]}{dt} = \frac{V_e}{\left(1+\frac{K_{A_{ae}}}{[D]}\right)\left(1+\frac{[C]}{K_{I_{ce}}}\right)} - k_e[E] + k_2[AE]$$

$$\frac{d[AE]}{dt} = k_1[A][E] - k_2[AE] - k_3[AE]$$

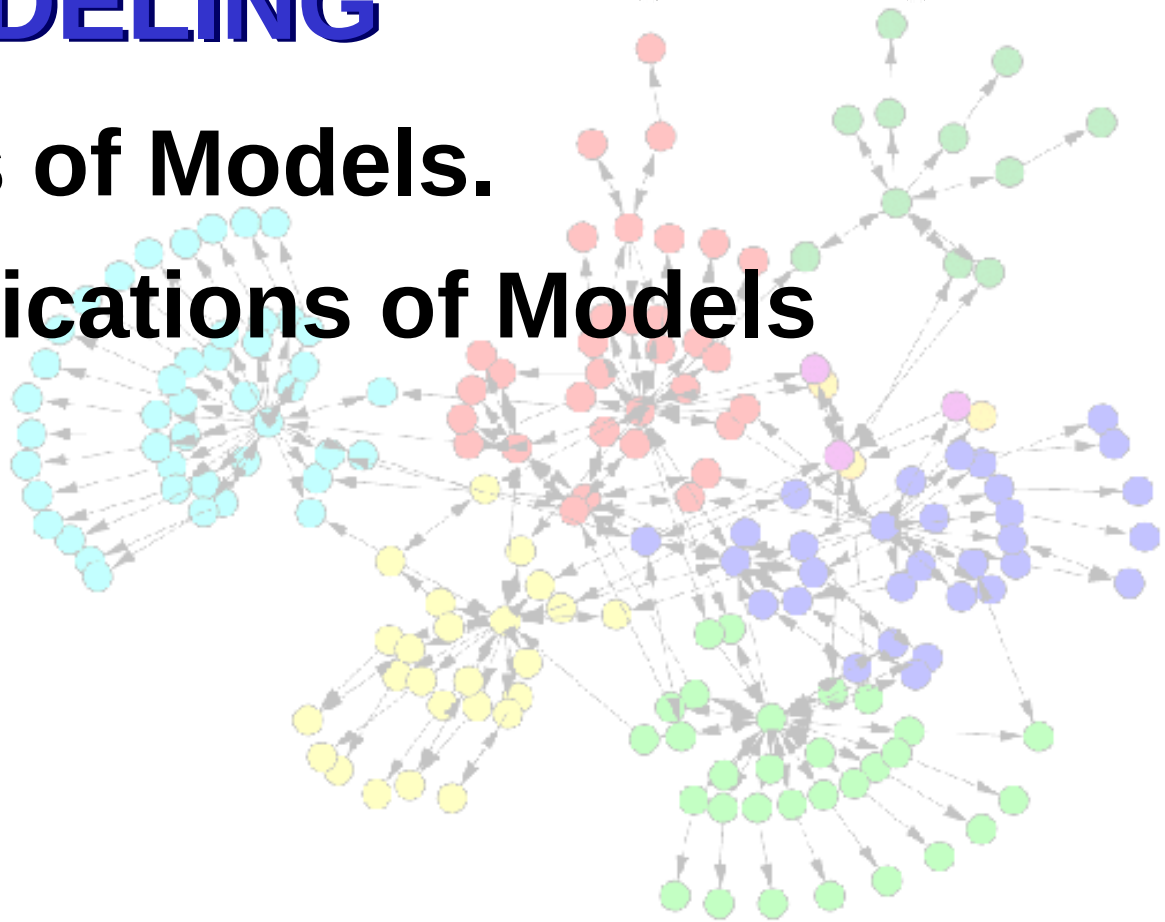
$$\frac{d[BD]}{dt} = k_4[B][D] - k_5[BD] - k_6[BD]$$



MODELING

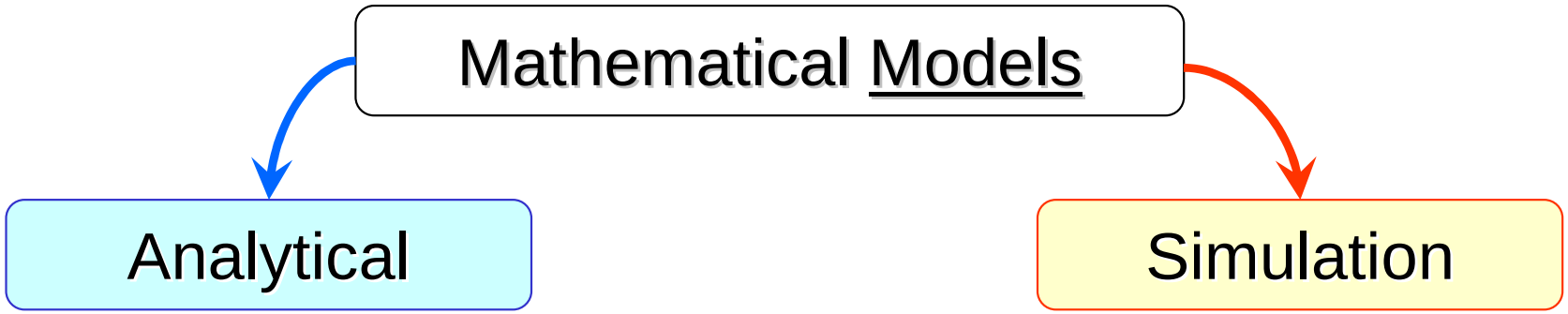
Types of Models.

General Applications of Models



TYPES OF MODELS

By the Method of Mathematical Description



Analytical

+
- general
- fast
- can be verified by analytical methods

−
- cannot be applied to complex systems
- problems of interpretation of obtained results

Simulation

+
- complex systems can be studied
- interpretation of the results is straight forward
- parallel computing can be applied

−
- computational resource intensive

STAGES OF SIMULATION MODELLING

1. **Experimental system**

2. Extraction of the precise object of study

3. Abstracting, idealization and **formalization**

Mathematical model

4. Algorithm developing

Simulation model

5. Realization in code

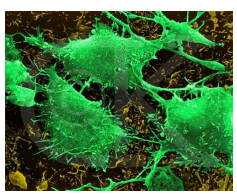
6. Computer (*in silico*) experiment

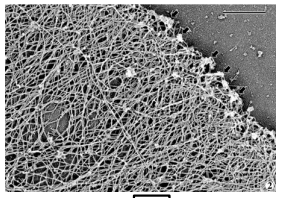
7. Model verification

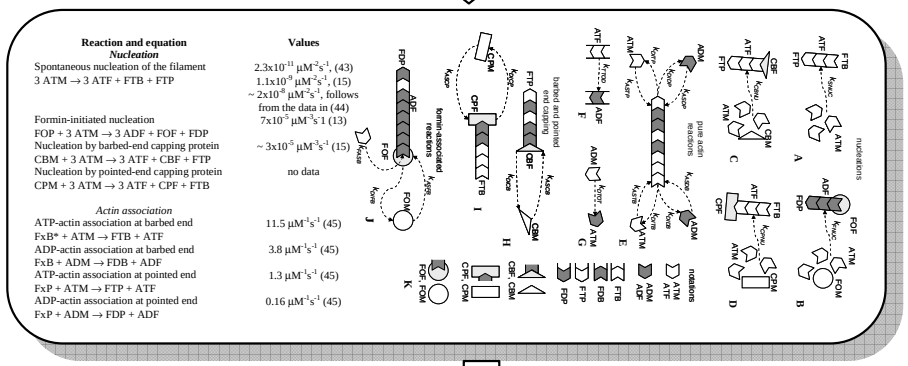
10. *Experimental data*

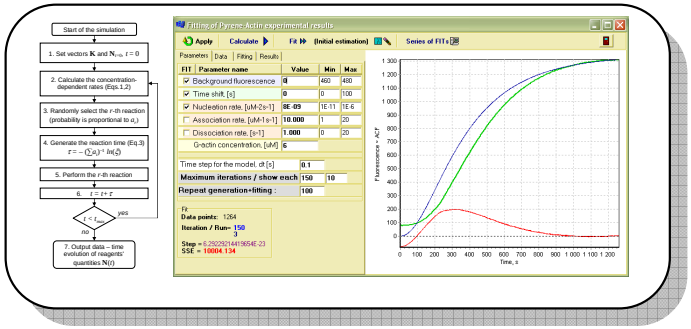
9. Inverse task: determination of hidden parameters of the considered object

8. Direct task: prediction of the properties / behavior of the experimental system

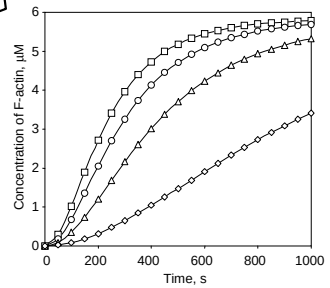




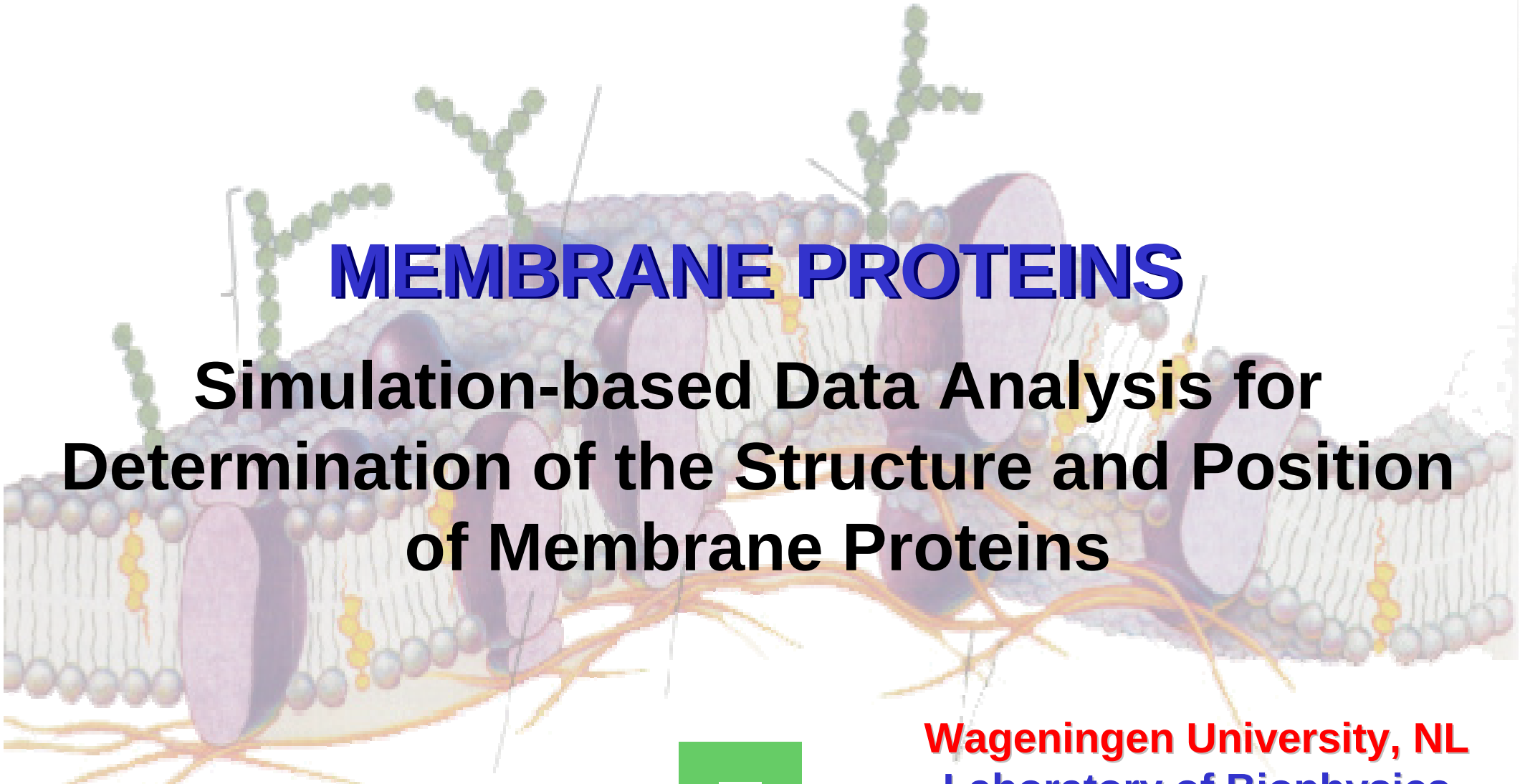




$k_{snuc} = 2.3 \times 10^{-11}\ \mu M^{-2}s^{-1}$
$k_{ARP} = 8.8 \times 10^{-6}\ \mu M^{-1}s^{-1}$
$[A] = 4\ \mu M$



PART II



MEMBRANE PROTEINS

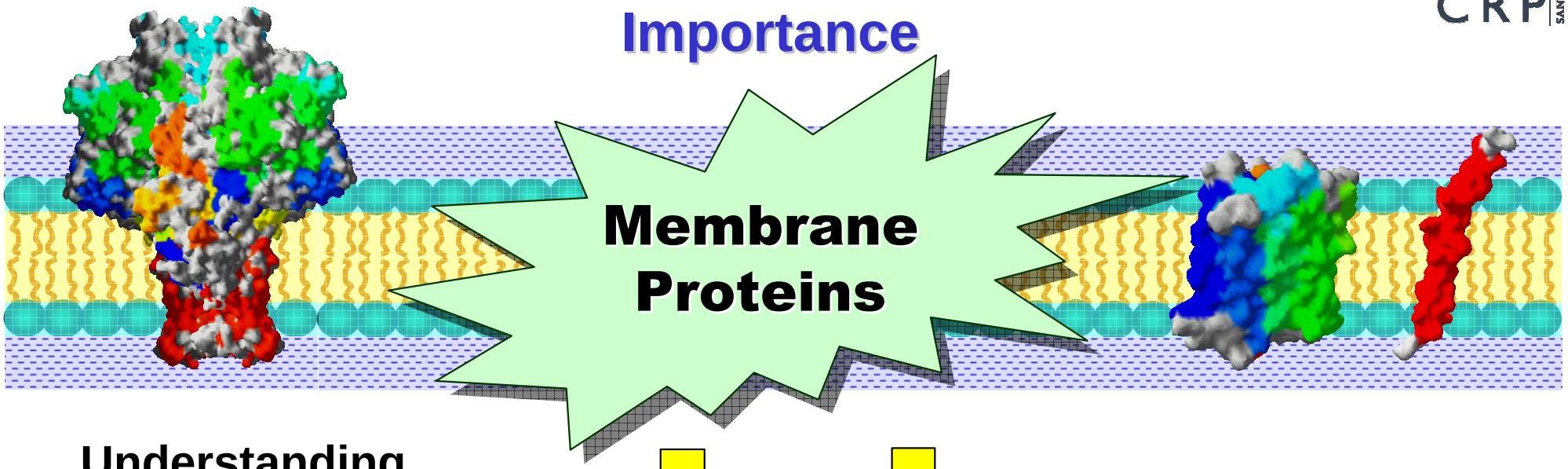
Simulation-based Data Analysis for Determination of the Structure and Position of Membrane Proteins

Wageningen University, NL
Laboratory of Biophysics

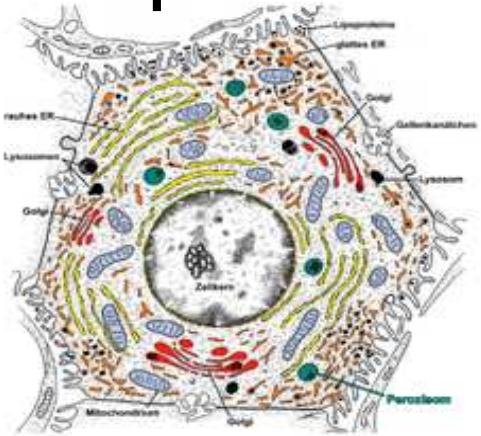
- Dr. Marcus Hemminga
- Rob Koehorst

MEMBRANE PROTEINS

Importance



Understanding cell processes



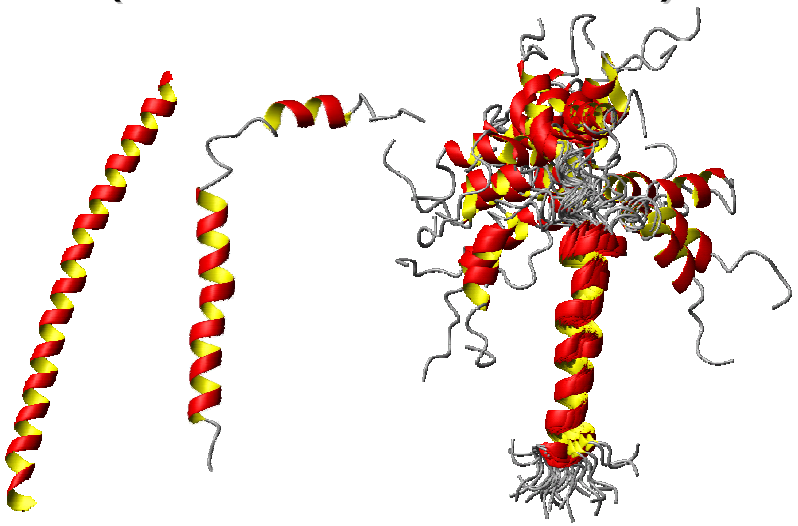
New drug development

- Participate in almost all cell activities
- Structure determination still at frontier structural biology

GOAL

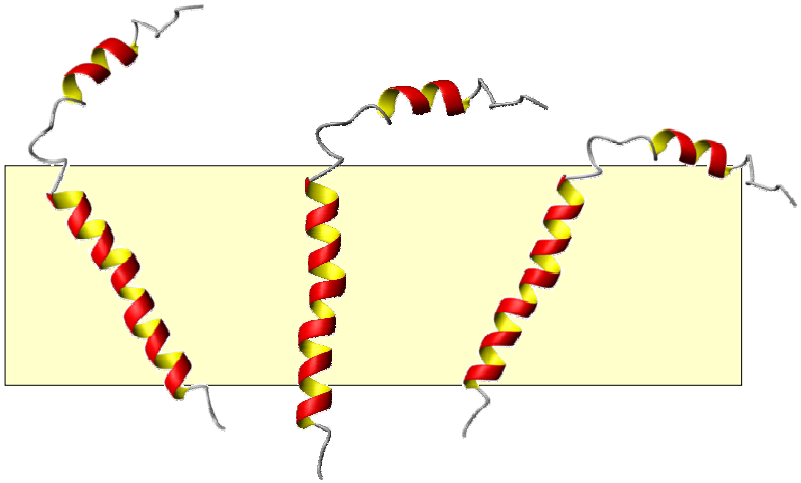
To Develop a Methodology, Providing:

- **Protein structure**
  (low-resolution model)

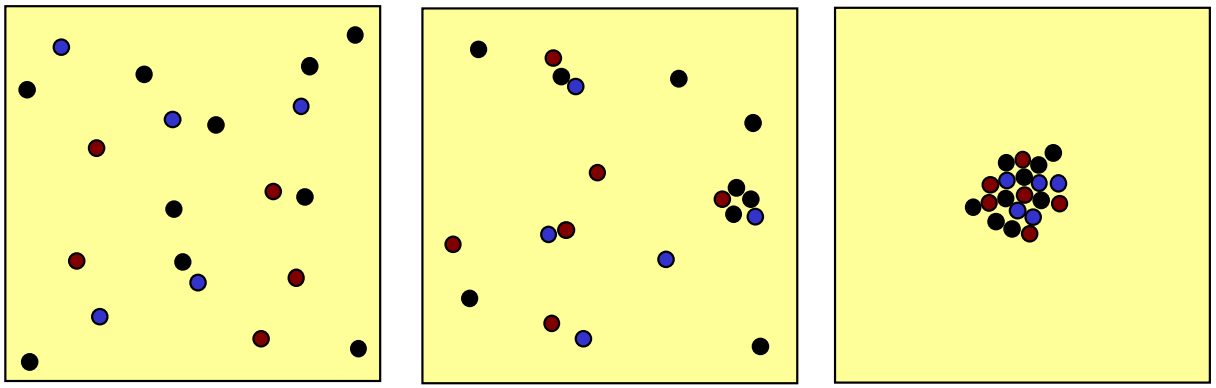


?

- **Protein embedment**

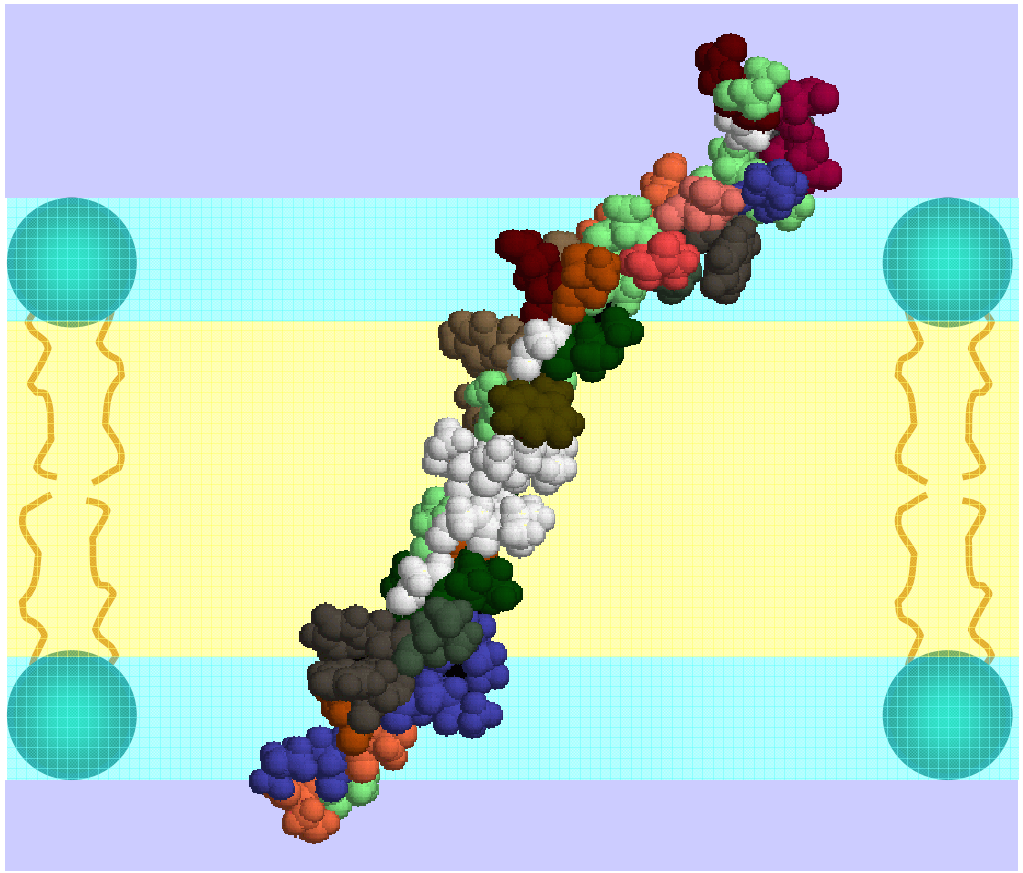


- **Protein aggregation**



OBJECT OF STUDY

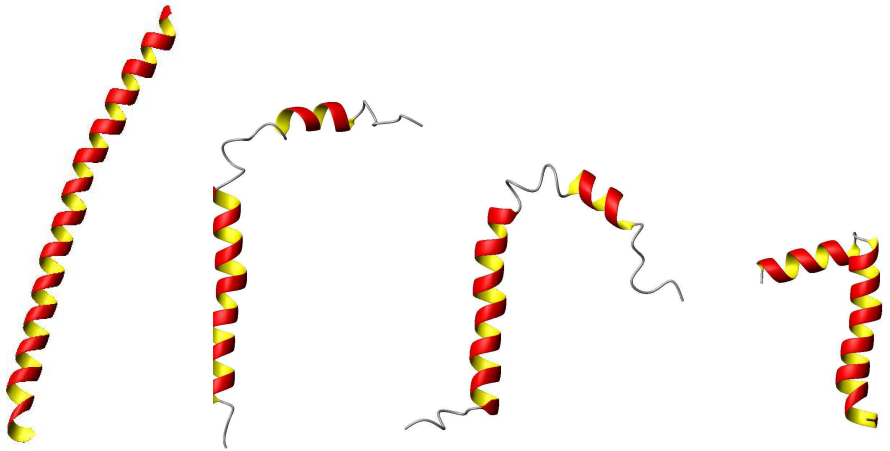
Bacteriophage M13 Major Coat Protein
(test system)



AEGDDPAKAAFNSLQA
SATEYIGYA**W**AMVVVIV
GATIGIKLFKKFCSKAS

Protein Features

- 50 amino acid residues
- Transmembrane protein
- Mainly α-helical

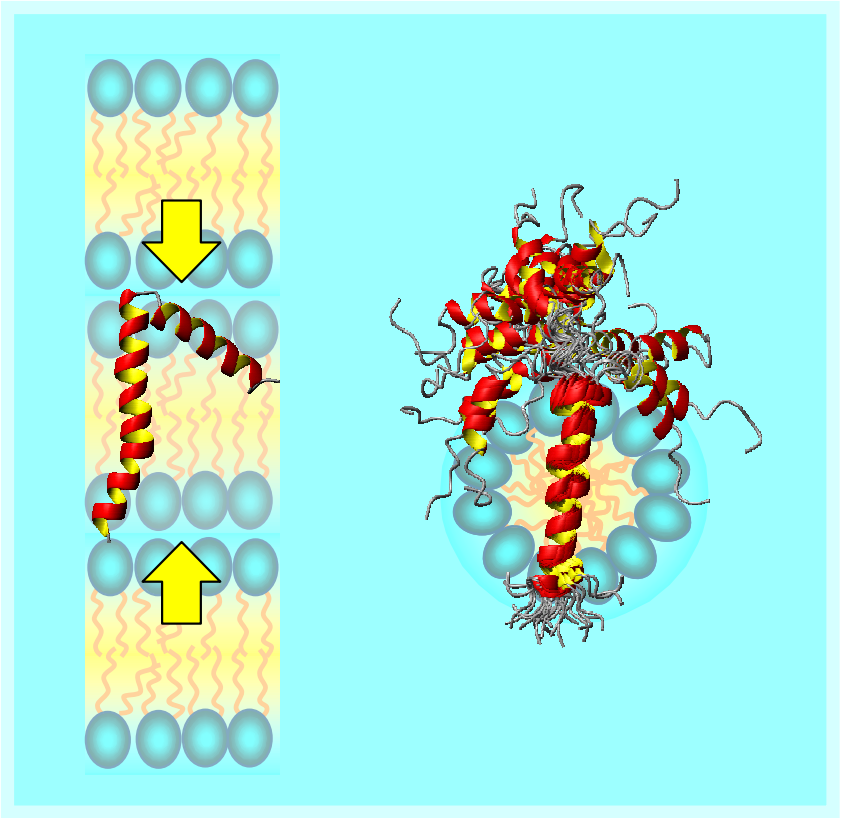


I-shape, L-shape, Banana-shape?..

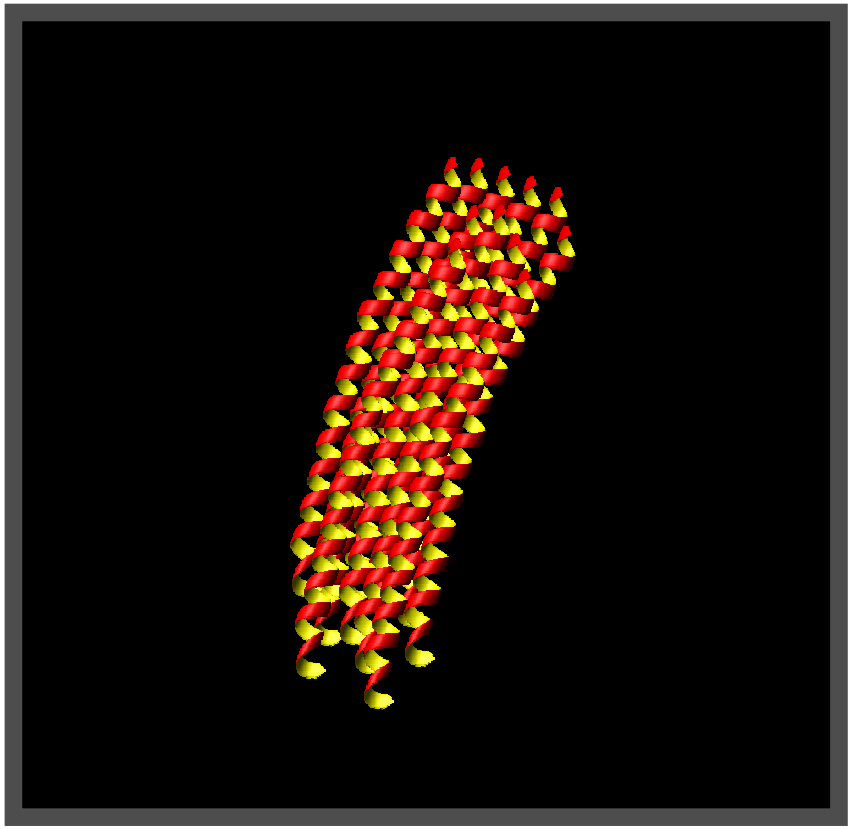
BIOPHYSICAL CHALLENGES

High-resolution Models

- NMR



- X-ray

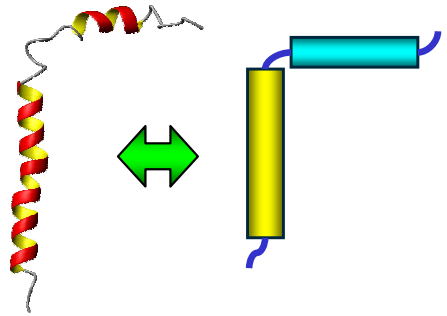


- Structure in micelles ≠ structure in membrane
- Structure oriented bilayers ≠ structure in membrane
- Crystals for membrane proteins are difficult to produce

ALTERNATIVE APPROACHES

Low-resolution Models

- ESR
- FRET

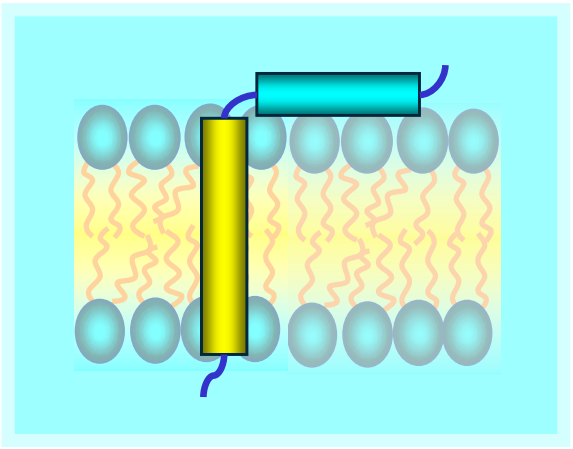


+ site-specific labelling +

- Distance determination at **5÷20** Å
- Dynamical information (ns)

- Distance determination at **10÷100** Å
- Dynamical information (ns)

- Protein structure
- Protein aggregation
- Mobility

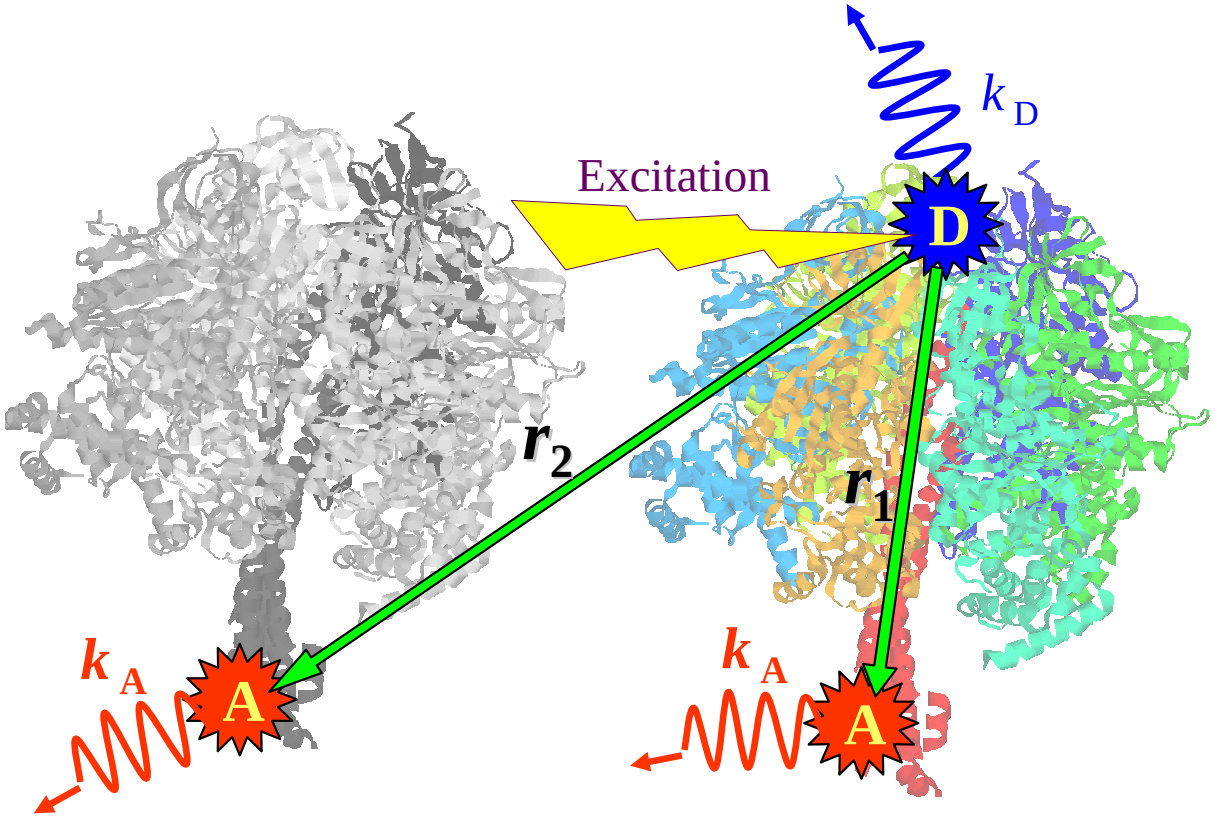


- Protein structure
- Protein embedment
- Protein aggregation

- **Structure in vesicles = structure in membranes**
- **NEED: Advanced data analysis**

EXPERIMENTAL APPROACH: FRET

Förster Resonance Energy Transfer Spectroscopy



Efficiency (probability) of energy transfer for donor-acceptor pair:

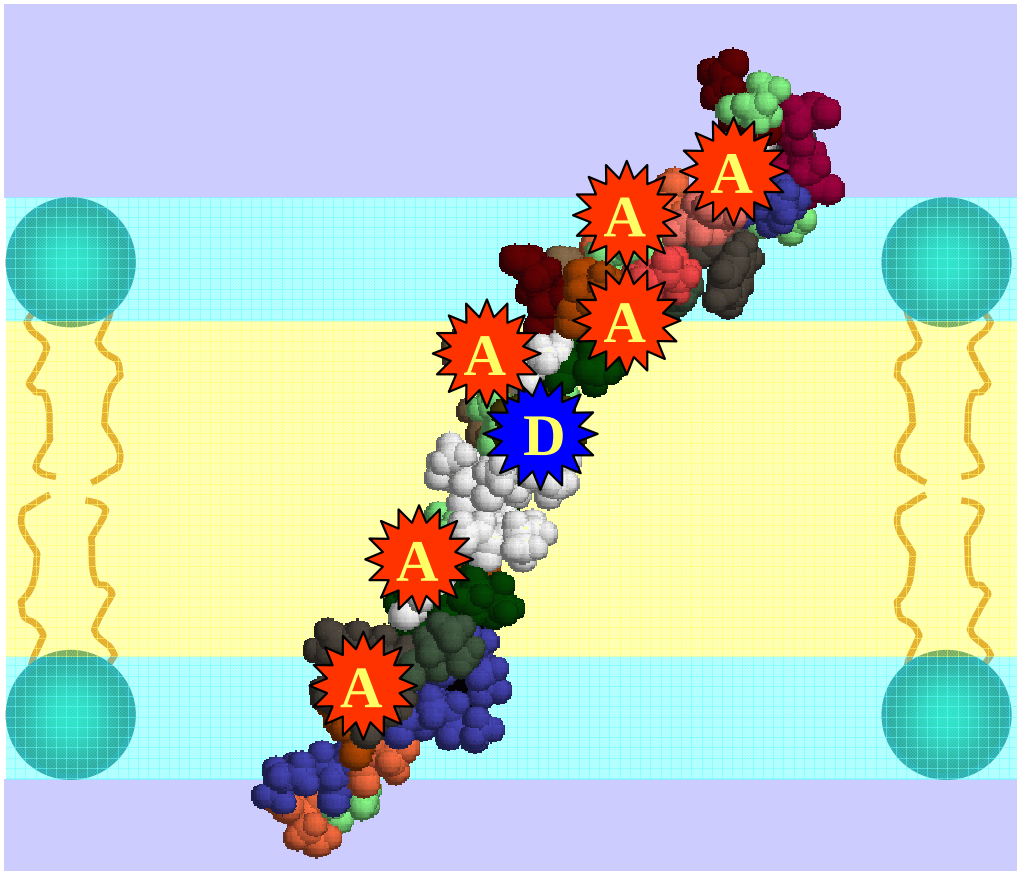
$$E = \frac{R_0^6}{R_0^6 + r^6}$$

- R_0 - Förster distance, a constant characterising donor-acceptor pair

Efficiency of energy transfer is related to donor-acceptor distances in the system
=> can be related to **STRUCTURE**

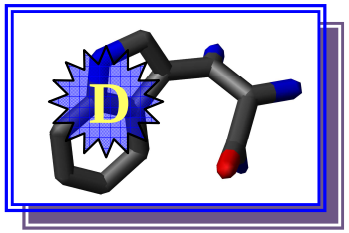
SITE-DIRECTED LABELLING

Bacteriophage M13 Coat Protein

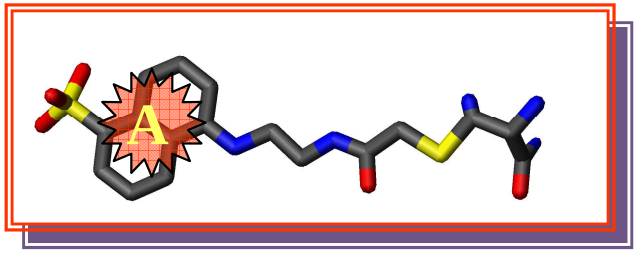


FRET Features

- Natural Trp(26) is used as donor
- Several Cys mutants are available
- Acceptor (AEDANS) covalently linked to Cys



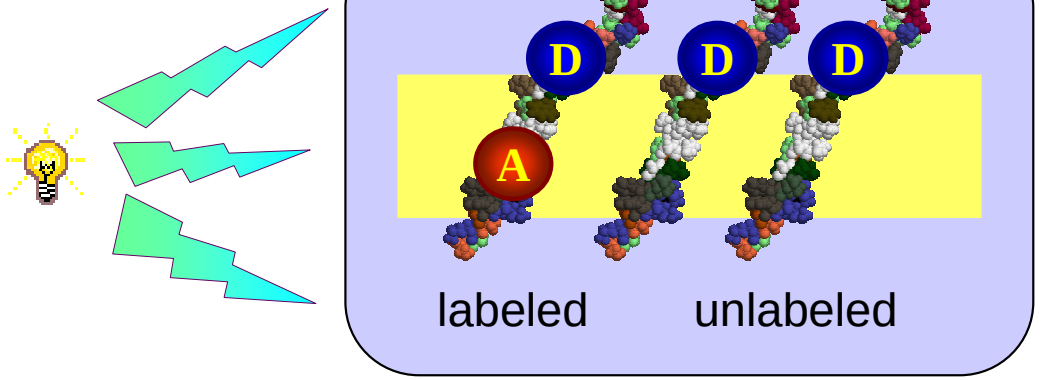
Trp – donor

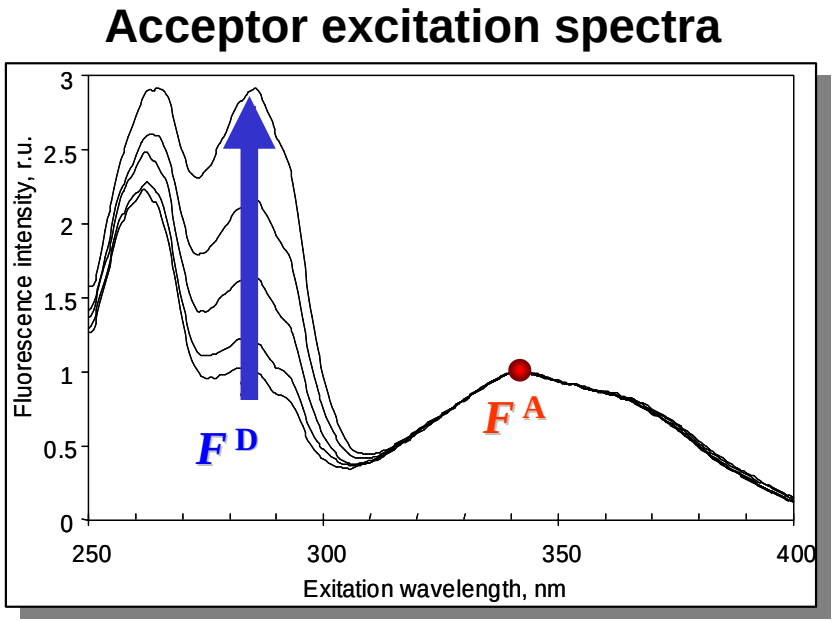


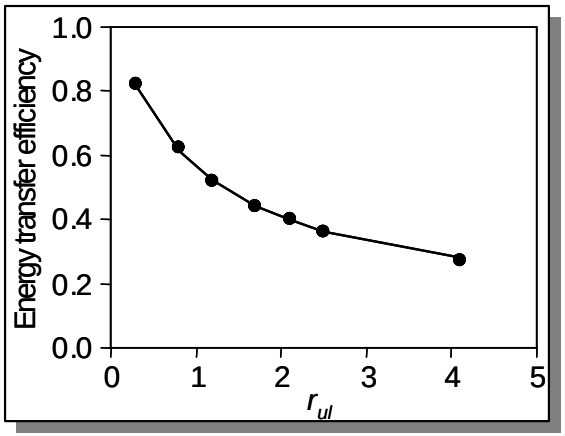
AEDANS – acceptor

EXPERIMENTS

Series of Titration Experiments







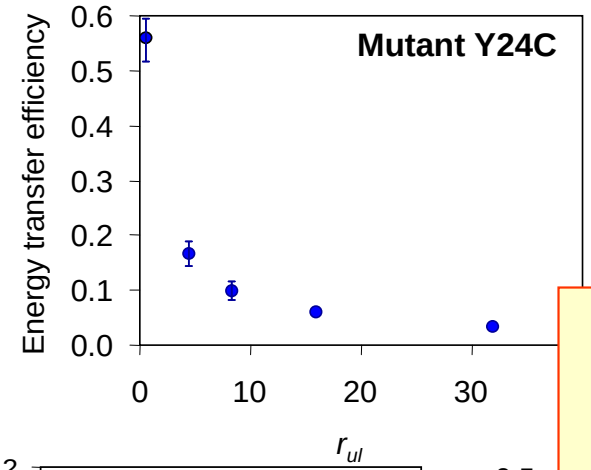
Energy Transfer Efficiency

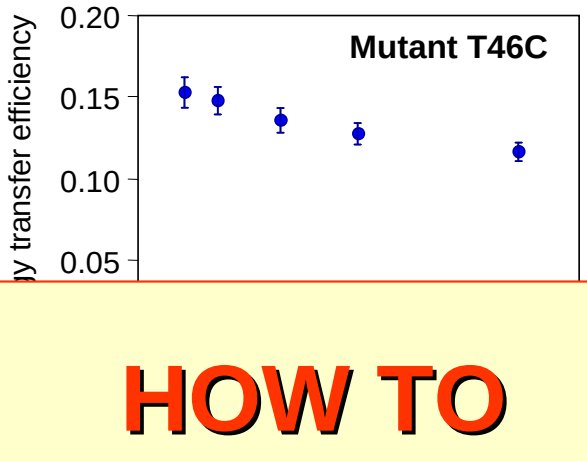
$$E = \frac{1}{1+r_{ul}}\left(\frac{F^D}{F^A} - \frac{\varepsilon_A^{290}}{\varepsilon_A^{340}}\right)\frac{\varepsilon_A^{340}}{\varepsilon_D^{290}}$$

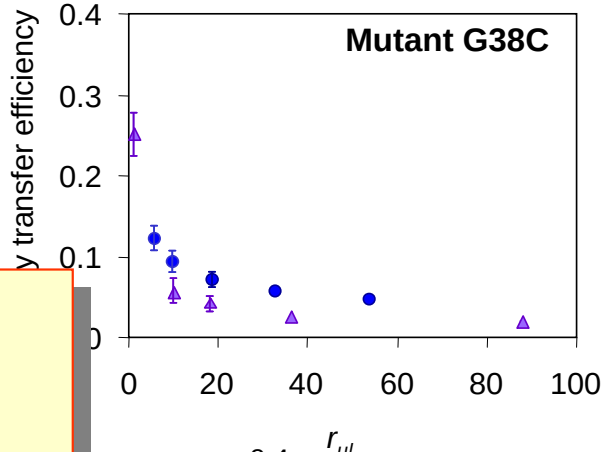
$$r_{ul} = \frac{[unlabeled]}{[labeled]}$$

EXPERIMENTAL DATA

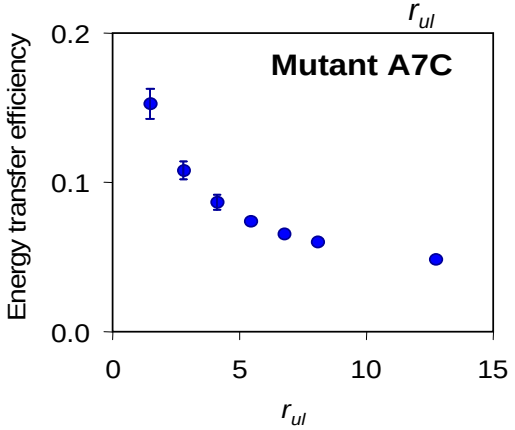
Experimental Energy Transfer Efficiencies

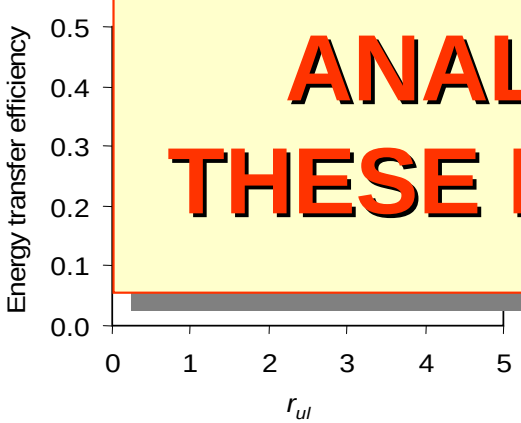


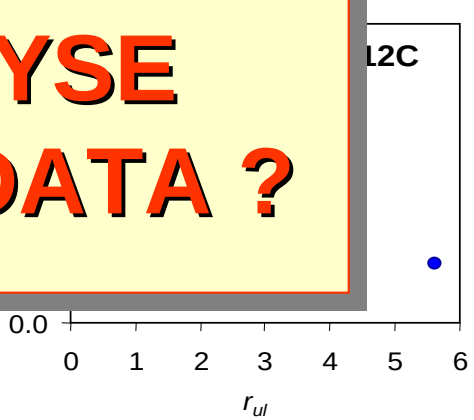


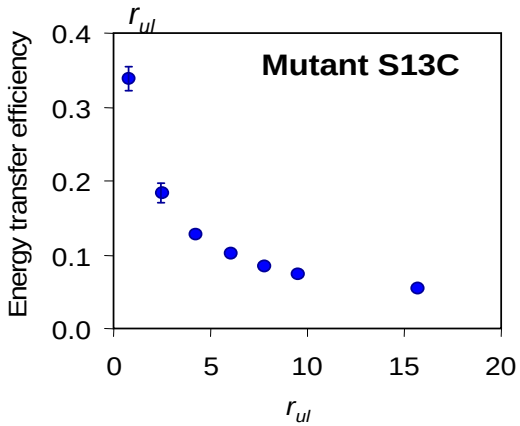


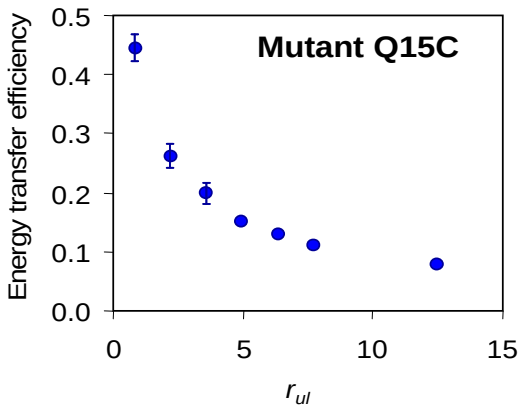
HOW TO ANALYSE THESE DATA ?

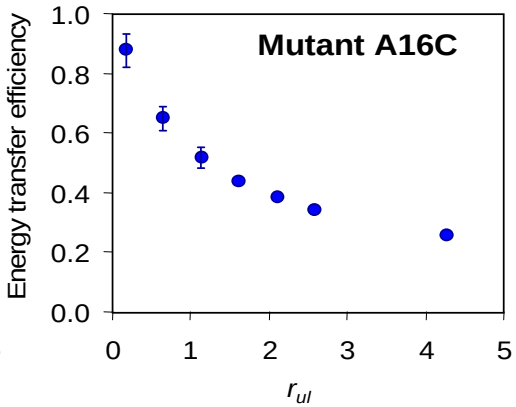


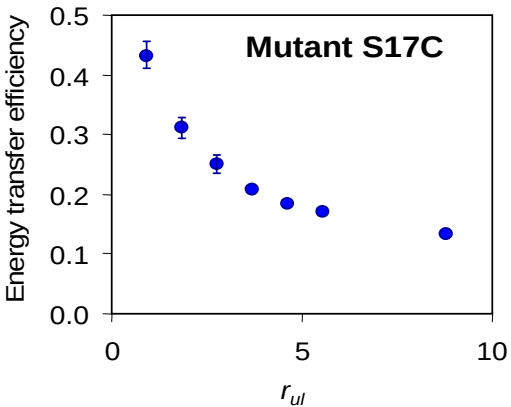


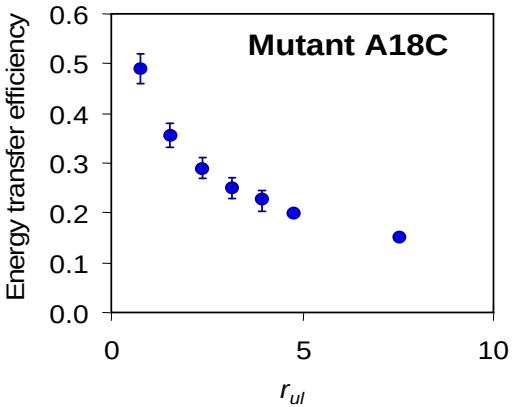






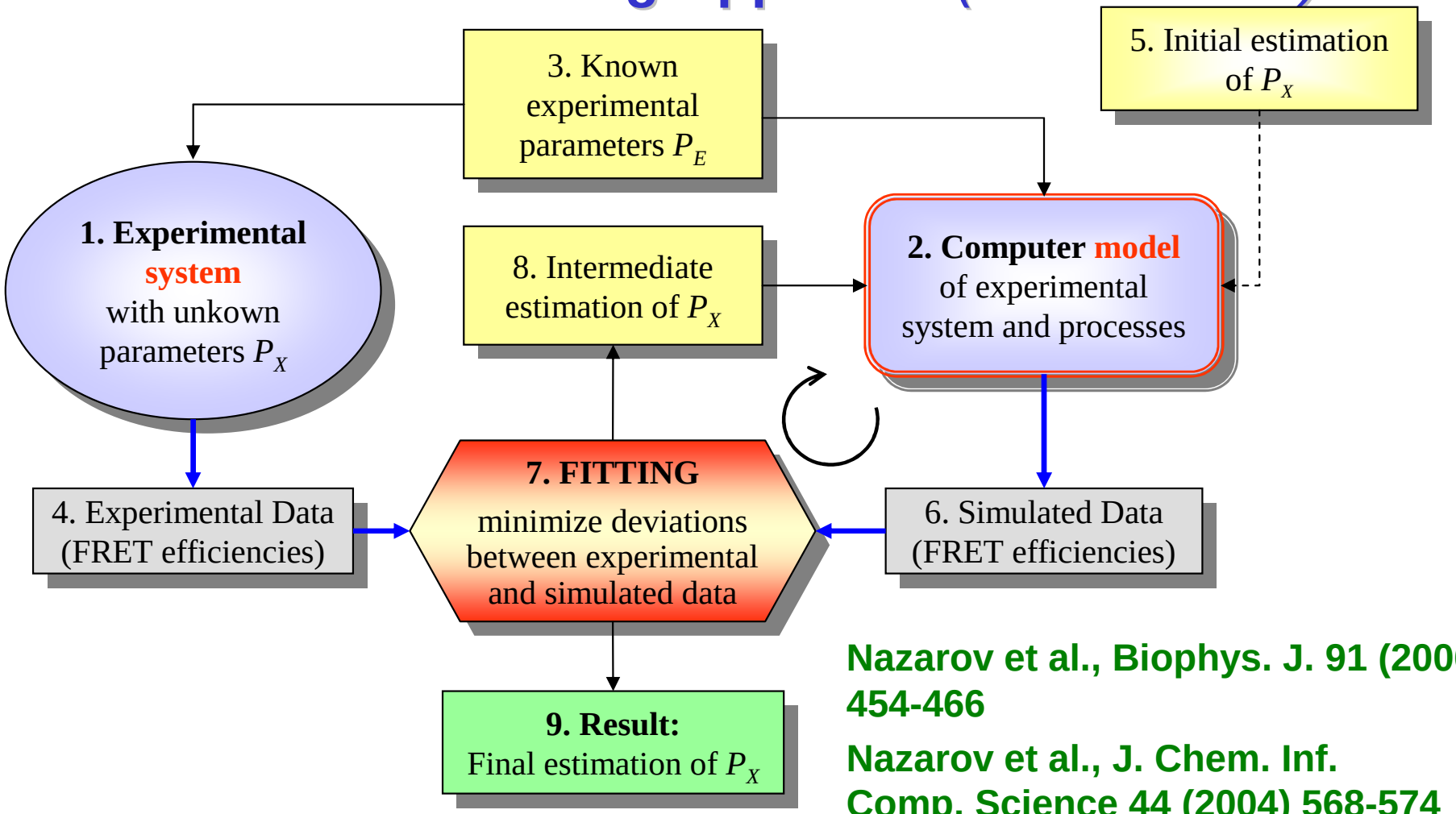






DATA ANALYSIS

Simulation-based Fitting Approach *(Inverse Task)*



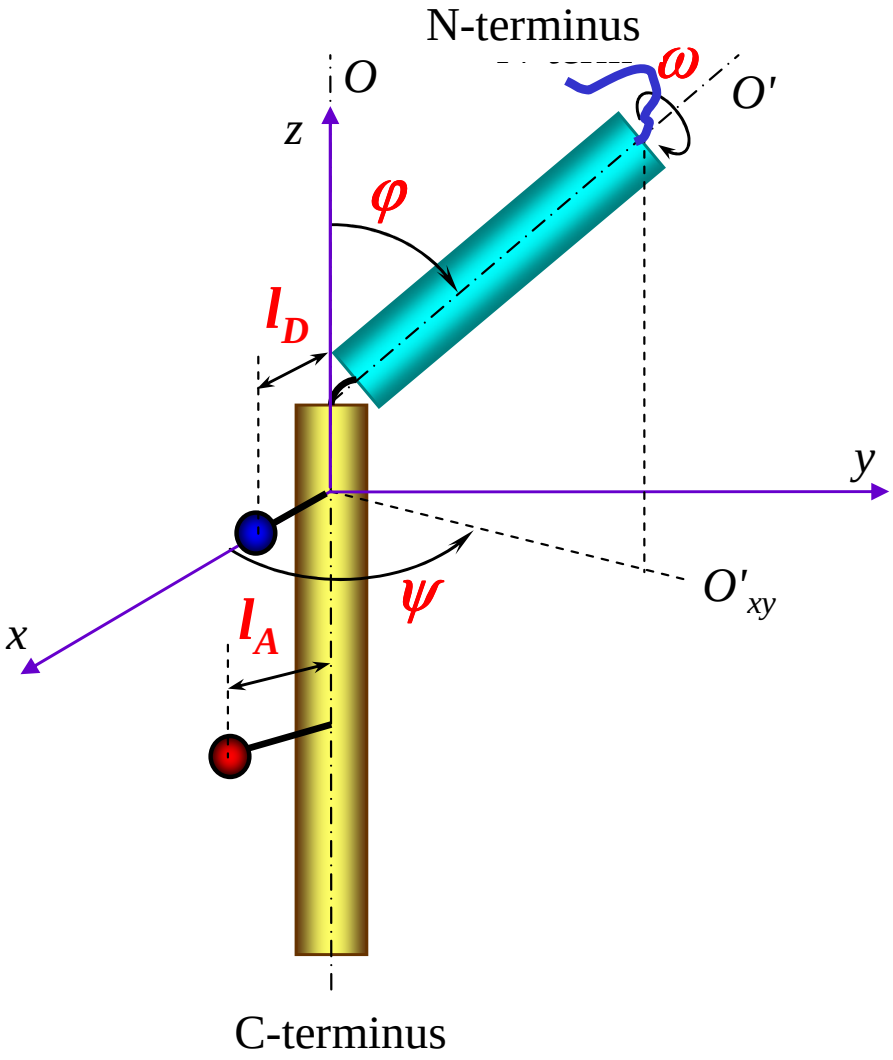
Nazarov et al., Biophys. J. 91 (2006) 454-466

Nazarov et al., J. Chem. Inf. Comp. Science 44 (2004) 568-574

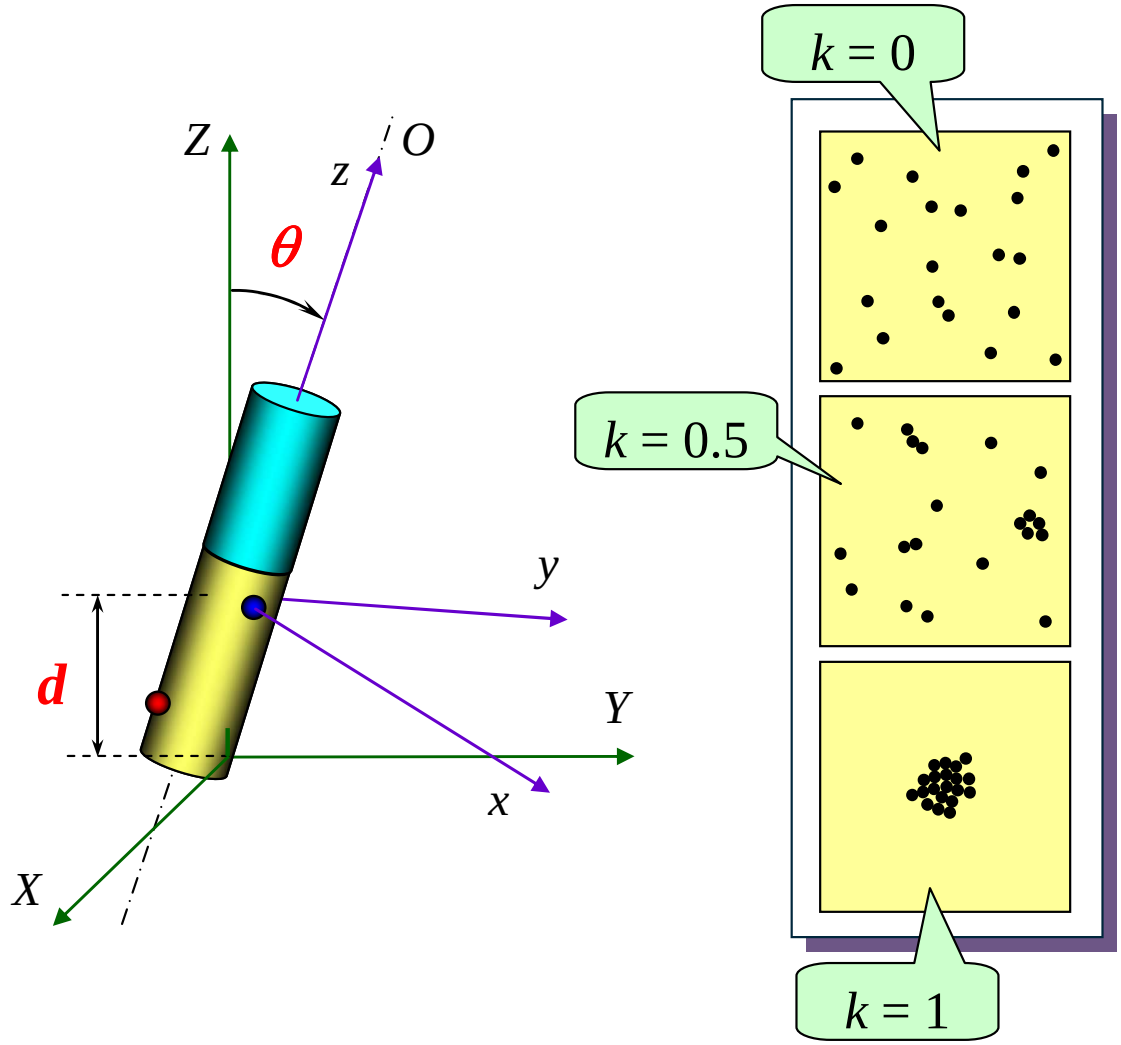
- Physical parameters are directly fitted and estimated
- Complex system is analyzed
- Global analysis of experimental data is applied

MODEL

Protein Model

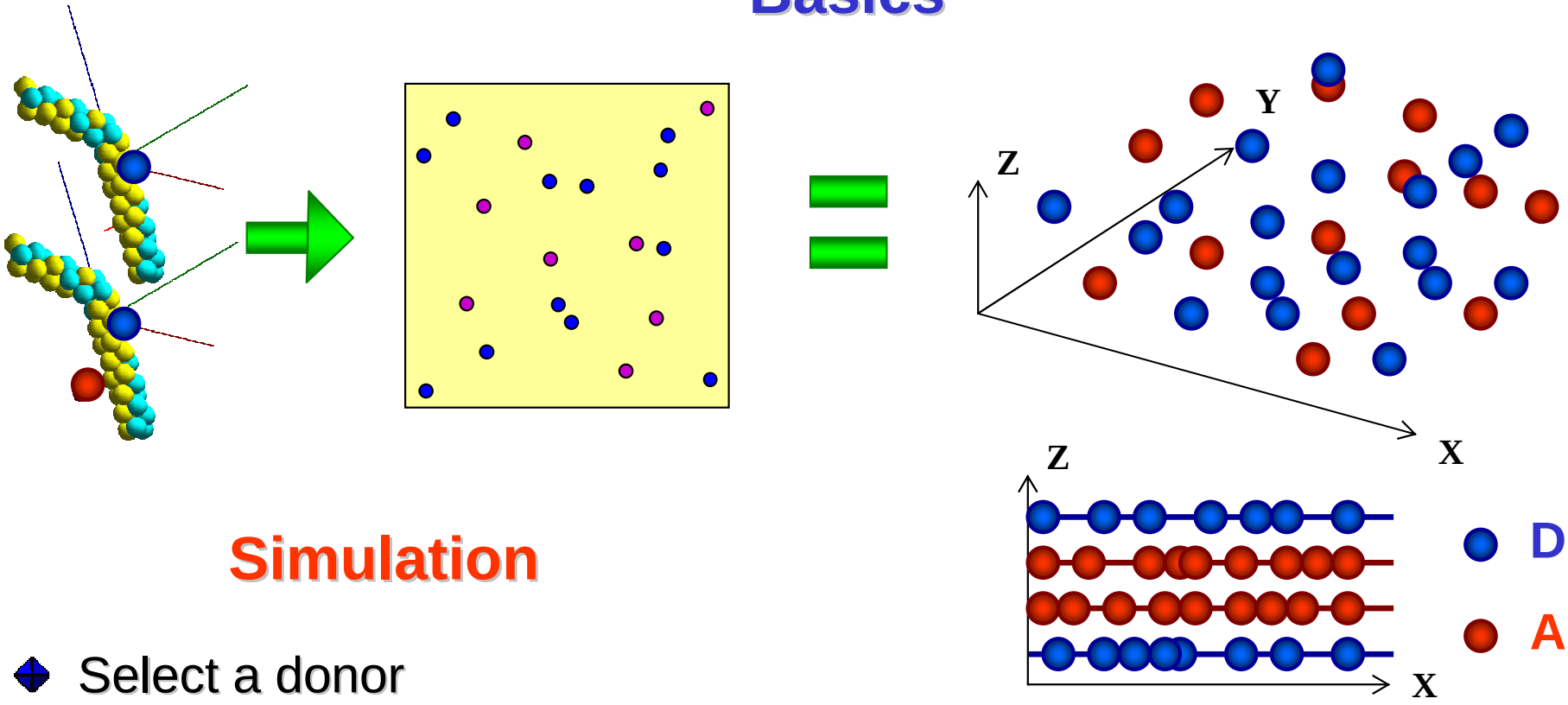


Bilayer Embedment Model



SIMULATION

Basics



Simulation

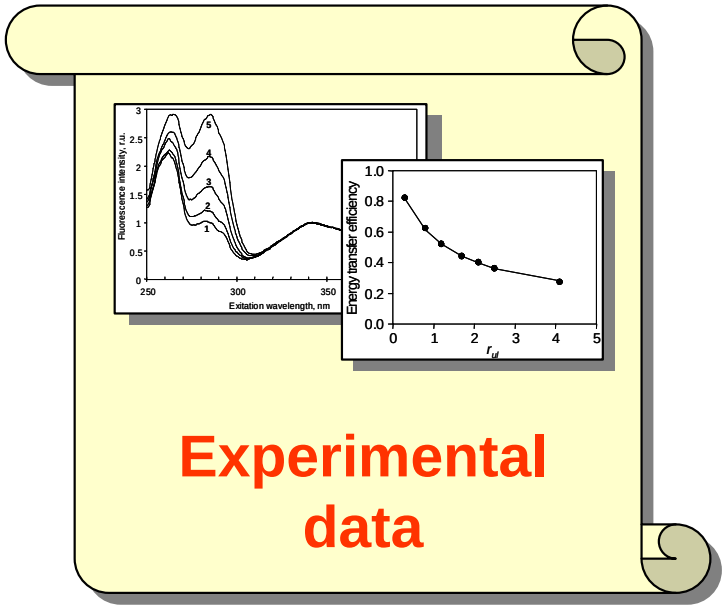
- Select a donor
- Calculate distances to all acceptors
- Calculate probability of energy transfer
- Calculate efficiency from an average of probabilities

E is a function of donor-acceptor distances !

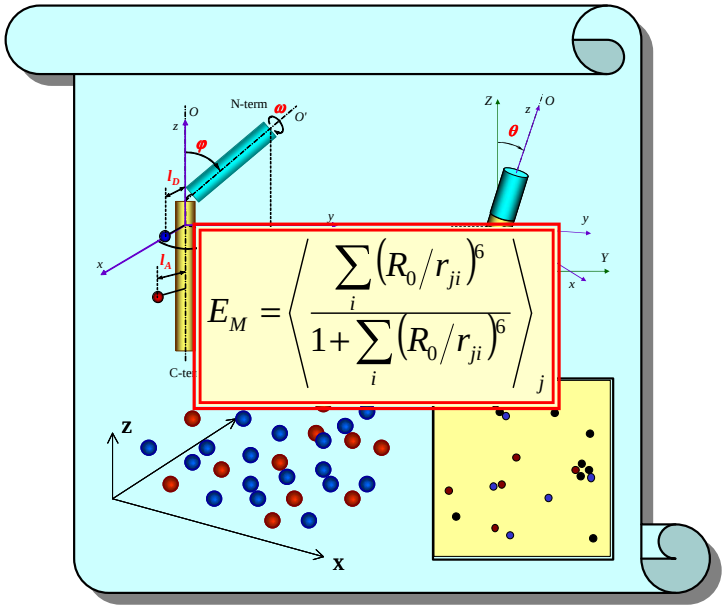
$$E = \left\langle \frac{\sum_i \left(R_0 / r_{ji}\right)^6}{1 + \sum_i \left(R_0 / r_{ji}\right)^6} \right\rangle_j$$

ANALYSIS

Summary

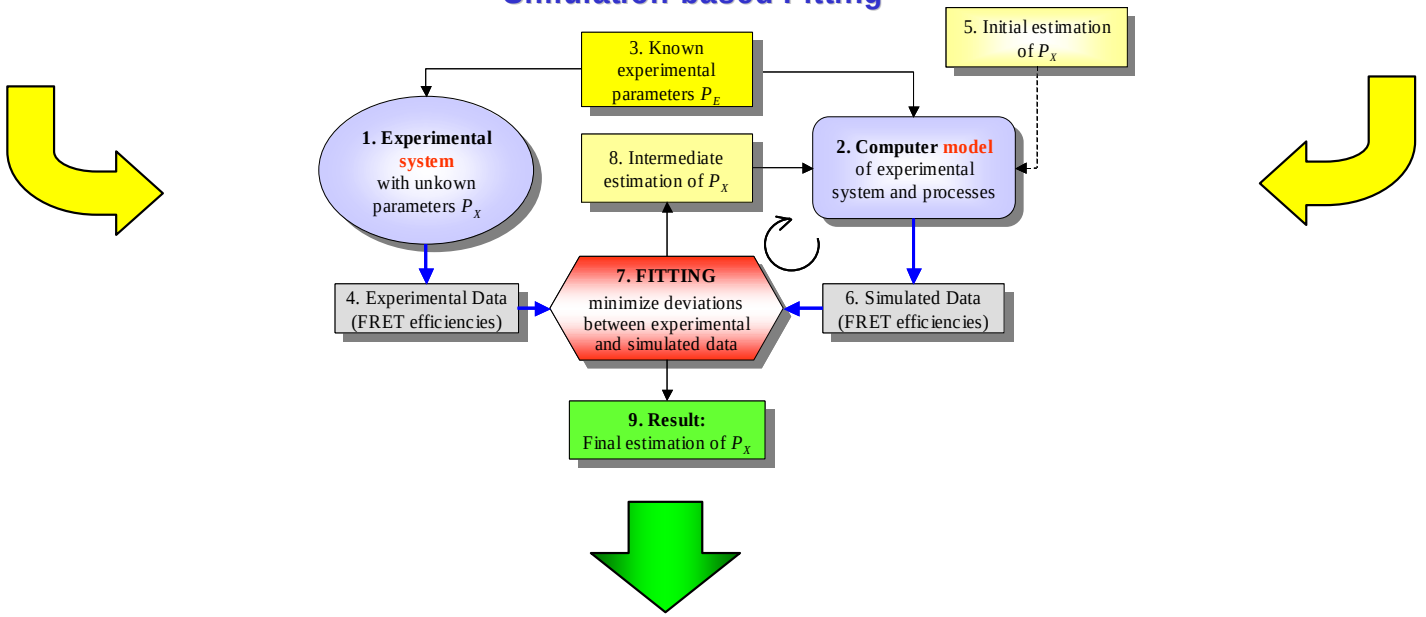


Experimental data

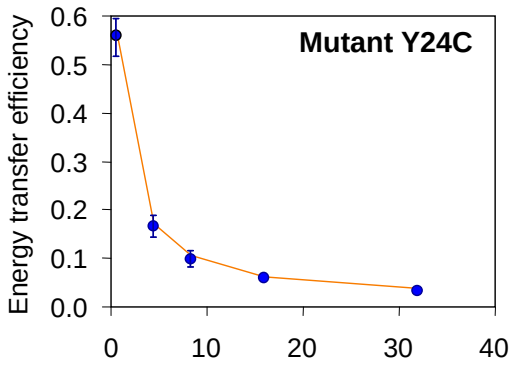


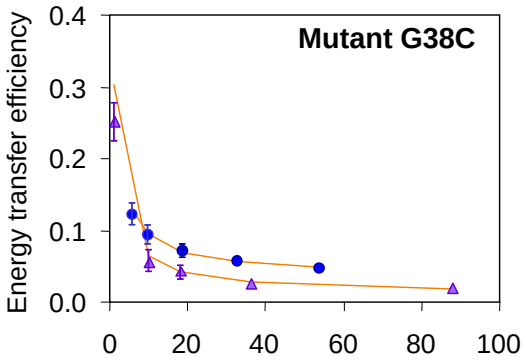
Global (simultaneous) analysis

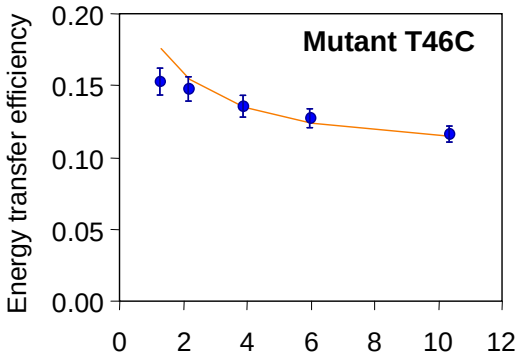
Simulation-based Fitting

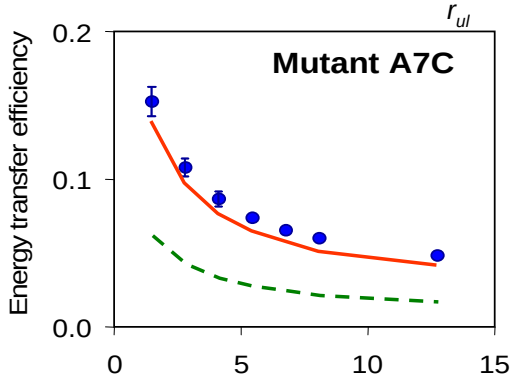


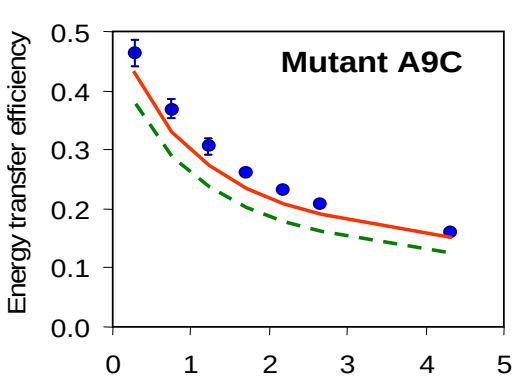
RESULTS: GLOBAL FIITING

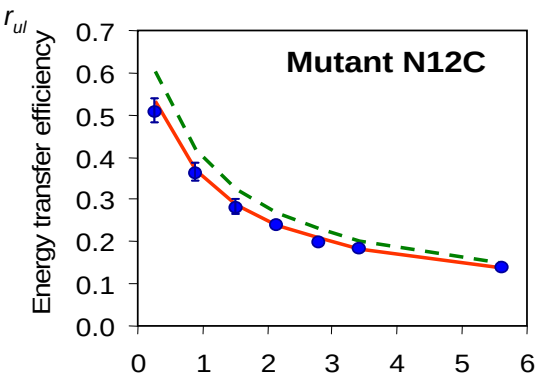


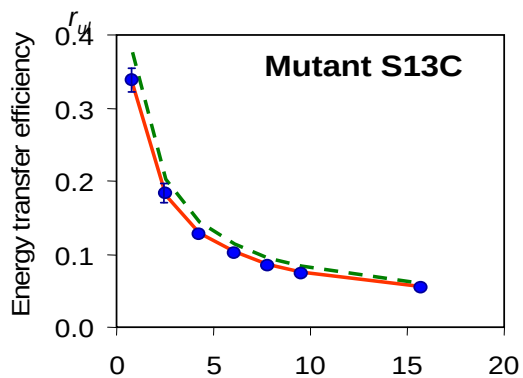


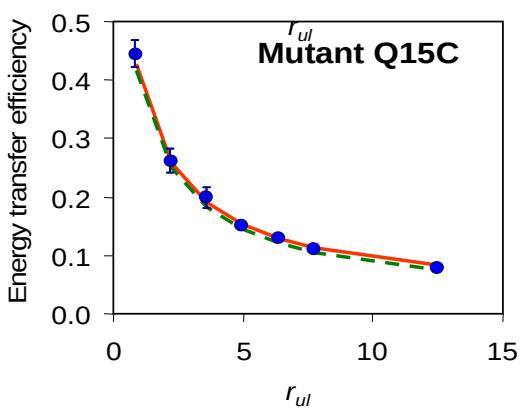


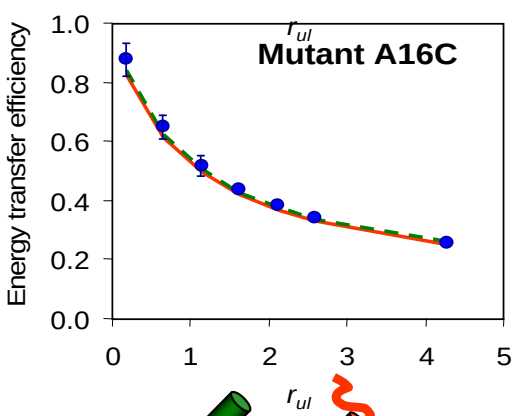


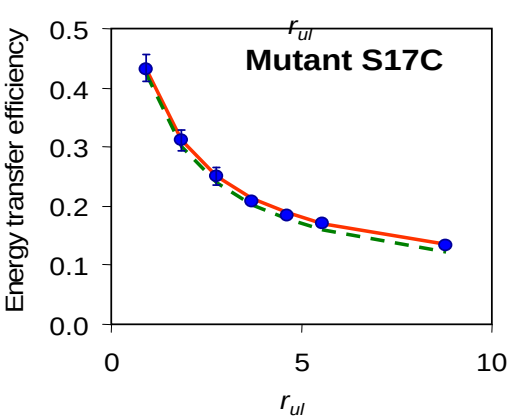


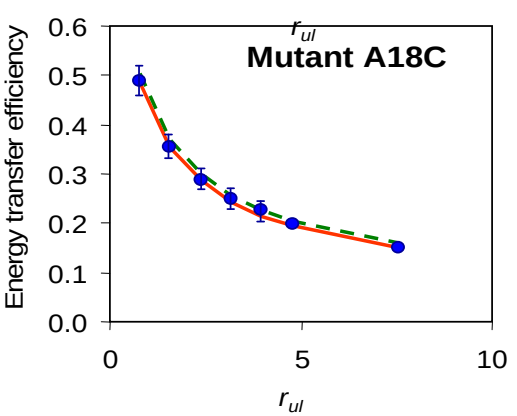


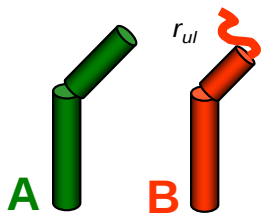








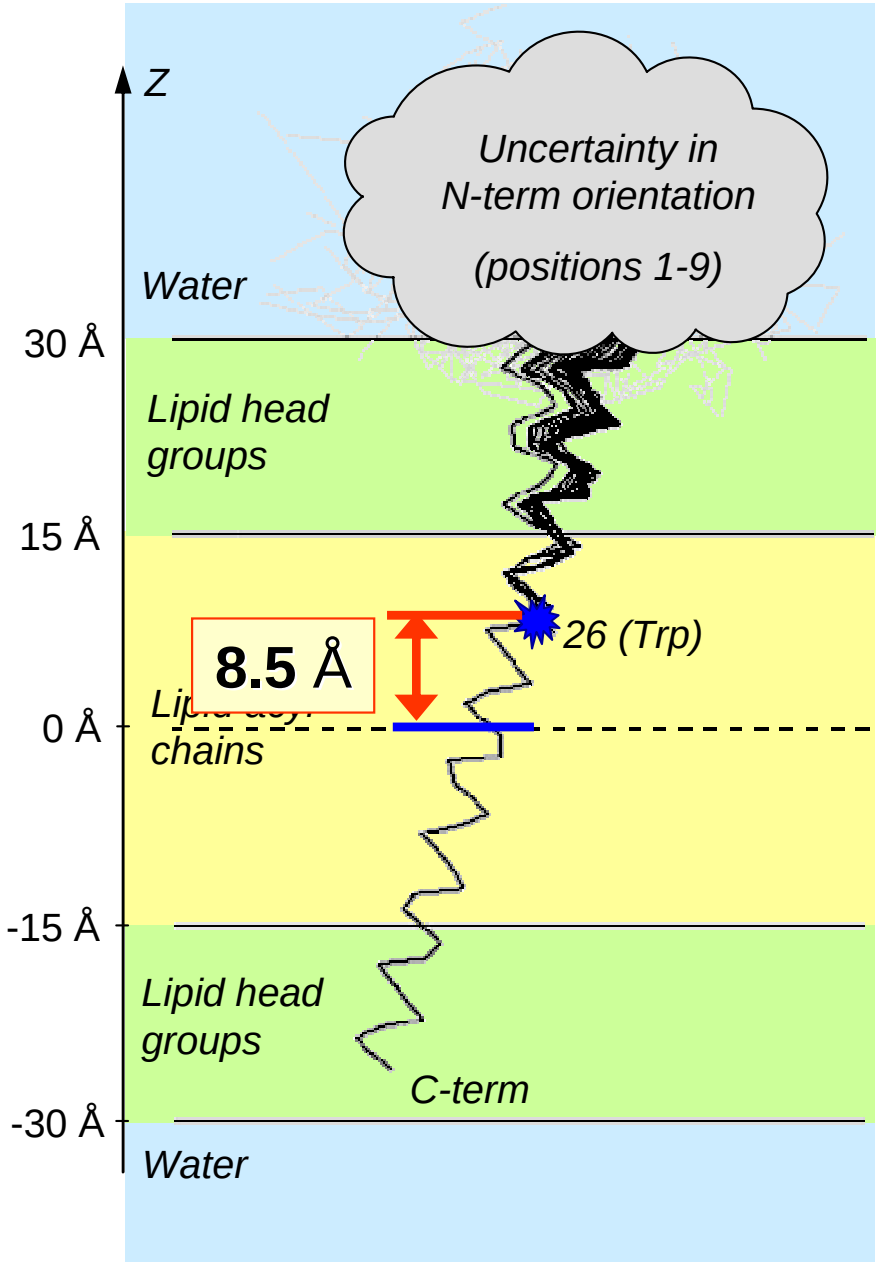




- experimental data
- Model A: 2 α-helixes
- Model B: 2 α-helixes + unstructured region at 1-9

RESULTS

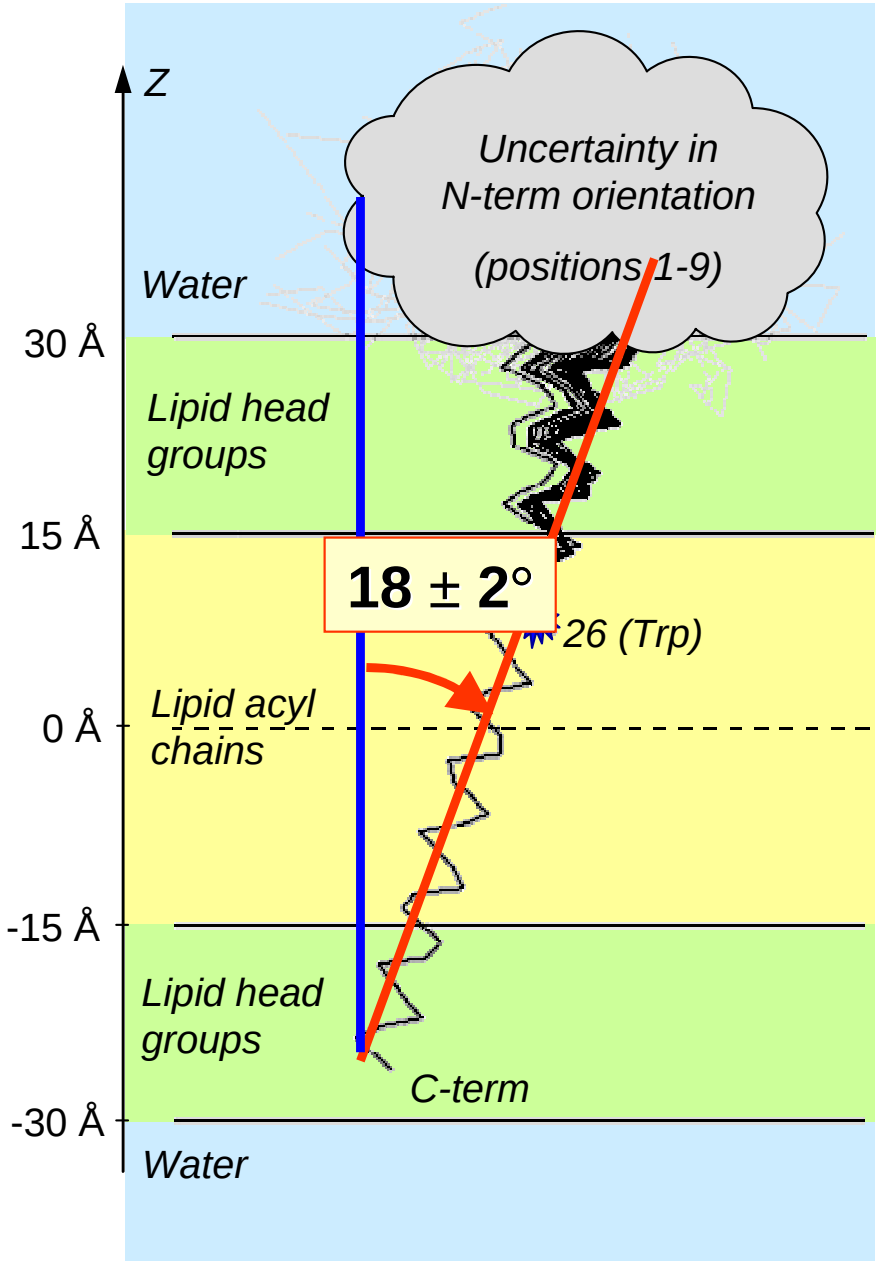
Final Structure and Membrane Embedment



Nazarov et al. (2007)
Biophys. J., 92,
p.1296-1305

RESULTS

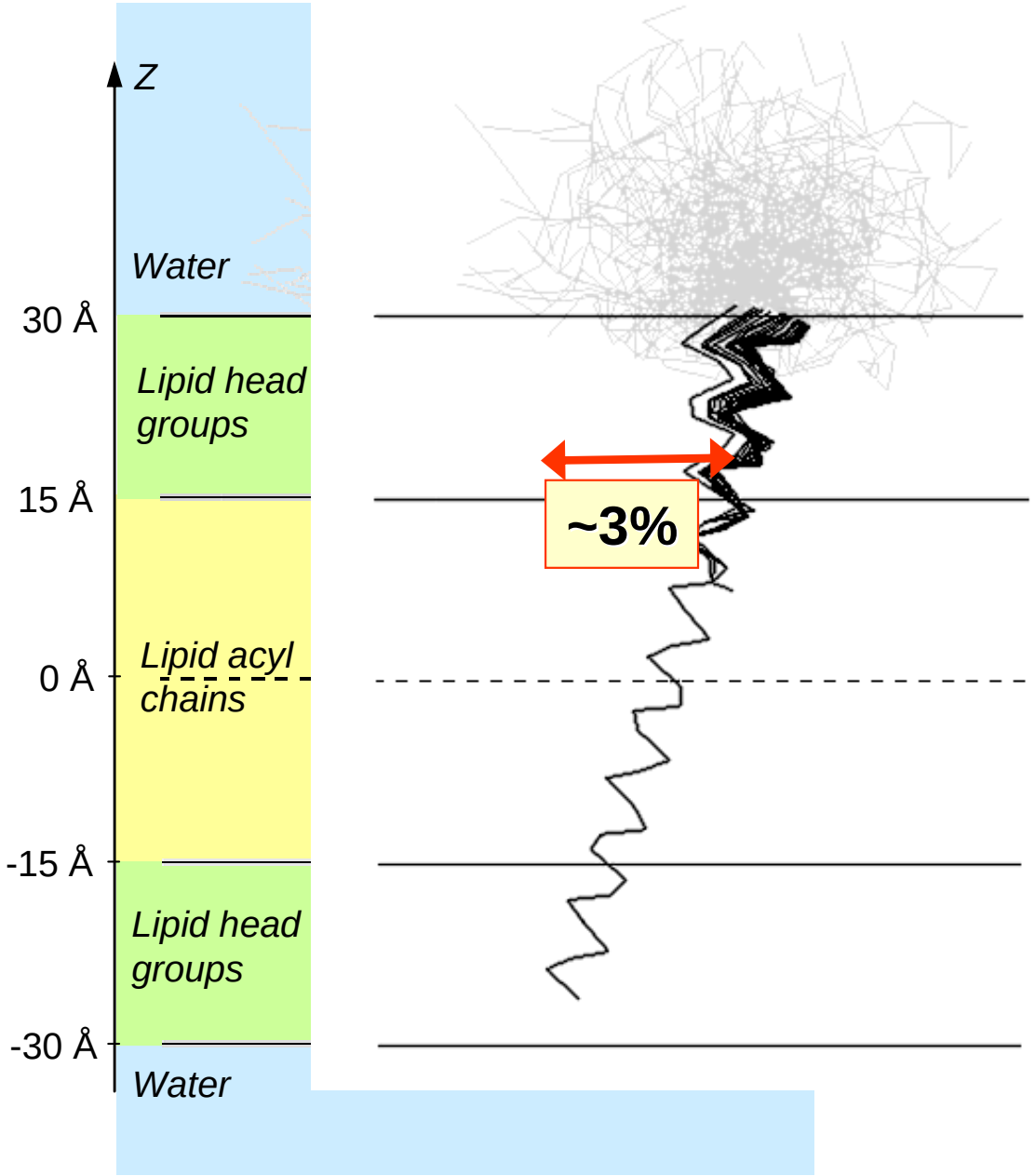
Final Structure and Membrane Embedment



Nazarov et al. (2007)
Biophys. J., 92,
p.1296-1305

RESULTS

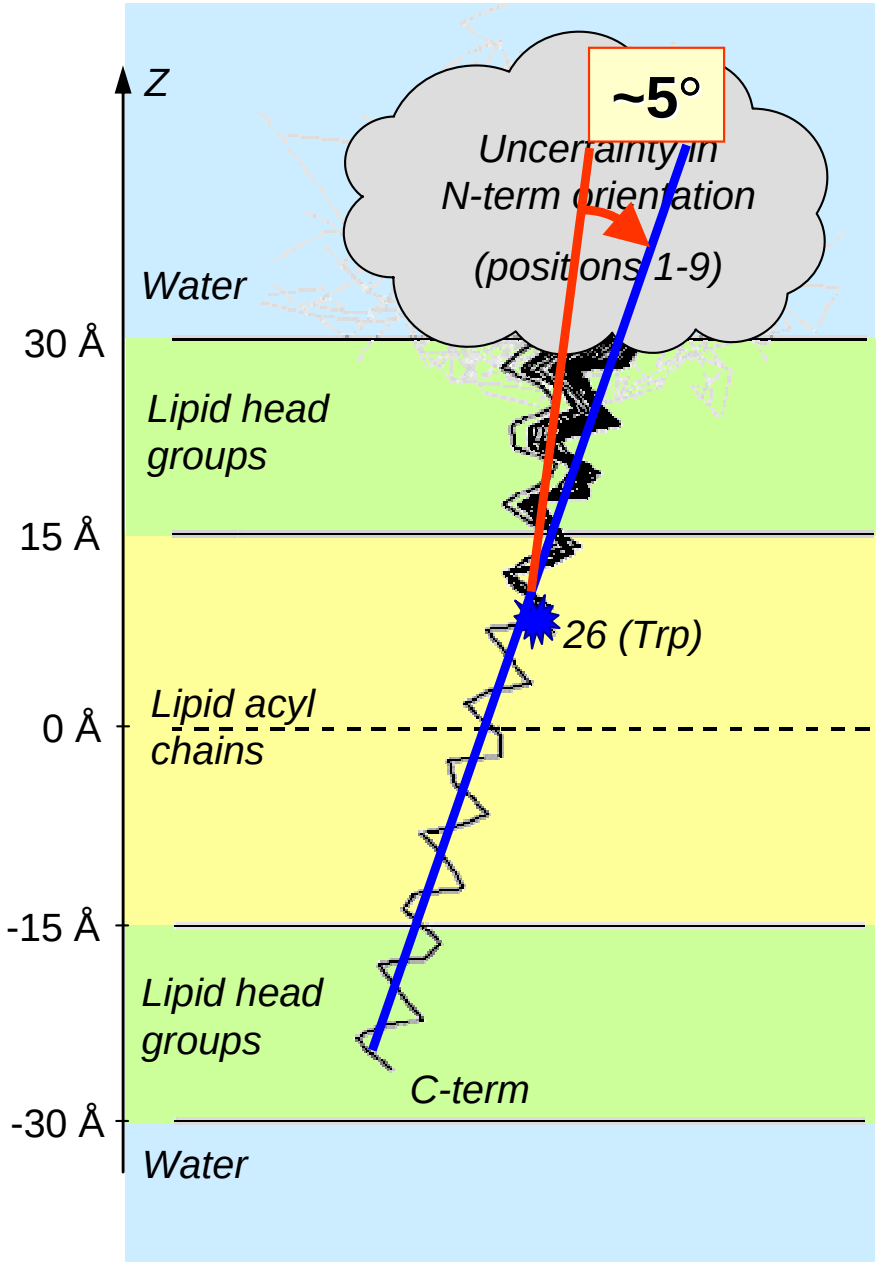
Final Structure and Membrane Embedment



zarov et al. (2007)
phys. J., 92,
296-1305

RESULTS

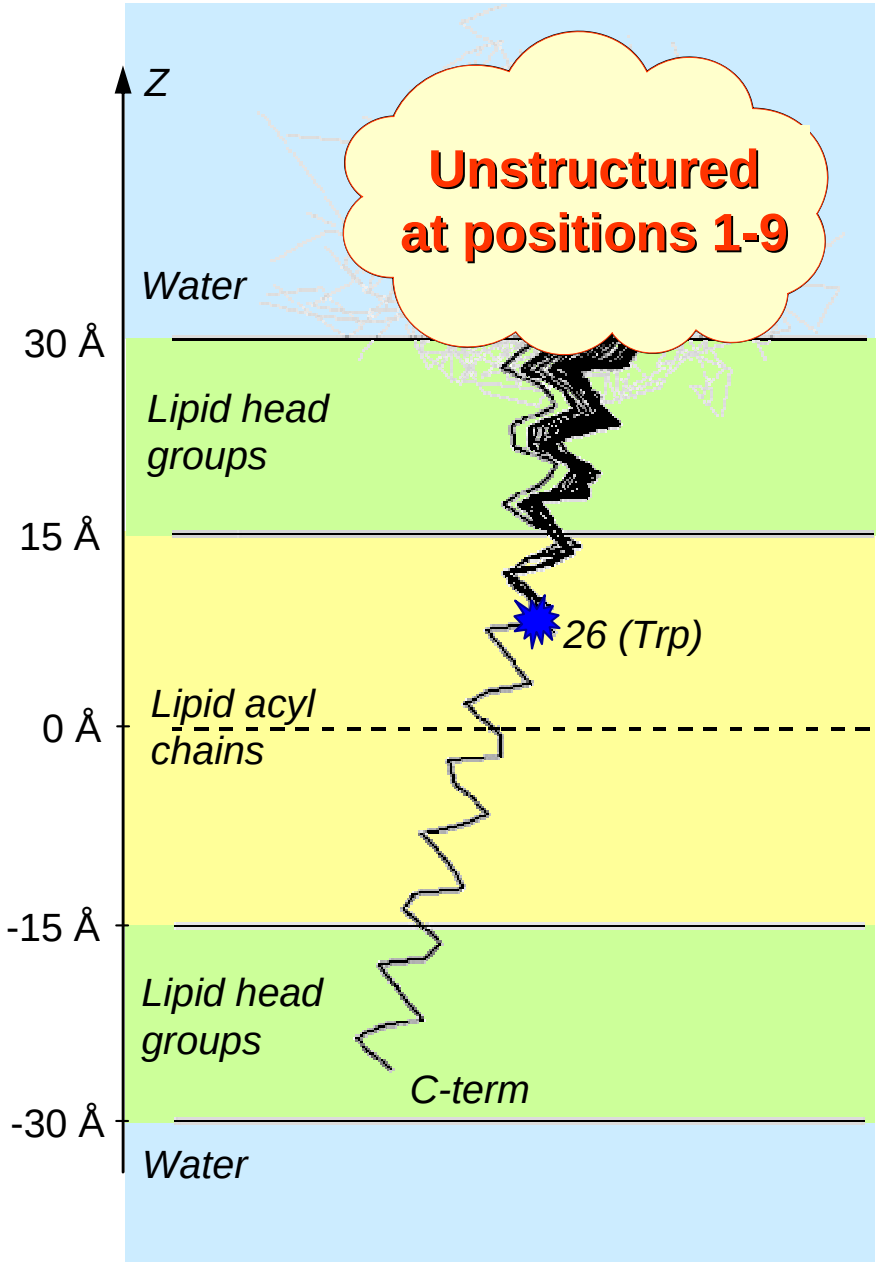
Final Structure and Membrane Embedment



Nazarov et al. (2007)
Biophys. J., 92,
p.1296-1305

RESULTS

Final Structure and Membrane Embedment

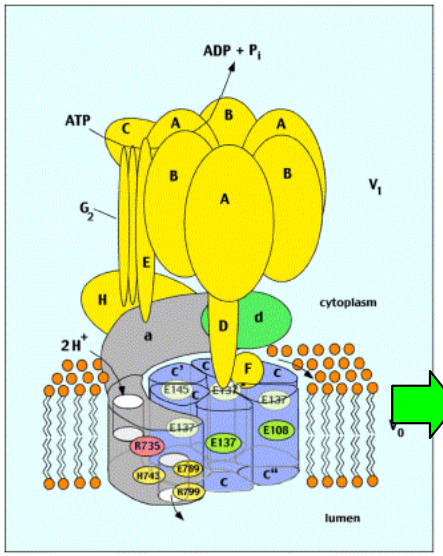


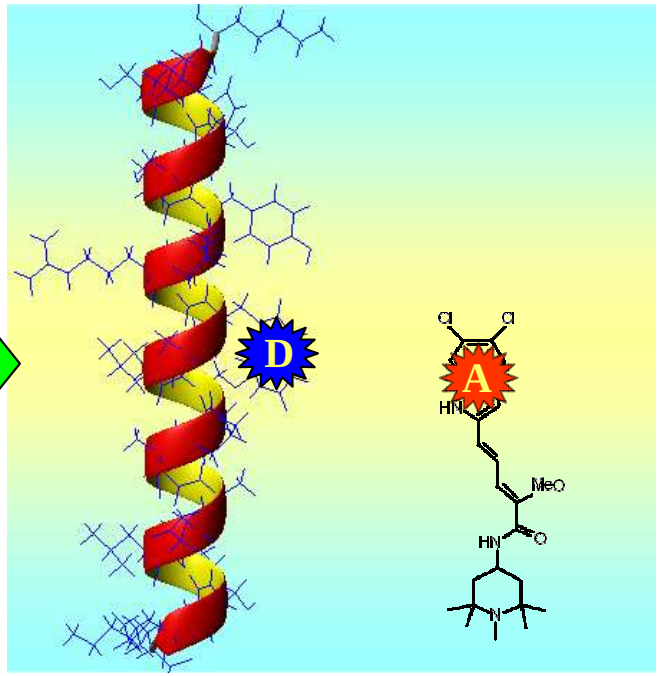
Nazarov et al. (2007)
Biophys. J., 92,
p.1296-1305

APPLICATIONS TO OTHER SYSTEMS

Peptide of subunit *a* of H^+ vacuolar ATPase

(responsible for osteoporosis)



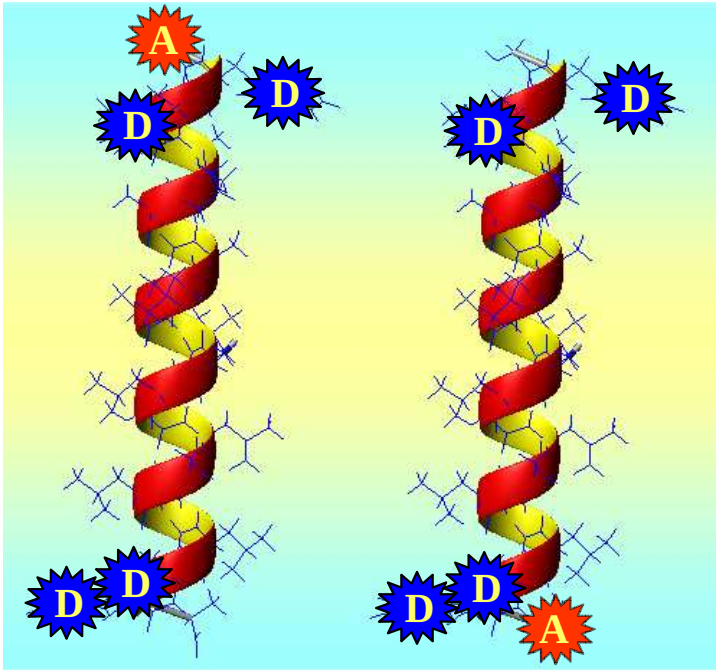


peptide **Ind3** inhibitor

Hesselink R.W., et al.,
Biophys. Biochem. Acta 1716 (**2005**) 137-145

WALP

(collaboration with group of Prof. A. Killian)



Sparr E., et al.,
J. Biol. Chem. 280 (**2005**) 39324-39331

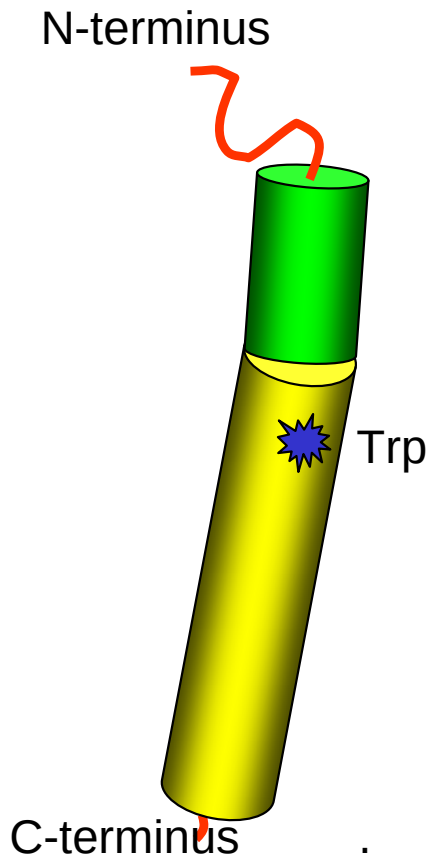
CONCLUSIONS

- Combination of fluorescence technique and advanced data analysis methods allows to obtain novel structural information about membrane proteins

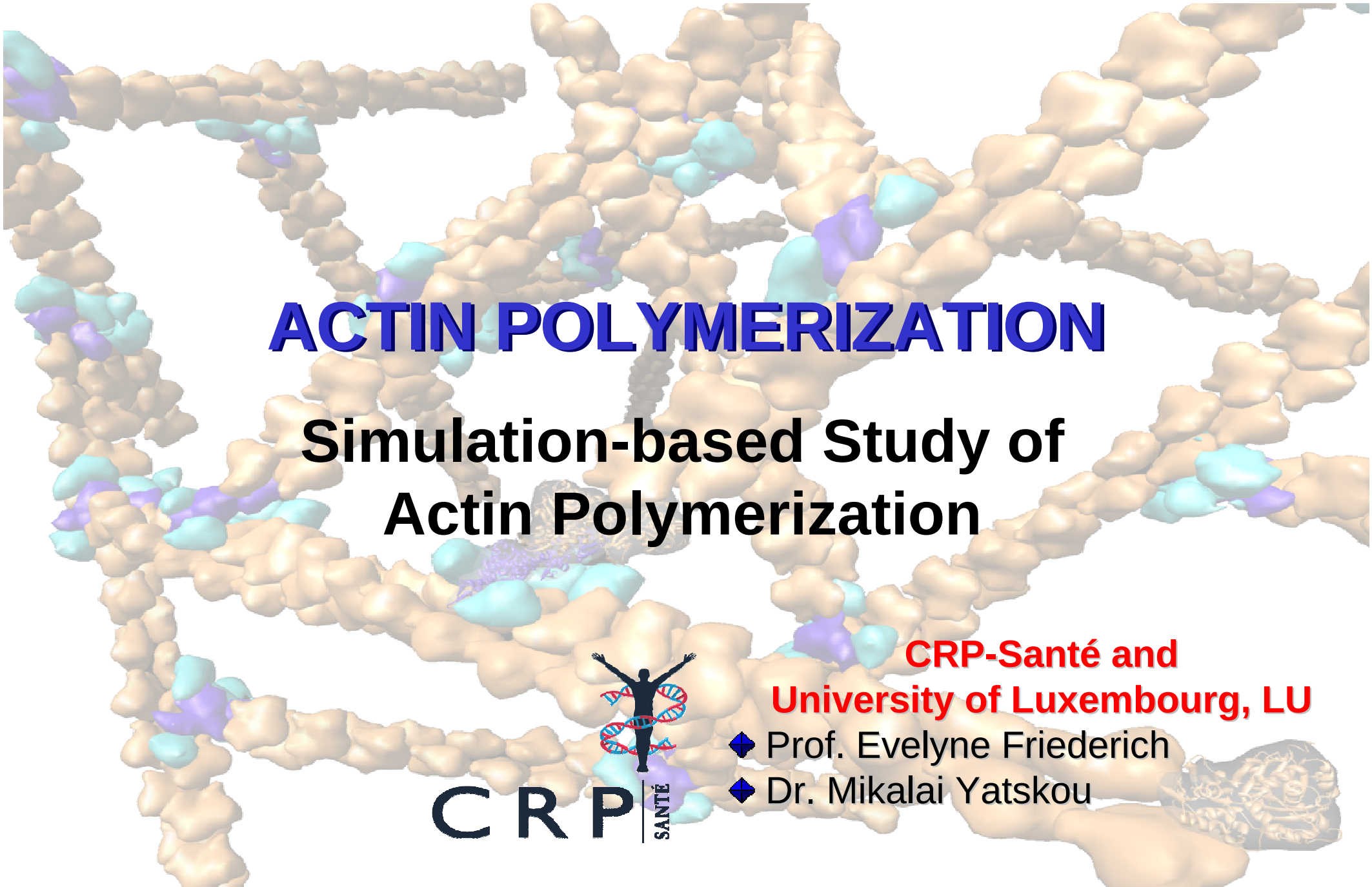
- Simulation-based fitting plus global analysis of FRET data allows simultaneous determination of protein structure, membrane embedment and aggregation

- The resulting structure of membrane-embedded M13 coat protein is →

- The same approach can be used to study other membrane proteins



PART III



ACTIN POLYMERIZATION

Simulation-based Study of Actin Polymerization

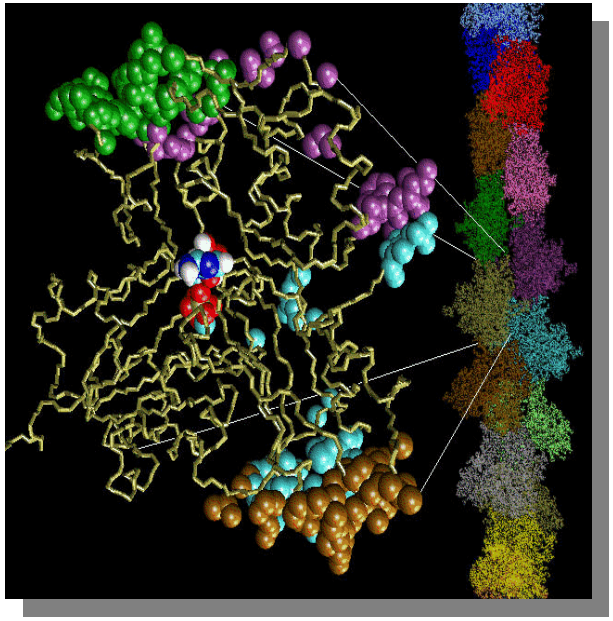
CRP-Santé and
University of Luxembourg, LU

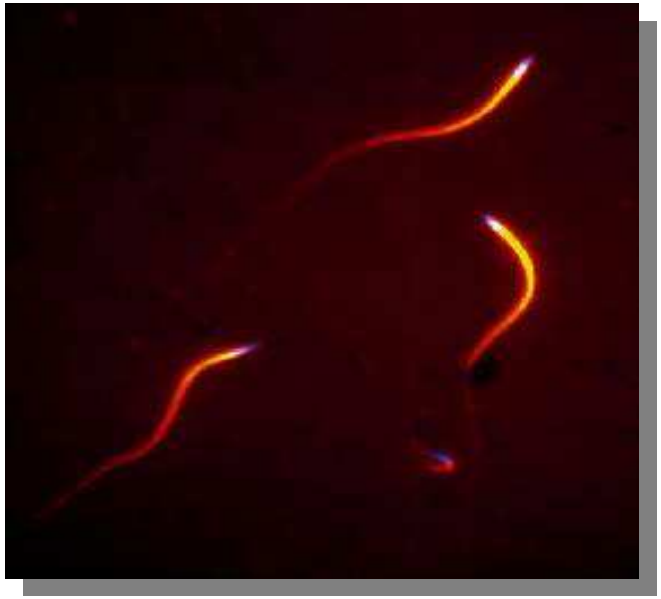
- Prof. Evelyne Friederich
- Dr. Mikalai Yatskou

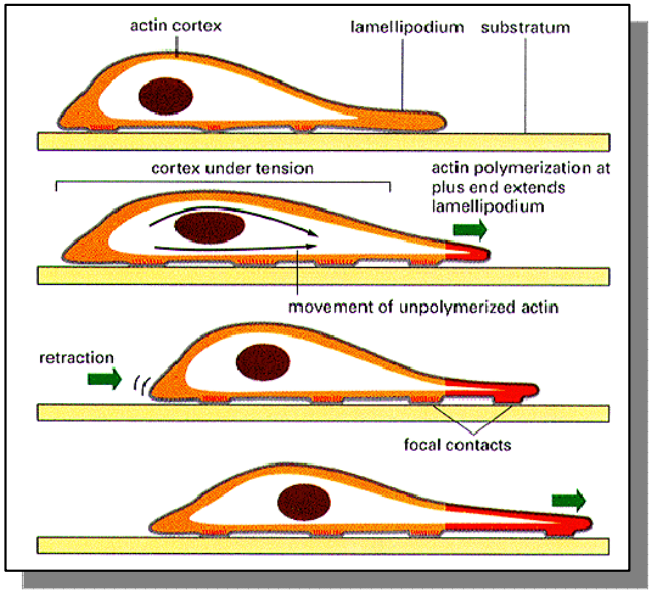
IMPORTANCE

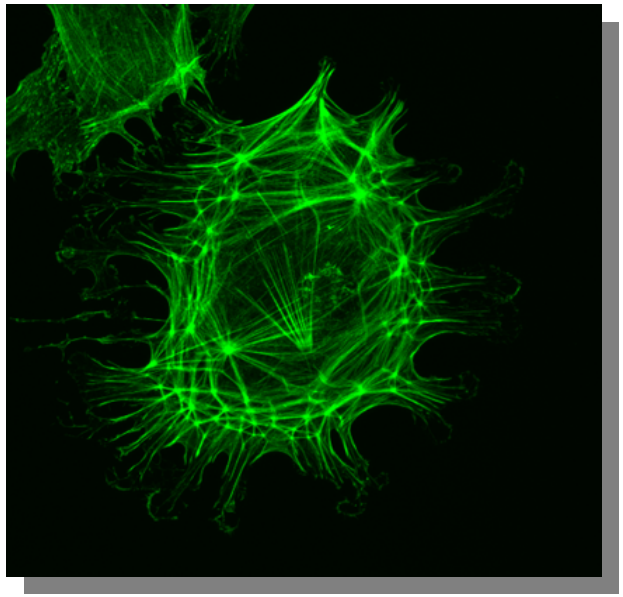
Cell Processes Involving Actin Polymerization

- Cytoskeleton formation
- Active transport of molecules
- Cell movement (filopodia and lamellipodia)
- Wounds healing, *etc.*
- Metastasis propulsions
- Bacteria propulsion (*Listeria monocytogenes*)



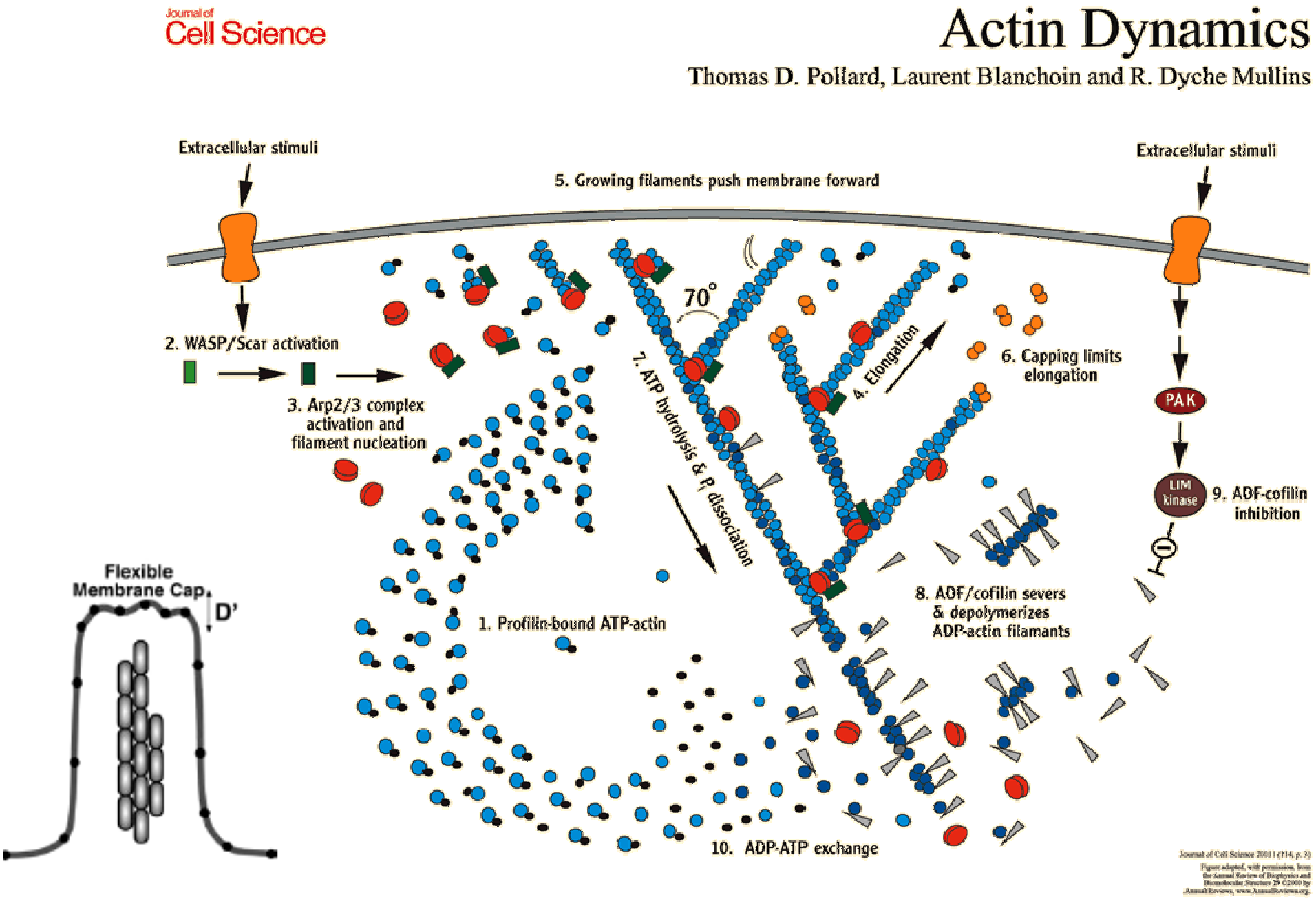






GENERAL OVERVIEW

Biochemical Reactions



BRIEF OVERVIEW OF EXPERIMENTS

Fluorescence Techniques

- Actin labelling with GFP + FCM (fluorescence confocal microscopy)
- Actin labelling by pyrene and detecting the fluorescence only from filaments (pyrene-actin experiments)
- FRAP (fluorescence recovery after photobleaching)
- Low concentration labelling with fluorescence speckle microscopy

Analytical models give rough approximation, when are applied for the data analysis in biological systems

INTRODUCTION

Goal and Tasks for the Project

- Developing an advanced computer-simulation approach, based on stochastic and analytical modelling algorithms, for the simulation and analysis of the actin filament formation and its effect on small bodies motility.

  - Build and test models for molecular reactions
  - Adapt the models to analysis of experimental data (e.g. actin-pyrene, FRAP data, biophysical experiments with bead motility, etc.)
  - Try to build the Monte Carlo model for actin-based motility of small bodies (beads, bacteria)

MODELS

Levels of Modelling

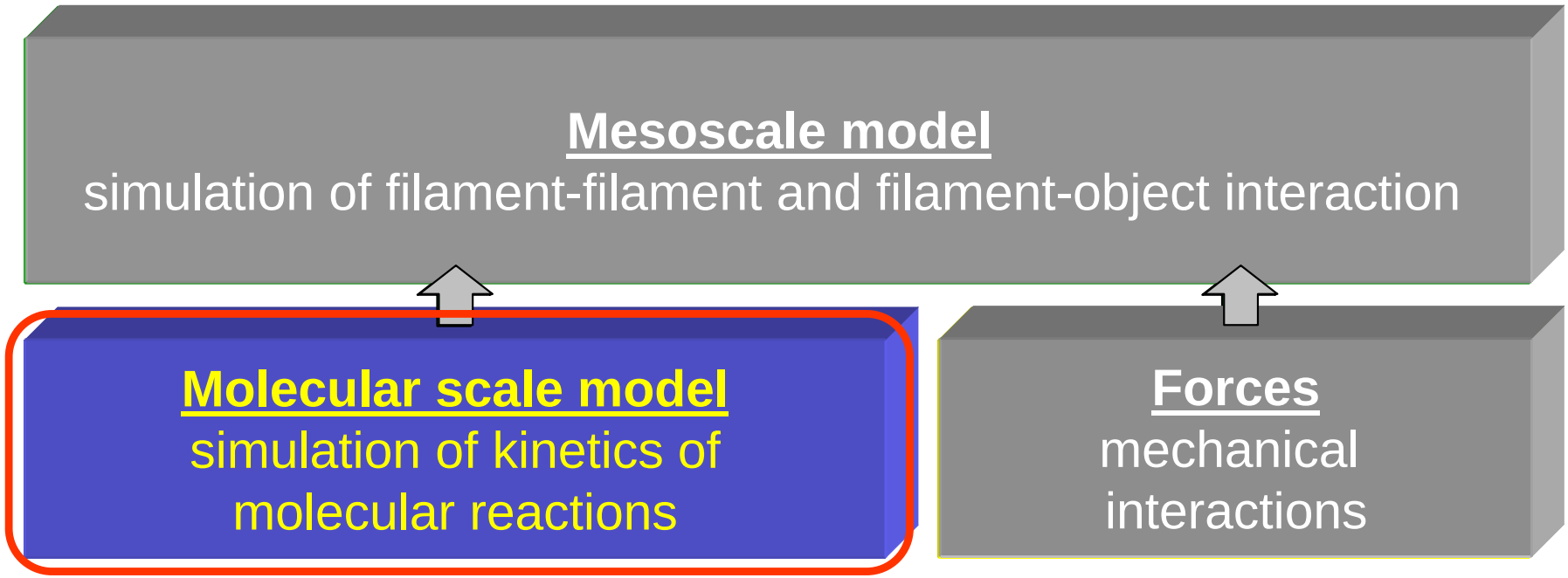


Figure. *Hierarchical organization of the model being developed*

BIOCHEMICAL REACTIONS

Formalization

- The model for the reactions includes 21 reaction and 14 reagents

- **Reagents (14)**
  - Actin is G- an F-form (ATM, ADM and ATF, ADF)
  - Free filament barbed and pointed ends (FTB, FTP, FDB, FDP)
  - Capping proteins in free and bound forms (CBM, CBF, CPM, CPF)
  - Formin in free and bound forms (FOM, FOF)

- **Reactions (21)**
  - Nucleations (spontaneous, induces)
  - Barbed and pointer end association for ATP- and ATD-containing actins
  - Barbed and pointed end dissociation for ATP- and ATD-containing actins
  - Capping and uncapping of barbed and pointed ends
  - Formin-related reaction (binding, unbinding, formin-initiated association)
  - Actin aging and ATP-recharge

BIOCHEMICAL REACTIONS

Formalization: Reactions

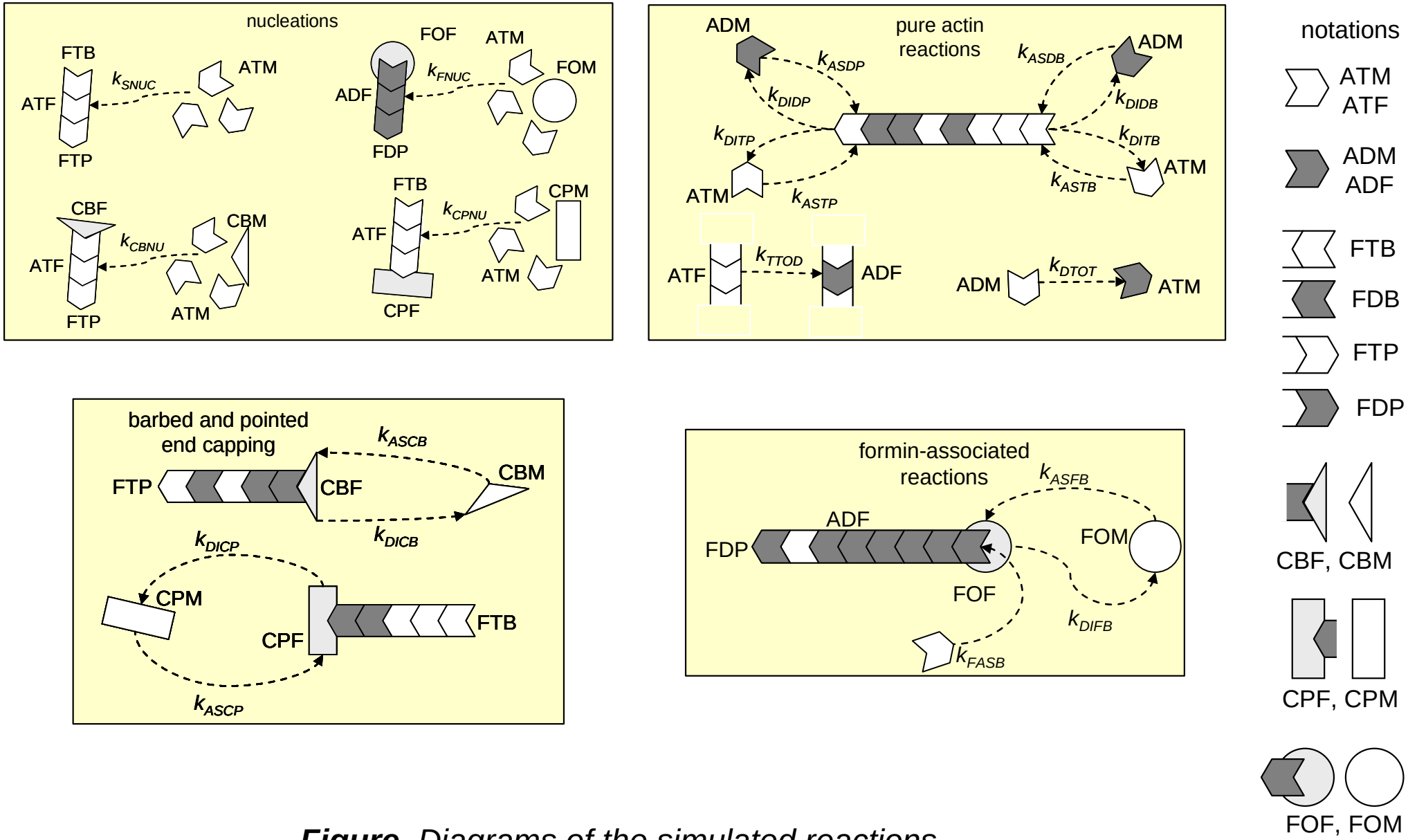
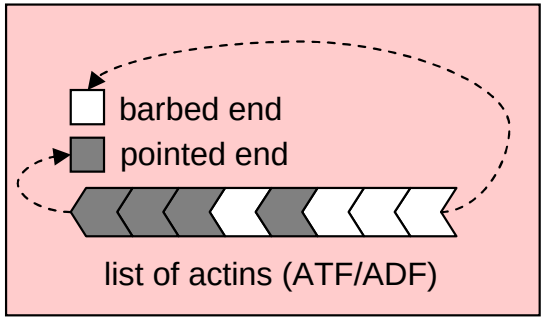


Figure. *Diagrams of the simulated reactions*

BIOCHEMICAL REACTIONS

Actin System Representations

- Structurally-resolved filament model



number of molecules

ATM
ADM
CBM
CPM
FOM

ATF
ADF
FTB
FDB
FTP
FDP
CBF
CPF
FOF

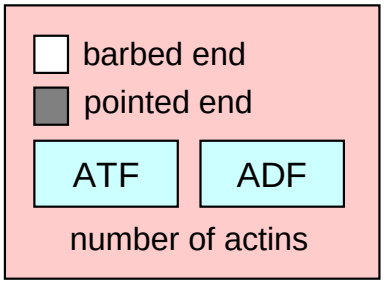
Filament 1
- number of ATF
- number of ADF
- type of the barbed end
- type of the pointed end
- bidirectional list of actins (ATF or ADF), representing filament structure
- pointer to the barbed end of the list
- pointer to the pointed end of the list

…

Filament *n*
- number of ATF
- number of ADF
- type of the barbed end
- type of the pointed end
- bidirectional list of actins (ATF or ADF), representing filament structure
- pointer to the barbed end of the list
- pointer to the pointed end of the list

considered volume v

- Simplified filament model



number of molecules

ATM
ADM
CBM
CPM
FOM

ATF
ADF
FTB
FDB
FTP
FDP
CBF
CPF
FOF

Filament 1
- number of ATF
- number of ADF
- type of barbed end
- type of pointed end

Filament 2
- number of ATF
- number of ADF
- type of barbed end
- type of pointed end

…

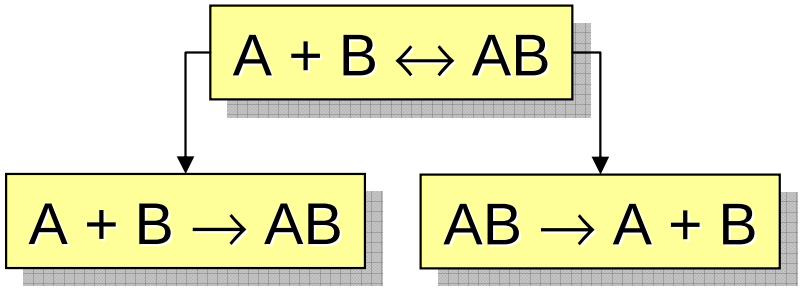
Filament *n*
- number of ATF
- number of ADF
- type of barbed end
- type of pointed end

considered volume v

BIOCHEMICAL REACTIONS

Simulation of Reaction: Monte Carlo Approach

- Gillespie algorithm (stochastic discrete-event simulation)
  - separate complex interaction into unidirectional simplest ones;
  - introduce concentration and experimental rate constants, k_i not dependent on concentration;
  - calculate occurrence times of events (reactions), based on k_i and number of molecules
  - take the case with minimal t_i



k_1, N_A, N_B $\qquad$ k_2, N_{AB}

$$t_1 = -\left(k_1 N_A N_B\right)^{-1} v \cdot \ln\left(\xi_1\right)$$

$$t_2 = -\left(k_2 N_{AB}\right)^{-1} \ln\left(\xi_2\right)$$

$\xi_{1,2}$ – uniform random [0..1]

v – considered volume

IMPLEMENTATION

Simulation & Analysis Software Tools

- ActinSimChem, ActinPyreneFit – developed in Borland C++ Builder 6.0
- Features: fast simulation of actin polymerization without structural representation of filaments
- Analysis of actin-pyrene experiments

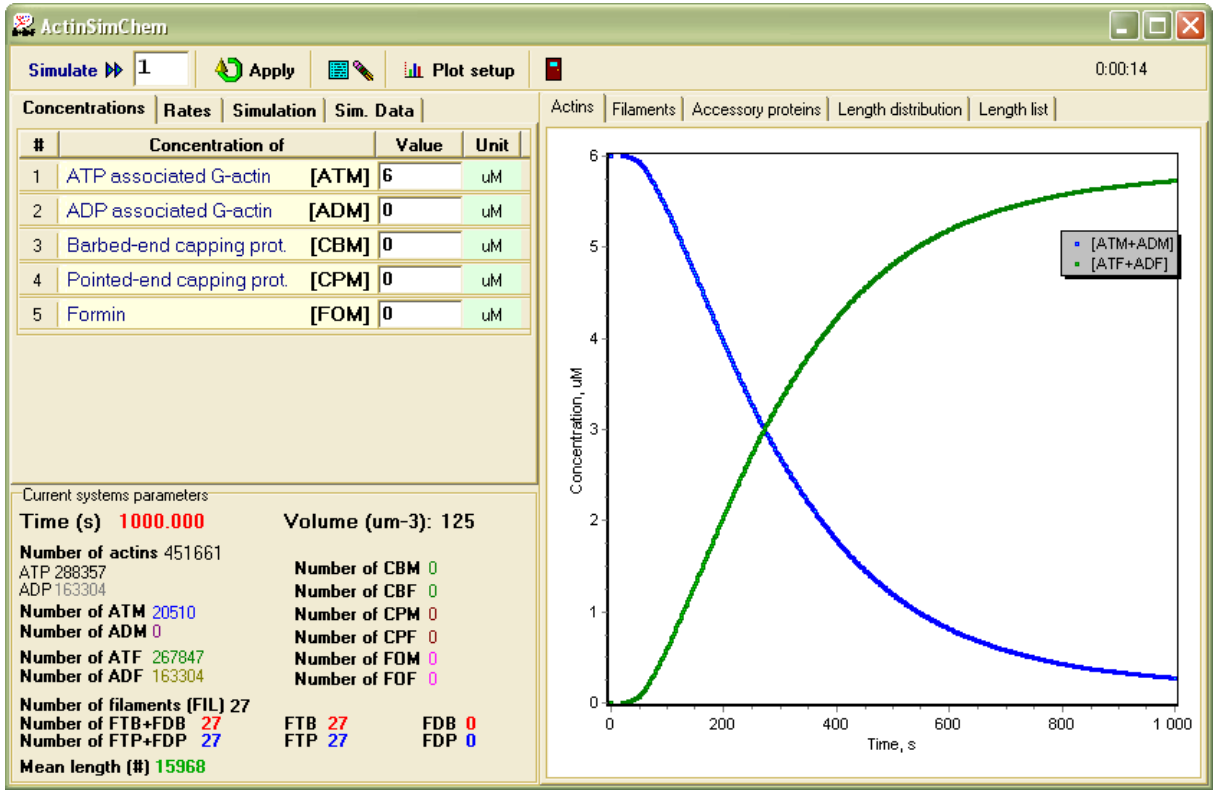
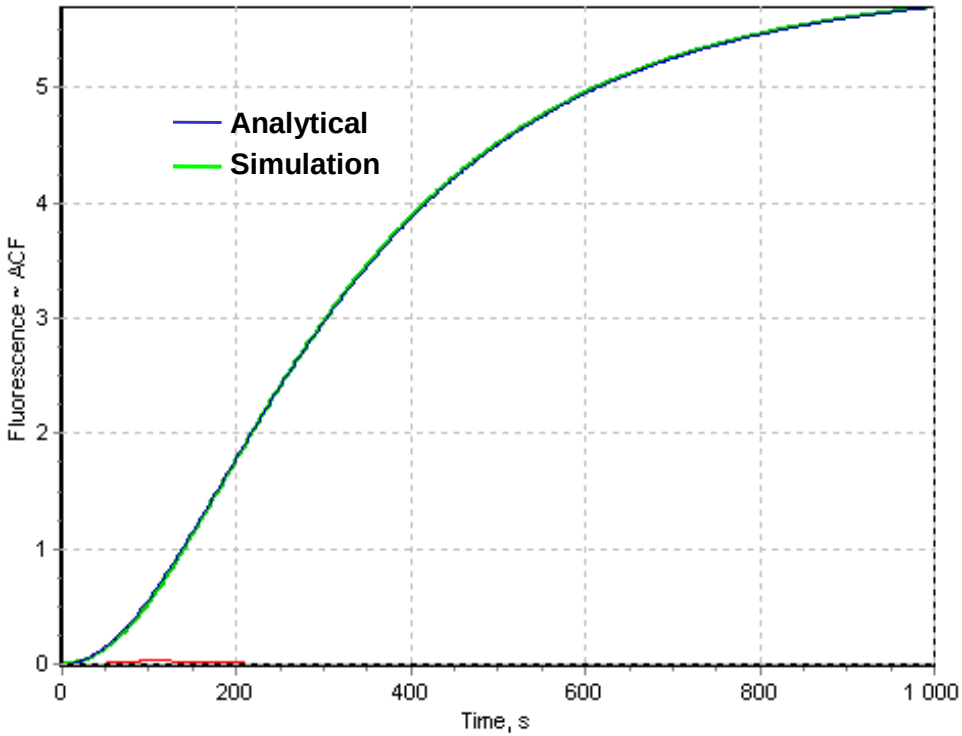
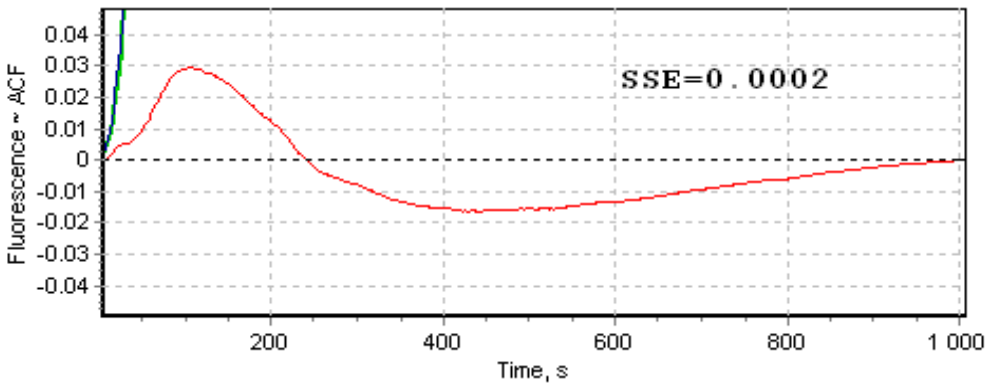


Figure. ActinSimChem screenshot with G- and F-actin concentration dynamics

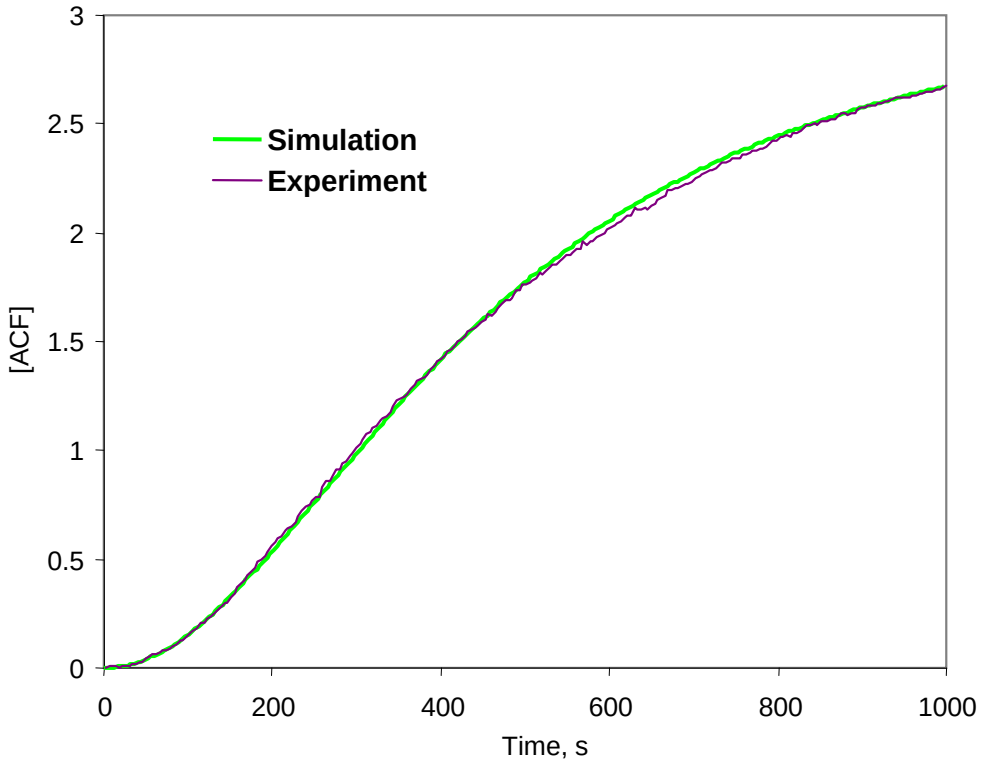
TESTS

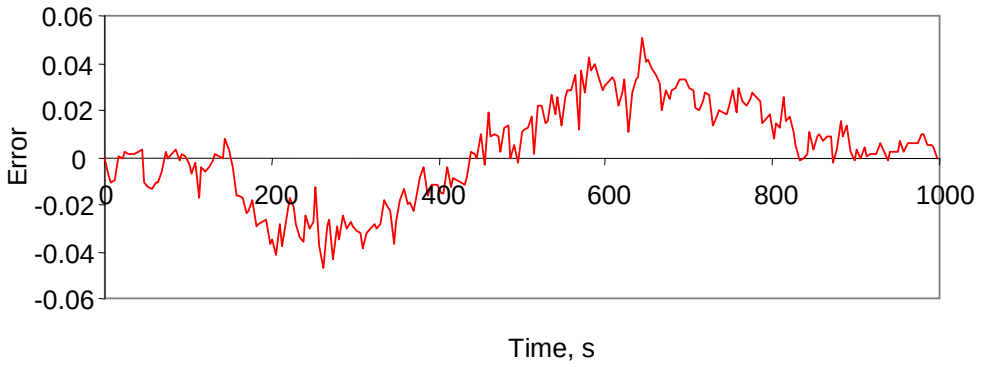
Analytical vs. Simulation





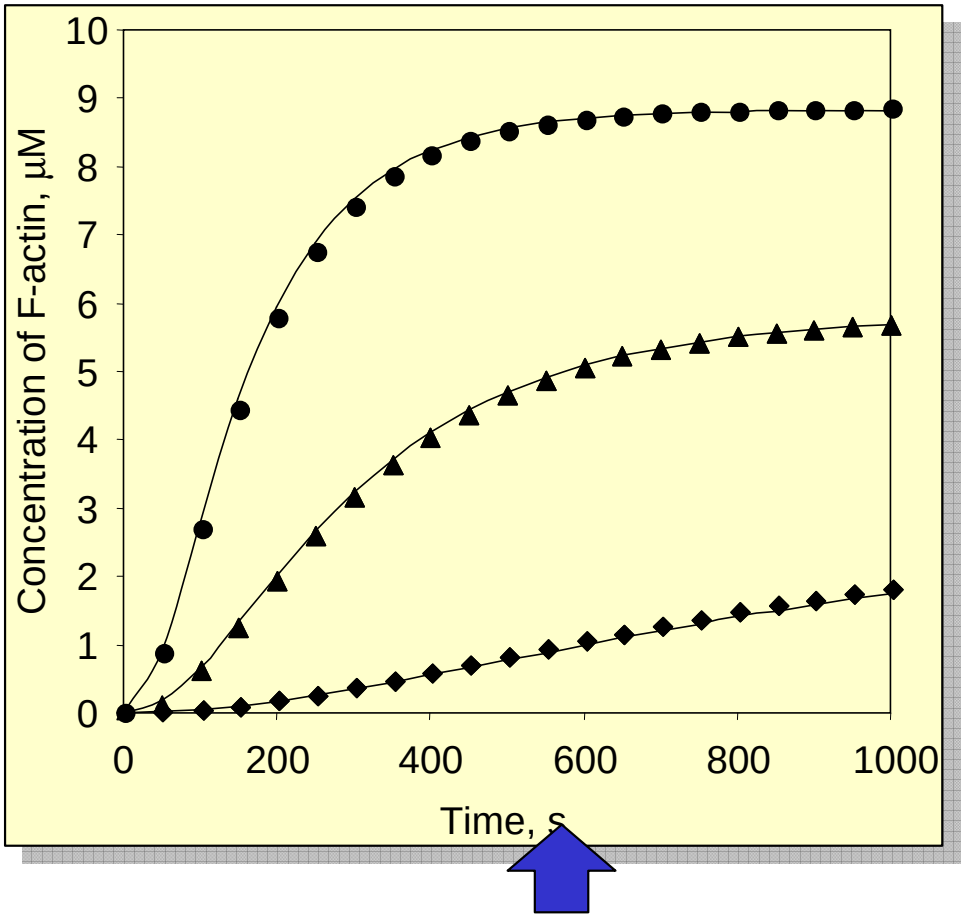
Simulation vs. Experiment

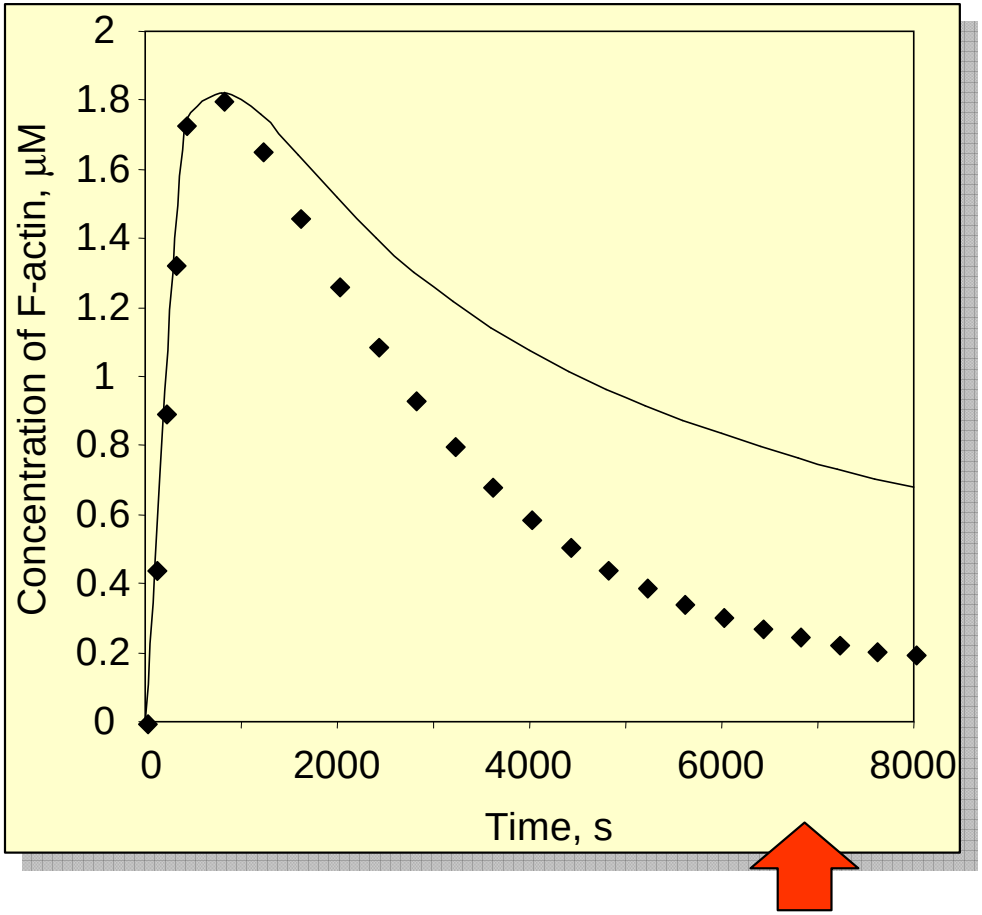




TESTS

Structure-resolved Filament Model vs Non-structure-resolved Filament Model





- Results are the **same** in most of the experimental conditions. **Difference** can be observed only at long time, with the absence of actin ATP recharge

PRELIMINARY RESULTS

Study of the Effects of Different Proteins

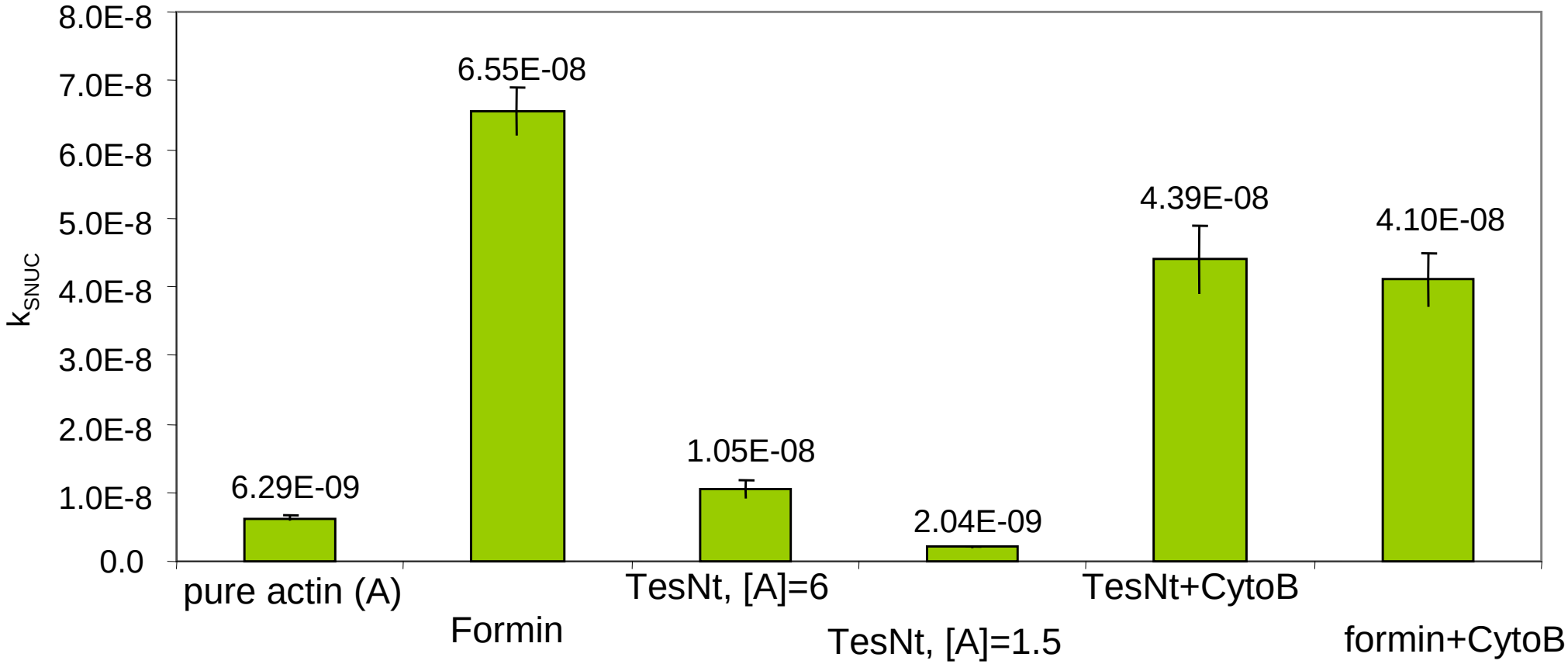


Figure. The determined k_{SNUC} for different experimental systems. *Pure actin* the generalization of several experiments wirh pure actin, [A] = 1.5, 3, 6 μM. *Formin* – known nucleator. *Testin-Nt* (6μM of *actin*), *testin-Nt* (1.5μM of *actin*), *testin-Nt* + *CytoB*, *formin* + *CytoB* – studied systems.

MECHANICAL MODEL

Levels of Modelling

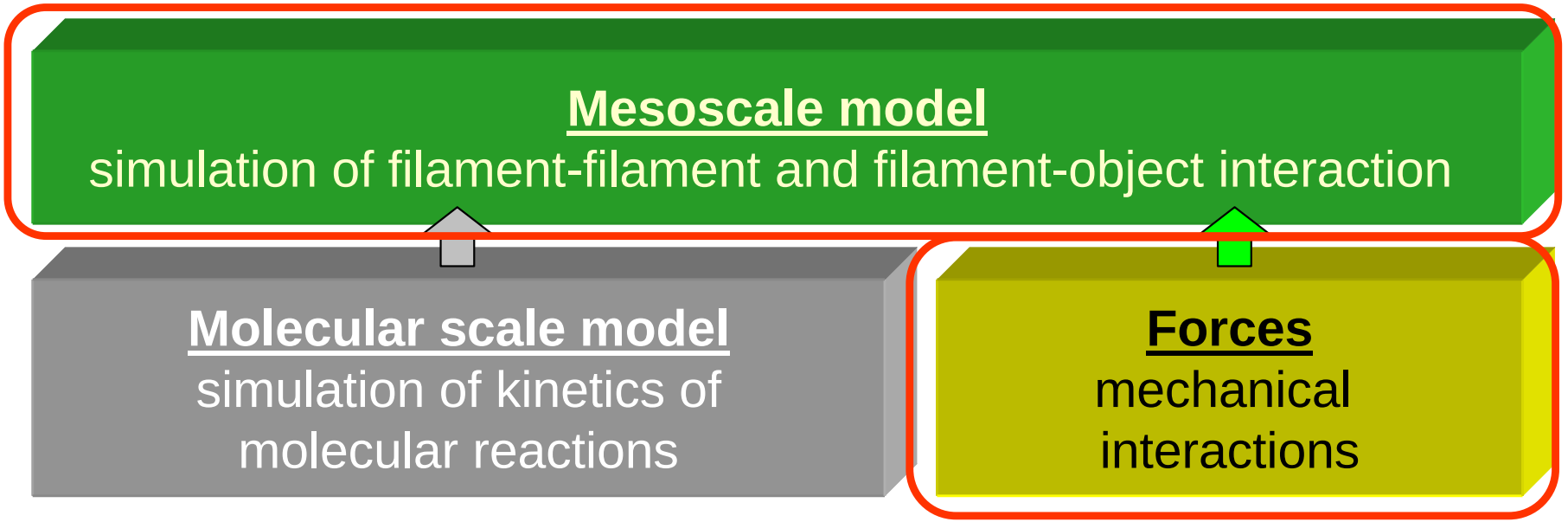


Figure. *Hierarchical organization of the model being developed*

- Features of the mesoscale model
  - filaments and beads are considered as physical objects with mass, coordinates, sizes, velocities, moments, etc.
  - molecules considered in terms of concentrations.
  - **possibility to apply the model directly for the FRAP data analysis**.

MECHANICAL MODEL

Dynamics and Kinematics

- Filaments and bead are considered to be solid bodies.
- Simulation of mechanical interaction during small time *dt* (~10^{-4} s) in accordance with the force model using Newton laws.

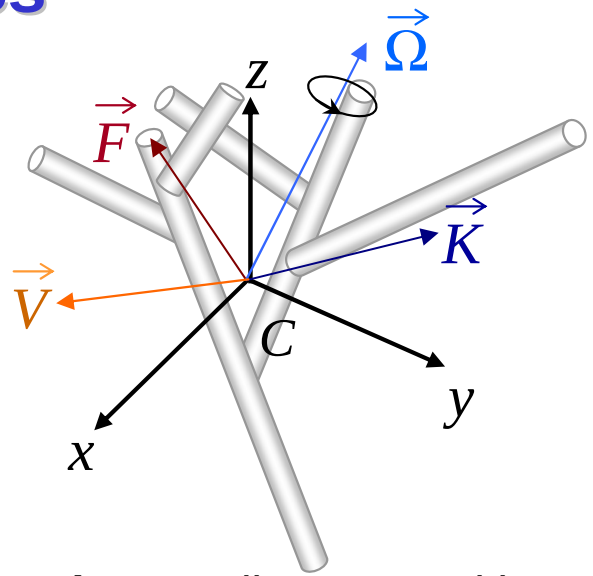


Figure. *Filament and its dynamical parameters*

- Translational motion:

$$\begin{cases} m\vec{a}_c = \vec{F} \\ \vec{a}_c = \dfrac{d\vec{V}_c}{dt} \\ \vec{V}_c = \dfrac{d\vec{R}_c}{dt} \end{cases}$$

- Rotational motion:

$$\begin{cases} \dfrac{d\vec{M}}{dt} + \left[\vec{\Omega} \times \vec{M}\right] = \vec{K}; \\ \vec{M} = \mathrm{I}\vec{\Omega}; \\ \vec{\Omega} = \dfrac{d\vec{\varphi}}{dt}; \end{cases}$$

m, C – mass and mass center;
F – net force;
a – acceleration;
V – linear velocity;
R_c – translation of mass center;
M – moment of force;
K – moment of impulse;
I – inertia tensor;
Ω – angular velocity;
φ – angle of rotation.

MECHANICAL MODEL

Mechanical Interactions

- Types of interactions:
  - Viscous friction
  - Brownian effect (for filaments and bead);
  - Repulsion due to filament crossing
    - Filament-filament crossing;
    - Filament-bead crossing
    - *Attraction due to ActA-filament linkage.*

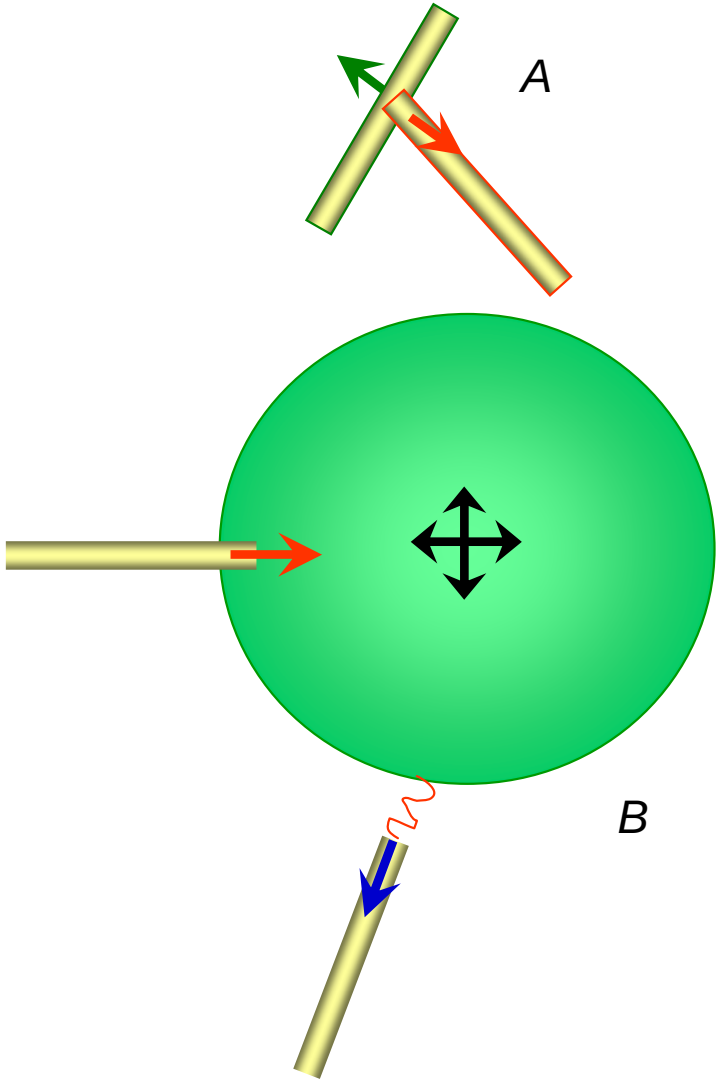


Figure. *Interacting filaments (A) and forces affecting the bead (B)*

MECHANICAL MODEL

Simulation Software Tool ActinSim3D

- Developed in Borland C++ Builder 6.0 environment
- Visualization – OpenGL
- Features:
  - simulation of actin polymerization (simplified reactions)
  - spatial structure of actin network
  - mechanical interaction

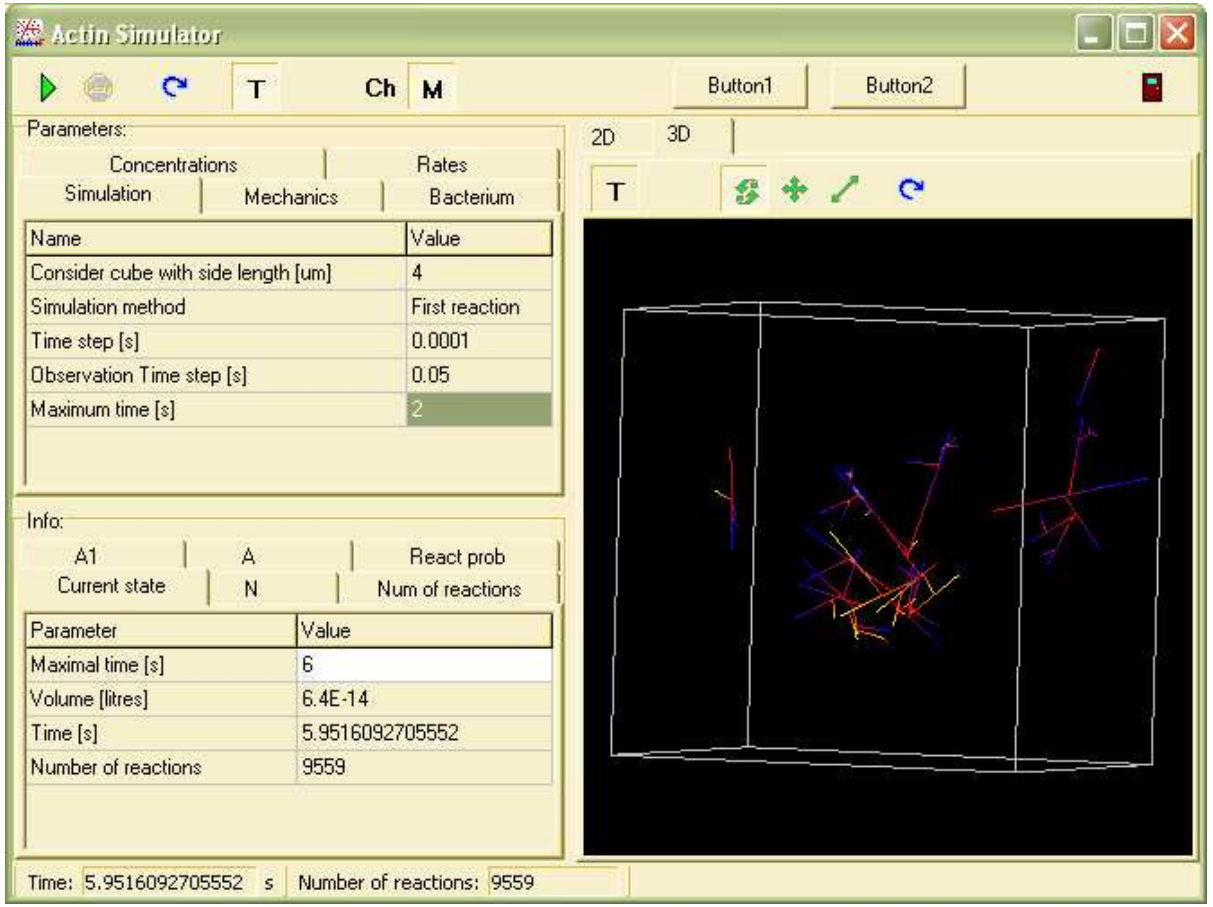


Figure. ActinSim3D screenshot with branched 3D filament structure

MECHANICAL MODEL

Demonstration of ActinSim3D Simulation

- **Parameters of simulation**
  - ACM = 4 μM
  - ARP = 0 μM
  - CBM = 0.5 μM
  - Bead size = 0.4 μm
  - Considered volume 64 μm^3
  - Filament-bead interactions
  - Simulation time 20 s
  - Calculation took ~ 30 minutes

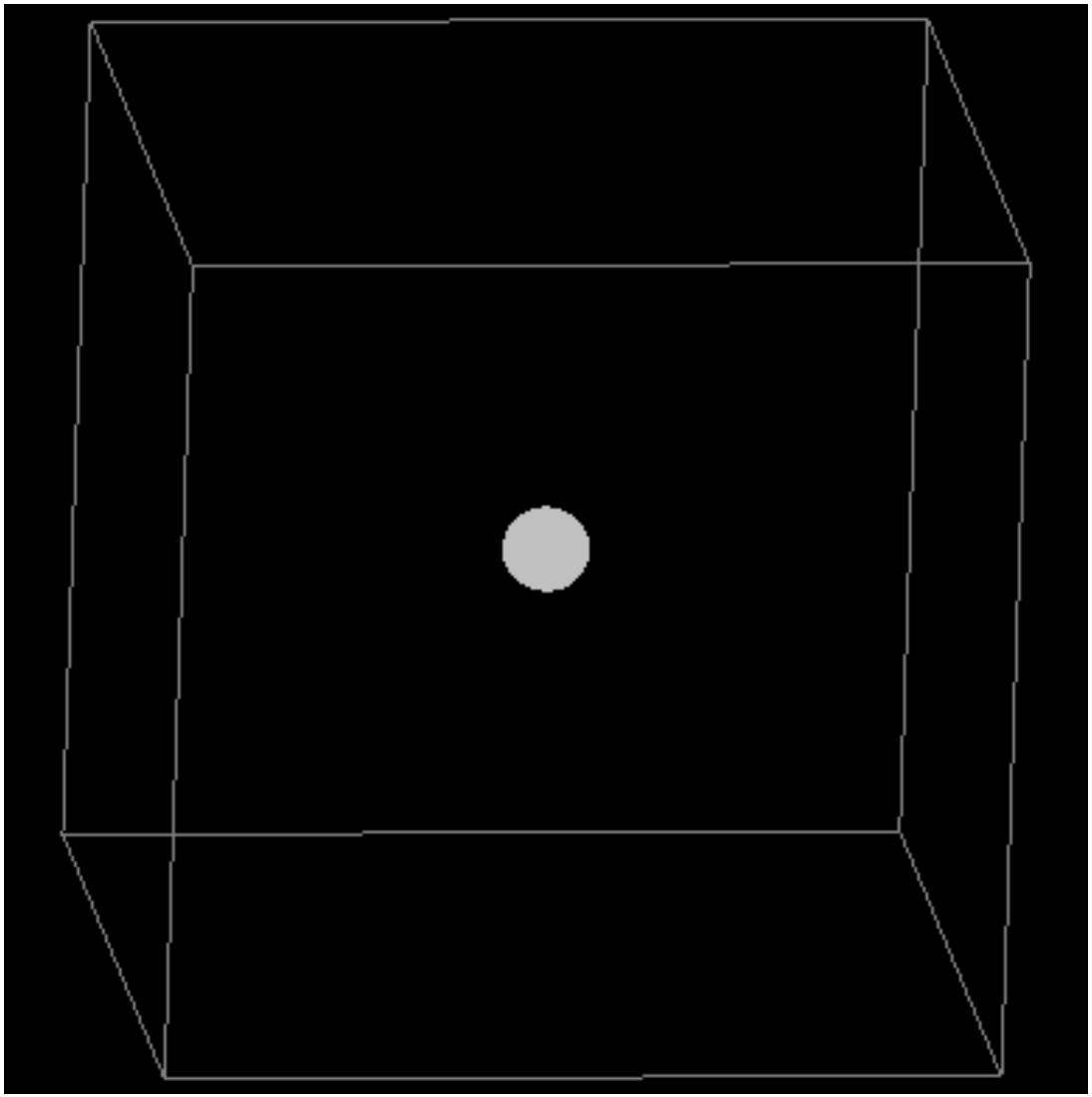


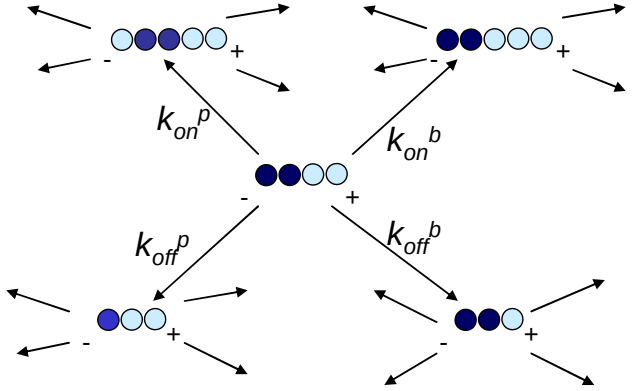
Figure. Animated bead propulsion for the simplified model of reactions

FRAP

Application of Developed Models for FRAP Analysis

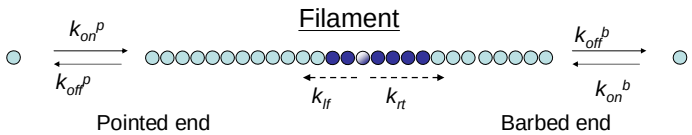
Polymerization model – ways of formalization

- 1. Events on the ends



- Considering all reactions as they do
- A quantity of system states is averaged to obtain an estimation of experimental characteristics
- Used in simulation method

- 2. Bleach state virtual migration



- Looking on actin reactions as virtual migration of bleached actin in average length filament
- Used in analytical model

- The shape of FRAP recovery for bleached actin filaments depends on filament length, rate constants for barbed and pointed ends, filament concentration
- Size of the bleach spot can influence in the case of specific ordering of filaments
- In some cases polymerization processes can be approximated by binding and diffusion models

- Performed by **Alexander Halavatyi** under the supervision of dr. M. Yatskou and prof. E. Friederich

SUMMARY

- The simplified actin filament representation is valid and can be used for analysis of actin-pyrene experiments via simulation-based fitting approach

- Being properly analyzed, actin-pyrene experiments can provide important information about actin systems (*knuc*, *kon, koff*)

- Monte Carlo modeling of actin polymerization and actin-based motility are challenging, but realizable tasks

- Current state: application of the developed structure-resolved model for the analysis of FRAP data (A. Halavatyi, M.Yatskou)

CONCLUDING REMARKS

- Combination of fluorescence techniques (FRET, FRAP, FLIM, etc) and advanced data analysis methods allows to obtain novel information about proteins and cellular processes

- Simulation-based fitting plus global analysis is a powerful tool to study complex biomolecular systems and processes

- **The integration of Biology and Informatics is mutually beneficial**. Examples are – systems biology, bioinformatics and biostatistics from one side, and neural networks and genetic algorithms from another ☺.

ACKNOWLEDGMENTS

Belarusian State University, BY
Department of Systems Analysis
- Prof. Vladimir Apanasovich
- Eugene Barsukov
- Alexander Halavatyi

Wageningen University, NL
Laboratory of Biophysics
- Dr. Marcus Hemminga
- Rob Koehorst

Luxembourg University, LU
Faculté des Sciences, de la Technologie et de la Communication
- Prof. Evelyne Friederich
- Dr. Mikalai Yatskou