Short Visit Report (18 March - 2 April 2007)

# Actin Polymerization Project

## Model validation. ActinPyreneFit and its application to the data analysis

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### 1. Tasks for the visit

The following tasks have been set for the visit 18 March – 2 April 2007.

1. Validate developed models for the reactions on experimental data. Check for technical inaccuracies and possible correlations between parameters.
2. Work on the article. Write down the urgency and novelty of the developed approach.
3. Develop the numerical tests for the models
4. Bridge the current project with future research (ESF, etc.)

### 2. Novelty of the approach (part of the article)

This work is devoted to the developing of models and simulation-based formalism of data analysis to study the actin polymerization. The research is stimulated by two reasons: (1) developing of the adequate simulation model for prediction of the effects of different actin-related proteins (capping proteins, nucleators, Arp2/3, severing proteins, etc); (2) application of the developed models for the analysis of experimental time-resolved data (pyrene-actin experiments).

Despite a big number of theoretical works related to actin polymerization, the formalism for the modeling of the processes in not completely developed. The existing analytical models are obtained for the special cases and preserve some natural limitations (however they are successfully used for to obtain bright results for these special cases). For instance the model for filament capping and branching of Carlsson (Carlsson, 2005; Carlsson et al., 2004) clarify the way of filament branching. In this works three models were considered and validated: barbed-end branching, side-branching and side branching with aging of F-actins (the last models showed best prediction). However, the strict quantitative correspondence between analytical predictions and experimental data for the system with capping and branching is questionable (see Fig. 7 in (Carlsson et al., 2004)). Most of analytical models were developed to estimate parameters in specific systems and cannot by used in a general case (see (Romero et al., 2004), etc). Moreover, the specificity of the developed analytical models significantly hampers their validation: the observed effect can be the real property of the studied system, as well as the result of a logical inaccuracy.

Therefore it is important to develop the computational simulation models, which can be used in a general case to predict the behavior of actin systems from the first principle. Although such models have been presented in some works, for instance (Alberts and Odell, 2004), (Carlsson,

2001), the lack of a systematic consideration of the simulation modeling of actin polymerization still exists. The model of Alberts and Odell (Alberts and Odell, 2004) has been developed mainly to study the mechanical properties of actin filament-bacteria interactions, and their paper does not contain the description of the simulation model for the related biochemical processes (polymerization, branching, capping). Therefore it is too approximate and rough to be used to study this processes in detail. The model presented by Carlsson (Carlsson, 2001) is based on a rather time-consuming simulation algorithm (which is by a factor of ~100 slower then the method used here), and can be significantly enhanced using Gillespie's (Gillespie, 1977) and Gibson-Bruck (Gibson and Bruck, 2000) approaches.

Important and still open task is developing methodology for application of the simulation models directly for data analysis. This task is used in biophysics, when the studied systems and processes cannot be described analytically with satisfactory precision. In such a case computational simulation models can be used to fit the data as was described early (Nazarov et al., 2004; Nazarov et al., 2006; Yatskou et al., 2001). In the current paper we are aimed at models development for the analysis of the experimental data coming from actin-pyrene experiments: the time-resolved evolution of pyrene fluorescence, which is proportional to the concentration of F-actin in a solution (Mahaffy and Pollard, 2006). There is no evidence in literature of such application of the simulation models of actin polymerization.

In the current work we also propose rather general formalism to simulation of actin polymerization, which, being realized as a software tool, should in principle allow to a biologist to build his own systems by fast and vivid combination of proposed reagents and interactions.

## 3. Simplest model for actin polymerization and its validation

### *3.1. Analytical model*

The simplest possible model for actin polymerization includes 3 reagents (G-actin, F-actin and filament nucleation sides) and 3 reactions (nucleation, association, dissociation). Using the standard names (see Appendix) this reactions can be presented by the following differential equations[1]:

$$\begin{cases} \dfrac{d(FIB)}{dt} = k_{SNUC} \cdot ACG^3 - p_3 \cdot k_{DISB} \cdot FIB \\ \dfrac{d(ACF)}{dt} = 3k_{SNUC} \cdot ACG^3 + k_{ASSB} \cdot FIB \cdot ACG - k_{DISB} \cdot FIB \cdot (1 + 2\,p_3) \end{cases} \quad (1)$$

[1] Note that instead of $k_{FORM}$ in this report the notation $k_{SNUC}$ is used for the rate of spontaneous nucleation to avoid similarities and inconsistency with nucleation due to formin.

where $p_3$ is the probability to find a filament with length of 3 actins (minimal allowed value for the filament). Therefore $p_3 \cdot FIB$ defines the concentration of such filament in the solution. The expression for the parameter $p_3$ was empirically determined in the following form:

$$p_3 \approx k_{SNUC} \cdot ACG^3 + 2\frac{\sqrt{k_{DISB}}}{k_{ASSB}} \cdot \frac{FIB}{ACF}. \qquad (2)$$

The first item of the Eq. 2 corresponds to the newly nucleated filaments, and the second is in inverse proportion to the average filament length <*l*> =ACF/FIB.

Eqs. 1 describes the following processes: spontaneous nucleation, addition of a monomer to the filament, dissociation of an actin, and complete destruction of the short (length equals to 3) filament due to dissociation of one of the 3 actins.

### *3.2. Comparison of the analytical model with simulation one*

We compared the analytical model (Eqs. 1) with the simulation model developed early. The result is presented in Fig. 1 A, B.

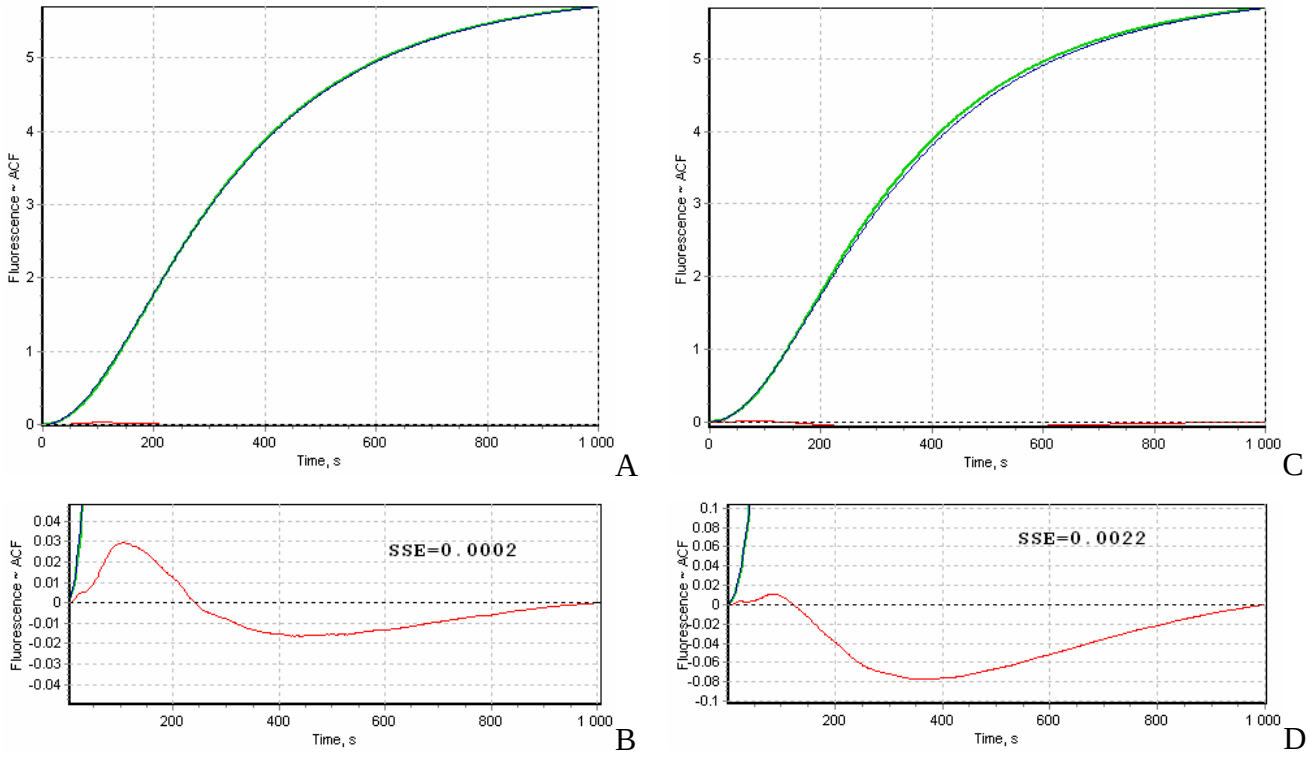



Fig. 1. The comparison of the numerical simulation results (green line in (A) and (B)) and analytical modeling (dark blue lines in (A) and (B)). The experimental conditions: $ACG_0$=6 μM, $k_{SNUC}$=$10^{-8}$ $\mu M^{-2}s^{-1}$, $k_{ASSB}$ =10 $\mu M^{-1}s^{-1}$, $k_{DISB}$ =1 $s^{-1}$, considered volume for the simulation model – cube with side of 15 μm.

(A) shows the results for analytical model given by Eqs. 1, (B) shows the deviations between analytical and simulation models. On plots (C) and (D) the same data are presented for the analytical model from (Carlsson et al., 2004).

The most intricated parameter of the analytical model is the probability $p_3$. To obtain the precise value of this parameter we have to know the filament length distribution at each time moment. To describe this parameter usually the following approximation is used in literature

(Carlsson et al., 2004): in the nucleation reaction ACG concentration is replaced by (ACG – $k_{DISB}/k_{ASSB}$), where the subtrahend $k_{DISB}/k_{ASSB}$ is so called critical concentration. We tested this approach, and found that it leads to a certain inaccuracy of the model (see Fig. 1 C, D). The sum square error (SSE) for the Eqs. 1 is ~ 10 times smaller then for the approximated analytical model.

### *3.3. Comparison of the models with experimental results*

The validity of the models (both simulation and analytical) was tested on the experimental data for actin polymerization. The experimental data was kindly provided by Sandrine Medves. The pyrene-actin data has been fitted by the model as described below ($k_{snuc}$ is modified), and the results of the best fit of the $k_{snuc}= 6.3\times10^{-09}$ is given in Fig. 2. The deviations (Fig. 2 B) are approximately at the order of magnitude of the stochastic noise.

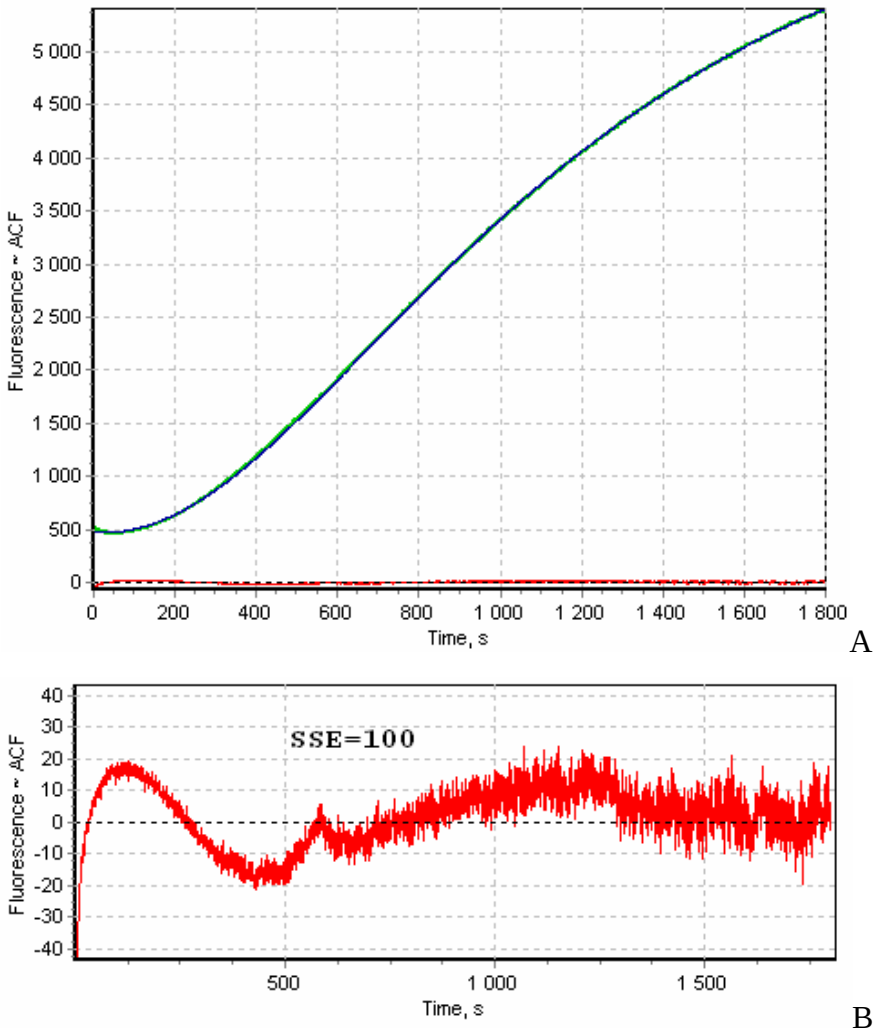



Fig. 2. (A) The comparison of the analytical modeling (dark blue) and experimental data (green) for pure actin system (3 μM actin, data series 16 from Sandrine Medves). Plot (B) presents deviations between the model and experimental results.

## 4. ActinPyreneFit – new software tool and its application to experimental data

### *4.1. Brief characterization*

The analytical model described in section 3.1 was implemented in a software tool ActinPyreneFit for the fast analysis of experimental pyrene-actin fluorescence. The screenshot of the program is given in Fig. 3. To assign the F-actin concentration with the fluorescence 2 additional fitted parameters has been introduced: background fluorescence B and time shift T. The relation between ACF and fluorescence then is:

$$f(t) = (F_0 - B)\frac{ACF(t-T)}{\max(ACF)} + B \qquad (3)$$

where $F_0$ – maximal experimental fluorescence, max(*ACF*) – maximal obtained *ACF*. For $t<T$ *ACF*(*t*) was set to 0.

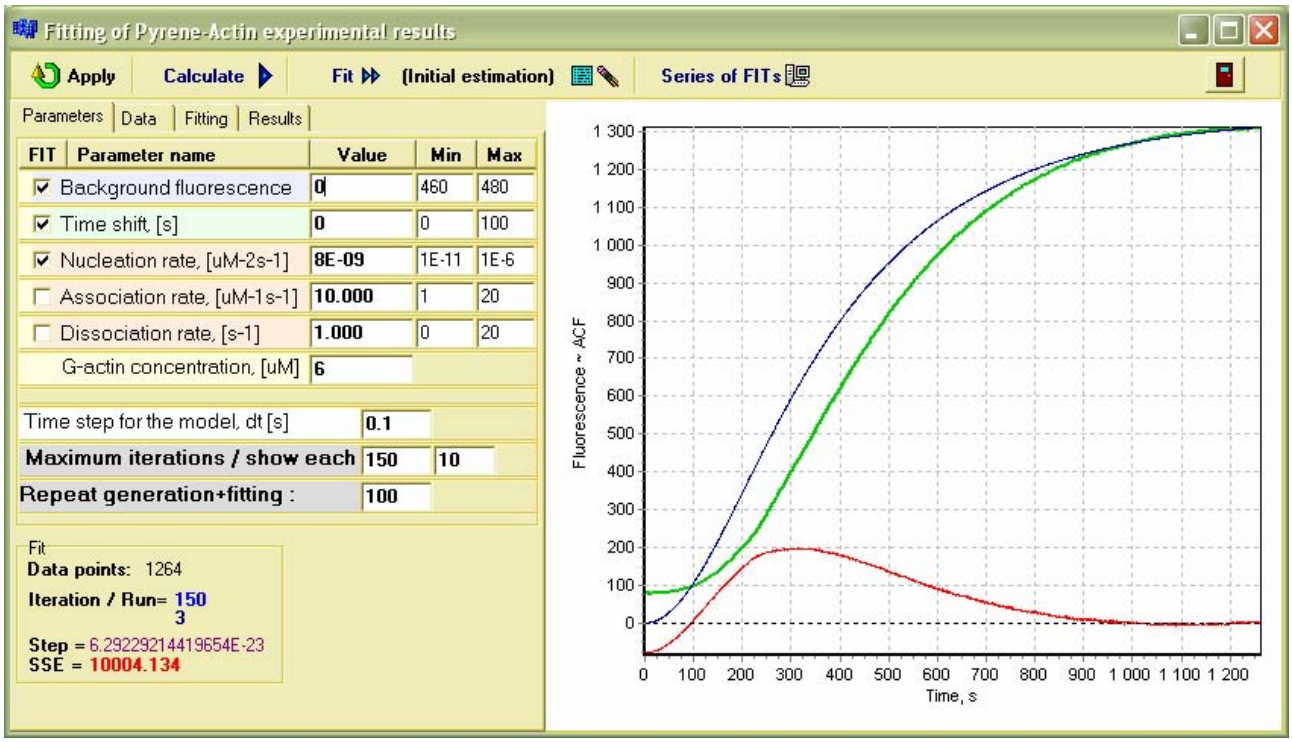



Fig. 3. Screenshot of ActinPyreneFit. On plots green line shows experimental data, blue line – model, red line – deviations (residuals).

The general scheme of work with the software is the following:

- The experimental data (time and fluorescence) should be pasted in the proper windows at the page “Data”. Press [Apply].
- Select the fitted parameters, modify their boundaries and set constant parameters at page “Parameters”. To apply changes press [Apply] or [Calculate].

- Set the number of fit iterations (100 – 200 is enough) and number of independent fits if necessary (“Repeat generation+fitting”).
- To start single fit – press [Fit]. But we suggested to start a series of fits by pressing [Series of FITs].
- Collect the results at the page “Results”. The first value in each row gives SSE for the fit, other gives *B*, *T*, $k_{SNUC}$, $k_{ASSB}$, $k_{DISB}$ respectively. The parameters with minimal SSE should be selected.

### *4.2. Correlation between parameters*

To find possible correlation between fitted rate constants the fitting was performed on the data obtained by the simulation modeling with known rates. The following conclusions have been made after analysis of the results of 200 independent fitting runs.

1. Association rate ($k_{ASSB}$) is noticeably correlated with nucleation rate ($k_{SNUC}$). The correlation is depicted by scatter diagram of the found 80 best solutions in Fig. 4. The relation between the parameters can by approximated by the expression $k_{ASSB} \approx \alpha \cdot k_{SNUC}^{-1}$ .
2. The dynamics of F-actins is not sensitive to the dissociation rate ($k_{DISB}$), therefore its determination via this type of experiments is questionable.

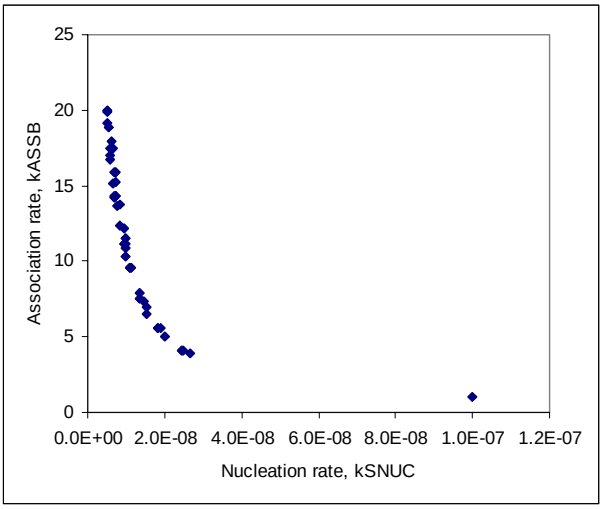
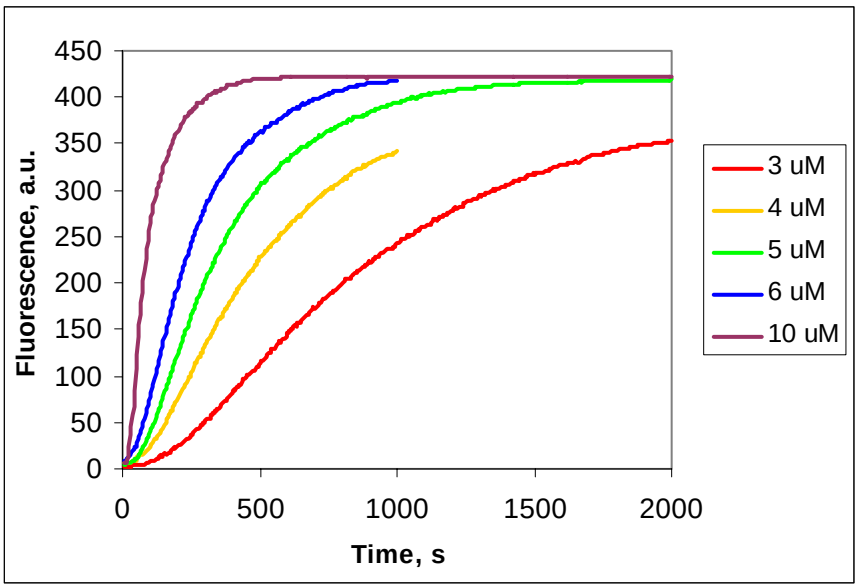


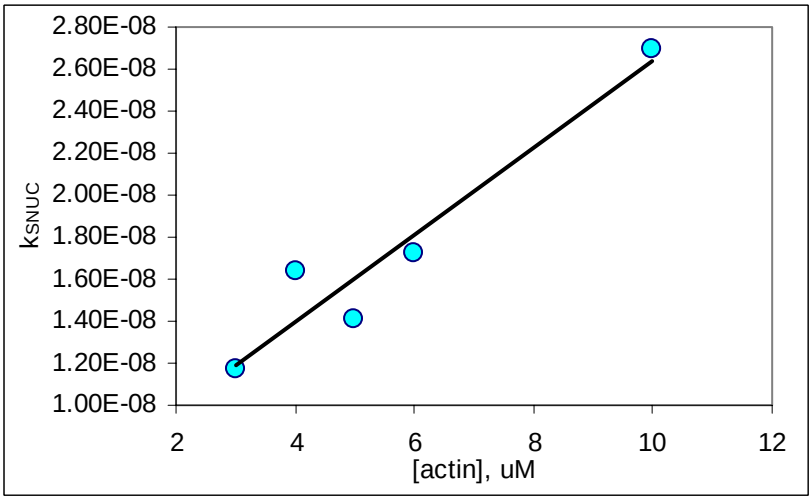

Fig. 4. Scatter diagram of 2 parameters ($k_{ASSB}$ and $k_{SNUC}$) obtained in 80 independent fits.

### *4.3. Some preliminary results of data analysis*

**Sept’s data.** The first series of the experimental data for the analysis was obtained from (Sept and McCammon, 2001) (see Fig. 5 A). The data was fitted by the model with $k_{ASSB}$ =10 $\mu M^{-1}s^{-1}$ and $k_{DISB}$=1 $s^{-1}$.




A




B

Fig. 5. Experimental data for pure actin systems with different concentrations from (Sept and McCammon, 2001) (A).And found $k_{SNUC}$ after fitting of the data with constant $k_{ASSB}$ and $k_{DISB}$. uM stands for μM.

It can be seen that the nucleation rate linearly dependents on the concentration. The reason of this effect is not yet understood. It might be caused by some impurities in the actin stock solution.

**Sandrine's data.** The data were obtained by Sandrine during her experiments with forming, testin and CytoB proteins. To estimate the nucleation rate for the pure actin samples the following data sets have been used: 8, 10, 16, 18, 59. The resulted value and standard deviation are

$$k_{SNUC} = (6.29 \pm 0.46)\times 10^{-9}\ \mu M^{-2}s^{-1}$$

The found nucleation rate was compared with the results coming from data series 1 (formin), 3, 4 (testin-Nt), 7 (testin-Nt + CytoB), and 2 (formin + CytoB). The association and dissociation rates were hold constant $k_{ASSB}$ =10 $\mu M^{-1}s^{-1}$ and $k_{DISB}$=1 $s^{-1}$. The results are summarized in Fig. 6.

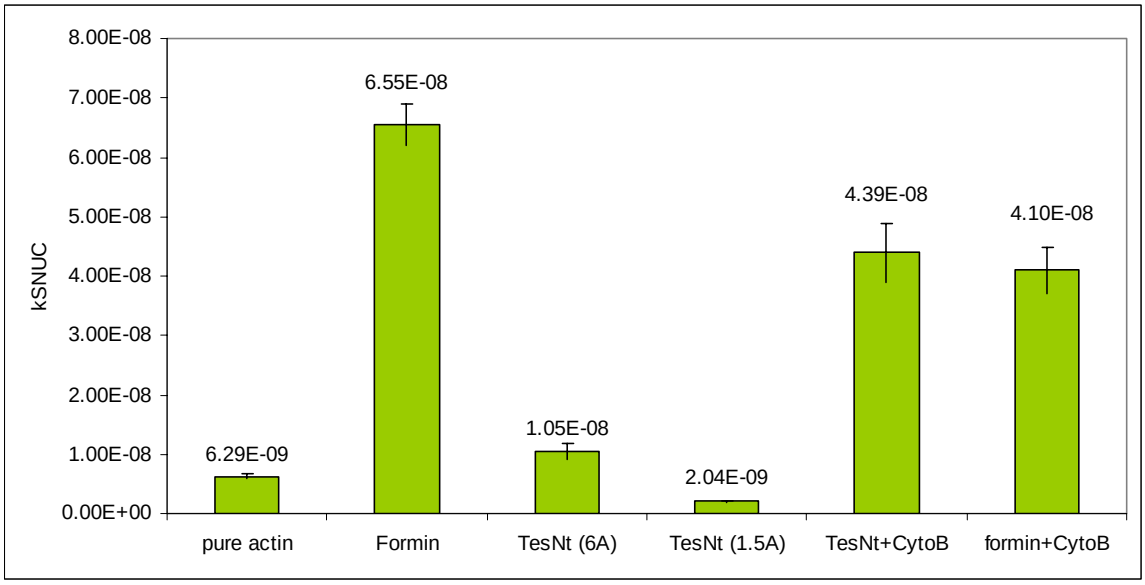



Fig. 6. The determined $k_{SNUC}$ for different experimental systems.

For *pure actin* the 8, 10, 16, 18, 59 series were used, *formin* – series 1, *testin-Nt* (6μM of *actin*) – series 4, *testin-Nt* (1.5μM of *actin*) – series 3, *testin-Nt* + *CytoB* – series 7, and finally *formin* + *CytoB* – series 2.

From the presented results it can be conclude that testin-Nt does not change the nucleation rate. However formin and CytoB have both significant effects on the nucleation.

**Romero's data.** The data obtained by (Romero et al., 2004) on the system containing actin 1.5 μM, and formin (FH2) 40 nM, was fitted, assuming that formin change only the nucleation speed and does not influence the polymerization rate (the association and dissociation rates were hold constant $k_{ASSB}$ =10 $\mu M^{-1}s^{-1}$ and $k_{DISB}$=1 $s^{-1}$). Interestingly the obtained fit was quite good, see Fig 7, with SSE=2.1. Determined $k_{SNUC}$ =1.24×$10^{-7}$ $\mu M^{-2}s^{-1}$. To distinguish between formin effect as a nucleator and stimulator of association, the reference experiment with the same concentration of actins should be performed and analyzed simultaneously.

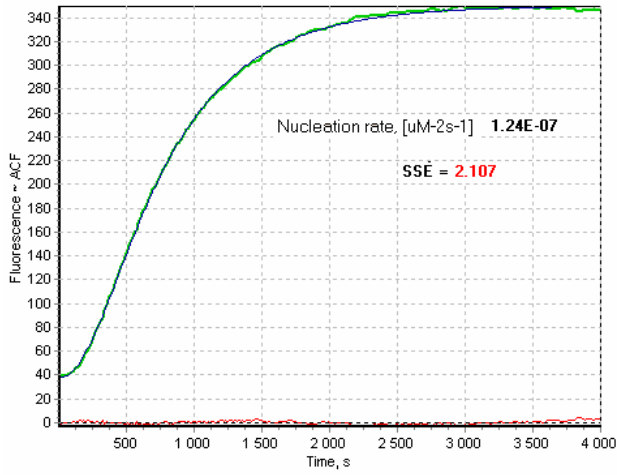



Fig. 7. The obtained fit of the Romero's data (Romero et al., 2004) with $k_{SNUC}$ =1.24×$10^{-7}$ $\mu M^{-2}s^{-1}$ and constant $k_{ASSB}$ =10 $\mu M^{-1}s^{-1}$ and $k_{DISB}$ =1 $s^{-1}$.

## 5. Appendix. Table of reaction rates for actin polymerization processes

Table 1. Notation

| | |
|---|---|
| ***Reagents*** | |
| ACG | G-actin (any) |
| ADG | G-actin with ADP |
| ATG | G-actin with ATP |
| ACF | F-actin (any) |
| ADF | F-actin with ADP |
| ATF | F-actin with ATP |
| FIB | Barbed end (any) |
| FDB | Barbed end with ADP-actin at the end |
| FTB | Barbed end with ATP-actin at the end |
| FIP | Pointed end (any) |
| FDP | Pointed end with ADP-actin at the end |
| FTB | Pointed end with ATP-actin at the end |
| CAP | Capping protein, free |
| CAF | Capping protein, bound at barbed |
| CPF | Capping protein, bound at pointed |
| ARP | Arp2/3 complex, free |
| ARA | Arp2/3 complex, activated |
| ARF | Arp2/3 complex, bound |
| ACT | ActA |
| CAD | Cofilin-ADP-actin |
| PAD | Profilin-ADP-actin |
| PAT | Profilin-ATP-actin |
| PRO | Profilin |
| FOP | Formin, free |
| FOF | Formin in a bound form |
| ***Reactions*** | |
| SNUC | Spontaneous nucleation |
| FNUC | Nucleation by formin |
| ASSB | Association at barbed end |
| ASSP | Association at pointed end |
| DISB | Dissociation at barbed end |
| DISP | Dissociation at pointed end |

Table 2. Reaction speeds

| Reaction | Value | Units | Reference |
|---|---|---|---|
| ***Nucleation*** | | | |
| Spontaneous nucleation<br>3 ACG $\rightarrow$ 3 ACF + FIB + FIP | $2.3\times10^{-11}$ | $\mu M^{-2}s^{-1}$ | (Samarin et al., 2003) |
| Spontaneous nucleation<br>3 ACG $\rightarrow$ 3 ACF + FIB + FIP | $1.05\times10^{-9}$ | $\mu M^{-2}s^{-1}$ | (Carlsson et al., 2004) |
| Capping protein –induced nucleation<br>CAP + 6ACG $\rightarrow$ FIP + CAF + 6 ACF | $2.9\times10^{-5}$ | $\mu M^{-6}s^{-1}$ | (Carlsson et al., 2004) |
| Arp2/3 –induced nucleation<br>ARP + 2ACG $\rightarrow$ ARF + 2ACG | $6.8\times10^{-6}$<br>$8.7\times10^{-5}$ | $\mu M^{-2}s^{-1}$ | (Carlsson et al., 2004) |

| | | | |
|---|---|---|---|
| ***Barbed end actin-filament reactions*** | | | |
| Association ATP-actin at barbed end<br>FIB + ATG → FIB + ATF | 11.5 | $\mu M^{-1}s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Dissociation of ATP-actin from barbed end<br>FTB → FIB + ATG | 1.4 | $s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Association ADP-actin at barbed end<br>FIB + ADG → FIB + ADF | 3.8 | $\mu M^{-1}s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Dissociation of ADP-actin from barbed end<br>FDB → FIB + ADG | 7.2 | $s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Barbed end association<br>FIB + ACG → FIB + ACF | 8.7 | $\mu M^{-1}s^{-1}$ | c. at (Carlsson et al., 2004) |
| Formin-enhanced barbed end grow<br>FOF+ACG → FOF + ACF | 110 | $\mu M^{-1}s^{-1}$ | (Romero et al., 2004) |
| ***Pointed end actin-filament reactions*** | | | |
| Association ATP-actin at pointed end<br>FIP + ATG → FIP + ATF | 1.3 | $\mu M^{-1}s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Dissociation of ATP-actin from pointed end<br>FTP → FIP + ATG | 0.8 | $s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Association ADP-actin at pointed end<br>FIP + ADG → FIP + ADF | 0.16 | $\mu M^{-1}s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Dissociation of ADP-actin from pointed end<br>FDP → FIP + ADG | 0.27 | $s^{-1}$ | (Pollard, 1986)<br>cited at (Alberts and Odell, 2004) |
| Pointed end association<br>FIP + ACG → FIP + ACF | 1.3 | $\mu M^{-1}s^{-1}$ | c. at (Carlsson et al., 2004) |
| ***Capping*** | | | |
| Barbed end capping 1<br>FIB + CAP → CAF | 3.0 | $\mu M^{-1}s^{-1}$ | (Schafer et al., 1996)<br>cited at (Alberts and Odell, 2004) |
| Barbed end uncapping 1<br>CAF → FIB + CAP | $4.0x10^{-4}$ | $s^{-1}$ | (Schafer et al., 1996)<br>cited at (Alberts and Odell, 2004) |
| Barbed end capping 2<br>FIB + CAP → CAF | 8.0 | $\mu M^{-1}s^{-1}$ | (Carlsson et al., 2004) |
| Barbed end uncapping 2<br>CAF → FIB + CAP | 4.2 | $s^{-1}$ | (Carlsson et al., 2004) |
| Pointed end capping<br>FIP + CAP → CPF | ~ 1<br>~0.25 | $\mu M^{-1}s^{-1}$ | (Carlsson et al., 2004) |

| | | | |
|---|---|---|---|
| ***Hydrolysis*** | | | |
| Hydrolysis stage 1 (vectorial model)<br>ATP → ADP-Pi | 12.3 | $s^{-1}$ | (Carlier et al., 1987)<br>cited at (Alberts and Odell, 2004) |
| Hydrolysis stage 2 (vectorial model)<br>ADP-Pi → ADP | 0.0026 | $s^{-1}$ | (Melki et al., 1996)<br>cited at (Alberts and Odell, 2004) |
| Aging of filamentous actins<br>ATF → ADF | 0.0087<br>0.0059 | $s^{-1}$ | (Carlsson et al., 2004) |
| Hydrolysis (by Carlier)<br>ATF → ADF | 60 | $s^{-1}$ | (Romero et al., 2004) |
| Formin-initiated hydrolysis<br>FOP (+ATF) → FOP (+ADF) | 340 | $s^{-1}$ | (Romero et al., 2004) |
| ***Branching*** | | | |
| Arp2/3 activation<br>ACT + ARP → ARA (+ ACT ?) | 0.07 | $\mu M^{-1}s^{-1}$ | (Alberts and Odell, 2004) |
| Arp2/3 – ActA unbinding (deactivation?)<br>ARA → ARP (+ ACT ?) | 3.0 | $s^{-1}$ | (Alberts and Odell, 2004) |
| Active Arp2/3 binding<br>ARA + ACG → ARF + ACF (+ ACT ?) | 0.4 | $\mu M^{-1}s^{-1}$ | (Alberts and Odell, 2004) |
| Inverse reaction for Active Arp2/3 binding<br>???? | 30.0 | $s^{-1}$ | (Alberts and Odell, 2004) |
| Branching at end<br>ARA + FIB + 2ACG → ARF + 2FIB + 2ACF | ~0.43<br>~0.01 | $\mu M^{-3}s^{-1}$ | (Carlsson et al., 2004) |
| Branching at side<br>ARA + ACF + 2ACG → ARF + FIB + 3ACF | $\sim 1.4 \times 10^{-3}$<br>$\sim 4.9 \times 10^{-4}$ | $\mu M^{-3}s^{-1}$ | (Carlsson et al., 2004) |
| ***Actin recharge (ATP-ADP)*** | | | |
| Profilin replaces cofilin in CAD complex<br>CAD (+PRO) → PAD (+ADC) | 2 | $s^{-1}$ | (Mogilner and Edelstein-Keshet, 2002) |
| Cofilin replaces profilin in PAD complex<br>PAD (+ADC) → CAD (+PRO) | 10 | $s^{-1}$ | (Mogilner and Edelstein-Keshet, 2002) |
| Profilin-initiated actin recharge<br>PAD → PAT | 20 | $s^{-1}$ | (Mogilner and Edelstein-Keshet, 2002) |
| ***Severing*** | | | |
| Severing rate (assumption), in 1/μm<br>ACF → ACG + FIB + FIP | 0.5 | $\mu m^{-1}s^{-1}$ | (Mogilner and Edelstein-Keshet, 2002) |
| Severing<br>ACF → ACG + FIB +FIP | $2.3 \cdot 10^{-6}$ | $s^{-1}$ | (Carlsson, 2006) |

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