

# Simulating signaling pathways: the motile photoresponse of *H. salinarum* as a case study

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## outline

- 1 Why signaling pathways?
- 2 The approach
- 3 Why *Halobacterium salinarum*
- 4 The models
- 5 Results

We adopted a **System Biology** approach with *in silico* stochastic simulation **to compare** the two most representative qualitative **models** proposed to explain the molecular basis of the phototile responses of *H. salinarum*.

The simulation of **signaling pathways** is an important current challenge in Systems Biology.

They show some characteristics difficult to manage by means of the simulation approaches usually adopted for constitutive metabolic pathways.

In particular, they are **multi-scale** with respect to:

- reaction rates
- metabolite concentrations (signal amplification)

The classic simulation approaches are mainly based on **differential equations** systems.

- Difficult or even impossible to solve analytically
- **Critically** sensitive to initial conditions
- If you add new components to the system, it changes qualitatively
- They rely on bulk reactions that **require** the interactions of **millions of molecules**

The **Gillespie's** direct-method approach is more suitable for biochemical simulation.

### Exact Stochastic Simulation of Coupled Chemical Reactions

Daniel T. Gillespie\*

Research Department, Naval Weapons Center, China Lake, California 93555 (Received May 12, 1977)

*The Journal of Physical Chemistry*, Vol. 81, No. 25, 1977

- **Stochastic**
- It obeys to the chemical master equation
- Unlike the case of differential equations, it works correctly for very **small quantities** of molecules

We have developed a **simulator** that provides an optimized exact implementation of the Gillespie's direct-method.

- **Compositional**: several metabolic pathways of a network can be **separately modeled** and **then compounded** without the need of any additional remodeling
- It is **friendly** to be used for rapid perturbative "*what-if*" approaches

The **input** of the simulator is a **formal description** of the model, composed by:

- the **chemical species**
- declaration of every **reaction** with **kinetic coefficient**
- initial **number of molecules** for each species

The input also contains user-specified **dynamic parameters**, whose values can change during the simulation: this allows to represent the **time course of experimental stimuli** in the simulation.

```
587_m, 373_m, 587_mm, 373_mm, 518, 518_m, 587, chey, chey_p,
libero, occupato, intermedio, chea, chea_p, cheb, cheb_p
```

```
$orange, 587_m > 373_m
$blue, 373_m > 518_m
0.5, cheb_p + 518_m > cheb + 518
0.25, 518 > 587
0.25, 587 > 587_m
0.25, 373_m > 373_mm
$r, 373_mm > 587_mm
0.25, cheb_p + 587_mm > cheb + 587_m
4.5, 373_m+chea_p > 373_m+chea
1.99, 587_mm+chea > 587_mm+chea_p
1.99, 518_m+chea > 518_m+chea_p
4.5, 587+chea_p > 587+chea
0.49, chea_p + chey > chea + chey_p
0.49, chea_p + cheb > chea + cheb_p
1.0, chey_p > chey
0.05, chey > chey_p
0.007, libero + chey_p > occupato
0.49, occupato > libero + chey_p
1.0, occupato > libero + chey
-, 80 occupato > 80 intermedio
0.5, intermedio > libero + chey_p
```

```
587_m, 4200
chey, 8500
chea, 4250
cheb, 2000
libero, 80
```

```
$orange, $r, $blue
```

```
0, $orange, 0.1
0, $r, 0.2
0, $blue, 0.01
1, 15.0, $orange, 2.0
1, 15.0, $r, 0.09
1, 35.0, $orange, 0.1
1, 35.0, $r, 0.2
```

The **output** is composed by the time course of:

- the **number of molecules** of each metabolite
- the **propensity** coefficient of each reaction
- the effective **firing** of each reaction

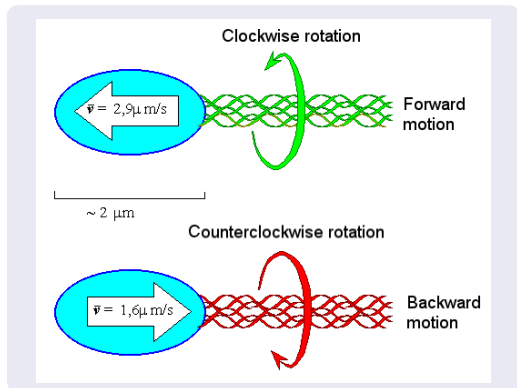
These three time courses allow us to monitor the simulation and check for possible artifacts.

*H. salinarum* belongs to *Archæa* and it is more **simple** with respect to higher organisms. It **swims** by means of flagellar bundles.

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By **switching the rotation verse** of the flagella *H. salinarum* makes a **reversal**, changing the swim direction.

In normal conditions, it makes a spontaneous reversal every 5-50 seconds.



*H. salinarum* reacts to **light stimuli** by altering its motile behavior, significantly **increasing** (**repellent** stimuli) or **decreasing** (**attractant** stimuli) the **frequency** of reversals.

It is structurally simple, but its **photomotile behaviour is very complex** and, for this reason, the **photoperception** in this organism has been widely studied in the past years.

The photoresponse depends on several features of the stimulus:

- **Intensity**
- **Duration**
- **Spectral composition**

We have selected the two most representative qualitative models proposed to explain the molecular basis of *H. salinarum* photoperception:

- the model by **Marwan** *et al.* (M1).

**Mechanism of Photosensory Adaptation in  
*Halobacterium salinarum***

Wolfgang Marwan, Sergei I. Bibikov, Marco Montrone and  
Dieter Oesterhelt

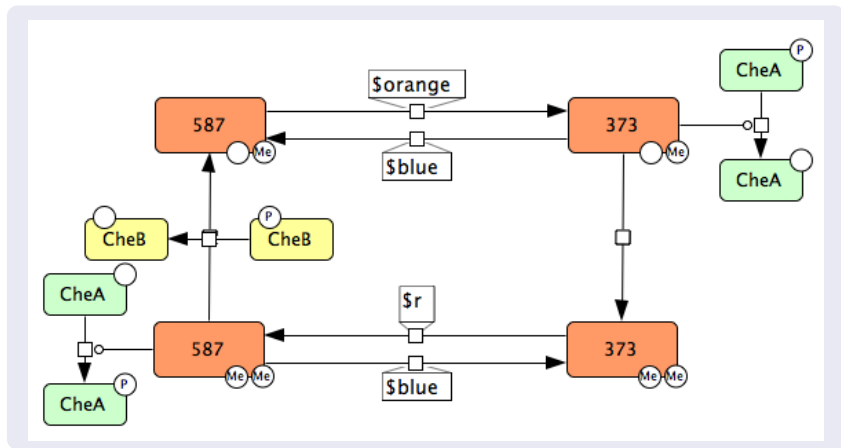
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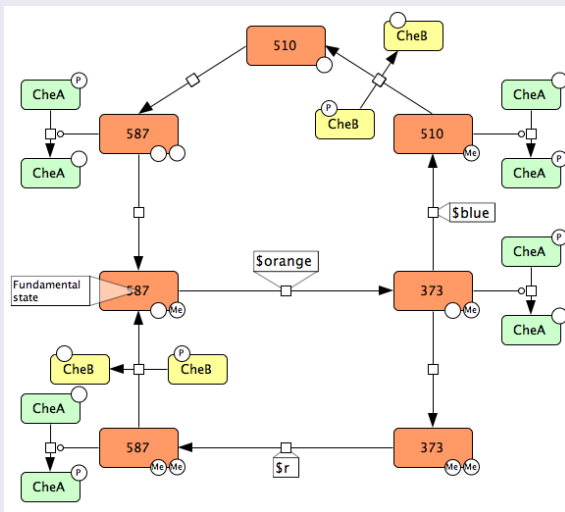
- the model by **Hoff** *et al.* (M2).

**MOLECULAR MECHANISM OF  
PHOTOSIGNALING BY ARCHAEAL  
SENSORY RHODOPSINS**

*Wouter D. Hoff, Kwang-Hwan Jung, and John L. Spudich*

*Annu. Rev. Biophys. Biomol. Struct.* 1997. 26:223–58





```

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libero, occupato, intermedio, chea, chea_p, cheb, cheb_p

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0.25, 587 > 587_m
0.25, 373_m > 373_mm
$r, 373_mm > 587_mm
0.25, cheb_p + 587_mm > cheb + 587_m
4.5, 373_m+chea_p > 373_m+chea
1.99, 587_mm+chea > 587_mm+chea_p
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$orange, $r, $blue

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```

The two models describe in depth the **proper photoperception**, but they do not describe the **transduction chain** of the signal.

We have derived the kinetic constants for the transduction **by analogy** with those measured by **Spiro *et al.*** for the chemotaxis in *E. coli*, the source of data closest to our case study.

### **A model of excitation and adaptation in bacterial chemotaxis**

PETER A. SPIRO<sup>\*</sup>, JOHN S. PARKINSON<sup>†</sup>, AND HANS G. OTHMER<sup>\*‡</sup>

Departments of <sup>\*</sup>Mathematics and <sup>†</sup>Biology, University of Utah, Salt Lake City, UT 84112

*Proc. Natl. Acad. Sci. USA*  
Vol. 94, pp. 7263–7268, July 1997  
Biochemistry

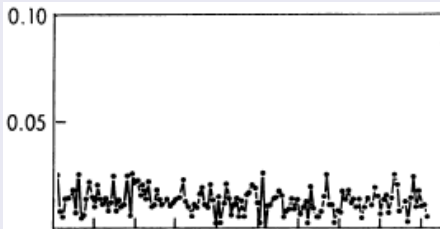
We simulated the behavior of both M1 and M2 in several *in vivo* tested **different irradiation regimes**:

- **dark** (absence of light stimulation)
- **orange flash**
- **long-lasting orange illumination**
- **blue flash during a long-lasting orange illumination**

*In vivo* measurements are executed by counting the number of reversals performed by a population in a given time-interval.

In order to obtain comparable data with *in vivo* measurements, we have integrated the results of 100 single cell simulations for each irradiation regime.

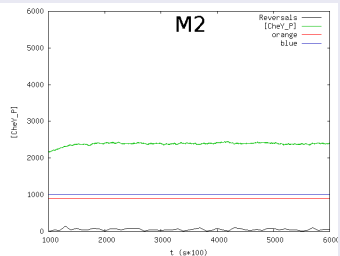
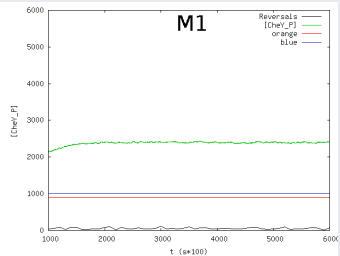
dark



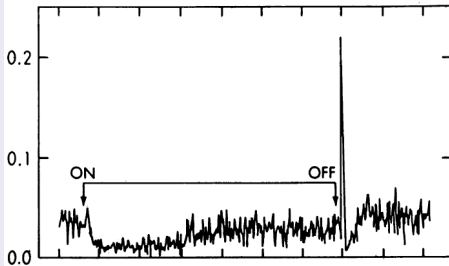
Kinetically Resolved States of the *Halobacterium halobium* Flagellar Motor Switch and Modulation of the Switch by Sensory Rhodopsin I

DONALD A. MCCAIN,<sup>1</sup> LOUIS A. AMICI,<sup>1</sup> AND JOHN L. SPUDICH<sup>1,2\*</sup>

*Department of Anatomy and Structural Biology<sup>1</sup> and Department of Physiology and Biophysics,<sup>2</sup> Albert Einstein College of Medicine, Bronx, New York 10461*



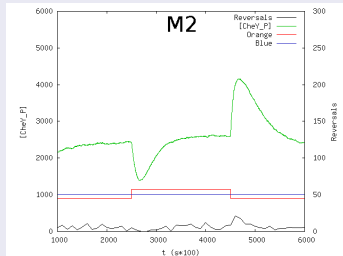
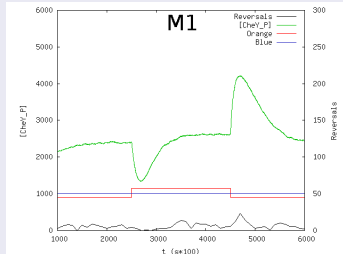
## long-lasting orange illumination



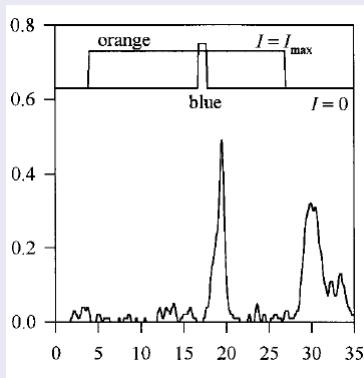
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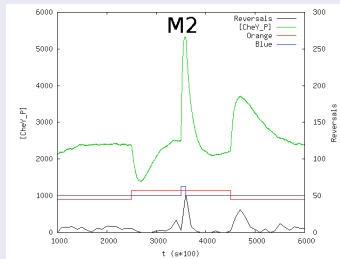
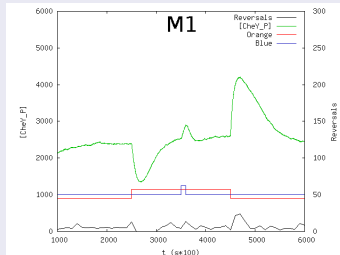


## blue flash during a long-lasting orange



Competition-Integration of Blue and Orange Stimuli in *Halobacterium salinarum* Cannot Occur Solely in SRI Photoreceptor

Giovanni Cercignani,<sup>\*</sup> Andrea Frediani,<sup>†</sup> Sabina Lucia,<sup>†</sup> and Donatella Petracchi<sup>†</sup>  
<sup>\*</sup>Università di Pisa, Dipartimento Fisiologia e Biochimica, I-56126 Pisa, and <sup>†</sup>Istituto di Botanica del CNR, I-56127 Pisa, Italy



In all these cases M1 and M2 have shown a **similar behavior**, qualitatively in **agreement** with *in vivo* observations.

Currently, work is in progress to study **more complex** irradiation regimes (white flash, orange flashes integration, paradox effect) to further validate both the models.

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Giovanni Cercignani <sup>1,3</sup>  
Davide Chiarugi <sup>6</sup>  
Nicoletta Deiana <sup>1,5</sup>  
Salvatore Fabbiano <sup>4</sup>

Claudio Felicioli <sup>2,4</sup>  
Luca Freschi <sup>4</sup>  
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1. CNR – Institute of Biophysics
2. CNR – Institute of Informatics and Telematics
3. Pisa University – Department of Biochemistry
4. Pisa University – Department of Computer Science
5. Scuola Superiore Sant'Anna
6. Siena University – Department of Computer Science

Thank you for your attention!