Biological synchronization
&
The geometry of bacteria colonies

The statistical physics of biological systems

October 12, 2020

Technical information

Simulation ideas:

- Vicsek model & its variants (coll. mot.)
- Reynold model & its variants (coll. mot.)
- Couzin model & its variants (coll. mot.)
- Any hierarchy measure (with examples graphs; you can also *propose* one)
- Voter model, Deffuant model, Axelrod model, etc.
- Kuramoto model

Input: parameters, graph (where applicable)

Topic ideas for presentations:

(~15 min)

- Hierarchy among species (Göksel)
- Neural synchronization

First example of spontaneous synchronization

- Huygens, 1665
- Inventor of pendulum clocks
- Hang two clocks to the same wall
- In half an hour they always regained synchrony
- Opposite wall: one loosing 5 sec a day relative to the other
- *Theory of coupled oscillators*

nature

Synchronized swinging

Ancient pendulum conundrum solved.

SCIENTIFIC REPORTS

OPEN Huygens synchronization of two clocks

Not so obvious: https://www.youtube.com/watch?v=SGgbRkix_hY

First explanation

- Huygens wrote about “sympathy of two clocks” in a letter to his father
- He also provided a qualitative explanation of this effect of *mutual synchronization*;
- he correctly understood that the conformity of the rhythms of two clocks had been caused by an imperceptible motion of the beam.

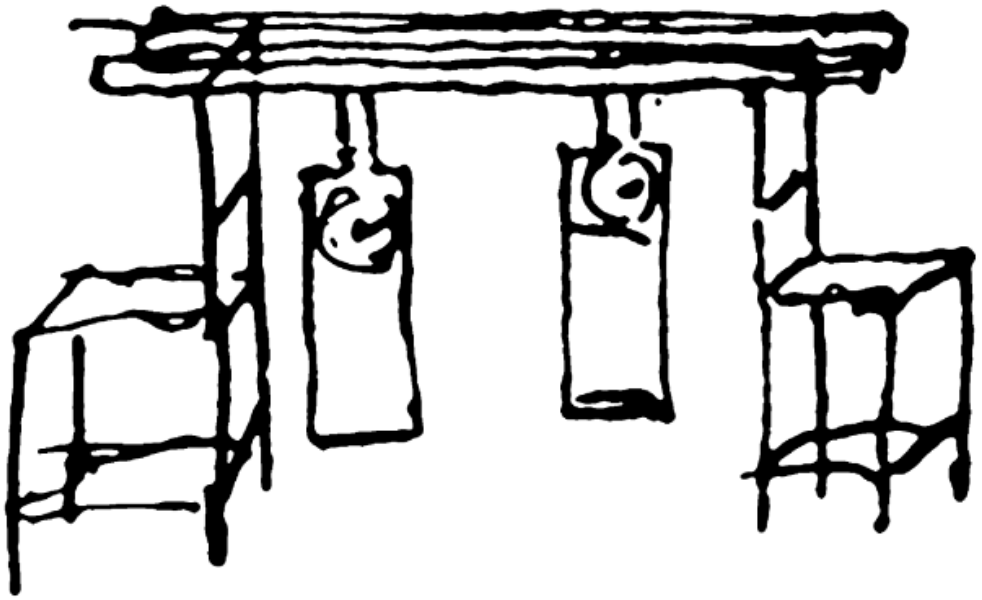

Figure 1.2. Original drawing of Christiaan Huygens illustrating his experiments with two pendulum clocks placed on a common support.

Oscillating metronomes – a demonstration

https://www.youtube.com/watch?v=bl2aYFv_978

- The burst into spontaneous applause
- Human physiology: walking, breathing
- Neuron network
- Pacemaker cells in the heart
- Chirping of crickets
- Fireflies
- Etc.

https://www.youtube.com/watch?v=ZGvtnE1Wy6U

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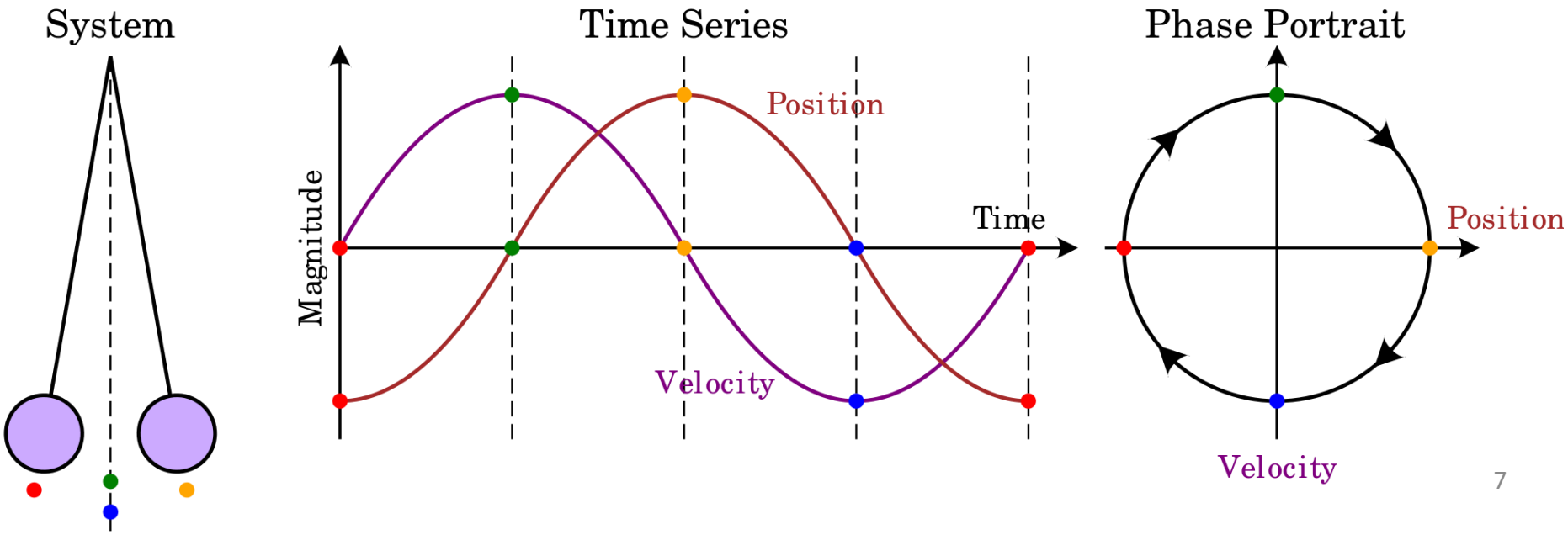
What is an “oscillator”?

- **Definition**: An oscillator is any system that executes periodic behavior.
  - A swinging pendulum: returns to the same point in space at regular intervals; its velocity also rises and falls with clockwork regularity
  - Their trajectories in the phase space are closed curves



First models of biological oscillators

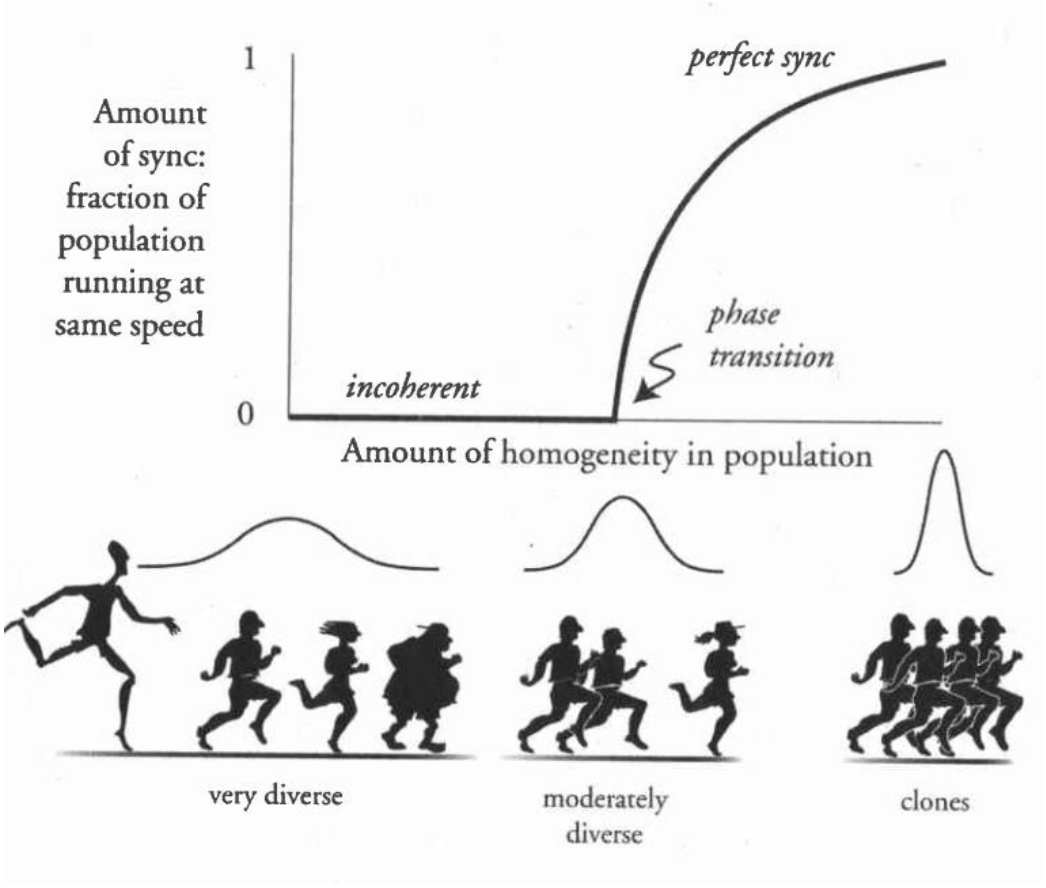
- Arthur Winfree, late 1960s
  - Ignored *all* biological differences and focused on the only common things: the ability to *send* and *receive signals*
  - Complication: both of these are often a function of phase
    - "Influence function" – what signal it sends
    - "Sensitivity function" – how an oscillator responds to the signals it receives
    - ➢ Oscillators can advance or delay, depending on where they are in their cycle when they receive a pulse. (Experiments show that most biological oscillators are like this)
  - ❖ Assumptions:
    - ❖ All the oscillators in a given population have the same influence and sensitivity function
    - ❖ But the natural frequencies can vary, according to a bell shape
    - ❖ Connectivity (the way the oscillators are connected)

Winfree's model - continuation

- Assumed that the oscillators are globally coupled
- Instead of solving the differential equations, he used computer models ("experiment")
  - For some sensitivity-influence function pairs he always got incoherence
  - For other sensitivity-influence function pairs he always got synchronicity

- Another aspect: the distribution of natural frequencies
  - Very diverse: no synch
  - Low diversity: synchronization
  - There is a threshold
    Phase transition
  - Connection between nonlinear dynamics and statistical physics

„frequency pulling"



Kuramoto model

- 1975: solved a simpler, more abstract version of Winfree's model
- Replaced Winfree's influence and sensitivity function with a *sine* function: highly symmetrical rule for Winfree's concept of "frequency pulling"
  - (analogy: jogging friends)
- The model makes several assumptions:
  - the oscillators are identical or nearly identical (bell-shaped distribution of natural frequencies)
  - the interactions depend sinusoidally on the phase difference between each pair of objects.
- Later it has found widespread applications in other fields too (neuroscience, physical systems, etc.)

The Kuramoto model (KM)

- Continuous time and phase
- Consists of a population of *N* coupled oscillators
- Each tries to run independently at its own frequency, while the coupling tends to synchronize it to all the others
  - ϕ_i : the phase of oscillator *i* (in the sense of mod 2π)
  - t : time
  - T_i : periodic time
  - $\nu_i = \frac{1}{T_i}$: frequency
  - $\omega_i = \frac{2\pi}{T_i}$: natural angular frequency
- One oscillator (an oscillator without interaction):

$$\frac{d\phi}{dt} = \omega$$

The Kuramoto model in mean field approximation

- IN GENERAL: *N* coupled oscillators interacting with each others pairwise :

$$\frac{d\phi_i}{dt} = \omega_i + \sum_{j=0}^{N-1} \Gamma_{ij}(\phi_j - \phi_i)\,, \qquad (i, j = 0,1, \dots, N-1)$$

- $\Gamma_{ij}(\Delta\phi)$: interaction, a function with 2π periodicity
- All the oscillators interact with each other the same way (this was the simplifying assumption of Kuramoto):

$$\Gamma_{ij}(\phi) = \frac{K}{N}\sin(\phi)\,, \qquad (i, j = 0,1, \dots, N-1)$$

- K : strength of the coupling
- If $\mathrm{K} > 0 \;\rightarrow \Gamma$ minimizes the phase difference

The Kuramoto model in mean field approximation

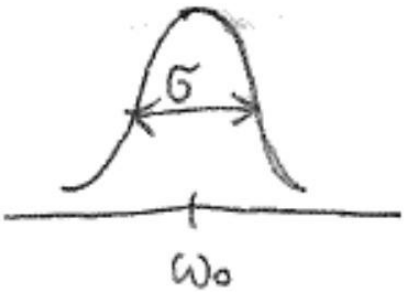
- The basic formula of the KM with MF approximation:

$$\frac{d\phi_i}{dt} = \omega_i + \frac{K}{N}\sum_{j=0}^{N-1}\sin(\phi_j - \phi_i), \qquad (i, j = 0,1, \dots, N-1)$$

- How do such oscillators synchronize?
- The interplay between the coupling strength and the distribution of the natural frequencies determines how many oscillators are synchronized.
- How can we measure the level of synchronization?
  - Order parameter: An order parameter is a measure of the degree of order across the boundaries in a phase transition system; it normally ranges between zero in one phase and nonzero in the other.
- A trivial order parameter can be: $R = \frac{N_S}{N}$, where N_S is the number of synchronized units

Order parameter for the Kuramoto model

- The “Kuramoto order parameter” is more appropriate to monitor the transition towards synchronization)
- Let us assume that
  - the ω_i natural frequencies are taken from a Gaussian distribution $g(\omega)$
  - The expected value of the $g(\omega)$ density function is ω_0, with σ standard deviation



$$g(\omega) = \frac{1}{N}\sum_{i=0}^{N-1}\delta(\omega_i - \omega) = \frac{1}{\sigma\sqrt{2\pi}}e^{-\frac{(\omega-\omega_0)^2}{2\sigma^2}}$$

Defining the order parameter

- Parameter transformation:

$$\Psi_i \coloneqq \phi_i - \omega_0 t$$
$$\omega_i \leftarrow \omega_i - \omega_0$$

(ω_0 : average natural frequency)

- The Kuramoto formula is invariant to the above transformation:

$$\frac{d\psi_i}{dt} = \omega_i + \frac{K}{N}\sum_{j=0}^{N-1}\sin(\psi_j - \psi_i)\ , (i, j = 0,1, \dots, N-1)$$

- $\theta(t)$: the vectorial average of the (transformed) ψ_i unit vectors
- Now we can define the order parameter as next (as the *complex mean field* of the population):

$$z(t) \coloneqq Z(t)e^{i\theta(t)} = \frac{1}{N}\sum_{j=0}^{N-1} e^{i\psi_j(t)}$$

(here i is not the index of an oscillator, but $\sqrt{-1}$)

Defining the order parameter – cont.

$$z(t) := Z(t)e^{i\theta(t)} = \frac{1}{N}\sum_{j=0}^{N-1} e^{i\psi_j(t)}$$

Complex order param. Real part $\frac{1}{N} N \left| e^{i\psi_j(t)} \right|$ =1

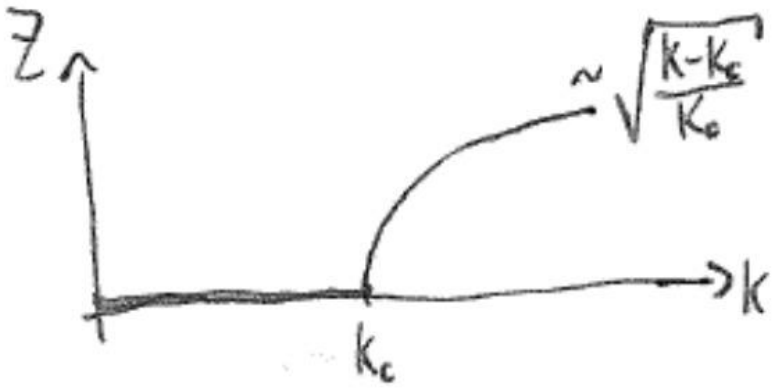
- $Z(t)$ is the real part of $z(t)$, $\rightarrow Z = |z|$
- $Z(t)$ is the *order parameter* with the following properties:
  - Expresses the "closeness" of the ψ_i unitvectors
  - If $Z \approx 1 \rightarrow$ the ψ_i phases are close to each other
  - If $Z \approx 0 \rightarrow$ the ψ_i phases point in random direction

Bifurcation

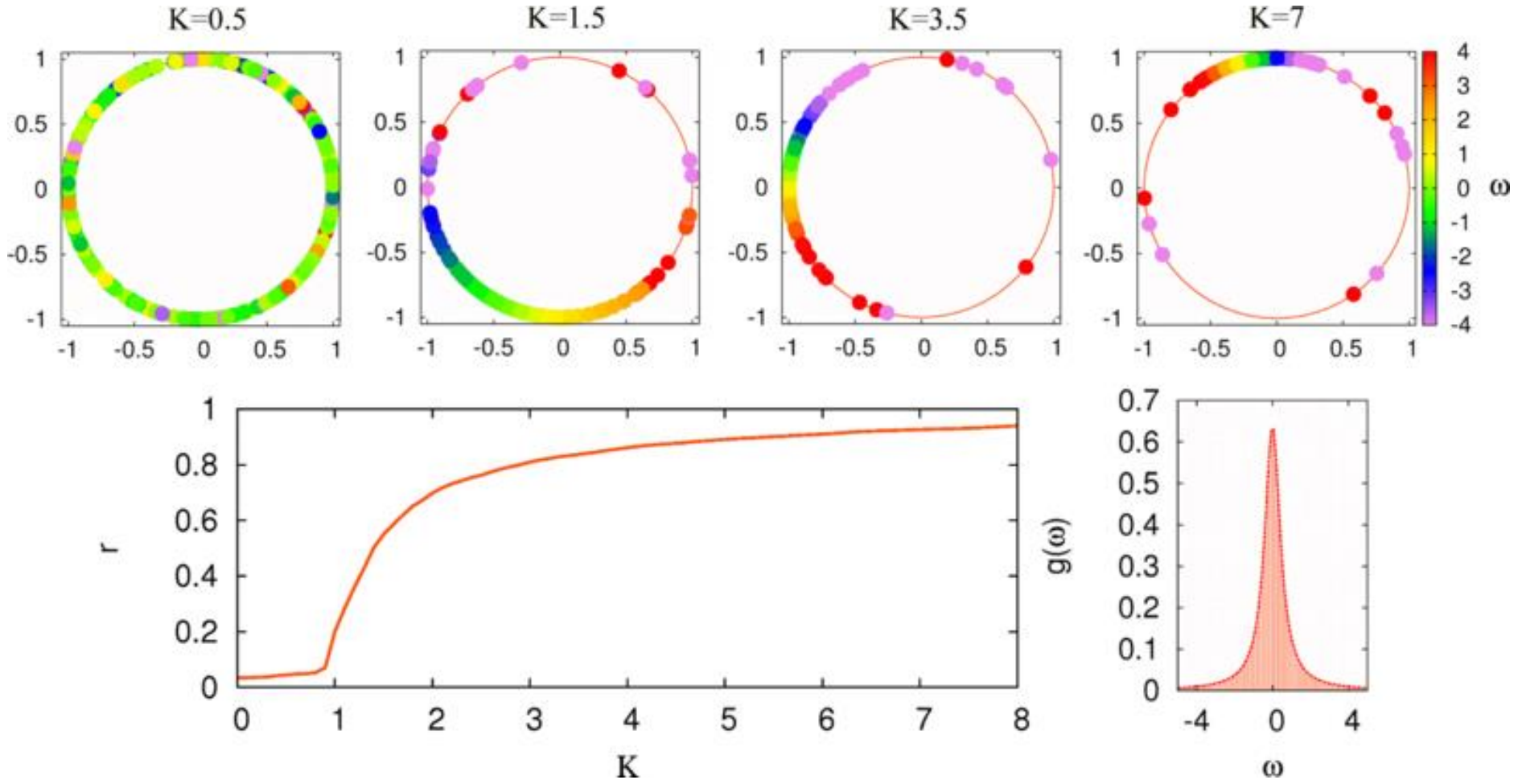
- In the uncoupled limit (*K*=0) each element i describes limit-cycle oscillations with characteristic frequency ω_i.
- Kuramoto showed that, by increasing the coupling *K* the system experiences a transition towards complete synchronization, i.e. , a dynamical state in which $\psi_i(t) = \psi_j(t)$ for $\forall i, j$ and $\forall t$.
- This transition shows up when the coupling strength exceeds a critical value whose exact value is

$$K_C = \frac{2}{\pi \cdot g(\omega_0)}$$

(ω_0 is the mean frequency of the $g(\omega)$ frequency distribution)



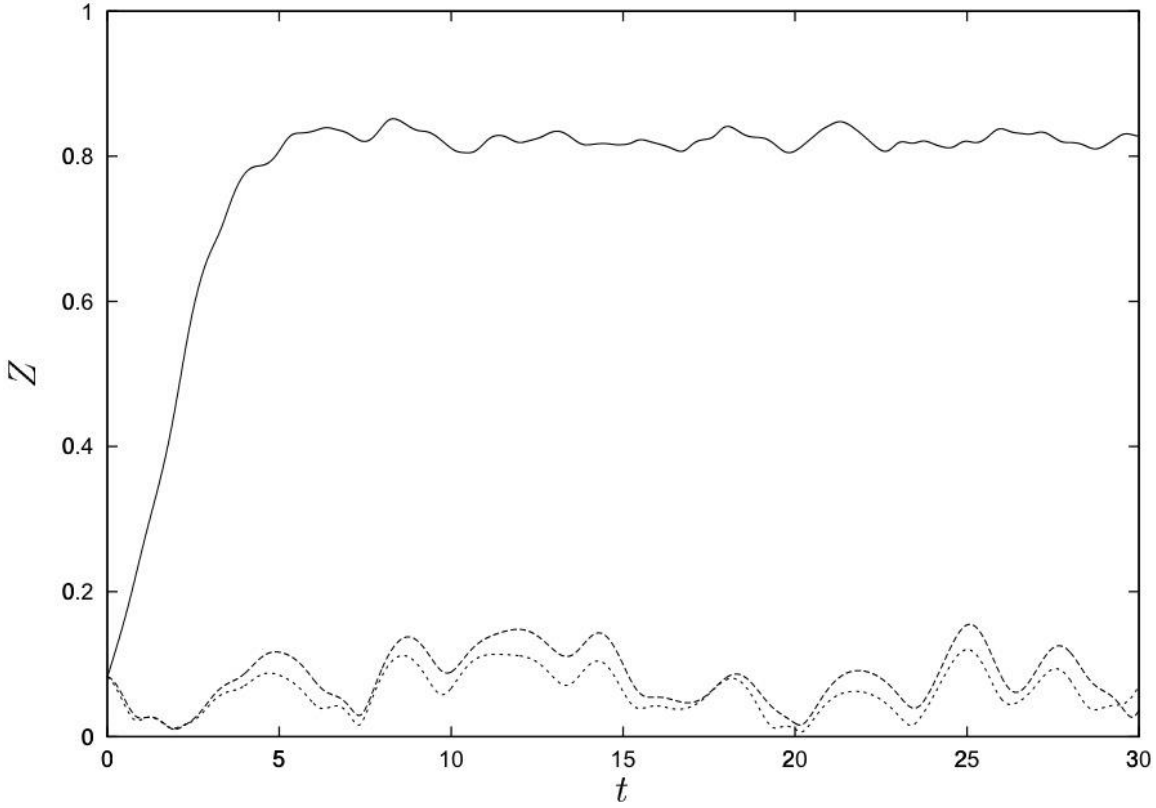
From: Mendoza et al., 2014, Synchronization in a semiclassical Kuramoto model.



Synchronization in the classical Kuramoto model. Each panel on the top shows the collection of oscillators situated in the unit circle (when each oscillator *j* is represented as $e^{i\psi_j(t)}$).
The color of each oscillator represents its natural frequency. From left to right we observe how oscillators start to concentrate as the coupling *K* increases. In the panels below we show the synchronization diagram, i.e. , the Kuramoto order parameter *Z* as a function of *K* . It is clear that $K_c = 1$.

From: Mendoza et al., 2014, Synchronization in a semiclassical Kuramoto model.

Simulation results



Z : order parameter
t : time
N = 200 coupled oscillators
σ = 1
K = 2.5 (top curve),
0.5 (middle curve)
0 (bottom curve)

→ K=0 and K=0.5 (weak coupling) results in similar order parameter

For the region where *Z* is constant

- According to Kuramoto's analysis, based on the definition of the order parameter and on the time evolution of the phases, we get:

$$\frac{d\psi_i}{dt} = \omega_i + KZ\sin(\theta - \psi_i)$$

- A set of one-dimensional uncoupled system!
- In other words: the particle is just interacting with the mean-field (produced by the average)

- But for this you need *Z* to be independent of *t*
  - **Q**: How can it be, given that there are drifting oscillators?
    (*Z*<1 → the synchronization is not perfect → there are "drifting" oscillators)

(Original form was: $\frac{d\psi_i}{dt} = \omega_i + \frac{K}{N}\sum_{j=0}^{N-1}\sin(\psi_j - \psi_i)$)

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  - **A**: The oscillators form a stationary distribution on the circle

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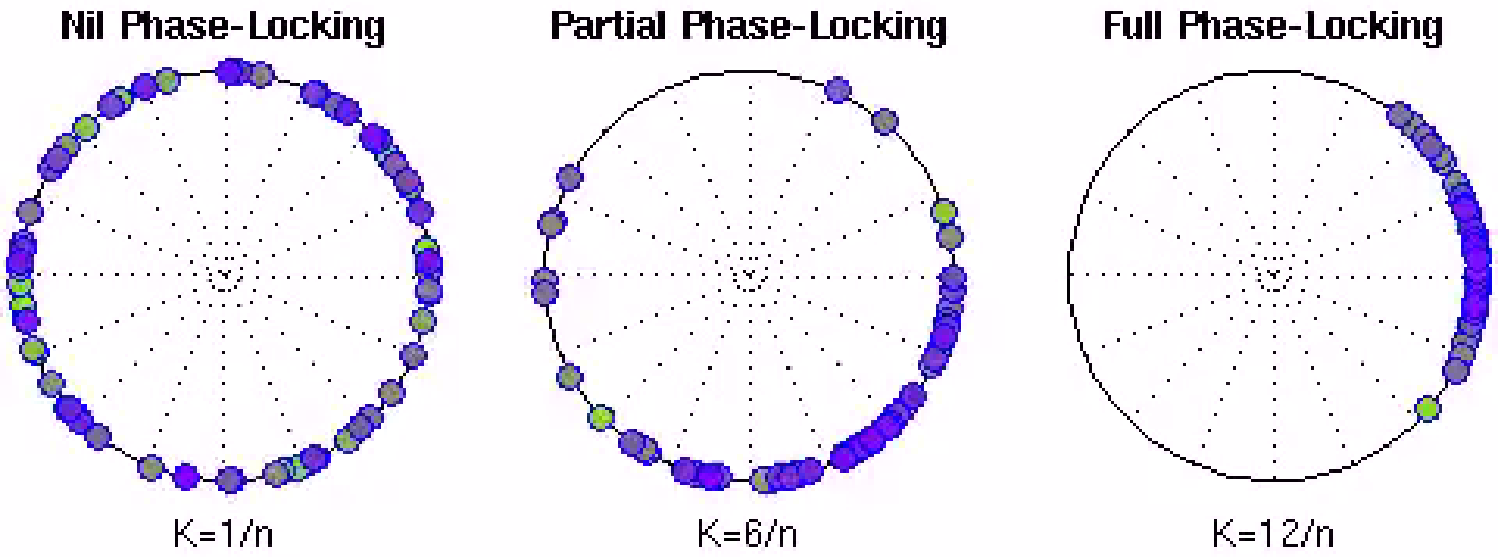
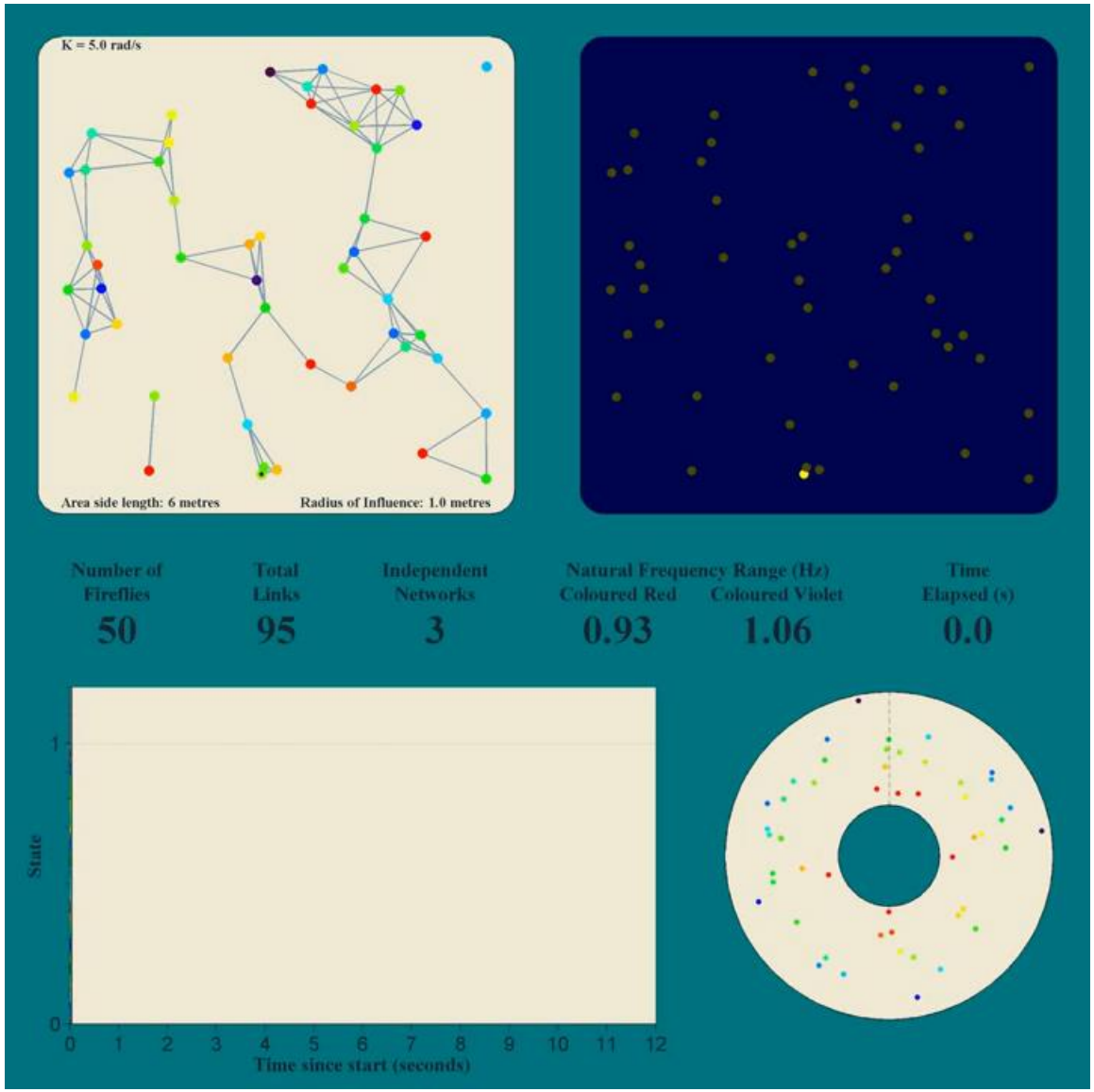
Phase-Coupled Oscillators



Nil, partial and full phase-locking behavior in a network of phase-coupled oscillators with all-to-all connectivity. The natural frequencies of the oscillators are normally distributed SD=±0.5Hz. The phase-locking behaviour is dictated by the strength of the global coupling constant K.

https://www.youtube.com/watch?v=9zrOoVlN8tg

Outlook: Kuramoto model on networks.



The all-to-all coupling considered originally by Kuramoto can be trivially generalized to any connectivity structures by introducing other coupling forms (via (weighted) adjacency matrices, graphs, etc.)

This allows for the study of the synchronization properties of a variety of real-world systems for which interactions between constituents are better described as a complex networks.

https://www.youtube.com/watch?v=hzRhdUkZc-s

Distance dependency

- In some cases dependency on the distance is more realistic than MF
- Assumptions:
  - Oscillators sit on a grid
  - $r_{i,j}$ is the distance between oscillators *i* and *j*
  - α is an exponent determining the strength of the distance dependency
  - η is a renormalizing factor
- The time evolution of the oscillator phases:

$$\frac{d\phi_i}{dt} = \omega_i + \frac{K}{\eta}\sum_{i \neq j} \frac{\sin(\phi_j - \phi_i)}{r_{i,j}^{\alpha}}$$

- Can not be handled analiticaly
- Dependency on α:
  - $\alpha = 0$: no dependency, gives back the mean field approach
  - $\alpha \to \infty$: the interaction decays fast, interaction only with the first neighbor

Noise in the discrete Kuramoto model

- The KM with the above defined noise:

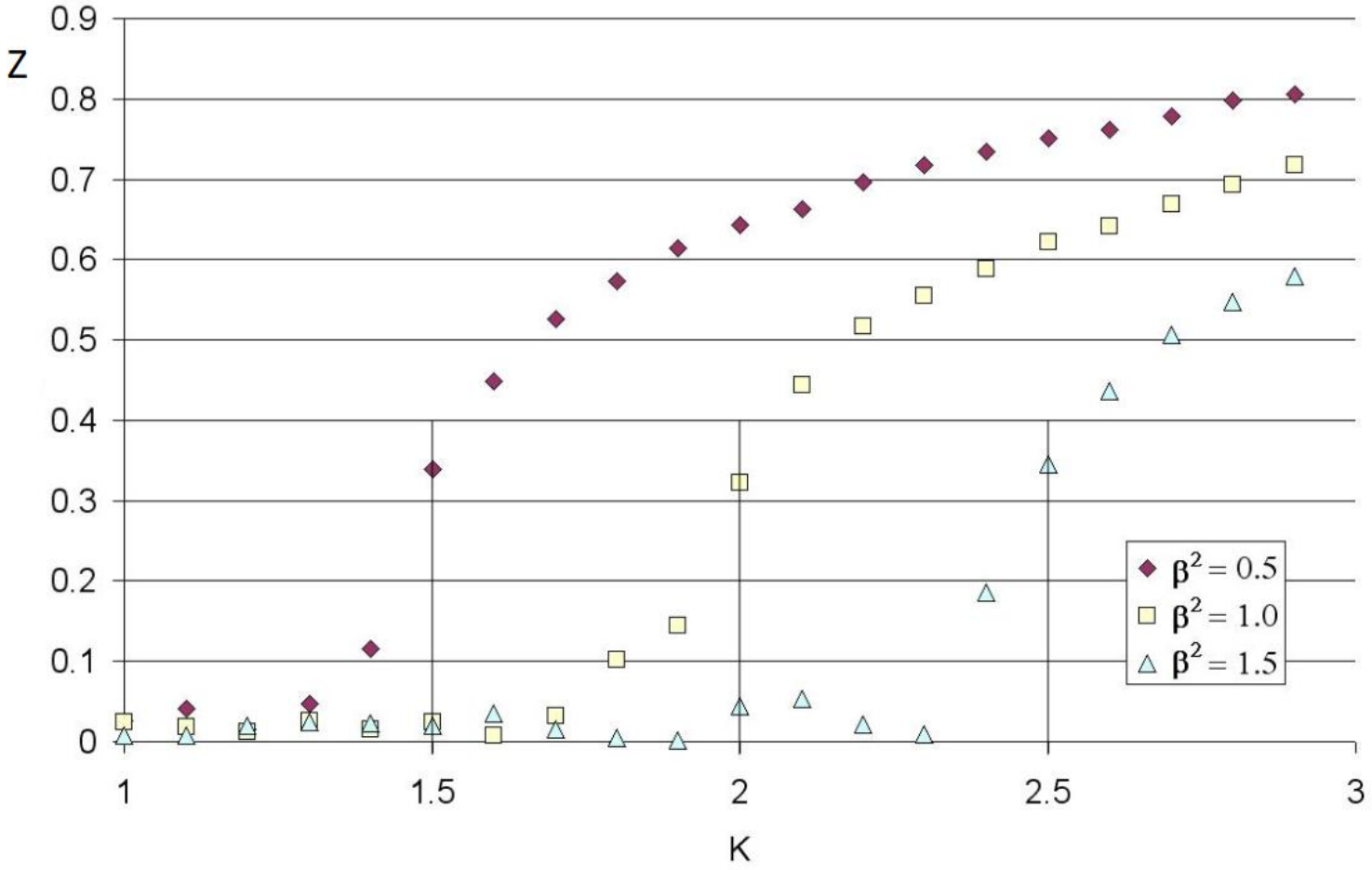
$$\frac{d\phi_i}{dt} = \omega_i + \frac{K}{N}\sum_{j=0}^{N-1}\sin(\phi_j - \phi_i) + \xi_i$$

- Or in other form:

$$\frac{d\psi_i}{dt} = \omega_i + KZ\sin(\theta - \psi_i) + \xi_i$$

- ξ: a random value chosen from a normal (Gaussian) distribution of mean zero and width $\beta^2/\Delta t$, where
- β^2 defines the strength of the noise, and
- Δt is the time of the time-steps used in the simulations

Simulation results with white noise introduced to the discrete KM



The dependency of the magnitude of the order parameter Z on the coupling K in presence of noise. β^2 sets the strength of the noise. From theoretical results K_C is predicted to occur at $\beta^2 + 1$, shown as three vertical lines at 1.5, 2.0, and 2.5.

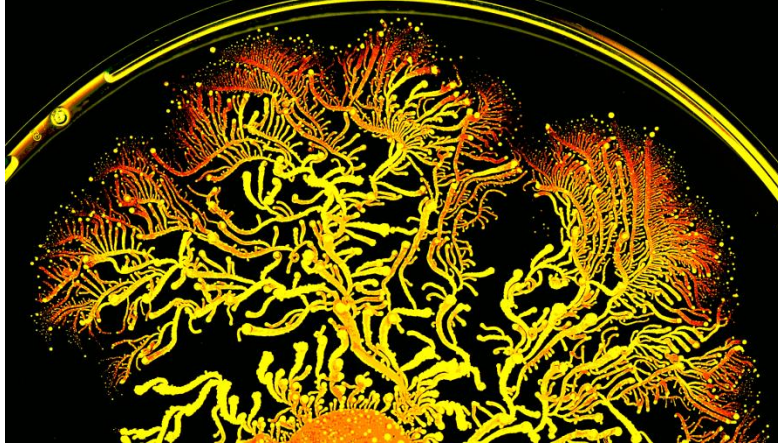
From: Bryan C. Daniels: Synchronization of Globally Coupled Nonlinear Oscillators: the Rich Behavior of the Kuramoto Model, Fig 4.2.

The geometry of bacteria colonies

The microbiological background of motion, morphology diagram, Fisher equation. Self-affine surfaces, branching morphology and models for individual bacteria.

2020

Why exactly bacteria colonies?

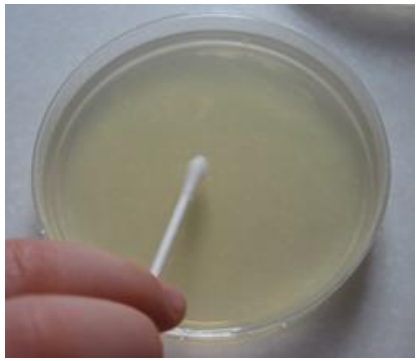


Colony of *Paenibacillus* vortex bacteria

- Unicellular organisms with
- Their interactions are mostly understood
- Various spatio-temporal patterns

- They provide easy-to-handle experiments
- A system whose collective behavior can be explored with computational models
  (Theories can be modeled and tested via computer simulations)
- Experiments can be reproduced
- They can give an insight into the formation of self-organized biological structures

The set-up of the simplest experiments for colony formation



Bacteria are grown on the surface of agar gel (an alga)

- "Dry" surface (= big agar concentration)
  - The cells can not move (to spread over the substrate can take even weeks)
  - The duplication time is much smaller

    → *proliferation* is the key factor in determining the morphology
- "Soft" gel (= small agar conc.)

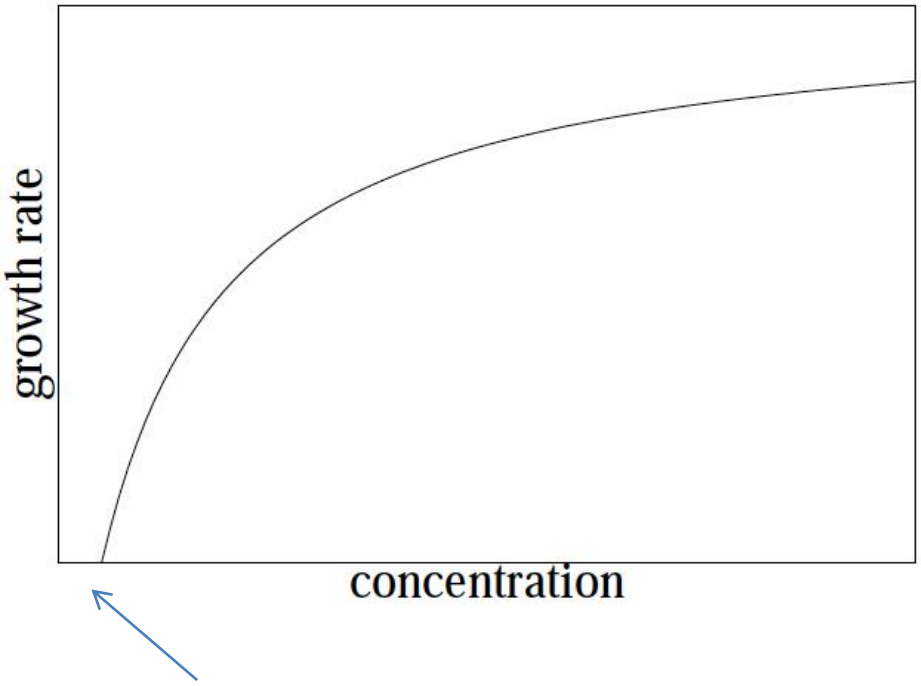
  Or: the bacteria produce surfactant
  - The colony spreads over the substrate in a few hours

    → *bacterial motion* and *chemotaxis* are the main factors

Microbiological background - Proliferation

- Growth (the increase of the number and total mass of bacteria) strongly depends on the nutrient concentration

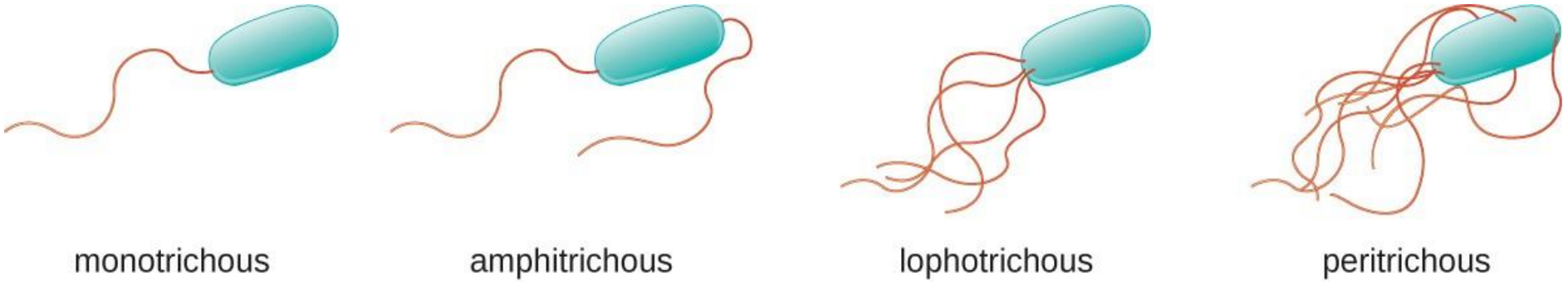
- Rate of growth (number of cell divisions within a population of unit size during a unit time interval) increases with the nutrient concentration in a hyperbolic manner.



A certain amount of nutrient is required to maintain the intracellular biochemical processes

Microbiological background - Motility

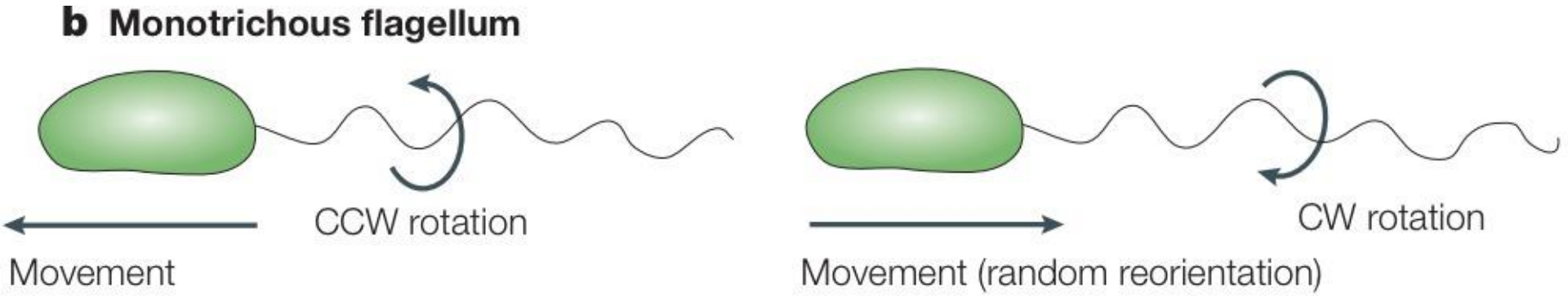
Procaryotes move in aquatic environment by rotating their flagella



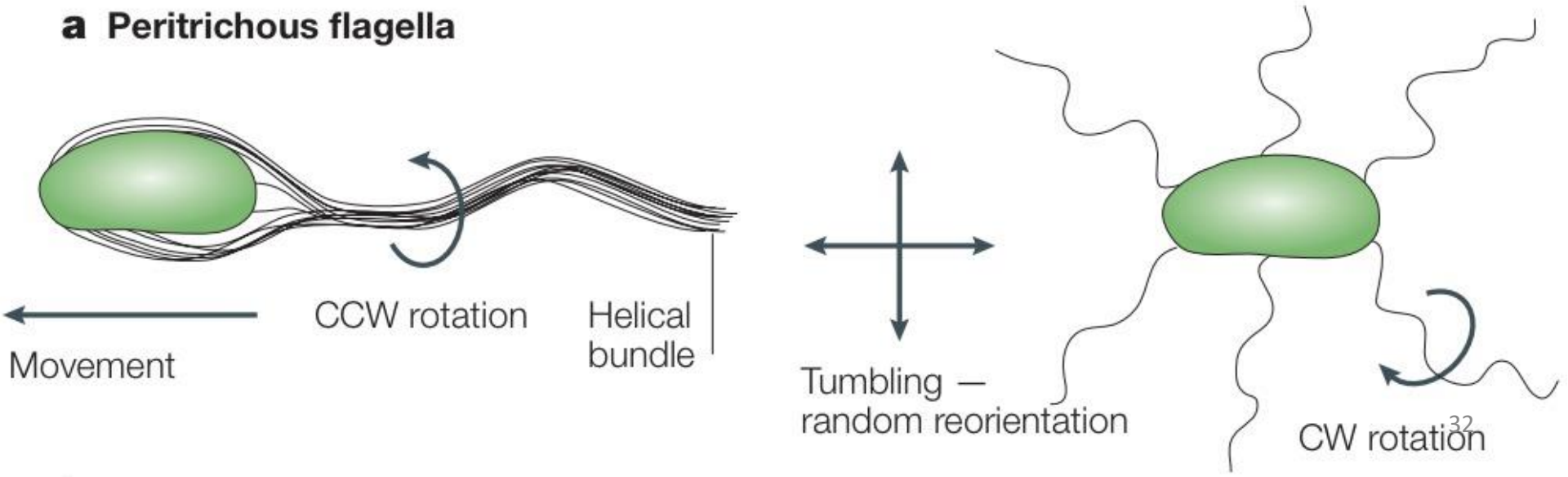
Bacteria can have

- One flagellum, “monotrichous”
- A pair of flagella at the opposite cell poles, “amphitrichous”
- Clusters of flagella at the poles, “lophotrichous”
- Uniformly distributed flagella over the cell membrane, “peritrichous”

The direction of flagellar rotation determines the motion



The forward motion is interrupted by short intervals of “tumbling”



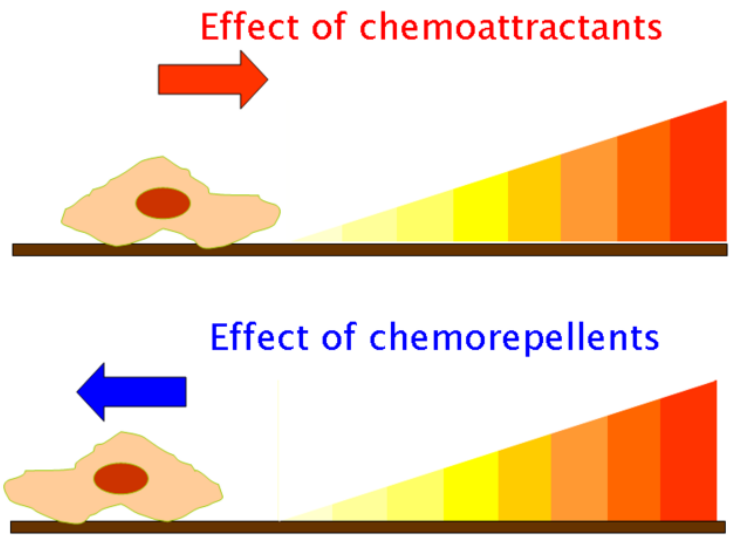
Bacterial Motility - Gliding

- Entirely different type of motility (flagella-independent)
- Slower and smoother than swimming
- Requires surface contact
- Employed by many strains when moving on surfaces
- No visible cellular structures associated – little is known about it
- Slime secretion
- Motion types varies greatly → probably more than one mechanisms exist
  - Gliding along the direction of the long axis of the cell (e.g. Myxococcus or Flexibacter)
  - Screw-like motion (e.g. Saprospira)
  - Direction perpendicular to the long axis (Simonsiella)

Microbiological background - Chemotaxis

- Bacteria are attracted by nutrients (sugar, amino acids, etc.) and repelled by harmful substances and metabolic waste products.
- Other environmental factors, e.g. temperature, light, oxygen concentration

- Stochastic process: chemical gradients modulate the tumbling frequency: repressed when moving towards chemoattractants
- A molecular machinery compares the changes of the chemical concentration *in time*.



Morphology diagram

- A diagram showing the shape (morphology) of the bacterium colony as a function of certain environmental parameters
  - temperature, humidity, chemical composition of the substrate, etc.
  - Can result in different morphologies even for the same strain
- Characteristic colony shapes are assigned to the parameter pairs

- Most systematic experiments explore the relation between the concentration of the *agar* and *nutrients*.

- Agar concentration (consistency of the gel) determines:
  - motility of the bacteria and
  - diffusibility of the nutrient

- Nutrient concentration determines:
  - the proliferation rate

Morphology diagram of *Bacillus Subtilis*

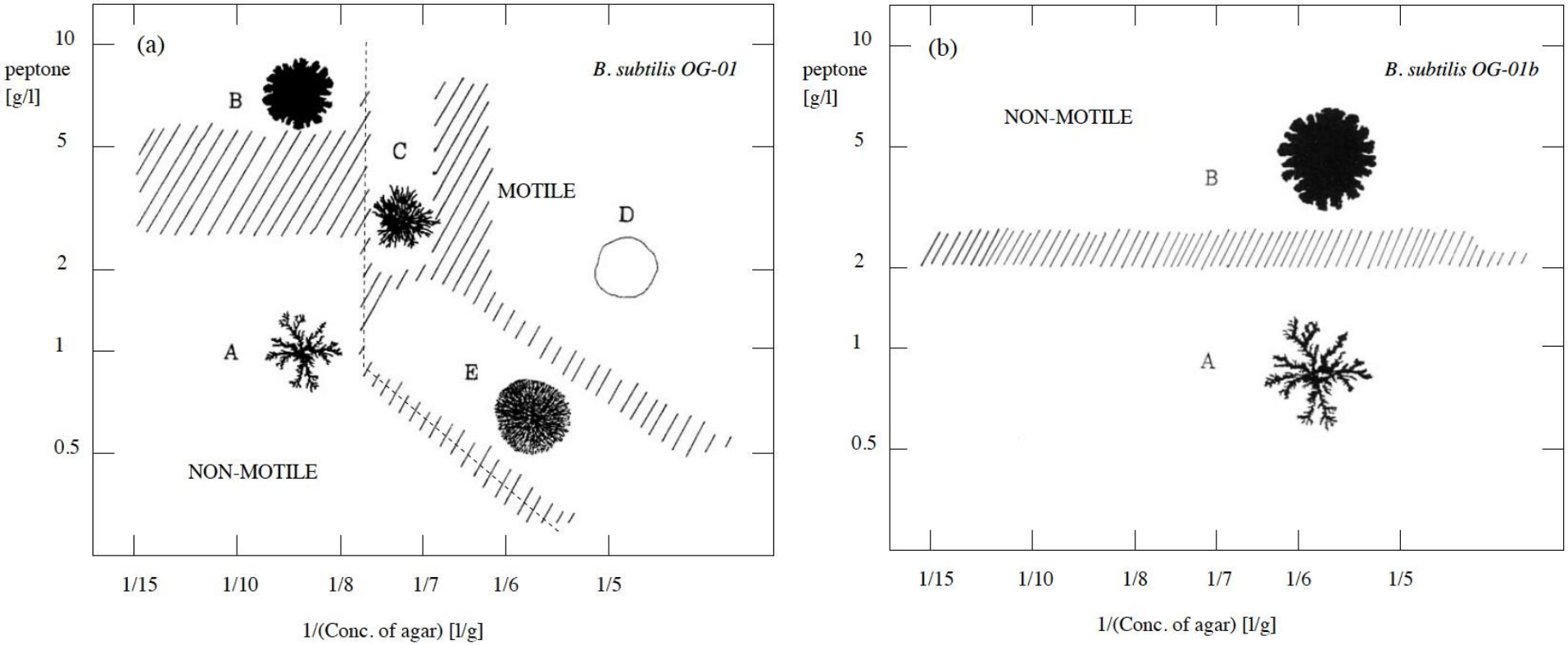
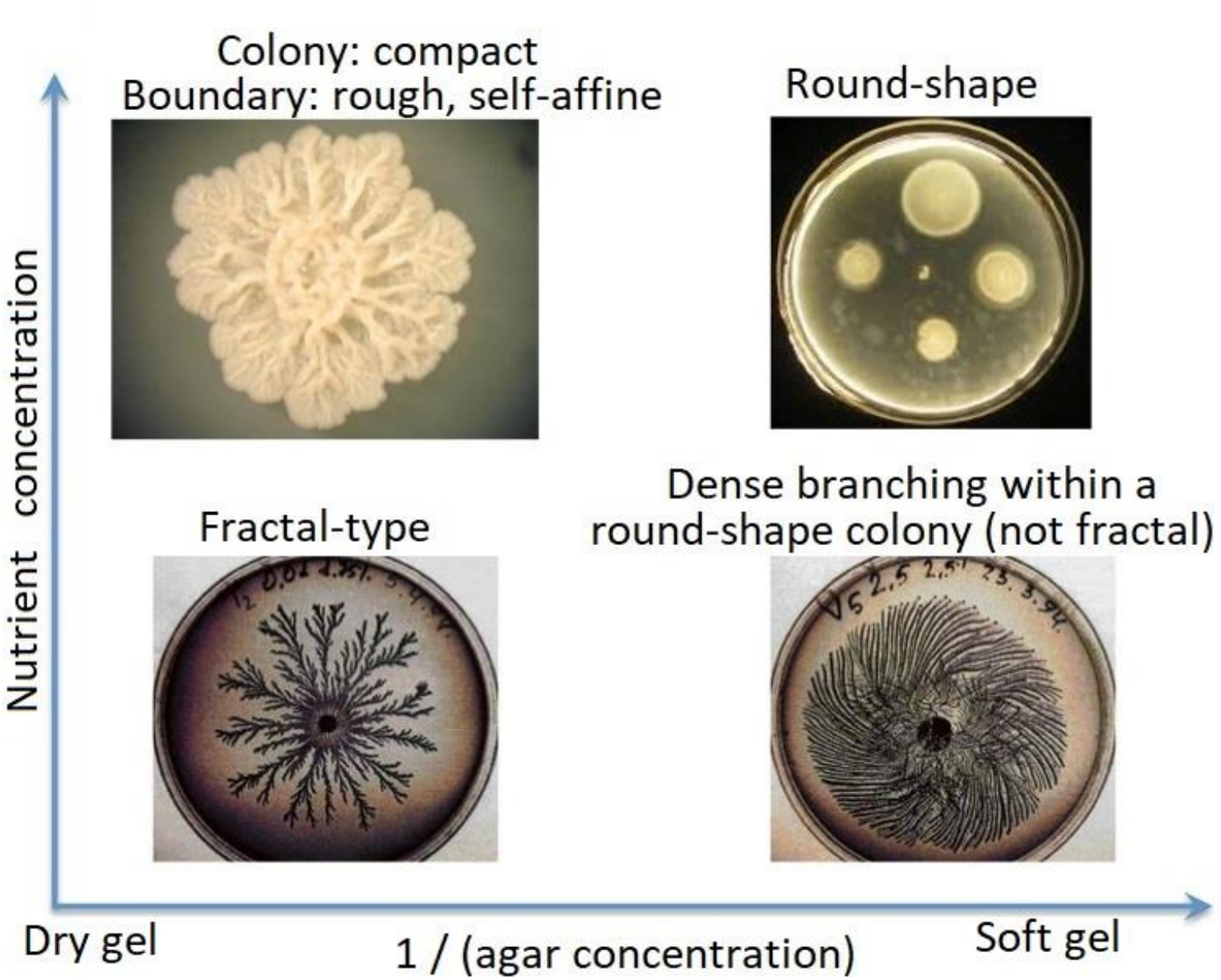


Figure 4.4: Morphology diagram of the strain *Bacillus subtilis* OG-01 (a) and a non motile mutant OG-01b (b) as a function of peptone and agar concentration. The dashed line in (a) indicates the boundary of the active movement of bacterial cells inside the colonies. The morphologies are classified as follows: fractal (A), compact with rough boundary (B), branching with periodic growth phases (C), compact with diffuse boundary (D) and dense branching (E). In the case of the non motile strain the regions A and B seen in (a) expand laterally, while regions C, D and E disappear.

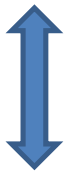
“Summary” of the morphology diagrams



Compact morphology

Abundant nutrient → compact colony

Either smooth or irregular perimeter



Soft gel dry gel

Compact morphology

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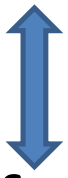

Soft gel → - Bacteria can move

- Takes a few hours to migrate across the dish

- Random walk trajectory

Compact morphology

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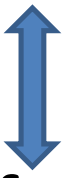
Either smooth or irregular perimeter



Soft gel → - Bacteria can move

- Takes a few hours to migrate across the dish
- Random walk trajectory

⇒ Inter-cellular interactions are negligible

⇒ Time dependence of the bacterial density ρ can be described by the Fisher-Kolmogorov equation

Fisher-Kolmogorov equation

Starts as a small spot

– Diffuses due to random translation, and multiplicates

$$\frac{\partial \rho}{\partial t} = D_\rho \nabla^2 \rho + f(\rho, c)$$

Notations:

$\rho = \rho(\vec{r}, t)$: bacterial density

D_ρ : Diffusion coefficient (can be measured as the average displacement of the cells within a given time interval – see later)

∇ : Partial derivative with respect of the space coordinates

$f = f(\rho, c)$: Bacterial multiplication

c : Nutrient concentration

Fisher-Kolmogorov equation – cont.

$$\frac{\partial \rho}{\partial t} = D_\rho \nabla^2 \rho + f(\rho, c)$$

- D_ρ, (diffusion coefficient) can be determined from the (measurable) squared displacements $d^2(t)$ of the individual cells during a time period t as :

$$\overline{d^2(t)} = 2D_\rho t$$

(overline: averaging among the cells)

- $f(\rho,c)$: bacterial multiplication
  - When ρ is small, cells proliferate with a fixed rate
    → exponential growth
  - In practice, even with unlimited nutrient supply, there's a certain threshold ρ^* for the density (e.g., accumulation of toxic metabolites)
  - We choose cell density units such that $\rho^* = 1$

Compact morphology

Abundant nutrient → compact colony

Either smooth or irregular perimeter

- Dry gel and/or un-motile bacteria
  - Bacteria exert mechanical pressure on their environment (in order to expand to their preferred size)
  - Inter-cellular interactions
  - Modified Fisher-Kolmogorov equation
  - Irregular (self-affine) surface

Cell-cell interaction

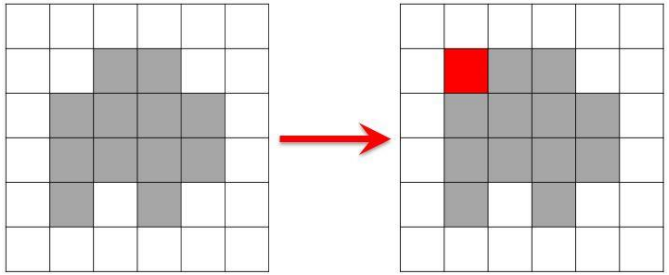
- When the bacteria are *not* independent during the spreading of the colony (e.g. non-motile cells)
  - Abrupt change in the cell density at the border of the colony
- Propagation of the boundary: expansions of the cell volumes inside the colony
  - The bacteria can not expand to their preferred size, they exert mechanical pressure on their surroundings
  - Large densities: $p \sim \rho - \rho_0$ (ρ_0 threshold density for close-packed colonies)
- For large density values the displacement is: $v = D_0 \nabla(\rho - \rho_0)$
  (D_0: diffusion coef., similarly to D_ρ in the F-K. eq.)
- Modified F-K. eq: $$\partial_t \rho = \begin{cases} D_0 \nabla^2 \rho + f(\rho) & \text{for } \rho > \rho_0 \\ f(\rho) & \text{otherwise} \end{cases}$$
- In such cases the colony boundary is self -affine

The formation of self-affine boundaries – the Eden model

- One of the earliest method to generate self-affine objects (1961)
- Cells grown on a lattice
- One single rule for growing the colony:

  – In each step, one of the lattice sites next to the populated areas is chosen randomly and occupied.

  - Or: in each time step, a randomly chosen (non-motile) bacterium proliferates.



- Primitive, but universal model

Eden-model

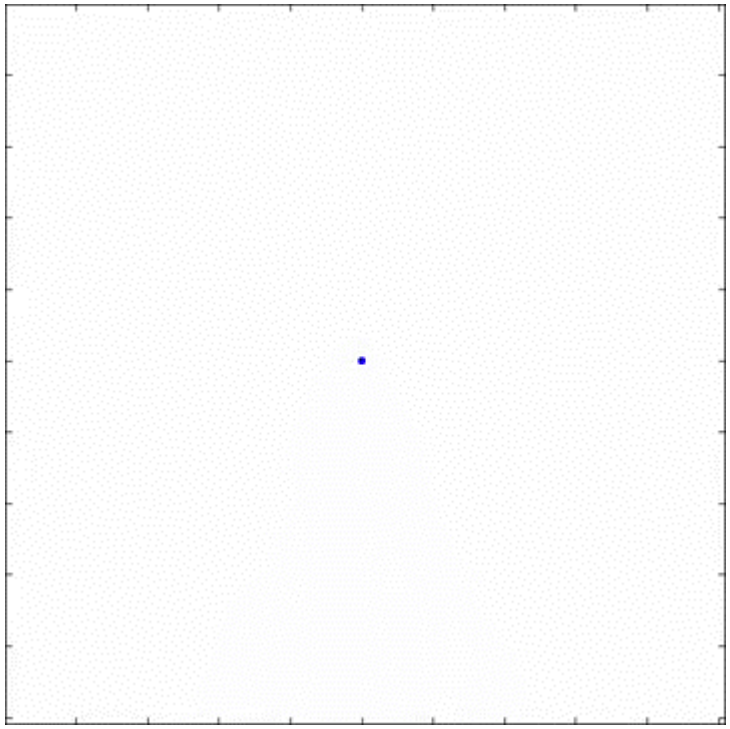
A typical colony in the Eden model grown on a strip of 256 lattice units.

- Initial step:

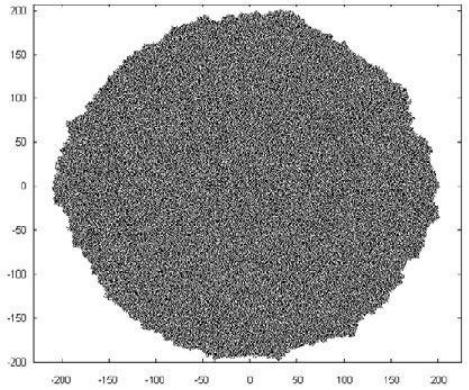
  1 occupied cell

- Variants:
  - Each position with same probability
  - Higher number of occupied neighbors increase the probability
- Variants of the model leave the statistical features of the developing clusters invariant in the asymptotic limit.

Simulations of the Eden model in 2D



- The lattice can destroy the rotational symmetry
- Continual model is more realistic



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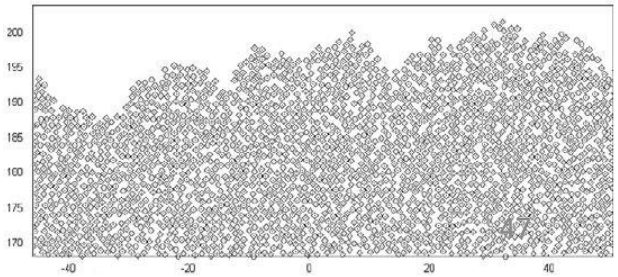
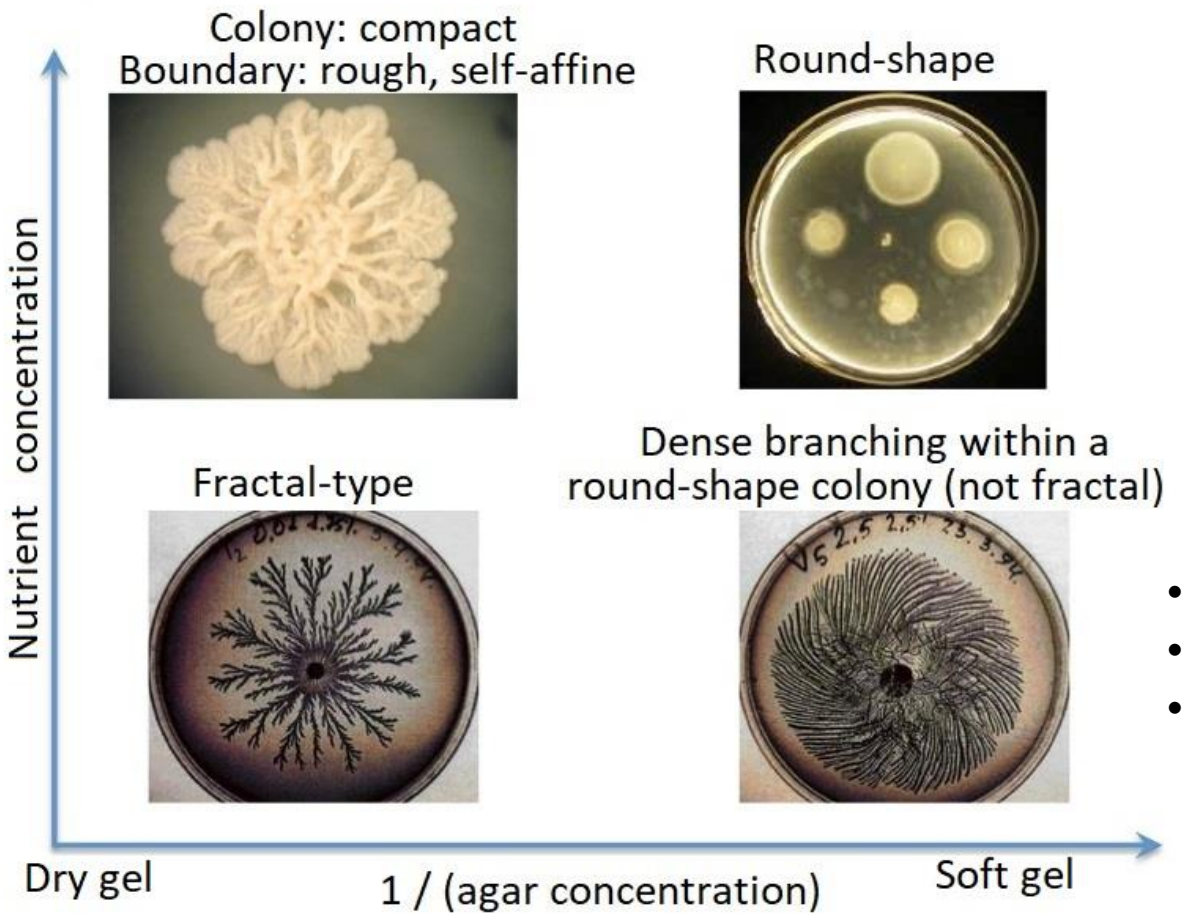


Branching morphology

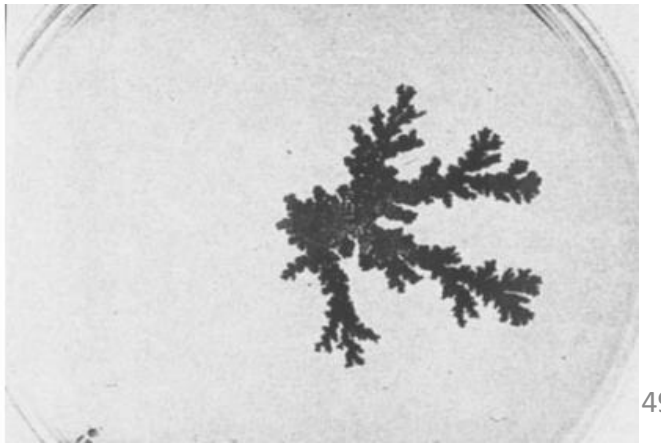


Baillus subtilis colony, under nutrient-poor conditions. 8 and 19 days after inoculation.

- Nutrient-poor agar substrate
- Complex, branching colonies
- Not exhibited by all strains (but by many)

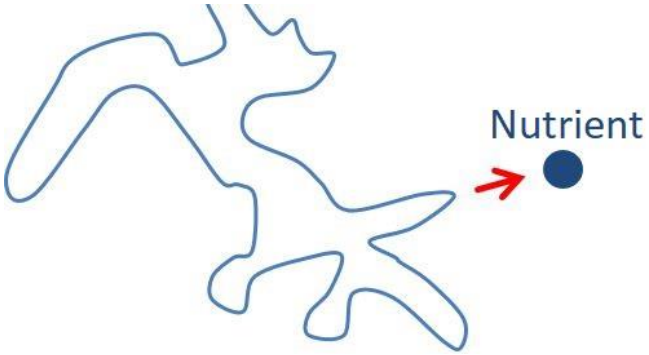
Branching morphology – colony formation

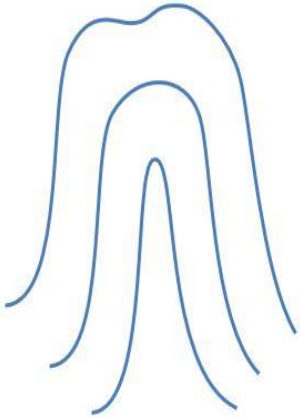
- Basic assumption:
  - the growth of the colony is *diffusion-limited*:
  - The multiplication of the bacteria is determined by the locally available nutrient
    - At the beginning: local nutrient is enough to maintain the growth
    - After some bacterial multiplication, nutrient deprivation progresses in and around the colony
    - Further growth depends on the diffusive transport from distant regions of the petri-dish
  - Experimental support
    - Non motile *B. subtilis* grows only towards nutrient-rich regions



Branching morphology – colony formation

- The speed of the growth is determined by the nutrient diffusion
- The colony develops towards the nutrient
- Instability:
  - Due to some random perturbation a small part of the colony advances “ahead” (towards some nutrient)
  - This part of the colony gets closer to the nutrient
  - Can multiply faster
- This process stops at a certain curvature
  - Certain amount of neighboring cells are needed
  - A certain “steady shape” is set
- New perturbation: new branch

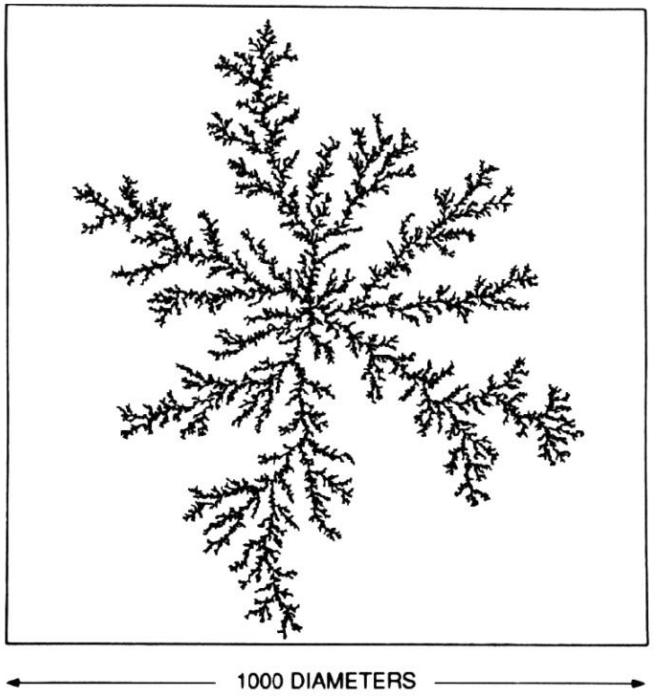




Diffusion-Limited Aggregation (DLA)

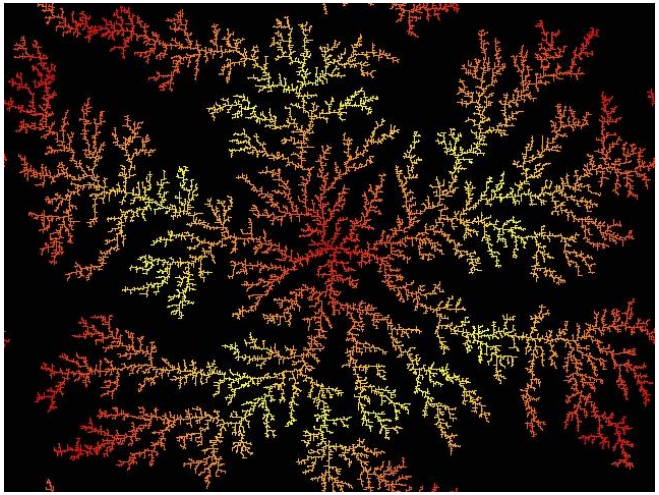
- The definition of the basic DLA algorithm:
  - Start: 1 cell
  - In each time step:
    - A particle (performing random walk) departs from infinity

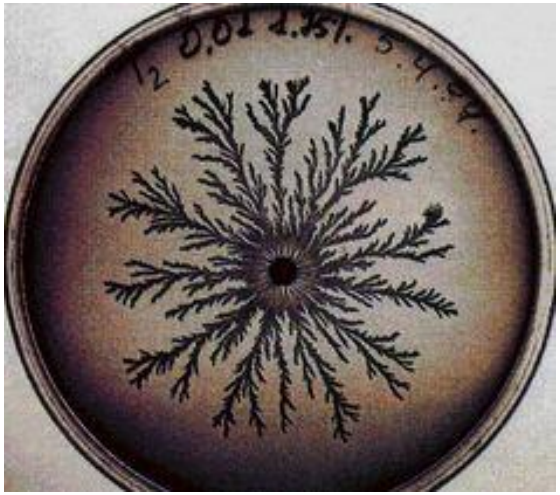
      (in simulations from finite distance)
    - Sticks to the colony upon graze
- Result: Fractal-type clusters



Typical DLA cluster with 50, 000 particles

Relation to bacterium colonies





- Random walk of the particle ~ diffusion of the nutrient
- Sticking to the colony ~ bacterium proliferation

- Non-motile bacteria!
- Very simple model (1 “nutrient-unit” = 1 multiplication) generating realistic formations → “universality”

Refinement of the DLA model – Modeling non-motile bacteria

- Assumptions:
  - Bacteria interact with each other
  - Each particle (cell) is characterized by
    - Space coordinate x_i
    - Energy state E_i (or cell cycle state)
      - $E_i < 0$: spore state. Without nutrient, remains in this state
      - $0 < E_i < 1$: right after multiplication
      - $E_i > 1$: has enough energy to multiply
- Notations:
  - ω_i : nutrient consumption rate
  - κ : conversion factor relating the maximal nutrient consumption rate with the shortest cell cycle time

    (nutrient → energy conversion)
  - ϵ : generic "maintenance" term (not directly contributing to growth)

The energy-level of bacterium *i*: uptake - consumption

$$\frac{dE_i}{dt} = \kappa \cdot \omega_i - \epsilon$$