MSc Computational Science
joint programme UvA/VU

MODELLING DIFFUSIVE SIGNALLING IN *ASPERGILLUS* SPP. GERMINATION INHIBITION

INTERMEDIATE PRESENTATION - MAY

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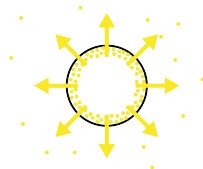
OUTLINE

SINGLE-SPORE EXPERIMENTS: REVISION

- The goal is to test if **interface permeability theory** (spore as a "leaky balloon") can be used to estimate meaningful properties of a non-replenishable germination inhibitor released by the spore.



SINGLE-SPORE EXPERIMENTS: REVISION

Revision of diffusivities/permeabilities

» A lower limit (200 nm) and an upper limit (400 nm) of the cell wall thickness are considered, of which 10 nm is the rodlet-melanin layer, the rest considered mainly polysaccharides (membrane considered separately):

1-octen-3-ol	D	P_{eff}
water (400 nm)	$6.9016 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}$	$0.172\,54\ \text{cm}\ \text{s}^{-1}$
agarose-like layer (190 nm)	$6.9016 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}$	$0.363\,24\ \text{cm}\ \text{s}^{-1}$
agarose-like layer (390 nm)	$6.9016 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}$	$0.176\,96\ \text{cm}\ \text{s}^{-1}$
cellulose-like layer (190 nm)	$1.9555 \times 10^{-7}\ \text{cm}^2\ \text{s}^{-1}$	$0.010\,292\,1\ \text{cm}\ \text{s}^{-1}$
cellulose-like layer (390 nm)	$1.9555 \times 10^{-7}\ \text{cm}^2\ \text{s}^{-1}$	$0.005\,014\,1\ \text{cm}\ \text{s}^{-1}$
lipid bilayer membrane (3.8 nm)	$1.086 \times 10^{-13}\ \text{cm}^2\ \text{s}^{-1}$	$9.04 \times 10^{-5}\ \text{cm}\ \text{s}^{-1}$
hydrophobin layer (10 nm)	$1.086 \times 10^{-13}\ \text{cm}^2\ \text{s}^{-1}$	$3.43 \times 10^{-5}\ \text{cm}\ \text{s}^{-1}$

Table: Diffusion coefficient (D) and effective permeability (P_{eff}) of 1-octen-3-ol through various media.

SINGLE-SPORE EXPERIMENTS: REVISION

Revision of diffusivities/permeabilities

» A lower limit (200 nm) and an upper limit (400 nm) of the cell wall thickness are considered, of which 10 nm is the rodlet-melanin layer, the rest considered mainly polysaccharides (membrane considered separately):

heat-labile peptide	D	P_{eff}
water (400 nm)	$4.548\,55 \times 10^{-7}\ \text{cm}^2\ \text{s}^{-1}$	$0.011\,37\ \text{cm}\ \text{s}^{-1}$
agarose-like layer (190 nm)	$4.548\,55 \times 10^{-7}\ \text{cm}^2\ \text{s}^{-1}$	$0.023\,939\,7\ \text{cm}\ \text{s}^{-1}$
agarose-like layer (390 nm)	$4.548\,55 \times 10^{-7}\ \text{cm}^2\ \text{s}^{-1}$	$0.011\,662\,9\ \text{cm}\ \text{s}^{-1}$
cellulose-like layer (190 nm)	$1.29 \times 10^{-8}\ \text{cm}^2\ \text{s}^{-1}$	$6.789 \times 10^{-4}\ \text{cm}\ \text{s}^{-1}$
cellulose-like layer (390 nm)	$1.29 \times 10^{-8}\ \text{cm}^2\ \text{s}^{-1}$	$3.3 \times 10^{-4}\ \text{cm}\ \text{s}^{-1}$
lipid bilayer membrane (3.8 nm)	N/A	N/A
hydrophobin layer (10 nm)	N/A	N/A

Table: Diffusion coefficient (D) and effective permeability (P_{eff}) of the heat-labile peptide through various media.

SINGLE-SPORE EXPERIMENTS: REVISION

Barrier scenarios

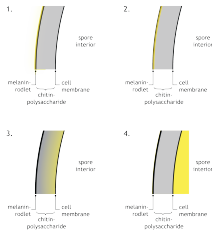
» When combining layers with different permeabilities, the net permeation constant P_{eff} can be computed via the formula [1]

$$\frac{1}{P_{\text{eff}}} = \frac{1}{P_1} + \frac{1}{P_2} + \ldots + \frac{1}{P_n}, \tag{1}$$

where $P_i,\ i = 1, 2, \ldots n$ are the permeabilities of the separate layers.

» Some possible permeation-defining configurations are:

1. an inhibitor source and receiver expressed in the **outermost layer** of the cell wall, virtually unobstructed by any layers, only limited by diffusion in water;
2. an inhibitor source and receiver **embedded between the inner and outer cell wall layers**, obstructed only by the hydrophobin-melanin layer;
3. an inhibitor source and receiver embedded **deep in the inner cell wall layer**, obstructed by polysaccharide network and the hydrophobin-melanin layer;
4. an inhibitor source and receiver **inside the spore interior**, obstructed by the cell membrane and the entire cell wall.

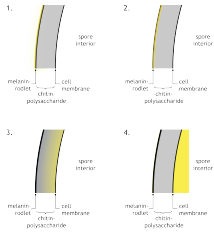


SINGLE-SPORE EXPERIMENTS: REVISION

Barrier scenarios

» Disregarding the effect of unstirred water around the spore, these scenarios can be translated into permeabilities as follows:

1. $P_{\text{eff}} \approx \infty$,
2. $P_{\text{eff}} \approx P_{\text{hp}}$,
3. $P_{\text{eff}} = \left(\frac{1}{P_{\text{hp}}} + \frac{1}{P_{\text{agar}}}\right)^{-1}$ or $P_{\text{eff}} = \left(\frac{1}{P_{\text{hp}}} + \frac{1}{P_{\text{cel}}}\right)^{-1}$,
4. $P_{\text{eff}} = \left(\frac{1}{P_{\text{hp}}} + \frac{1}{P_{\text{agar}}} + \frac{1}{P_{\text{lb}}}\right)^{-1}$ or $P_{\text{eff}} = \left(\frac{1}{P_{\text{hp}}} + \frac{1}{P_{\text{cel}}} + \frac{1}{P_{\text{lb}}}\right)^{-1}$.



SINGLE-SPORE EXPERIMENTS: REVISION

Table of permeabilities

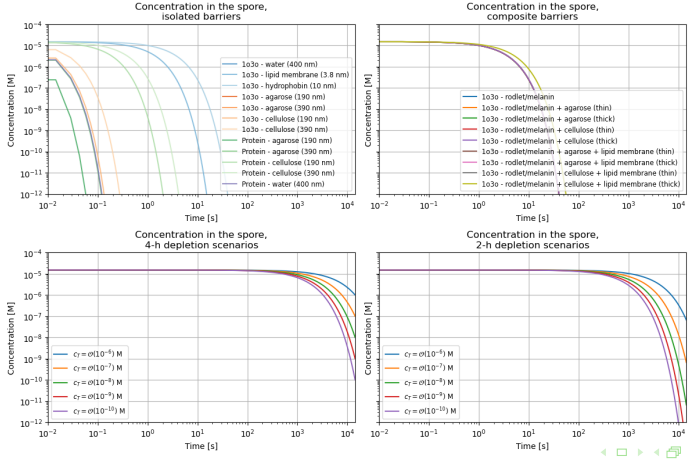
» As the outermost layer is impermeable for large proteins, the permeation of a heat-labile peptide can only be considered during the onset of swelling, i.e. when the rodlet layer begins losing its integrity.

Barrier type	$P_{\text{eff}}(\text{1-octen-3-ol})$
superficial (0 nm)	∞
full water-like cell wall (400 nm)	$0.172\,54\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin layer (10 nm)	$3.43 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + agarose-like polysaccharides (200 nm)	$3.43 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + agarose-like polysaccharides (400 nm)	$3.429 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + cellulose-like polysaccharides (200 nm)	$3.419 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + cellulose-like polysaccharides (400 nm)	$3.407 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + agarose-like polysaccharides + lipid membrane (203.8 nm)	$2.486 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + agarose-like polysaccharides + lipid membrane (403.8 nm)	$2.486 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + cellulose-like polysaccharides + lipid membrane (203.8 nm)	$2.481 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$
rodlet-melanin + cellulose-like polysaccharides + lipid membrane (403.8 nm)	$2.474 \times 10^{-5}\,\text{cm}\,\text{s}^{-1}$

Table: Effective permeability (P_{eff}) of 1-octen-3-ol through different barrier configurations.

SINGLE-SPORE EXPERIMENTS: REVISION

Single-spore release scenarios



SINGLE-SPORE EXPERIMENTS: REVISION

Single-spore release scenarios

- The results confirm that, under the assumed initial concentration, the permeation of 1-octen-3-ol and of the hypothetical protein through any of the barrier candidates, isolated or composite, occurs **too fast** to meet any realistic threshold at a time mark longer than 60 seconds.
- The permeation constants needed for the attainment of the different thresholds are listed in the table below:

c_T	$c_0 \times 10^{-1}$	$c_0 \times 10^{-2}$	$c_0 \times 10^{-3}$	$c_0 \times 10^{-4}$	$c_0 \times 10^{-5}$
$P_s(4\,\mathrm{h})$ [cm/s]	$1.567\,158\,68 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$	$2.899\,673\,2 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$	$4.232\,187\,72 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$	$5.564\,702\,25 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$	$6.897\,216\,77 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$
$P_s(1\,\mathrm{h})$ [cm/s]	$3.134\,317\,36 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$	$5.799\,346\,4 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$	$8.464\,375\,45 \times 10^{-8}\,\mathrm{cm\,s^{-1}}$	$1.112\,940\,449 \times 10^{-7}\,\mathrm{cm\,s^{-1}}$	$1.379\,443\,353 \times 10^{-7}\,\mathrm{cm\,s^{-1}}$

- The release mechanism possibly involves stronger interaction between the inhibitor and the cell wall.

SINGLE-SPORE EXPERIMENTS: REVISION

Single-spore release scenarios

- In a potential cell wall adsorption model, the permeation constant could be shaped by the Langmuir isotherm equation:

$$P_{\text{eff}} = \frac{P_s}{1 + KB_{\max}}. \tag{2}$$

- The implications of such a mechanism are yet to be studied.

OUTLINE

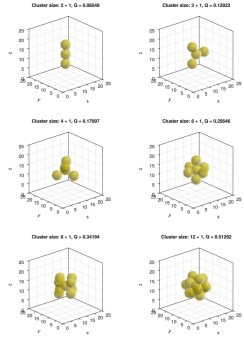
1 Single-spore experiments: revision
- Introduction
- Revision of diffusivities/permeabilities
- Barrier scenarios
- Table of permeabilities
- Single-spore release scenarios
- Cell wall adsorption model

2 Spore cluster experiments: revision
- Introduction
- Homogeneous vs. cell-wall-bound inhibitor
- Extreme cases
- Functional relationship

3 From a deterministic to a statistical model
- Deriving the germination probability
- Parameter estimation

SPORE CLUSTER EXPERIMENTS: REVISION

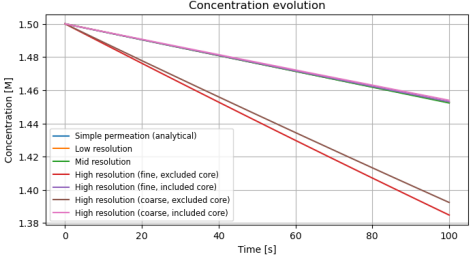
- Previously, it was found that neighbour clusters affect diffusivity, but this effect could not be reproduced across all models.
- An error was found in the high-resolution solver and was corrected.
- Hence, the cluster simulations were repeated.

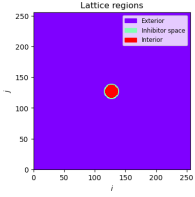


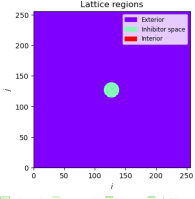
SPORE CLUSTER EXPERIMENTS: REVISION

Homogeneous vs. cell-wall-bound inhibitor

- It was found that, if the inhibitor is **only in the cell wall**, it is released faster compared to a **homogenous distribution** in the spore volume.
- The reason for this is that there is a higher concentration "pressure" for the same amount of molecules.







SPORE CLUSTER EXPERIMENTS: REVISION

Extreme cases

- The inhibitor release was compared between a **single spore** and a **full cluster of 12+1 spores**.
- The results show that the neighbours *in direct contact* still obstruct inhibitor release, but to a much lesser degree (7% more residual concentration at $t = 4$ h).
- If the inhibitor is only in the cell wall and the spore interiors are inaccessible, the blocking effect is stronger (20% more residual concentration at $t = 4$ h).

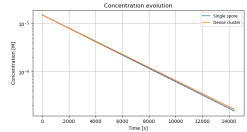


Figure: Empty spore interior.

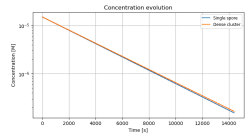


Figure: Full spore interior.

SPORE CLUSTERS VS. RELEASE EXPONENT

Functional relationship

- The neighbour arrangements produce fluctuating results in the measured release exponent.
- The results are sensitive to the arrangement of neighbours around the central spore (symmetric vs. asymmetric).
- Finite lattice size with absorbing boundary possibly distorts the results.
- A fitted power-law yields a relationship $c(t) \sim e^{\alpha t/\tau}$, where $\alpha \sim M^{-0.004}$, i.e. a very slow decrease with increasing number of neighbours M.
- In general, ast diffusion through the cluster gaps still depletes the inhibitor locally. Thus, **clustering is not critical in slowing down inhibitor release**.
- It is to be investigated whether the cluster effect is amplified by the combination of density-driven inhibitor saturation.

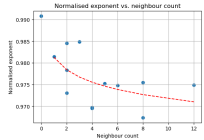


Figure: Empty spore interior.

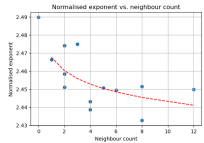


Figure: Full spore interior.

OUTLINE

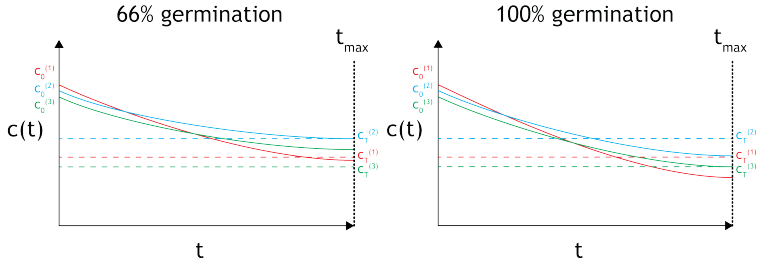
FROM A DETERMINISTIC TO A STATISTICAL MODEL

Deriving the germination probability

» Spores are heterogeneous. This means that the parameters of the deterministic formula

$$c_{\text{in}}(t) = \phi c_0 + (1-\phi)\left[c_{\text{ex}} + (c_0 - c_{\text{ex}})\, e^{-\frac{t}{\tau(1-\phi)}}\right], \quad \tau = \frac{V_s}{P_s A}, \tag{3}$$

may vary randomly, with specific distributions.



FROM A DETERMINISTIC TO A STATISTICAL MODEL

Deriving the germination probability

- Each spore is approximately spherical, so the surface-to-volume ratio can be expressed through the **spore radius** R: $\frac{V_s}{A} \approx \frac{\frac{4}{3}\pi R^3}{4\pi R} = \frac{R}{3}$. The radius can be modelled by a **random variable** ξ.
- The **spore density** can be considered a **constant**, so the volume fraction ϕ varies only depending on the radius via the spore volume ($\phi = \rho_s V_s$).
- The **permeation coefficient** is a **constant**.
- The **initial concentration** c_0 and the **concentration threshold** c_T for germination inhibition vary **randomly**.

FROM A DETERMINISTIC TO A STATISTICAL MODEL

Deriving the germination probability

- The onset of germination can be observed at slightly varying times, but this randomness can be absorbed by the threshold variable c_T
- Thus, expressing

$$c_{\text{in}}(t_{\text{germ}}) = \beta c_0, \tag{4}$$

where $\beta = \phi + (1-\phi)e^{-\frac{t_{\text{germ}}}{\tau(1-\phi)}}$, and $c_T = \gamma c_0$, where γ is a random variable with an unknown distribution, one can express the condition for germination as

$$\beta c_0 < \gamma c_0, \tag{5}$$

or, simplified,

$$\beta(\xi, \rho_s) < \gamma. \tag{6}$$

FROM A DETERMINISTIC TO A STATISTICAL MODEL

Deriving the germination probability

- When changing the main control parameter of the model, the spore density ρ_s, the percentage of germinated spores is expressed through the probability

$$p(\rho_s) = P(\beta < \gamma \mid \rho_s). \tag{7}$$

- Accounting for the distribution of ξ,

$$p(\rho_s) = \int_0^\infty P(\beta < \gamma \mid \rho_s, \xi) f_\xi(\xi)\, d\xi, \tag{8}$$

where $f_\xi(\xi)$ is the probability distribution funciton (PDF) for the spore radius. For simplicity, it can be assumed that γ and ξ are **normally distributed**. Then,

$$P(\beta < \gamma \mid \rho_s, \xi) = 1 - \Phi\left(\frac{\beta(\xi) - \mu_\gamma}{\sigma_\gamma}\right). \tag{9}$$

FROM A DETERMINISTIC TO A STATISTICAL MODEL

Deriving the germination probability

- The PDF of ξ is then the Gaussian function

$$f_\xi(\xi) = \frac{1}{\sqrt{2\pi}\sigma_\xi} \exp\left(-\frac{(\xi - \mu_\xi)}{2\sigma_\xi^2}\right). \tag{10}$$

- Thus, the germination probability can be expressed as

$$p(\rho_s) = \int_0^\infty \left[1 - \Phi\left(\frac{\beta(\xi) - \mu_\gamma}{\sigma_\gamma}\right)\right] \frac{1}{\sqrt{2\pi}\sigma_\xi} \exp\left(-\frac{(\xi - \mu_\xi)^2}{2\sigma_\xi^2}\right) d\xi. \tag{11}$$

- The mean and standard deviation of the spore radius can be inferred from literature. For instance, the diameter of *A. niger* is $5.3(6)$ µm ($\mu_\xi = 2.65$ µm and $\sigma_\xi = 0.3$ µm [3], while the diameter of *A. nidulans* is observed to vary between 2.4 and 2.7 µm [5] ($\mu_\xi = 1.275$ µm and $\sigma_\xi = 0.075$ µm).

FROM A DETERMINISTIC TO A STATISTICAL MODEL

Deriving the germination probability

- If an inhibitor concentration is **exogenously added**, the germination condition is

$$\beta c_0 + \chi c_{\text{ex}} < \gamma c_0 \tag{12}$$

- c_0 does not cancel, can be modelled by a random variable ψ. The probability formula becomes

$$p(\rho_s, c_{\text{ex}}) = \int_0^\infty \int_0^\infty \left[1 - \Phi\left(\frac{\beta(\xi) + \chi\frac{c_{\text{ex}}}{\psi} - \mu_\gamma}{\sigma_\gamma}\right)\right] f_\psi(\psi)\, f_\xi(\xi)\, d\psi\, d\xi, \tag{13}$$

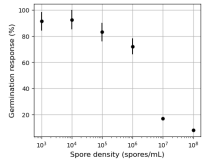
where the two PDFs are

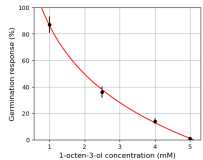
$$f_\psi(\psi) = \frac{1}{\sqrt{2\pi}\sigma_\psi} e^{-\frac{(\psi-\mu_\psi)^2}{2\sigma_\psi^2}}, \quad f_\xi(\xi) = \frac{1}{\sqrt{2\pi}\sigma_\xi} e^{-\frac{(\xi-\mu_\xi)^2}{2\sigma_\xi^2}}. \tag{14}$$

FROM A DETERMINISTIC TO A STATISTICAL MODEL

Parameter estimation

- Using the probability formula, the unknown parameters P_s, μ_γ and σ_γ are fit on data by Herrero-Garcia et al. [2], including
  - germination response under varying spore density,
  - germination response under added concentrations of 1-octen-3-ol.
- The fitting process aims to investigate whether
  - the inhibition effects of spore density and added 1-octen-3-ol have the same underlying mechanism,
  - the model can capture both of these effects.





FROM A DETERMINISTIC TO A STATISTICAL MODEL

Parameter estimation

- Fitting the model to the density-driven germination data only, the transition in germination rates around 10^5 spores/mL is captured.

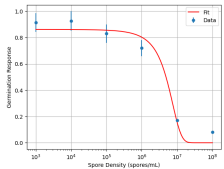


Figure: Model fitted to density-driven germination data.

- Fixing the P_s, μ_γ and σ_γ from the last fit, μ_ψ and σ_ψ are fit again under varying c_{ex}. The match is unsatisfactory.

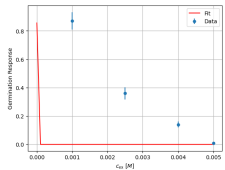


Figure: Model fitted to exogenously driven germination data.

- Fitting P_s, μ_γ, σ_γ, μ_ψ and σ_ψ on both models does not produce a good match.

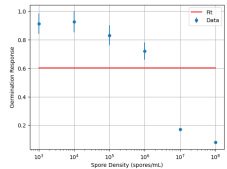
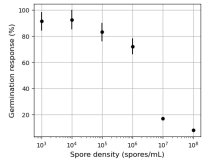


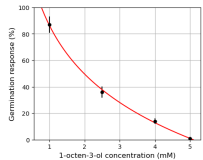
Figure: Model fitted to both data simultaneously.

FROM A DETERMINISTIC TO A STATISTICAL MODEL

Parameter estimation

- The model can represent density-driven inhibition somewhat accurately (under $c_{\text{ex}} = 0$).
- The model cannot represent both density-driven inhibition and exogenously driven inhibition simultaneously.
- The mechanisms behind the two phenomena may indeed be different:
  - It is known that the thresholds for exogenous inhibition are higher than the presumed spore concentration [4],
  - so 1-octen-3-ol likely does not act alone.

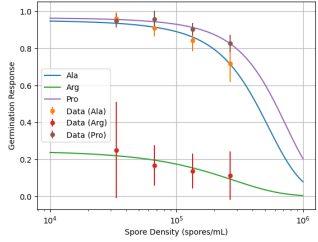




FROM A DETERMINISTIC TO A STATISTICAL MODEL

Parameter estimation

- The parameters P_s, μ_γ and σ_γ are fit again on data by Ijadpanahsaravi et al. [3], including density-driven germination rates under different carbon sources.
- The time-frame is set to 100 hours, since $P_{\max}$ is a long-time asymptotic limit.
- The results are consistent with
  - **conserved permeability** - rate of inhibitor release not altered by environmental changes;
  - **varying inhibition threshold** across the different environmental scenarios - carbon signals modulate the inhibition threshold in the spore culture.



Case	P_s [cm s^{-1}]	μ_γ	σ_γ
Ala	3.176×10^{-9} cm s^{-1}	3.780×10^{-5}	1.819×10^{-5}
Arg	2.741×10^{-9} cm s^{-1}	1.000×10^{-10}	2.213×10^{-5}
Pro	3.109×10^{-9} cm s^{-1}	5.284×10^{-5}	2.345×10^{-5}

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