Optimal methylation noise for best chemotactic performance of *E. coli*

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Introduction

- Behavior of a cell is controlled by the intracellular biochemical reactions in its signaling pathway
- These reactions crucially depend on the expression levels of each protein involved
- Any fluctuations in the numbers of protein molecules have important consequences on the cell performance
- Small number of protein molecules inside a single cell: flucutations are expected
- How the variability in protein numbers affect the cell behavior?

E.coli Chemotaxis

- Chemotaxis pathway of E. coli bacteria: a model system to study cellular behavior
- Bacterial cells sense and respond to the changes in the nutrient concentration in its environment
- In presence of a concentration gradient, it moves up the gradient to reach a region of higher nutrient concentration
- The motion of an E. coli is controlled by flagellar motors
- CCW $\rightarrow$ run: cell moves in one direction with speed $\sim 20\mu m/s$
- CW $\rightarrow$ tumble: random rotation with no net displacement
- By modulating the rotational bias of the motors the cell can modulate its run and tumble durations

Chemotaxis pathway: adaptation

- Chemoreceptors in the cell membrane binds to nutrient molecules
- In a bound state the receptors suppress the phosphorylation of cytoplasmic protein CheA
- The methylation level of the receptors is controlled by two proteins CheR and CheB
- CheR raises the level and phosphorylated CheB-P causes demethylation
- In a phosphorylated state, CheA transfers the phosphate group to CheY and CheB
- When the phosphorylation of CheA is suppressed, CheB-P concentration decreases, demethylation process stops
- The receptor then reaches a highly methylated state, which in turn raises the activity of CheA
- Adaptation in the network

Chemotaxis pathway: response

- CheY which also receives phosphate group from CheA, controls the sensing mechanism of the network
- CheY-P binds to flagellar motors and increases its CW bias, causing the cell to tumble
- In absence of phosphorylation CheY-P concentration goes down which reduces CW bias and the cell swims smoothly

Noise in the biochemical pathway

- In absence of any noise in the signaling pathway, the switching of rotational bias of the flagellar motors is expected to be a Poisson process
- Duration of a particular run or tumble should follow an exponential distribution
- Switching events of a single cell in an isotropic medium were monitored in experiment and the residence time of the motors in the CCW bias was found to follow a power law distribution [Korobkova *et al.* Nature (2004)]
- Noise present in the signaling network of a single cell makes it possible to have large fluctuations in the CCW lifetimes and consequently, the cell can execute really long runs with significant probability

Fluctuations in CheY-P level

- CCW and CW bias states of the motors modelled as a two-level system whose energy levels depend on the concentration of the motor protein CheY-P [Tu and Grinstein, PRL (2005)]
- As the noise present in the network causes this protein number to fluctuate, the energy levels also fluctuate with time
- Such fluctuations give rise to power law distribution for the lifetime of the CCW state
- For a single bacterial motor, when CW bias is large, which corresponds to higher CheY-P level, and hence smaller fluctuations, the CCW intervals show exponential distribution [Korobkova *et al.* PRL (2006)]
- For small CW bias, when fluctuations in CheY-P level are more significant, CCW intervals show power law distribution

Noise and chemotactic performance

- Large fluctuations present at the single cell level do not impair the chemotactic response or robust adaptation observed at the population level
- Overall chemotactic performance improves as a result of interplay between the signaling noise and multiple flagellar motors [Sneddon *et al.* PNAS (2012)]
- Presence of multiple motors brings down the motor response time—beneficial in steep gradient of chemoattractant concentration
- Presence of noise in the signaling pathway generates longer runs and improves the chemotactic performance in shallow gradients
- Large variability in a cellular population ensures that different cells behave in different ways and each type of behavior may be suitable for one particular type of environment [Frankel *et al.* eLife (2014)]

Methylation noise

- How noise affects the chemotactic efficiency of a single cell?
- For an efficient chamotactic performance, the cell should be able to find the nutrient-rich regions quickly and localize there
- The most important source of noise is the methylation demethylation reactions in the network
- Time-scales of these reactions are order of magnitude larger than all other time-scales present in the pathway
- Slow methylation flucutations cannot be integrated out by the downsteam processes in the network

Different aspects of chemotactic performance

- Chemotactic drift velocity: average velocity with which the cell climbs up the chemical concentration gradient
- Larger values of drift velocity indicates a better performance
- Localization: Nutrient concentration, averaged over the steady state distribution of the cell position
- Localizaton takes a high value, when in the long time limit most of the cells are present in the regions which contains maximum nutrient
- High values of localization and drift velocity ensures a good chemotactic performance in the long time limit
- Non-monotonic variation of localization and drift velocity as a function of methylation noise strength
- An optimal noise strength at which each of these quantities becomes maximum

Explanation from detailed CheY-P level statistics

- Chemotactic response is the result of differential behavior of the cell up and down the nutrient concentration gradient
- For large signaling noise, the tumble frequency is almost totally controlled by the stochastic fluctuations of the methylation level, and not by the local nutrient concentration
- Above difference becomes small $\Rightarrow$ poor performance
- Why the performance gets worse for very low noise level?
- When CheY-P level falls below a certain threshold value, the cell shows a tendency to migrate towards regions of low nutrient concentration
- This affects its overall chemotactic performance adversely
- This threshold value decreases with noise strength, and hence the performance improves with noise
- Within our model, we give a clear explanation behind this threhsold behavior

- In a shallow ligand gradient, the chemotactic drift velocity shows a peak at a specific noise strength, while the localization remains constant at low noise level [Flores *et al.* PRL (2012), He *et al.* Biophys J (2016)]
- Existence of a threshold behavior has never been studied before
- Threhsold of CheY-P level below which the behavior of the cell is detrimental to its chemotactic performance, plays a crucial role when noise is low

Model description

- $a(t)$: activity of the receptor complex
  $m(t)$: methylation level
  $y_P(t)$: CheY-P level
- probability that a given receptor complex is in an active state

$$a = \frac{1}{1 + \exp(N\epsilon(m, [L]))}$$

$\epsilon(m, [c])$ is the free energy difference between the active and inactive states:

$$\epsilon(m, [L]) = f_m + f_{[L]} = \alpha(m_0 - m) - \log\left(\frac{1 + c(x)/K_A}{1 + c(x)/K_I}\right)$$

- $N = 6$, $K_A = 3mM$, $K_I = 18.2\mu M$, $\alpha = 1.7$, $m_0 = 1$
  [Jiang *et al.* PLoS Comp Biol (2010)]

Stochastic methylation dynamics

$$\frac{dm}{dt} = k_R(1-a) - k_B a + \eta(t)$$

- $< \eta(t)\eta(t') >= \lambda(k_R(1-\bar{a}) + k_B\bar{a})\delta(t-t')$, and $\bar{a} = 1/2$
- Dimensionless parameter λ controls noise strength
- $k_R = k_B = 0.015s^{-1} \Rightarrow$ methylation fluctuation is a slow process $\Rightarrow \eta(t)$ cannot be integrated out
- Fluctuations in methylation level cause fluctuations in activity which in turn affects the phosphorylation of CheY proteins

$$\frac{dy_P}{dt} = k_Y a(1-y_P) - k_Z y_P$$

y_P: fraction of phosphorylated CheY proteins, $k_Y = 1.7s^{-1}$ and $k_Z = 2s^{-1}$

- In the phosphorylated state, CheY-P proteins bind to the flagellar motors and cause the cell to tumble
- Tumbling rate $\omega(y_P)$ is a sigmoidal function of y_P

$$\omega(y_P) \sim y_P^{10}$$

- Instantaneous tumbling considered: a finite tumbling duration does not affect our conclusions
- Motion of the cell in one dimension
- Recent experiments study bacterial chemotaxis in narrow micro-fluidic channel whose width is comparable to the average run length of the cell [Li *el.* PRL (2017), Blinz *et al.* Microelectron. Eng. (2010)]

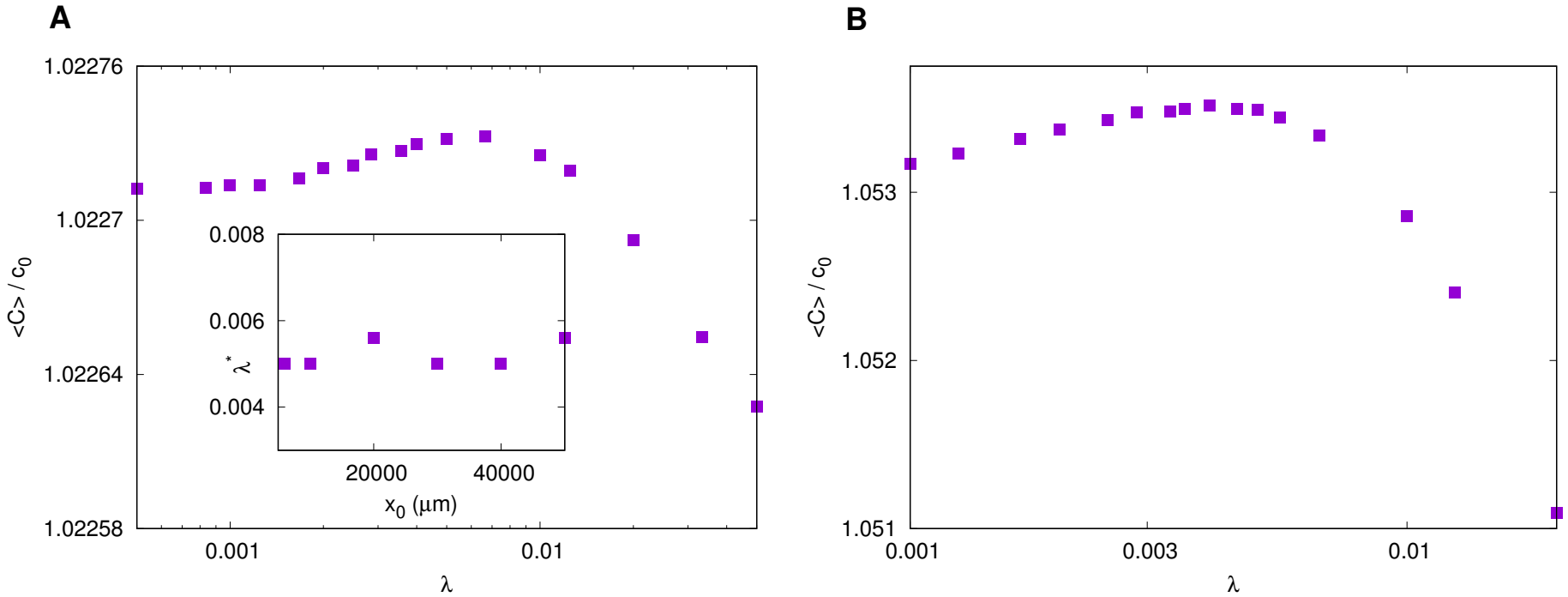
Simulation details

- $1d$ box of length L, with reflecting walls at two ends
- In a time-step dt, the cell moves a distance vdt
- $a(t)$, $m(t)$ and $y_P(t)$ are updated
- the tumbling probability $\omega(y_P)dt$ is calculated
- If a tumble does take place, the sign of v is reversed with probability q
- $L = 1000\mu m$, $v = 10\mu m/s$, $dt = 0.01s$
- Finite size effects negligible

Steady state distribution of cell position

- $\mathcal{P}_\lambda(x)$: steady state probability to find the cell at position x, for a given noise strength λ
- $\mathcal{P}_\lambda(x)$ should be large whenever $c(x)$ is large and $\mathcal{P}_\lambda(x)$ should be small where nutrient is sparse
- Average nutrient concentration experienced by the cell population in steady state : $\langle C \rangle = \int_0^L dx c(x) \mathcal{P}_\lambda(x)$
- Integrand has a large value only when both $c(x)$ and $\mathcal{P}_\lambda(x)$ are large, indicating strong localization in favorable region
- $\langle C \rangle$ shows a non-monotonic variation with noise strength λ
- There is an optimum level of the signaling noise when the chemotactic performance, as meaured by $\langle C \rangle$, is at its best

Localization vs noise



- For linear concentration profile best chemotaxis is observed for $\lambda = \lambda^* \simeq 0.007$
- λ^* does not depend strongly on the concentration gradient
- For a Gaussian $c(x)$ also a similar λ^* obtained

Chemotactic drift velocity in steady state

- Runs in the direction of increasing concentration of the chemo-attractant are extended and those in the opposite direction are shortened
- An overall drift motion up the concentration gradient
- Large drift velocity indicates good chemotactic performance
- Chemotactic drift velocity of the cell in presence of a linear concentration profile of the nutrient
- Consider an arbitrary position x where the cell tumbles and a new run begins
- $N_R(x)$ and $N_L(x)$ are total number of rightward and leftward runs starting at x, within an observation time window t_{obs}
- $d_R(x)$ and $d_L(x)$ are total durations of these rightward and leftward runs

Average run duration (in either direction) starting at x

$$\tau(x) = \frac{d_R(x) + d_L(x)}{N_R(x) + N_L(x)}$$

Probability that a run starts from the position x

$$Q_{tum}(x) = \mathcal{N}^{-1}[N_R(x) + N_L(x)]$$

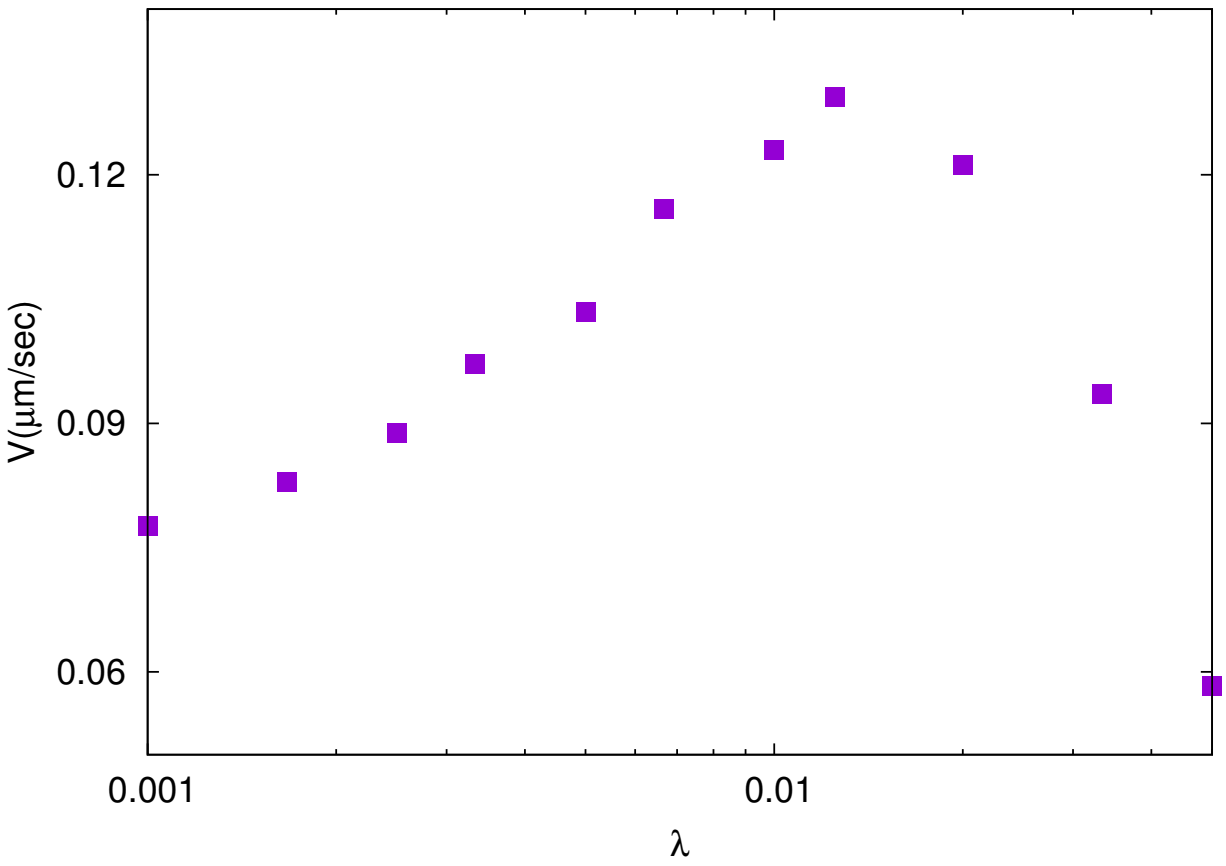
Average displacement in a run

$$\Delta = \int dx Q_{tum}(x) v \frac{d_R(x) - d_L(x)}{N_R(x) + N_L(x)}$$

$$\text{Chemotactic drift velocity} = \frac{\text{average displacement in a run}}{\text{average run duration}}$$

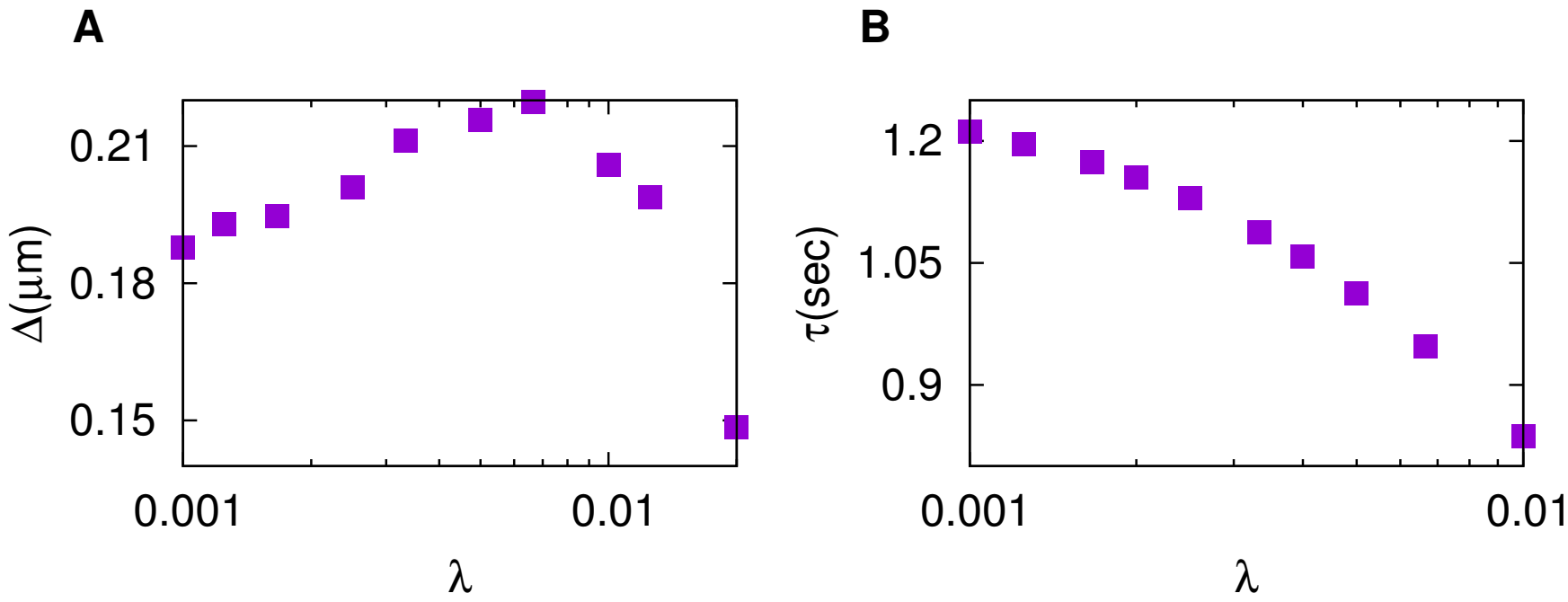
$$V = \frac{\Delta}{\tau} = \frac{v \int dx [d_R(x) - d_L(x)]}{\int dx' [d_R(x') + d_L(x')]}$$

Drift velocity vs noise



- Position of the peak does not match exactly with that for localization $\langle C \rangle$

Δ and τ for different noise strengths



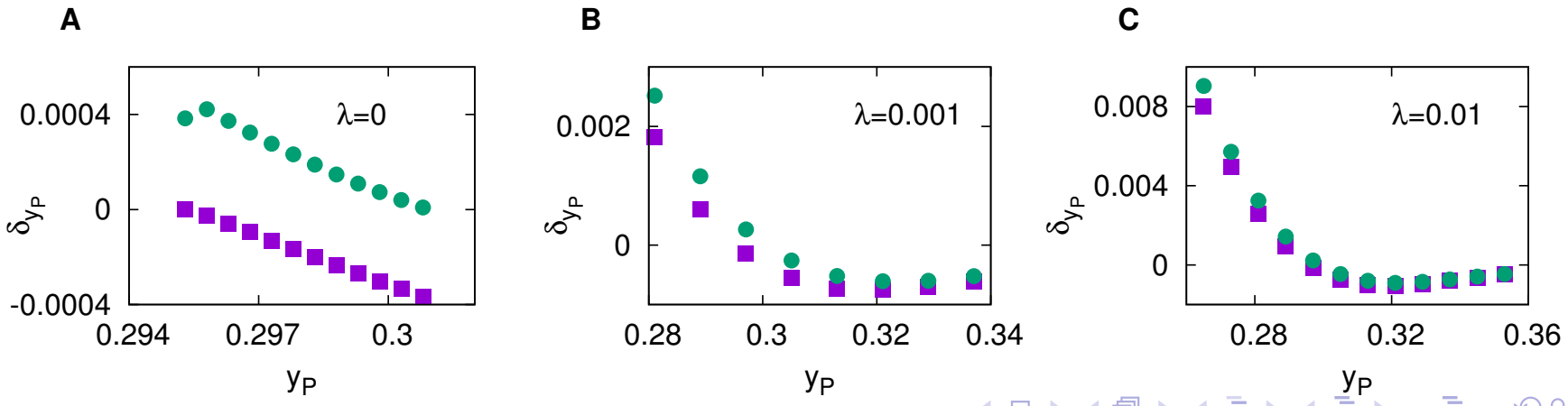
- Δ shows a peak at a λ, which matches with λ^* for $\langle C \rangle$
- τ decreases monotonically with noise, as in a homogeneous nutrient environment

Peak for V shifts to a higher noise value

- At the peak position λ_o one must have $\tau\Delta' - \Delta\tau' = 0$
- Since $\tau' < 0$ for all λ values, $\Delta' < 0$ at $\lambda = \lambda_o$
- Peak of Δ occurs at a smaller λ value and at λ_o it is decreasing with λ
- How the non-monotonic variation of Δ with noise can be explained from a detailed analysis of the biochemical pathway?
- Detailed study of CheY-P level statistics needed

Explanation of optimal noise strength

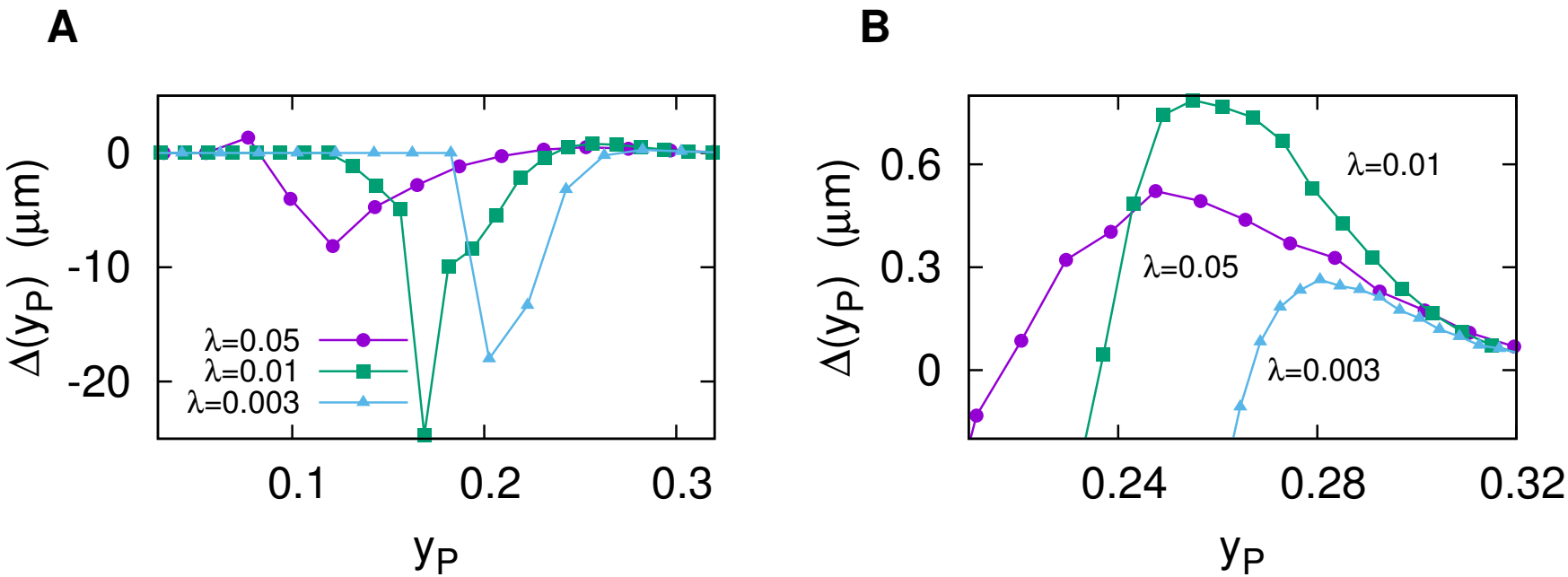
- For very low methylation noise, the only source of fluctuations in activity, methylation or CheY-P level is the stochastic change in the cell position
- As the cell moves rightward, the ligand concentration increases, and the free energy $f_{[L]}$ increases, causing the activity to decrease
- In a leftward run, activity increases
- CheY-P level goes down (up) in a rightward (leftward) run
- Measure the average change in CheY-P level in between two tumbles, when the intervening run is directed rightward (leftward).



Average displacement in a run that starts with y_P

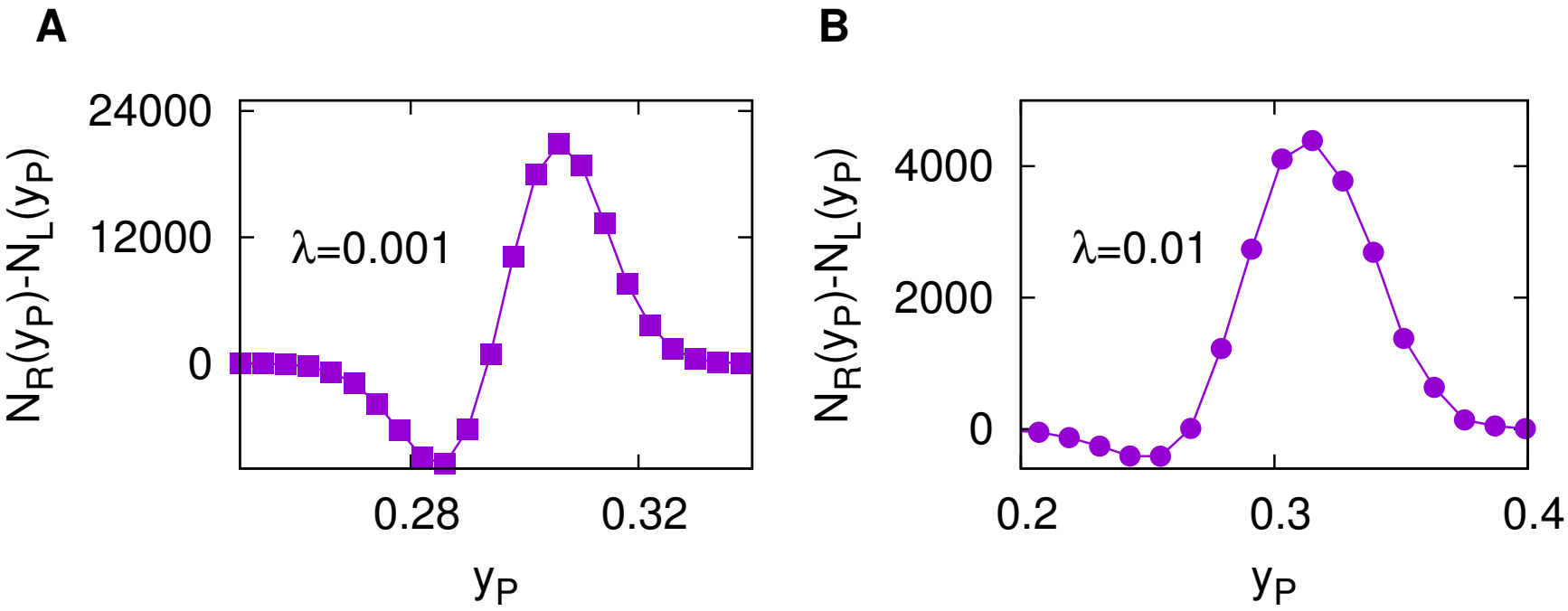
$$\Delta(y_P) = \frac{d_R(y_P) - d_L(y_P)}{N_R(y_P) + N_L(y_P)}$$

A negative peak at small y_P, followed by a positive peak at large y_P



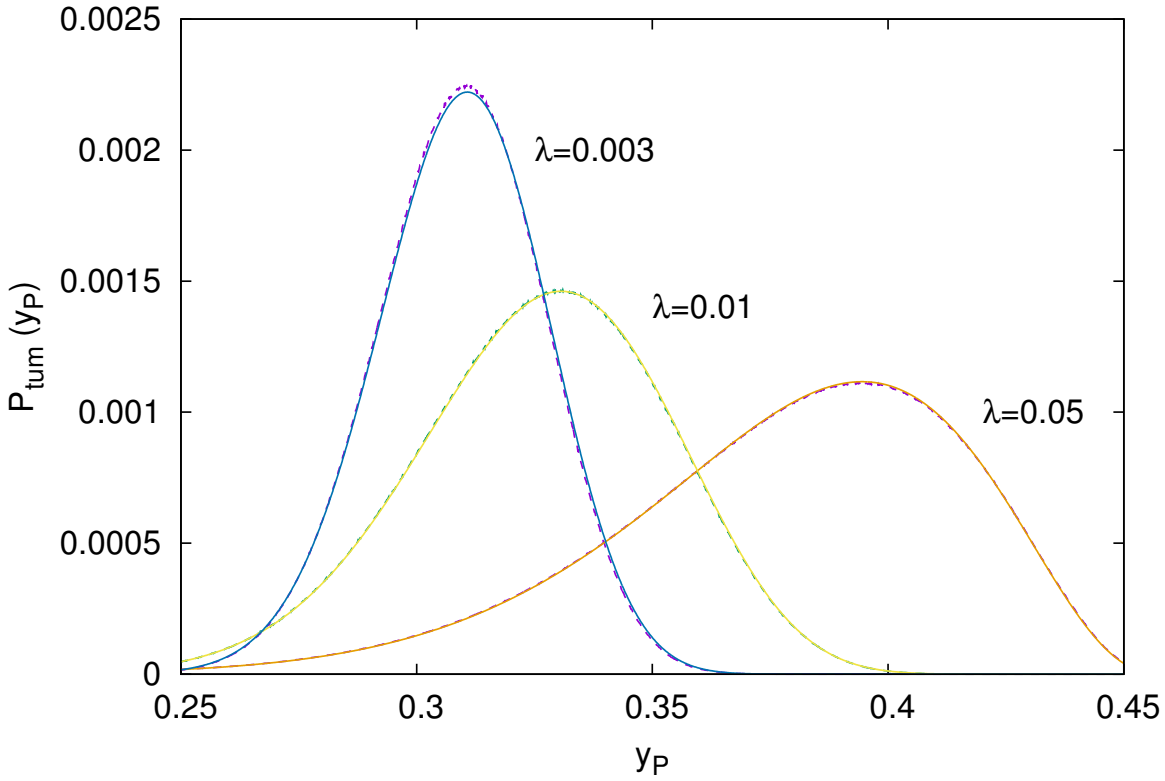
- For low noise, a rightward run starting with a given y_P must be preceeded by a leftward run which terminates at the same y_P
- This leftward run must have originated from a lower y_P value since for low noise, y_P value can only increase during a leftward run
- This event becomes particularly unlikely when y_P values are already small, near the left-tail of the distribution $P_{tum}(y_P)$
- Therefore, for small y_P values, $N_R(y_P) < N_L(y_P)$ and as a result, $d_R(y_p) < d_L(y_p)$, which makes $\Delta(y_P)$ negative
- As y_P increases and comes out of the tail region, $N_R(y_P)$ gradually increases and overtakes $N_L(y_P)$, and $\Delta(y_P)$ becomes positive
- However, as y_P becomes very large, run durations become rather small and while $N_R(y)$ remains above $N_L(y)$, their individual values start decreasing for large y_P
- $\Delta(y_P)$ decreases again for large y_P

$N_R(y_P) < N_L(y_P)$ for small y_P values



- For very small and large y_P both $N_R(y_P)$ and $N_L(y_P)$ vanish
- For intermediate y_P values, $N_R(y_P) - N_L(y_P)$ shows a positive and negative peak

CheY-P distribution for different noise



- While averaging $\Delta(y_P)$ over the distribution $P_{tum}(y_P)$, small y_P values give negative contribution and reduces Δ
- Negative $\Delta(y_P)$ values are near the left tail of $P_{tum}(y_P)$ and hence occur with low probability
- Thus overall drift velocity still remains positive

Threshold shifts with noise

- As noise increases, the distribution $P_{tum}(y_P)$ becomes wider and the left tail becomes much longer than the right tail
- For very small y_P both rightward and leftward runs raise the y_P level, but leftward runs do so by a larger magnitude
- As a result, a leftward run that preceeds a rightward run and that terminates at a small y_P must have to start from an even smaller y_P, which has a low probability associated with it
- $N_R(y_P) < N_L(y_P)$, but at a much smaller y_P value, when λ is relatively large
- Zero-crossing of $\Delta(y_P)$ and its positive peak are both shifted towards smaller y_P

- Starting from a large value, as y_P is decreased, $\Delta(y_P)$ keeps increasing and this trend continues till a much smaller y_P value, after which it finally starts declining again
- Averaging over such a curve yields a higher value of Δ than what was observed for small noise
- Hence Δ increases as noise increases
- But when noise becomes too large, the cell cannot distinguish between rightward and leftward runs
- The change in activity or y_P in a run is completely controlled by methylation level fluctuations now, and ligand concentration plays an insignificant role
- This again reduces Δ

Noise induced sensitivity [Flores *et al.* PRL (2012)]

- In a shallow gradient, chemotactic drift velocity shows a peak with noise, while localization remains flat at low noise and decreases to zero as noise increases
- (a) Internal state of the signaling pathway is described just in terms of activity and both methylation level and CheY-P level are expressed as a function of activity
- (b) Sigmoidal nature of dependence of tumbling rate on activity was approximated by making the tumbling rate zero as the activity level falls below some value
- Drift motion results from the difference in the amount of time a right-mover and a left-mover spends in the small activity state
- With increasing noise these small activity states are reached more often and hence drift velocity also increases
- For large noise, the difference between right- and left-mover again decreases, causing the drift velocity to go down

Detrimental response below a threhold

- In comparison, we find high activity level gives negligible contribution to noise
- When activity decreases, contribution increases
- When activity becomes lower than a certain threshold, contribution becomes negative
- The threhold value decreases as noise increases
- A crucial factor in explaining the noise induced enhancement of chemotactic drift velocity

Effect of signaling noise with time-varying nutrient concentration

- In many physical situations the chemical environment experienced by the cell changes with time
- Diffusion or degradation of chemo-attractant
- Short time behavior of the cell more important here

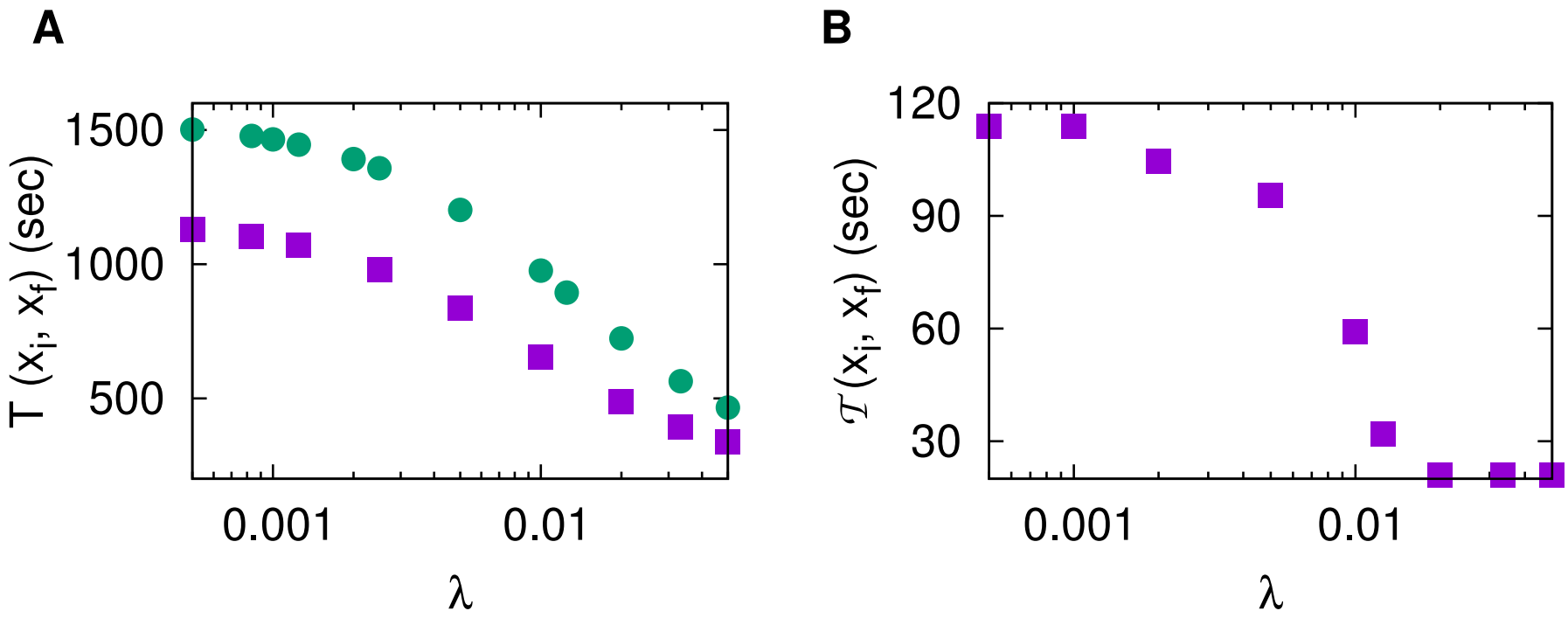
$$c(x,t) = c_0 e^{-t/\tau_d} \left[1 + \frac{\exp\left(-\frac{(x-\overline{x})^2}{\sigma_0^2 + 4\mathcal{D}t} \right)}{\sqrt{2\pi(\sigma_0^2 + 4\mathcal{D}t)}} \right]$$

- In a harsh chemical environment, the cell needs to find the favorable spot quickly and its trajectory should encounter large number of nutrient molecules
- First passage time of the cell measured at a region close to the peak of the Gaussian where the nutrient concentration is highest
- Uptake $\mathcal{U} = \int_0^{t_{obs}} dt \int_0^L dx c(x,t) \mathcal{P}_\lambda(x,t)$
- Mean amount of nutrient encountered by the cell along its trajectory upto a large enough observation time

Decaying nutrient profile

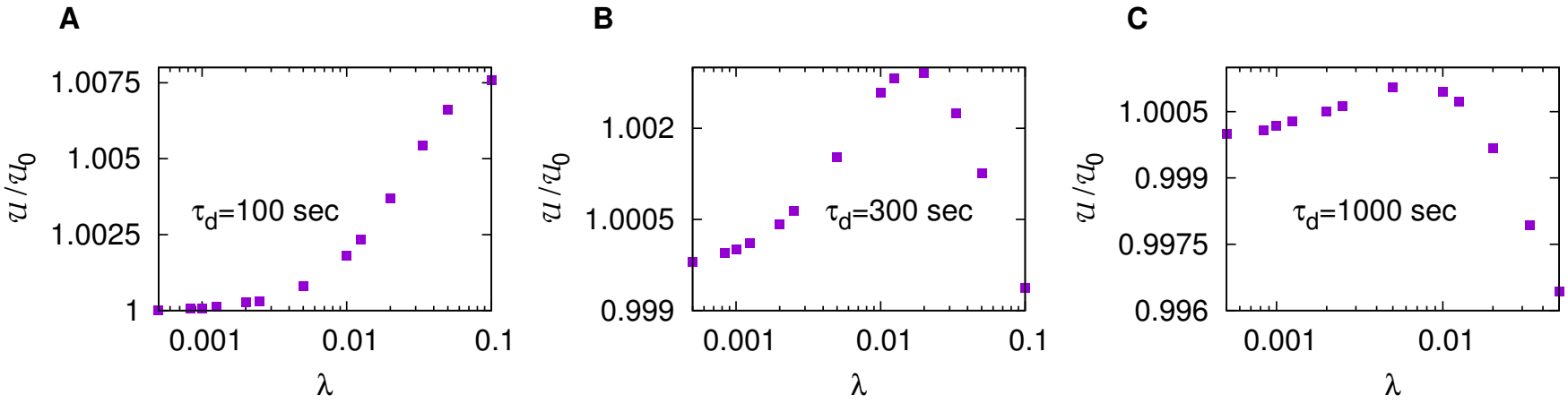
- Nutrient diffusivity $\mathcal{D}$ very small
- Cell experiences a Gaussian concentration profile with almost fixed width σ_0, and an exponential decay of the overall concentration level
- Mean and typical first passage time decreases with λ, as in a homogeneous medium without degradation
- However, degradation increases the first passage time for a given λ
- When nutrient degrades, even when the cell is moving in a homogeneous medium, it experiences a decreasing concentration along its trajectory, which makes it tumble more
- Average run durations shorter and hence the mean first passage time longer

Mean and typical first passage time vs λ



- The circles show the data for $c(x,t) = c_0 e^{-t/\tau_d}$ and squares are for degrading Gaussian profile with $\mathcal{D} = 0$
- $\tau_d = 500sec$, $\sigma_0 = 100\mu m$
- For small λ, typical first passage time is more useful

Uptake variation with noise depends on τ_d

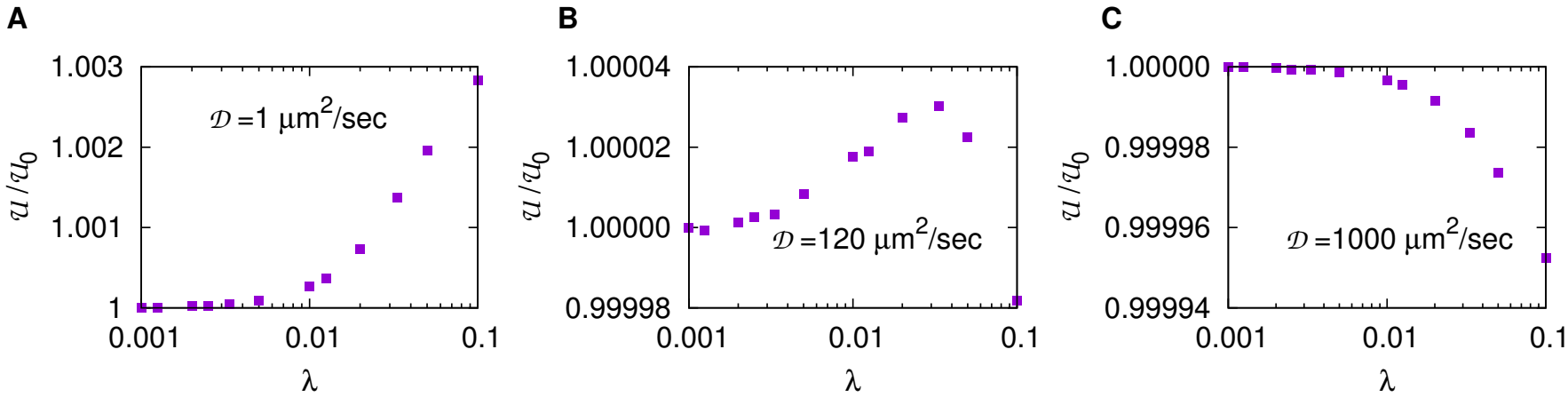


- $t_{obs} = 1000s$
- For small τ_d, nutrient decays rapidly, only those ttrajectories with very long runs contribute
- As λ increases, such trajectories become more probable
- For large τ_d, nutrient degrades slowly, time-independent limit recovered
- Uptake peak approaches localization peak λ^*

Nutrient profile with decay and diffusion

- Same qualitative behavior of first passage time
- Uptake behavior depends on the interplay between degradation and diffusion time-scales
- For large $\mathcal{D}$ concentration gradient in the medium disappears fast and the uptake does not depend on cell trajectory anymore, except for very small times
- The more time the cell is able to spend close to the peak of the Gaussian profile before the profile flattens or nutrient degrades, larger will be its uptake
- For large λ, cells execute long runs which decreases the residence time near the peak: uptake is low

Uptake vs noise with degradation and diffusion

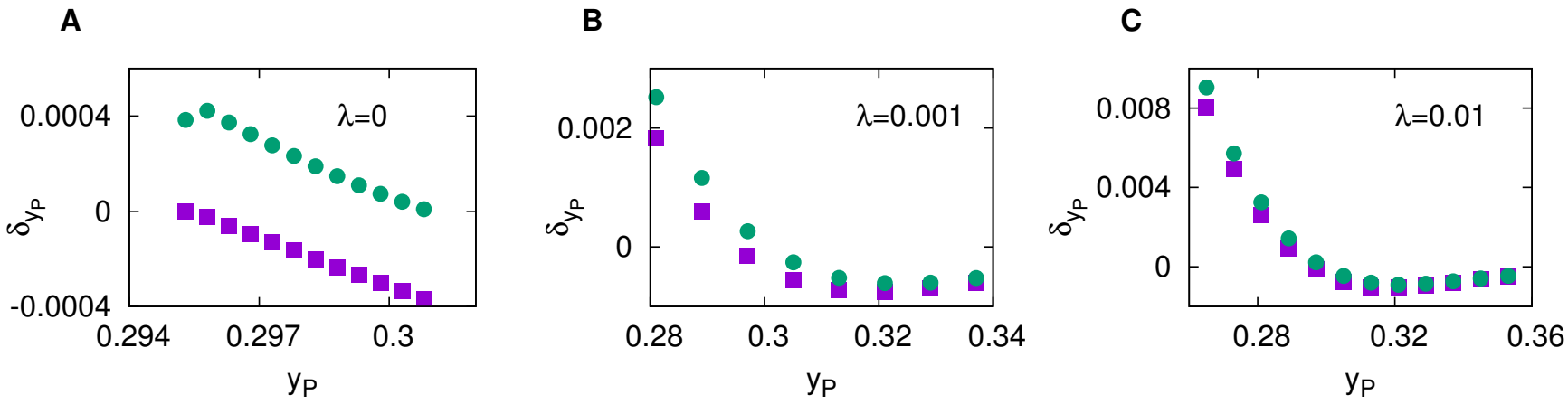


- $\tau_d = 100sec$, $\sigma = 10\mu m$, $t_{obs} = 200sec$
- For small λ, FPT is larger
- Cell spends most of its short time trajectory trying to climb up the concentration gradient, reaching the peak of the Gaussian
- Uptake is larger
- A peak for intermediate $\mathcal{D}$ value

Conclusions

- Effect of methylation noise on the chemotactic performance of a single *E. coli* cell
- An optimum noise strength for the best performance
- Explanation from CheY-P level fluctuations for cell motion up and down the gradient
- Detrimental behavior below noise-dependent threshold
- Adaptation of flagellar motors or spatial organization of chemo-receptors neglected
- Fluctuatins can originate from clustering of chemo-receptors [Colin *et al.* (2017)]

Change in CheY-P level during a run



- as λ increases, the change in activity is also controled by the methylation level fluctuations and the feedback it produces on the reaction network
- When activity becomes too low (high), the methylation level increases, which in turn causes the activity to increase (decrease)
- For large λ, when the activity varies over an wider range, the feedback effect is more prominent and can easily override the change in activity due to change in cell position

- When λ becomes high, activity can also increase during a rightward run, especially when its value at the start of the run is sufficiently small
- Similarly, in a leftward run activity may decrease when its value is high enough
- In terms of CheY-P level, this means that during a rightward (leftward) run the CheY-P level y_P can increase (decrease) when y_P has small (large) values