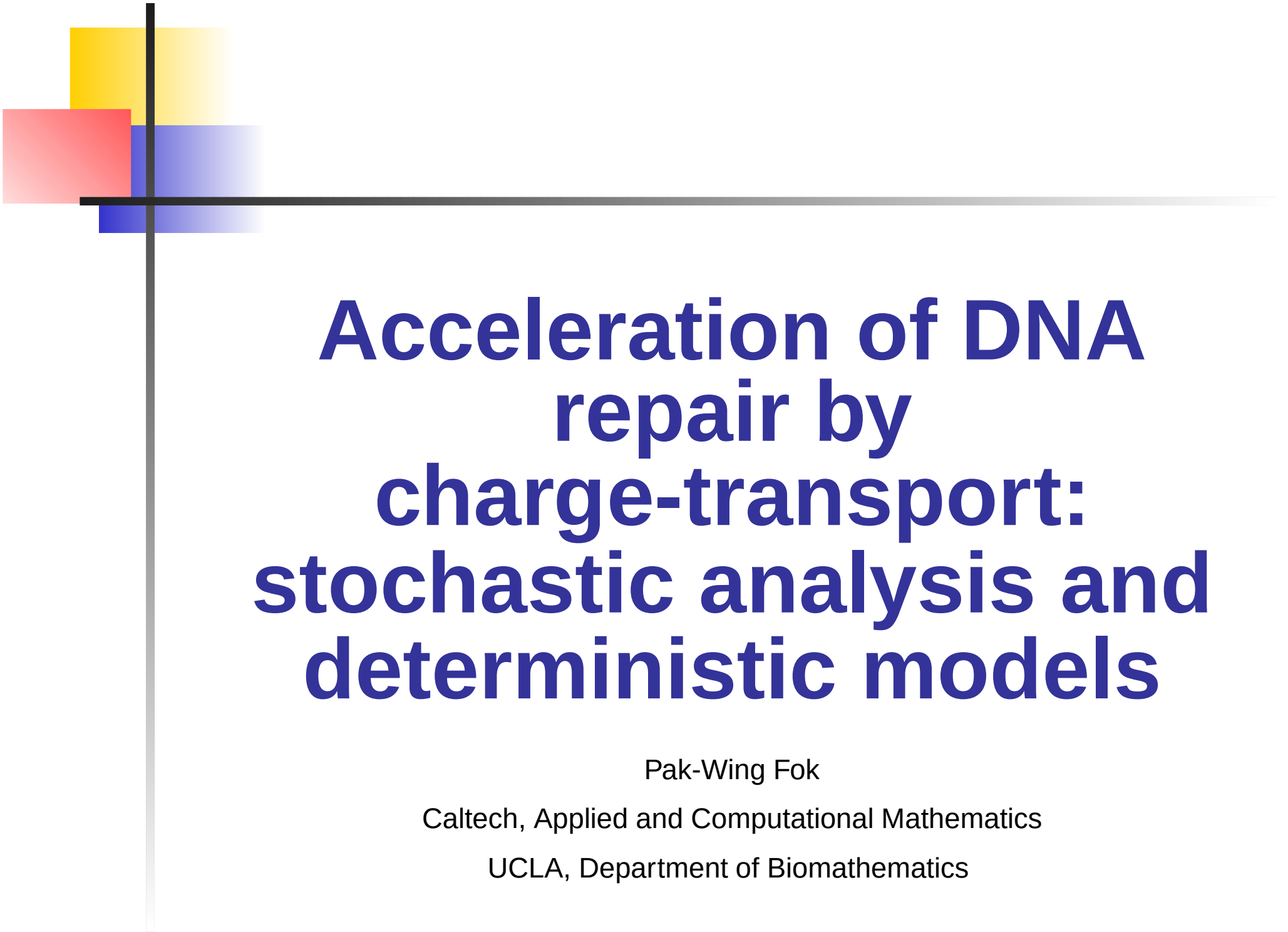

# Acceleration of DNA repair by charge-transport: stochastic analysis and deterministic models

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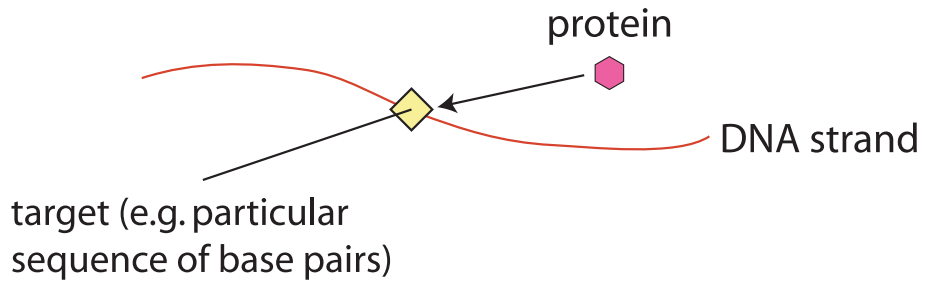
# Background and Motivation

- DNA-protein interactions are important in gene transcription and protein production




- But: rates of DNA-protein reactions are faster than the theoretical upper limit predicted by 3D diffusive (Debye-Smoluchowski) theory. [Riggs *et al.* 1970]
- And: rates of reactions also faster than typical 1D diffusive sliding time. For *E. coli*, $L \sim 10^6$ bp $D \sim 5 \times 10^6$ bp$^2$/s $\Rightarrow T \sim L^2/D \approx$ 2 days
- Question: how do proteins/enzymes find their targets on DNA so quickly? [Berg *et al.* 1981, Von-Hippel and Berg, 1987]

# Proposed solution

- Facilitated diffusion [Berg 1981]: combination of 1D sliding and 3D diffusion $\Rightarrow$ rates predicted to increase up to $100\times$.


Proteins find their targets through a combination of 1D diffusive sliding, attachment/detachment and 3D diffusion

- However, acceleration requires $D_{1D}$ and $D_{3D}$ to be comparable and equal time spent in 1D and 3D diffusion.
- This is not true in most situations!

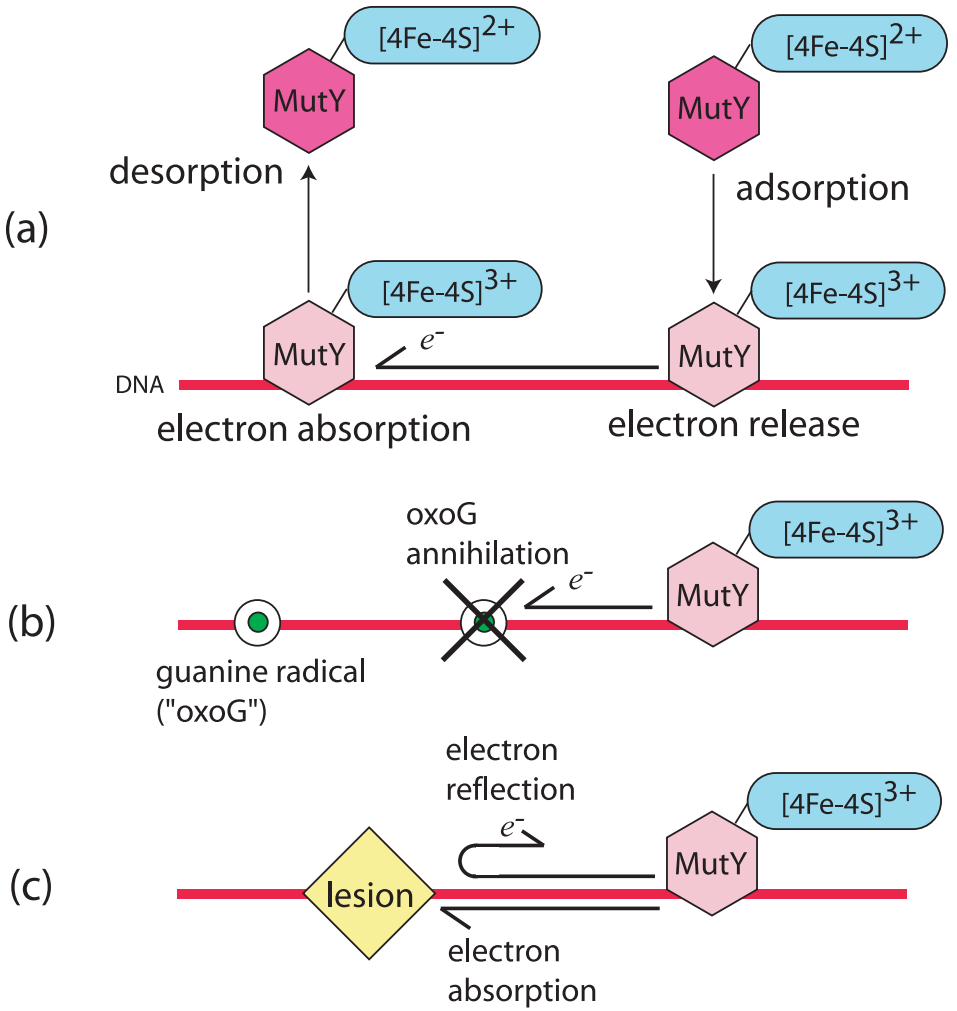
# Other mechanisms

- Fast intersegmental transfers [Sheinman and Kafri 2008]
- Effect of DNA conformation [Hu *et al.* 2006]
- Protein cooperativity [Cherstvy *et al.* 2008]
- Charge Transport [Yavin *et al.* 2005, Boon *et al.* 2003]: applicable to a particular protein called MutY, a Base Excision Repair enzyme.

# Base Excision Repair (BER) enzymes

- The genome of all living organisms is constantly under attack by mutagenic agents e.g. ionizing radiation
- Mutagenic agents give rise to damaged base pairs in DNA ("lesions") $\Rightarrow$ miscoded proteins, possibly cancer.
- BER enzymes locate lesions on DNA, remove them, maintain integrity of genome.
- MutY searches for lesions via a Charge-Transport (CT) mechanism [Yavin *et al.* 2005, Boon *et al.* 2003]

# Charge Transport (CT)

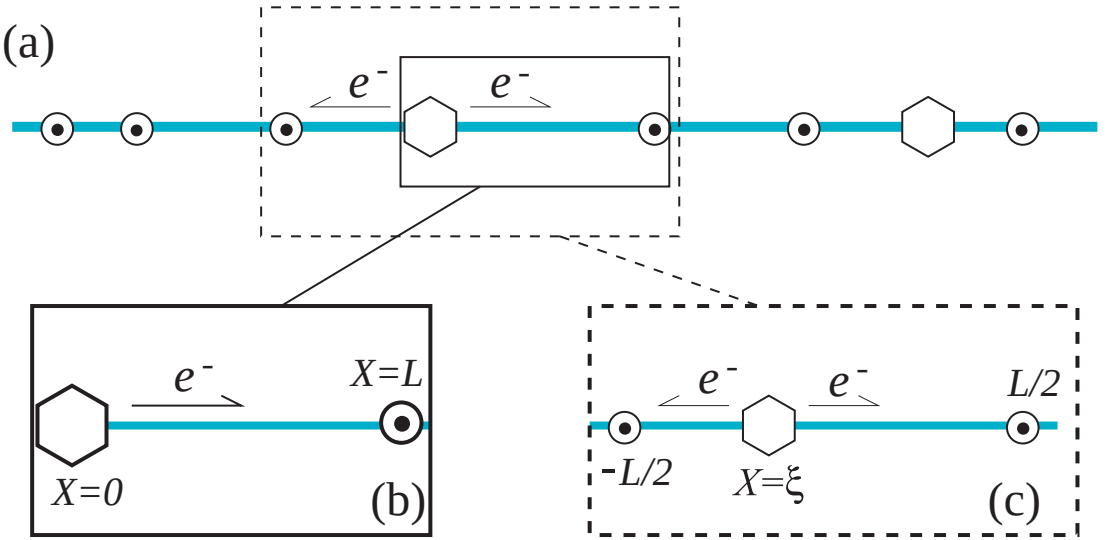



(a) Iron-Sulfur cofactors oxidize when MutY adorbs to DNA. Release/absorption of electrons $\Leftrightarrow$ adsorption/desorption of enzyme.

(b) Guanine radicals ("OxoGs"): damaged bases that annihilate upon absorbing an electron.

(c) Lesions prevent passage of electrons by reflection/absorption. They require presence of MutY to be excised from DNA.

# Stochastic Broadwell Model




(a) Enzyme is deposited on DNA and releases an electron to either side

(b) “One-sided” Broadwell problem: electron released to right with probability 1

(c) “Two-sided” Broadwell problem : electron released left or right with probability 1/2.

Note: electron return probability $= 1$ in absence of guanine radicals

# One-sided Broadwell problem

- Governing equations [Bicout, 1997, Fok *et al.* 2008]:

$$\frac{\partial P_+}{\partial T} + V\frac{\partial P_+}{\partial X} = -FP_+ + FP_- - MP_+$$
$$\frac{\partial P_-}{\partial T} - V\frac{\partial P_-}{\partial X} = FP_+ - FP_- - MP_-$$

  $P_\pm(X,T)$: pdfs of rightward and leftward electron, $V$: electron speed, $F$: flip rate, $M$: decay rate

- Boundary conditions:

$$P_+(0,T) = P_-(L,T) = 0$$

- Initial conditions:

$$\begin{aligned} P_+(X,0) &= \delta(X) \\ P_-(X,0) &= 0 \end{aligned}$$

# Dimensionless eqns

Non-dimensionalize space by $1/\rho$, time by $1/\rho V$ where $\rho =$ OxoG density:

$$x = \rho X, \qquad t = \rho V T,$$

$$\Rightarrow \frac{\partial \mathbf{Q}}{\partial t} = \mathbf{L}\mathbf{Q}, \quad \mathbf{Q} = \begin{pmatrix} Q_+(x,t) \\ \\ Q_-(x,t) \end{pmatrix},$$

where $Q_\pm = P_\pm/\rho$ and

$$\mathbf{L} = \begin{bmatrix} -\dfrac{\partial}{\partial x} - f - \mu & f \\ f & \dfrac{\partial}{\partial x} - f - \mu \end{bmatrix},$$

and

$$f = \frac{F}{\rho V}, \qquad \mu = \frac{M}{\rho V}.$$

# Adsorption/desorption probabilities

Take $\mu = 0$ always (no electron decay)

Desorption prob: $$\int_0^\infty Q_-(0,t')dt' = \frac{f\ell}{1+f\ell}$$

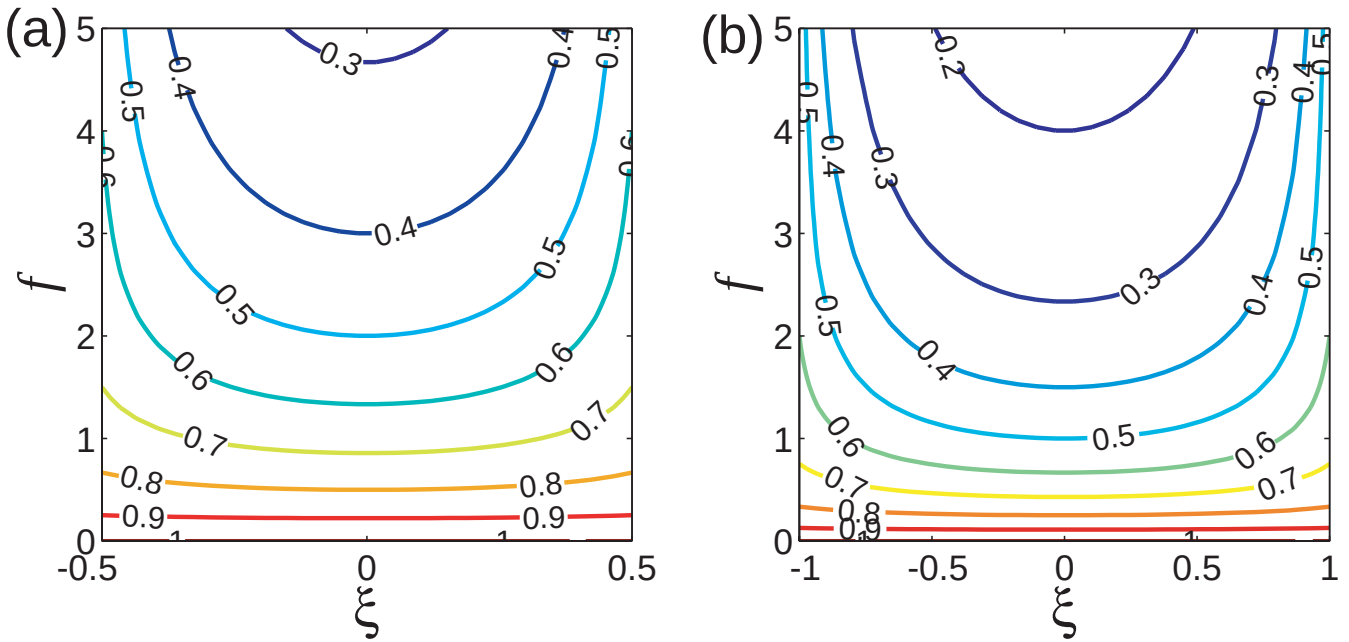
Adsorption prob: $$\int_0^\infty Q_+(L,t')dt' = \frac{1}{1+f\ell}$$

Extension to two-sided problem:

$$\Pi_{\text{desorb}} = \frac{1}{2}\left[\frac{f(\ell/2-\xi)}{1+f(\ell/2-\xi)} + \frac{f(\ell/2+\xi)}{1+f(\ell/2+\xi)}\right]$$

$$\Pi_{\text{adsorb}} = \frac{1}{2}\left[\frac{1}{1+f(\ell/2-\xi)} + \frac{1}{1+f(\ell/2+\xi)}\right]$$

# Sticking probability




Dependence of enzyme sticking probability $\Pi_{\text{adsorb}}$ on its landing position $\xi$ and flipping rate for gap size (a) $\ell = 1$ and (b) $\ell = 2$.

$f \ll 1$: ballistic limit, $f \gg 1$: diffusive limit

# Adsorption Statistics away from lesions

1. Average over landing position $\xi$:

$$\begin{aligned}\bar{\Pi}_{\text{adsorb}} &= \frac{1}{2\ell}\int_{-\ell/2}^{\ell/2}\Pi_{\text{adsorb}}(\xi,\ell;f)d\xi \\ &= \frac{2}{f\ell}\tanh^{-1}\left(\frac{f\ell}{2+f\ell}\right).\end{aligned}$$

2. Consider gaps with discrete distribution $\ell_1,\ell_2,\ell_3,....$ Assume a fraction $\phi_j$ of gaps have size $\ell_j$. Deposit a single enzyme onto the DNA. The probability of landing in gap of size $\ell_j$ is $\phi_j\ell_j/\sum_{j=1}^{\infty}\phi_j\ell_j$. Fraction that *stays adsorbed* in gap of size $\ell_j$ is

$$\frac{2}{f\ell_j}\tanh^{-1}\left(\frac{f\ell_j}{2+f\ell_j}\right)\times\frac{\phi_j\ell_j}{\sum_{j=1}^{\infty}\phi_j\ell_j}$$

Continuous gap size distribution: $\ell_j\to\ell$, $\phi_j\to\phi(\ell)d\ell$:

$$\text{Ensemble average}\quad\langle\bar{\Pi}_{\text{adsorb}}\rangle=\frac{2}{f\langle\ell\rangle}\int_0^{\infty}\phi(\ell)\tanh^{-1}\left(\frac{f\ell}{2+f\ell}\right)d\ell.$$

# Form of $\phi(\ell)$?

If OxoGs randomly appear anywhere on an infinite DNA, what is the pdf of the gap size (distance between 2 consecutive OxoGs)?

Consider a lattice of length $L_0$ with $n$ sites (each site has width $a$) where OxoGs can appear with rate $\Omega$ radicals per unit time $T$ per lattice site. Time taken for $G$ radicals to appear is $T_0 = G/n\Omega$.

Let $N(m,T)$ be the pdf of the number of gaps of size $m$. Then $N$ obeys [D'Orsogna and Chou 2005]

$$\frac{1}{\Omega}\frac{\partial N(m,T)}{\partial T} = 2\sum_{m'=m+1}^{n} N(m',T) - mN(m,T)$$

Define $\rho \equiv G/L_0$ as the OxoG density and the dimensionless variables

$$y = \rho a m, \qquad t = T/T_0, \qquad p = N/Gt = \text{gap fraction}$$

Take continuum limit $n \to \infty$, $a\rho \to 0$ and $G, L_0 \to \infty$ such that $\rho$ remains fixed.

$$p(y,t) \to \text{probability density for continuous gap size } y$$

# Gap distribution

Setting $q(y,t) = tp(y,t)$ where $p(y,t)$ is the continuous pdf of gaps of size $y$:

$$\frac{\partial q}{\partial t} = 2\int_y^\infty q(y',t)dy' - yq(y,t)$$

Solve by Laplace transform in time:

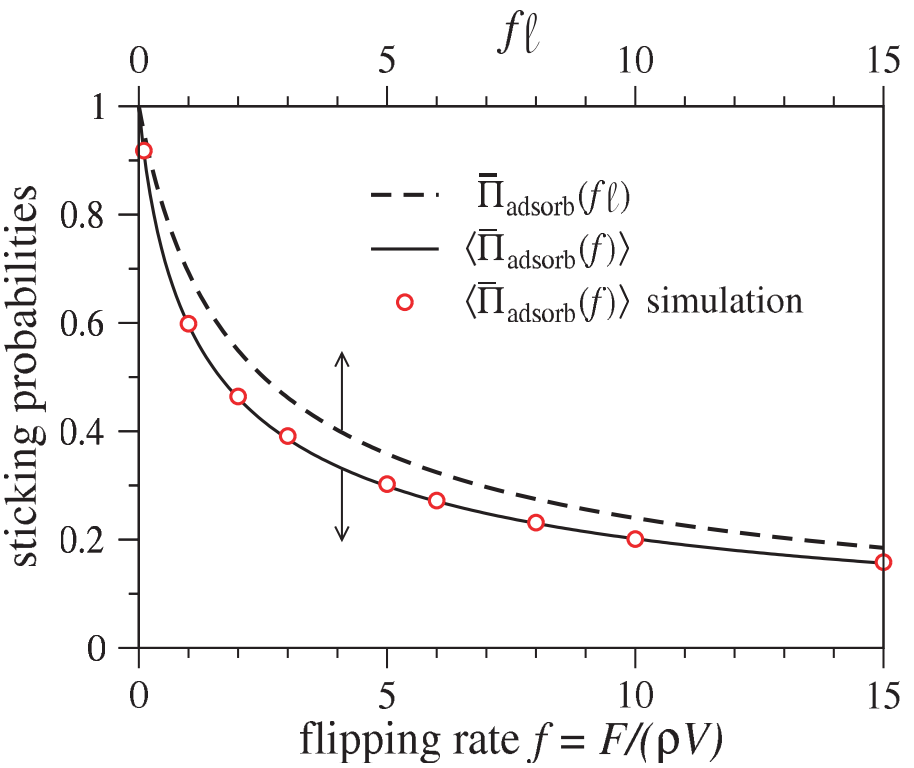
$$\begin{aligned} \Rightarrow q(y,t) &= t^2 e^{-yt} \\ \Rightarrow p(y,t=1) &= e^{-y} \\ \Rightarrow \text{Prob}(y \le Y \le y+dy) &= e^{-y}dy \equiv \phi(y)dy \end{aligned}$$

$Y$: non-dimensional gap length at $t = 1 \Leftrightarrow G$ radicals have appeared. Hence,

$$\langle \bar{\Pi}_{\text{adsorb}} \rangle = \frac{e^{1/f}\,\text{Ei}(1/f)}{f}$$

where $\text{Ei}(x) = \int_x^\infty \frac{e^{-t}}{t}dt$

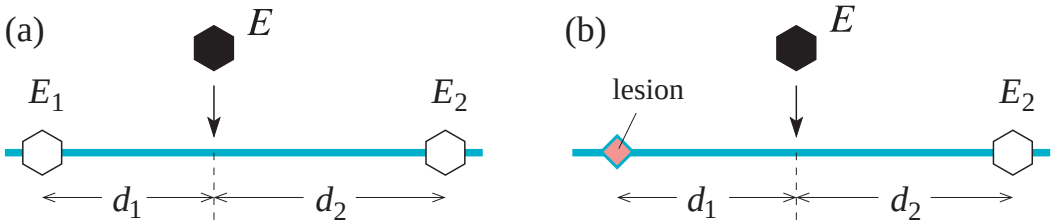
# Verification




Sticking probabilities for deposition of a single enzyme onto an infinite DNA with gap distribution $\phi(\ell) = e^{-\ell}$. Also expect probabilities to be approximately valid when the fraction of OxoGs annihilated is $\ll 1$.

# Colocalization of enzymes near lesions

Analyze using Monte Carlo with adsorption/desorption probabilities $\Pi_{\text{adsorb}}$ and $\Pi_{\text{desorb}}$:



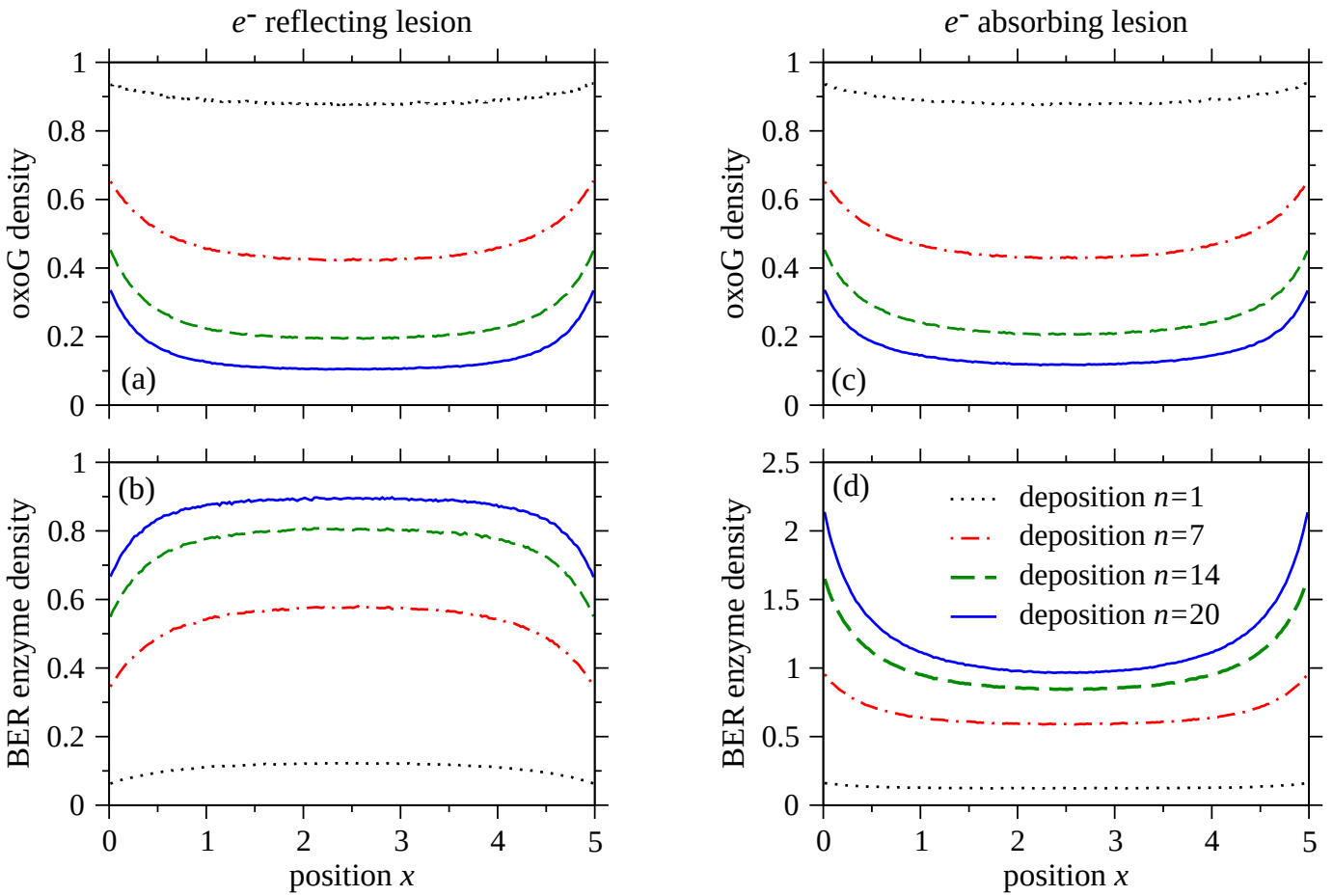

(a)

| Event: | $E$ self-desorbs | $E$ adsorbs, $E_1$ desorbs | $E$ adsorbs, $E_2$ desorbs |
|---|---|---|---|
| Probability: | $\frac{1}{2}\left(\frac{fd_1}{1+fd_1}+\frac{fd_2}{1+fd_2}\right)$ | $\frac{1}{2}\frac{1}{1+fd_1}$ | $\frac{1}{2}\frac{1}{1+fd_2}$ |

(b)

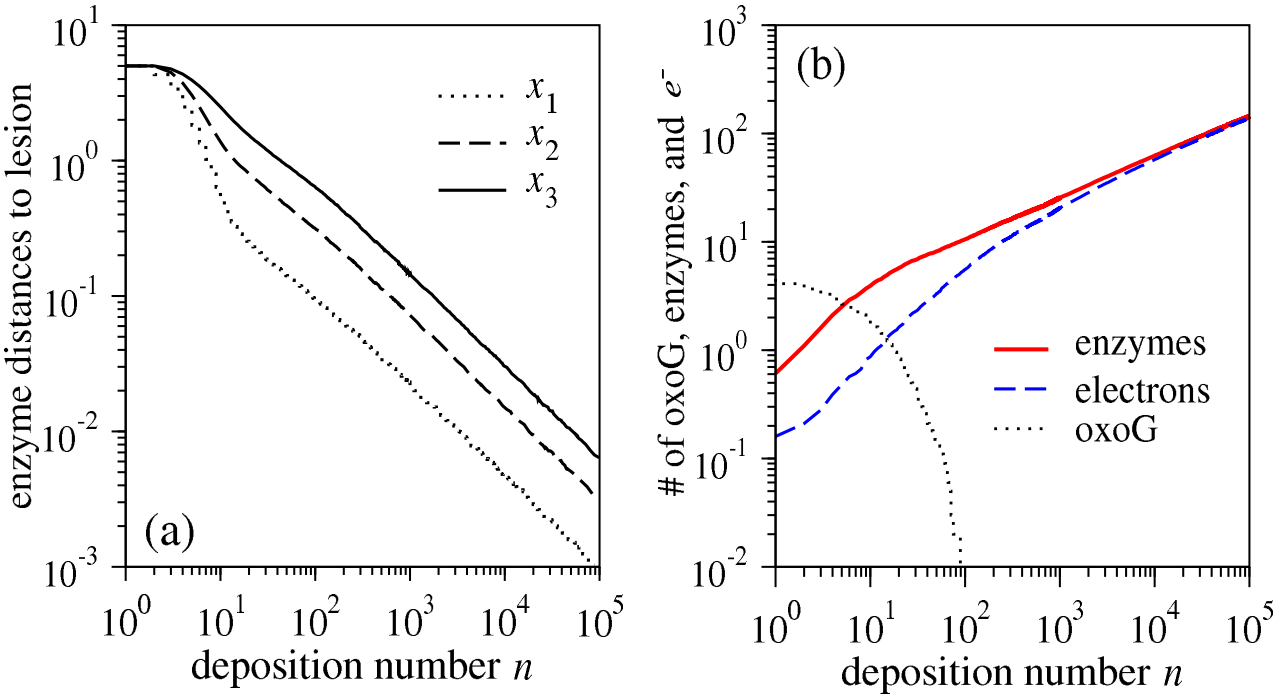
| Event: | $E$ self-desorbs | $E$ adsorbs, $E_2$ stays adsorbed | $E$ adsorbs, $E_2$ desorbs |
|---|---|---|---|
| Prob: (reflecting lesion) | $\frac{1}{2}+\frac{1}{2}\left(\frac{fd_2}{1+fd_2}\right)$ | $0$ | $\frac{1}{2}\frac{1}{1+fd_2}$ |
| Prob: (absorbing lesion) | $\frac{1}{2}\left(\frac{fd_1}{1+fd_1}+\frac{fd_2}{1+fd_2}\right)$ | $\frac{1}{2}\frac{1}{1+fd_1}$ | $\frac{1}{2}\frac{1}{1+fd_2}$ |

# MC Results (1/2)




Evolution of enzyme density as enzymes are *adiabatically* deposited onto DNA. Results came from averaging $10^7$ trials using flip rate $f = 1$.

# MC Results (2/2)




Scaling results in large $n$ limit. (a) $x_i$ = distance from lesion to $i$th closest enzyme. Convergence of repair enzymes $= O(n^{-2/3})$. (b) Accumulation of enzymes $= O(n^{1/3})$.

Enzyme deposition within 5 base pairs of a lesion requires $n \approx 6 \times 10^6$ deposition attempts. If each deposition takes $0.0005$s $\Rightarrow$ total search time $\approx 50$ minutes.

# Discussion

- Compare with randomly deposited “passive” enzymes that always stick to the DNA. $n \gg 1$: number of depositions

| | Passive | CT |
|---|---|---|
| Enzyme number : | $O(n)$ | $O(n^{1/3})$ |
| enzyme-lesion distance : | $O(n^{-1})$ | $O(n^{-2/3})$ |

- Passive enzymes converge more quickly but search is very redundant/wasteful.
- CT search strategy more effective when number of enzymes in system is limited.

# Weaknesses of model

- Search time of 50 minutes is an improvement, but it is still too slow.
- Enzymes are stationary on DNA; they do not diffuse along strand.
- MC simulations keep bulk chemical potential constant. Number of enzymes on DNA can grow without bound.
- Assumed adiabatic depositions.

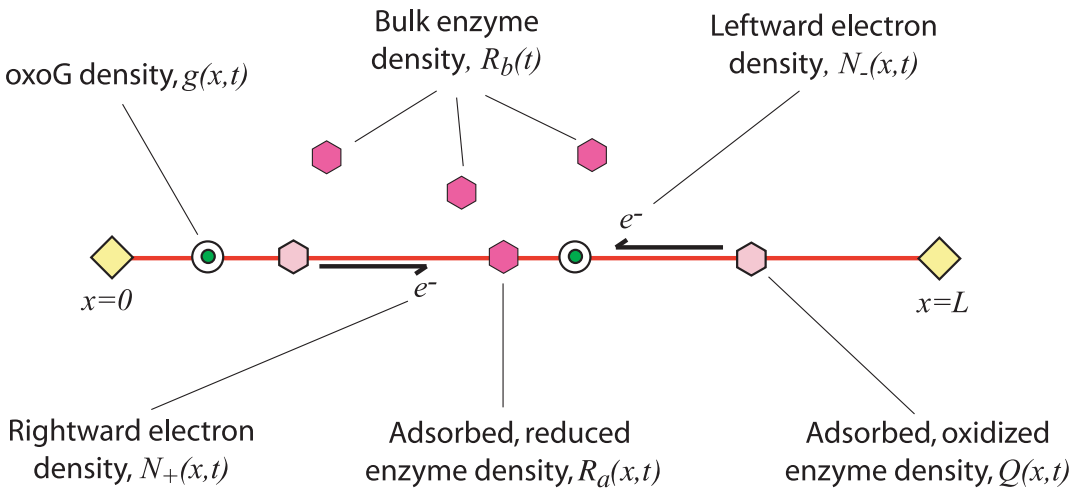
All these factors make the discrete model rather unrealistic.

Improvements:

- Use a continuum PDE model
- Model enzyme binding more carefully

# Binding kinetics

(a)




(b)

| | | | | |
|---|---|---|---|---|
| MutY-[4Fe-4S]$^{2+}$ (aq) | $\underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}}$ | MutY-[4Fe-4S]$^{2+}$ (adsorbed) | $\underset{v\,(N_+ + N_-)}{\overset{m}{\rightleftharpoons}}$ | MutY-[4Fe-4S]$^{3+}$ + $e^-$ (adsorbed) |
| $R_b$ | $\rightleftharpoons$ | $R_a$ | $\rightleftharpoons$ | $Q + e^-$ |

Discrete model assumed electron release prob = 1 upon contact with DNA, desorption prob = 1 upon electron absorption. This is **not** the same as taking $m, k_{\text{off}} \to \infty$.

# PDE model for CT enzymes

$$
\begin{aligned}
\frac{\partial Q}{\partial t} &= D_+ \frac{\partial^2 Q}{\partial x^2} - v(N_+ + N_-)Q + mR_a, \\
\frac{\partial R_a}{\partial t} &= D_- \frac{\partial^2 R_a}{\partial x^2} + v(N_+ + N_-)Q - k_{\text{off}} R_a + k_{\text{on}} \left(\frac{\Omega}{L}\right) R_b - mR_a, \\
\frac{dR_b}{dt} &= -k_{\text{on}} R_b + \frac{k_{\text{off}}}{\Omega} \int_0^L R_a \, dx, \\
\frac{\partial N_+}{\partial t} + v\frac{\partial N_+}{\partial x} &= fN_- - fN_+ - vN_+(Q+g) + \frac{mR_a}{2}, \\
\frac{\partial N_-}{\partial t} - v\frac{\partial N_-}{\partial x} &= -fN_- + fN_+ - vN_-(Q+g) + \frac{mR_a}{2}, \\
\frac{\partial g}{\partial t} &= -v(N_+ + N_-)g.
\end{aligned}
$$

$Q$: Oxidized enzyme on DNA, $R_a$: reduced enzyme on DNA, $R_b$: reduced enzyme in solution, $N_\pm$: rightward and leftward electrons, $g$: guanine radicals

$D_\pm$: 1D diffusivities, $f$: flip rate, $v$: $e^-$ speed, $\Omega$ reservoir volume, $L$: DNA length, $m$: oxidation rate, $k_{\text{on}}$: deposition rate, $k_{\text{off}}$: desorption rate of reduced enzymes on DNA.

# Model reduction

Drop derivatives in equation for $R_a$; obtain "outer" solution in $x$ and $t$:

$$R_a \approx \frac{1}{m + k_{\text{off}}} \left( v(N_+ + N_-)Q + k_{\text{on}} \left( \frac{\Omega}{L} \right) R_b \right)$$

Reduced, non-dimensional equations are:

$$\begin{aligned}
\frac{\partial Q}{\partial t} &= -U(1-\sigma)(N_+ + N_-)Q + \nu \frac{\partial^2 Q}{\partial x^2} + \sigma R_b, \\
\frac{dR_b}{dt} &= U(1-\sigma) \int_0^1 (N_+ + N_-) Q dx - \sigma R_b, \\
\frac{\partial N_+}{\partial t} + U \frac{\partial N_+}{\partial x} &= \left[ F + \frac{\sigma}{2} UQ \right] N_- - \left[ F + \left( 1 - \frac{\sigma}{2} \right) UQ \right] N_+ - gUN_+ + \frac{\sigma R_b}{2}, \\
\frac{\partial N_-}{\partial t} - U \frac{\partial N_-}{\partial x} &= -\left[ F + \left( 1 - \frac{\sigma}{2} \right) UQ \right] N_- + \left[ F + \frac{\sigma}{2} UQ \right] N_+ - gUN_- + \frac{\sigma R_b}{2}, \\
\frac{\partial g}{\partial t} &= -U(N_+ + N_-)g,
\end{aligned}$$

$\sigma = \frac{m}{m + k_{\text{off}}}$: effective binding rate per enzyme (competition between electron release $m$ and desorption $k_{\text{off}}$)

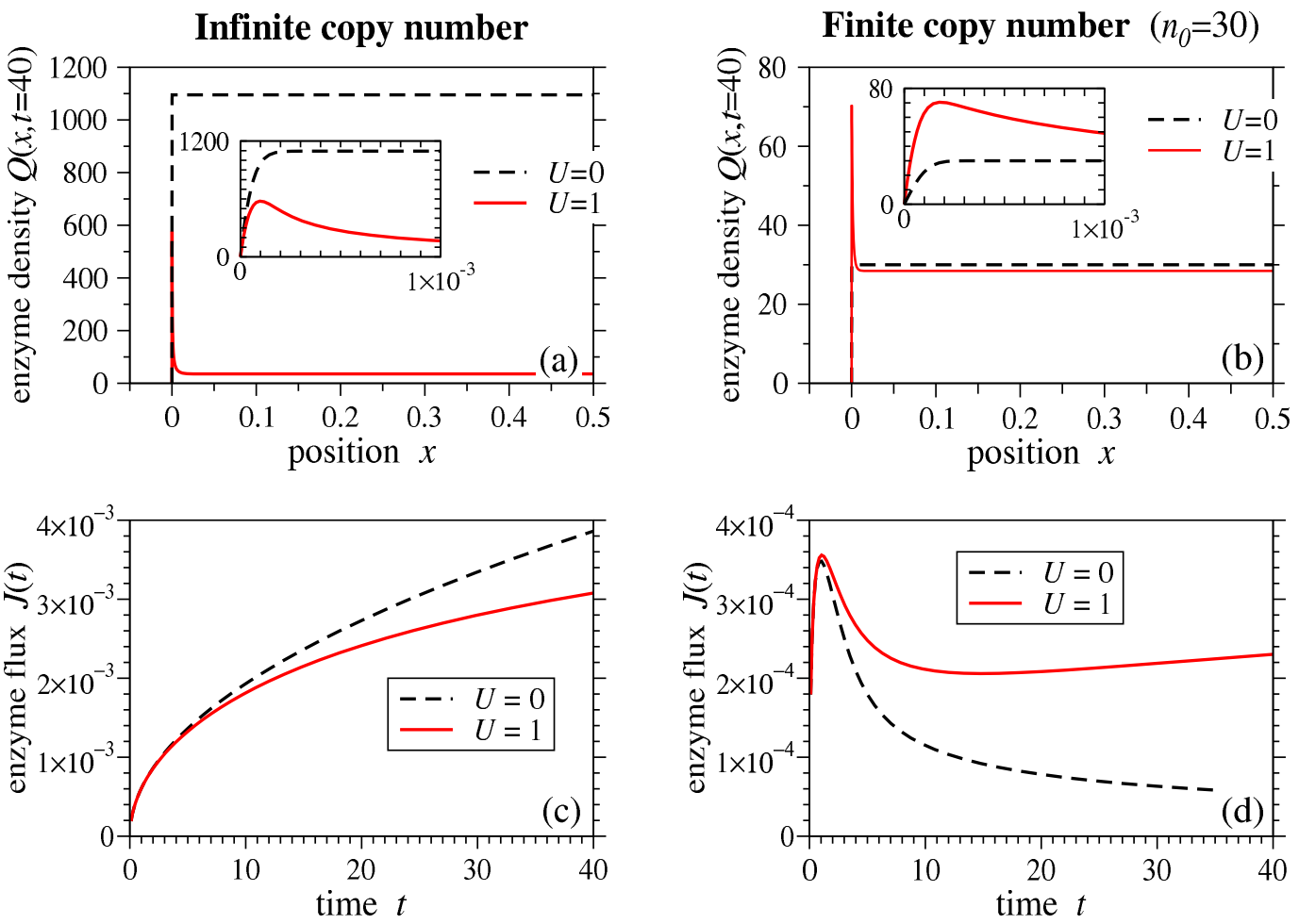
# Reduced model

- Dimensionless diffusivity, electron speed, flip rate: $\nu = \frac{D_+}{k_{\text{on}} L^2}$, $U = \frac{v}{k_{\text{on}} L}$, $F = \frac{f}{k_{\text{on}}}$
- Modified flip rates due to enzyme re-attachment: $F \to F + \frac{1}{2} UQ$ when $\sigma = 1$.
- Numerical scheme: Finite differences on non-uniform grid. Typical $\nu \sim 10^{-10}$, $F \sim 10^5$, $\sigma \sim 1 \Rightarrow$ cluster mesh points near boundary, use stiff solver in time.
- Reservoir dynamics: $R_b(0) \equiv n_0$: copy number of MutY ( $\approx 30$ in *E. coli*). Infinite copy number limit: $R_b(t) = n_0 \ \ \forall t$.
- $U = 0$: passive enzyme limit (no CT)
- Estimate time $\tau_s$ for enzyme to reach lesion by

$$\int_0^{\tau_s} J(t) dt = 1$$

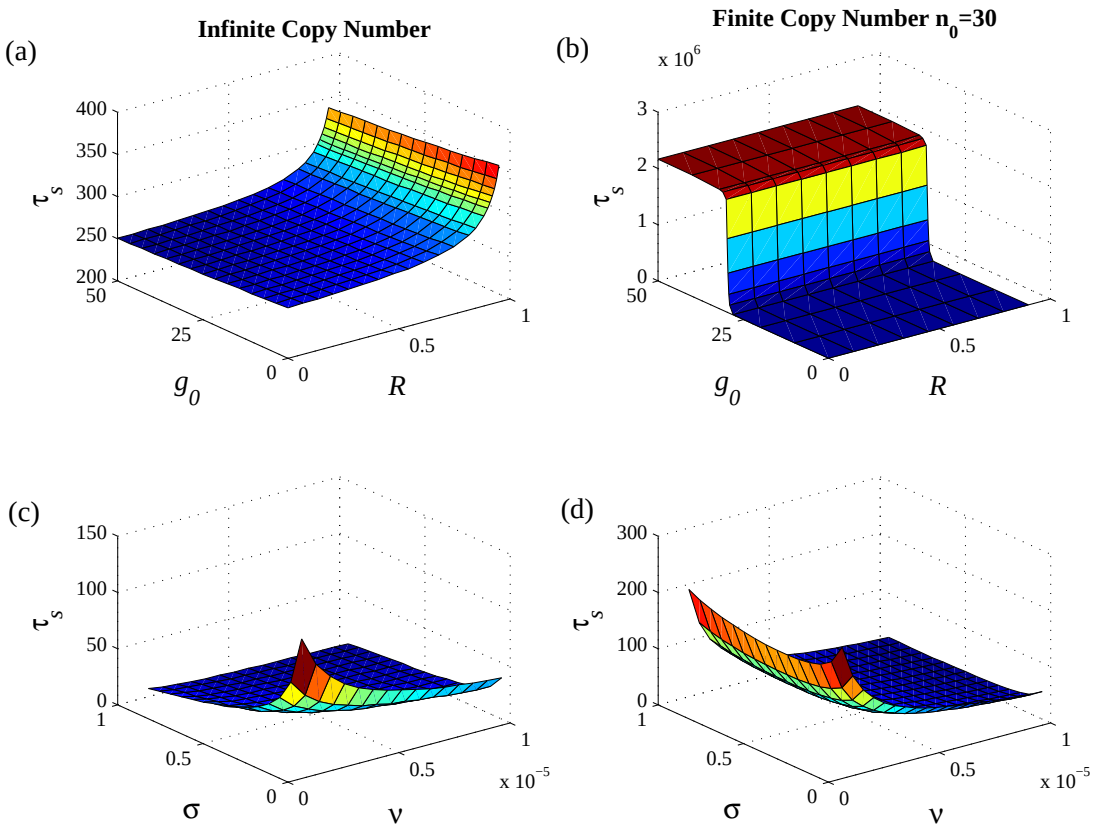
where $J(t) = 2\nu \left. \frac{\partial Q}{\partial x} \right|_{x=0}$.

# Enzyme Profiles/Fluxes



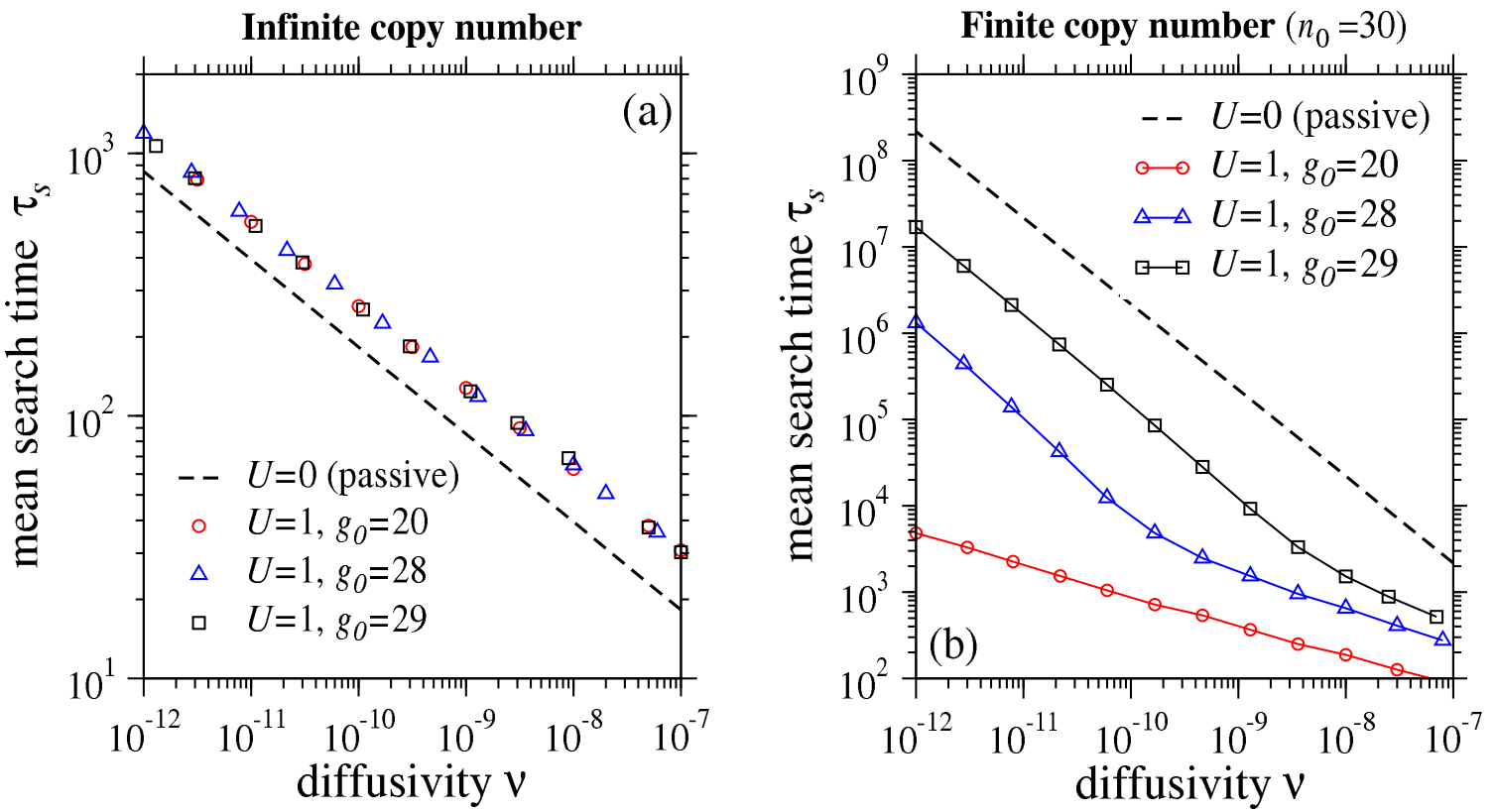

Enzymes colocalize near lesions due to spatially dependent desorption $\propto (N_+ + N_-)Q$.

# Search Times




$\tau_s$: search time, $g_0$: initial OxoG number, $R$: lesion's electron reflectivity, $\sigma$: enzyme binding affinity, $\nu$: enzyme diffusivity along DNA.

# Search Time Dependence on $\nu$

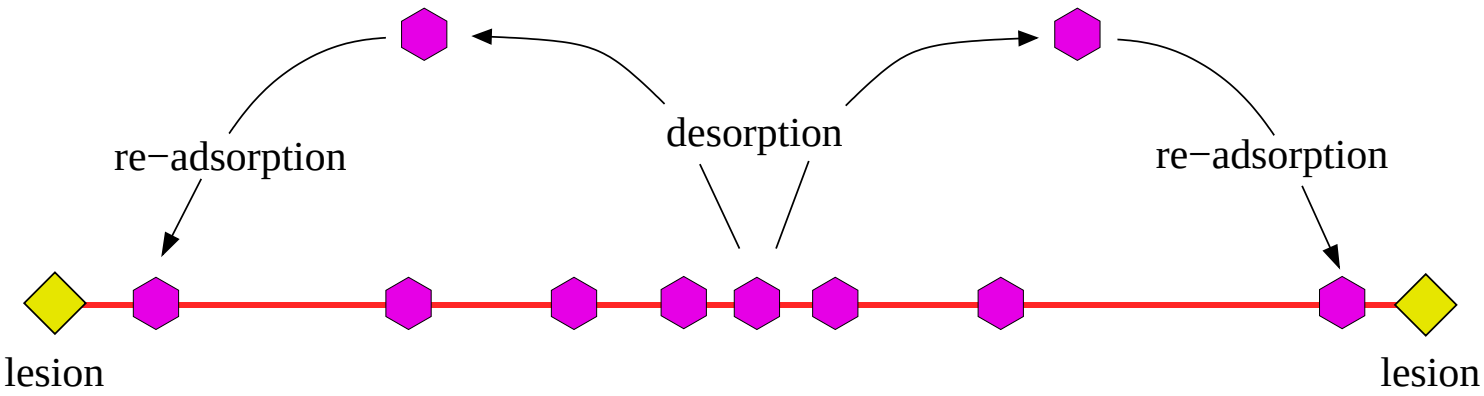



For wide range of diffusivities $\nu$, (a) Infinite CN: $\tau_s = O(\nu^{-1})$ insensitive to $g_0$. (b) Finite CN: $\tau_s$ *extremely* sensitive to $g_0$. Scaling switches from $O(\nu^{-1}) \to O(\nu^{-1/3})$

CT accelerates search only in finite CN case.

# Conclusions

- Studied discrete and PDE models of Charge Transport (CT) mediated enzyme kinetics.
- Discrete broadwell model improves over facilitated diffusion
  - Statistics of enzyme binding for lesion-free DNA
  - Density profiles/scaling results from MC simulations for DNA with lesions
- PDE model improves over discrete model. Included diffusion along DNA, redox-dependent binding kinetics and “reservoir” effects.
  - Can yield search time on order of seconds
  - CT acceleration due to spatially dependent desorption and enzyme “recycling”



# Extensions

- Comparison of discrete and PDE models
- Effect of small copy number $\Leftrightarrow$ chemical master equation
- PDE asymptotics for $F \gg 1$, $\sigma \sim 1$, $\nu \ll 1$
- Other enzyme binding mechanisms with spatially dependent desorption

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