# Describing the motion of cellular proteins at individual and population levels
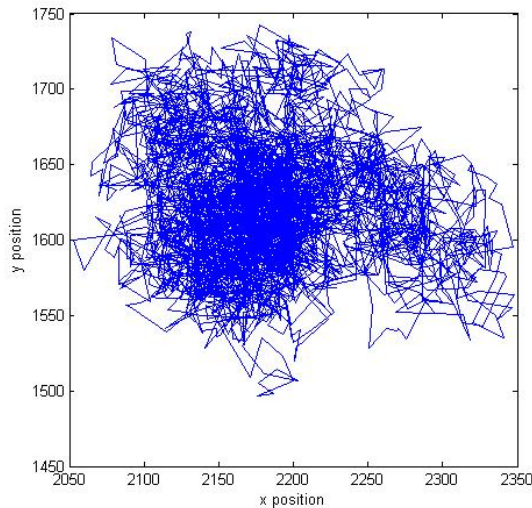
Gustavo Carrero
Athabasca University
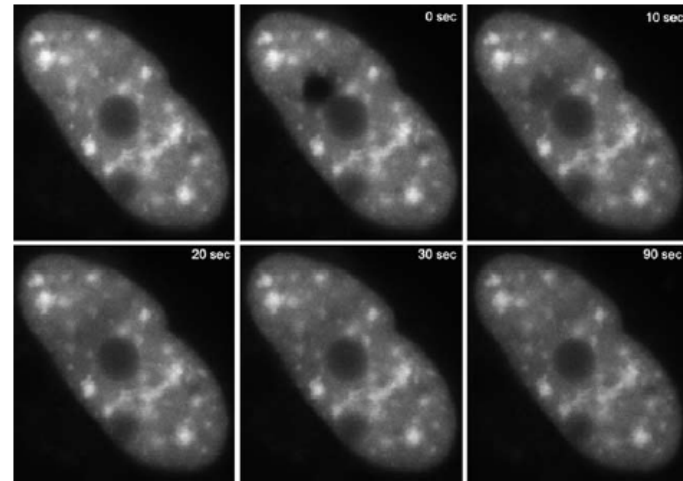
MITACS Annual Conference
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Athabasca University

# Outline

I.  Experimental population data & experimental individual data at the cellular level:  Fluorescence Recovery After Photobleaching (FRAP) data and Single Particle Tracking (SPT) data

II.  Population-based modeling example: Modeling self-organization of nuclear proteins.

III.  Individual-based modeling example: Testing a correlated random walk for cellular receptors.

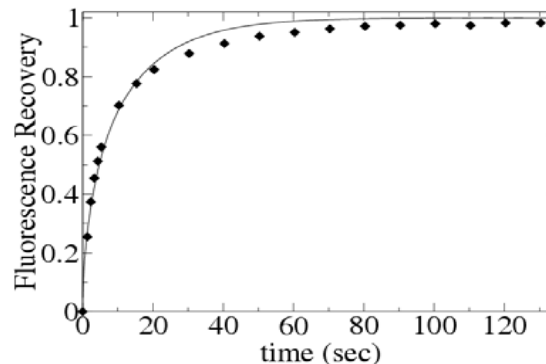# Fluorescence Recovery After Photobleaching (FRAP) experiments: protein population data
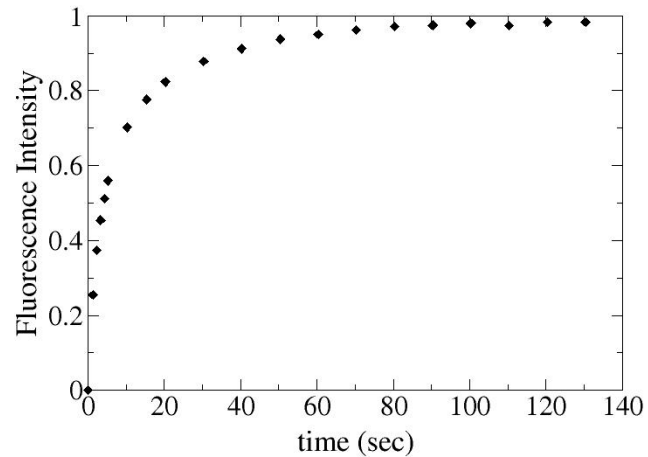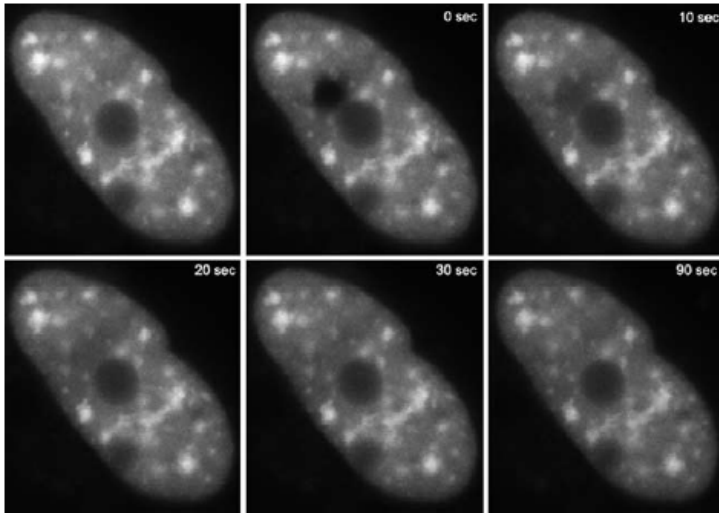


FRAP experiment of SC-35 in an Indian Muntjac Fibroblast cell nucleus
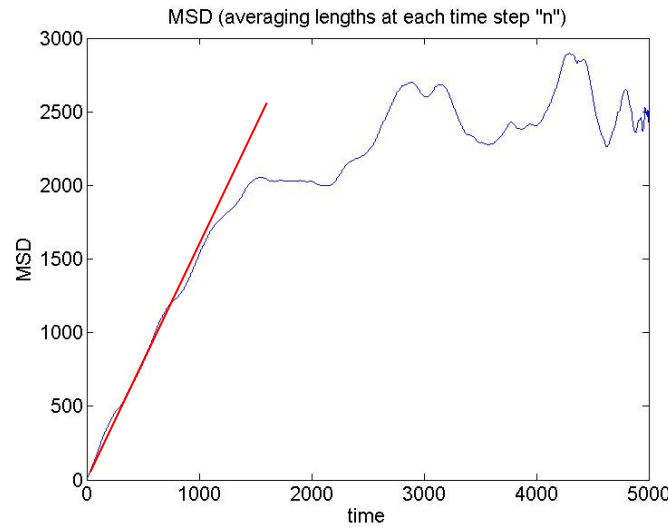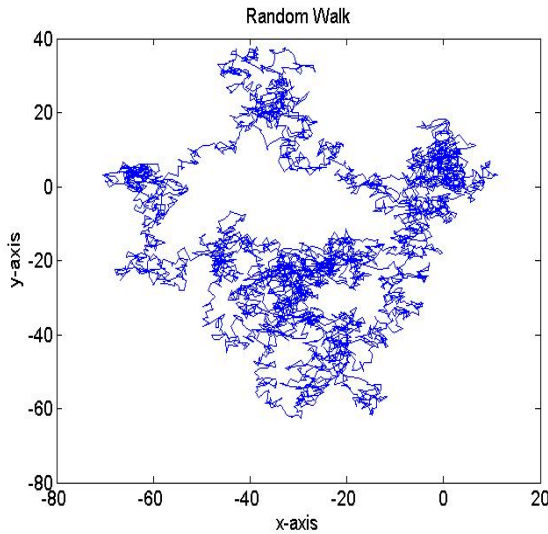
The data are used to quantify the mobility by determining an effective diffusion coefficient $D_{eff}$ via a diffusion approximation of a random walk

$$\frac{\partial}{\partial t} u(x,t) = D_{eff} \frac{\partial^2 u(x,t)}{\partial x^2}$$

$$R(t) = \int_\Lambda u(x,t)dx$$

# Single Particle Tracking (SPT) experiments: individual protein data


Random Walk


MSD (averaging lengths at each time step "n")

MSD is related to the diffusion coefficient via a random walk approach

$$\rho(t) = 4Dt$$


control    cytoD

PMA
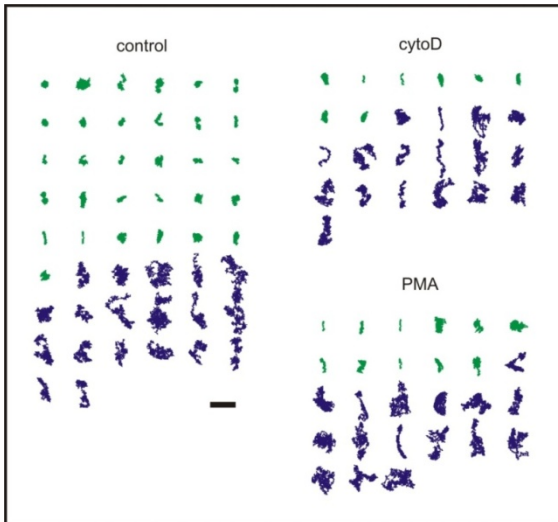
MSD: $\rho(t) = \left\langle (r(t) - r(0))^2 \right\rangle$

$r(t) = (x(t), y(t));$   position of the particle at time *t*

$\langle \ \rangle$: Averaging over time or over an ensemble of similar particles

# QUESTIONS

1) Using population data, can we describe the individual mechanism driving the dynamics of the population?
   *Pop. data → Ind. mech. → Pop. dyn.*

2) Using individual data, can we describe the dynamics of the whole the population?
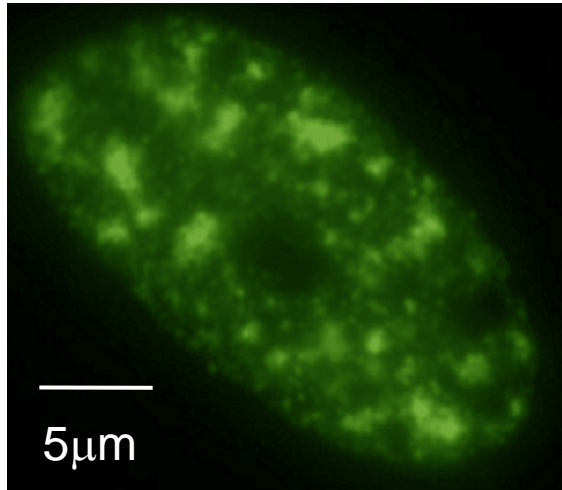   *Ind. data → Ind. mech. → Pop. dyn.*

# ANSWER

Yes and No ?? … Let us illustrate this non-contradictory answer with a couple of examples:
I.Population data of splicing factors
II.Individual data of membrane receptors

# Population data of splicing factors



Fluorescence microscopy image of SC-35
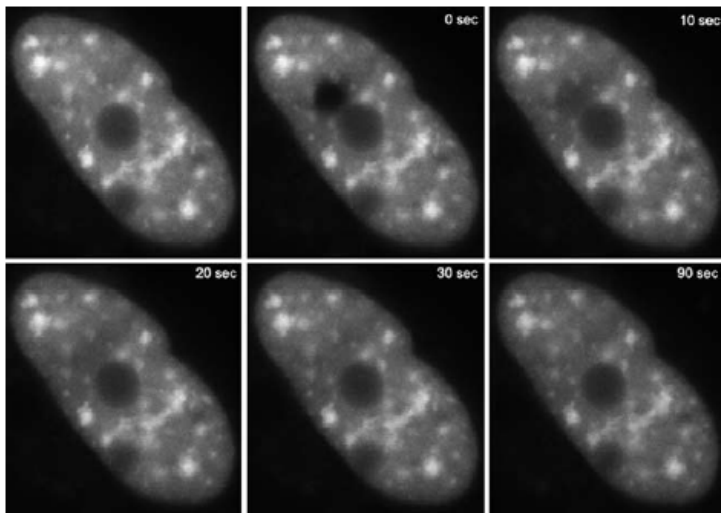distribution in an Indian Muntjac Fibroblast cell nucleus

Splicing factor compartments (speckles)

## Question:
What is the mechanism responsible for
the formation of speckles?



**Fluorescence Microscopy experiments
(FRAP) have shown that:**

1. SFs are in continuous flux between
   speckles and nucleoplasm.
2. SFs move randomly within the nucleus
   at a rate two orders of magnitude lower
   than expected.

# Self-organization of splicing factors

1.  **Self-organization** is responsible for the formation of speckles (modulated by phosphorylation and dephosphorylation)

2.  The existence of an **underlying nuclear scaffold** (nuclear matrix) plays a major role in the organization of SFs (it slows down the mobility of SFs via binding)

**Phosphorylation**

**Nuclear Scaffold**

p

p

p

p

p

p

**Self-organization**

**Dephosphorylation**
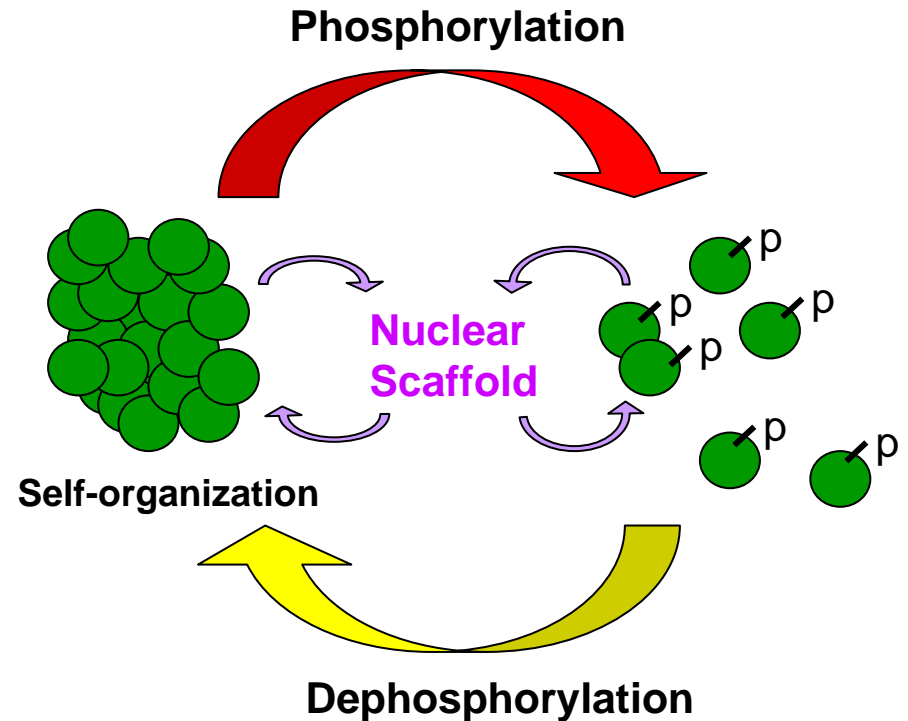
# **Full** Schematic Model

(*T. Misteli, J. Cell Sci.*, 113: 1841-1849, 2000)

(*G. Carrero, M.J. Hendzel, G. de Vries, J. Theor. Biol.*, 239: 298-312, 2006)

# A population model for self-organization of SFs

$$\frac{\partial v}{\partial t} = \qquad D\frac{\partial^2 v}{\partial^2 x} \qquad -\delta v + \rho u$$

$$\frac{\partial u}{\partial t} = \quad \text{(motion and self-interaction term)} \quad +\delta v - \rho u$$

$v\,(\,x\,,\,t\,)$ : phosphorylated splicing factors density

$u\,(\,x\,,\,t\,)$ : unphosphorylated splicing factors density

$\delta$ : dephosphorylation rate
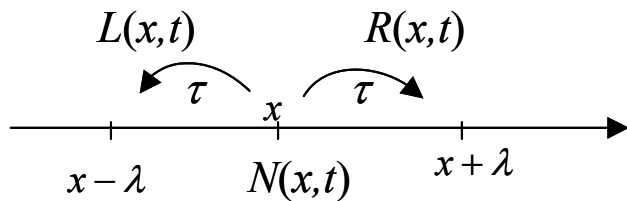
$\rho$ : phosphorylation rate

$D = (1-k)D_b$ : effective diffusion coefficient of SFs

   $k$: proportion of SFs bound to the nuclear scaffold

   $D_b$: diffusion coefficient describing the Brownian motion of SFs.

# Motion and Self-Interaction Term
## (for unphosphorylated SFs)

Random Walk Analysis

$L(x,t)$    $R(x,t)$

$\tau$    $x$    $\tau$

$x - \lambda$    $N(x,t)$    $x + \lambda$

Diffusion Approximation

No bias

$$\frac{\partial u}{\partial t} = \frac{\partial^2 (\mu u)}{\partial x^2} - \frac{\partial (\beta u)}{\partial x}$$

Fokker-Planck Equation

$$\mu = \frac{\lambda^2}{2\tau}(R+L) = D(1-N) \quad \text{[Motility]}$$

$$\frac{\partial u}{\partial t} = \frac{\partial^2 (\mu u)}{\partial x^2}$$

Diffusion Approximation

$$\beta = \frac{\lambda}{\tau}(R-L) \quad \text{[Bias]}$$

Task: To find the motility $\mu = D(1-N)$

( i.e., to find the probability $N(x,t)$ ):

# Motion and Self-Interaction Term

**Developing the probability $N(x,t)$:**

$$N(x,t) = \frac{\kappa}{\omega} \int_{-\infty}^{\infty} H(s)\, u(x+s,t)\, ds$$

$N(x,t)$ is proportional to the average density of SFs bound to the underlying structure.

$\omega$: critical density dictated by space limitations

$\kappa$: aggregative sensitivity $= \alpha\, k$, $\alpha$: affinity of the self-interaction

$k$: proportion of bound SFs

$$H(s) = \begin{cases} \dfrac{1}{2\sigma} & \text{for} \quad |x| \leq \sigma \\ 0 & \text{for} \quad |x| > \sigma \end{cases}$$

(Kernel function)

$$\int_{-\infty}^{\infty} H(s)\, ds = 1$$

$\sigma$: range of influence of the self-interaction

# Motion and Self-Interaction Term

$$N(x,t) = \frac{\kappa}{\omega} \int_{-\infty}^{\infty} H(s)\, u(x+s,t)\, ds$$

Task: To find the motility

$$\mu = D\left(1 - N\right)$$

Substituting the Taylor expansion $u(x+s,t) = u(x,t) + \frac{\partial u}{\partial x} s + \frac{\partial^2 u}{\partial x^2} \frac{s^2}{2} + \frac{\partial^3 u}{\partial x^3} \frac{s^3}{6} + O(s^4)$

in $N(x,t)$, calculating the integral, and neglecting 4th order and higher terms with respect to σ, we obtain the motility

$$\mu = D - D\frac{\kappa\, u}{\omega} - D\frac{\kappa\, \sigma^2}{6\,\omega} \frac{\partial^2 u}{\partial x^2}$$

[Motility]

Plug in μ

$$\frac{\partial u}{\partial t} = \frac{\partial^2 (\mu\, u)}{\partial x^2}$$

[Diffusion Approximation]

# Motion and Self-Interaction Term

**Aggregation-Diffusion Equation**

$$\frac{\partial u}{\partial t} = \frac{\partial}{\partial x}\left[\left(D - 2D\kappa\,\frac{u}{\omega}\right)\frac{\partial u}{\partial x}\right]D - \frac{\partial^2}{\partial x^2}\left[\left(\frac{D\,\kappa\,\sigma^2}{6}\,\frac{u}{\omega}\right)\frac{\partial^2 u}{\partial x^2}\right]$$
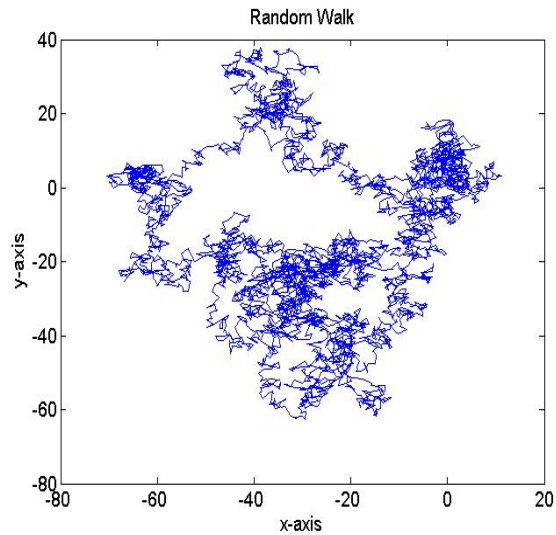
**Aggregation-Reaction-Diffusion Equation**

$$\frac{\partial v}{\partial t} = D\frac{\partial^2 v}{\partial^2 x} \qquad -\delta\,v + \rho\,u$$

$$\frac{\partial u}{\partial t} = \frac{\partial}{\partial x}\left[\left(D - 2D\kappa\,\frac{u}{\omega}\right)\frac{\partial u}{\partial x}\right] - \frac{\partial^2}{\partial x^2}\left[\left(\frac{D\,\kappa\,\sigma^2}{6}\,\frac{u}{\omega}\right)\frac{\partial^2 u}{\partial x^2}\right] + \delta\,v - \rho\,u$$

The model describes the onset of the compartmentalization of splicing factors

(*G. Carrero, M.J. Hendzel, G. de Vries, J. Theor. Biol.*, 239: 298-312, 2006)
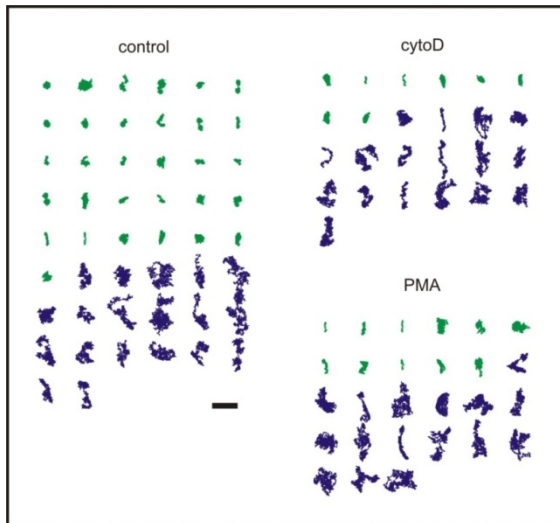
# Individual data of membrane receptors



**Single Particle Tracking (SPT):**

The study of a trajectory or a collection of single trajectories

**Question:**

1. Can we characterize the dynamics of the population with an individual trajectory?

2. Can we characterize the dynamics of the population with a collection of trajectories?

Cairo et al. (2006)

# Mean Square Displacement (MSD) and Diffusion

MSD: $\rho(t) = \left\langle (r(t) - r(0))^2 \right\rangle$

$r(t) = (x(t), y(t))$ ; The position of the particle at time *t*

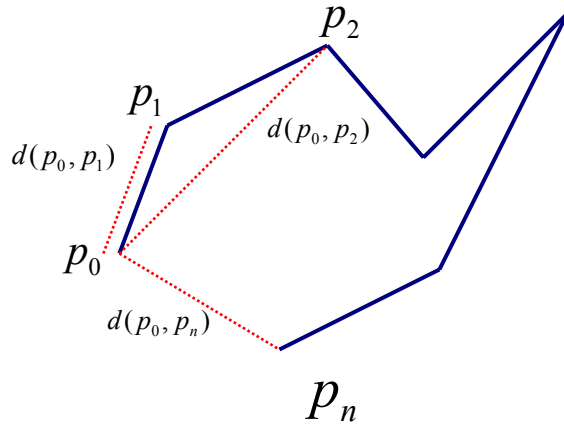$\langle \ \rangle$ : Averaging over time or over an ensemble of similar particles

The mean square displacement is related to the diffusion coefficient via

$$\rho(t) = 4Dt$$

Thus, an accurate estimation of this parameter requires an accurate MSD calculation.

# Estimating the MSD for one trajectory

**I.** *Considering one length for each time step "n"*



$$\begin{cases} p_0 = (x_0, y_0) \\ p_1 = (x_1, y_1) \\ \vdots \\ p_n = (x_n, y_n) \end{cases}$$

$$R_1^{\ 2} = (x_1 - x_0)^2 + (y_1 - y_0)^2 = d^2(p_0, p_1)$$

$$R_2^{\ 2} = (x_2 - x_0)^2 + (y_2 - y_0)^2 = d^2(p_0, p_2)$$

$$\vdots$$

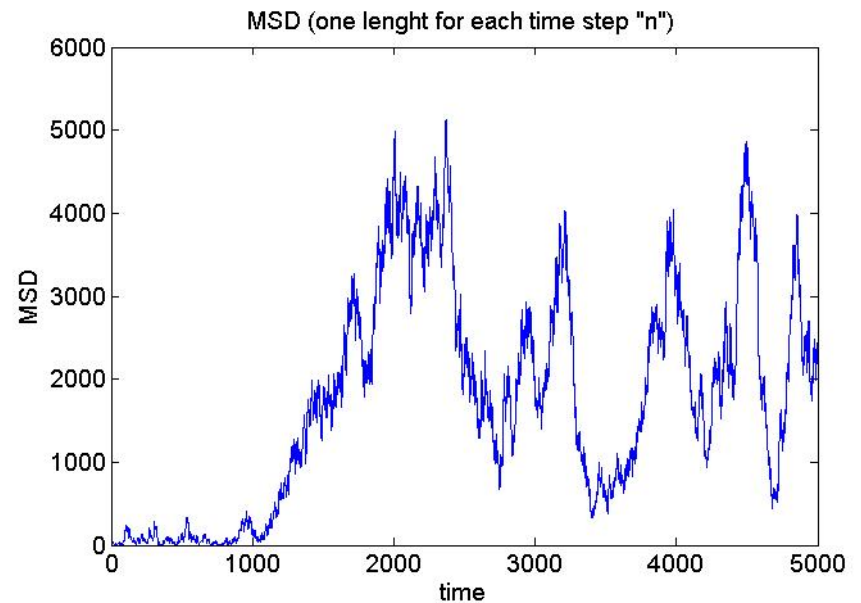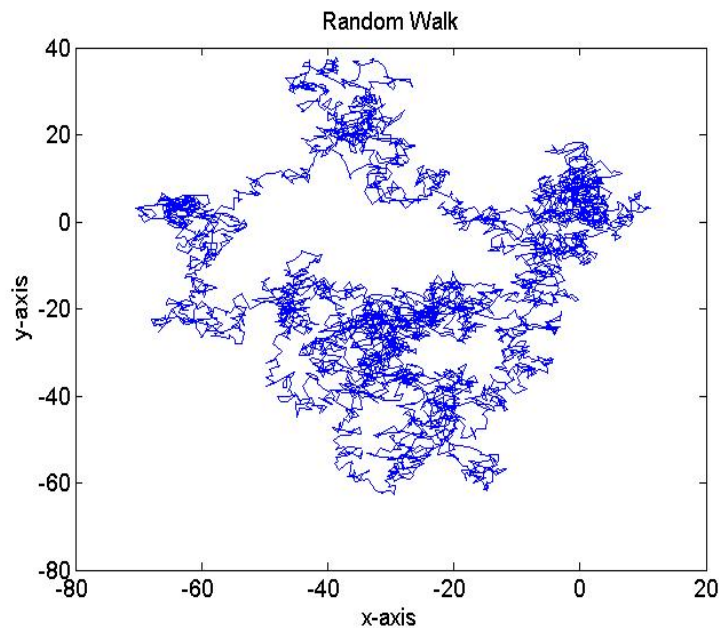$$R_n^{\ 2} = (x_n - x_0)^2 + (y_n - y_0)^2 = d^2(p_0, p_n)$$

MSD:

$$\rho(t) \sim R_n^{\ 2}$$

$$t = \tau n$$

$\tau$ : time step size

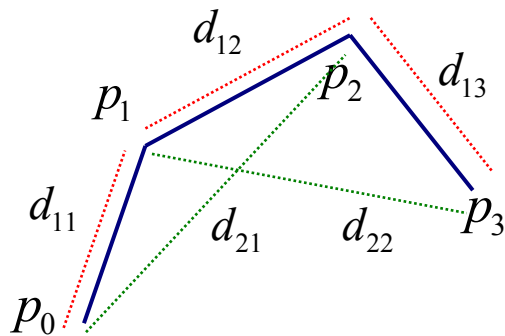# Estimating the MSD for one trajectory

**I.** *Considering one length for each time step:  Example*

# Estimating the MSD for one trajectory

**II.** *Averaging lengths given by position pairs obtained with the time separation $n\tau$*

(Qian et. al, 1991)



$$\overline{R_1}^2 = \frac{d_{11}^2 + d_{12}^2 + d_{13}^2}{3}$$

$$d_{11} = (x_1 - x_0)^2 + (y_1 - y_0)^2$$
$$d_{12} = (x_2 - x_1)^2 + (y_2 - y_1)^2$$
$$d_{13} = (x_3 - x_1)^2 + (y_3 - y_1)^2$$

(1)

$$\overline{R_1}^2 = \frac{(x_1 - x_0)^2 + (x_2 - x_1)^2 + (x_3 - x_2)^2}{3} + \frac{(y_1 - y_0)^2 + (y_2 - y_1)^2 + (y_3 - y_2)^2}{3}$$

$$\overline{R_1}^2 = \rho_x(1\tau) + \rho_y(1\tau)$$

$$\begin{cases} p_0 = (x_0, y_0) \\ p_1 = (x_1, y_1) \\ p_2 = (x_2, y_2) \\ p_3 = (x_3, y_3) \end{cases}$$

(2)

$$\overline{R_2}^2 = \frac{d_{21}^2 + d_{22}^2 +}{2}$$

$$d_{21} = (x_2 - x_0)^2 + (y_2 - y_0)^2$$
$$d_{22} = (x_3 - x_1)^2 + (y_3 - y_1)^2$$

$$\overline{R_2}^2 = \frac{(x_2 - x_0)^2 + (x_3 - x_1)^2}{2} + \frac{(y_2 - y_0)^2 + (y_3 - y_1)^2}{2}$$

$$\overline{R_2}^2 = \rho_x(2\tau) + \rho_y(2\tau)$$

# Estimating the MSD for one trajectory

**II.** *Averaging lengths given by position pairs obtained with the time separation $n\tau$*

(Qian et. al, 1991)

For a general $n$: $\overline{R_n}^2 = \rho_x(n\tau) + \rho_y(n\tau)$, where

$$\rho_x(n\tau) = \sum_{i=0}^{N(n)} \frac{(x_{i+n}-x_i)^2}{N(n)+1}$$

$$\rho_y(n\tau) = \sum_{i=0}^{N(n)} \frac{(y_{i+n}-y_i)^2}{N(n)+1}$$

and $N(n) = $ (# of position pairs obtained with the time separation $n\tau$) - 1

<u>Note</u>: It is practical to express $N(n)$ in terms of the # of snapshots $N_s$ : $N(n) + 1 = N_s - n$ . Thus,

$$\rho_x(n\tau) = \sum_{i=0}^{N_s-n-1} \frac{(x_{i+n}-x_i)^2}{N_s-n}$$

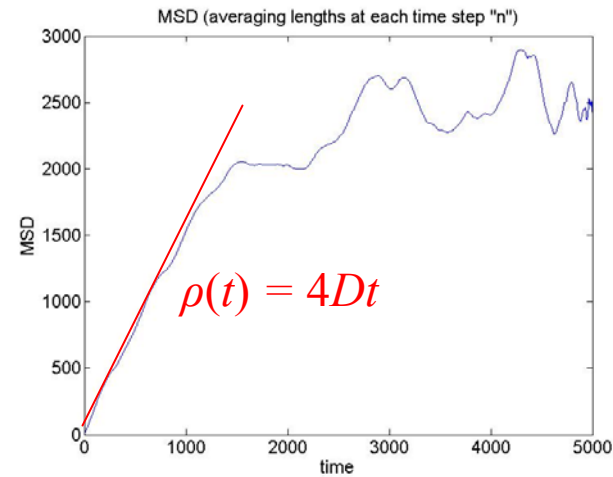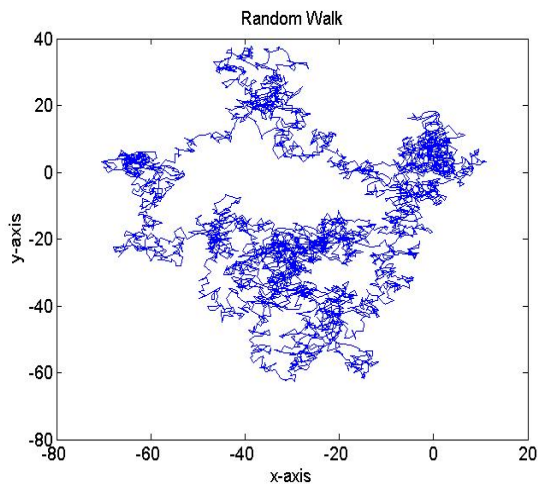$$\rho_y(n\tau) = \sum_{i=0}^{N_s-n-1} \frac{(y_{i+n}-y_i)^2}{N_s-n}$$

MSD:

$$\boxed{\rho(t) \sim \overline{R_n}^2 = \rho_x(n\tau) + \rho_y(n\tau)}$$

$t = \tau n$

$\tau$ : time step size

# Estimating the MSD for one trajectory

**II.** *Averaging lengths given by position pairs obtained with the time separation nτ : Example*
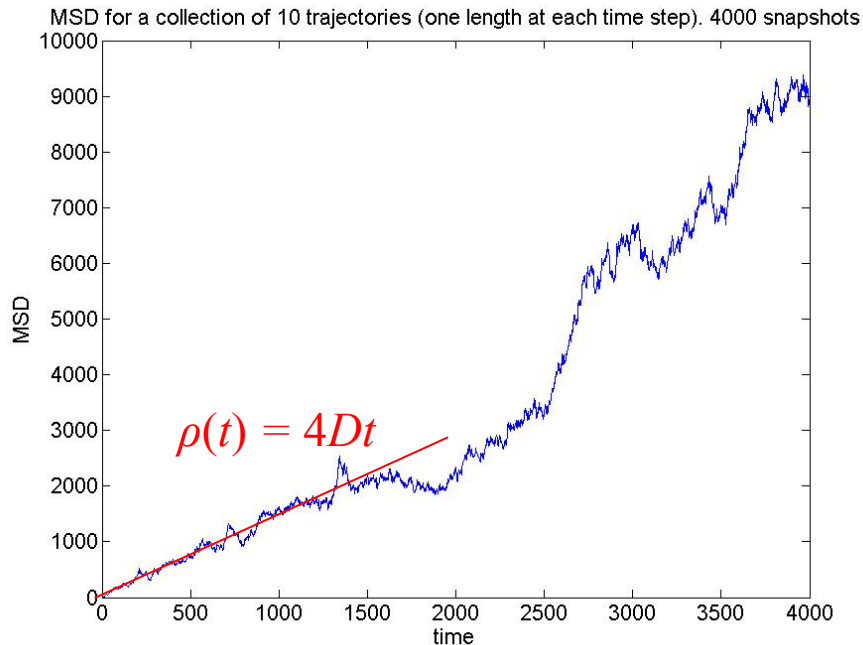


Q: Is this diffusion coefficient representative of the whole population?

A: ??

# Estimating the MSD for a collection of trajectories

I.  *Considering one length for each time step in each trajectory and then averaging them over the collection of trajectories:*
    *Example (10 trajectories of 4000 time steps)*



MSD for a collection of 10 trajectories (one length at each time step). 4000 snapshots

MSD:

$$\boxed{\rho(t) \sim \left\langle R_n^2 \right\rangle}$$

$$\left\langle R_n^2 \right\rangle = \sum_{j=1}^{k} \frac{R_{nj}^2}{k} \; ; \; k = \# \text{ of traj.}$$

$$t = \tau n$$

$\tau$ : time step size

# Estimating the MSD for a collection of trajectories

II. *Averaging lengths for each time step in each trajectory and then averaging them over the collection of trajectories:*
*Example (10 trajectories of 4000 time steps)*
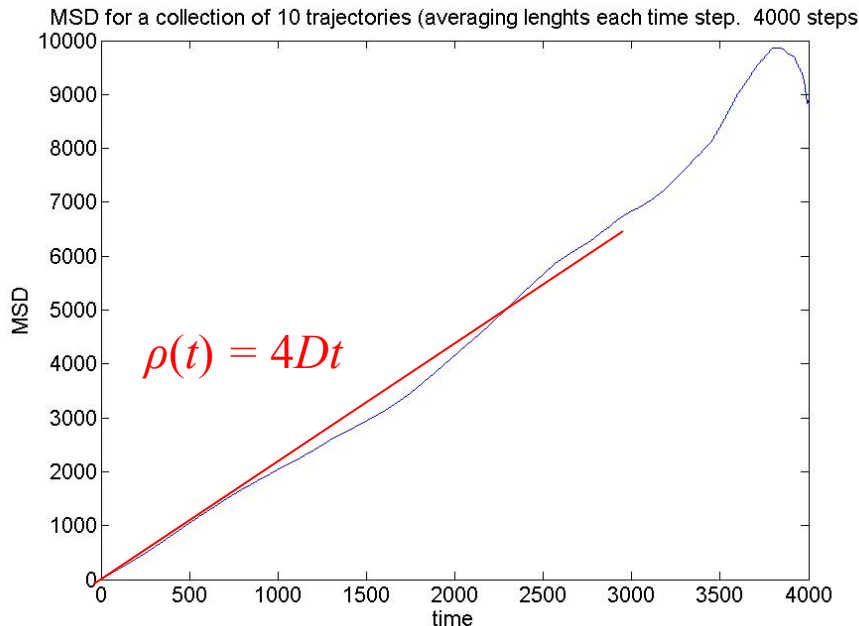
MSD:



$$\rho(t) \sim \left\langle \overline{R}_n^2 \right\rangle$$

$$\left\langle \overline{R}_n^2 \right\rangle = \sum_{j=1}^{k} \frac{\overline{R}_{nj}^2}{k} \; ; \; k = \# \text{ of traj.}$$
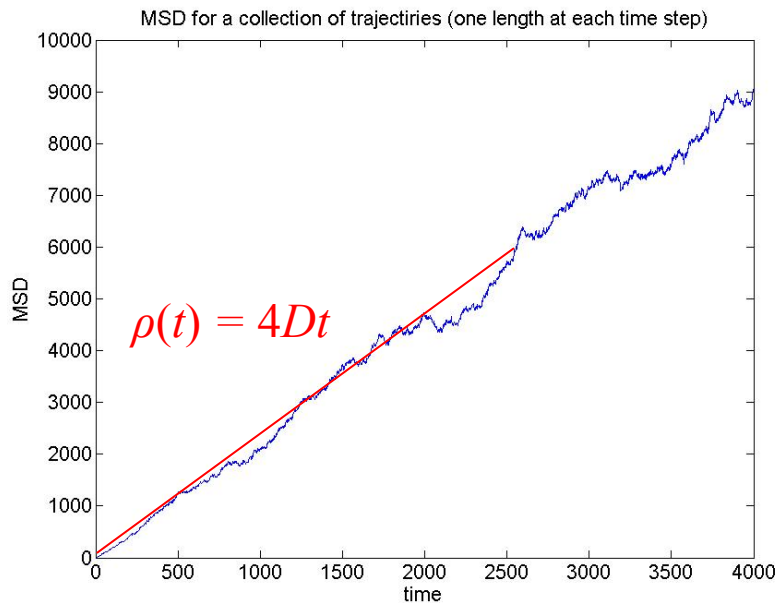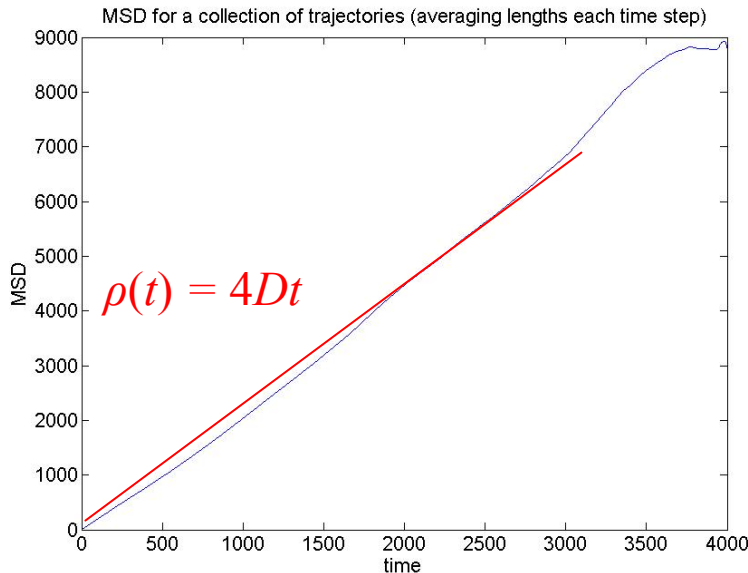
$$t = \tau n$$

$\tau$ : time step size

# Estimating the MSD for a collection of  trajectories

III. *Considering one length for each time step in each trajectory and then averaging them over the collection of trajectories:*
*Example (50 trajectories of 4000 time steps)*

MSD for a collection of trajectiries (one length at each time step)



$\rho(t) = 4Dt$

MSD:

$$\boxed{\rho(t) \sim \left\langle R_n^2 \right\rangle}$$

$$\left\langle R_n^2 \right\rangle = \sum_{j=1}^{k} \frac{R_{nj}^2}{k} \ ; \ k = \# \text{ of traj.}$$

$t = \tau n$

$\tau$ : time step size

# Estimating the MSD for a collection of  trajectories

*IV.    Averaging lengths for each time step in each trajectory and then averaging them over the collection of trajectories:*
*Example (50 trajectories of 4000 time steps)*



MSD for a collection of trajectories (averaging lengths each time step)

$\rho(t) = 4Dt$

MSD:

$$\boxed{\rho(t) \sim \left\langle \overline{R}_n^2 \right\rangle}$$

$$\left\langle \overline{R}_n^2 \right\rangle = \sum_{j=1}^{k} \frac{\overline{R}_{nj}^2}{k} \; ; \; k = \# \text{ of  traj.}$$

$$t = \tau n$$

$\tau$ : time step size

# Questions

- Can we assume that the population of particles is diffusing? How do we test a random walk model?

- Can we use the MSD of each trajectory to statistically differentiate their behaviour?

# Correlated Random Walk (CRW)

**CRW**: Movement sequences are described in terms of move
lengths ($l_i$) and turning angles ($\theta_i$) distributions.

**Expected MSD**:

Skellam (1973);
Kareiva & Shigesada (1983)

$$\overline{R_n^{\,2}} = nm_2 + 2m_1^{\,2}\left[\frac{(c - c^2 - s^2)n - c}{(1-c)^2 + s^2} + \frac{2s^2 + (c + s^2)^{\frac{n+1}{2}}}{\left[(1-c)^2 + s^2\right]^2}\gamma\right]$$
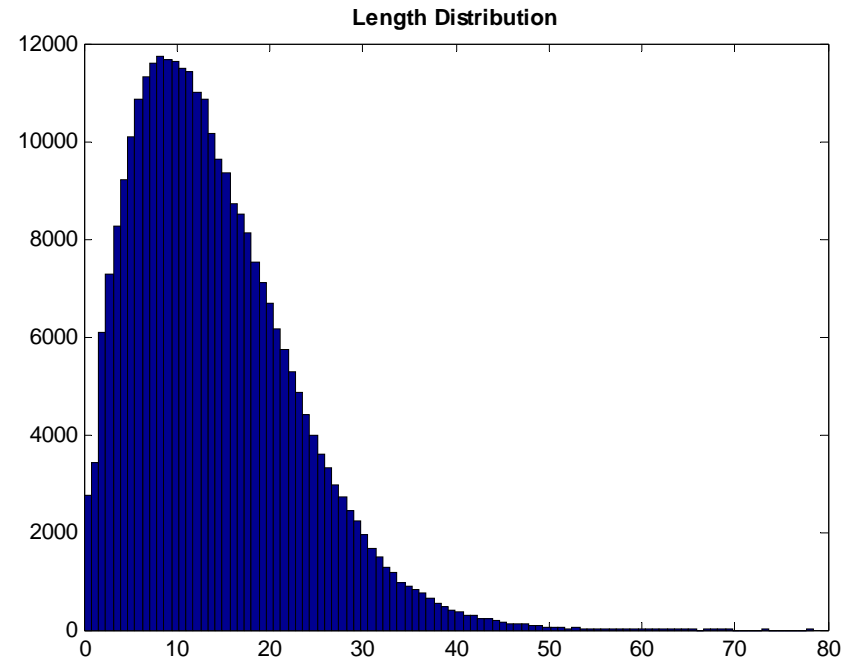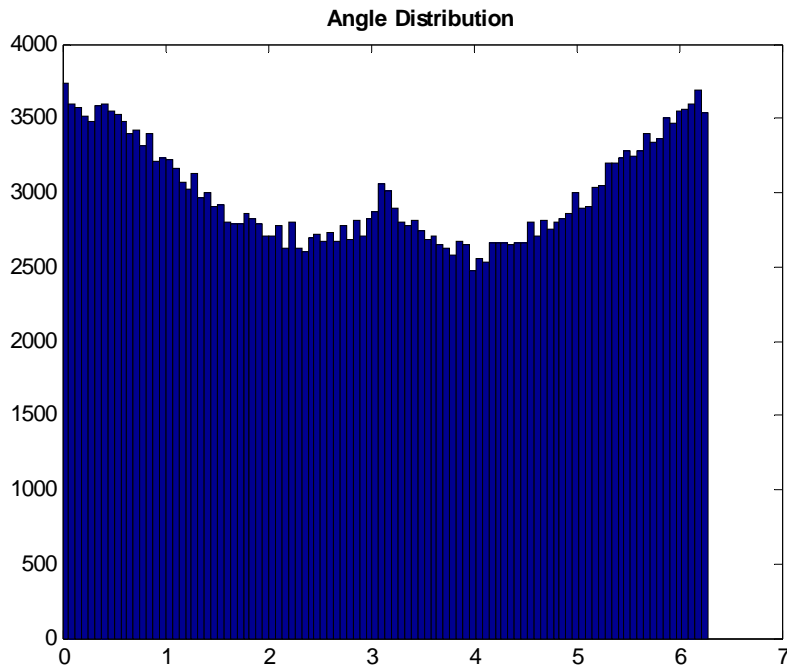
$$\gamma = \left[(1-c)^2 - s^2\right]\cos((n+1)\alpha) - 2s(1-c)\sin((n+1)\alpha) \; ; \quad \alpha = \arctan\left(\frac{s}{c}\right)$$

$$m_1 = \frac{1}{k}\sum_{i=1}^{k}\ell_i \; ; \quad m_2 = \frac{1}{k}\sum_{i=1}^{k}\ell_i^{\,2} \; ; \quad c = \frac{1}{k}\sum_{i=1}^{k}\cos\theta_i \; ; \quad s = \frac{1}{k}\sum_{i=1}^{k}\sin\theta_i$$

**Note**: If the distribution of turning angles has a uniform density, then

$$\overline{R_n^{\,2}} = nm_2$$

# CRW.   Angle and Length Distributions



**Main Question:**

Is the movement of receptors driven by a CRW?

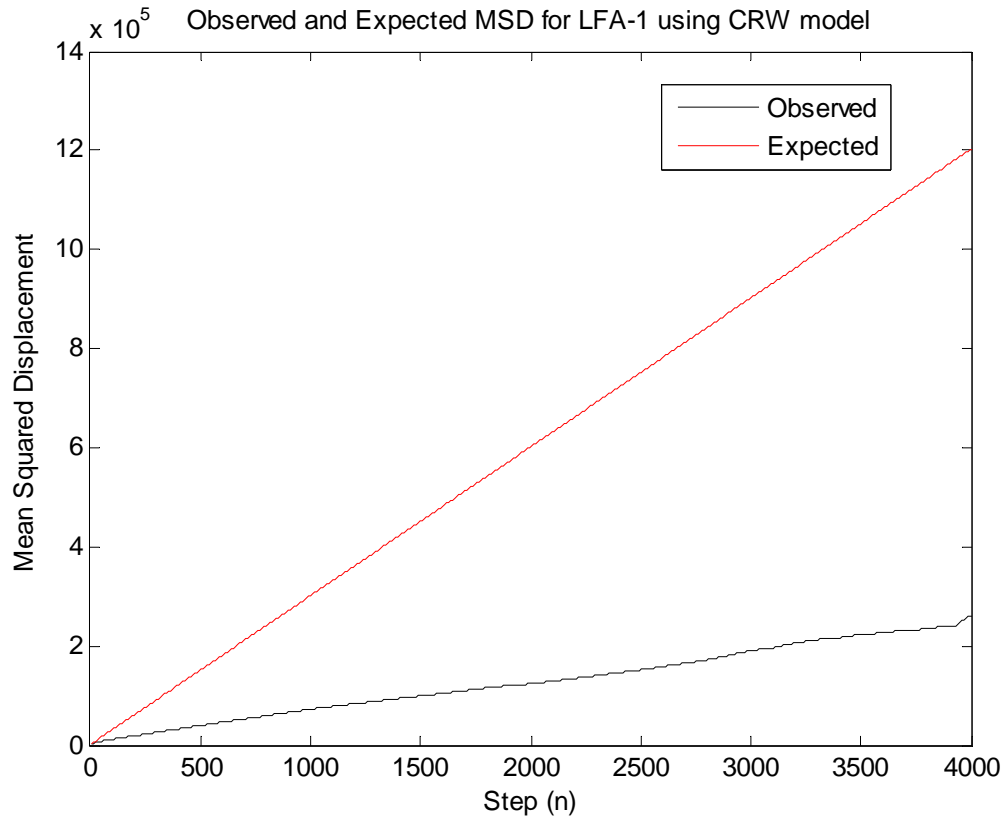# Testing the Data for Correlated Random Walk

$H_0$ : The population of particles moves according to a CRW
$H_1$:  CRW is rejected

**Bootstrapping Steps (**Kareiva et. al, 1983):

1. Calculate observed and expected MSD (from all the population)
2. Prepare the distributions of lengths and angles from experimental data (from all the population)
3. a. Using these distributions generate the same # of pseudotrajectories as experimental trajectories.
   b. Calculate and plot the MSD using these pseudotrajectories
   c. Repeat this procedure about 100 times.
4. For each time step truncate the five highest and lowest values for the MSD. This will create, for example, a 90% confidence interval.

5. If the observed MSD does not fall into the confidence interval the null hypothesis is rejected.  Otherwise, it $H_0$ cannot be rejected.

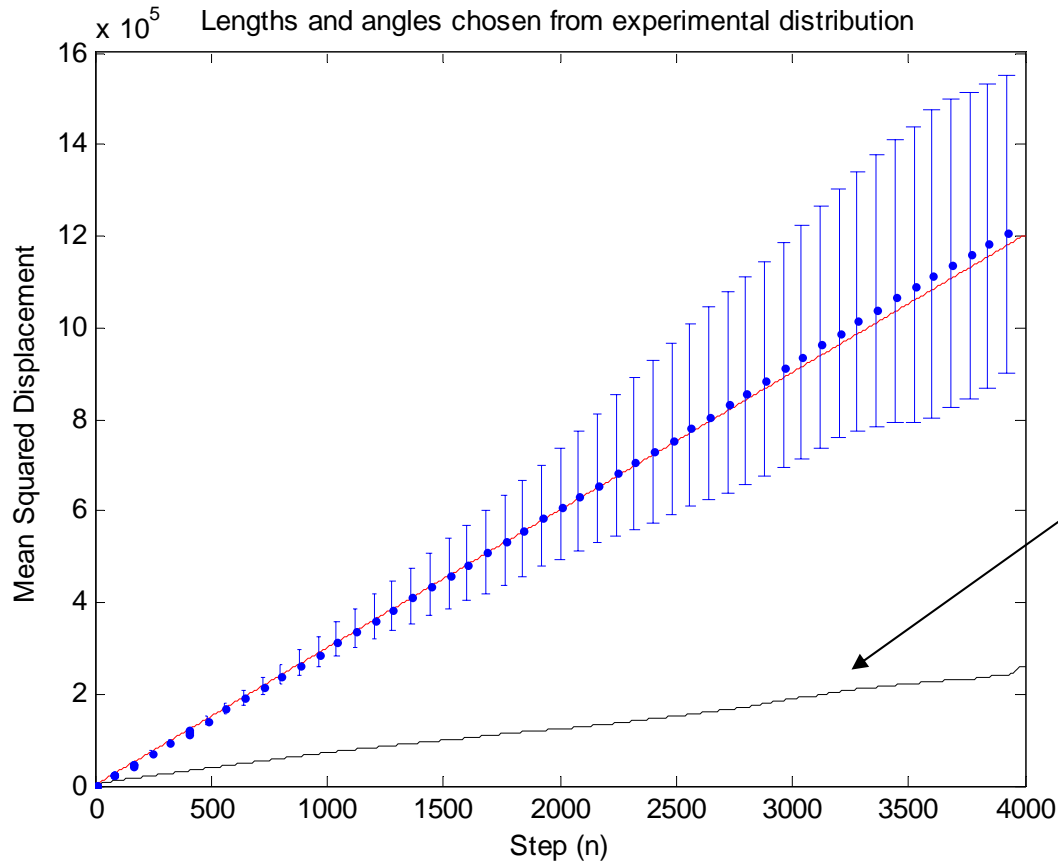# Testing the Data for Correlated Random Walk



Observed and expected MSD

# Testing the Data for Correlated Random Walk
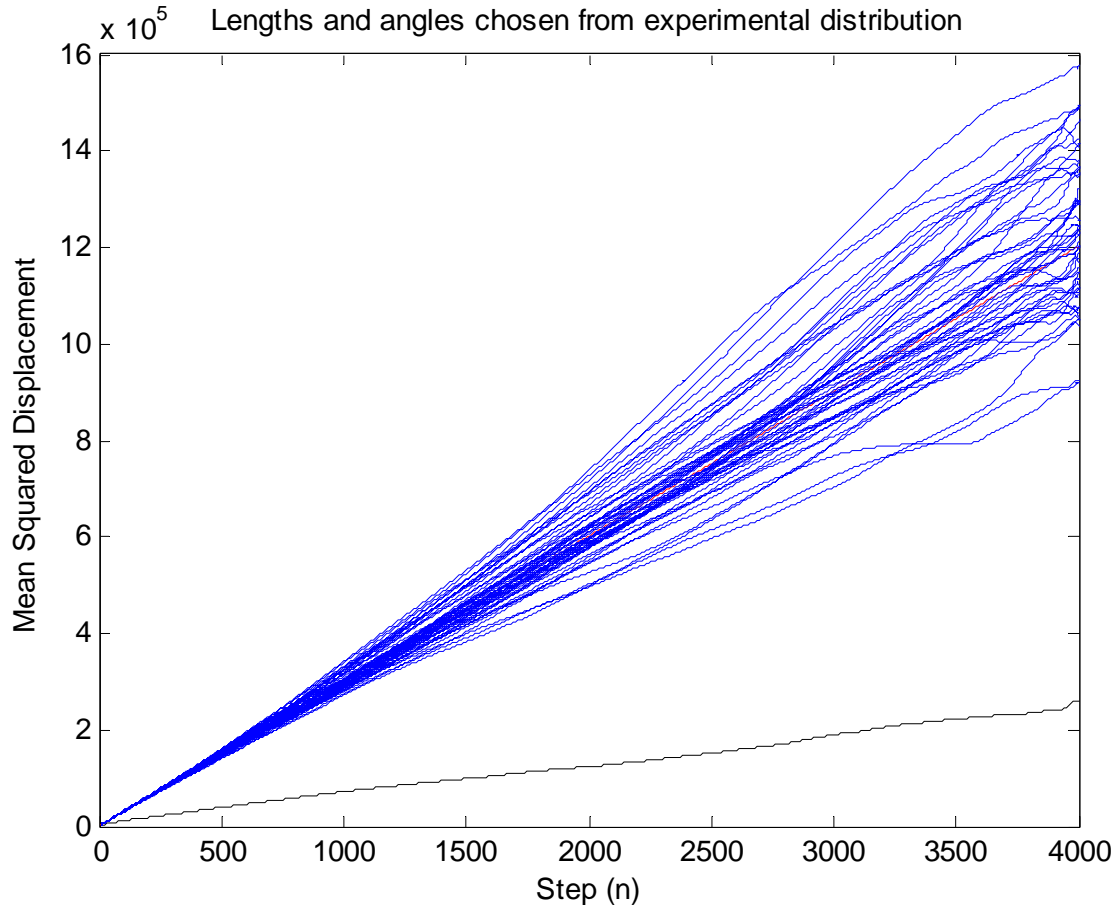


Each blue MSD curve is obtained From 75 pseudotrajectories generated from the angle and length distributions. There are 100 blue MSD curves.

# Testing the Data for Correlated Random Walk



Lengths and angles chosen from experimental distribution

The observed MSD does not fall into the confidence interval

$H_0$ is rejected! CRW is rejected!

# Question:

What is causing the receptors to disperse slower than expected from a CRW ?



Lengths and angles chosen from experimental distribution

# Possible answers:

- Existence of angle-length correlations?
- Existence of autocorrelations?
- The existence of two or more populations moving at different rates (possibly caused by cytoskeleton interaction) ?
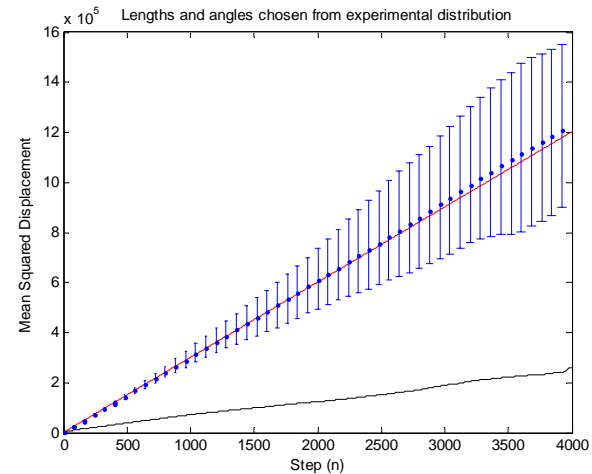
# Acknowledgements
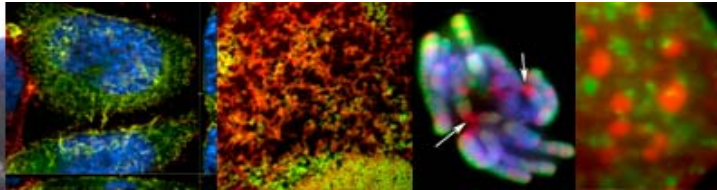


Hendzel's Lab members, and Dr. Michael Hendzel, Cross Cancer Institute, UofA


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Math, UofA


Dr. Christopher Cairo,
Dept. of Chemistry  UofA


Athabasca University

- A&PDF (Academic & Professional
Development Fund) Award
- RIG (Research Incentive Grant)