What is a random event?
What is Probability?

Probability is a numerical measure of uncertainty about an event.
Can you single out a major concept, experiment, or result in science as THE ONE?
Why is Normal Distribution so central?
The Central Limit Theorem
The Central Limit Theorem

Let $X_1, X_2, X_3, \ldots, X_n$ be identical and independently distributed random variables with mean $m$ and variance $s^2$.

Let $\sum X$ be the sum of the values of the $n$ variables.

When $n$ is very large $\sum X$ follows approximately a Normal distribution with mean $n \cdot m$ and variance $n \cdot s^2$
As a corollary

A sample mean tends to be normally distributed irrespective of the distribution of the population one is sampling from
\[
\frac{d[X]}{dt} = -k_{on} [X][Y] + k_{off} [XY]
\]
Collision Theory of Reaction

Collision frequency
(collisions per molecule per unit of time)

\[ z = \frac{2^{1/2} \sigma \bar{c} N}{V} = \frac{2^{1/2} \sigma \bar{c} p}{kT} \]

Collision density
(total collisions per unit of volume)

\[ Z_{AA} = \sigma \left( \frac{4kT}{\pi m} \right)^{1/2} N_A^2 [A]^2 \]
Collision Theory of Reaction

\[
\frac{d[X]}{dt} = -k_{on} [X][Y] + k_{off} [XY]
\]

Valid when we have many, many molecules
WHAT IS LIFE?
ERWIN SCHRODINGER

PHYSICAL LAWS REST ON ATOMIC STATISTICS AND ARE THEREFORE ONLY APPROXIMATE
And why could all this not be fulfilled in the case of an organism composed of a moderate number of atoms only and sensitive already to the impact of one or a few atoms only? Because we know all atoms to perform all the time a completely disorderly heat motion, which, so to speak, opposes itself to their orderly behaviour and does not allow the events that happen between a small number of atoms to enrol themselves according to any recognizable laws. Only in the co-operation of an enormously large number of atoms do statistical laws begin to operate and control the behaviour of these assemblies with an accuracy increasing as the number of atoms involved increases. It is in that way that the events acquire truly orderly features. All the
Being based on pure chance, its validity is only approximate. If it is, as a rule, a very good approximation, that is only due to the enormous number of molecules that co-operate in the phenomenon. The smaller their number, the larger the quite haphazard deviations we must expect and they can be observed under favourable circumstances.
How many copies of each molecule does a cell have?
The bacterium *E. coli* as a standard ruler for characterizing spatial scales

*E. coli* as a standard ruler for characterizing spatial scales
Volume $\approx 1 \, \mu m^3$
Area $\approx 6 \, \mu m^2$

(A) Atomic force microscopy image of an *E. coli* cell (courtesy of C. T. Lim),
(B) Electron micrograph of *E. coli* bacterium,
(C) the *E. coli* ruler.

Figure 2.1 Physical Biology of the Cell (© Garland Science 2009)

[Courtesy of Filipa Alves]
Powers of ten representation of biological length scales

Figure 2.7 (part 1) Physical Biology of the Cell (© Garland Science 2009)

Figure 2.7 (part 2) Physical Biology of the Cell (© Garland Science 2009)

[Courtesy of Filipa Alves]
The cartoon on the left shows the crowded cytoplasm of the bacterial cell.
The cartoon on the right shows an order-of-magnitude molecular census of the E. coli bacterium with the approximate number of different molecules in E. coli.

[Courtesy of Filipa Alves]
Molecular contents of the bacterium *E. coli*

<table>
<thead>
<tr>
<th>Substance</th>
<th>% of total dry weight</th>
<th>Number of molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macromolecule</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>55.0</td>
<td>2.4 \times 10^6</td>
</tr>
<tr>
<td>RNA</td>
<td>20.4</td>
<td></td>
</tr>
<tr>
<td>23S RNA</td>
<td>10.6</td>
<td>19,000</td>
</tr>
<tr>
<td>16S RNA</td>
<td>5.5</td>
<td>19,000</td>
</tr>
<tr>
<td>5S RNA</td>
<td>0.4</td>
<td>19,000</td>
</tr>
<tr>
<td>Transfer RNA (4S)</td>
<td>2.9</td>
<td>200,000</td>
</tr>
<tr>
<td>Messenger RNA</td>
<td>0.8</td>
<td>1,400</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>9.1</td>
<td>22 \times 10^6</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>3.4</td>
<td>1.2 \times 10^6</td>
</tr>
<tr>
<td>DNA</td>
<td>3.1</td>
<td>2</td>
</tr>
<tr>
<td>Murein</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Glycogen</td>
<td>2.5</td>
<td>4,360</td>
</tr>
<tr>
<td><strong>Total macromolecules</strong></td>
<td><strong>96.1</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Small molecules</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolites, building blocks, etc.</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Total small molecules</strong></td>
<td><strong>3.9</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 Observed macromolecular census of an *E. coli* cell. (Data from F. C. Neidhardt et al., *Physiology of the Bacterial Cell*, Sunderland, Sinauer Associates Inc., 1990 and M. Schaechter et al., *Microbe*, Washington DC, ASM Press, 2006.)

Table 2.1 Physical Biology of the Cell (© Garland Science 2009)
Concentration in *E. coli* units

Volume ≈ 1 μm³
Area ≈ 6 μm²

Number of copies of a given molecule in a volume the size of an *E. coli* cell as a function of the concentration

Figure 2.4a Physical Biology of the Cell (© Garland Science 2009)

[Courtesy of Filipa Alves]
How are the data produced?

Cell Tissue → Blending → Homogenate
How are the data produced?
Flow cytometer (FACS)
Noise in protein expression scales with natural protein abundance

Arren Bar-Even\textsuperscript{1}, Johan Paulsson\textsuperscript{2,3}, Narendra Maheshri\textsuperscript{4}, Miri Carmi\textsuperscript{1}, Erin O’Shea\textsuperscript{4}, Yitzhak Pilpel\textsuperscript{1} & Naama Barkai\textsuperscript{1,5}

\[\text{Figure 1} \] Single-cell distributions of fluorescence levels. (a,b) Cells expressing the high-abundance protein PGM2 were shifted from synthetic complete medium to medium containing 3% ethanol. (c,d) Cells expressing the low-abundance protein ARX1 were diluted from stationary phase. The cells were subjected to flow cytometry analysis at different time points after the transfer. Fluorescence distributions are shown on linear (a,c) and on logarithmic (b,d) scales. Blue, green, red, turquoise, magenta and yellow lines correspond to fluorescence distributions after 0, 30, 60, 90, 120 and 150 min from perturbation start, respectively. For low-abundance proteins, the fluorescence values appeared to follow normal distributions. By contrast, at high abundances, we more often observed a deviation from normality, with overrepresentation of high fluorescence values (see Supplementary Note and Supplementary Fig. 6 online).

\[\text{Figure 2} \] Scaling of noise with mean protein abundance. (a) Noise as a function of mean protein abundance. All genes in all conditions and time points are shown. Thick curve corresponds to \(\log(n^2) = 117.5 - \log(p)\). Green filled circles represent initial, steady-state time points of stress perturbations (\(t = 0\)). Gray points were excluded from the fitting process (see Methods). The fit to the autofluorescence region (thin curve) corresponds to \(\log(n^2) = 9.9 \cdot 10^5 - 2 \cdot \log(p)\). (b-e) Noise versus mean protein abundance shown separately for genes belonging to a common module.
Figure 5

Polyclonal

Monoclonal

Full

0 h

24 h

48 h

72 h

TCRβ-A647

[Courtesy: Thiago Guzella]
Super resolution microscopy:
PALM, STORM beyond the Rayleigh diffraction limit

But before …
Airy disc is the central bright disc present in the diffraction pattern generated by a perfect, aberration-free lens.
Radius of the Airy Disc

\[
\text{\textit{radius}}_{\text{Airy}} = \frac{1.22 \lambda}{2 \text{NA}_{\text{obj}}}
\]

\(\lambda\) wavelength and \(\text{NA}\) is the numerical aperture of the objective
Numerical aperture

Wavelength
Reyleigh criterion is used to establish the minimum resolvable distance between two light sources (independent of the magnification)

http://www.microscopyu.com/tutorials/java/imageformation/airyna/
Resolution: Rayleigh criterion

Laterally: \[ \sim \frac{\lambda}{2NA} \quad (x, y) \]

Axially: \[ \sim \frac{2\lambda \eta}{(2NA)^2} \quad (z) \]

Conventional fluorescence microscope

with visible light: \[ 450 \text{ nm} < \lambda < 700 \text{ nm} \]

and high numerical aperture objective: \[ NA = 1.4 \]

\[ \sim 200 \text{ nm} \quad (x, y) \quad 500 - 800 \text{ nm} \quad (z) \]

The central limit theorem

Let $X_1$, $X_2$, $X_3$, ..., $X_n$ be identical and independently distributed random variables with mean $m$ and variance $s^2$.

Let $\Sigma_n$ be the sum of values of the $n$ variables.

When $n$ is very large $\Sigma_n$ follows approximately a Normal distribution with mean $nm$ and variance $ns^2$. 
Resolution in PALM, STORM

\[
\frac{\sigma}{\sqrt{N}} \quad (x,y)
\]

**Statistics:** Standard error of the sample mean

Unresolved by conventional wide-field microscopy

But would be resolved if one could look at one fluorophore at a time
Photo-activated Localization Microscopy (PALM)

TIRF image (left) and PAL-M image (right) of antibody staining for tubulin in a cultured cell. Specimen: S. Niwa, University of Tokyo, Japan.
“Mathematics is Biology’s next microscope, only better. Biology is Mathematics’ next Physics, only better.”

— Joel E. Cohen PLoS Biology
Diffusion of molecules and particles
Brownian motion

On his scientific poem “De Rerum Natura” (c. 60BC) Lucretius makes a remarkable description of the (Brownian) motion of dust particles.

He uses this observation as a proof for the existence of atoms.

[Courtesy of Filipa Alves]
Observe what happens when sunbeams are admitted into a building and shed light on its shadowy places. You will see a multitude of tiny particles mingling in a multitude of ways... their dancing is an actual indication of underlying movements of matter that are hidden from our sight... It originates with the atoms which move of themselves. Then those small compound bodies that are least removed from the impetus of the atoms are set in motion by the impact of their invisible blows and in turn cannon against slightly larger bodies. So the movement mounts up from the atoms and gradually emerges to the level of our senses, so that those bodies that we see in sunbeams are in motion, moved by blows that remain invisible.

—Lucretius (c. 60 BC) “De Rerum Natura”
Brownian motion

**Robert Brown** (Scottish botanist, 1773 -1858)

In 1827, while examining pollen grains suspended in water under a microscope, Brown observed the particles expelled when the pollen grains burst, now known to be amyloplasts (starch organelles) and spherosomes (lipid organelles). These **small particles** were “executing a continuous jittery motion”.

*Clarkia pulchella* pollen bursting (the grain’s diameter is around 50µm)

Brownian motion of the contents of *Clarkia pulchella* pollen

[Courtesy of Filipa Alves]
Brownian motion

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In 1827, while examining pollen grains suspended in water under a microscope, Brown observed the particles expelled when the pollen grains burst, now known to be amyloplasts (starch organelles) and spherosomes (lipid organelles). These small particles were “executing a continuous jittery motion”.

Are these particles moving because they are “alive”? Brown observed the same motion in particles of inorganic matter, enabling him to rule out the hypothesis that the effect was life-related. The motion doesn’t arise from a “vital force”, it’s a purely physical phenomenon, common to both living and non-living things.

[Courtesy of Filipa Alves]
Brownian motion

**Albert Einstein** (1879 - 1955)

In 1905, Einstein proposes that the small particles are pushed around by **collisions** with water molecules **moving randomly** in all directions. In the light of this hypothesis, Brown’s observations would be a way to indirectly confirm the existence of atoms and molecules.
Brownian motion

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In 1905, Einstein proposes that the small particles are pushed around by *collisions* with water molecules *moving randomly* in all directions.

In the light of this hypothesis, Brown’s observations would be *a way to indirectly confirm the existence of atoms and molecules*.

**If this is true**, then:

- the motion should obey the **Newton's laws of motion** (inertia, force=mass×acceleration, action-reaction)
- the forces acting on the particle movement should be random
- the **diffusion coefficient** of the particles could be calculated and measured
- the particles moving in a fluid would also be affected by **frictional forces** (Stokes formula)

[Courtesy of Filipa Alves]
ON THE MOVEMENT OF SMALL PARTICLES SUSPENDED IN STATIONARY LIQUIDS REQUIRED BY THE MOLECULAR-KINETIC THEORY OF HEAT

by A. Einstein

[Annalen der Physik 17 (1905): 549-560]

It will be shown in this paper that, according to the molecular-kinetic theory of heat, bodies of microscopically visible size suspended in liquids must, as a result of thermal molecular motions, perform motions of such magnitude that these motions can easily be detected by a microscope. It is possible that the motions to be discussed here are identical with the so-called "Brownian molecular motion"; however, the data available to me on the latter are so imprecise that I could not form a definite opinion on this matter.

If it is really possible to observe the motion to be discussed here, along with the laws it is expected to obey, then classical thermodynamics can no longer be viewed as strictly valid even for microscopically distinguishable spaces, and an exact determination of the real size of atoms becomes possible. Conversely, if the prediction of this motion were to be proved wrong, this fact would provide a weighty argument against the molecular-kinetic conception of heat.
Brownian motion

Jean Perrin (1870 - 1942)

Einstein’s statement that thermal molecular motions should be easily observed under a microscope stimulated Jean Perrin to make quantitative measurements.

“I did not believe that it was possible to study the Brownian motion with such a precision.”

(letter from Albert Einstein to Jean Perrin, 1909)

Tracings of the motion of 3 colloidal particles of radius 0.53 µm, as seen under the microscope. Successive positions every 30 seconds are joined by straight line segments (the mesh size is 3.2 µm).


[Courtesy of Filipa Alves]
Brownian motion and diffusion

• Why do the particles “spread”?
• What is the shape of their distribution during the first time steps?
• How does this distribution vary with time?
• What is the shape of the particle distribution at equilibrium?
• Why are the particles normally distributed in space?

[Courtesy of Filipa Alves]
Brownian motion simulation
(uniform distribution of particles)
Brownian motion simulation
(start as a Dirac pulse distribution of particles’ coordinates)

$t = 433$
Brownian motion simulation
(start as a Dirac pulse distribution of particles' coordinates)
Brownian motion simulation
(start as a Dirac pulse distribution of particles’ coordinates)
Brownian motion simulation
(start as a Dirac pulse distribution of particles' coordinates)

(x,y) scatterplot

Probability density of x
Brownian motion

The expected square displacement is given by:

\[ E[\Delta X^2] = kDt \]

where \( D \) is the diffusion coefficient, \( t \) is time and \( k \) is a constant that depends on the dimension of the system. For one dimension \( k=2 \).

The expected distance travelled per unit of time is therefore:

\[ E[|\Delta X|] = \sqrt{kDt} \]

The diffusion coefficient \( D \) is given for a pair of molecule types (e.g. solute and water) in the units area/time (usually \( cm^2/s \)).

How long will a protein with a diffusion coefficient of 10µm²/s take to move 10µm?

A protein with a diffusion coefficient of 10µm²/s takes, on average, 5 seconds to move 10µm, and 500 seconds to move 100µm.
Steady state diffusion: Fick’s first law

\[ J_i = -D_i \frac{\partial c_i}{\partial x} \]

The \textbf{diffusive flux} of a molecule \( i \) along the direction \( x \) is proportional to its concentration gradient (in this case, the flux doesn’t change with time) (in number of molecules per unit area per unit time)

\begin{itemize}
  \item Flux of molecules across a plane with area = 1
  \item (the minus sign in the equation means that diffusion is down the concentration gradient)
\end{itemize}

Adolf Fick (1829-1901)

[Courtesy of Filipa Alves]
Diffusion by Brownian motion

Solute molecules do not move down a gradient of concentration.

There is net flux of solute molecules down a gradient of concentration.
Proton hopping in water

Protons (H$^+$) move much faster than Hydrogen atoms!

The Grotthuss mechanism (“proton-hopping”), along with the relative lightness and small size of the proton, explains the unusually high diffusion coefficient of protons relative to other ions, for which the movement is due simply to random thermal motion (Brownian motion).

\[ D \approx 3.6 \times 10^5 \, \mu m^2/s \]

[Courtesy of Filipa Alves]
The cytoplasm is not a homogeneous solution

Eukaryotic cell and its organelles

The schematic shows a eukaryotic cell and a variety of membrane bound organelles. A thin-section electron microscopy image shows a portion of a rat liver cell approximately equivalent to the boxed area on the schematic. A portion of the nucleus can be seen in the upper left corner. The most prominent organelles visible in the image are mitochondria, lysosomes, the rough endoplasmic reticulum and the Golgi apparatus. (adapted from Fawcett, 1966)

[Courtesy of Filipa Alves]
The cytoplasm is **crowded**

There are many physical barriers that restrict free particle movement (internal compartments, cytoskeleton, large proteins, organelles, etc).

This simulation shows a dynamic molecular model of the bacterial cytoplasm, giving us a spectacular glimpse of the crowded conditions of the interior of a cell over a brief 15 microsecond time span. The model includes 50 of the most abundant types of macromolecules reported in *Escherichia coli*, for a total concentration of 275g/L. This "full energy" simulation shows a model of macromolecular diffusion based on Brownian dynamics and intermolecular interactions including electrostatic and hydrophobic interactions.

[Courtesy of Filipa Alves]
Many molecules are transported by the cytoskeleton.

Pollen tube growth in a germinating pollen grain (speed of growth can vary from $\mu$m/sec to $\mu$m/min, depending on the species)

Nuno Moreno, Plant Development Lab, IGC

[Courtesy of Filipa Alves]
Some ions and molecules are transported across membranes by **active transport**, against their concentration gradient.

K-Cl cotransporters (KCCs) control transmembrane electrolyte flux in a variety of physiologic settings, including the acute response to altered extracellular osmolarity. In this issue of Cell, Rinehart et al. (pp. 525–536) use targeted phosphoproteomics to reveal how phosphorylation at two conserved sites in KCCs controls their activity. The image depicts the activation of KCC3 in red blood cells in response to extracellular hypotonicity. KCC3 (blue) is shown embedded in the red blood cell membrane. Cotransporters that are phosphorylated at T991 and T1048 in the C terminus (highlighted in a white “flash”) are inactive, while those that are dephosphorylated at these sites are active, allowing K-Cl efflux from the cell and preventing cell swelling due to influx of water. Image concept by E. Gulcicek, J. Rinehart, and R. Lifton. Design and artwork by Xvivo.

[Courtesy of Filipa Alves]
Several mechanisms can be reasonably approximated to diffusion, and described by the same type of equations.

**Dispersion** of molecules can be equivalent to diffusion.

---

**Figure 1**: Models of morphogen dispersal. Source cells harbour vesicles filled with morphogen molecules (red), which fuse with the cell membrane and release their contents. a. Yu et al. propose that Brownian motion of molecules in the extracellular space leads to dispersal of the FGF8 morphogen. Two tracks of random walk by single FGF8 molecules are shown. b. Kicheva and colleagues suggest that repeated release and uptake by cells (transcytosis) leads to dispersal of the morphogen Dpp in the fly wing. c. A few slowly diffusing FGF8 molecules are associated with carbohydrates at the cell surface. This cell-surface pool may contribute to long-range dispersal of FGF8.


[Courtesy of Filipa Alves]