1. Basics

1.1 Macroscopic diffusion

- Fick’s first law: \( v = \frac{dN}{dt} \frac{1}{A} = -D \frac{dn}{dx} \)

\( D \) \text{ \textasciitilde average “speed” of particles, determined by thermal motion}

in a gas: \( D = \frac{1}{2} \lambda \bar{v} \) \text{ mean free path between collisions}

in a liquid: \( D = \frac{kT}{\zeta} \) \text{ Stokes-Einstein law}

\( \bar{v} = \sqrt{\frac{3kT}{m}} \) \text{ avg. velocity (Maxwell)}

\( \zeta = 6\pi \eta_0 R_h \) \text{ drag/friction coefficient}
1. Basics  

1.1 Macroscopic diffusion

- relation between flux and concentration/density change

\[ \text{volume } V = A \Delta x \]
\[ \text{density } \bar{n} = \frac{\Delta N}{\Delta V} \]

between \( t \) and \( t + \Delta t \):

\[
\frac{J(x) \cdot A \cdot \Delta t - J(x + \Delta x) \cdot A \cdot \Delta t}{\Delta V \Delta t} = \Delta N_x - \Delta N_{x+\Delta x} = \Delta \Delta N
\]

\( \Rightarrow \) continuity equation

\[ \frac{\Delta \Delta N}{\Delta V} = \Delta \bar{n} \quad \text{(density change), } \Delta x \to 0; \Delta t \to 0 \]

\[ \Rightarrow \frac{d\bar{n}}{dt} = -\frac{dJ}{dx} \]

1. Basics  

1.1 Macroscopic diffusion

- combine Fick’s first law and continuity equation:

\[ \Rightarrow \frac{d}{dt} \bar{n}(x, t) = D \frac{d^2}{dx^2} \bar{n}(x, t) \]

Fick’s second law, diffusion equation

- second-order differential equation!
- solution with appropriate initial and boundary conditions

\( \bar{n}(x, t) \) - is a function of space and time
- replace by \( c(x,t) \) in a solution (= concentration)

- point source, 1D solution:

initial condition: \( \bar{n}(t = 0) = \delta(x) \)
boundary conditions: \( \bar{n}(x = \pm \infty, t) = 0; \int_{-\infty}^{\infty} \bar{n}(x,t)dx = 1 \)

\[ \bar{n}(x, t) = \frac{1}{\sqrt{4\pi Dt}} \exp \left\{ -\frac{x^2}{4Dt} \right\} \]

\[ \langle x^2(t) \rangle = \sigma = 2Dt \]

3D: \( \langle r^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle + \langle z^2 \rangle \)

\[ \Rightarrow \langle r^2(t) \rangle = 6Dt \]
1. Basics

1.2 Brownian motion

- describe the random motion of individual particles ("self motion" or "self diffusion")
- first observed by Brown on cell organelae

simple 1D derivation for end distance:

\[ \langle x^2(t) \rangle = 2Dt \]

\[ \langle x^2(t) \rangle = 6Dt \]

random step, uniform length per time \( \Delta t \):

\[ \bar{l}_i = \pm l \]

distance traveled:

\[ x_n = \bar{l}_1 + \bar{l}_2 + \bar{l}_3 + \ldots + \bar{l}_n \]

average:

\[ \langle \bar{x} \rangle = \langle \bar{l}_1 \rangle + \ldots = 0 \]

but:

\[ \langle \bar{x}^2 \rangle = \left( \langle \bar{l}_1^2 \rangle + \langle \bar{l}_2^2 \rangle + \ldots + \langle \bar{l}_n^2 \rangle \right) + \langle \bar{l}_1 \bar{l}_2 \rangle + \ldots + \langle \bar{l}_{n-1} \bar{l}_n \rangle \]

\[ \langle \bar{x}^2 \rangle = n l^2 \]

0 (uncorrelated random numbers!)

compare with Einstein-Smoluchowski equation (full statistical/kinetical description of single-particle motion):

\[ \sigma = \langle x^2 \rangle = 2D_{\text{self}} t, \quad D_{\text{self}} = \frac{kT}{\zeta} \]

here: \( D_{\text{self}} \to \frac{l^2}{2\Delta t}, \quad t \to n\Delta t \)
1. Basics

1.3 Correlation functions

\( \chi(a) \) \( \chi \): general property, e.g., density, intensity
\( a \): variable, e.g., \( r \) (space), \( t \) (time), ... 

- define **auto**correlation function (ACF) of \( \chi(a) \):

\[
C_\chi(a) = \langle \chi(b) \chi(c) \rangle_{b,c} = \langle \chi(b) \chi(b + a) \rangle_b \\
= \lim_{B \to \infty} \frac{1}{2B} \int_{-B}^{B} \chi(h) \chi(h + a) dh \\
= \lim_{N \to \infty} \frac{1}{N} \sum_{i=1}^{N} \chi(b_i) \chi(b_i + a)
\]

- in a system, where \( \chi(a) \) varies *randomly*, \( C_\chi(a) \) is often a simple, analytical function that describes the **basic statistical properties**
  - e.g. for
  - instantaneous positions of molecules in liquids
  - random location or velocity of Brownian particles

- \( C_\chi(a) \) is often **directly measured**, e.g., in scattering experiments, NMR, or...

\[
\sum_{b} \left( \langle \chi(b) \rangle - \langle \chi \rangle \right) \left( \langle \chi(b + a) \rangle - \langle \chi \rangle \right) \\
\text{very often: } C_\chi(a) \sim e^{-a/\tau}
\]

\( \tau \): decay constant, correlation time

\( \text{long times: different sign as likely as equal sign } \Rightarrow \text{cancellation!} \)

\[
C_\chi(a) = \langle (\chi(b) - \langle \chi \rangle)(\chi(c) - \langle \chi \rangle) \rangle_{b,c} \\
= \langle \chi(b) \chi(c) \rangle_{b,c} - 2\langle \chi(b) \rangle \langle \chi(c) \rangle + \langle \chi \rangle^2 \\
= \langle \chi(b) \rangle \langle \chi(c) \rangle - \langle \chi \rangle^2 \\
\Rightarrow \text{so you can correlate the differences from the average!}
\]

\[
\text{white noise ("most random" variation of } \nu \text{): } C_{\nu}(t) = \langle \nu(t') \nu(t + t') \rangle_{t'} = \delta(t)
\]

\[
\text{example: random hopping (Markov process)}
\]
2. Dynamic light scattering (DLS)

2.1 The light scattering experiment

2.2 Rayleigh scattering

- interaction of an incoming (vertically polarized) wave
  \[ \vec{E}_{iv} = \vec{E}_{0iv} \cos(\omega t - \vec{k} \vec{x}) \]
  with a molecule (or a fragment) with polarizability \( \alpha \)
  \( \Rightarrow \) induced dipole moment
  \[ \vec{\mu} = \alpha \cdot \vec{E}_{iv} = \alpha \vec{E}_{0iv} \cos(\omega t - \vec{k} \vec{x}) = \alpha \vec{E}_{0iv} \cos(\varphi) \]
- oscillating dipole emits a scattered spherical wave

According to the Maxwell eqs. (accelerated charge!):

\[ \vec{E}_{sv} = \sin \phi_z \frac{1}{4\pi\varepsilon_0 c^2} \left( \frac{d^2 \vec{\mu}}{dt^2} \right) \]

\[ = \frac{\alpha \omega^2 \sin \phi_z}{4\pi\varepsilon_0 c^2} \frac{\vec{E}_{0iv}}{R} \cos(\omega t - \vec{k} \vec{R}) \]

\( \Rightarrow \) intensity ratio (scattered vs. incoming), use \( \omega = 2\pi \nu = 2\pi c / \lambda_0 \)

\[ \frac{i_{sv}}{I_{iv}} = \frac{|\vec{E}_{sv}|^2}{|\vec{E}_{iv}|^2} = \frac{\pi^2 \alpha^2 \sin^2 \phi_z}{\varepsilon_0^2 \lambda_0^4 R^2} \propto \frac{\alpha^2}{\lambda_0^4} \] (!! blue sky, red sunset...)

- modifications when scattering center moves:
  \( \Rightarrow \) internal dynamics (vibrations etc.): polarizability changes, \( \alpha = f(t) \), Raman effect!
  \( \Rightarrow \) molecule moves: Doppler shift/broadening (\( \Delta \omega \sim \omega \nu > c \) for absorption lines)
2. Dynamic light scattering (DLS)

2.3 Interference and scattering vector

\[ E_{s1} = E_0 e^{i(\omega t - \vec{k} \vec{R})} \]

\[ E_{s2} = E_0 e^{i(\omega t - \vec{k} \cdot \vec{R})} \cdot e^{i \Delta \phi} \]

- phase shift \( \Delta \phi \) depends on path length difference \( \delta = a - b \) (simple trigonometry, \( |\vec{k}| = 2\pi/\lambda_0 \)):
  \[ \Delta \phi = \frac{2\pi \delta}{\lambda_0} = \frac{2\pi}{\lambda_0} (a - b) = \vec{r}_{12} \cdot (\vec{k}_0 - \vec{k}) = -\vec{q} \cdot \vec{r}_{12} \]

includes the definition of the \textbf{scattering vector} (quantifies momentum transfer)

\[ |\vec{q}| = |\vec{k} - \vec{k}_0| = \frac{4\pi \sin \theta/2}{\lambda_0} \]

- note: for constructive interference, \( \Delta \phi = n 2\pi \Rightarrow n = 2\pi \sin \theta/2 \equiv \text{Bragg} \)
- interference = superposition

\[ F_{det} = F_{s1} + F_{s2} = F_0 e^{i(\omega t - \vec{k} \vec{R})} \cdot (1 + e^{-i\vec{q} \cdot \vec{r}_{12}}) \]

\[ = E_0 e^{i(\omega t - \vec{k} \cdot \vec{R})} \sum e^{-i\vec{q} \cdot \vec{r}_i} \]

for many particles, independent of origin (e.g., \( r_1 = 0 \))

2.4 Basic phenomenon and spectroscopic approach

- Doppler effect of diffusing scattering centers, \( r_i(t) \):
  \[ E_{det}(t) = E_0 e^{i(\omega t - \vec{k} \vec{R})} \sum e^{-i\vec{q} \cdot \vec{r}_i(t)} \]

  \[ \Rightarrow i_{det}(t) \propto E_{det}^2(t) \propto e^{-q^2 D_c t} \]

  \text{time-dependent scattered intensity (damped)!}

- line width from FT\{i_{det}(t)\}: \( \delta \omega \sim q^2 D_c \)

- energy change \( \delta \omega \sim q^2 D_c \) related to Doppler broadening:
  \( \sim 10 \text{ meV in a liquid at room temperature} \)
- typical vis. photon energy: \( 1.7 - 3 \text{ eV} \)

\[ \Rightarrow \text{very small effect! measurable only with} \]

- narrow-banded lasers
- Fabry-Perot interferometers

\( \sim \text{ since 1960} \)
2. Dynamic light scattering (DLS)

2.5 Time correlation

- **idea:** direct observation of intensity fluctuations, possible for small scattering volume
  (⇔ static LS: large volume, fluctuations are averaged!)

\[
Ci(t) <idet>^2 <i_{det}^2> t <i_{det}^2> 1/2q^2Dc
\]

- **DLS terminology:**
  \[G_2(t) = C_i(t) = \langle i_{det}(t) i_{det}(t + \tau) \rangle_T \quad \text{intensity ACF}\]
  \[= A + Bg_1^2(t), \quad \text{Siegert relation}\]
  \[g_1(t) = \frac{\langle E_{det}(t) E_{det}(t + \tau) \rangle_T}{E_{det}^2(0)} = e^{-q^2Dc\tau} \quad \Gamma: \text{“first cumulant”}\]

2. DLS

2.5 Time correlation

- in practice for polymers (large molecules, many scattering centers):
  \[\Gamma = q^2D_{app}\]
  apparent diffusion coefficient, depends on
  - **intra**molecular interferences, intramolecular motions ⇒ size effect, \(q\) dependence
  - hydrodynamic interactions between particles ⇒ concentration (c) dependence

series expansion in \(c\) and \(q\):

\[D_{app} = \frac{\Gamma}{q^2} = D_{self}(1 + k_d c)(1 + C q^2 \langle R_g^2 \rangle)\]

⇒ do extrapolation for \(c \to 0, q \to 0\)
2. DLS

2.6 DLS on cellulose solutions

Cellulose (cotton) = \[
\begin{array}{c}
\text{in solution:} \\
\text{random coil}
\end{array}
\]

\[ R_h \sim \text{molecular weight} \]

in bulk:
- semicrystalline polymer
- hardly soluble
- chain length?

Cuoxam: “Schweizers reagent”, copper sulfate - ammonia solution

Cd-tren: new high-tech coordinating solvent

Abbildung 4.2: Korrelationsfunktionen \( G(t) \) zweier Bakterienzellulosen in Cuoxam (oben) und Cd-tren (unten), aufgenommen für \( c = 0.2 \text{ g/l bei } 95^\circ \).


2. DLS 2.6 DLS on Cellulose solutions

“dynamic Zimm plots”
to remove \( c \) (concentration) and \( q \) (angle) dependencies:

Abbildung 4.3: Dynamische “Zimm-”Auftragung der Bakterienzellulosen BC122.2 in Cuoxam (A, via Kumulantenfit) und BC122.3 in Cd-tren (B, via Fit nach Williams-Watts).

3. Fluorescence correlation spectroscopy

3.1 Basic concepts

- fluorescence: red-shift of absorbed light

- confocal detection/imaging

3.2 Apparatus
3. Fluorescence correlation spectroscopy

3.3 Intensity fluctuations and normalized autocorrelation function

- remove offset: normalized autocorrelation function
  \[ G(t) = \frac{C_i(t)}{\langle i \rangle^2} 1 = \frac{\langle i(t+\tau) i(\tau) \rangle}{\langle i \rangle^2} \frac{\langle i \rangle^2}{\langle i \rangle^2} \]
  \[ G(t) = \frac{\Delta i(t+\tau) \Delta i(\tau)}{\langle i \rangle^2}, \text{ where } \Delta i(t) = i(t) - \langle i \rangle \]
  \[ G(t \to 0) = \frac{1}{\langle N \rangle} \text{ inverse number of particles in the focus} \]

- theory assuming Brownian motion (free diffusion) and Gaussian detection volume
  \[ G(t) = \langle N \rangle (1+t/\tau_D) \frac{1}{\sqrt{1+\omega^2 t/\tau_D}} \]
  \[ \omega = w_z/w_{xy} \text{ 1/e^2-widths of detection volume} \]
  \[ \tau_D = w_{xy}^2/D_{\text{self}} \text{ lateral diffusion time} \]
  \[ \langle N \rangle = \langle c \rangle \tau^3/2 w_{xy}^2 w_z \text{ average particle number in focus} \]

- including triplet dynamics (triplet fraction \( f_T \), triplet lifetime \( \tau_T \))
  \[ G(t) = \frac{1+f_T (exp(-t/\tau_T) - 1)}{\langle N \rangle (1-f_T) (1+t/\tau_D)} \frac{1}{\sqrt{1+\omega^2 t/\tau_D}} \]
  particles in triplet state are invisible for a certain time \( \tau_T \)
3. FCS  3.3 Autocorrelation function

- RNA: mediates between genetic code (DNA) and protein synthesis
- virus replication (e.g., AIDS) is based on certain RNAs
- replication (binding of reverse transcriptase) can be hindered by hybridization (=binding and blocking) with "anti-sense"-DNA
- understanding of hybridization dynamics (accompanied by unfolding of RNA)
  \[ \Rightarrow \text{powerful drugs on the basis of DNA!} \]

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3. FCS  3.4 Hybridization dynamics of RNA with DNA probes

- RNA: mediates between genetic code (DNA) and protein synthesis
- virus replication (e.g., AIDS) is based on certain RNAs
- replication (binding of reverse transcriptase) can be hindered by hybridization (=binding and blocking) with "anti-sense"-DNA
- understanding of hybridization dynamics (accompanied by unfolding of RNA)
  \[ \Rightarrow \text{powerful drugs on the basis of DNA!} \]
2-component fit:

\[ G(t) = \frac{1}{N_1+N_2} \sum_{i=1,2} \frac{Y_i}{1+t/\tau_i} \frac{1}{\sqrt{1+\omega^2 t/\tau_i}} \]

known: \( \tau_1 \) (free DNA) < \( \tau_2 \) (hybr. DNA),
determined: rel. population \( Y_{1,2} = N_{1,2}/(N_1+N_2) \)

Fig. 4: Increase in correlation decay time during the time course of hybridization of a labeled probe (HS6) to the folded α-1 RNA target. The average diffusion time increases due to higher fractions of \( \tau_2 \).

Fig. 5: Hybridization kinetics (fraction \( Y \) against time) of five different DNA oligonucleotide probes to the RNA target. \( Y \) is determined by evaluating the measurements (e.g. Fig. 4) with known \( \tau_1, \tau_2 \). The differences reflect the accessibility of binding sites at the folded target sequence.

⇒ details on binding kinetics!

P. Schwille et al., *Biochemistry* 35 (1996), 10182