Universal dynamics of pore formation in membranes.

E.I. Kats [1]

L.D.Landau Institute for Theoretical Physics RAS, Moscow, Russia

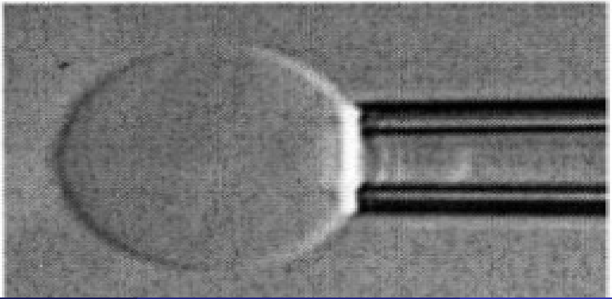
[1] Collaboration with D.J.Bicout.

Fluid membranes are indispensable constituents of all living things ($\simeq 200\, km^2$ in a human body). They are made of self-assembling amphiphilic molecules, mostly lipids, which aggregate to bilayer sheets in an aqueous environment.

- ”The entire preoccupation of a physicist is with things that contain within themselves a principle of movement and rest”, Aristotle (340 BC).
- Pores in membranes are such things !

INTRODUCTION on DTS TECHNIQUE.

Image of a $20 - mm$ vesicle aspirated in a micropipette



DTS experiments may be easy to conduct but difficult to interpret

- Rupture tests on different types of giant lipid vesicles.
- Loading rate from 0.01 and up to 100 $(mN/m)/s$.
- Output : (i) rate of membrane rupture ; (ii) distributions of breakage tensions governed by the kinetic process of membrane failure.
- One might naively expect that lipid membranes will rupture at tensions close to the hydrocarbon-water surface tension as lipids are held together by hydrophobic interactions.
- However biomembranes rupture at a much lower tension. Rupture is a dynamical property and the level of strength depends on the time frame of loading.

Our objective is to develop a minimal theoretical framework of the DTS method.

There are two steps :

- First, pore nucleation is described as an activated process following a first order kinetics with a rate q. It means that the distribution of times for the membrane to remain free of pores is given by the exponential distribution with the rate q. The pore nucleation rate $q(\sigma)$ is a function of the membrane surface tension σ.
- Second, based on the Kramers reaction rate theory we describe the pore growth and membrane rupture dynamics as a Markovian stochastic process crossing a time-dependent energy barrier.

Pore diffusion

- Once the pore is already formed, the net energy $V(r)$ of such a membrane of thickness l

$$V(r) = 2\pi\gamma r - \pi\sigma r^2$$

- The surface tension σ favoring the pore expansion, and energy cost γ of forming a pore edge (line tension) favoring the closure.
- Assuming $\sigma > 0$ and $\gamma > 0$ and both are constant, $V(r)$ predicts :

$$a = \frac{\gamma}{\sigma}$$

  where a is the pore radius for the maximum energy $V(r)$.
- If $r < a$ the radial force tends to reseal the pore and the membrane remains stable against pore growth.
- However if $r > a$ pore will grow without bound and, ultimately, will rupture the membrane.

Pore diffusion for DTS technique

- γ remains constant but

$$\sigma = \sigma_0 + Ft$$

  where σ_0 is the unstressed membrane tension and F is the loading rate constant.
- In this case, the critical radius $a(t)$ becomes a decreasing of time. Therefore any pore initially with radius $r < a(0)$ will ultimately lead to membrane rupture at a time such that $r > a(t)$ as a result of the decreasing of both the critical pore radius and associated barrier energy.
- Incorporating thermal fluctuations we view the rapture of the membrane as a Brownian process crossing the time-dependent energy barrier $V[a(t)]$.

Kramers theory

- Dynamics of a pore radius r is governed by the Langevin equation with the time dependent potential

$$\zeta \frac{dr}{dt} = -\frac{dV(r,t)}{dr} + f(t)$$

- where

$$V(r,t) = 2\pi\gamma r - \pi(\sigma_0 + Ft)r^2$$

  $\zeta = 4\pi\eta_m l$ is the friction coefficient with η_m the internal 2D membrane viscosity, and $f(t)$ is a Gaussian random force of zero mean with

$$\langle f(t)f(t')\rangle = 2\zeta k_B T\delta(t-t')$$

Parameters and dimensionless variables. The index "0" denotes quantities for unstressed membrane

Symbols	Definition
γ	line tension (*energy/length*)
σ_0	unstressed surface tension (*energy/surface*)
F	tension loading rate (*energy/surface/time*)
$D = \mathrm{k_B T}/\zeta$	pore diffusion coefficient (*length*^{2}*/time*)
$r_0 = \gamma/\sigma_0$	critical pore radius (*length*)
$\tau = r_0^2/D$	diffusing time scale of the critical pore (*time*)
$F_0 = \sigma_0/\tau$	critical tension loading rate (*energy/surface/time*)
q_0	reduced unstressed pore nucleation rate
$x = r/r_0$	reduced pore radius
$y = \sigma/\sigma_0$	reduced membrane surface tension
$\varepsilon = \pi\gamma^2/\sigma_0 \mathrm{k_B T}$	reduced energy barrier for unstressed membrane
$v = F/F_0$	reduced tension loading rate : $\begin{cases} v < 1 & : \text{diffusing limit} \\ v > 1 & : \text{drift limit} \end{cases}$

Dimensionless equations

- In dimensionless variables $x = r/r_0$, $y = \sigma/\sigma_0$, and $t \to t/\tau$ with with $x \in [0, 1]$ and $y \in [1, \infty[)$.
- This operation leads us to define the control parameter

$$v = \frac{F\tau}{\sigma_0} \equiv \frac{\text{diffusing time scale}}{\text{surface tension time scale}} .$$

- Two regimes in the dynamics of the membrane rupture : the diffusion-controlled regime $v \ll 1$ and the drift regime for $v \gg 1$ limit.
- Then dynamic equations

$$\begin{cases} \dfrac{dx}{dt} = -\dfrac{dU(x,y)}{dx} + X(t) \\ \\ \dfrac{dy}{dt} = v \end{cases}$$

where $\langle X(t)X(t')\rangle = 2\delta(t - t')$.

Potential landscape for DTS

- $$U(x|y) = \frac{\varepsilon}{2}[2x - yx^2]$$

  with $\varepsilon = V(r_0)/(k_B T) = (\pi\gamma^2)/(\sigma_0 k_B T)$.
- The potential $U(x|y)$ is maximum at $x^* = 1/y$ corresponding to the energy barrier $U^* = U(x^*|y) = \varepsilon/2y$. Both the position x^* and height U^* of the energy barrier decrease as y becomes larger as a result of the membrane stress.

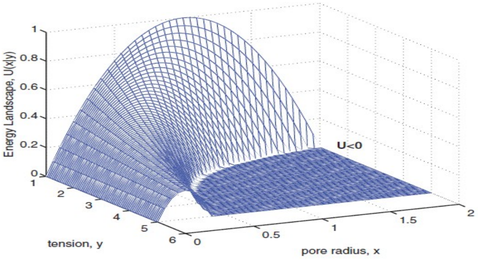


FIG.: Energy landscape with $\varepsilon = 2$ (flat area $x > 2/y$ corresponds to $U < 0$)

Our goal

- As the barrier crossing to both pore nucleation and membrane rupture are stochastic processes, both the membrane life time and the membrane tension at rupture are distributed.
- Our goal is to calculate the two quantities that characterize the kinetics of membrane rupture in DTS experiments : the rate of membrane rupture and the distribution of tension at membrane rupture.

Simplest example

- For a membrane with a pore in the absence of mechanical stress $v = 0$, the distribution of tension at membrane rupture is the delta function

$$Q(y) = \delta(y-1)$$

and the rate of the membrane rupture can be obtained by using the first passage time approach

$$\frac{1}{k(\epsilon|0)} = \int_0^1 \frac{dx}{p_{eq}(x|1)} \left(\int_0^x p_{eq}(z|1)dz \right)^2$$

We assume that the membrane is initially prepared with the distribution $p_{eq}(x|1)$, where

$$p_{eq}(x|y) = \frac{\exp(-U(x|y))}{Z(y)} \; ; \; Z(y) = \int_0^{1/y} \exp(-U(x|y))dx$$

then $1/k(\epsilon|0)$ is obtained analytically in terms of the error function *erf*(...) and $erfi(z) \equiv erf(iz)/i$.

Rate of unstressed membrane rupture

- $$\frac{1}{k(\epsilon|0)} = \frac{\sqrt{\pi/(2\epsilon)}}{erfi[\sqrt{\epsilon/2}]} \int_0^1 dx \exp(-\epsilon(x-1)^2/2)$$
  $$\times \left\{ erfi[\sqrt{\epsilon/2}] - erfi[(1-x)\sqrt{\epsilon/2}] \right\}^2$$

DIGRESSION to EXPLAIN FIRST PASSAGE TIME APPROACH.

First passage time approach

- There are a variety of problems where one wishes to calculate the average time τ_1 required for a particle, generated at some point and diffusing under the influence of a potential to reach a certain target. τ_1 is related to the probability $\Sigma(t)$ that a system is still unreacted at time t

$$\Sigma(t) \simeq \Sigma_{approx}(t) = \exp(-t/\tau_1)$$

- Approximation of a single exponential decay of $\Sigma(t)$ means that $\tau_1 = \int_0^\infty dt \Sigma(t)$.
- $\Sigma(t)$ in turn is related to the distribution $p(r,t)$ of finding the system at position r at time t : $\Sigma(t) = \int drp(r,t)$.
- $p(r,t)$ obeys the Smoluchowski equation

$$\frac{\partial p(r,t)}{\partial t} = \nabla \boldsymbol{j}(r,t)\,;\; \boldsymbol{j}(r,t) = D(r)[\nabla p(r,t) + \beta p(r,t)\nabla U(r)]$$

where $\boldsymbol{j}$ is the corresponding flux, $D(r)$ is the position dependent diffusion coefficient, and $U(r)$ is an external potential.

Technical details to the 1-st passage time approach.

- Notations in particle terminology : If the particle was at r_0 at $t = 0$, the probability that it has not been absorbed at time t is

$$\Sigma(r_0, t) = \int drp(r, t|r_0, 0)$$

and the average time required for adsorption

$$\tau_1(r_0) = \int_0^\infty dt\, \Sigma(r_0, t)$$

- Instead of solving the equation for $p(r, t|r_0, 0)$ and then integrating over r to obtain $\Sigma(r_0, t)$ one can derive a differential equation which determines $\Sigma(r_0, t)$ directly.
- The adjoint to Smoluchowski equation which holds for the adjoint operator

$$L^* = \nabla D(r)\nabla - \beta D(r)(\nabla U)\nabla$$

Technical details (continuation)

- The differential equation for $\Sigma(r_0, t)$ (L.Pontryagin, JETP, 1933)

$$\frac{\partial \Sigma(r_0, t)}{\partial t} = L^*(r_0)\Sigma(r_0, t)$$

- Then differential equation for $\tau_1(r_0)$ by virtue $\int_0^\infty dt(\partial/\partial t)\Sigma(r_0, t) = -1$

$$L^*(r_0)\tau_1(r_0) = -1$$

- The first passage time theory addresses the case of the Smoluchowski boundary condition for which every encounter at $r = a$ leads to reaction, making $\tau_1(r_0)$ the average time required to reach $r = a$ for the 1-st time starting from $r = r_0$. We enclose our system and prevent any particles from escaping by erecting a reflective barrier at $r = 0$ ($j(0, t) = 0$).
- While this equation is general, it can be solved analytically only when boundary conditions, the potential, and the diffusion coefficient depend solely on a single coordinate.

Back to DTS in membranes.

DTS observables for a stressed membrane

- For a membrane initially free of pore but stressed $v > 0$ the rate of membrane rupture and the distribution of tension at membrane rupture can be determined in terms of the survival probability $\Sigma(t)$. The membrane rupture rate $k(\epsilon|v)$ (inverse of the membrane lifetime)

$$\frac{1}{k(\epsilon|v)} = \int_0^\infty t\left(-\frac{d\Sigma(t)}{dt}\right)dt = \int_0^\infty \Sigma(t)dt$$

- Likewise, the distribution $Q(y)$ of tensions y at which membrane rupture is related to the distribution of rupture time and, as $y = 1 + vt$

$$Q(y) = \left|\frac{dt}{dy}\right|\left(-\frac{d\Sigma}{dt}\right)_{y=1+vt}$$

Survival probability to describe membrane fate

- The DTS spectrum for rupture tensions

$$\langle y(\epsilon|v)\rangle = \int_1^\infty yQ(y)dy$$

and it is related to the rupture rate by

$$\langle y(\epsilon|v)\rangle = 1 + \frac{v}{k(\epsilon|v)}$$

- In the case $\Sigma(t)$ satisfies a 1st order rate equation with the effective time dependent rate $\Gamma(t)$ (not the same as the bare function $q(t)$), i.e., $\Sigma(t) = \exp(-\int_0^t \Gamma(t')dt')$, the distribution $Q(y)$

$$Q(y) = \frac{\Gamma((y-1)/v)}{v} \exp\left[-\int_0^{(y-1)/v} \Gamma(z)dz\right]$$

Thus $\Sigma(t)$ is the key function to find !

Analytical theory

- Instead of stochastic equations for x and y one can write Fokker-Planck equation for the joint probability density $P(x, y, t)$

$$\frac{\partial P(x,y,t)}{\partial t} = -v\frac{\partial P(x,y,t)}{\partial y} - \frac{\partial J(x,y,t)}{\partial x}$$

  where the 1st term in the rhs -ballistic drift caused by the applied loading rate, and the 2d term is the diffusive flux for a given y

$$J(x,y,t) = -\exp(-U(x|y))\frac{\partial}{\partial x}\exp(U(x|y))P(x,y,t)$$

  it reduces to Smoluchowski equation for $v = 0$.
- $P(x, y, t)$ satisfies the reflecting boundary condition at $x = 0$ and the adsorbing boundary condition at $x = 1/y$:

$$J(x,y,t) = 0 \;\; x = 0\,; \; P(x,y,t) = 0 \;\; x = 1/y$$

  and initial condition $P(x, y, t = t_0|x_0, y_0) = \delta(x - x_0)\delta(y - y_0)$.

Formal, yet numerically computable solution

- Green's function

$$P(x,y,t|x_0,y_0,t_0) = \left(\frac{p_{eq}(x|y)}{p_{eq}(x_0|y_0)}\right)^{1/2} \delta[y - y_0 - v(t-t_0)]$$

$$\times \sum_{n=1}^{\infty} \psi_n(x_0|y_0)\psi_n(x|y)\exp[-(1/v)\int_{y_0}^{y} \lambda_n(z)dz]$$

where $\psi_n(x|y)$ and $\lambda_n(y)$ are normalized eigenfunctions $\int_0^{1/y} dx\psi_n(x)\psi_{n'}(x) = \delta_{n,n'}$ and eigenvalues for the Hamiltonian

$$H\psi = \frac{d^2\psi}{dx^2} - \left[\frac{v\epsilon x^2}{4} + \frac{\epsilon^2(1-yx)^2}{4} + \frac{\epsilon y}{2}\right]\psi = -\lambda\psi$$

- satisfying the reflecting and absorbing boundary conditions at $x = 0$ and $x = 1/y$

$$\exp(-U(x)\frac{\partial}{\partial x}[\exp(U(x)/2)\psi(x)]_{x=0} = 0\,;\ \psi(x = 1/y) = 0$$

Change of variables

- Let $z = (v\epsilon + \epsilon^2 y^2)^{1/4}(x - \epsilon y/(v + \epsilon y^2))$ with $z_0 \leq z \leq z_1$, where

$$z_0 = -\frac{\epsilon y(v\epsilon + \epsilon^2 y^2)^{1/4}}{(v + \epsilon y^2)}\ ;\ z_1 = \frac{v(v\epsilon + \epsilon^2 y^2)^{1/4}}{y(v + \epsilon y^2)}$$

- Then the equation $H\psi = -\lambda\psi$

$$\frac{d^2\psi}{dz^2} - \left[E - \frac{z^2}{4}\right] = 0$$

where

$$E = \frac{1}{(v\epsilon + \epsilon^2 y^2)^{1/2}}\left(\lambda - \left(\frac{\epsilon y}{2} + \frac{v\epsilon^2}{4(v + \epsilon y^2)}\right)\right)$$

Formal solution (continuation)

- The general solution satisfying the reflecting and absorbing boundary conditions

$$\psi(z) = A[D_\nu(-z_1)D_\nu(z) - D_\nu(z_1)D_\nu(-z)]\,;\ \nu = E - \frac{1}{2}$$

  where D_ν is the Weber (parabolic cylinder) function.
- A is obtained from the normalization, and the eigenvalue by using BC

$$D_\nu(-z_1)\left(\frac{dD_\nu(z_0}{dz_0} + \frac{\epsilon}{2(v\epsilon + \epsilon^2 y^2)^{1/4} D_\nu(z_0)}\right)$$

$$+D_\nu(z_1)\left(\frac{dD_\nu(z_1)}{dz_0} - \frac{\epsilon}{2[v\epsilon + \epsilon^2 y^2]^{1/4}} D_\nu(-z_0)\right) = 0$$

Last efforts

- Assuming that the system is initially prepared with the distribution function $g(x, y, t)$, then

$$\Sigma(t) = \int_1^\infty dy_0 \int_1^\infty dy \int_0^{1/y_0} dx_0 \int_0^t dt_0 P(x, y, t|x_0, y_0, t_0) g(x_0, y_0, t_0)$$

  where

$$g(x, y, t) = p_{eq}(x|y)\delta(y - 1 - vt) \left[q(t) \exp\left(- \int_0^t q(t')dt' \right) \right]$$

  and the term [....] stands for the distribution of times for pore nucleation.
- This is exact expression of $\Sigma(t)$ from which the rupture rate $k(\epsilon|v)$ (the DTS spectrum), and the distribution $Q(y)$ of rupture tension and also time dependent rate $\Gamma(t)$ can be found. Unfortunately, the derivation is very tedious, and analytical expressions unpractical for explicit calculations.

OUR RESULTS.

Distribution $Q(y)$ of rupture tensions y

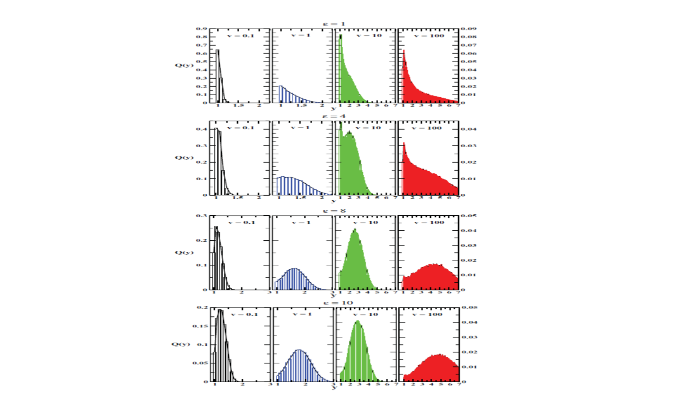


FIG.: $q(y) = q_0 \exp(\alpha(y-1))$ with $q_0 = 0.1$ and $\alpha = 1$.

DTS spectrum $\langle y(\epsilon|v)\rangle$ as a function of the barrier height ϵ

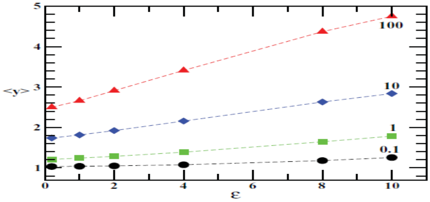


FIG.: Loading rates v are quoted numbers

DTS spectrum $\langle y(\epsilon|v)\rangle$ as a function of the loading rate v

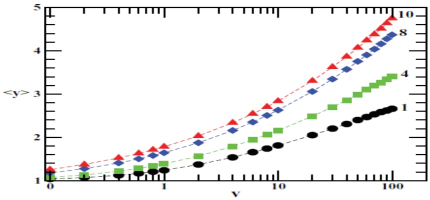


FIG.: Barrier heights ϵ are quoted numbers.

$Q(y)$ for various tension independent pore nucleation rate q_0

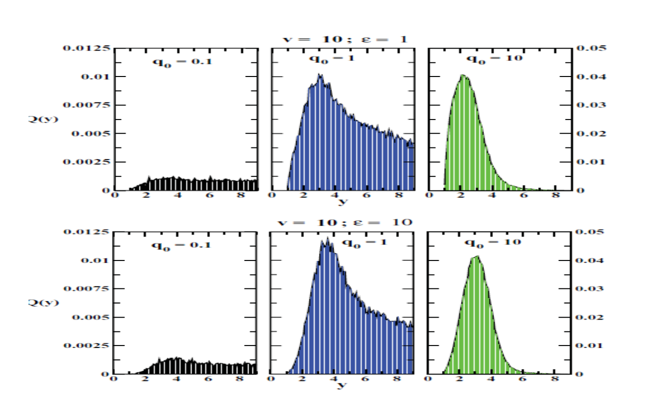


FIG.: $\alpha = 0$