CATCH-U-DNA

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FETOPEN MEETING at Valencia

- Marc Meléndez Schofield
- Adolfo Vazquez-Quesada
- Raul Pérez Peláez
- Proteins in collab with Ivan Korotin and Sergei Karavasov, Queen Mary University (London)

Objectives

- M30. QCM analysis of liposome-DNA complex
- M20. Molecular dynamics simulations QCM for streptavidin under GHz flow.

M30. QCM analysis of liposome-DNA complex

- Theoretical understanding of dissipation and frequency shifts.
- Ways to increase the acoustic ratio
- Predictions at higher frequencies (up to $135\mathrm{MHz}$)

Liposome

Radius. R = [15-100] nm

Bending rigidity. κ_{LIPO} ~ [10-100] k_BT

lipid phase ($T-T_m$) (gel / fluid)

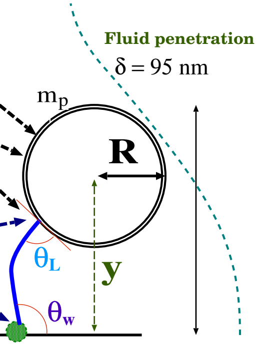
DNA-STRAND

L_{DNA} = [7-50] nm

$\lambda_{persistence}$ = 50 nm

θ_L : Stiffness of the DNA-liposome anchor, $k_{DNA\text{-}LIPO}$

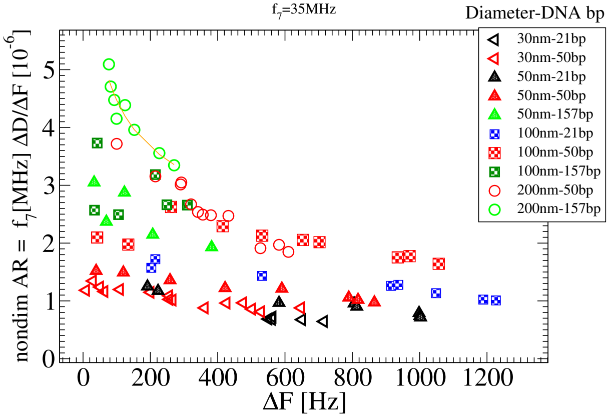
θ_w : Stiffness of the DNA-linker-wall contact



Linker (protein)
(MD SIMULATIONS)

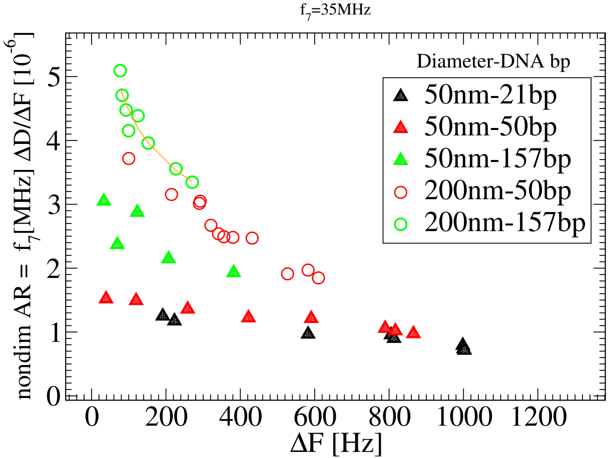
Experimental acoustic ratio

AR_{exp} is the value of $\Delta D/\Delta f$ at the limit $\Delta f \to 0$ (very low coverage).

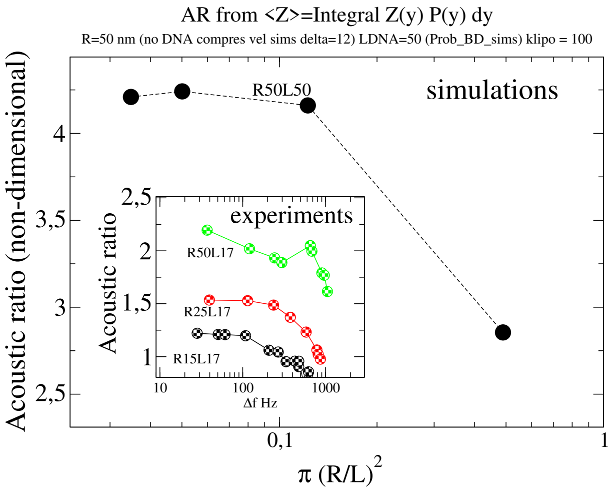


Outlook

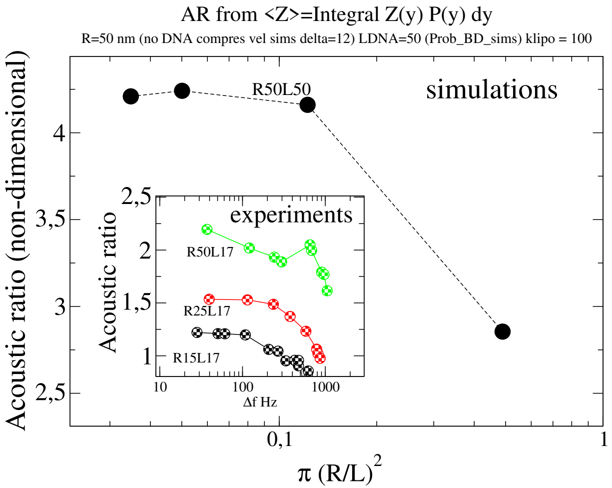
Observed values of Δf are larger for smaller DNA strands.



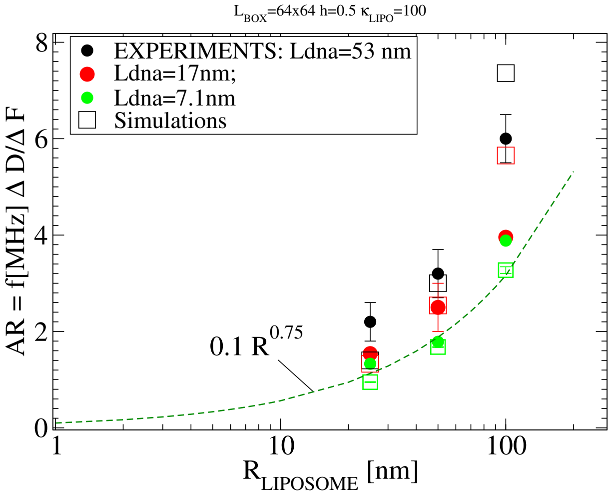
Simulations: AR converges for large boxes ("small concentration").



Objective of simulations: Relate Δf and AR with liposome surface fraction ϕ.

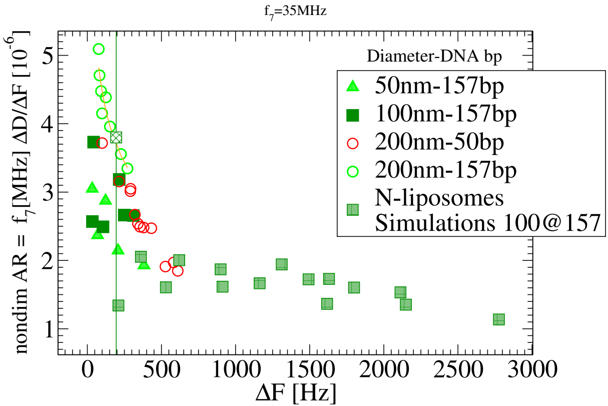


Achieved: relatively good **quantitative** agreement with experiments (POPC lipids).



Acoustic ratio: comparison with experiments

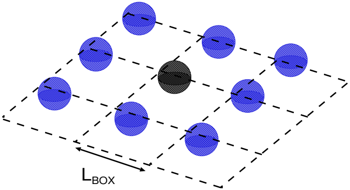
Soon: theoretical prediction of lipid coverage from Δf and AR

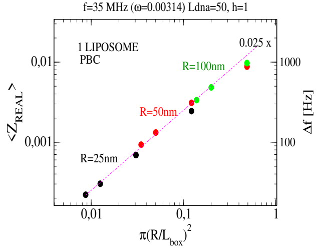


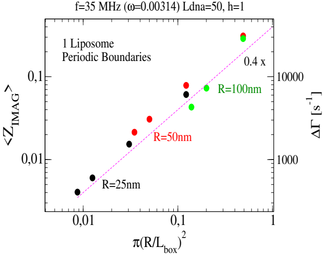
ONE LIPOSOME in a periodic box: hydrodynamic interaction with its own images

surface fraction

$$\phi = \pi R^2 / L_{BOX}^2$$



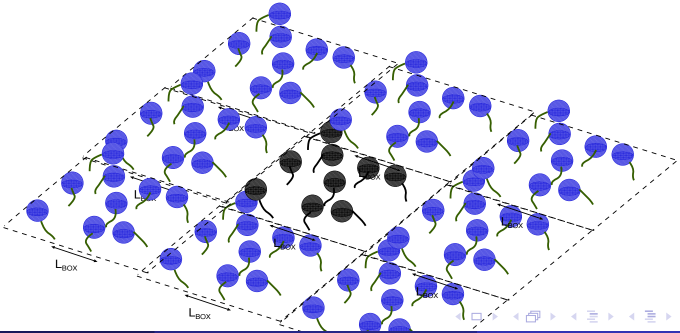




N LIPOSOMES in PBC: hydrodynamic interactions between randomly distributed liposomes

number of liposomes in primary box $N = [1, 16]$

surface fraction $\phi = N \pi R^2 / L_{BOX}^2$



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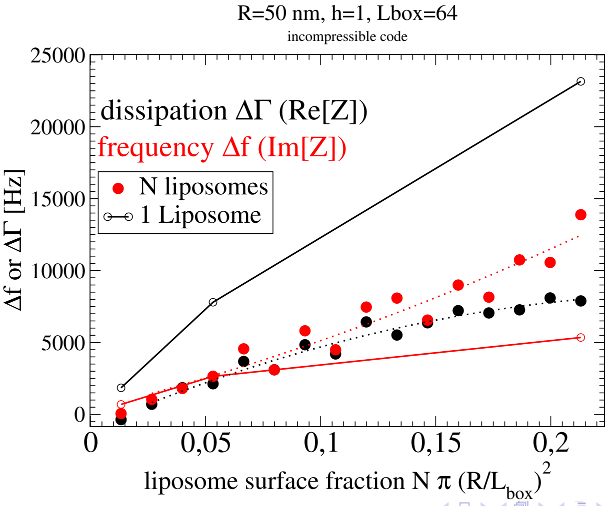
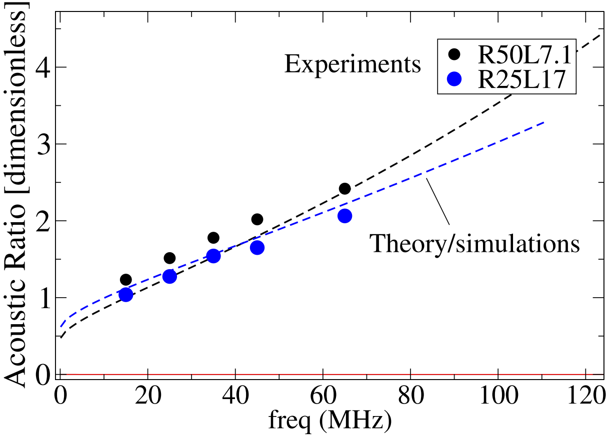
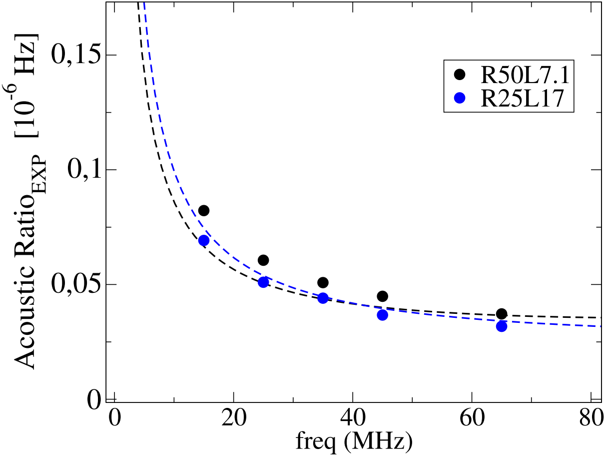


Achieved: AR at different frequencies

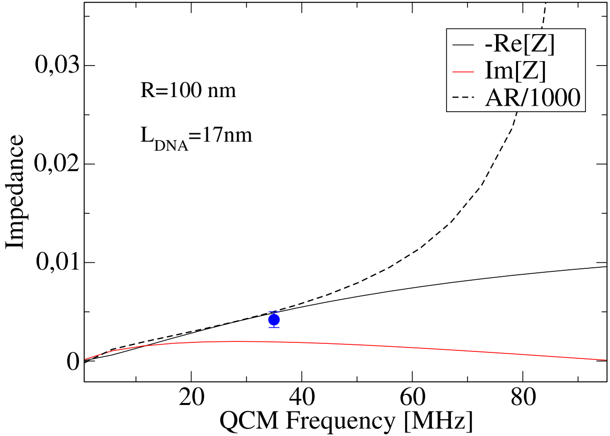


Prediction at higher QCM frequencies

Achieved: AR at different frequencies



Prediction: Divergence of AR for the largest liposomes $R = 100\mathrm{nm}$



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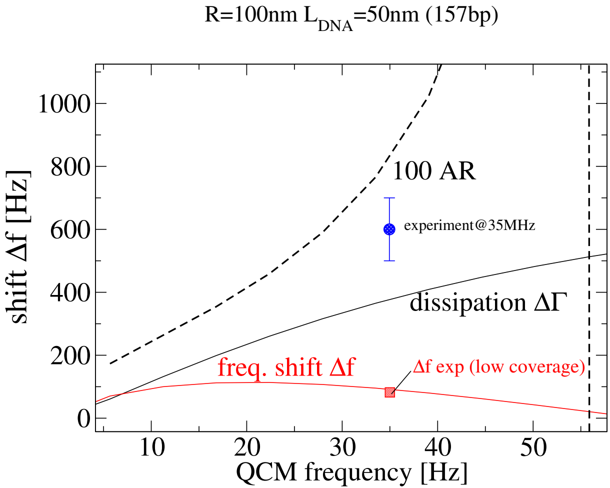
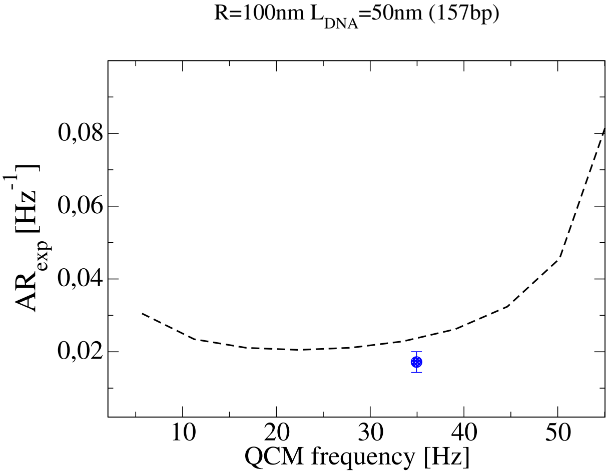
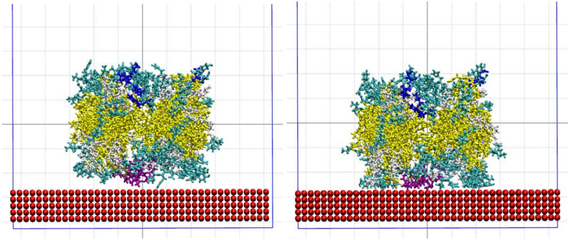




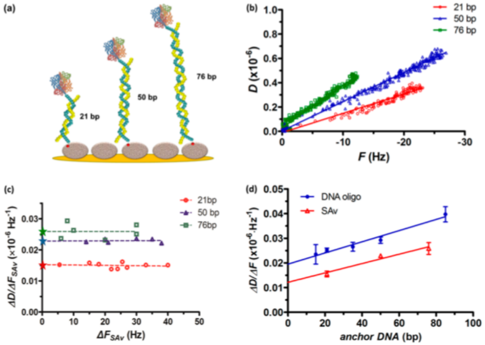
R=100nm L_DNA=50nm (157bp)
0,02
0,04
0,06
0,08
AR_exp [Hz^-1]
10
20
30
40
50
QCM frequency [Hz]

Molecular simulations of streptavidin close/adsorbed to the resonator surface



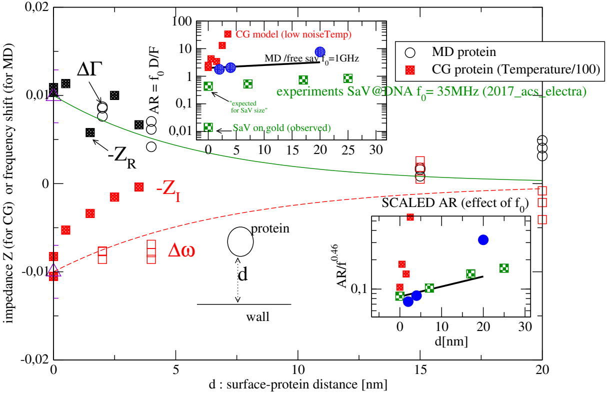
Molecular simulations of streptavidin close/adsorbed to the resonator surface

Chemistry



) Schematic representation of the 21, 50, and 76bp DNA anchors used to achieve SAv binding through a single point (b) typical D versus F plots of SAv (20 μg/mL) obtained for each one of the three DNAs; (c) plot of SAv ac ıg surface coverage (indicated by ΔF) for each DNA anchor; (d) plot of the acoustic ratio of SAv (red) and 47b :he DNA anchor length.

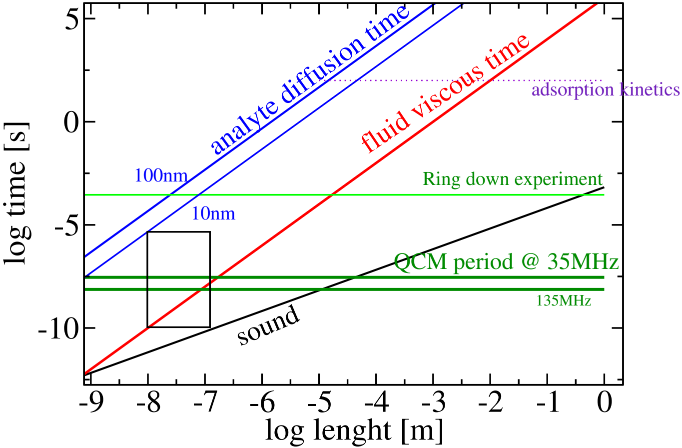
Molecular simulations of streptavidin close/adsorbed to the resonator surface



Achieved issues

- Reproduce the experimental acoustic ratio ✓
- Predict QCM response at higher frequencies ✓
  - Divergence of AR induced by hydrodynamics (feasible for $R = 100\mathrm{nm}$)
- Predict the relation between lipid coverage and Δf and $\Delta\Gamma$ (Soon)
- Effect of **liposome bending rigidity** ✓
  - Number of carbons in lipid chains (quantitative analisis yet to be done)
  - Membrane in fluid/gel phase (microscopic relation with the **stifness of the DNA-Liposome link**?)
- Propose ways to increase the dissipation and the AR
  - Increase the liposome height $\implies$ increase AR (electric fields?, modifying ph?)
  - Numerical estimation of coverage $\Delta f(\phi)$ permits calibration with larger impedance complex $\implies$ (working with smaller DNA strands)
  - Other ideas ready to be tested

Time scales and mapping with experimental units



analyte diffusion time
fluid viscous time
adsorption kinetics
100nm
10nm
Ring down experiment
QCM period @ 35MHz
135MHz
sound
log time [s]
log lenght [m]
5
0
-5
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0

Code reference unit lenght: $\ell = 7.917\mathrm{nm}$

Magnitude	**Simulation**	**International Units**
Lenght	1	$7.917\mathrm{nm}$
Kin. viscosity	0.226	$10^{-6}\mathrm{m}^2/\mathrm{s}$
fluid density	1	$10^3\mathrm{kg}/\mathrm{m}^3$
sound velocity	2.68	$1.5 \times 10^3\mathrm{m}/\mathrm{s}$
QCM frequency	$\mathbf{0.003145}/(2\pi)$	$35\mathrm{MHz}$
$k_B T$	6.6×10^{-5}	$4 \times 10^{-21}\mathrm{J}$

Basic definitions

Phasor

$$x(t) = x_R \cos(\omega t) + x_I \sin(\omega t)$$

$$x(t) = \hat{x} \exp[-\mathrm{i}\omega t]$$

$$\hat{x} \equiv x_R + \mathrm{i} x_I$$

Definitions

- **Resonator position (Ring down)**

$$x(t) = \Delta x \exp[-\Gamma t] \cos(2\pi f t + \phi)$$

- Angular frequency $\omega = 2\pi f$
- Decay rate Γ
- **Phasor**

$$x(t) = \mathrm{Re}\left[\hat{x} \exp[-\mathrm{i}(\omega - \mathrm{i}\Gamma)t]\right]$$

- Complex frequency $\tilde{\omega} = \omega - \mathrm{i}\Gamma$
- Dissipation

$$D = \frac{\Gamma}{\pi f} = \frac{2\Gamma}{\omega}$$

- **Reference state: (R)**

$$x_R(t) = \mathrm{Re}[\hat{x}_R \exp[-\mathrm{i}(\omega_R - \mathrm{i}\Gamma_R)t]]$$

- **Loaded state: (A)**

$$x_A(t) = \mathrm{Re}[\hat{x}_A \exp[-\mathrm{i}(\omega_A - \mathrm{i}\Gamma_A)t]]$$

- **Complex frequency shift**

$$\Delta\tilde{\omega} = \tilde{\omega}_A - \tilde{\omega}_R$$

- **Shifts**

$$F \equiv \Delta f = f_A - f_R \tag{1}$$

$$\Delta\Gamma = \Gamma_A - \Gamma_R \tag{2}$$

$$D \equiv \Delta D = D_A - D_R \tag{3}$$

Acoustic ratio: two definitions

$$\mathrm{AR} \equiv -\frac{2\Delta\Gamma}{\Delta\omega} \;\; \text{No dimensions} \tag{4}$$

$$\mathrm{AR}_{exp} \equiv \frac{D}{(-F)} \times 10^{-6} = \frac{1}{f_0[\mathrm{MHz}]}\frac{2\Delta\Gamma}{\Delta\omega} \;\; [10^{-6}\mathrm{Hz}^{-1}] \tag{5}$$

$$\boxed{\mathrm{AR}_{exp} = \frac{\mathrm{AR}}{f_0[\mathrm{MHz}]}}$$

Impedance = stress at the wall / wall velocity

- Total stress at the wall: $\hat{\sigma}_{tot} = \sigma_A + \sigma_R$
- Analyte's impedance:

$$Z_A = \frac{\hat{\sigma}_A}{\hat{v}_{wall}} = \frac{\hat{\sigma}_{tot} - \hat{\sigma}_R}{\hat{v}_{wall}}$$

- Impedances sum up:

$$Z_A = Z_{tot} - Z_R$$

Reference load	Reference impedance
Simple fluid	$Z_R = -\mathcal{Z}_f(1 - \mathrm{i})$

Simple fluid impedance (modulus)

$$\mathcal{Z}_f \equiv \frac{\eta}{\delta} \quad (6)$$

$$\delta = \left(\frac{2\eta}{\rho\omega}\right)^{1/2} \quad \text{penetration lenght} \quad (7)$$

The Small Load Approximation (SLA)

- If the resonator "mass" (per area) is much larger than that of any other load..

$$m_Q >> m_{Fluid} = \rho\delta$$

$$m_Q >> m_{Load}$$

- Then.. the impedance is proportional to the complex frequency shift:

$$\boxed{\begin{aligned} \frac{\Delta f}{f_0} &= -\frac{Z_{Im}}{\pi Z_Q} \\ \frac{\Delta\Gamma}{f_0} &= -\frac{2Z_{Re}}{Z_Q} \end{aligned}}$$

with **Unloaded resonator impedance**

$$Z_Q = (C\rho_Q)^{1/2} \approx 8.8 * 10^6 \mathrm{kg}/(\mathrm{m}^2\mathrm{s})(\approx 15.745\,\mathrm{Simulation\,Units})$$

C is the quartz elastic moduls and ρ_Q its density.

- If the resonator "mass" (per area) is much larger than that of any other load..

$$m_Q >> m_{Fluid} = \rho\delta$$

$$m_Q >> m_{Load}$$

- Then.. the impedance is proportional to the complex frequency shift:

Acoustic Ratio

$$\mathrm{AR} = \frac{\Delta\Gamma}{\pi\Delta\omega} = -\frac{2Z_{Re}}{Z_{Im}}$$

Impedance analysis

$$Z = -\frac{\sigma}{\hat{v}_w} = -\frac{\sigma}{\mathrm{i}\omega\hat{x}}$$

- Imposing the wall motion: $x(t) = x_0 \cos(\omega t)$ or $\hat{x} = x_0 \in \mathcal{R}$
- Imposing the wall stress: $\sigma_{ext}(t) = \sigma_e \cos(\omega t)$ or $\hat{\sigma}_e \in \mathcal{R}$

Simple analytic models: impedance

$$Z = \frac{\hat{\sigma}}{\hat{v}_0}$$

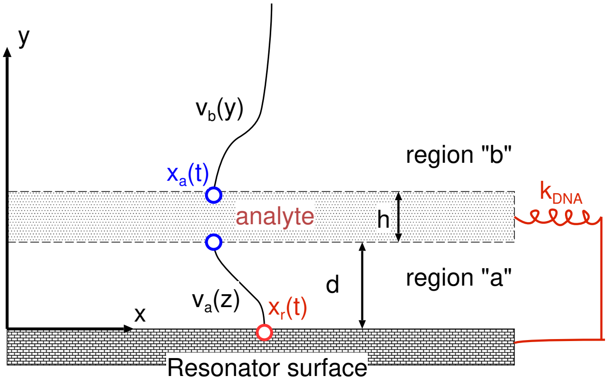
1. Impose the wall velocity $\hat{v}_0 = -\mathrm{i}\omega x_0$
2. Solve (or model) the stress at the wall, as a function of the analyte motion: $\hat{\sigma} = \hat{\sigma}(\hat{x}_a)$
3. Solve the analyte motion:

$$-\mathrm{i} m_a \omega^2 \hat{x}_a = F_f(\hat{x}_a, x_0) - k_{DNA}(\hat{x}_a - x_0) \tag{8}$$

with

- F_f: Fluid force on the analyte
- k_{DNA}: effective spring for the DNA (could be complex)

Plane model



y
$v_b(y)$
region "b"
$x_a(t)$
k_{DNA}
analyte
h
region "a"
d
$v_a(z)$
$x_r(t)$
x
Resonator surface

$$-\mathrm{i}m_a\omega^2\hat{x}_a = F_f(\hat{x}_a, x_0) - k_{DNA}(\hat{x}_a - x_0)$$

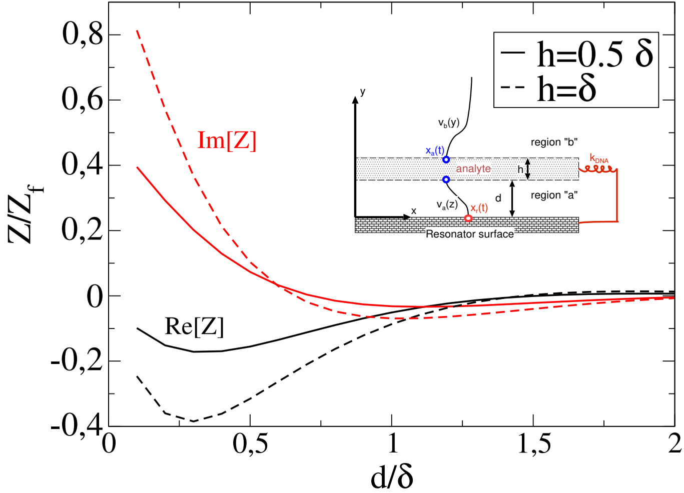
- Mass per unit surface: $m_a = \rho_a h$
- Fluid force (per unit area):

$$F_f = \eta\left(\frac{\partial v}{\partial y}\right)_{y=d+h} - \eta\left(\frac{\partial v}{\partial y}\right)_{y=d}$$

- Fluid

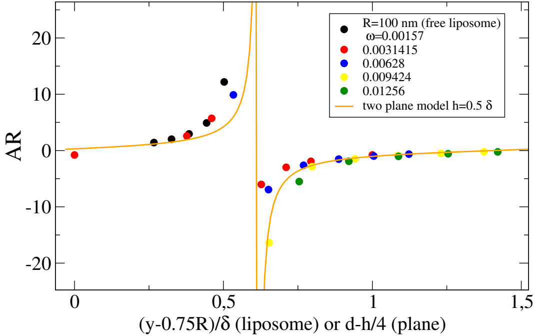
$$\begin{aligned}
\frac{\partial v}{\partial t} &= \nu\frac{\partial^2 v}{\partial z^2} \\
v(0,t) &= \frac{dx_r}{dt} \\
v(d,t) &= \frac{dx_a}{dt} \\
v(d+h,t) &= \frac{dx_a}{dt} \\
v(\infty,t) &= 0
\end{aligned}$$

Plane model

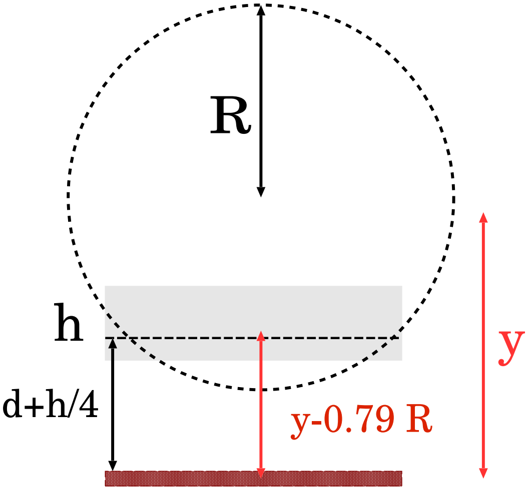

0,8
0,6
0,4
0,2
0
-0,2
-0,4
0
0,5
1
1,5
2
Z/Z_f
d/δ
Im[Z]
Re[Z]
h=0.5 δ
h=δ
y
x
v_b(y)
x_a(t)
region "b"
analyte
h
k_DNA
d
region "a"
v_a(z)
x_f(t)
Resonator surface

Zero crossing of $\mathrm{Im}[Z]$ leads to AR divergence at some height y_c.

Plane model



Height y_c for the zero crossing of $\mathrm{Im}[Z]$ (divergence of AR)



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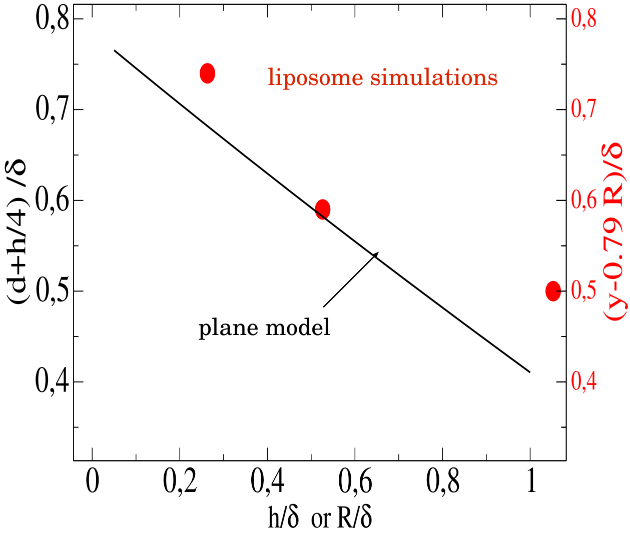
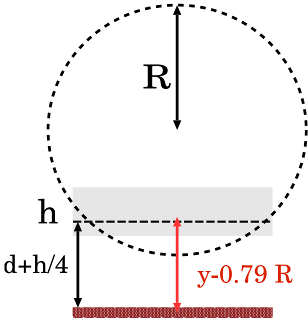
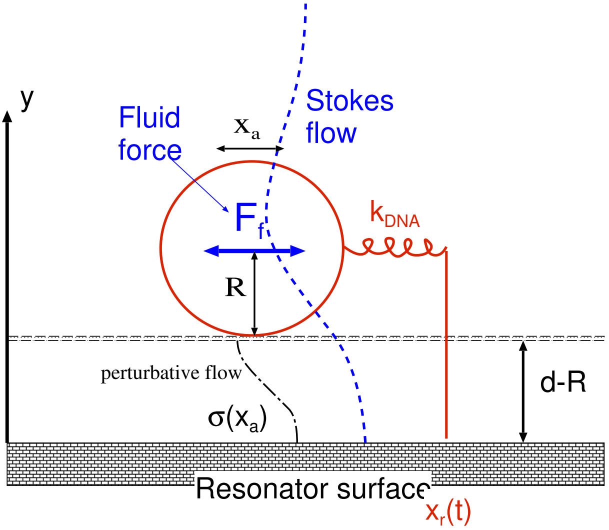





y
Fluid
force
x_a
Stokes
flow
F_f
k_{DNA}
R
perturbative flow
$\sigma(x_a)$
d-R
Resonator surface
$x_r(t)$

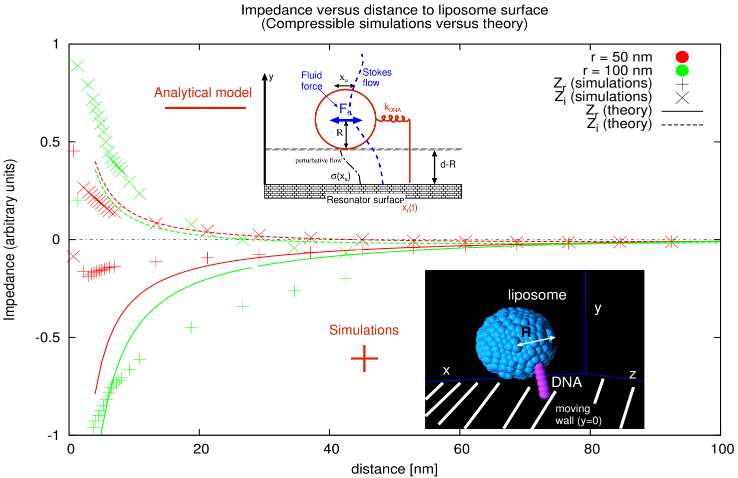
Sphere-Plane model

- Analyte mass: $m_a = (4/3)\pi\rho_a R^3$
- Fluid force (Mazur and Bedeaux 1979)

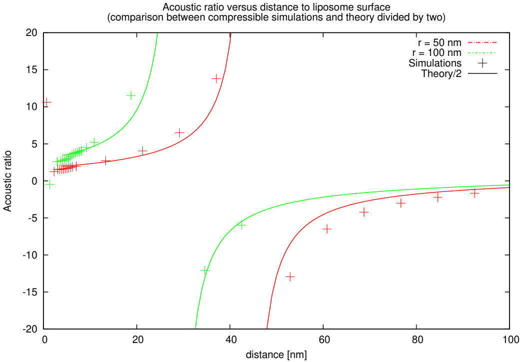
$$\text{with}\, \alpha = \frac{(1-\mathrm{i})}{\delta} \text{and} \;\; \hat{u} = -\mathrm{i}\omega\hat{x}_a \text{ analyte velocity}$$

$$\begin{aligned}
\frac{F_f(\omega)}{6\pi\eta R} &= \left[1 + \alpha R(\hat{v}_s - \hat{u}) + (1/3)(\alpha R)^2(\hat{v}_v - \hat{u}) - (2/9)(\alpha R)^2\hat{u}\right] \\
\hat{v}_s &= \frac{1}{4\pi R^2}\oint \hat{v}(\mathbf{r})d^2\mathbf{r} \text{ Surface average} \\
\hat{v}_v &= \frac{3}{4\pi R^3}\int \hat{v}(\mathbf{r})d^3\mathbf{r} \text{ Volume average} \\
\hat{v}(y) &= -\mathrm{i}\omega x_0 \exp[-\alpha y] \text{ Stokes base flow}
\end{aligned}$$

Sphere-Plane model



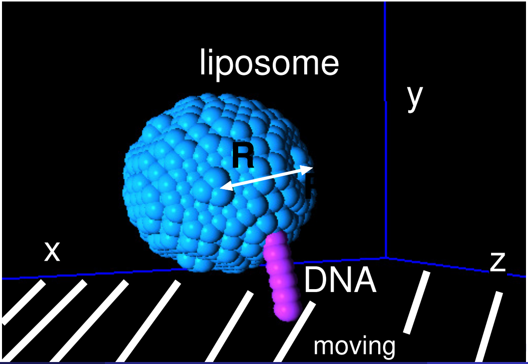
Sphere-Plane model



Simulations of QCM dynamics of free liposomes

- Immersed boundary method: mesh size h
- Compressible fluid (sound velocity of water).
- Resolution: number of cells per penetration layer. $N_\delta = \delta/h \geq 12$
- Numerical evaluation of the wall stress (second order accurate).

$$\sigma = \eta \left(\frac{\partial v_x}{\partial y}\right)_{y=0}$$



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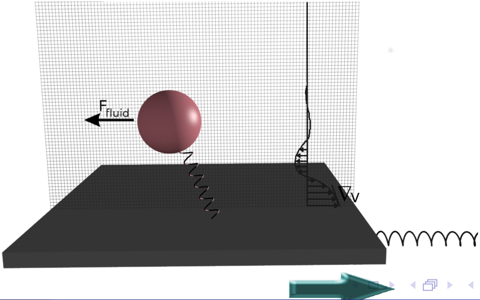

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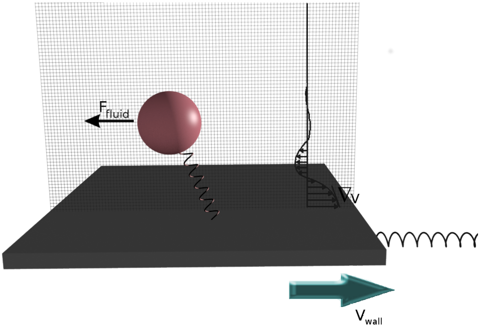
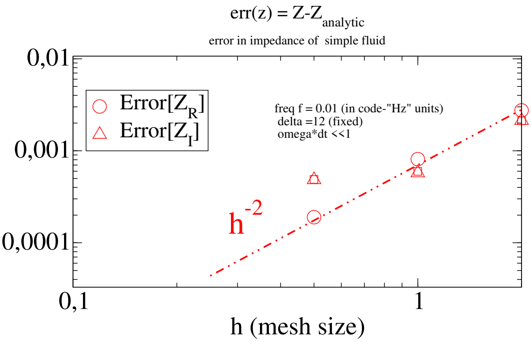
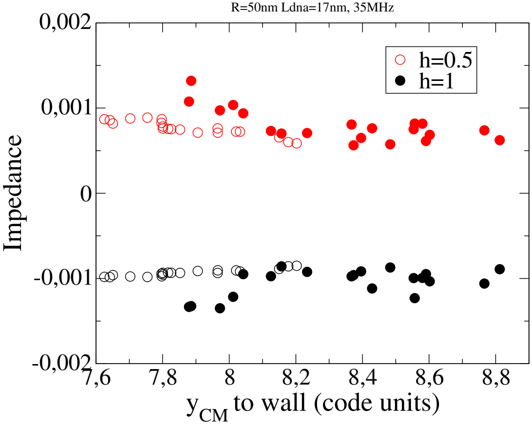


- Convergence tested for simple fluid and liposome.



- Double resolution needed for liposomes close to the wall.
  $h = 0.5\ell = 3.95\text{nm}$



Coarse-grained model: some movies

1GHz

1GHz

CODE PARAMETERS

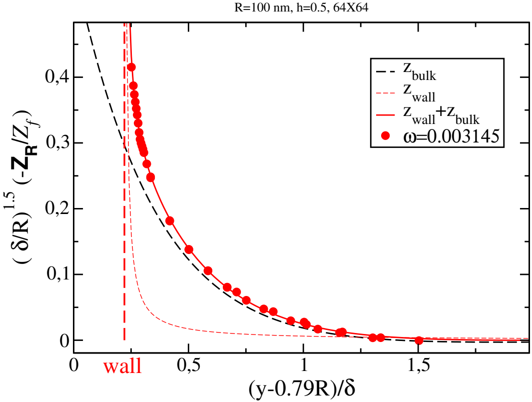
$\delta = \sqrt{\frac{2\eta}{\rho\omega}}$	6, 6.9282, 8.48528, **12**, 16.9706 (47.5, 54.9, 67.2, **95.0**, 134.4 nm)
Radius R	3.16, 6.32, 9.0, 12.63 (25, 50, 75, 100 nm)
DNA length L_{DNA}	0.88, 2.15, 6.32 (7, 17, 50 nm) (or without DNA)
Box length $L_x = L_y$	8, 16, 24, 32, 60, 64 (63.3, 126.0, 190.0, 253.0, 475.0, 506.0 nm)
Box length L_z	64 (506 nm)
Liposome thickness	1.26 (10 nm)
Cell size h	0.5, 1.0, 2.0 (0.06, 0.13, 0.25 nm)
DNA rigidity	$k_{\mathrm{DNA}}^{\mathrm{spring}} = 100$ $k_{\mathrm{DNA}}^{\mathrm{3spring}} = 63.16$
Liposome rigidity	$k_{\mathrm{Lipo}}^{\mathrm{spring}} = 1, 10, 20, 100, 300, 1000$
Type of oscillations	Forcing wall Imposing velocity
Angular frequency ω	between 0.0005 and 0.0157

TABLE I. Parameters of the model.

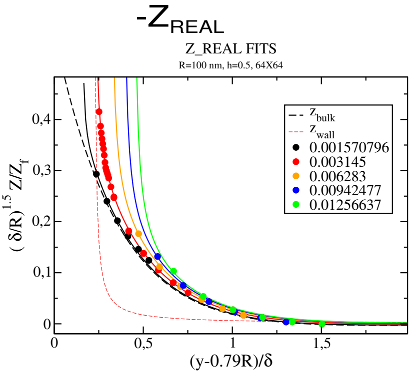
COLOR CODE

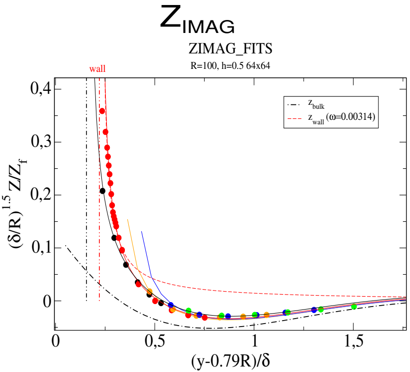
	ω	$f\,[\mathrm{MHz}]$	$\delta[nm]$
• BLACK	0.0015708	17.5	134.4
• RED	0.0031416	35	95
• GREEN	0.0062832	70	67.2
• BLUE	0.0094248	105	54.8
• YELLOW	0.0125664	140	47.5

Impedance scaling with y far from wall



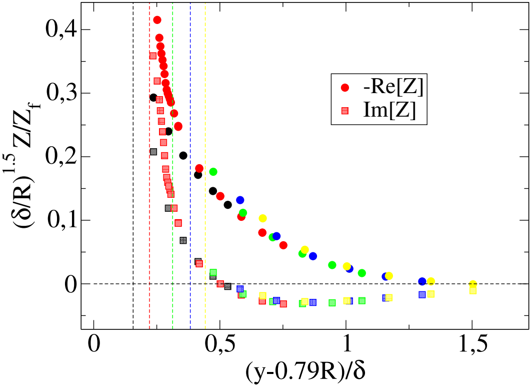
Free liposomes (without DNA)



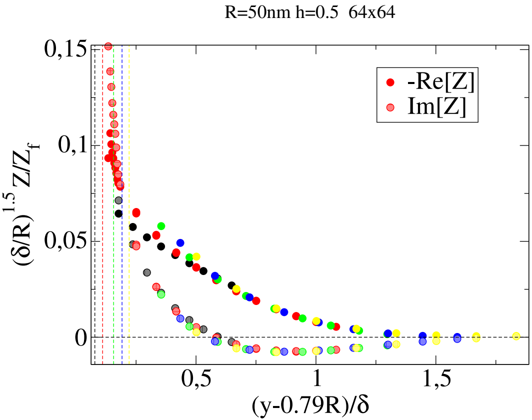


Free liposomes (without DNA)

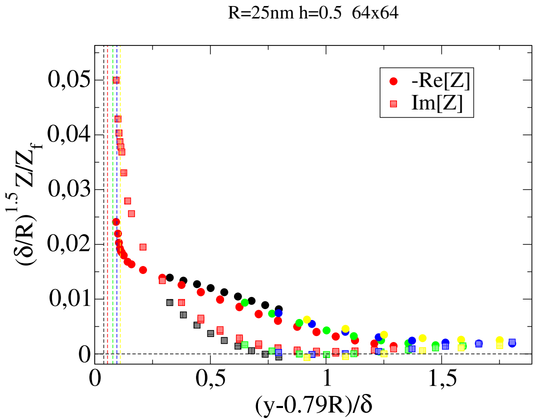
R=100nm h=0.5 64x64



Free liposomes (without DNA)



Free liposomes (without DNA)



Free liposomes (without DNA)

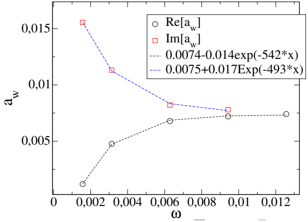
Fits to $Z = Z(y)$, impedance relation with liposome heigth above surface y

$$Z(y) = \left(\frac{R}{\delta}\right)^{1.5}\left[z_w\left(\frac{y-R}{\delta}\right) + z_b\left(\frac{y-0.79R}{\delta}\right)\right]$$

$$z_w(\xi) = \frac{a_w(\omega)}{\xi}$$

$$z_b(\xi) = ae^{-b\xi}\cos(c\xi + d)$$

	$Re[z_b]$	$Im[z_b]$
a	3.51646	0.21900
b	2.20985	1.54217
c	0.115397	1.99065
d	1.40748	0.92781

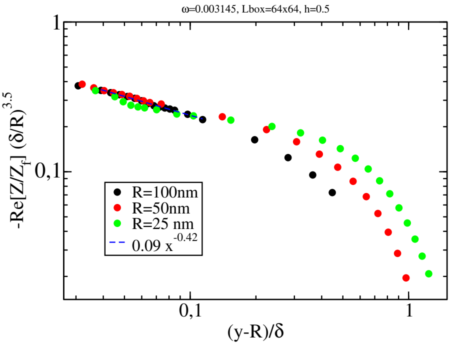


Free liposomes (without DNA)

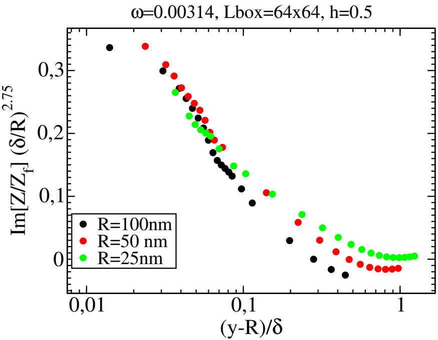
Scaling near the wall

$$\mathrm{AR} = -\frac{2Z_R}{Z_I} \sim R^{0.75} \quad \text{observed in experiments}$$

Real part



Imaginary part



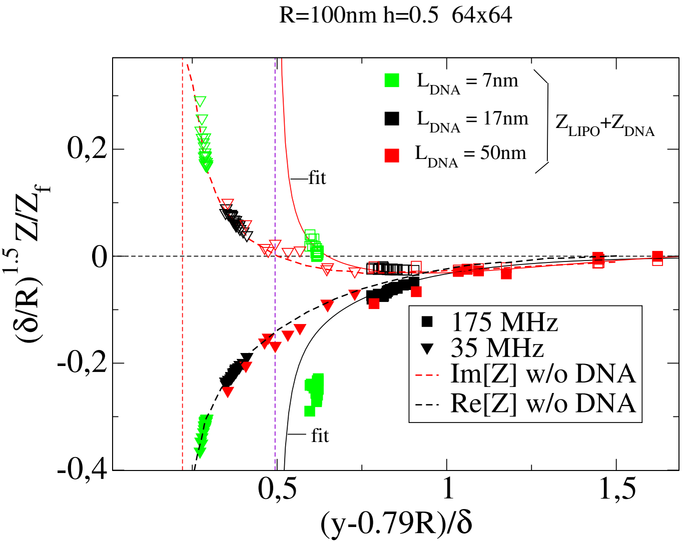
- Z_{LIPO}. DNA constraints liposome motion and alters the perturbative flow,
    - Fluid velocity

$$v = v_{\text{base}} + v_{\text{perturbation}}$$

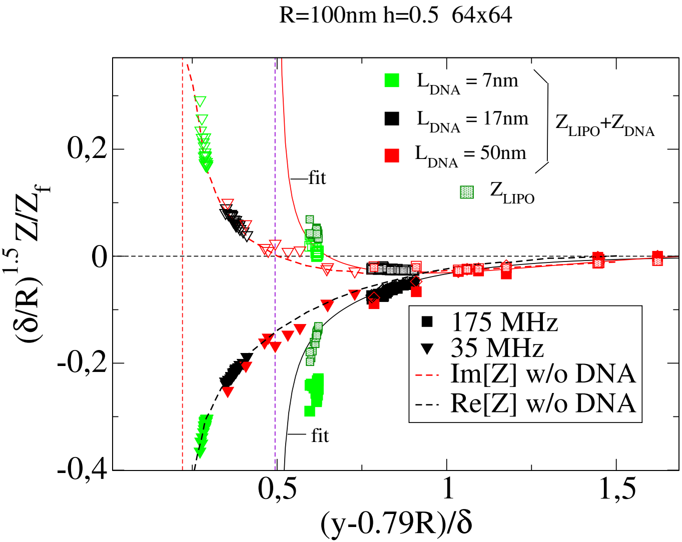
$$\sigma = \eta \left(\frac{\partial v_{\text{pertubation}}}{\partial z} \right)_{wall}$$

- Z_{DNA}: DNA explicit stress on the surface.

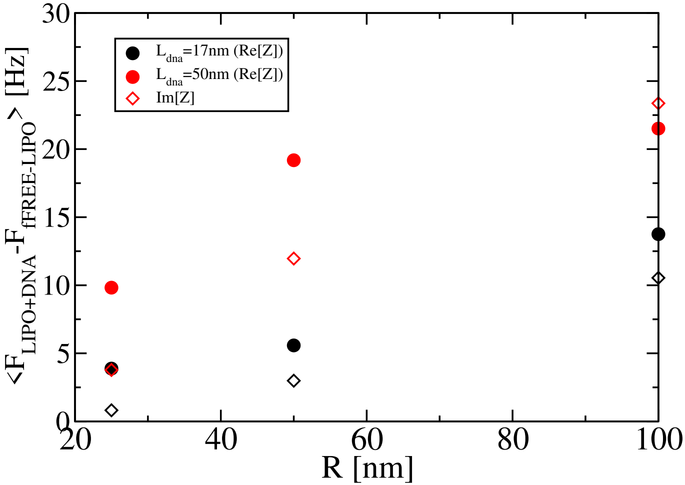
Effect of DNA strand on the impedance



Effect of DNA strand on the impedance

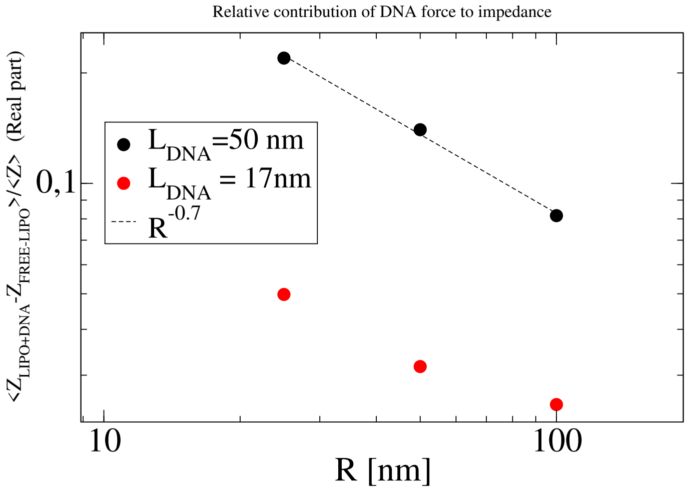


Effect of DNA strand on the frequency shift and impedance



Frequency shift

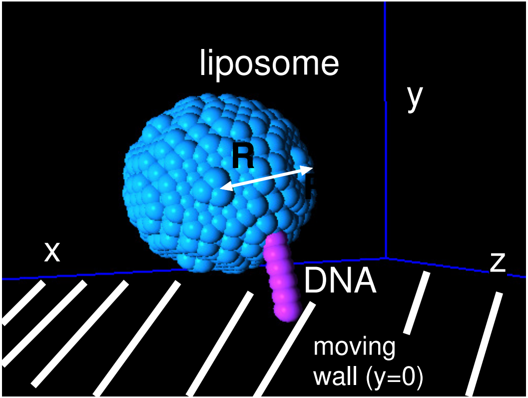
Effect of DNA strand on the frequency shift and impedance



Relative variation

Bending rigidity of the liposome

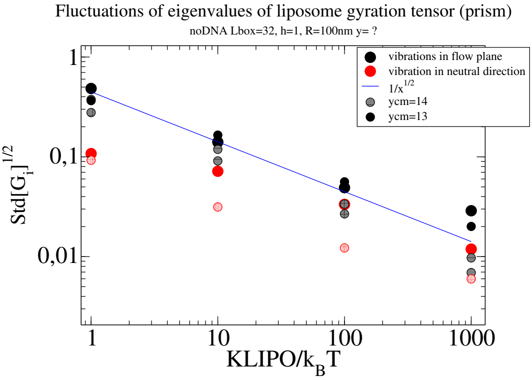
- Modelled using the elastic network model: spring between neighbours liposome beads κ_{LIPO} (the effective κ_{BEND} needs to be calibrated)



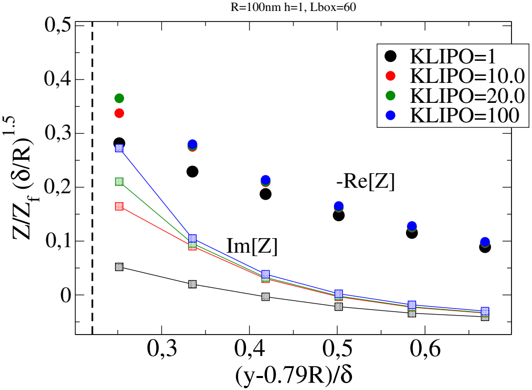
- The liposome membrane vibrates more easily as $\downarrow \kappa_{\mathrm{LIPO}}$

$$\downarrow \kappa_{\mathrm{LIPO}} \implies \downarrow \mathrm{impedance}(Z)$$

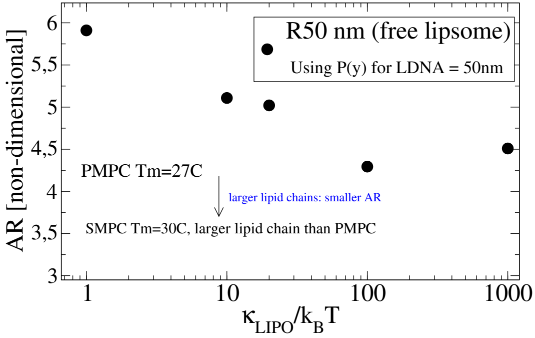
.



- But as $\uparrow \kappa_{\text{LIPO}}$
  frequency shift $Im[Z]$ increases more than dissipation $-Re[Z]$.



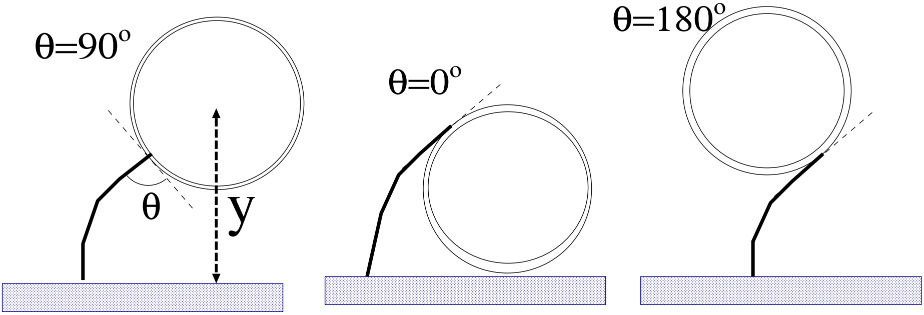
- **Fact:** Liposome bending rigidity increases with lipid length.
- **Experiments:** AR decreases with lipid length (for similar T_m).
- Theory and experiments in qualitative agreement



DNA-Liposome link affects the liposome height y above the surface, $P(y)$.

Modelled by bending energy $\kappa_{\mathrm{DNALIPO}}$

$$P_\theta(\theta) = \exp[-\kappa_{\mathrm{DNA\text{-}LIPO}} (\theta - 90^o)^2]$$

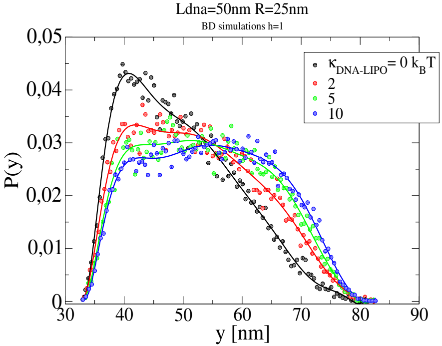


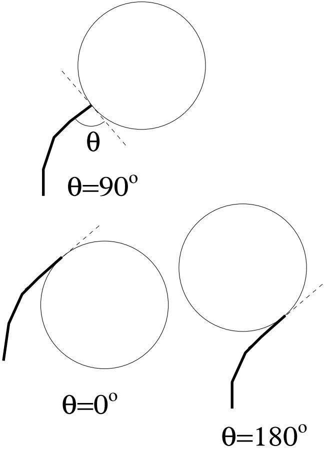
$\kappa_{\mathrm{DNA\text{-}LIPO}} >> k_B T$ $\kappa_{\mathrm{DNA\text{-}LIPO}} \sim 0\, k_B T$

DNA-LIPOSOME molecular link

DNA-Liposome link affects the liposome height y above the surface, $P(y)$.

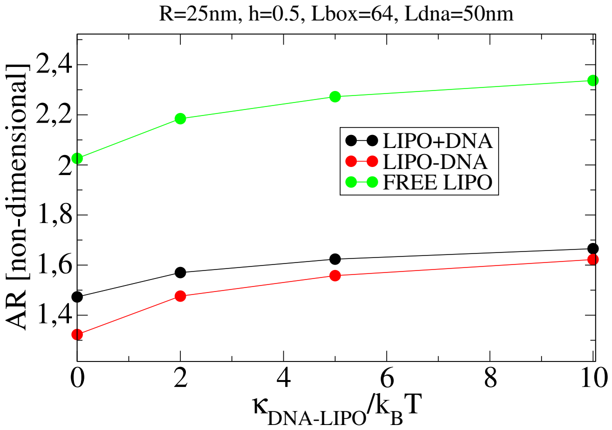
Modelled by bending energy $\kappa_{\mathrm{DNALIPO}}$





DNA-Liposome link affects the liposome height y above the surface, $P(y)$.

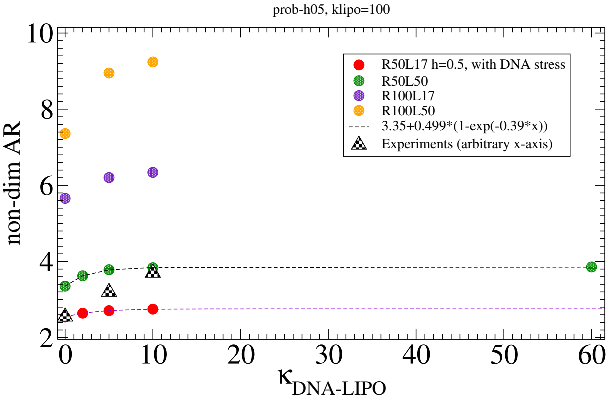
AR saturates at large $\kappa_{\mathrm{DNALIPO}}$



DNA-LIPOSOME molecular link

DNA-Liposome link affects the liposome height y above the surface, $P(y)$.

Just qualitative agreement with experiments; AR increases with $(T - T_m)$ (larger in gellified lipid phase)



Achieved issues

- Reproduce the experimental acoustic ratio ✓
- Predict QCM response at higher frequencies ✓
  - Divergence of AR induced by hydrodynamics (feasible for $R = 100\mathrm{nm}$)
- Predict the relation between lipid coverage and Δf and $\Delta\Gamma$ (Soon)
- Effect of **liposome bending rigidity** ✓
  - Number of carbons in lipid chains (quantitative analisis yet to be done)
  - Membrane in fluid/gel phase (microscopic relation with the **stifness of the DNA-Liposome link**?)
- Propose ways to increase the dissipation and the AR
  - Increase the liposome height $\implies$ increase AR (electric fields?, modifying ph?)
  - Numerical estimation of coverage $\Delta f(\phi)$ permits calibration with larger impedance complex $\implies$ (working with smaller DNA strands)
  - Other ideas ready to be tested

Thanks for your attention