# AZO-modified lipids bilayer and their dynamics associated with optical stimulation: self-organization at a molecular level, mechanical properties and membrane permeability


Cintia A. Menéndez[1], Arash Manafirad[2], Gustavo P. Lemus[1], Anthony Dinsmore[2] and Juan J. de Pablo[1].

1- Pritzker school of Molecular Engineering, The University of Chicago, Chicago IL 60637.

2- Department of Physics, University of Massachusetts, Amherst, Massachusetts 01003, United States.


PRITZKER SCHOOL OF MOLECULAR ENGINEERING
THE UNIVERSITY OF CHICAGO

## 1-Introduction

In this work we have studied the dynamics of membranes with azo-modified lipids from molecular scales (atomistic molecular dynamics simulations) to vesicle scale (experimental work), with the aim of increasing understanding regarding steady states, elastic constants and membrane permeability. In this way, we have found that vesicles dilate and become flexible and much more permeable as a result of trans-to-cis conversion. Therefore, to the best of our knowledge, these are the first studies to look at the membrane dynamics associated with optical stimulation. Finally, it is worth to highlight that this combined studies (computational and experimental work) open new avenues regarding rational molecular design in order to tune response.

## 2- Experimental Results

### 2.1- Quantitative Response: Irradiating azo-PC/DOPC bilayer

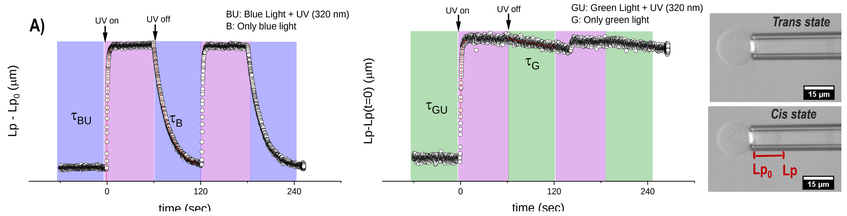


**Figure 2.1-A**: Membrane dynamics of AZO-PC 100% vesicles under optical stimulation explored by means of Micropipette aspiration experiments: vesicles are held in the micropipette at fixed pressure, continuously illuminated with either blue (left) or green (right) and then exposed to UV for short periods (as shown on the plots). The tongue length is plotted here as function of time.

From **figure 2.1-A** and **B** we observed that vesicles dilate as a result of *trans-to-cis* isomerization process. UV causes dilation, blue light causes contraction, and green light does not have any effect. These correlate with the absorbance of azobenzene functional group. Therefore, since the direction of response depends on light frequency, these responses cannot be attribute to simple heating phenomena.

In addition, the equilibrium area changes by up to 10% depending on excitation level and percentage of AZO-PC lipids. The process is reversible and repeatable. It is worth to highlight the rapid rate of response, where the membrane dilatation phenomena occurs within seconds.

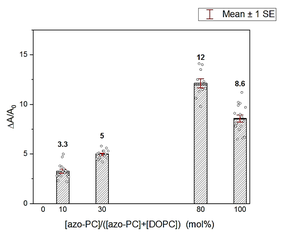


**Figure 2.1-B**: Relative area per lipid increase (consequence of AZO-PC trans-cis isomerization) as function of AZO-PC content for AZO-PC: DOPC vesicles.

### 2.2-Water Permeability Response

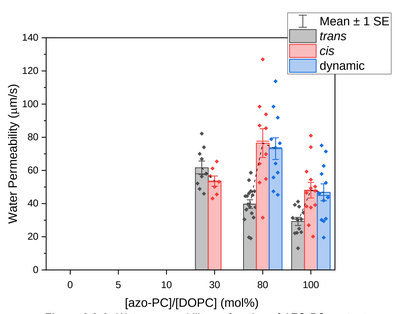


**Figure 2.2-A**: Water permeability as function of AZO-PC content for AZO-PC: DOPC vesicles. .

From **figure 2.2-A** we observed that vesicles become more permeable as a result of trans-to-cis conversion. Water-permeability of cis increases with mol% of AZO-PC lipids until 100% AZO-PC system, where the permeability drops by 40%. Permeability of trans-doped membranes is close to that of DOPC and decreases slightly with added azo-PC. Simultaneous excitation from cis-to-trans and trans-to-cis is qualitatively the same as the response with a constant fraction of cis and trans. Therefore, it seems that the permeability can be viewed as a steady-state average of the two configurations.

### 2.3-Mechanical Response

From **figure 2.3-A** we observed that vesicles become more flexible as a result of trans-to-cis conversion. Under stretching, cis is slightly more flexible than trans and maintains a roughly constant modulus at all compositions. Trans increases slightly with composition, and then shoots up by 3.5x greater at 100% azo-PC.

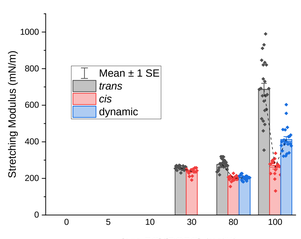


**Figure 2.3-A**: Streching modulus as function of AZO-PC content.

## 3- Atomistic Molecular Dynamics Simulations

Molecular dynamics simulations (MD) were performed using the all atom general Amber force field (GAFF) and the Amber force field for lipids, Lipid17. The initial system size was 64 lipids per leaflet. The distance between the Z edge of the water box and the closest atom of the solutes was at least 20 Å (roughly 90 water molecules per each lipid). NPT production simulations were conducted for each system (different AZO-PC: DOPC mixtures) .

### 3.1- Quantitative Response: AZO-PC trans-cis isomerization

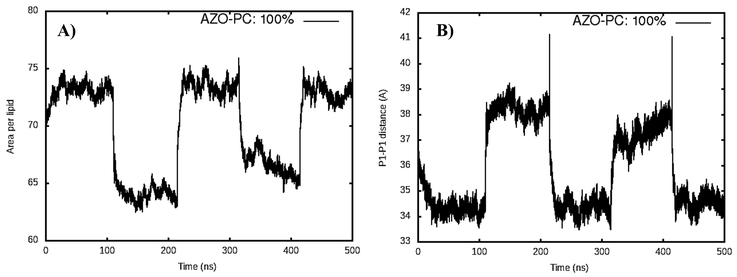


**Figure 3.1**- Membrane dynamics of AZO-PC 100% bilayer under trans-cis isomerization. **A**: Area per lipid in Å$^2$ as a function of time (ns). **B**: Membrane thickness (Å), determined as the average distance among phosphate atoms belonging to different leaflet, as function of time (ns)

We have applied a torque force over azo-bencene in order to achieve the trans-to-cis isomerization for such functional group.

From **figure 3.1-A** we observed that simulations are able to reproduce the system behavior qualitatively and quantitatively. In addition, from **figure 3.1-B**, we observed an exactly inverse response for membrane thickness as a consequence of the trans-to cis conversion. **Figure 3.1-C** included an schematic representation of the aforementioned.

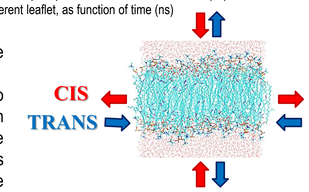


**Figure 3.1-C** Schematic representation of structural properties for AZO-PC 100% system under trans-cis isomerization.

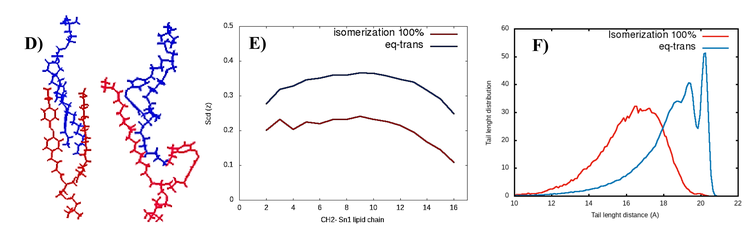


**Figure 3.1-D** Schematic representation of AZO-PC lipids in the trans steady state (left) and cis steady state (right). **E)** Scd order parameter for the AZO-PC aliphatic chain. We observed that the bilayer is much more less order in the cis state. **F)** Tail length (Å) frequency for the AZO-PC lipids aliphatic chain.

### 3.2-Water Permeability Response

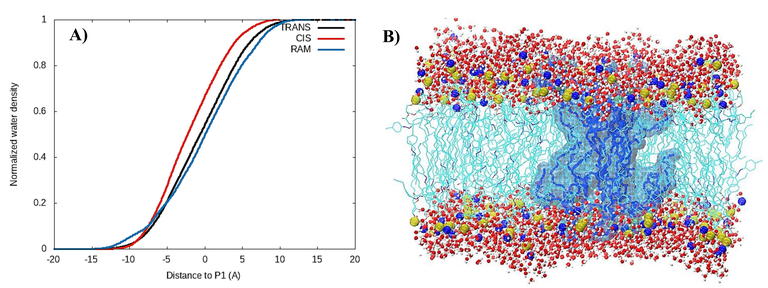


**Figure 3.2-A** Normalized Water density distribution evaluated along a direction normal to the membrane surface. Origin was set to the average position of phosphate atoms in the same leaflet, in this way, negative values correspond to the inner bilayer region and positive values to the outer region and bulk solution. **Red**: AZO-PC 100% lipid bilayer, lipids on cis state; **Black**: AZO-PC 100% lipid bilayer, lipids on trans; **Blue:** AZO-PC 100% lipid bilayer, 80:20 trans:cis, lipids on cis state performed a domain like configuration. **B)** Schematic representation of 80:20 trans:cis AZO-PC lipids. Color: blue for N atoms, red for O atoms, white for H atoms, cyan for C atoms, yellow for P atoms. VMD Representation method: licorice + surface in shaded blue was used for AZO-PC lipids on the cis state; lines was used for AZO-PC on trans state; VDW was applied to nitrogen and phosphate atoms belonging to the head group. CPK was used for water molecules.

### 3.3-Mechanical Response

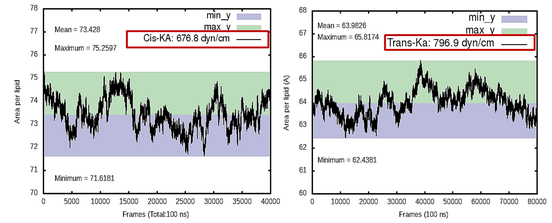


**Figure 3.3-** Area per lipid in Å$^2$ as a function of time for AZO-PC bilayer on cis state (left) and AZO-PC on trans state (right).

Membrane compressibility constant, $K_A$, were theoretically determined taking into account area per lipid averaged values and their mean squared fluctuations. Whereas we observed good agreement between $K_A$ values and the experimentally determined stretching modulus for the membrane on trans state, exist a significant discrepancy between both elastic constant for AZO-PC on cis state. Finally, such disparity could be due to system size.

### 3.4- Relative energies for AZO-PC on cis and trans states:

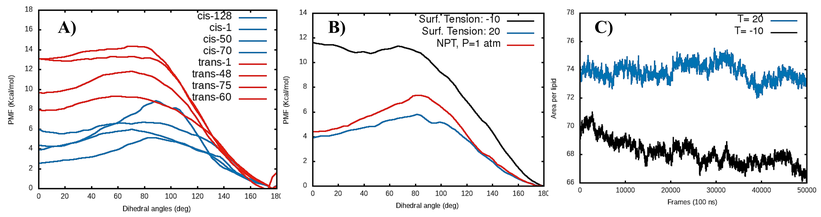


**Figure 3.4- A)** Free energy as function of azo-bencene dihedral angle, where dihedral angles equal to 0 represent cis state, while 180 apply for trans state. Red: lipids belonging to the environment are in trans state (this means that all lipids in the membrane are in Trans state except the 'target' molecule). Blue: lipids belonging to the environment are in cis state. We have performed this studies over 4 different 'target' molecules in each case. B) Free energy as function of azo-bencene dihedral angle, where dihedral angles equal to 0 represent cis state, while 180 apply for trans state. Red: lipids belonging to the environment are in trans state and there is no external surface tension applied. Black: lipids belonging to the environment are in trans state and there is an external surface tension applied of -10 mN/m. Blue: lipids belonging to the environment are in trans state and there is an external surface tension applied of 20 mN/m **C**: Area per lipid in Å$^2$ as a function of time (ns). Blue: surface tension was set to 20 mN/m. Black: surface tension was set to -10 $\mathrm{mN/m}$.

In order to determine the impact of membrane global properties over the free energy profile for trans-to-cis isomerization process, we performed a series of Umbrella Sampling simulations where the azo-bencene dihedral angle was restricted at different values between 0 (cis) and 180 (trans). The free energy difference between cis and trans goes from roughly 12 kcal/mol to 4 kcal/mol when the lipids belonging to the environment are in Trans or Cis state. In addition, the free energy difference between cis and trans goes from roughly 12 kcal/mol to 4 kcal/mol when surface tension goes from -10 to 20 mN/m (in both cases all the lipids belonging to the environment are in trans state).

In summary, there is a clear impact of the membrane global properties over Cis-Trans relatives energies, where lower area per lipid destabilized Cis state and increase the energy barrier going from trans to cis configuration.

## 4- Summary

In this work we have studied the dynamics of membranes with azo-modified lipids from molecular scales (atomistic molecular dynamics simulations) to vesicle scale (experimental work), with the aim of increasing understanding regarding steady states, elastic constants and membrane permeability.

In summary, we have characterized the membrane dynamics associated with optical stimulation. In this regard, it is important to highlight the qualitatively and quantitatively agreement between simulations and experimental work.

SCIENCE TO SHAPE THE FUTURE OF THE ARMY — ARMY RESEARCH OFFICE — 1951

THE UNIVERSITY OF CHICAGO | Research Computing Center

MURI — Stimuli and response. Inspired by nature.