

## **AFM STUDIES ON BILAYER INTERACTIONS AND FUSION**

Midhat H. Abdulreda\*, Vincent T. Moy

Department of Physiology and Biophysics, University of Miami Miller School of Medicine, 1600 NW 10<sup>th</sup> Ave, Miami, Florida 33136, USA ([ab\\_m@msn.com](mailto:ab_m@msn.com))\*

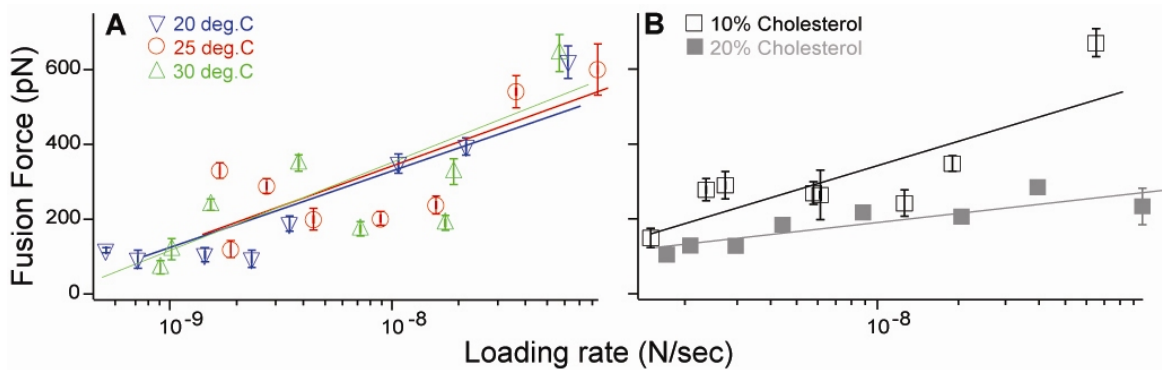
**INTRODUCTION.** Reconstituted membrane model systems have been used extensively to investigate the structure of bilayers and the dynamics of their interactions and fusion. Fusion is a physiologic process necessary for organism homeostasis. In humans, basic physiologic processes such as exocytosis and endocytosis involve membrane fusion. Conversely, certain pathologic conditions may also be due to fusion or defects in fusion mechanisms. In essential hypertension, the accumulation of neutrophils followed by release of proinflammatory factors from primary vesicles is an important factor in vascular damage (1). In cancer immunotherapy, fusion cell-hybrid vaccines provide a promising therapeutic approach with high target specificity and minimal cytotoxicity (2,3). In this study, we report on a membrane system that uses the atomic force microscope (AFM) to investigate lipid bilayer fusion.

**METHODS.** Chicken egg L- $\alpha$ -phosphatidylcholine (EggPC) vesicles were prepared by detergent depletion. Bilayers were formed by vesicle adsorption and fusion to hydrophilic glass surfaces. AFM measurements of forces required to induce fusion between two apposed bilayers were carried out at different temperatures and lipid compositions.

**RESULTS.** Estimates of force required to generate fusion at different scan velocities were obtained in different conditions. A clear dependence of the fusion force to the loading rate or scan velocity was observed. Forces collected in EggPC alone at 20, 25, and 30°C, and EggPC containing 10% cholesterol (25°C), ranged from ~150 to ~700pN between loading rates of ~517 and ~84500pN/sec (Figure1). When 20% cholesterol was added, fusion forces were reduced (25°C); they ranged from ~100 to ~450pN for the same loading rate range (Figure1B). Calculated fusion time ranged from the sub-milliseconds to ~5 msec between scan velocities of 6.79 and 0.136 $\mu$ m/sec.

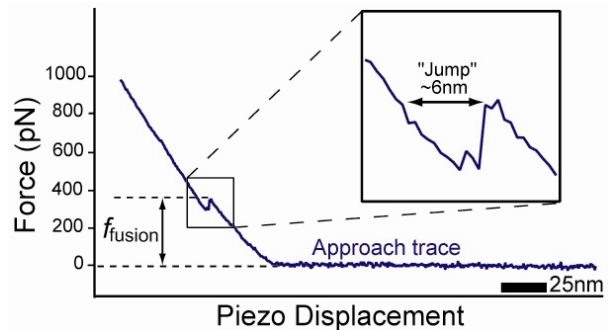
**DISCUSSION.** Our data demonstrate that this novel approach offers the temporal and spatial resolution and sensitivity to detect fusion (Figure2). It enabled us to estimate fusion time which was well within reported limits (4,5). We characterized the energy barrier for the fusion reaction in the specified experimental conditions. A significant shift

in the energy landscape was observed in EggPC/20% cholesterol (Figure1B) showing a decrease of  $0.73KT$  in the fusion activation potential compared to that in EggPC alone at 25°C. In conclusion, we established a membrane system that allows direct measurement of interaction forces between lipid bilayers in varied experimental conditions.



**Figure1:** (A and B) Plots of fusion force vs. loading rate showing force dependence on the loading rate. Force measurements were carried out, at different loading rates/scan velocities, between bilayers prepared from EggPC alone at 20, 25, and 30°C (A), and EggPC containing 10 and 20% cholesterol (B).

**Figure2:** AFM force scan during approach showing the increase in force as the lipid bilayers are compressed against one another. Force increased from zero to ~400pN ( $f_{\text{fusion}}$ ) before a “jump” was observed. The jump is due to the sudden movement of the AFM tip toward the substrate. The jump distance (inset) is on the order of a lipid bilayer thickness and is interpreted as the result of the coalescence of the two apposed bilayers into one (i.e. fusion).



## REFERENCES

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