ESI Comments S1

Interpretation of FRET Data and relation to Longitudinal Waves

A mechanical agitation of the air-water interface always leads to propagation of water waves. In the experiments of Mann and Hansen and later Lucassen, they justify that the observed perturbation are localized at the lipid interface by observing that the propagation characteristics depend on the interfacial elasticity. Similarly the primary proof here that the observed pulses are localized at the lipid interface and not just water waves (capillary waves) comes from the observation that the propagation velocity slows down in the transition region and has a minimum where the compressibility has a maximum. Moreover since we measure these pulses in different states, while keeping all other measurement parameters constant the difference in the signal in different state can only be attributed to change in state. In that sense given we don't see any signal in the liquid expanded state, while a strong sharp pulse in the transition state for the same strength of excitation, is already a kind of control one would look for. In addition to this capillary pulses only get weaker on increasing surface pressure where as we see an increase in amplitude on increasing pressure. The argument with the FRET measurements is secondary and is based on the microscopic interpretation of FRET data. Still within this microscopic picture a discussion on its interpretation is helpful. A simple mathematical treatment addresses the particular concerns one can have regarding FRET artifacts due to tilting of the individual dye molecules. Forster energy transfer is a function of the orientation of the donor transition dipole relative the acceptor dipole and the distance between them. This can be represented mathematically as $f(r, \overleftarrow{\mu_d} . \overleftarrow{\mu_a})$. The curvature of the interface can affect the relative angle between the dipoles only when the wavelengths are comparable to the typical Forster distances between the dye molecules. Here, for an order estimate of the minimum wavelengths in the system, the maximum frequency of ~

400Hz at a minimum velocity of ~40cm/s suggests a λ ~ 1mm which is >> than Forster radius which is of the order of 10Å. Therefore the curvature induced by the water waves underneath the monolayer should not affect the FRET based measurements itself. But the effect due to independent tilting of the individual acceptor and donor dye molecules and that the corresponding intensity changes might also produce a FRET artifact needs to be ruled out. The following generalized analysis of the FRET parameter addresses these concerns, where we only look at the effect of tilting of individual dipoles with a general orientation due to a capillary wave of wavelength >> the Forster distance. The FRET parameter has been defined as;

$$\theta = \frac{I_d}{I_a}$$

where …

$$dln\theta = dlnI_d - dlnI_a$$

$$\frac{d\theta}{\theta} = \frac{dI_d}{I_d} - \frac{dI_a}{I_a}$$

$$\frac{\Delta\theta}{\theta} = \frac{\Delta I_d}{I_d} - \frac{\Delta I_a}{I_a}$$

Let φ be the local tilt of the interface. Instead of rotating the interface one can simplify the calculations by rotating the optical field by the same angle. Therefore in the absence of compression and any relative changes in the angle between donor and acceptor,

$$I_d = E\mu_d \cos{(\alpha + \varphi)}$$

$$I_a = E\mu_a \cos{(\beta + \varphi)}$$

$$\frac{\Delta\theta}{\theta} = \frac{\cos\,(\alpha+\varphi)}{\cos(\alpha)} - \frac{\cos\,(\beta+\varphi)}{\cos(\beta)}$$

$$\frac{\Delta\theta}{\theta} =$$

$$\frac{\cos\,(\alpha).\cos(\beta).\cos(\varphi) - \sin(\alpha).\sin(\varphi)\,.\cos(\beta) - \cos\,(\alpha).\cos(\beta).\cos(\varphi) + \sin(\beta).\sin(\varphi)\,.\cos(\alpha)}{\cos(\alpha)\,.\cos\,(\beta)}$$

$$\frac{\Delta\theta}{\theta} = \frac{-\sin(\alpha).\sin(\varphi)\,.\cos(\beta) + \sin(\beta).\sin(\varphi)\,.\cos(\alpha)}{\cos(\alpha)\,.\cos\,(\beta)}$$

$$\frac{\Delta\theta}{\theta} = \frac{\sin(\varphi)\,.\cos(\beta-\alpha)}{\cos(\alpha)\,.\cos\,(\beta)}$$

The expression gives a generalized expression for the effect of optical field's orientation on the FRET parameter defined here. For capillary waves φ would be very small giving

$$lim_{\varphi\to 0}\frac{\Delta\theta}{\theta} = 0$$

Therefore, such artifacts although present, would be negligible.

Finally the aim is not to avoid the excitation of capillary (transversal) modes, but to observe the solitary phenomenon at the interface due to nonlinear compressibility. The decoupling achieved by our detection method (in measurement only, capillary pulses still exist weather we chose to look at them or not) helps us to better appreciate the relation between nonlinear compressibility and the solitary phenomenon.