In the past several years our group has been exploring protein energy landscapes in pigment-protein complexes involved in photosynthesis. These complexes offer a unique opportunity to explore native protein environments using optical spectroscopy methods, as chromophores are built into them by nature, without any extraneous manipulations that could potentially alter the structure or dynamics of the protein. Single Molecule (or single complex) Spectroscopy has recently been a technique of choice for studying spectral dynamics in photosynthetic complexes. However, here I am going to demonstrate that Spectral Hole Burning (SHB) is capable of providing additional or competing information. In particular, most of the spectral shifts observed in single complex experiments are in fact light-induced (and not occurring anyway whether one observes them or not) and thus constitute SHB on a single-molecule level [1]. Analysis of the hole broadening allows us to claim that fast-small shift spectral dynamics in the LH2 complex is specific only to single-complex scenarios and not to the bulk sample. And so on.

Inspired by these results we undertook a detailed SHB study of spectral dynamics in several photosynthetic complexes, with the main focus on the CP43 antenna complex [2] of Photosystem II and dimeric Cytochrome b₆f [3]. We also developed a unified approach to modeling SHB and spectral hole recovery, at fixed (burn) temperature and upon thermocycling. This approach relies on the argument that in the presence of “spectral memory” (holes recovering mostly due to burnt systems returning to the pre-burn configuration) the barrier distributions encoded into the non-saturated spectral holes and manifesting during the hole recovery differ from the full true barrier distributions. These partial barrier distributions are vastly different for different shapes of the true full distributions, and one can easily distinguish their manifestations. Quantitatively, all complexes we have explored so far exhibit similar barrier distribution parameters, distinct from those of some simple organic glasses. Qualitatively, however, barrier distribution shapes show great variability. Unlike in CP43 [2], the distributions of barriers between protein sub-states involved in light-induced conformational changes (SHB) in Cytochrome b₆f are more likely glass-like $\sim V^{0.5}$ ($V$ is the barrier height), and not Gaussian. There is a high degree of correlation between the heights of the barriers in the ground and excited states in the individual pigment-protein systems, as well as nearly perfect spectral memory. Both spectral hole burning and recovery are due to phonon-assisted tunneling associated with the increase of the energy of a scattered phonon. As the latter is unlikely for simultaneously both the hole burning and hole recovery, proteins must exhibit a NPHB mechanism involving diffusion of the free volume towards the pigment. Entities involved in the light-induced conformational changes are characterized by $md^2$
value of about $1.0 \cdot 10^{-46}$ kg·m$^2$. Thus, these entities are protons or, alternatively, small groups of atoms experiencing sub-Å shifts. However, explaining all SHB and recovery data simultaneously, employing just one barrier distribution, requires a drastic decrease in the attempt frequency to about 100 MHz. This decrease may occur due to cooperative effects.


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**Monday**

**September 28, 2015**

Starts at 12:15 PM  
Coffee at 12:00 PM  
Physics Conference Room, SB B326