Studying the self-association and distribution of annexin A4 at the plasma membrane utilizing brightness analysis and photoactivated localization microscopy

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Annexins make up a pervasive, structurally related class of proteins found in most eukaryotic species. They are characterized by a highly conserved core consisting of four (or eight, in the case of annexin A6) calcium binding domains of approximately 70 residues known annexin repeats [1]. A shared trait of almost all annexins is their ability to bind negatively-charged phospholipids in a calcium dependent manner. These proteins are thought to be involved in a myriad of cellular functions such endo/exocytosis, actin configuration, signaling, and plasma membrane repair [2, 3]. However, their precise mechanistic roles in many of these processes has yet to be completely elucidated.

The mammalian annexin family member A4 (AnxA4) is found primarily in epithelial cells and has been shown to self-assemble into ordered 2D arrays on model membranes in vitro, a characteristic that is believed to be a key factor in the proposed roles that this protein may play in processes that require reorganization of membrane [4]. The self-association of AnxA4 was tracked in living cells utilizing FRET microscopy while FRAP experiments showed that once bound to cellular membrane, AnxA4 showed very little mobility and restricted the diffusion of other membrane components [5].

Molecular brightness analysis has allowed us to quantitatively characterize the self-association of AnxA4 in living cells and visualize the trimeric form of AnxA4, which we propose is the predominate mobile form of the protein at the membrane. PALM revealed a heterogeneous distribution, suggesting a micro-level of organization beyond the self-association into trimers, although any higher order aggregates are likely to be largely immobile. This assembly of AnxA4 may serve to stabilize membrane domains, perhaps creating signaling platforms and/or serving as a functional scaffold for other membrane bound proteins.