S1d1-7
DNA and Estrogen Receptor Interaction Revealed by the Fragment Molecular Orbital Method
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Estrogen receptor (ER) families belong to a superfamily of ligand-induced nuclear steroid hormone receptors. These families repress/enhance transcription by identifying and binding as a dimer to specific DNA target sequences: estrogen response element (ERE). DNA binding domain (DBD) of ER controls both the DNA binding and its dimerization. The sequence specific DNA binding is found to be due to the contact interaction between the bases of DNA-ERE and three amino acids called as “P-box” in ER-DBD [1]. ER-DBD can not make the stable DNA-binding as monomer, but as dimer [2]. We apply the FMO method [3] to analyze the DNA binding of ER-DBD. Analysis of the interfragment interaction energy (IFIE) revealed that each ER-DBDs strongly bind to the phosphate groups of -2T:A and 2T:A of DNA-ERE. These two interactions can fix the position and direction of the ER-DBD dimer, although the single interaction of the ER-DBD monomer can not fix the direction. Dimerization is indispensable to stable DNA-binding of ER-DBD. The sequence specific DNA binding of ER-DBD was examined by IFIEs. The sequence specific interaction is mainly due to the electrostatic interaction although the contact interactions between the bases of DNA-ERE and P-box also exist. References [1] K. Umesono, R. M. Evans, Cell, 57, 1139 (1989), [2] M. A. L. Eriksson, L. Nilsson, Eur. Biophys. J., 28, 102 (1999), [3] K. Kitaura et al., Chem. Phys. Lett., 312, 319 (1999).

S1e1-1
Evolutionary analysis and molecular dissection of caveolae biogenesis
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Caveolae are striking morphological features of the plasma membrane of mammalian cells. Caveolins, the major proteins of caveolae, play a crucial role in the formation of these invaginations of the plasma membrane but the precise mechanisms involved are only just starting to be unravelled. Modeling of caveolin-membrane interactions have provided new insights into how caveolin-lipid interactions could generate the unique architecture of the caveolar domain. These studies have been complemented by analysis of caveole formation in caveolin-1 null fibroblasts. Evolutionary analysis shows high conservation of caveolin-1 in vertebrates. In invertebrates caveolins are conserved in C. elegans and the honey bee, Apis mellifera. In view of the high conservation of caveolin primary sequence in evolution, we investigated whether the role of caveolins in caveole formation was similarly conserved. C. elegans caveolin is efficiently transported to the plasma membrane but does not generate caveoleae in a mammalian or insect model system. In contrast, all vertebrate caveolins tested form caveoleae. Using C. elegans caveolin as a template to generate hybrid caveolin-constructs, formation of caveoleae and trafficking of caveolin were analysed using multiple assay systems. We now define the domains of caveolin required for caveoleae formation and pinpoint specific regions required for membrane remodeling. This leads to a new model for caveoleae formation and novel insights into the role of caveolin in evolution.

S1e1-2
Plasma membrane signaling platforms
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The spatial organization of signaling complexes on the plasma membrane provides a theoretical additional level of control over signal output. We have used electron microscopy coupled with spatial statistics and mathematical modeling to map the microlocalization of H+, K-ras, N-ras and lipid raft marker proteins attached to intact plasma membrane sheets. We can show that the three different isoforms of Ras, H-, N- and K-ras operate in spatially discrete microdomains and that H- and N-ras undergo GTP-regulated movement between different types of microdomain. These results strongly support our general hypothesis that differential membrane microlocalization accounts for biological differences between the highly homologous Ras isoforms. We have investigated in detail the nature of these different microdomains including their dependence on the actin-cytoskeleton and cholesterol and the structural determinants on Ras that regulate microlocalization and can propose a mechanism whereby GTP-loading regulates H-ras lateral segregation. On a more general level our studies yield interesting insights into what may be common principles that regulate the surface organization of lipidated proteins. More recently, we have explored computationally and experimentally the role of plasma membrane microlocalization on the regulation of signal output from the Raf/MEK/Erk signalling pathway and designed simulators of protein diffusion on the plasma membrane to investigate the possible influence of lipid rafts on protein-protein interactions.

S1e1-3
Active segregation of nanoscale clusters of GPI-anchored proteins in living cell membranes: implications for rafts and endocytosis
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Glycosylphosphatidylinositol (GPI)-anchored proteins at the cell surface of living cells form cholesterol-sensitive nanoscale clusters. These are composed of at most four molecules and accommodate diverse GPI-anchored protein species (1). In conjunction with an analysis of the statistical distribution of these clusters, our observations suggest an active mechanism for lipid-dependent clustering of GPI-anchored proteins. Recent experiments from our laboratories suggest that these nanoclusters in turn are spatially segregated in membranes of living cells in collaboration with the actin cytoskeleton. These studies support a picture wherein these larger-scale domains are induced from pre-existing nanoscale complexes. These studies argue for active segregation of lipid components into functional domains, capable of facilitating endocytosis of GPI-anchored proteins (2). References (1) P. Sharma et al., Cell 116, 577 (2004); R. Varma and S. Mayor., Nature 394, 798 (1998); (2) S. Mayor, M. Rao, Traffic 5, 231 (2004)