Asymmetric quantum dots (QD) provide non-bleaching imaging probes yielding orientation-dependent optical signals from individual cell surface proteins. The Invitrogen QD655 measures 12.8 x 5.8 nm and exhibits an initial fluorescence anisotropy of about 0.1. Calculated rotational correlation times for rotation in water about the particle short and long axes, 0.27 μs and 0.18 μs respectively, suggest that the nanoparticle can probe molecular rotation down to the μs timescale. We have used QD655 conjugated to A2 DNP-specific IgE to explore slow rotation of the Type I Fcε receptor on variously-treated RBL-2H3 cells. We excite fluorescence from cell-bound QD with illumination polarized at 45 deg and use an image splitter and an EMCCD camera to record 100 fps image sequences containing simultaneous x- and y-polarized sub-images in each frame. Blinking of spots verifies imaging of individual QDs. Time-dependent fluorescence from regions containing individual QDs in image pairs is extracted and the time-autocorrelation function for polarization fluctuations calculated with correction for blinking-induced intensity fluctuations. Individual QDs exhibit peak polarization fluctuations with an RMS amplitude of ~0.04 which decay slowly over 100-300 ms. This behavior is exhibited on untreated cells and on cells treated with polyvalent DNP-BSA, methyl-β-cyclodextrin or cytochalasin D or fixed with paraformaldehyde. Previous time-resolved phosphorescence anisotropy (TPA) measurements showed substantial limiting anisotropies for these receptors, indicating that complete receptor orientational randomization required times beyond the 1 ms timescale of TPA experiments. Whether the slow decay observed in the present experiments represents the hindered receptor rotation implied by TPA results remains to be determined. Work on faster techniques aimed at single molecule rotation measurements in the microsecond timescale is underway. Supported by NSF grants MCB-1024668 and CHE-0628260.

3319-Pos Board B180
Optimal Diffusion Coefficient Estimation in Single-Particle Tracking
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Despite being widely and successfully applied to study transport in biophysical systems, the statistics of single-particle tracking data are only partially understood even in the simplest case of free diffusion. Here, we present the correct distribution of measurement results for a freely diffusing particle observed with localization error sigma and a finite camera integration time. We derive the fundamental limit (Cramer-Rao bound) on the error in estimating the diffusion coefficient D from such data, represented by a simple formula that can be applied to judge whether experimental data contains enough information to determine D. Two recently developed estimation procedures, a maximum-likelihood estimator [A. J. Berglund, Phys. Rev. E 82, 011917 (2010)] and an optimized least-squares fit to the mean-square displacement [X. Michalet, Phys. Rev. E 82, 041914 (2010)], are shown through numerical simulations to be nearly optimal in extracting D and sigma. These results can be applied to understand when D can be determined with reasonable confidence from short trajectories or in high-noise scenarios.

3320-Pos Board B181
The Nature of Constitutive Activation of HER2 at the Single Molecule Level
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HER2 is a highly active kinase that is distinguished from the other HER family members in that no ligand has been identified to directly bind the receptor. HER2 can be trans-activated by forming complexes with other receptors, but it also exhibits constitutive activation when it is over-expressed. In fact, this ligand-independent activation plays an important role in driving the growth of HER2 amplified tumors. To gain further mechanistic understanding of the constitutive HER2 activation, we performed single molecule tracking studies of HER2 and its mutants on the living cell membrane and developed new analysis tools. From these studies, we found that activation of HER2 is less regulated by the structural features of its ectodomain than that of EGFR as we previously demonstrated. Rather, HER2 activation may be largely related to its interaction with membrane subdomains, which in turn modulates its local density. Indeed, we found that cholesterol content and distribution pattern on the membrane altered the diffusion dynamics of HER2 and its phosphorylation status. The modulation of HER2 activation by cholesterol may have relation to tumor cell response to trastuzumab.

Auditory Systems

3321-Pos Board B182
An Active Mechanism for Signal Detection in the Mammalian Ear
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The ear's exquisite sensitivity, sharp frequency tuning, and broad dynamical range result from an active process known as the cochlear amplifier. Although outer hair cells play a central role in cochlear amplification, their mechanism of action remains uncertain. In non-mammalian ears hair bundles, the sensory organelles of hair cells, can perform mechanical work and account for the active process in vitro. Although active hair-bundle motility also occurs in the mammalian cochlea, a membrane-based piezoelectric effect known as electromotility is required for amplification in vivo.
We present a physical description of a segment of the mammalian cochlea that, by amplifying the power of an external input, acts as a sensitive, sharply tuned, and nonlinear signal detector. This model, which is based on the known physiology and morphology of the inner ear, couples active hair-bundle motility and electromotility through the geometric arrangement of hair cells in the organ of Corti. The model displays quantitative agreement with in vivo measurements of basilar-membrane movements. The model's predictions of the vibration pattern at different input levels and of the differences between wild-type and electromotility-deficient mice accord qualitatively with experimental observations.
We demonstrate how internal sources of energy in this system enhance the signal-detection properties of the mammalian ear in comparison with those of a passive cochlea. Although the hair bundle provides nonlinearity and feedback sufficient for detecting low-frequency signals in non-mammals, electromotility produces additional feedback that allows the mammalian cochlea to detect high-frequency inputs. This work supports an evolutionary scenario in which high-frequency hearing in mammals arose by supplementing a preexisting auditory amplifier with an additional source of mechanical energy.

3322-Pos Board B183
Otoacoustic Emission through Waves on Reissner's Membrane
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The cochlea not only acts as a detector of sound but can also produce tones itself. These otoacoustic emissions are a striking manifestation of the mechanical active process that sensitizes the cochlea and sharpens its frequency discrimination. It remains uncertain how these mechanical signals propagate back to the middle ear, from which they are emitted as sound. Although reverse propagation might occur through waves on the basilar membrane, experiments suggest the existence of a second component in otoacoustic emissions. We have combined theoretical and experimental studies to show that mechanical signals can also be transmitted by waves on Reissner's membrane, a second elastic structure within the cochea. We have developed a theoretical description of wave propagation on the parallel Reissner's and basilar membranes and its role in the emission of distortion products. By scanning laser interferometry we have measured traveling waves on Reissner's membrane in the gerbil, guinea pig, and chinchilla. The results accord with the theory and thus support a role for Reissner's membrane in otoacoustic emission.

3323-Pos Board B184
Magnetic Nanoparticles as Mechanical Actuators of Inner Ear Hair Cells
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Mechanical sensation by the auditory system is performed by hair cells, named in reference to the stereociliary bundles that protrude from their apical surface. They have been shown to exhibit active motility under in vitro conditions and a highly nonlinear dynamic response. To explore their mechanical properties, we developed a technique that utilizes paramagnetic beads to actuate the stereocilia. Steady-state deflections were imposed and seen to strongly affect the dynamic state of the bundle, inducing a transition from multi-mode to single-mode state, as well as the crossover from the oscillatory to the quiescent state. Numerical simulations capturing the behavior near the critical points will be presented.

3324-Pos Board B185
Effect of Non-Steroidal Anti-Inflammatory Drugs on the Outer Hair Cell Protein Prestin
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