104-Plat
Ultrastructure of Dynactin Complex: A Mediator of Cytoplasmic Dynein
Salkat Chowdhury1, Stephanie A. Ketcham1, Trina A. Schroer2, Gabriel C. Lander1.
1Integrative Structural and Computational Biology, The Scripps Research
Institute, La Jolla, CA, USA, 2Department of Biology, Johns Hopkins
University, Baltimore, MD, USA.

The ~1.0 megadalton dynactin complex interacts with cytoplasmic dynein to
increase its processivity during minus-end-directed transport of cargo along mi-
crotubules (MT). The detailed molecular understanding of how dynactin regu-
lates dynein motility is still elusive due to lack of structural information of
the complex. Here we present structure of vertebrate dynactin at 20Å resolution,
achieved by negative stain electron microscopy (EM). The reconstruction re-
veals the overall architecture of dynactin, allowing for delineation of the major
known subcomplexes of the molecule. We can clearly discern the individual
Arp subunits arranged in actin like helical fashion in the filament, along with the
capping density at the barbed-end, and the pointed-end complex. We can also
identify the shoulder domain above the filament observing extensive inter-
actions with the Arp subunits. Due to the flexibility of the extended p150Glued
coiled-coil arm, whose base interacts with dynein and whose globular tip binds
the MT surface, this region was not resolved in the 3D reconstruction. Howev-
er, focused 2D analysis of the p150Glued arm revealed its attachment point at
the shoulder domain, as well as structural details of the globular CAP-Gly
domain. This structural study of the dynactin complex establishes a strong
foundation for understanding how its architecture is adapted for concerted
interaction with dynein, cargo, and MTs during transport processes.

105-Plat
A Mechanical Switch from Diffusion to Directional Motion Activates
ATPase in Dynein Motor
Seichi Uchimura1, Takashi Fuji1, Hiroko Takazaki1, Rie Ayukawa1, Yousuke Nishikawa2, Iwushi Minoura3, You Hachikubo4, Genji Kurisu4, Kazuo Sutoh5, Takahide Kon6, Keiichi Namba3, Etsuko Muto4.
1Lab for Molecular Biophysics, RIKEN BSI, Wako, Saitama, Japan, 2RIKEN
QBiC, Suita, Osaka, Japan, 3Osaka University, Suita, Osaka, Japan, 4Institute
for Protein Research, Osaka University, Toyonaka, Osaka, Japan, 5Waseda
University, Toshima-ku, Tokyo, Japan, 6Hosei University, Koganei, Tokyo, Japan.

Dynein is a motor protein that moves along microtubule tracks via the energy
taken from ATP hydrolysis. Unlike other processive cytoskeletal motors, the dynein
step size is highly variable with a significant level of diffusion. To investigate
the molecular basis of the stochastic nature of dynein stepping, we here char-
acterize the structure, physical properties, and effects of site-directed muta-
tions of the dynein-microtubule interface.

We found that mutation of either the R403 or E416 residue of z-tubulin to
alanine changed the directional movement of the microtubules on a dynein-
covered surface to undirected thermal diffusion, resulting in a loss of dynein
ATPase activity. Biochemical and cryo-electron microscopy analyses of the
microtubule binding domain (MTBD)-microtubule complex revealed that these
residues switch dynein from diffusional to stationary binding by form-
ing salt bridges with the residue in H1 and H6 of the MTBD. The formation of two
salt bridges then triggers a registry change in the stalk coiled coil required for
ATPase activation, and thus leads to directional movement. In this mecha-

nism, the previously undescribed interaction between e-R403 and E3390 in H1
of the MTBD plays a key role, and is likely to explain the fact that the trans-
equivalent tubulin mutation in mammals (R402) can cause lissencephaly (Keays et
al., Cell 128, 45-).

Compared to kinesin-microtubule interactions, where the weak-to-strong state
transition is mediated by several contact sites involving a few tubulin residues
(Uchimura et al., EMBO J., 29, 1167-), for dynein, the mechanical switch from
diffusional to stationary binding is controlled by only two salt bridges. Because of
this pinpoint regulation, the stepping motion of dynein might be only loosely
related with the reaction of ATP hydrolysis, resulting in the variable step size.

106-Plat
Cytoplasmic Dynein Ring Tilting Detected by Combined polTIRF and
Sub-Pixel Particle Tracking of Semiconductor Quantum Rods
Lisa G. Lippert1, Tali Dadosh2, Benjamin T. Diroll3, Jeffrey T. Hallock4, Jeffrey T. Hallock4, Christopher B. Murray1, Erika L.F. Holzbaur1, Trina A. Schroer2.
1Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, USA, 2Weizmann Institute of Science, Rehovot, Israel, 3Chemistry, University of Pennsylvania, Philadelphia, PA, USA, 4Physics, University of Pennsylvania, Philadelphia, PA, USA, 5Cell Biology, Harvard Medical School, Boston, MA, USA.

The mechanism of cytoplasmic dynein, a microtubule-based motor responsible
for the majority of minus-end-directed transport in eukaryotes, remains
poorly understood compared to myosin and kinesin. While recent crystal
and EM structures have given insight into the conformational changes
that occur in the AAA+ ring of dynein during ATP hydrolysis, dynamic struc-
tural information is lacking. In order to better understand the dynein
translocation mechanism we combine polarized total internal reflection fluo-
rescence (polTIRF) microscopy and high precision localization to simulta-
neously track position and orientation of single dynein molecules in real
time. CdSe/CdS rod-in-nanoparticles with polarized emission are coated with mercaptoundecanoic acid (MUA) and functionalized with NeutrAvidin. The polarized nanorods are bifunctionally conjugated via
biotin-avidin linkages to biotinylation sites inserted in two positions in
dynein’s AAA+ ring. Both homo- and hetero-dimeric dynein constructs are
analyzed. Fluorescence emission of dynein-conjugated rods is split into four
channels based on polarization and imaged with an EMCCD camera.
The relative intensities of the four channels are used to determine the
three-dimensional orientation of the rod, and therefore the dynein AAA+
ring, during stepping. Using this combined polTIRF/tracking method we
detect tilting of the ring domain during stepping. Ring rotations are
ATP-dependent, highly irregular and are mainly in the plane of the microtubu-
be when correlated with translocation events. Supported by NIH grant
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Symposium: Systems Biology Approaches in Neuroscience

108-Symp
Mapping Behavior to Neural Anatomy Using Machine Vision and Thermog-
gene
Kristin Branson, Alice A. Robie.
HHMI Janelia Research Campus, Ashburn, VA, USA.

To understand the relationship between neural anatomy and behavior, the ul-
timate output of the nervous system, we performed a high-throughput, thermo-
geneic screen of 2,200 transgenic Drosophila lines from the Janelia GAL4
collection. Each GAL4 line drives expression in a different, sparse subset
of neurons in the fly nervous system. Using genetic techniques, we selectively
activated these sparse subsets of neurons, and measured the behavioral effects.
We developed a high-throughput, automated system for measuring the flies’
locomotion and social behavior with breadth and depth. We recorded
20,000 videos of groups of flies freely behaving in an open-field walking
arena, totaling ~400 TB of raw data. From the video, we tracked the flies’
body and wing positions using our tracking software, Ctrax. We used our ma-
chine learning-based behavior classification system, IAABA, to create 15
behavior classifiers (e.g. walking, chasing) that input trajectories from Ctrax
and output predictions for each frame of each fly’s behaviors (totaling ~175
billion annotations of behavior). For each line of flies, we computed ~200
behavior statistics, such as the fraction of time spent chasing, or average speed
while walking, summarizing the behavioral effects of activating the targeted
neurons in a concise, interpretable manner. Concurrent with our screen, the

Janelia Fly Light project imaged the expression pattern of each GAL4 line, producing image stacks indicating which neurons are likely being activated in each line. By jointly analyzing these behavioral and anatomy measurements, we created brain-behavior maps suggesting neural substrates for the generation and/or regulation of each behavior. This study is groundbreaking on two technological fronts. It represents the application of video-based behavior recognition to a highly phenotypically diverse data set of thousands of Drosophila genotypes, and the largest unbiased screen to assign function to neurons throughout the nervous system.

109-Symp Imaging the Connectome
Jeff Lichtman
Molecular/Cell Biology, Harvard University, Cambridge, MA, USA.

Connectional maps of the brain may have value in developing models of both how the brain works and how it fails when subsets of neurons or synapses are missing or interconnected. I am eager to obtain such maps in neonatal animals because of a longstanding interest in the ways neuropelemuscular circuitry is modified during early postnatal life as axonal input to muscle fibers is pruned. Work in my laboratory has focused on obtaining complete wiring diagrams (“connectomes”) of the projections of motor neuron axons in young and adult muscles. Each data set is large and typically made up of hundreds of confocal microscopy stacks of images that tile the 3-dimensional volume of a muscle. As a first step to analyze these data sets we developed computer assisted segmentation approaches and to make this task easier, have developed now second generation “Brainbow” transgenic mice that in essence segment each axon by a unique fluorescent spectral hue. This effort has led to new insights into the developmental processes which help the mammalian nervous system mold itself based on experience. In brain however, as opposed to muscle, the high density of neuropil is overwhelming, which has thus far precluded using the fluorescence approaches that have worked in the peripheral nervous system. We have thus developed an automated physical sectioning strategy that generates thousands of ultra-thin (~25-30 nm) sections on a firm plastic tape. This method makes large scale serial microscopic analysis of brain volumes more routine. We have generated “saturated” segmentations of cerebral cortex and wiring diagrams in thalamus to track every axon and annotate every synapse in neural circuits.

Platform: Voltage-gated K Channels I

110-Plat Interaction of Calmodulin with the EAG1 Potassium Channel
Maria J. Marques-Carvalho1,2, João H. Moraes-Cabral1
1IBMC, Porto, Portugal, 2ICBAS - University of Porto, Porto, Portugal.
The KCNH potassium channel family includes EAG (ether-a-go-go), ERG distinct binding modes to calmodulin and that the presence of the globular domain

Here I present biochemical and structural characterization of the interaction of calmodulin with the EAG1 potassium channel. Using calorimetry and X-ray crystallography I have determined that the three calmodulin BDs have very distinct binding modes to calmodulin and that the presence of the globular domain PAS and CNBhD affects the properties of the calmodulin binding sequences.

111-Plat Effects of the Accessory Subunit γ1 on the External Architecture of BK Channel
Willy Carrasquel-Ursulaez1,2, Juan P. Castillo1, Yenisleidy Lorenzo1, Romina Sepulveda1, Daniel Agasayo1, Francisco Bezanilla4, Fernando D. Gonzalez-Nilo1, Ramon Latorre1,2
1Centro de Neurociencias de Valparaiso, Valparaíso, Chile, 2Doctorado en Ciencias, Mención Neurociencias, Universidad de Valparaiso, Valparaíso, Chile, 3Centro de Bioinformatica y Biologia Integrativa, Universidad Andres Bello, Santiago, Chile, 4Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA.

Regulatory β and γ subunits are responsible for conferring functional diversity to BK channels but little is known about the detailed way that accessory subunits modulate the structure of the pore forming α subunit. It is known that the γ1 subunit produces a large leftward shift of the open probability vs. voltage curve in the absence of internal Ca2++ (Yan and Aldrich, 2010). To explore the external architecture of α subunit in the presence of γ1 subunit, we used lanthanide-based resonance energy transfer (LRET) as a molecular ruler to measure intra- and inter-molecular distances. We introduced a genetically encoded lanthanide binding tag (LBT) that binds Tb3++ (LRET acceptor). LRET sensitized emission (SE) decays were analysed using a nano-positioning system that determines the position of LBT-tagged sites with respect to the fixed acceptor near the pore axis. Interestingly, the external architecture of the BK α subunit is modified when co-expressed with the regulatory γ1 subunit indicating a conformational change of the BK voltage sensor domain of the BK channel. The largest changes was in S1 position (~25 Angstroms) followed by S0. In addition, all γ1-LBT positions were found peripherally positioned with respect to the α subunit.

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112-Plat Enhanced Voltage-Clamp Fluorometry Assigns Distance Constraints to BK Channel VSD Structural Transitions
Antonios Pantazis1, Riccardo Olcese1,2
1Anesthesiology, UCLA, Los Angeles, CA, USA, 2Physiology, UCLA, Los Angeles, CA, USA.

Voltage-clamp fluorometry (VCF) is a hybrid optical/electrophysiological approach allowing the simultaneous tracking of local protein movements (reported by a Cys-conjugated, environment-sensitive fluorophore) and membrane current. While VCF data reveal the voltage dependence of the labeled protein domain, the direction and distance of protein movements are not straightforward to extract. We recently used the ability of Trp to collisionally quench fluorophores to resolve the direction of activation-dependent helix movements in the BK channel VSD: Trp-203 (extracellular to S4) diverged from labels on S0, S1 and S2, and S2' subunit. Fluorescent probe BODIPY linked to a scorpion toxin was used as LRET acceptor. LRET sensitized emission (SE) decays were analysed using a nano-positioning system that determines the position of LBT-tagged sites with respect to the fixed acceptor near the pore axis. Interestingly, the external architecture of the BK α subunit is modified when co-expressed with the regulatory γ1 subunit indicating a conformational change of the BK voltage sensor domain of the BK channel. The largest changes was in S1 position (~25 Angstroms) followed by S0. In addition, all γ1-LBT positions were found peripherally positioned with respect to the α subunit. This information sufficed to compile a dynamic map of helix rearrangements; however, the distances of these movements were unknown.

Here we show that VCF can be enhanced to measure distances by using fluorophores of different lengths: tetramethylrhodamine (TMR) labels with variable links to a Cys-binding maleimide moiety. To account for fluorophore flexibility, we simulated the conformers of isolated Cys-fluorophore conjugates using molecular dynamics (MMFF94,50ps), generating distributions of the distance between the Cys α-C and a C-atom near the TMR center. Fluorochrome TMR-6'-M had the shortest distance distribution (mode=10.4Å), followed by TMR-6'-C2-M (14.0Å), TMR-5'-C2-M (14.8Å) and a mixture of TMR-5'/6'-C6-M isomers (16.8Å).

The extracellular flank of voltage-insensitive helix S1 (Cys-136) was labeled with each fluorophore and voltage-dependent ΔF were resolved, reporting the divergence of S4 Trp-203. TMR-5'-M produced the strongest ΔF (8.1 ± 1.7% mS, n=6), followed by TMR-6'-M (4.0 ± 0.53%, n=3), TMR-6'-C2-M (2.6 ± 0.67% n=4), TMR-5'-C2-M (0.97 ± 0.27%, n=4) and TMR-5'/6'-C6-M (0±0.01% ± 0.21, n=3). Given the Cy3/fluorophore distance distributions, these results are consistent with the extracellular portion of S4 being ~11Å away from S1 at rest and diverging to ~20Å upon activation. Thus, VCF can be used to determine state-dependent distances between assigned protein loci.