that the different domain organizations of the two proteins explained their different activities. Thus, Tmod has two tropomyosin (TM)- and two actin-binding sites, organized as: TMBS1-ABS1-TMBS2-ABS2. Lmod is longer, featuring a C-terminal extension that contains a Pro-rich region and a WH2 domain, which constitutes a third actin-binding site. The presence of the WH2 was considered to be the major feature distinguishing Lmod from Tmod. Here we show that this is not the case. Among the main findings are: 1) Lmod not only lacks TMBS2, but also ABS1, such that the entire region N-terminal to ABS2 has very little effect on nucleation, 2) The C-terminal extension of Lmod has also a limited effect on nucleation, and adding it to Tmod produces a very modest increase in nucleation, 3) Despite being relatively well conserved, the major feature distinguishing Lmod from Tmod is ABS2, consistent mostly of a Leu-rich repeat domain. Structural analysis shows that ABS2 can bind up to 3 actin subunits, and subtle differences between Lmod and Tmod dictate the affinities of their interactions with actin, and thus their roles in nucleation vs. capping. Understanding these differences allowed us to engineer an ABS2 Tmod-Lmod hybrid with nucleation activity equal to that of full-length Lmod.

## Symposium: Molecular Basis for Mitochondrial Signaling

**949-Symp**
**Systems Approaches to Mitochondrial Calcium Signaling**
Jennifer Wettmarshausen[1], Yiming Cheng[2], **Fabiana Perocchi**[1,2].
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Mitochondria are essential hubs of calcium-mediated signaling networks. The organelle can take up, buffer, and release calcium ions to effectively shape intracellular calcium transients, stimulate ATP production and regulate cell death. Although, the basic mechanisms of mitochondrial calcium homeostasis have been firmly established for decades, the molecular identity of the mitochondrial calcium signaling toolkit has evaded classical bottom-up approaches. Our previous studies (1,2) have provided a compelling example of the power of systems approaches applied to mitochondrial calcium signaling to discover hitherto unknown molecular components of the calcium uniporter. Currently, we are developing computational and experimental frameworks for a systematic reconstruction of calcium-dependent signal transduction cascades in mitochondria. By combining evolutionary genomics and loss-of-function genetic and chemical screens, our systems approach holds the potential to shed light on yet unanswered questions in the field of mitochondrial calcium signaling.
1. Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK (2011). Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature. 476(7360):341-5.
2. Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK (2010). MICU1 encodes a mitochondrial EF hand protein required for calcium uptake. Nature. 467(7313):291-6.

**950-Symp**
**The Mitochondrial Calcium Uniporter: Molecular Composition and Physiological Role**
**Rosario Rizzuto**.
Dept. Biomedical Sciences, University of Padua, Padua, Italy.
Mitochondria rapidly accumulate $Ca^{2+}$ through a low-affinity uptake system (the mitochondrial $Ca^{2+}$ uniporter, MCU) because they are exposed to high $[Ca^{2+}]$ microdomains generated by the opening of ER $Ca^{2+}$ channels. These rapid $[Ca^{2+}]$ changes stimulate $Ca^{2+}$-sensitive dehydrogenases of the mitochondrial matrix, and hence rapidly upregulate ATP production in stimulated cells. $Ca^{2+}$ also sensitizes to cell death mediators, e.g. ceramide. In my presentation, I will present the most recent molecular information on MCU, identified by our group in 2011, and the newly identified regulators (MCUb, MICU1, MICU2). I will also show how the availability of molecular tools for MCU now allows to carry out experiments in intact cells and whole organisms that highlight and clarify the importance of mitochondrial calcium homeostasis in physiology and pathophysiology.

**951-Symp**
**Molecular Mechanisms of Mitochondrial $Ca^{2+}$ Uptake: Role of MICU1 and its Paralogs**
**György Hajnóczky, MD**.
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Mitochondrial $Ca^{2+}$ uptake is central to cell metabolism, signaling and survival. Recent studies identified MCU as the pore of the mitochondrial $Ca^{2+}$ uniporter and MICU1 as its critical regulator. MICU1 and its paralogs, MICU2 and MICU3 are EF-hand proteins and are the primary candidates to confer $Ca^{2+}$ sensitivity to the $Ca^{2+}$ uniporter. We will present studies of the molecular mechanisms of the MICU-dependent closure of the uniporter at low $[Ca^{2+}]$ levels and its cooperative activation when $[Ca^{2+}]$ increases. Furthermore, we will present clues to the MICU-dependence of the tissue specific mitochondrial $Ca^{2+}$ uptake profiles.

**952-Symp**
**High-Affinity Interaction with VDAC Links Cytosolic Proteins to Mitochondrial Regulation in Health, Cancer, and Neurodegeneration**
**Tatiana K. Rostovtseva**[1], Philip A. Gurnev[2], David P. Hoogerheide[3], Eduardo N. Maldonado[4], John J. Lemasters[5], Olga Protchenko[6], Jennifer C. Lee[7], Sergey M. Bezrukov[1].
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We address the question of how mitochondria control cell survival and cell death by studying the voltage-dependent anion channel (VDAC). VDAC, the major channel of the mitochondrial outer membrane (MOM), is a well-recognized key conduit for ATP and other bioenergetics metabolites fluxes across MOM. We have found that dimeric tubulin, the subunit of microtubule, induces highly efficient reversible blockage of VDAC reconstituted into planar lipid membranes. Although the tubulin-blocked state still conducts small ions, it is virtually impermeable to ATP. We propose that by modulating VDAC permeability for ATP and other respiratory substrates, tubulin controls mitochondrial respiration. These findings are supported by experiments with isolated mitochondria and human hepatoma cells, thus uncovering a mechanism of regulation of mitochondrial energetics by free tubulin and also suggesting how cancer cells preferentially use inefficient glycolysis rather than oxidative phosphorylation (the Warburg effect).
We also found a functional interaction between VDAC and α-synuclein (α-syn), an intrinsically disordered neuronal protein intimately associated with Parkinson disease (PD) pathogenesis. Importantly, in addition to regulation of VDAC permeability by α-syn, our data indicate that VDAC facilitates translocation of α-syn across MOM where it could target complexes of the mitochondrial respiratory chain in the inner membrane. Supporting our *in vitro* experiments, a yeast model of PD shows that α-syn toxicity in yeast depends on VDAC. Considering that VDAC is a major conduit for respiratory substrates across the mitochondrial outer membrane, we conclude that the α-syn/VDAC functional interaction reveals the elusive physiological and pathophysiological roles for monomeric α-syn in PD and also in general neurodegeneration.

## Platform: Electron Microscopy and Solution Scattering

**953-Plat**
**GFP for EM: Site-Specific Labeling of Proteins for Electron Microscopy**
**Corey M. Dambacher**, Gabriel C. Lander.
Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA.
Structural analysis of macromolecules by electron microscopy (EM) has been facilitated by a recent technological revolution in instrumentation and data processing, which has led to the achievement of their visualization at atomic resolution. However, moderate resolution electron density maps of protein complexes can be misleading, resulting in ambiguity when ascribing subunits to particular locations within the architecture of complexes. To this end, investigators have traditionally performed subunit mapping by N- or C-terminal fusions with tags, such as maltose binding protein (MBP), with mixed success. Toward the accurate determination of the location and orientation of protein subunits, as well as large scale movements of protein complexes, the establishment of a highly specific labeling technique would be a major breakthrough. Here we present a site-specific labeling strategy that exploits a unique chemical handle introduced by the incorporation of the unnatural amino acid (UAA). The UAA permits a site-specific copper-free click reaction for labeling with a modified label, such as MBP or Nanogold. We use this method to label a subunit