FEBS Advanced Lecture Course 2019 | Biochemistry of Membrane Proteins – Structure, Trafficking, Regulation

Sunday, 2019/8/25

16:00 - 16:30 Lecture hall

Opening Networking

16:00 - 16:15 **Talk Title Example 1**
Presenter Y

16:15 - 16:30 **Talk Title Example 2**
Presenter Z

16:30 - 17:00 **Talk Title Example 3**
Presenter W

16:30 - 17:30 Lecture hall

Susan G. Amara: Close encounters: the intimate regulatory interactions between GPCR signaling events and neurotransmitter transporters Lectures

> > > >>Biogenic amine transporters are well-established as the primary targets for psychostimulant drugs of abuse and for drugs such as methylphenidate and amphetamines, which are used to treat attention deficit disorders. We have observed that once amphetamines enter neurons, they activate multiple intracellular signaling pathways. Within the cell, amphetamines activate the small GTPases, RhoA and Rac1 and trigger endocytosis of the dopamine transporter (DAT) and a neuronal glutamate transporter (EAAT3), which leads to enhanced dopaminergic and excitatory signaling. These events depend upon the expression of an intracellular G-protein coupled trace amine receptor (TAAR1) that signals through at least two types of G-protein alpha-subunit within the cell. Our results demonstrate that amphetamine-like drugs not only inhibit monoamine transport to potentiate neurotransmission, but they also activate G-protein signaling directly by acting as TAAR1 agonists. GPCRs also regulate transporter function in other ways. For example, G-protein beta gamma subunits released during GPCR activation bind directly to the DAT and facilitate a shift in DAT conformation towards an inwardly- oriented efflux mode. This lecture will highlight the interactions between GPCR signaling events and plasma membrane neurotransmitter transporters and will consider how they are linked to the action of a variety of drugs that modulate monoamine signaling. >> > > >

17:30 - 19:30 Atrium

Welcome buffet dinner Networking

FEBS Advanced Lecture Course 2019 | Biochemistry of Membrane Proteins – Structure, Trafficking, Regulation

Monday, 2019/8/26

09:00 - 10:00 Lecture hall

Satoshi Murakami: Structural analysis of membrane transporters at an atomic level

Lectures

> > > >>Multidrug resistance caused by drug efflux transporter is a serious problem in antibiotic treatment of numerous bacterial infections. The envelope of Gram-negative pathogens contains unique tripartite machineries that export noxious compounds from the cell. These machineries are composed of a plasma membrane transporter and outer membrane porin that are connected by a periplasmic adaptor protein. AcrB which belong to the Resistance-Nodulation-cell Division (RND) superfamily is one of the most characterized tripartite transporter complex>>1,2>>. Substrates are incorporated from periplasmic opening to the substrate translocation pathway, and exported in the manner of the “periplasmic vacuum cleaner” by the functional rotating mechanism. ATP-binding cassette (ABC) and major facilitator superfamily transporters can also be part of tripartite complexes, and share similar or identical components with RND transporters. The tripartite-type ABC transporter, MacB in complex with outer membrane porin, TolC and periplasmic adaptor protein, MacA, is an important efflux transporter that mediates the extrusion of macrolides, peptide toxins, virulence factors, siderophores, lipopolysaccharides and protoporphyrins. Although crystal structures of ABC transporters have been reported for both exporters and importers from various organisms, the ABC protein MacB is unrelated to AcrB, which form large trimeric complexes in the plasma membrane, raising questions about the structure and domain organization of MacB in the tripartite efflux transporter. Recently, we solved the crystal structure of MacB at 3.4 Å resolution>>3>>. MacB forms a dimer in which each protomer contains a nucleotide binding domain and four transmembrane helices that protrude in the periplasm for interaction with the periplasmic adaptor protein, MacA. The MacB structure exhibits significant differences with known structures of ABC proteins and provides a framework for further elucidation of the mechanisms of these important tripartite efflux transporters. The cryo- electron microscopy structure of MacA-MacB-TolC, the ABC tripartite assembly, solved at near-atomic resolution>>4 >>also gives various information for understanding molecular mechanism of transport in the tripartite type ABC transporters. >> >>As the tripartite transporters, MacB, a tripartite-ABC transporter, also seems to take substrates from the outer-leaflet of the bilayer (or periplasmic space), and export them into the connecting periplasmic adaptor proteins and the outer membrane channel proteins which form a tunnel like structure acting as an exhaust duct to the outside of the cell. >> > > >

10:00 - 11:00 Lecture hall

Bert Poolman: Can we build a minimal form of life from molecular components and control the physicochemistry of the cell?

Lectures

> > > >>The biochemical processes that characterize all living cells, such as energy provision, gene expression, and cell division take place in a confined and highly crowded space. High concentrations of macromolecules give rise to the phenomenon of macromolecular crowding, which impacts individual proteins, the formation of protein complexes and the structure of the cytoplasm. The interplay between macromolecular crowding, pH, ionic strength, water activity and osmotic pressure determines the physicochemical state of the cytoplasm. In addition, many processes in biological cells depend on the ability of macromolecules to find each other by translational diffusion, which can limit the tempo of processes. >> >>We have initiated a program to construct minimal forms of life from molecular components to achieve physicochemical homeostasis in synthetic cells. One of the grand challenges in synthetic biology is the construction of far-from-equilibrium molecular systems integrated into cell-like containers with control of solute fluxes and a constant supply of energy to fuel ATP-requiring processes. Such systems should enable long-term metabolic energy conservation and physicochemical homeostasis and a better understanding of how living cells perform these tasks. I will present the assembly of synthetic vesicles containing a pathway for sustained ATP production that performs at least an order of magnitude better than any system described so far. I will also show the bottom-up construction of a volume regulatory network to control the osmotic pressure, ionic strength, pH, and molecular crowding of the synthetic cells. >> > > >

11:00 - 11:30 Atrium

Coffee Break

Networking

11:30 - 12:30 Lecture hall

Richard Collins: Cryo-EM of membrane proteins in the modern era – from 2D crystals to high-throughput single particle averaging Lectures

> > > >>Up until around 2010, cryo-electron microscopy was viewed as the choice of last resort in the structural biology canon of methods; mainly because of the relatively limited resolution commonly obtained when compared to X-ray crystallography or NMR. The low dynamic quantum efficiency (sensitivity) of electron film made high resolution structure determination the exception rather than rule, essentially because of weak signal:noise in recorded data images. For enthusiasts of membrane protein structure the only pragmatic way to obtain structural information was to generate 2D crystals of your sample of interest and rely on redundancy of information from lattice repeats. With the development of new highly sensitive cameras ~10 years ago, improved sample preparation and much more accurate computer processing, single particle averaging improved out of sight and effectively made 2D crystal studies mostly redundant. Has it? >> >>In this lecture I will review how we used to make and use 2D crystals of membrane proteins and the limitations and problems we frequently encountered. I will discuss, using examples of membrane proteins and membrane protein complexes how single particle averaging can be used to investigate a variety of interesting biological questions. The field has evolved at a rapid rate and I will also review how we have started to use phase plates in these studies and how the next generation of 2D processing algorithms have improved processing with another step change. >> > > >

12:30 - 14:00 Atrium

Buffet lunch Networking

14:00 - 16:00 Atrium

Poster session 1 Poster Sessions

16:00 - 17:00 Lecture hall

Five minutes poster bullet presentations 1 Poster Sessions

17:00 - 18:30 Atrium

Dinner Networking

19:15 - 20:45 ##in the city##

Danube boat cruise "Legenda" Networking

Tuesday, 2019/8/27

09:00 - 10:00 Lecture hall

Poul Nissen: Structure and function of membrane ATPases Lectures

10:00 - 11:00 Lecture hall

Christine Ziegler: Membrane proteins and stress responses in bacteria Lectures

11:00 - 11:30 Atrium

Coffee break Networking

11:30 - 12:30 Lecture hall

Vassilis Koronakis: Tripartite efflux pumps: bacterial nanomachines for toxin export and antibiotic efflux Lectures

> > > >>In Gram negative bacteria such as Escherichia coli and Pseudomonas aeruginosa tripartite efflux pumps direct the efflux of heavy metals and antibiotics and the export of proteins including toxins. They are key determinants of bacterial survival and underpin the increasing threat of multidrug resistance. Pumps span the entire cell envelope and are composed of an outer membrane exit channel, a periplasmic adaptor protein and an inner membrane transporter typically a proton antiporter or ABC transporter. The assembled pump therefore spans the inner and outer membranes and intervening periplasmic space. >> >>For many years my research has focused on the structure including the elucidation of the structure of the TolC outer membrane channel almost twenty years ago. Here I will describe the efforts of my lab and others to understand the structure and function of tripartite efflux pumps focusing on two ABC transporters which both energise transport through TolC dependent-tripartite efflux pumps yet have different structures and mechanisms. >> >>HlyB powers export of a large (>1000 amino acid) hemolysin toxin from the cytoplasm across both membranes in a concerted step without a periplasmic intermediate. Conversely MacB drives export of antibiotics and peptide toxins from the periplasm across the outer membrane. MacB does not have a central cavity through which substrates are translocated but instead uses ATP hydrolysis to convey conformational changes from one side of the membrane to the other. Homologues of MacB that do not form tripartite pumps but share structural features include proteins underpinning lipoprotein trafficking and cell division. The structure of MacB can therefore be used as a blueprint to understand the structure of an entire ABC transporter superfamily responsible for fundamental cellular processes. >> > > >

12:30 - 14:00 Atrium

Buffet lunch Networking

14:00 - 16:00 Atrium

Poster session 2 Poster Sessions

16:00 - 17:00 Lecture hall

Five minutes poster bullet presentations 2 Poster Sessions

17:00 - 19:00 Atrium

Buffet dinner and mixer (speaker's corner for student - lecture discussions) Networking

Wednesday, 2019/8/28

09:00 - 10:00 Lecture hall

Giulio Superti-Furga: Complex regulation of human membrane transporters Lectures

10:00 - 11:00 Lecture hall

Robert Tampé: Illuminating macromolecular membrane complexes in adaptive immunity Lectures

> > > >>Identifying and eliminating infected or malignantly transformed cells are fundamental tasks of our adaptive immune system. For immune surveillance, the metastable proteome of the cell is displayed as broken bits (peptides) on major histocompatibility complex class I (MHC I) molecules to cytotoxic T-lymphocytes. Our knowledge about the track from the cellular proteome to the presentation of peptides has greatly expanded, leading to a quite comprehensive understanding of the antigen processing pathway. I will report on the mechanism of antigen translocation, chaperoning, editing, and ER quality control. Following an integrative approach, the full conformation landscape of ABC transporters under turnover conditions, the structure of ER quality control machineries, and MHC I chaperone and peptide-loading complexes will be addressed, also in the context of viral immune evasion. The seminar provides the framework for understanding the onset of an adaptive immune response. >> > > >

11:00 - 11:30 Atrium

Coffee break Networking

11:30 - 12:30 Lecture hall

Margarida Amaral: Membrane trafficking in cystic fibrosis Lectures

> > > >>Cystic Fibrosis (CF), the most common life-threatening genetic disease in Caucasians, is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. This encodes for an ABC transporter protein (ABCC7) expressed at the plasma membrane of epithelial cells functioning as a chloride/bicarbonate (Cl-/HCO3-) channel. Despite intensive symptomatic treatments, individuals with CF have progressive lung disease due to major airway mucus obstruction, recurrent bacterial infections and chronic inflammation, conducting to shortened life expectancy. >> >>It is known for long that F508del, the most common CF-causing mutation (occurring in ~85% of individuals with CF) leads to CFTR protein misfolding and recognition by the endoplasmic reticulum quality control (ERQC). The latter targets F508del-CFTR for premature degradation via the ubiquitin-proteasomal pathway (UPP), thus preventing it from trafficking to the cell surface. Nevertheless, we are still missing the global view of mechanisms and pathways involved in this process. >> >>We have used a functional genomics approach (high-content siRNA screen) for a global mechanistic characterization of F508del-CFTR traffic defect. This consisted in the development of a high-throughput microscopy assay in human bronchial epithelial cells to identify factors that rescue traffic of F508del-CFTR to the cell surface [1]. This assay an pipeline was applied to screen a library of 27,312 siRNAs targeting the druggable genome (~9,000 genes), i.e., about half of the human genome. >> >>The primary screen identified 227 hit genes that rescued F508del-CFTR traffic and, after validation by additional siRNAs, 35 genes were established as playing a role in F508del-CFTR traffic. In order to provide relevance and specificity of these traffic regulators as potential drug targets, further studies of these hits included: i) pathway classification; ii) overlap with general secretome [2]; iii) functional characterization according to ERQC checkpoints [3,4]; and iv) overlap with previous CFTR interactomes [5-7]. >> >>Results from these analyses pointed to a complex involvement of several cellular functions in the regulation of the CF physiopathology and led to the establishment of novel potential drug targets by correcting the primary cause of this disease. These potential drug targets can be potentially manipulated and combined with existing drugs in a synergistic way to increase traffic of mutant CFTR for the greater benefit of individuals with CF. Moreover, these novel regulators can also help defining basic cell and molecular biology traffic pathways. >> > > >

12:30 - 14:00 Atrium

Buffet lunch Networking

14:00 - 18:30 ##in the city##

Free afternoon Networking

18:30 - 21:30 ##in the city##

Dinner in "PÚDER" restaurant (Ruin pub) in Budapest Networking

Thursday, 2019/8/29

09:00 - 10:00 Lecture hall

Margaret S. Robinson: Trafficking of membrane vesicles Lectures

> > > >>In eukaryotic cells, much of the trafficking between membrane compartments is carried out by vesicles, which bud from one compartment and fuse with another. Vesicle budding requires coat proteins, which both shape the membrane into a vesicle and select the vesicle cargo. COPI and COPII coats facilitate trafficking between the ER and the Golgi, while heterotetrameric adaptor protein (AP) complexes facilitate various post- Golgi trafficking pathways. There are five APs, distantly related to COPI, which localize to different compartments. We are interested in the functions of the vesicle coats and in why mutations in some of them cause particular genetic disorders. >> > > >

10:00 - 11:00 Lecture hall

Erzsébet Ligeti: Role of extracellular vesicles in immune regulation Lectures

> > > >>A recently discovered common property of both pro- and eukaryotic cells is the ability to release – quasi continuously – membrane-surrounded vesicles, generically called extracellular vesicles (EV). EVs are heterogenous both in size, in composition and in biogenesis. Composition of these vesicles reflects the composition of the parent cell, containing components both from the plasma membrane, cytosol, even from certain subcellular organelles and different nucleic acids. EVs became accepted as an authentic pathway of intercellular communication in addition to soluble ligands and cell surface molecules. >> >>A plethora of different physiological functions have been associated with EVs, and they are also implicated in several pathological processes. Immunological functions were among the first which have been discovered. EVs are able to present antigens, to transfer different immunoreceptors from one cell to another one, and modulate the function of immunological synapses. On the other hand, EVs were also shown to present autoantigens and initiate autoimmune processes. >> >>Neutrophilic granulocytes are the most abundant population of white blood cells. Our group has shown earlier that under certain conditions they generate EVs which are able to impair bacterial growth. We have characterized in detail four different types of EVs formed from neutrophils under different conditions and showed that antibacterial EVs are very different from all other types. In our most recent work we have identified the plasma membrane receptors which serve as triggers for the generation of antibacterial EVs. Deciphering the receptor proximal signaling elements we demonstrate a unique pathway and present evidence for distinguishable molecular mechanisms in biogenesis of different EV types. Last but not least we show examples of selective effects exerted by the different types of EVs released from the same cell. >> >>The presentation will cover both general aspects of EV biogenesis and special roles in immunologic functions as well as unpublished results on formation and selectivity of neutrophil-derived EVs. >> > > >

11:00 - 11:30 Atrium

Coffee break Networking

11:30 - 12:30 Lecture hall

Sergio Grinstein: Phosphoinositide-gated ion fluxes drive endocytic traffic Lectures

> > > >>Despite ongoing endocytosis and (macro)pinocytosis of extracellular fluid, the overall volume of the endocytic pathway remains unchanged over time. Little is known about how the volume of cellular endomembranes is regulated. To investigate the underlying mechanisms, we used high-resolution video imaging to analyze the fate of macropinosomes formed by M-CSF-stimulated macrophages. This system was chosen because it can be monitored by diffraction-limited optical methods. We found that Na>>+>>, the primary cationic osmolyte internalized, exits the macropinocytic vacuoles via two- pore channels (TPC), accompanied by the parallel efflux of Cl>>- >>and osmotically-obliged water. Accordingly, the macropinosomes formed by macrophages isolated from TPC1- and TPC2-deficient mice failed to resorb. The shrinkage caused by ion flux-driven water efflux causes crenation of the membrane that, in turn, fosters the recruitment of curvature-sensing proteins that stabilize emerging tubules and promote their elongation, driving vacuolar remodeling and resolution. TPC channels are known to require PtdIns(3,5)P>>2 >>for their activation. This requirement likely accounts for the vacuolation caused by inhibitors of PIKfyve, the kinase that generates PtdIns(3,5)P>>2>>. We propose that increases in the surface-to-volume ratio of endomembranes driven by solute and water fluxes are an essential component of organellar size regulation and of traffic between compartments. >> > > >

12:30 - 14:00 Atrium

Buffet lunch Networking

14:00 - 15:30 Lecture hall

10+5 min talks of young investigators selected from participants (1-4) Lectures

> > > >>1. >>Wright Muelas, M>>, UK: Relationships between cellular metabolite and drug uptake and transporter expression profiles>2. >>Maksymowicz, M>,> Poland: Intracellular trafficking and signaling of lymphotoxin >>β >>receptor (LT>>β>>R) >>>3. >>Luginina, A>>, Russia: Structural insights into human CysLTR1 GPCR receptor>4. >>Farkas, B>>. Hungary: Characterization of the CFTR chloride channel using in silico methods >> > > >

15:30 - 16:00 Atrium

Coffee break Networking

16:00 - 17:00 Lecture hall

10+5 min talks of young investigators selected from participants (5-8) Lectures

> > > >>5. >>>Nicolàs i Aragó, A>, >>Spain: Transport Mechanism of LATs>6. >>Lengyel M>>, Hungary: TRESK background K+ channel regulates the excitability of capsaicin-sensitive somatosensory neurons>7. >>Bertovic, I>>, Croatia: Early endosomal GTPase Rab5 regulates platelet production>8.>>Vit, O>>, Czech Rep.: The “Pitchfork” approach to membrane proteome profiling of human pheochromocytoma and paraganglioma >> > > >

18:30 - 21:30 ##in the city##

Buffet dinner and mixer at Hotel Leonardo, poster awards and prizes Networking

Friday, 2019/8/30

09:00 - 10:00 Lecture hall

Barbara Ehrlich: The role of membrane proteins in cellular calcium regulation and signalling Lectures

> > > >>Neuronal Calcium Sensor 1 (NCS1) is a multi-functional calcium (Ca>>2+>>)-binding protein found in virtually all cell types. NCS1 interacts with many protein partners, and these interactions affect a number of cellular processes including motility, Ca>>2+>>-signaling, and cell survival. Although NCS1 is commonly studied in neurons, it also plays important regulatory roles in other cell types, specifically breast and liver cancer cells. Two aspects of NCS1 function will be addressed. First, starting with neuronal functions, NCS1 is a novel receptor for the common chemotherapeutic drug paclitaxel. The interaction between NCS1 and paclitaxel amplifies Ca>>2+ >>release from intracellular stores through the inositol trisphosphate receptor (ITPR), leading to calpain activation and subsequent protein degradation, including degradation of NCS1. From our studies in cells and mouse models, we found that this increased Ca>>2+ >>release initiates a signaling cascade leading to chemotherapy-induced peripheral neuropathy (CIPN). Prevention of CIPN can be accomplished by inhibiting Ca>>2+ >>release via the interaction between NCS1/ITPR in cells and mice. Second, when examining cancer cells derived from epithelia, we found that NCS1 levels in patient tumor samples vary 8-fold and are significantly elevated compared to non-tumor samples. High expression of NCS1 is associated with poor prognosis in both breast and liver cancer patients. NCS1-overexpressing cells have a marked increase in several of the features that are indicators of metastatic tumors, including cell motility and survival in both 2D and 3D assays. These functional changes seen with altered NCS1 expression can be linked to specific downstream signaling pathways related to motility and survival. We are using the results obtained from these studies to develop pharmacological interventions that target Ca>>2+>>-signaling to attenuate the NCS1-enhanced functions. >> > > >

10:00 - 11:00 Lecture hall

László Hunyady: Membrane receptor trafficking. The role of beta arrestins in receptor function Lectures

>G protein-coupled receptors (GPCRs) are membrane proteins with a common seven transmembrane architecture, which are major pharmaceutical targets for therapy. Agonist-induced activation of many GPCRs leads to G protein activation and signal transduction, and also causes translocation of beta-arrestin molecules to the activated receptor. Binding of beta-arrestin molecules cause desensitization by uncoupling the receptors from the G proteins. They can also serve ad adaptor proteins, which promote internalization of the activated receptors and also contribute to their signal transduction. Beta-arrestin molecules can promote signal transduction by organizing signaling complexes. The structural requirements of G protein activation and arrestin-mediated signaling are different, which present opportunity to selective activation of these pathways, since different agonists can stabilize different active conformation of the receptors. Ligands that can preferentially activate certain signaling pathways are also called bias agonists. Bias agonists present novel opportunities to selective activation of GPCRs, which may have important therapeutical consequences, since the side-effect profile of bias agonists may be more optimal than those of other agonists and antagonists of the given receptor. This presentation will focus on the role of beta-arrestin molecules in receptor internalization and their contribution to the pleiotropic signal transduction of GPCRs.>

11:00 - 11:30 Atrium

Coffee break Networking

11:30 - 12:30 Lecture hall

Anne Spang: Insights into organelle compartmentalisation Lectures

>Eukaryotic cells contain membrane-bounded organelles that communicate with each other. These interchange is essential to ensure the correct localization of proteins and lipids to their 'home' organelle and maintain the size and functionality of the cellular organelles. The same way membrane-bounded organelles fulfil different functions, these organelles generate domains or compartments in the membrane to even specify further the different tasks. For example, organelles along the secretory pathway form exit sites, from which transport containers emanate to bring cargo to the next organelle, which maintains an arrival site, which will recognize the correct transport containers. Likewise, a sorting endosome harbours different domains and compartments through which cargo is sorted either into various recycling pathways or into intraluminal vesicle. I will discuss underlying principles of compartmentalization in membrane traffic.>

12:30 - 14:00 Atrium

Closing & buffet lunch Networking