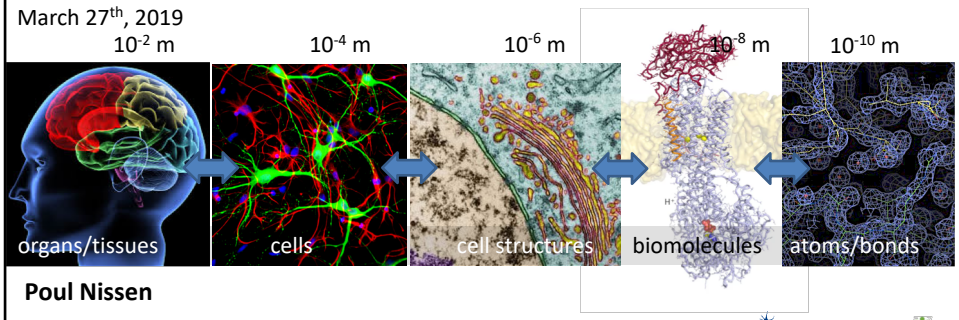
Structure and Dynamics of Biomembranes and Membrane Proteins

Workshop "New Scientific Capabilities at European XFEL"

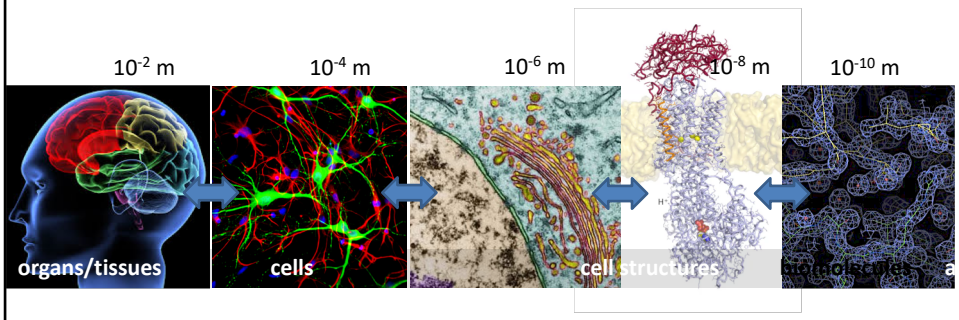
March 27th, 2019



Poul Nissen

Danish Research Institute of Translational Neuroscience - DANDRITE
Nordic-EMBL Partnership for Molecular Medicine & Lundbeck Foundation
Center for Membrane Pumps in Cells and Disease – PUMPkin 2007-2017
Center for Proteins in Memory – PROMEMO 2017 - (2027)
Danish National Research Foundation
BRAINSTRUC
Lundbeck Foundation
Department of Molecular Biology and Genetics
Aarhus University

Neuroscience from atomic to anatomic levels



- Animal models
- Clinical samples
- Patients
- Behavioral models
- Virtual arenas
- Morphology

- Stem cells
- Primary cells
- Organoids/Brain slices
- General microscopy
- Electrophysiology
- Two-photon microscopy

- Confocal
- Super-resolution microscopy
- High-throughput microscopy
- EM
- Histology

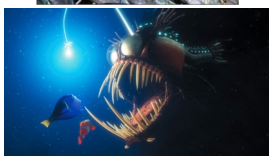
- Spectroscopy
- Microscopy
- Mass-spec
- Genetics
- Transcriptomics
- EM tomography

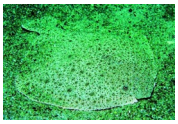
- Crystallography
- Single-particle CryoEM
- NMR
- Biochemistry
- Biophysics
- Time-resolved dynamics

Integrating structural biology

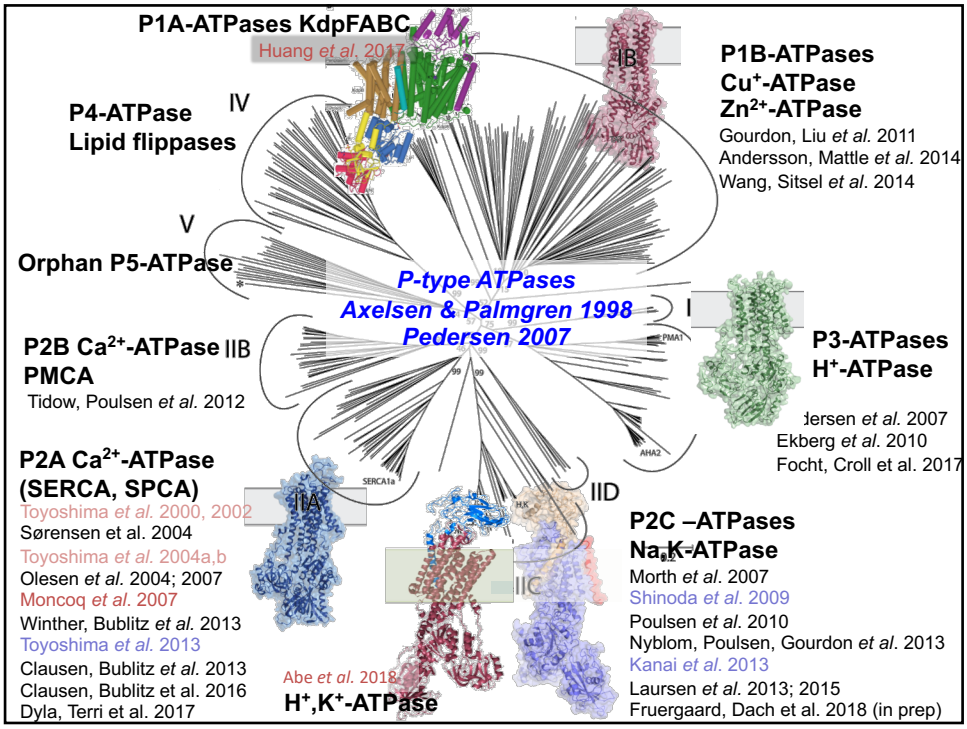
- **X-ray scattering/diffraction**
- **Neutron scattering/diffraction**
- **Electron microscopy**
- **Fluorescence microscopy/spectro**
- **Nuclear Magnetic Resonance**
- **Atomic Force Microscopy**
- **Modeling**



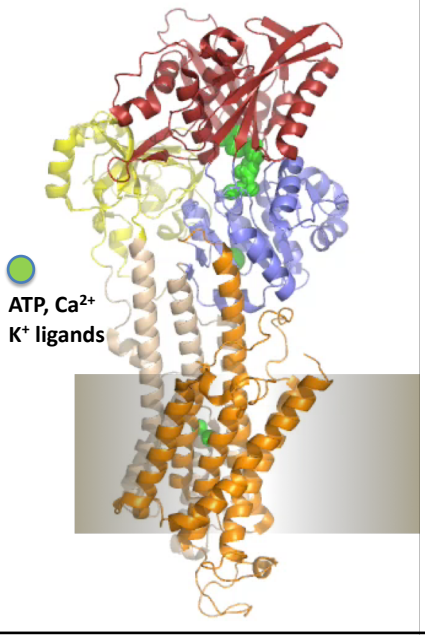



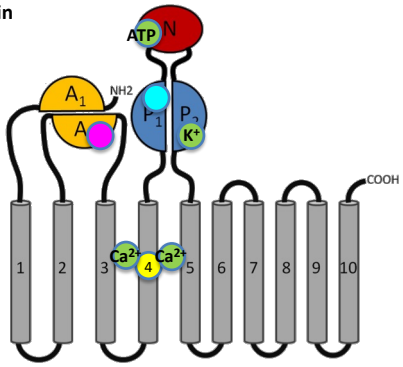
Location, location, location



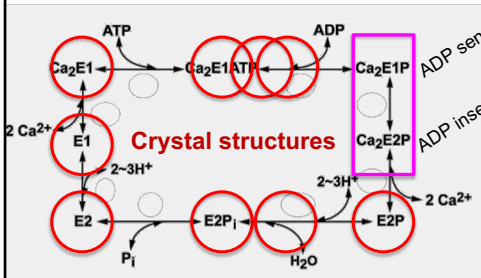
P-type ATPases



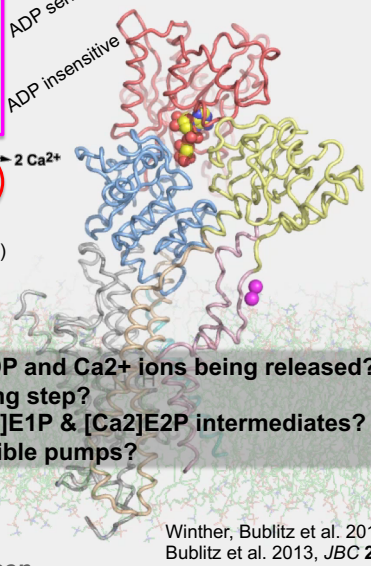
- ATPase with phospho-enzyme intermediate
- E1 and E2 states (interspersed by E1P and E2P)
- Conserved Asp-phosphorylation site
- Conserved TGES dephosphorylation motif
- 6 core TM segments, three cytoplasmic domains
- Conserved Proline residue in TM4
- Five subfamilies, I through V
- Spend about 35% of ATP in human, up to 80% in brain



SERCA Ca^{2+}-ATPase pump cycle - *Pump Fiction*



Ionic conditions (Ca2+, no Ca2+, pH, Mg2+)
ATP analogs (AMPPCP, AMPPNP)
Metallofluorides (MgF_x, AlF_x, BeF_3^-)
Inhibitors (TG, CPA, vanadate)



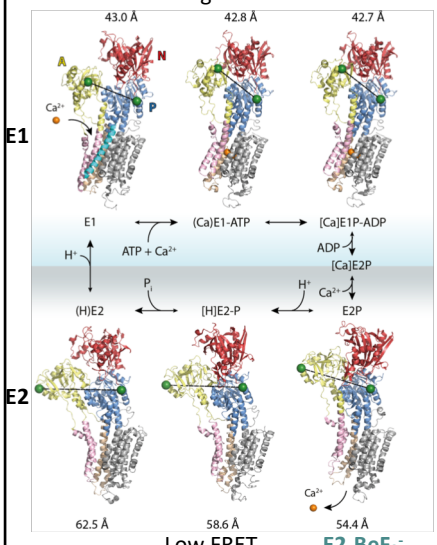
- **When and how are ADP and Ca2+ ions being released?**
- **What is the rate limiting step?**
- **Structures of the [Ca2]E1P & [Ca2]E2P intermediates?**
- **Reversible or irreversible pumps?**

Movie: Maike Bublitz, Jesper Karlsen

Winther, Bublitz et al. 2013, *Nature* **495**, 265
Bublitz et al. 2013, *JBC* **288**, 10759-65
Dyla et al. 2017, *Nature* **551**, 46-351

Identification of functional states in LMCA1

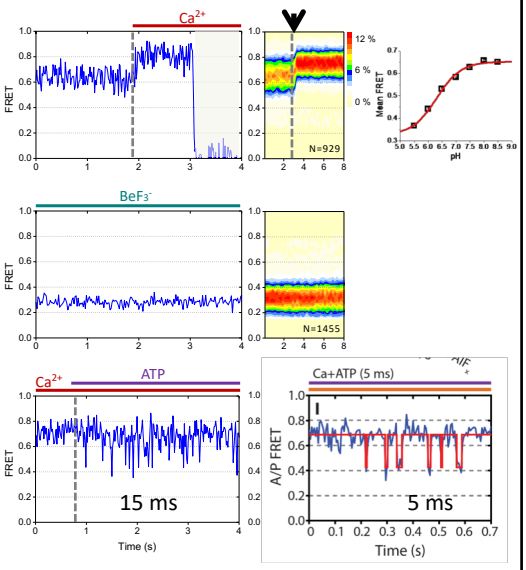
High FRET



Low FRET

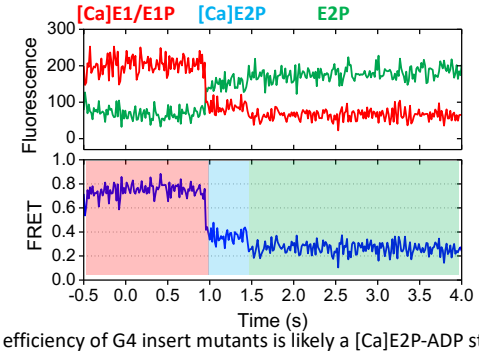
$E2\text{-}BeF_3^-$

Fast Ca^{2+} and ATP binding



G4 insert + E167Q mutant shows a ~500 msec dwell at a 0.4 FRET intermediate before reaching a dead-end 0.3 FRET/E2P

G4 + E167Q

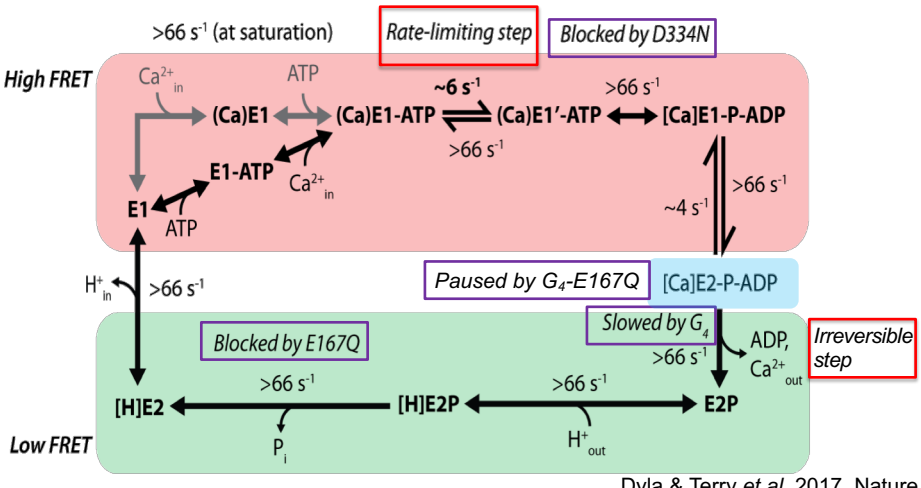


There must be an ADP releasing pathway or pocket that controls the ATPase cycle

- The 0.4 FRET efficiency of G4 insert mutants is likely a [Ca]E2P-ADP state - explains the high/low FRET dynamics combined with low ATPase activity, i.e. a reversible sampling of:
- **E1 + Ca^{2+} + ATP <–> CaE1-ATP <–>[Ca]E1P-ADP** <–> [Ca]E2P-ADP
- The transition of the 0.4 FRET dwell to the stable 0.3 FRET of G4-E167Q appears to show release of ADP/Ca^{2+} from the [Ca]E2P-ADP intermediate – **[Ca]E2P deocclusion is essentially** the **irreversible step** of the pump, **not phosphorylation or E1P-E2P**
- The A-domain of the [Ca]E2P-ADP state must dwell at an intermediate rotation, from where it can proceed to release ADP, but also return to [Ca]E1P-ADP →….E1 + Ca2+ + ATP

Dyla & Terry *et al.*, *Nature 2017*

Kinetic cycle of LMCA1



Dyla & Terry *et al.* 2017, Nature

When is ADP released?

Presumably before Ca2+, since SERCA has an ADP sensitive intermediate

Are there fundamental differences beween 1- and 2-site pumps?

Time-resolved X-ray solution scattering (TR-XSS) studies of conformational transitions

t=0 laser flash to release caged ATP

Simulating structures/fitting

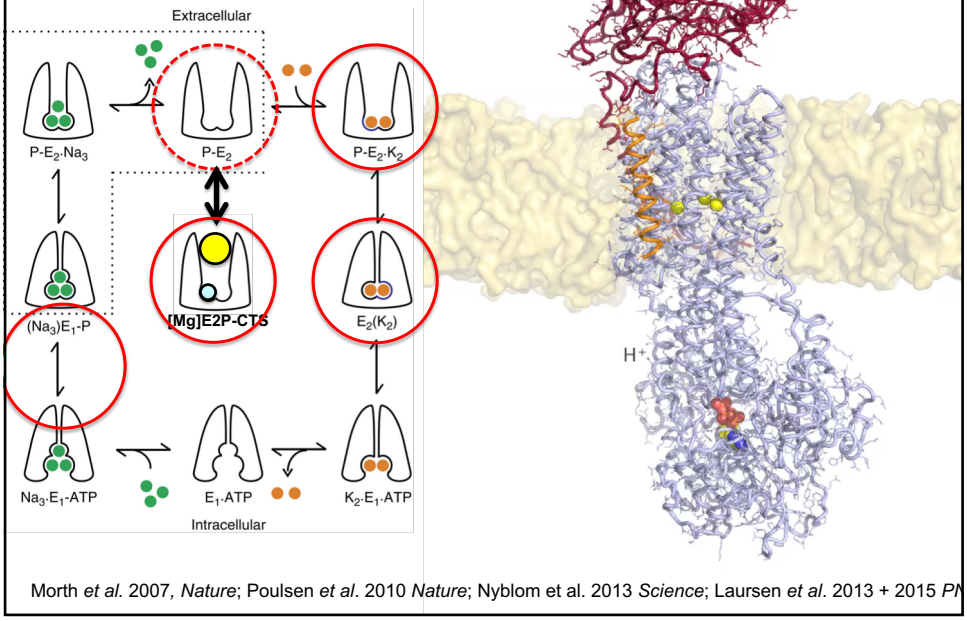
Active Inhibited

E1 E1-ATP *E2P*
pre inter *late*

3-state system

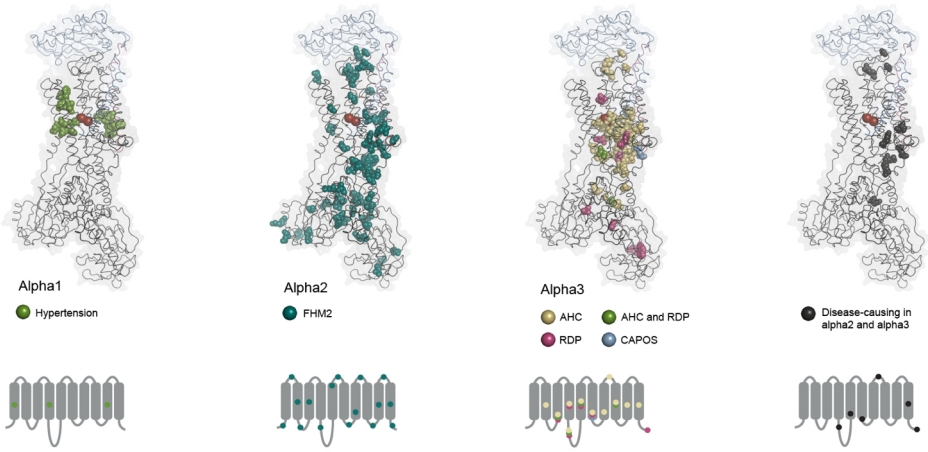
Magnus Andersson (Stockholm, Umeå)

Na^+,K^+-ATPase



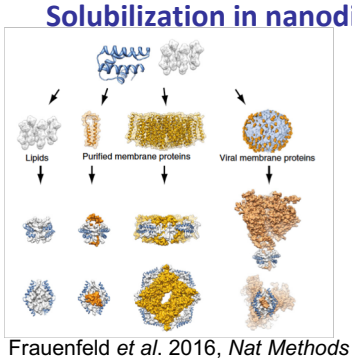
Morth *et al.* 2007, *Nature*; Poulsen *et al*. 2010 *Nature*; Nyblom et al. 2013 *Science*; Laursen *et al*. 2013 + 2015 *PN*

Disease mutations of Na,K-ATPase loss of function - and gain of function

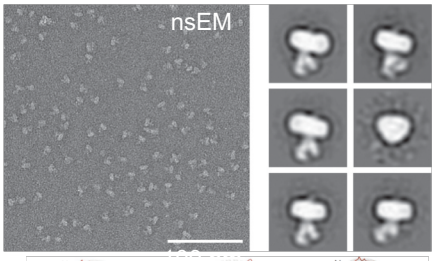


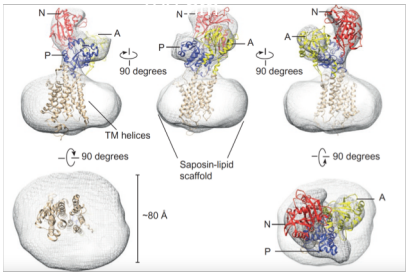
Hanne Poulsen et al.

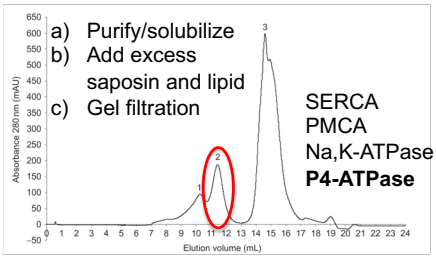
How can we release ourselves from detergents?
Solubilization in nanodiscs and saposin-lipid nanoparticles



Frauenfeld *et al*. 2016, *Nat Methods*

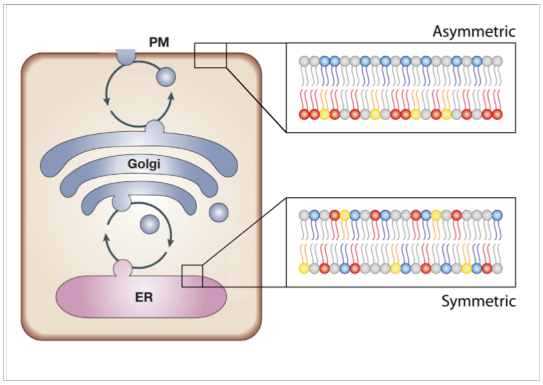


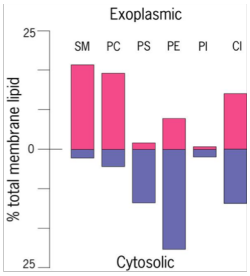




Lyons, Bøggild, Nissen & Frauenfeld 2017
Meth. Enzymol. **594**, 85-99

Membrane asymmetry





Adapted from Holthuis and Levine, *Nat Revs* (2005) 6:209

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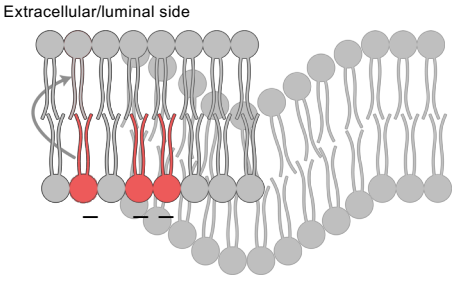
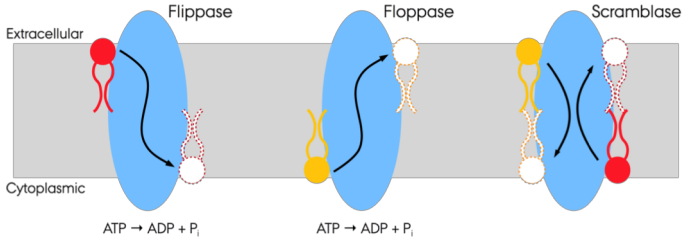
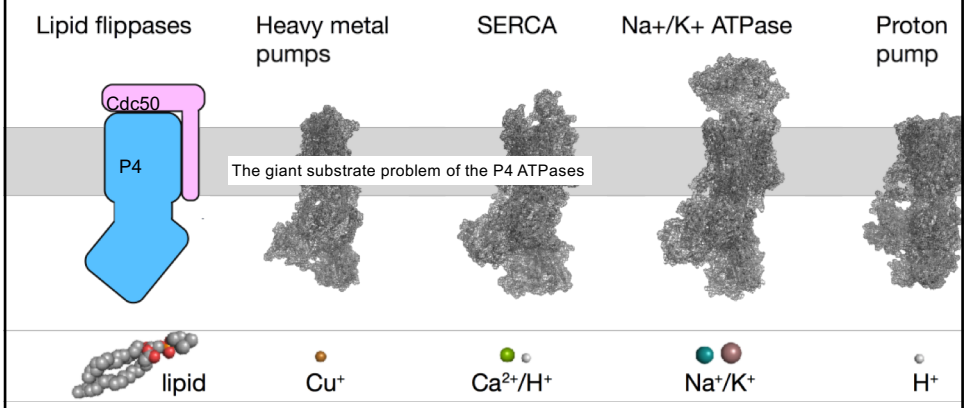
The lipid distribution in eukaryotic membranes is asymmetric and tightly controlled



- Membrane potential
- Function of membrane proteins
- Engulfment signal to macrophages
- Blood coagulation
- Membrane curvature



P4 ATPase are lipid flippases



Catalytic P4 ATPase:
ATP hydrolysis and lipid transport
- 14 human P4 ATPases
- Linked to numerous diseases

Complex with Cdc50-protein
- Chaperone for exit from ER
- Active role in regulation or transport?

Edited from figure by Miriam-Rose Ash

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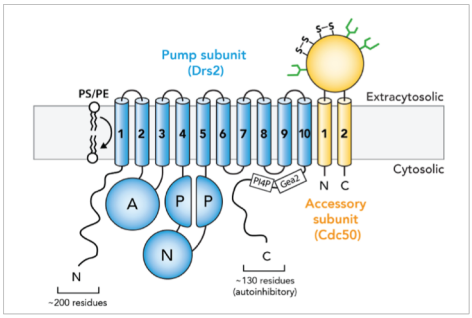
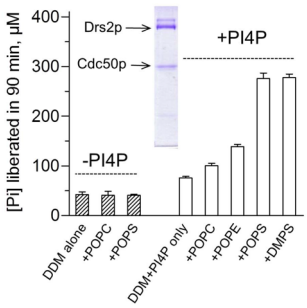
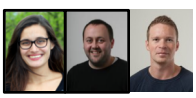
P4-type ATPases – yeast Drs2p-Cdc50p

Orthologue of human ATP8A/B
PS lipid flippase

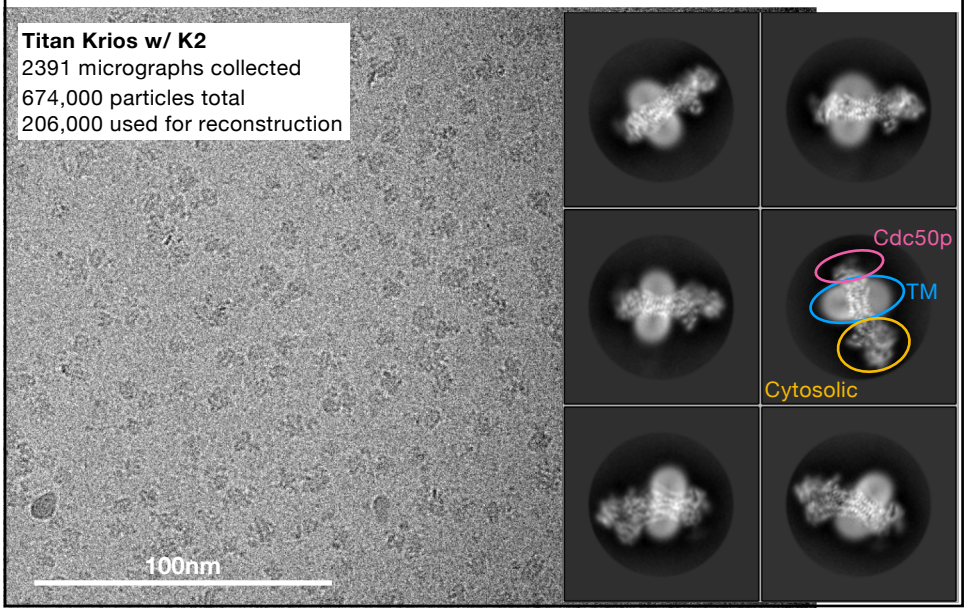






Milena Laban, Joe Lyons, Jakob Ulstrup Jensen
Collaboration with Guillaume Lenoir et al. CEA-Saclay
Arne Möller and & Werner Kühlbrandt, MPI-Biophysics, Frankfurt

Cryo-EM data of Drs2p/Cdc50p in LMNG



12.5 – 7 – 3.5 rmsd

TM4
Unwinding around
conserved proline

Binding of regulatory PI4P

Drs2-Cdc50 Lys1018 exposed in the transmembrane cleft – attracts a phospholipid headgroup?

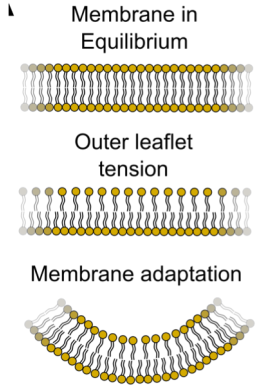
Autoinhibited **Activated**

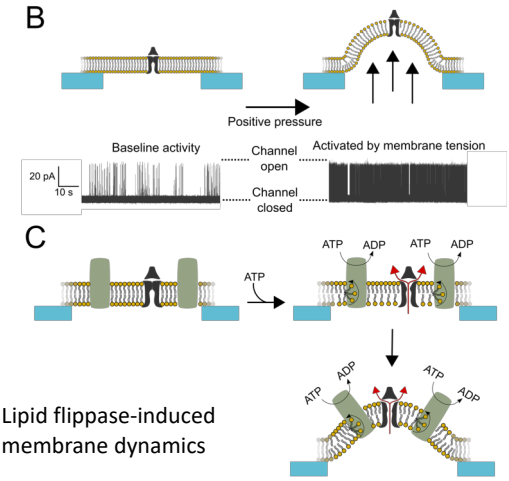
Release of Autoinhibition and Lipid Transport Opening Upon Regulatory PI4P Binding

Autoinhibited/activated

22

Structural dynamics of membranes induced by lipid flippases

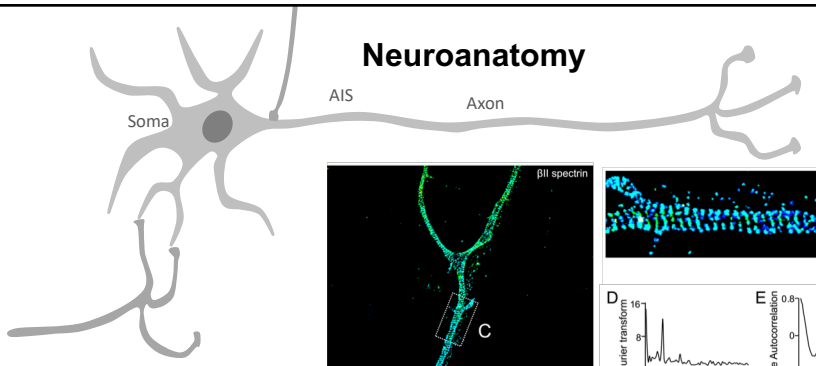




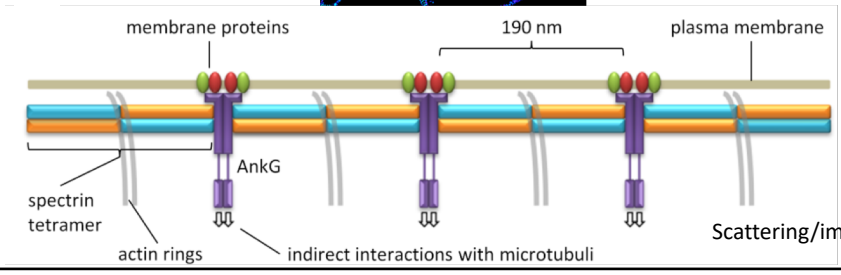
Lipid flippase-induced membrane dynamics

Photocaged ATP, liposomes or nanodiscs?

Neuroanatomy



Axon Initial Segment



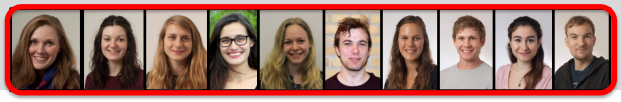
Scattering/imaging

Structural neurobiology lab

Postdocs (*fellowships*): **Antoni Kowalski** *(Polish Research Council)*, **Temitope Ayeotan**, **Michael Habeck** (*Marie Curie*), **Azadeh Shahsavar** (*EMBL-EI3POD*), **Montaña Bermejo** (*Extremadura*),

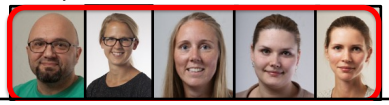
Assist. Prof. **Esben Quistgaard, Thomas Lykke-Møller Sørensen, Joseph Lyons** *(Lundbeck Foundation)*
Michael Clausen (*AIAS*)

PhD students (*scholarships*): **Sigrid Thirup** (*GSST*), **Jacob Ulstrup, Marlene Sørensen** (*GSST*), **Caroline Neumann** (*Lundbeck Foundation*), **Milena Laban** (*Boehringer-Ingelheim Fonds*), **Josephine Nissen** (*GSST*), **Jonathan Juhl** (*GSST*), **Sara Basse** (*Boehringer-Ingelheim Fonds*), **Samuel Hjort-Jensen** (*EU-ITN RAMP*), **Sofia Trampari** (*EU-ITN RAMP*), **Jeppe A. Nielsen** *(industry-PhD, Novo Nordisk* **Alya Kotsubei** (*FWO/IWT, KU Leuven*), **Paula Szalai** (*Lundbeck Foundation, Univ. Oslo*), **Lars Sørensen** (*industry-PhD Novo Nordisk*)



MSc and BSc students
Line Marie Christiansen, Mads Christensen, Helena Doherty-Bock, Line Cecilie Hansen, Christian Engelbrecht Larsen , Peter Schultz, Jens Brinch Larsen

Scientific, technical and administrative staff
Scientific staff: **Jesper L. Karlsen** (computing), **Christine J.F. Nielsen** (*contract research*)
Techs: **Anna Marie Nielsen, Tetyana Klymchuk**; *PA:*, **Karen Bech** (DANDRITE)



Alumni 2004-2018

- **Fellowships**

  Danish Research Councils
  Carlsberg Foundartion, Lundbeck Foundation,
  Polish Research Council
  Boehringer-Ingelheim Fonds,
  Aarhus Institute of Advanced Studies/AIAS,
  EMBL-EI3POD
  EMBO

- **Synchrotron beam time/facility access**

  EMBL-Hamburg, Diamond, SLS, ESRF, MAX-IV
  iNEXT, Molecular Machines COST, ESFRI-INSTRUCT
  LINX consortium – Innovation Funds, Denmark
  CryoNET