LBEM

Laboratory of Biological Electron Microscopy

Cryo-Electron Microscopy method development for Life Sciences

Henning Stahlberg

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EPFL

UNIL | Université de Lausanne

Short CV

Cryo-EM Method Development, Neurodegeneration

Henning Stahlberg (born 1965 in Germany. Swiss and German Nationality)

- 1993: Physics Diploma at the TU Berlin, Germany
- 1997: PhD in Cryo-EM of Membrane Proteins with **Dubochet** (UNIL) and **Vogel** (EPFL)
- 2002: Habilitation in Struct. Biology of Membrane Proteins with Andreas **Engel** (Biozentrum)
- 2003-2009: PATT & Assoc. Prof. of Molec. & Cell. Biology, UC Davis, CA, USA
- 2009-2021: Prof. of Struct. Biology, Biozentrum Basel and Director C-CINA, Uni Basel

- 2020– : Prof. of Physics at EPFL, Prof. Fund. Microbiol. at UNIL

  to set up the
  Laboratory of Biological Electron Microscopy
  and the *Dubochet Center for Imaging*

Transmission Electron Microscopy: TEM

- Transmission imaging
- Electrons at 300 keV energy.
- 3D reconstruction through image processing.
- Resolution on proteins: 3 Å
- Sample: <300 nm thin

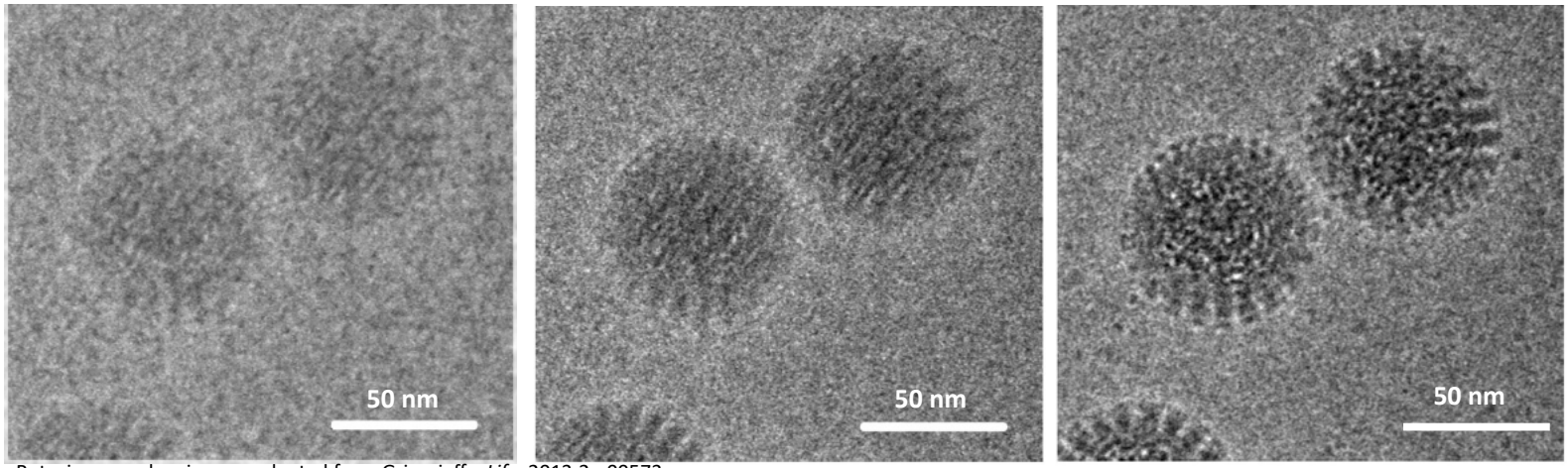
A Resolution Revolution in cryo-EM from Direct Electron Detector Cameras

Virus frozen in ice

until 2012
Photographed on a CCD camera or on film

2013
Photographed on a direct electron detector

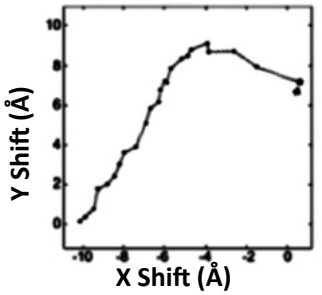
since 2014
***Filmed* on a direct electron detector**



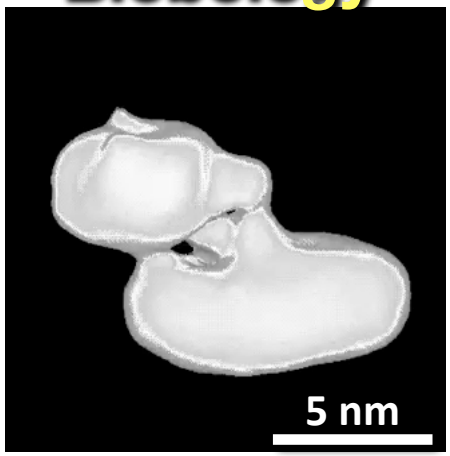
Rotavirus, mockup images adapted from Grigorieff, *eLife*, 2013;2:e00573.

Image after alignment and averaging of 100 movie-frames.

Movement profile:

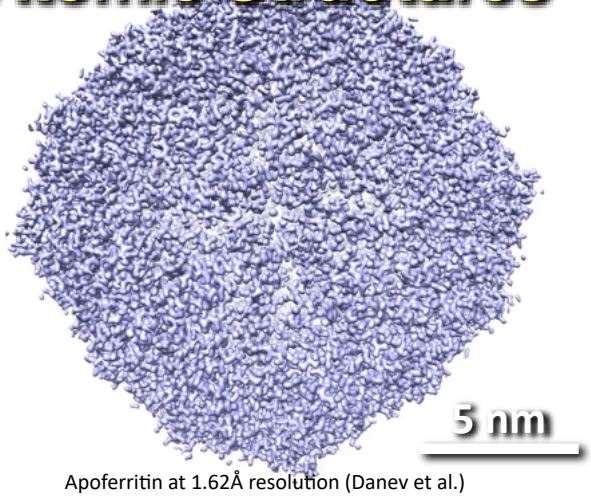


Blobology



FMO membrane protein complex at ~2 nm resolution

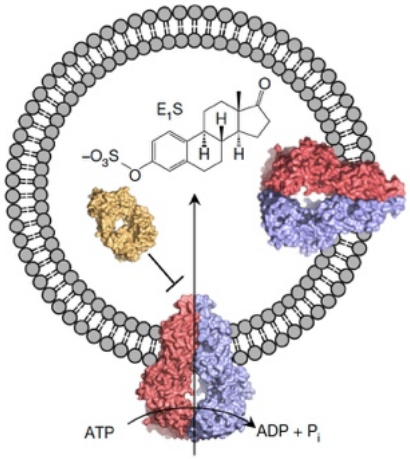
Atomic Structures

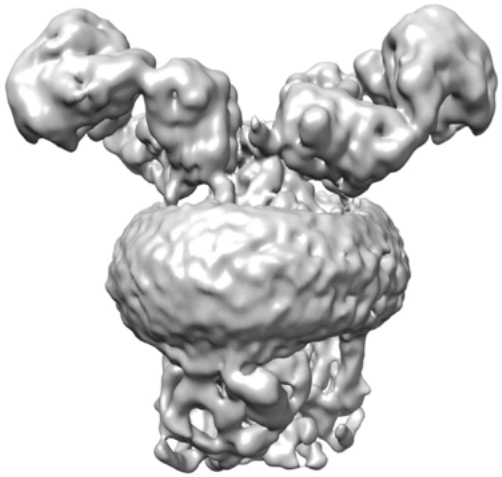


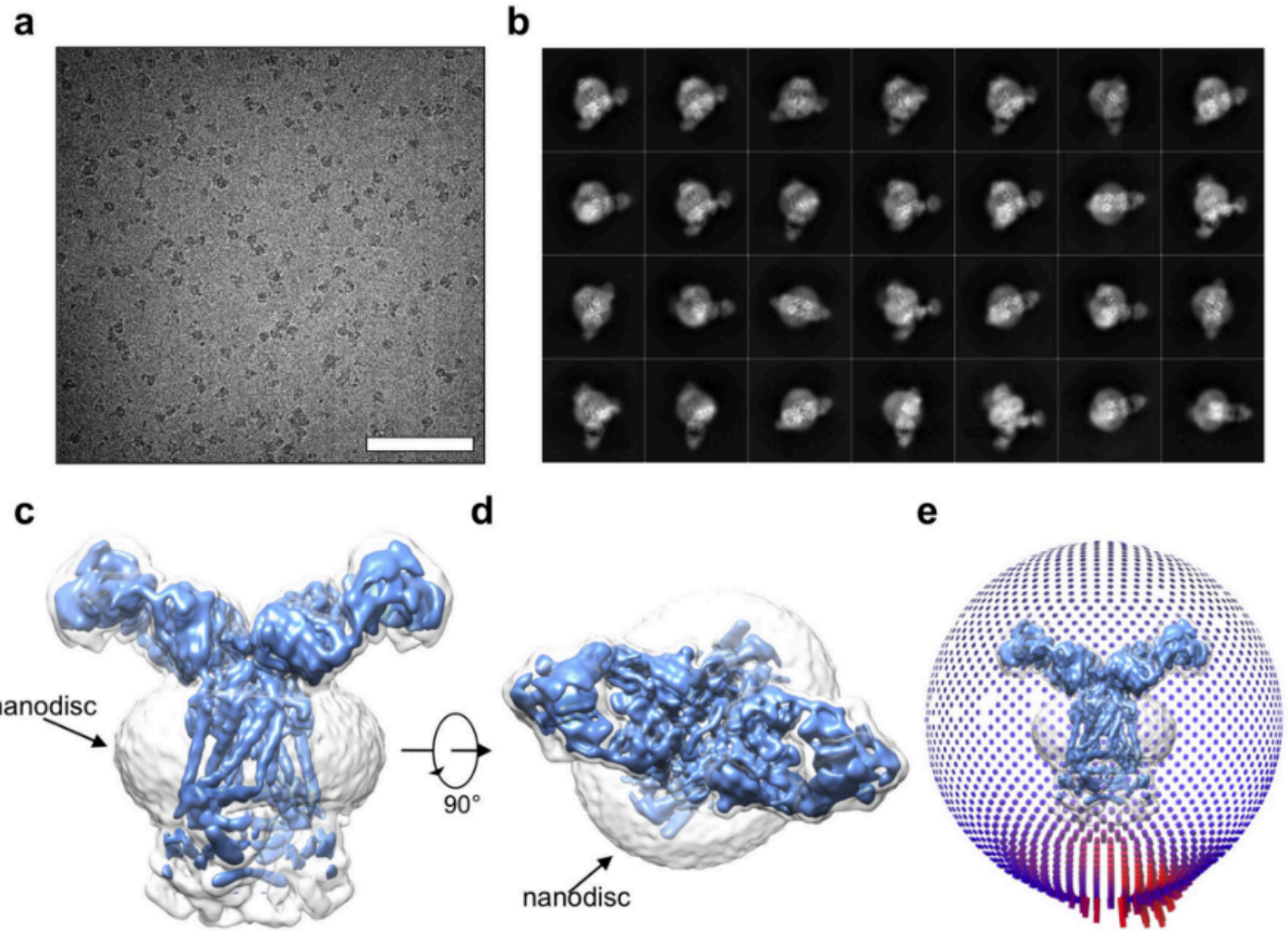
Apoferritin at 1.62Å resolution (Danev et al.)

Cryo-EM Structure of a human ABC Transporter

ABCG2 with Fab fragments in lipid nanodisk
Data collection: 3 days on Titan in C-CINA,
ca. 3000 movies, ca. 200'000 particles, ca. 30'000 particles in final map

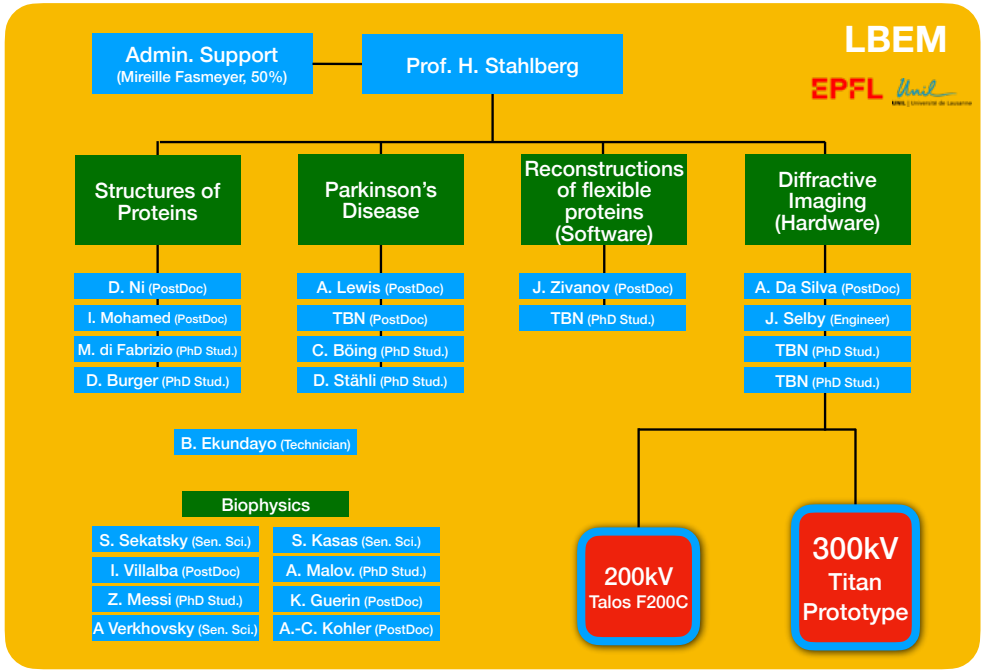


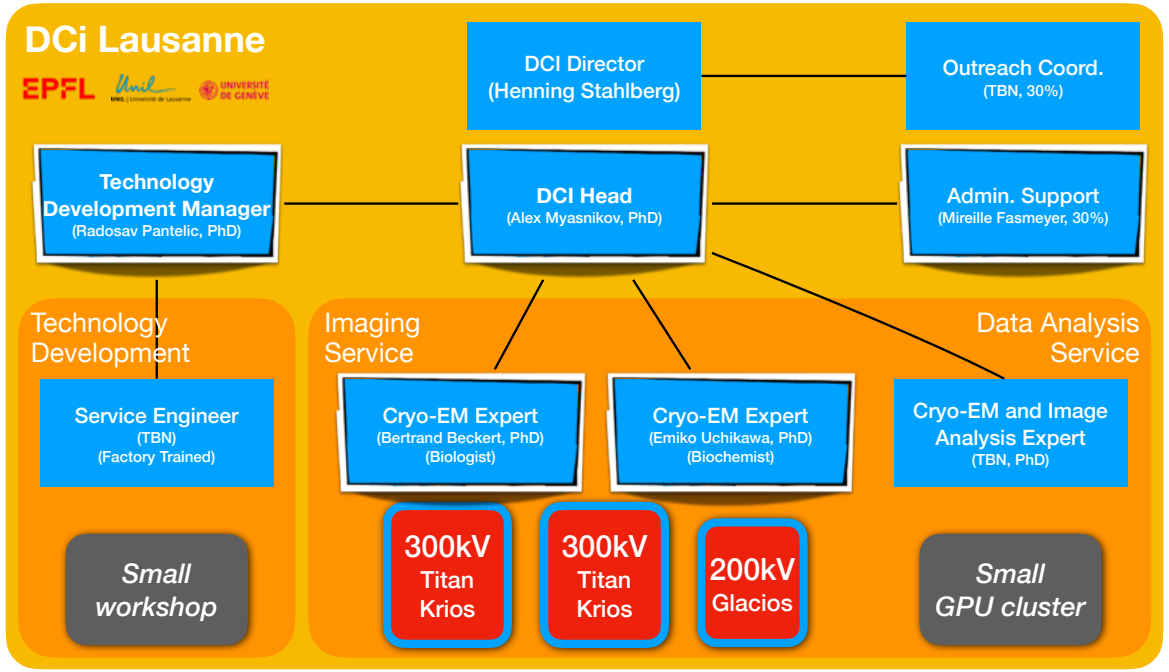




Taylor et al., Nature (2017), collaboration with Kaspar Locher (ETHZ)

LBEM & DCI





5/2021

Talos (200kV)
With STEM

1/2022

Prototype Titan Krios (300kV)
E-CFEG, RF-Cavity, Cs-probe corrector, Falcon4

5/2021

Titan Krios (300kV)
E-CFEG, SelectrisX filter, Falcon4

6/2021

Titan Krios (300kV)
E-CFEG, Falcon4

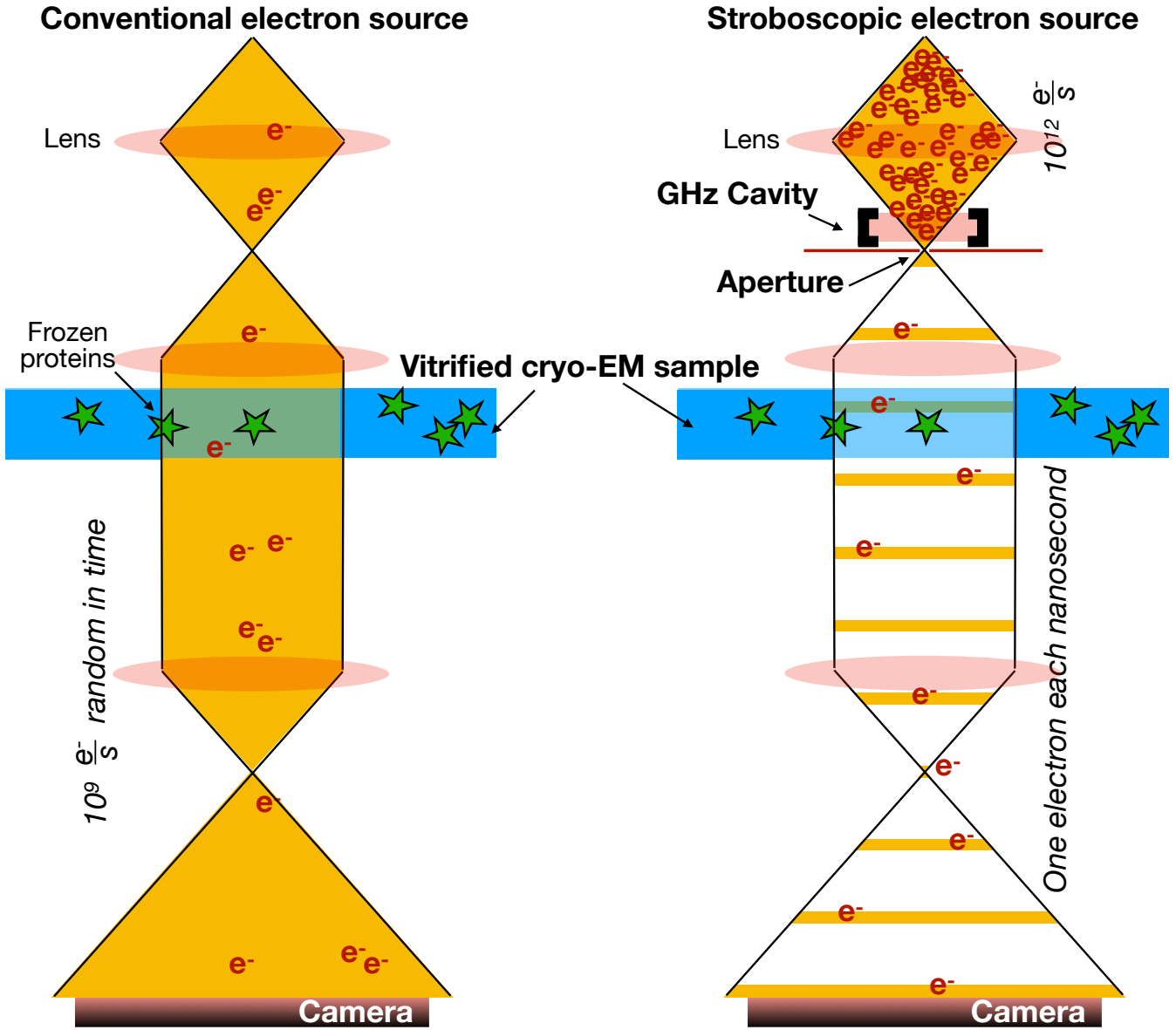
6/2021

Glacios (200kV)
X-FEG, Falcon4

The LBEM Instrument: RF Cavity: Producing single electrons at GHz rate

Titan Krios G4 — 300kV, CFEG, F4 camera, RF Cavity, Probe Cs corrector

Single electron illumination reduces the beam damage 2.5x or more (?)



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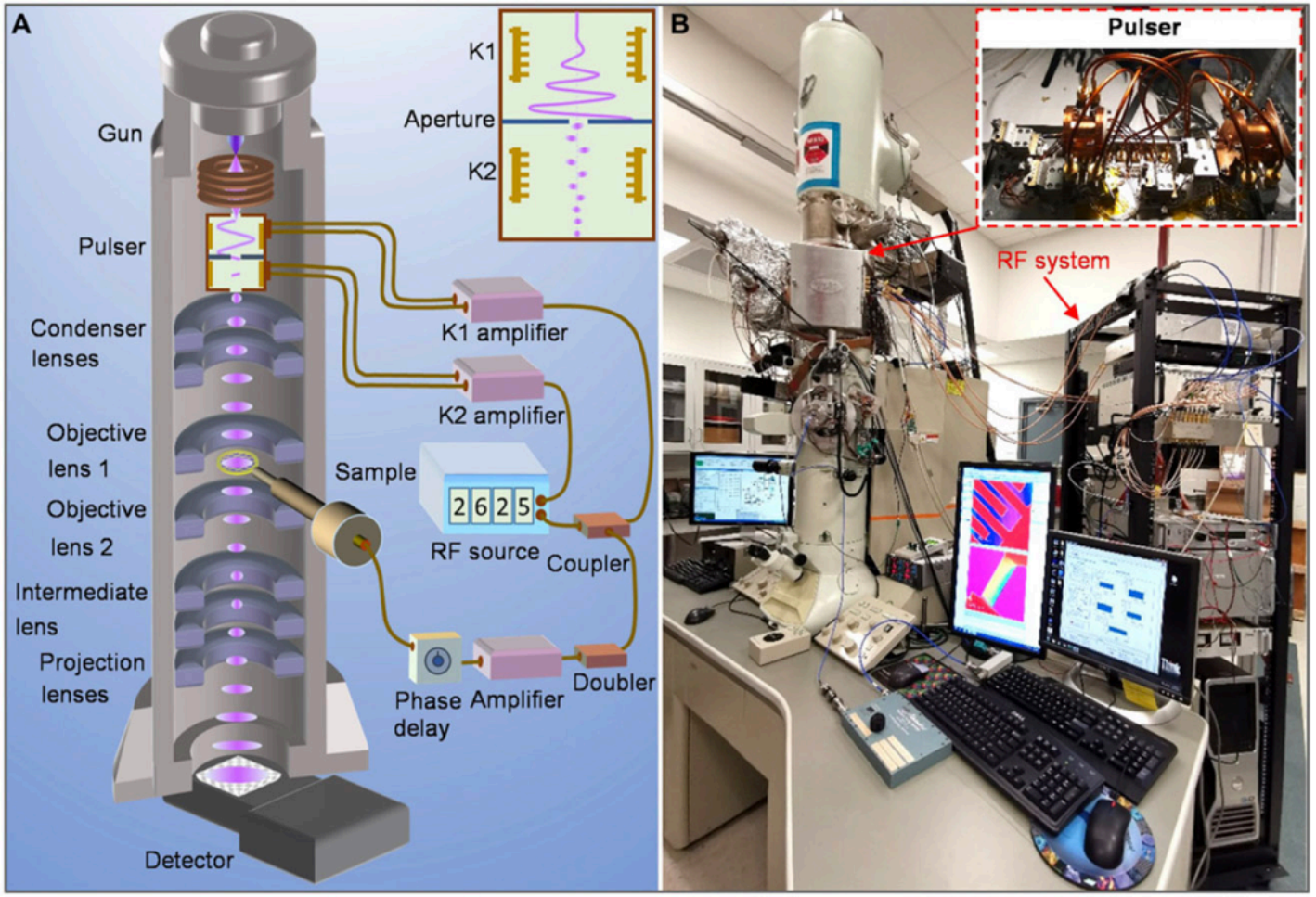

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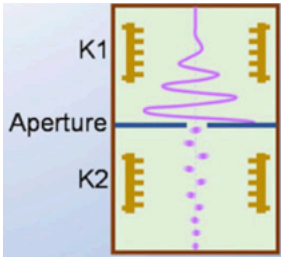
3 GHz frequency



Strobo 300 Pulser
Euclid Techlabs,
Bolingbrook, Illinois, USA

Lau et al., (2020)
Rev. Sci. Instrum. 91, 021301

Paraffin

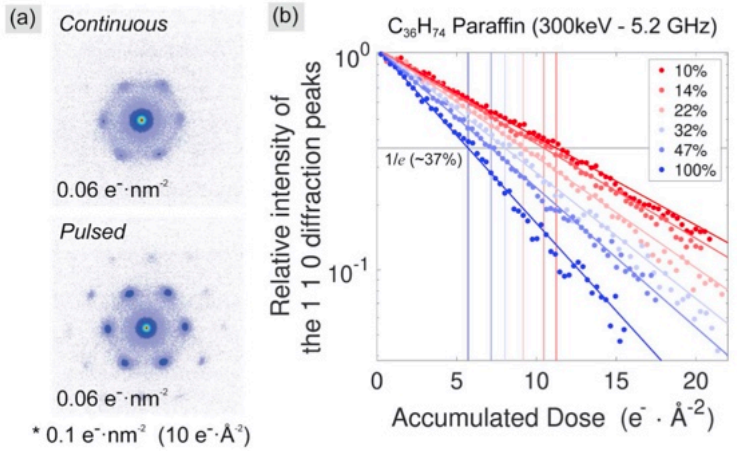


Figure 3. Mitigation of radiation damage at different duty factors of pulsed electrons. (a) Selected continuous- and pulsed-beam diffraction patterns of a paraffin crystal with the 110 family Bragg peaks highlighted. (b) Fading curves from the 110 family reflections and exponential decay fits as a function of pulsed beam duty cycle factor at 5.2 GHz repetition rate for the same dose rate (0.2546 $e^-/Å^2$/frame), grey guideline indicating critical dose (D_{cri}).

Bacteriorhodopsin

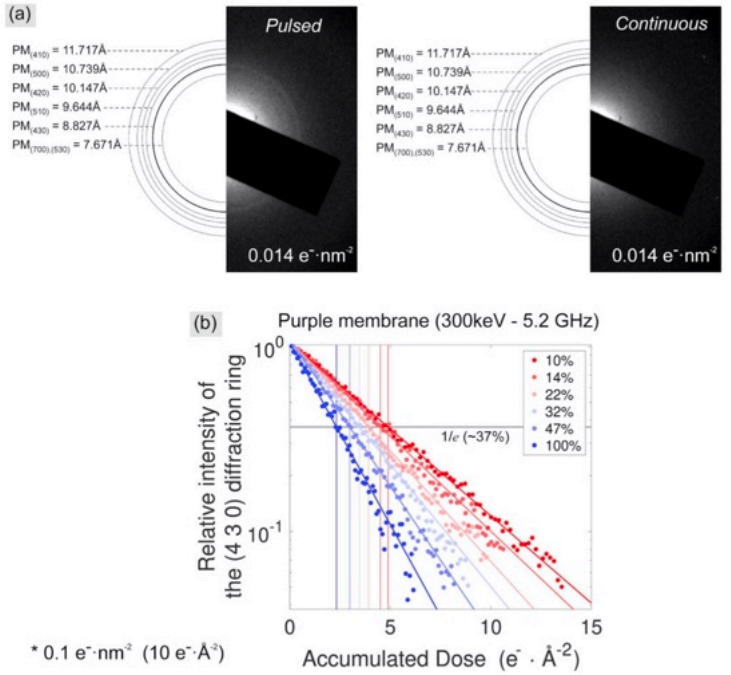


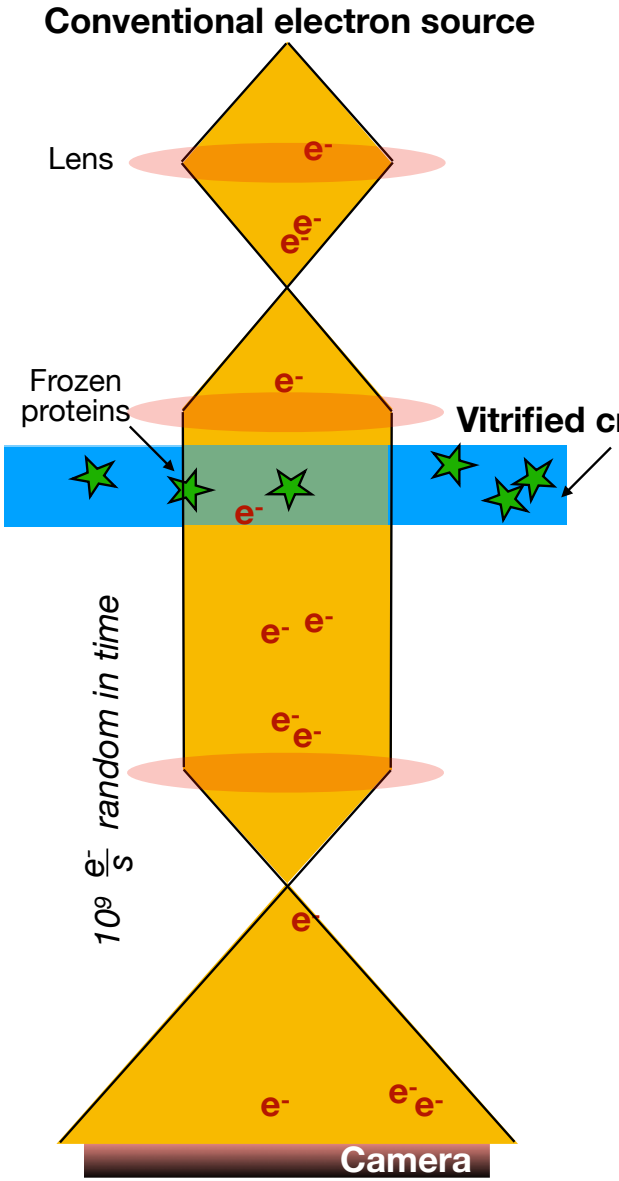
Figure 4. Comparison of the continuous random and pulsed random electron beam damage for the same dose rate (0.1045 $e^-/Å^2$/frame). (a) Selected continuous- and pulsed-beam powder diffraction patterns of a purple membrane with the (430) rings at total accumulated dose 0.014 e^-/nm^2 (1.4 $e^-/Å^2$). Rings are diffuse, possibly due to sample thickness or vibrational blurring from soft phonon modes at room temperature. (b) Fading curves from the (430) powder rings and non-linear exponential decay fits for each measured duty cycle factor from 10 to 100%.

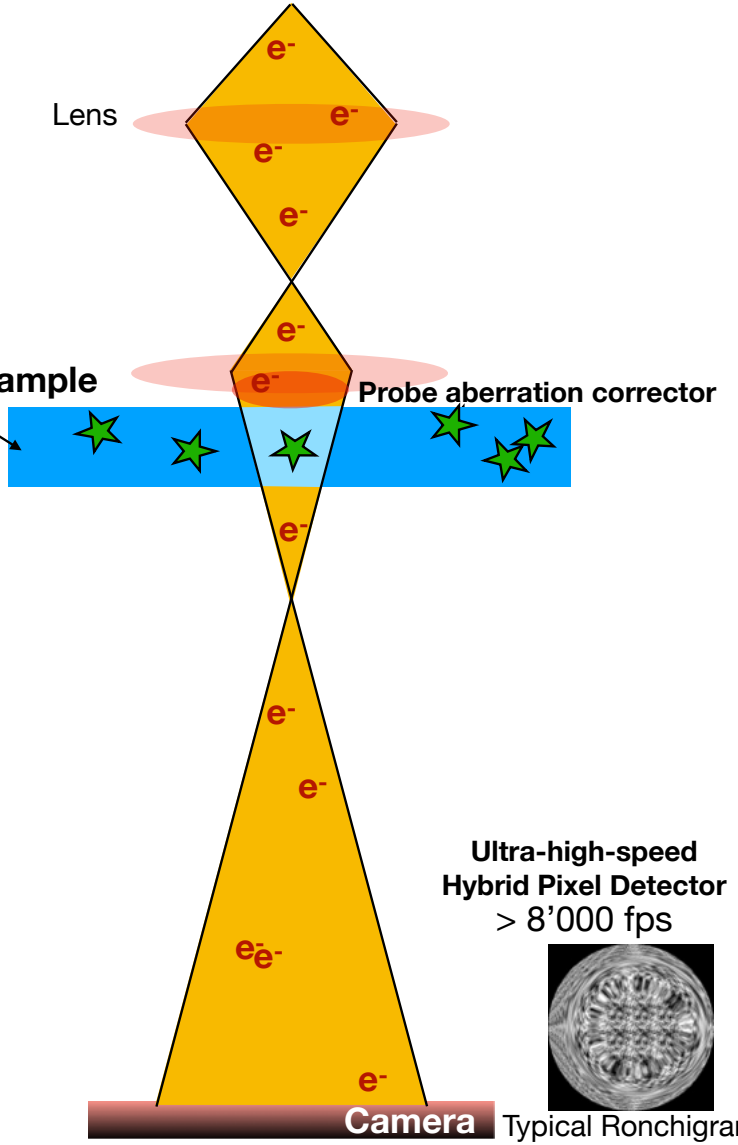
The LBEM Instrument: Coherent Electron Diffractive Imaging

Titan Krios G4

300kV, CFEG, F4 camera,
RF Cavity,
Probe Cs corrector

Electron diffraction
with one protein at a time



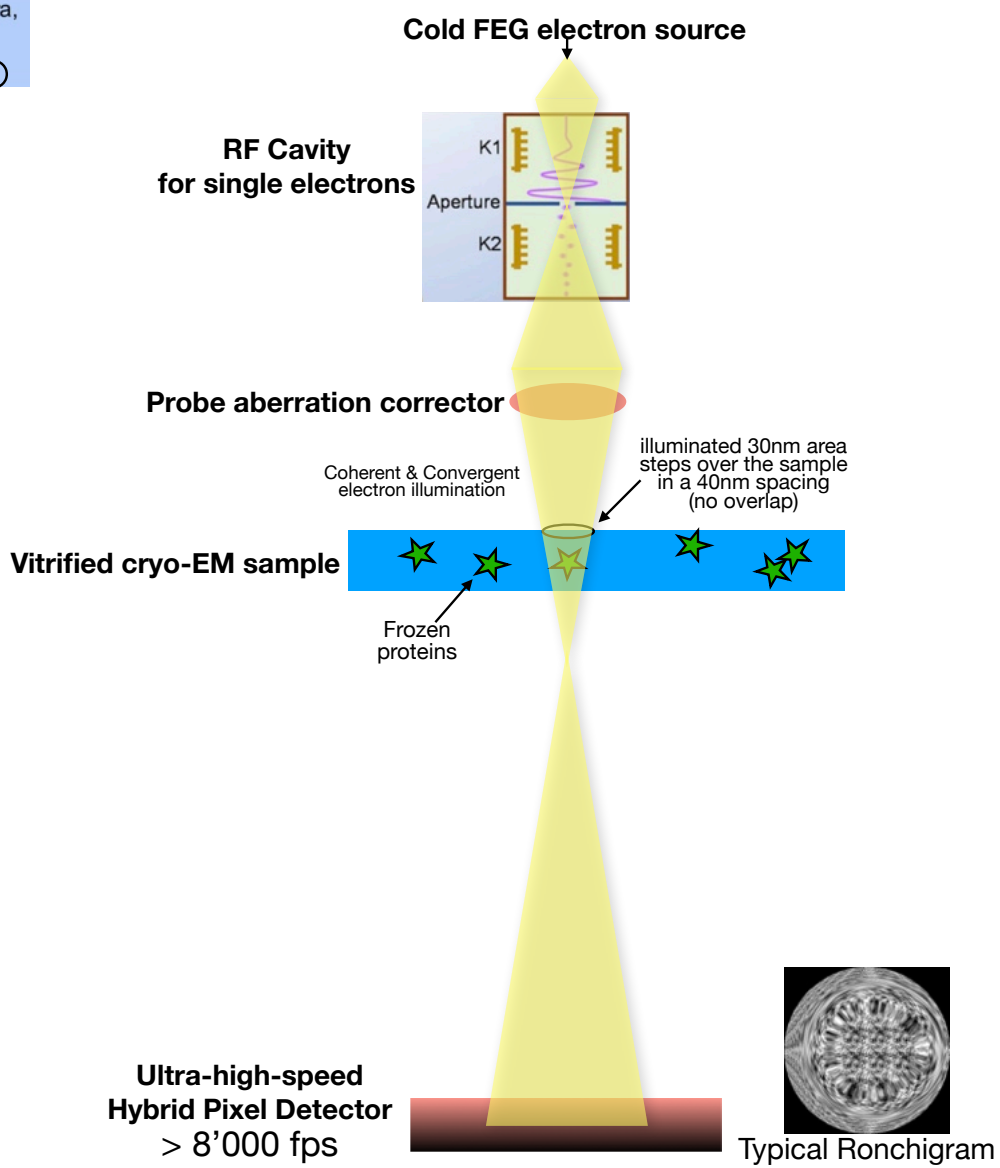


Ultra-high-speed
Hybrid Pixel Detector
> 8'000 fps

Typical Ronchigram

The LBEM Instrument: Coherent Electron Diffractive Imaging

Titan Krios G4 — 300kV, CFEG, F4 camera, RF Cavity, Probe Cs corrector



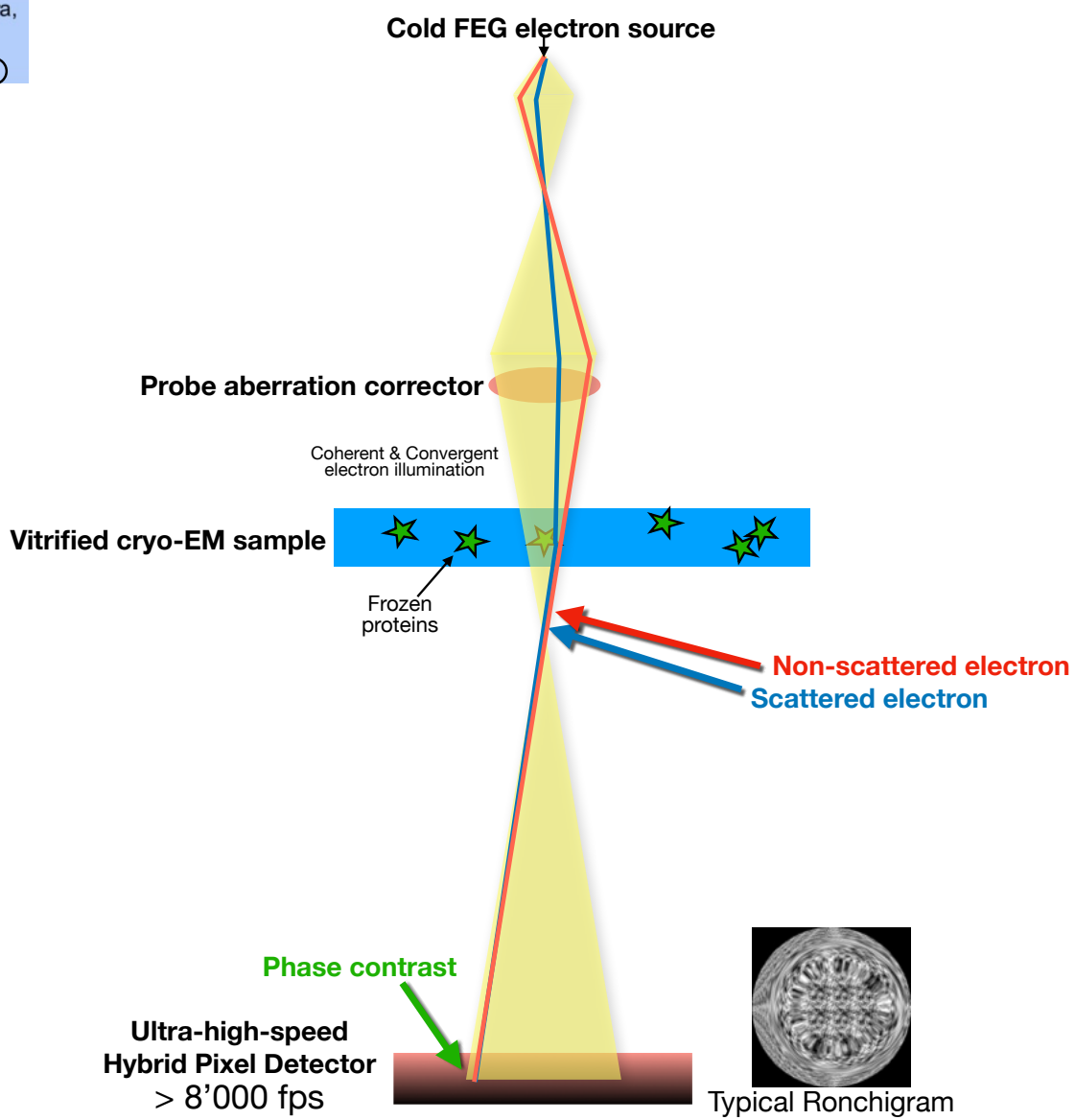
Typical Ronchigram

Goal: Diffractive Imaging to obtain *phase-contrasted* diffraction patterns, which give more SNR and no resolution loss from drift.

With
Radosav Pantelic, EPFL,
Bernd Schmitt, PSI

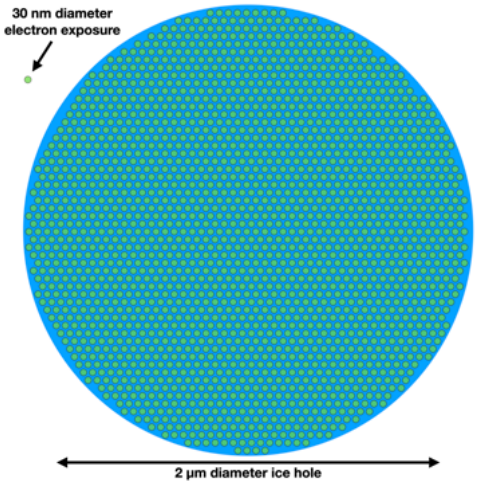
The LBEM Instrument: Coherent Electron Diffractive Imaging

Titan Krios G4 — 300kV, CFEG, F4 camera, RF Cavity, Probe Cs corrector



Typical Ronchigram

The sample is exposed at multiple locations with the electron beam:



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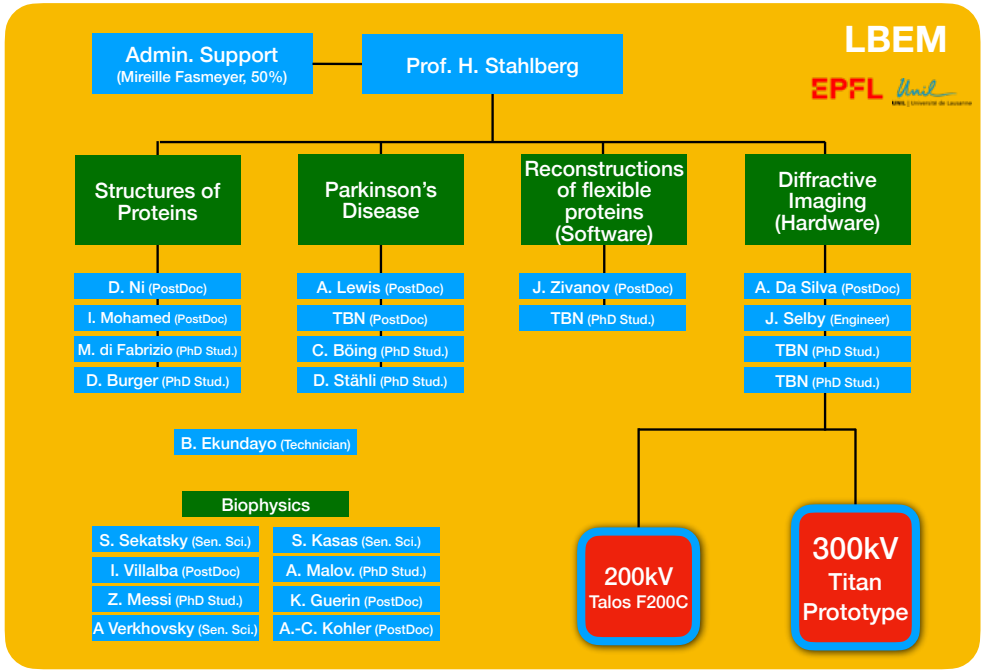

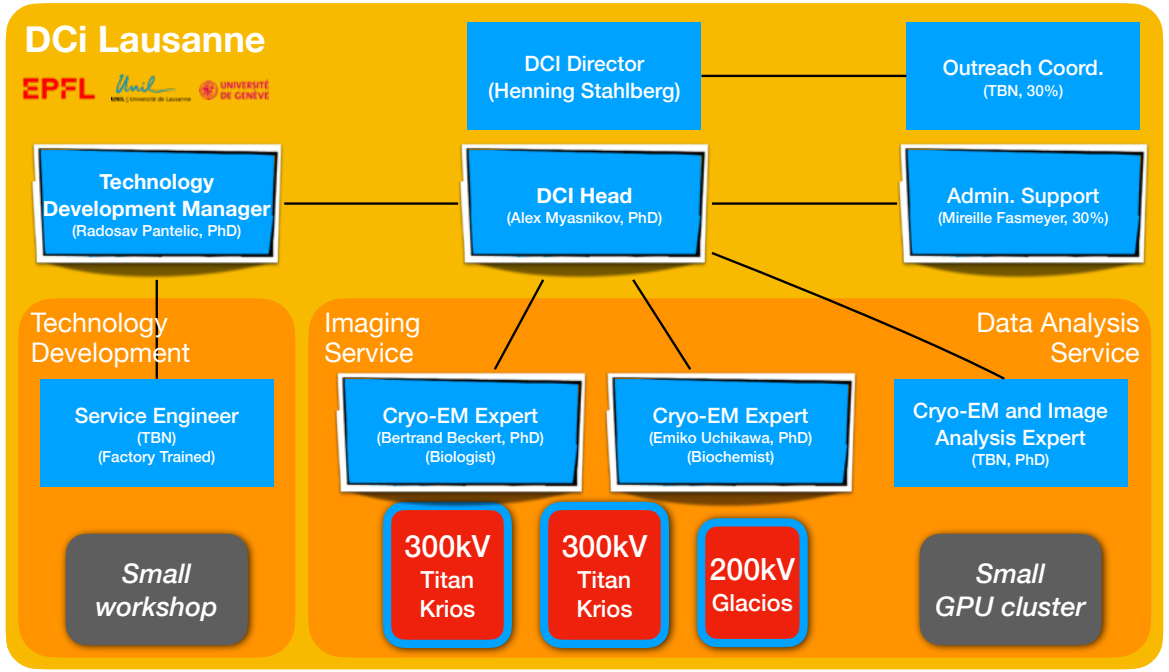

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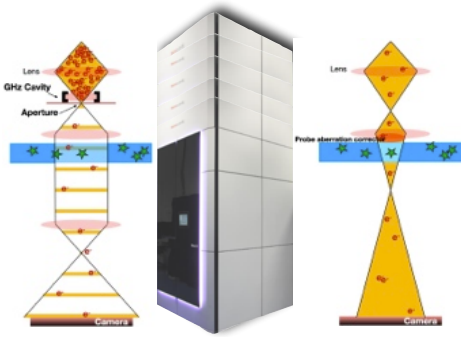

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Titan Krios (300kV)
E-CFEG, SelectrisX filter, Falcon4

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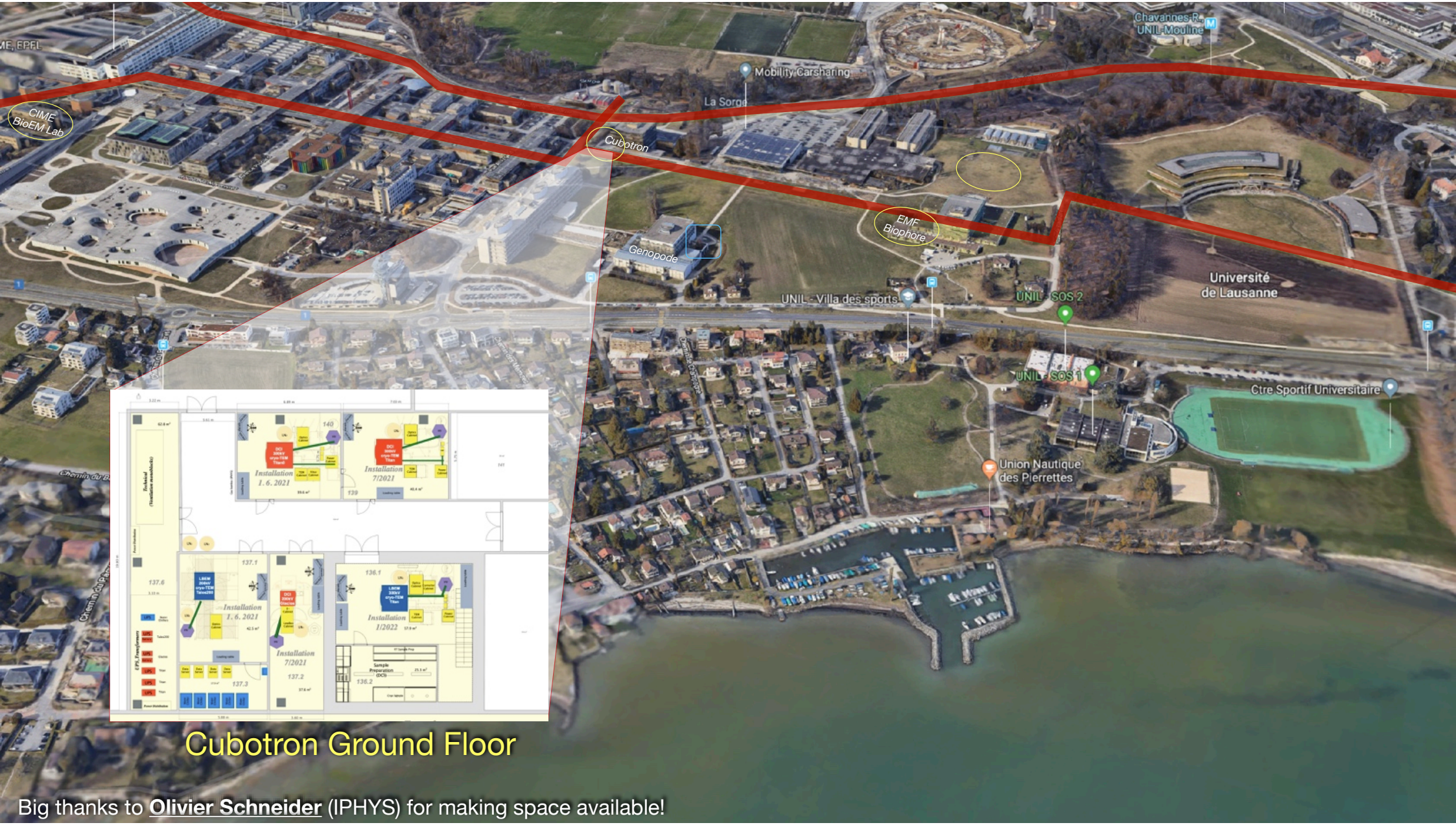
Glacios (200kV)
X-FEG, Falcon4



Cubotron Ground Floor

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