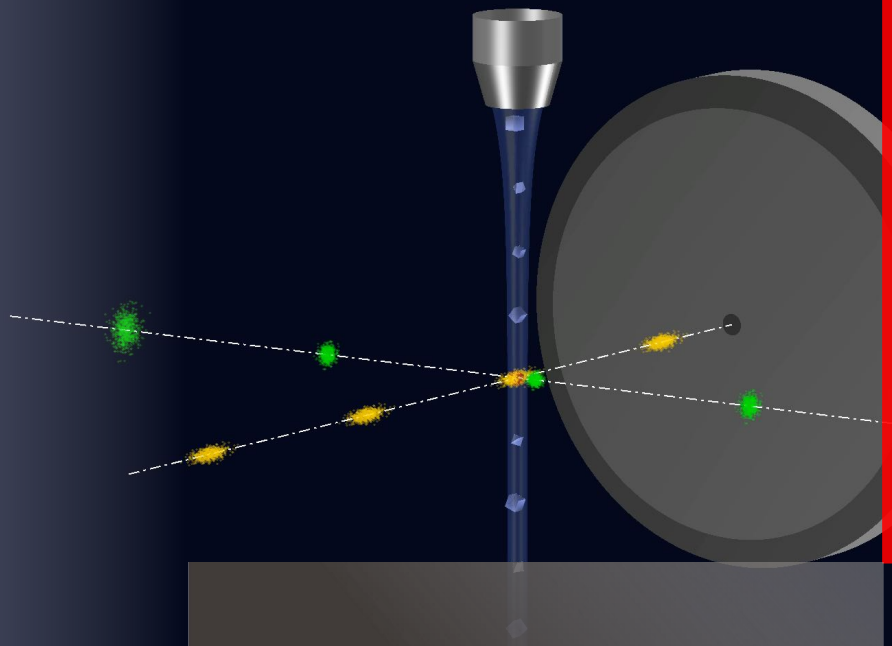
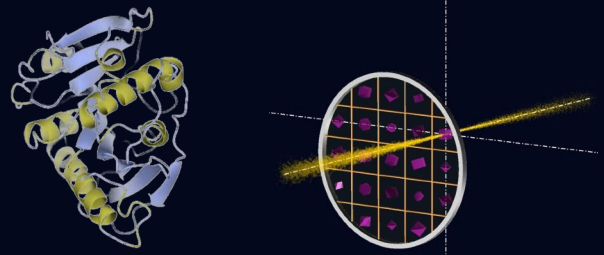
The SLS 2.0 upgrade & its impact on structural biology and drug discovery



SPG Jahrestagung

ETHZ

10.09.2024



Philip Willmott, Swiss Light Source, PSI

Contents

- What is a synchrotron?
- What defines a fourth-generation synchrotron?
- SLS 2.0 – the upgrade
- MX in today's milieu
- SLS 2.0 – the impact on MX and structural biology

Synchrotrons
Key features

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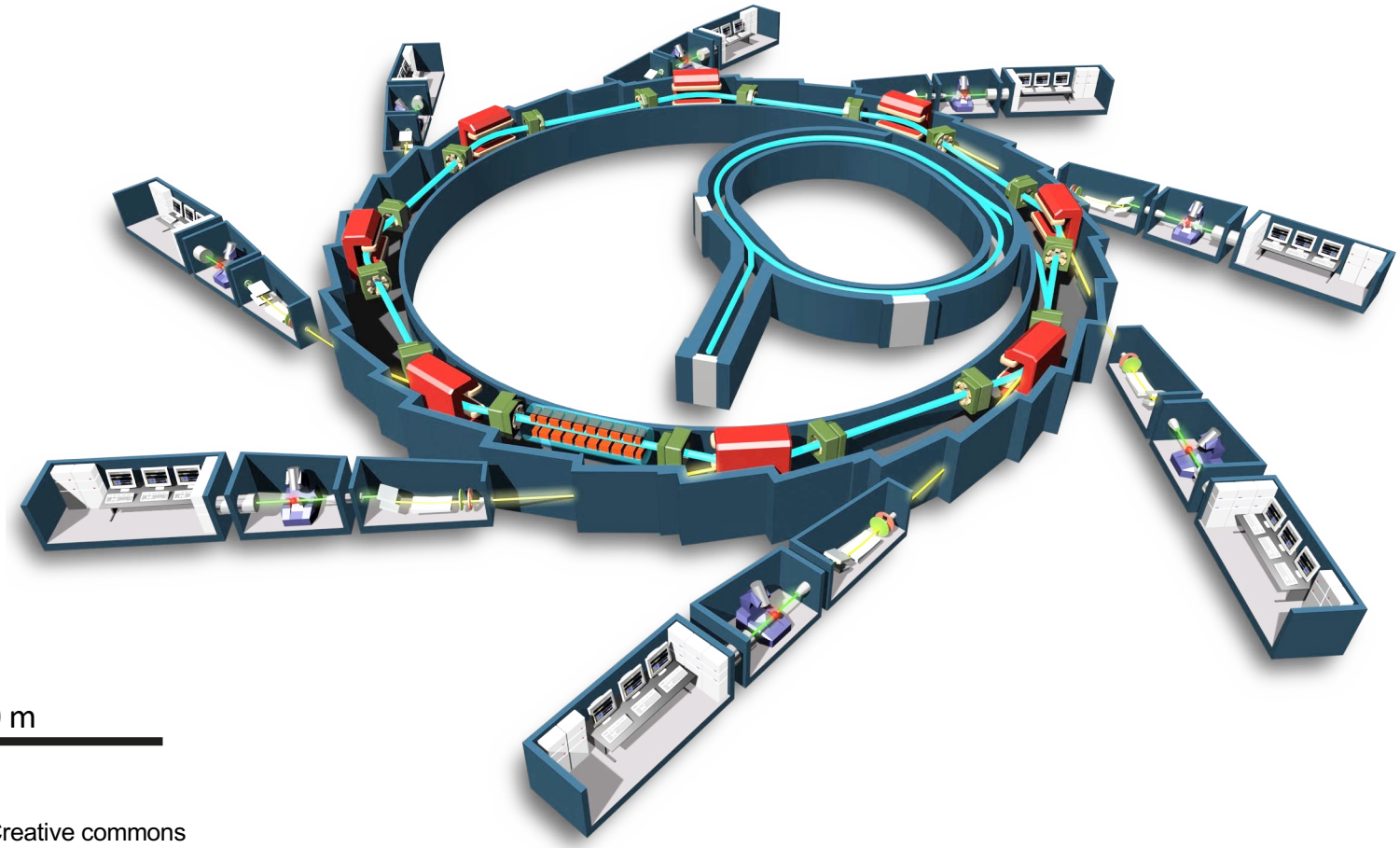
What is a synchrotron?

- Large-scale facility for generating high-intensity electromagnetic radiation
  - Most commonly in range of VUV to hard x-rays; also down to IR in some cases
  - “Synchrotron radiation”
- Key features of SR
  - Brightnesses many orders of magnitude greater than can be provided by lab-based x-ray sources
  - Extremely collimated beams
  - Extremely narrow beams
  - Tunability of the photon energy
    - e.g., SLS 2.0: ≃ 5 eV to > 80 keV
  - Multiple experiments (“beamlines”) around the closed-loop structure of a synchrotron ~ 10 – 100
- **SR used extensively in macromolecular structural studies** (“raison d’être” for SR!!)

Architecture of a synchrotron



~ 50 m

JF Santarelli, Creative commons

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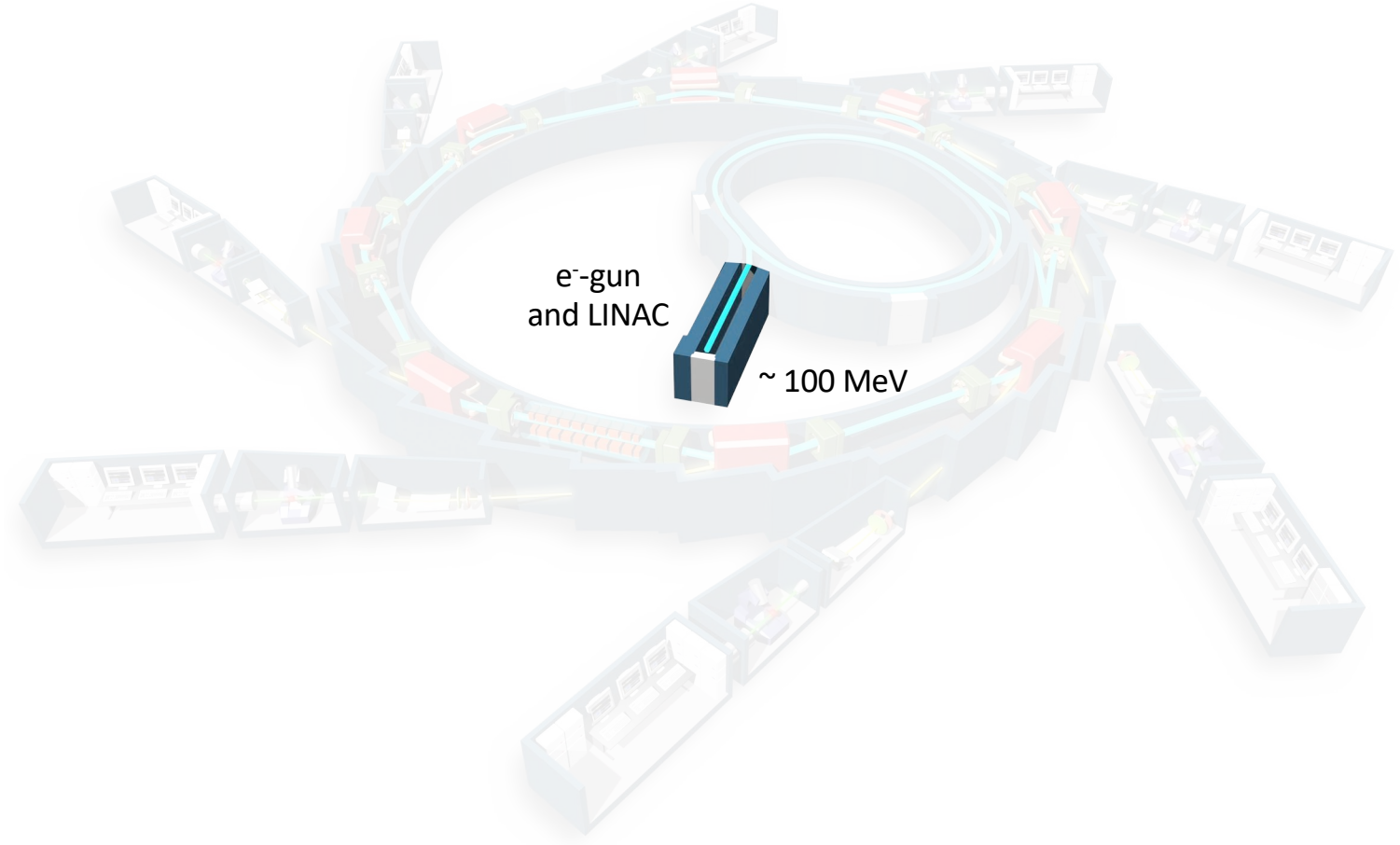
Architecture of a synchrotron



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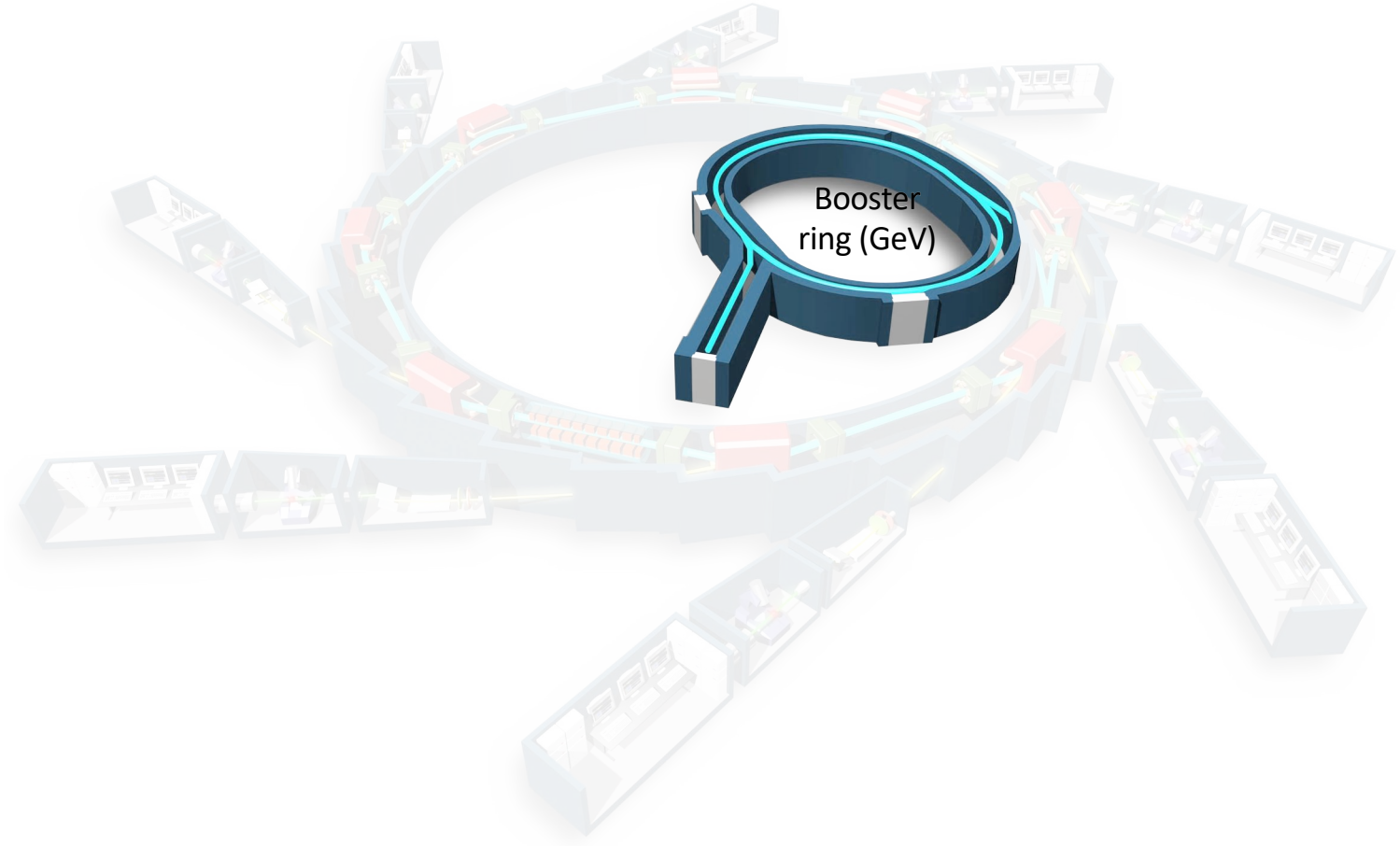
Architecture of a synchrotron



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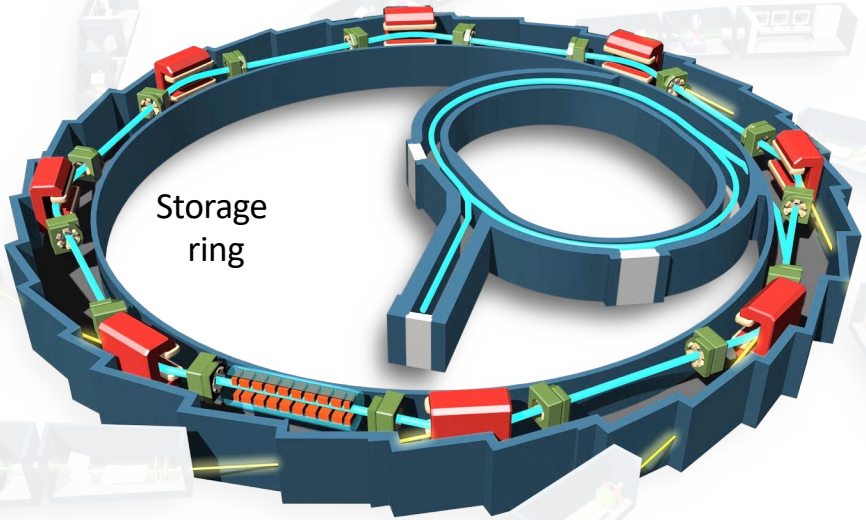
Architecture of a synchrotron



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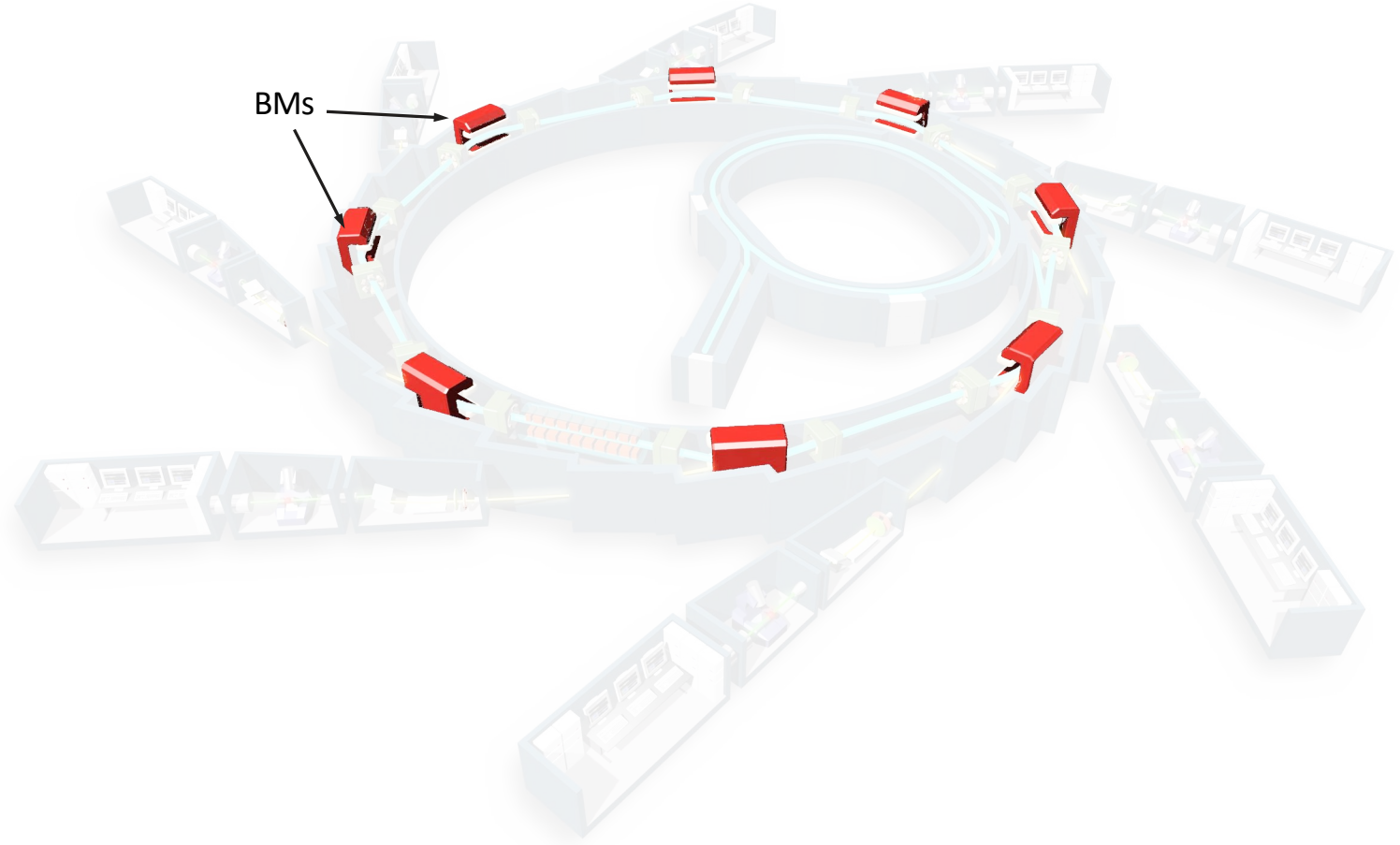
Architecture of a synchrotron



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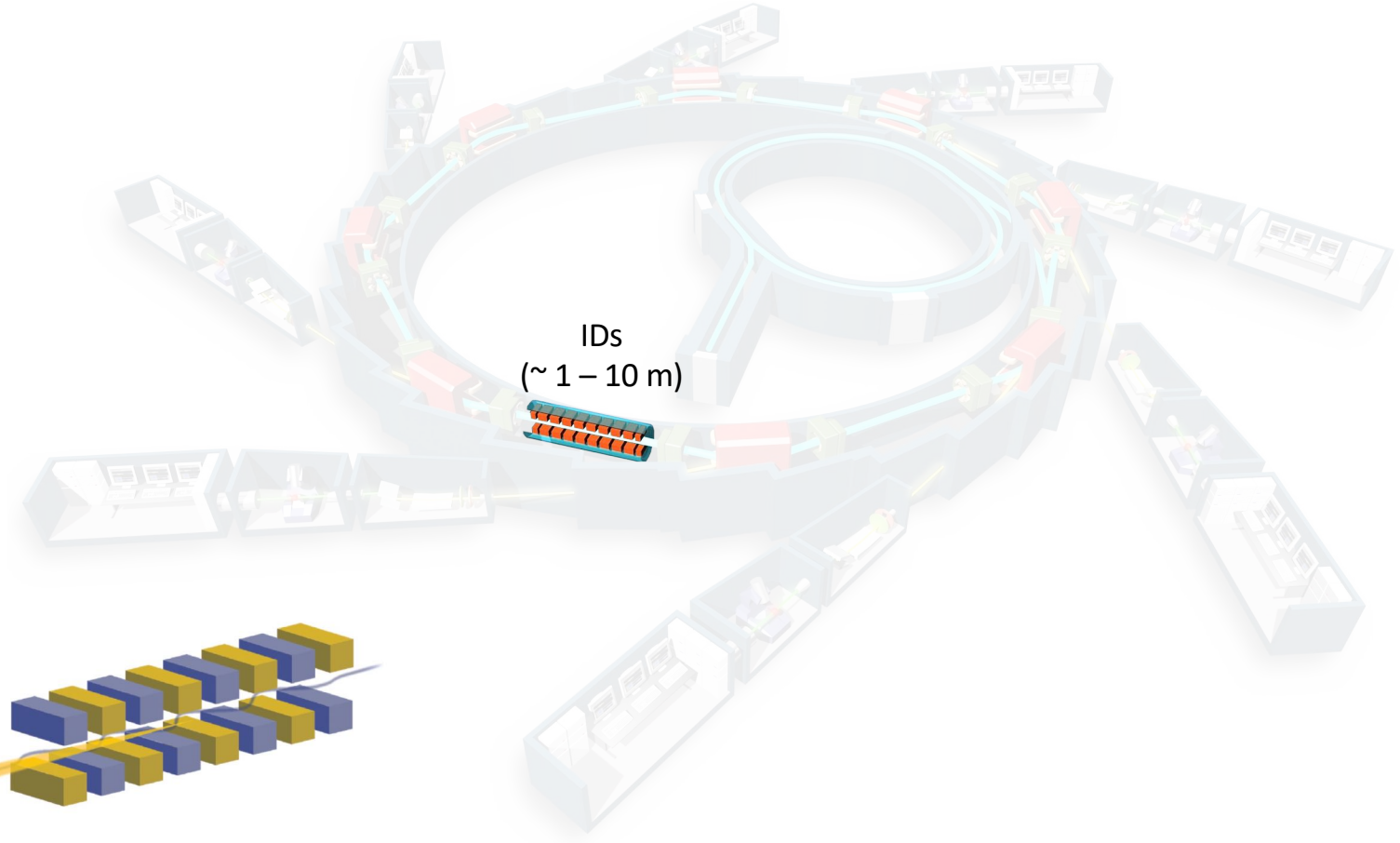
Architecture of a synchrotron



Also:
Quadrupoles
Sextupoles
Octupoles
RF power
BPMs
…

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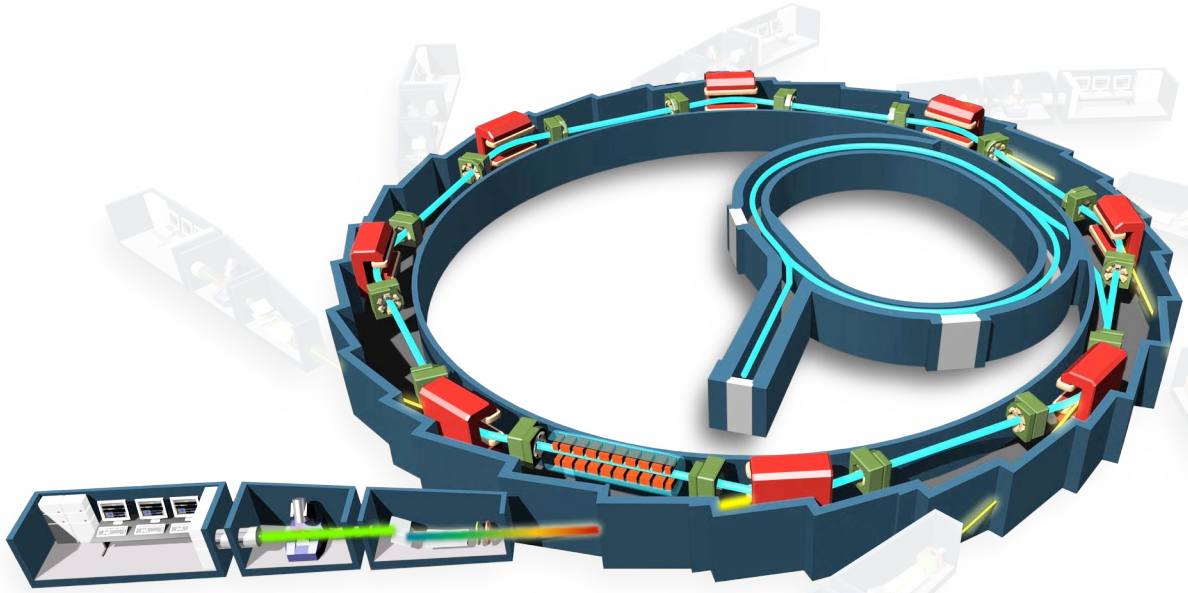
Architecture of a synchrotron



Beamlines

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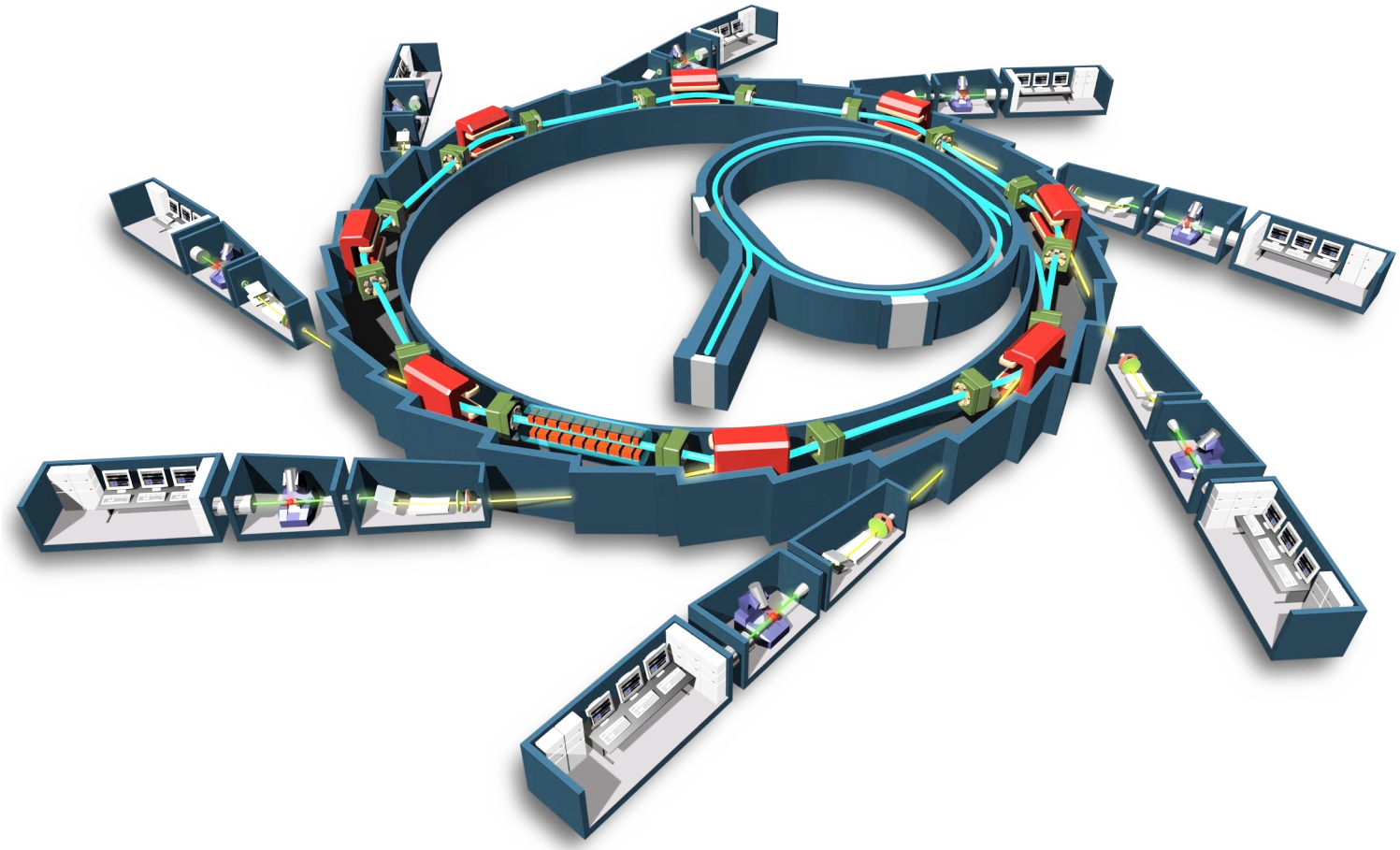
Architecture of a synchrotron



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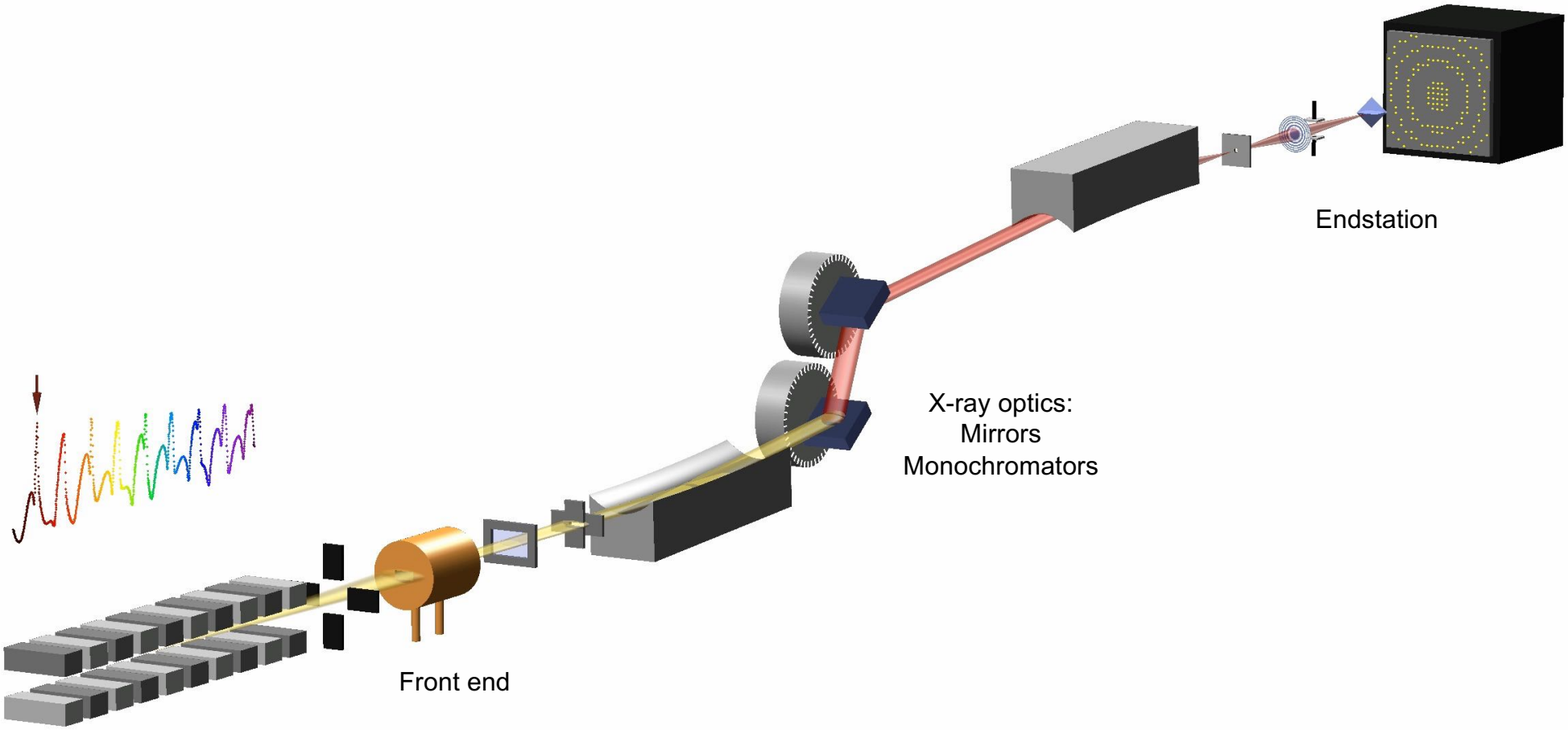
Beamlines



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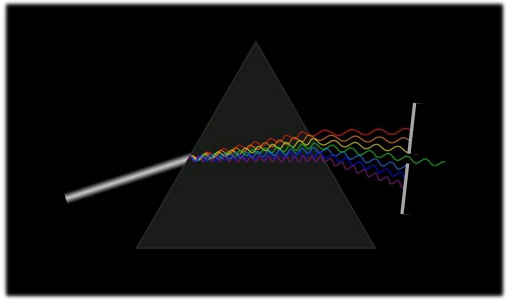
Brilliance – the synchrotron figure of merit

Units: $\frac{[\text{ph/s}]}{[\text{mm}^2\ \text{mrad}^2][0.1\ \%\ \text{BW}]}$

x

x



ε, emittance = size x divergence (both x- and y-directions)

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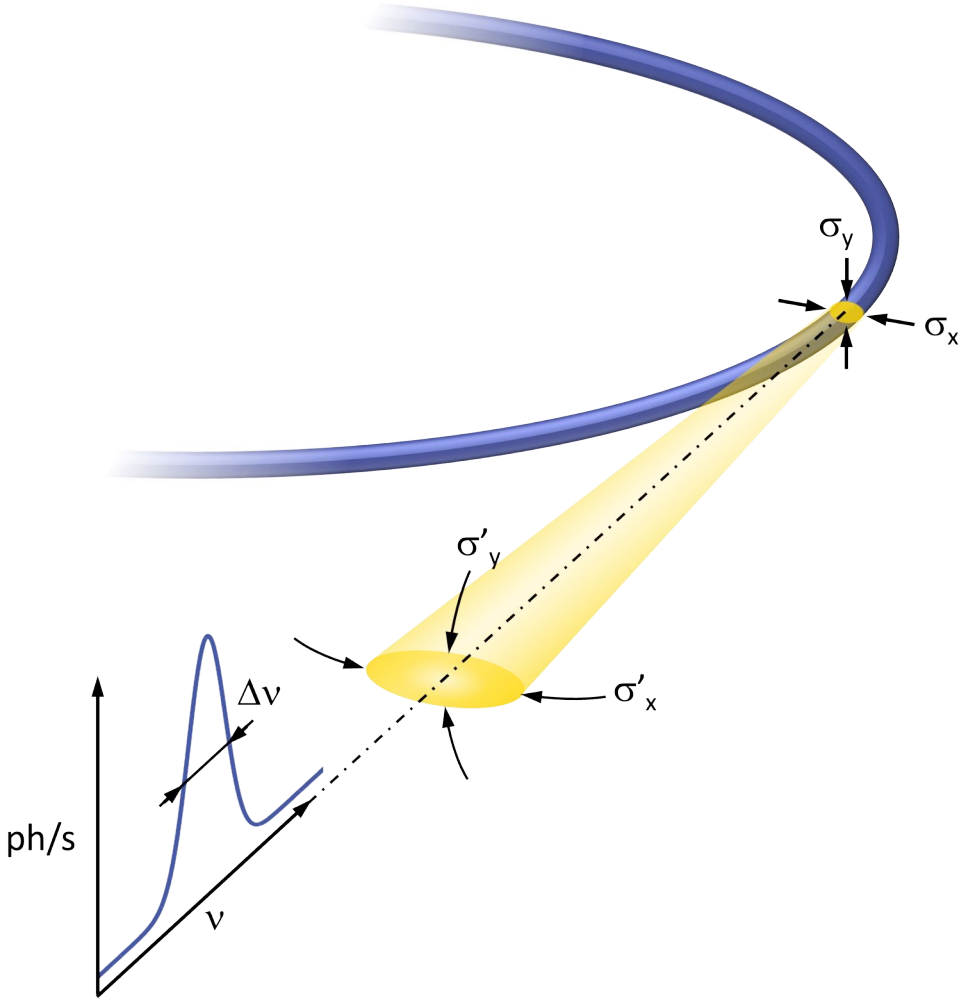
Brilliance (less whimsically)

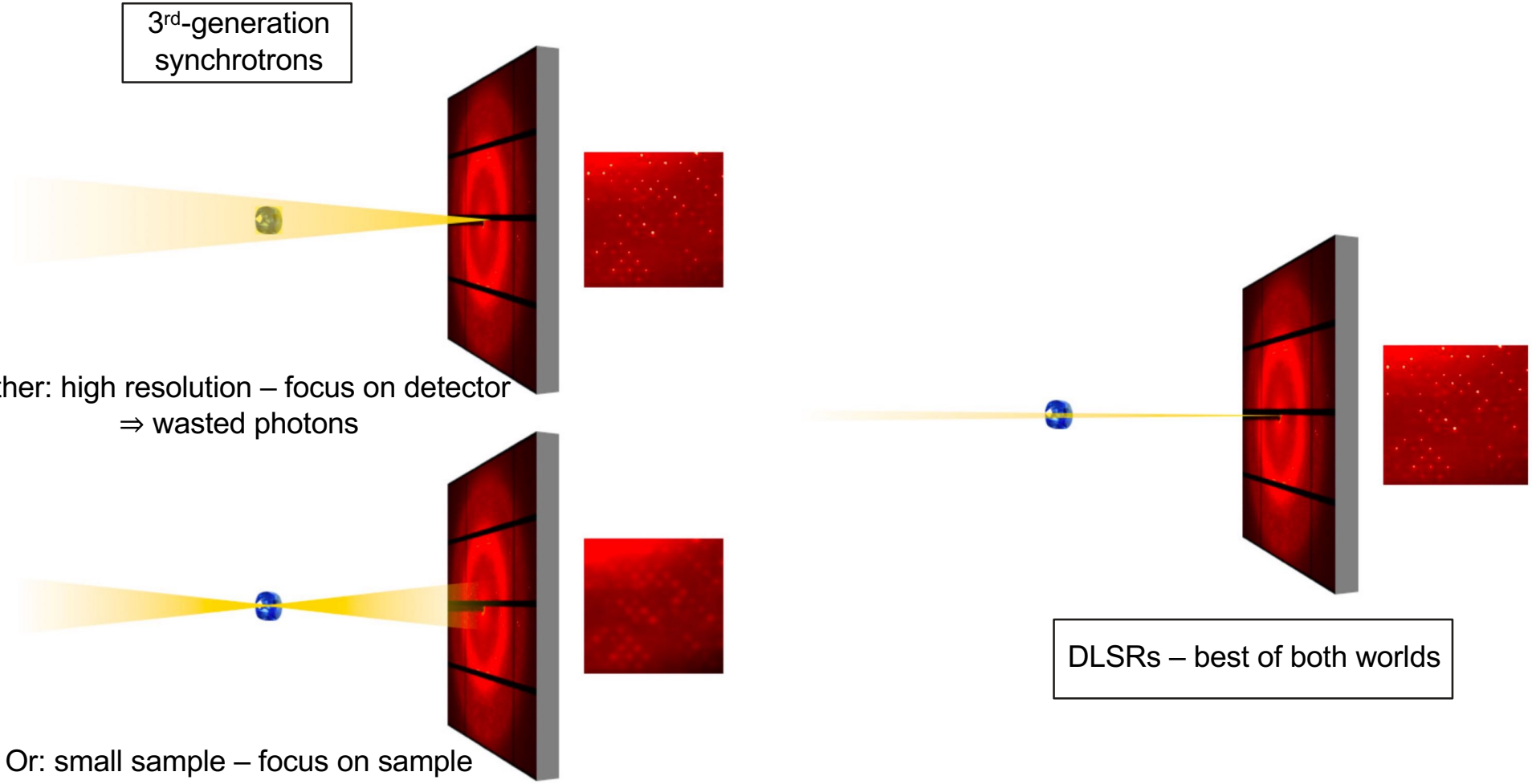


- σ (source size) and σ' (source divergence) have contributions from both the electron beam and the photon beam
  - Photon part fundamental (diffraction limit)

$$\epsilon_{h\nu} = \sigma_{h\nu} \cdot \sigma'_{h\nu} = \frac{\lambda}{4\pi} \sim 10\,\mathrm{pm} \cdot \mathrm{rad}$$

  - Electron contribution determined by storage-ring performance
- 3rd generation (SLS)
  - Dominated by electron beam: $\epsilon_e \gg \epsilon_{h\nu}$
- 4th generation (DLSR, SLS 2.0)
  - Electron and photon contributions similar
  - ⇒ collimated AND small x-ray beams

Small-emittance DLSR-beams for MX



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Fourth-generations synchrotrons
Diffraction-limited storage rings – SLS 2.0

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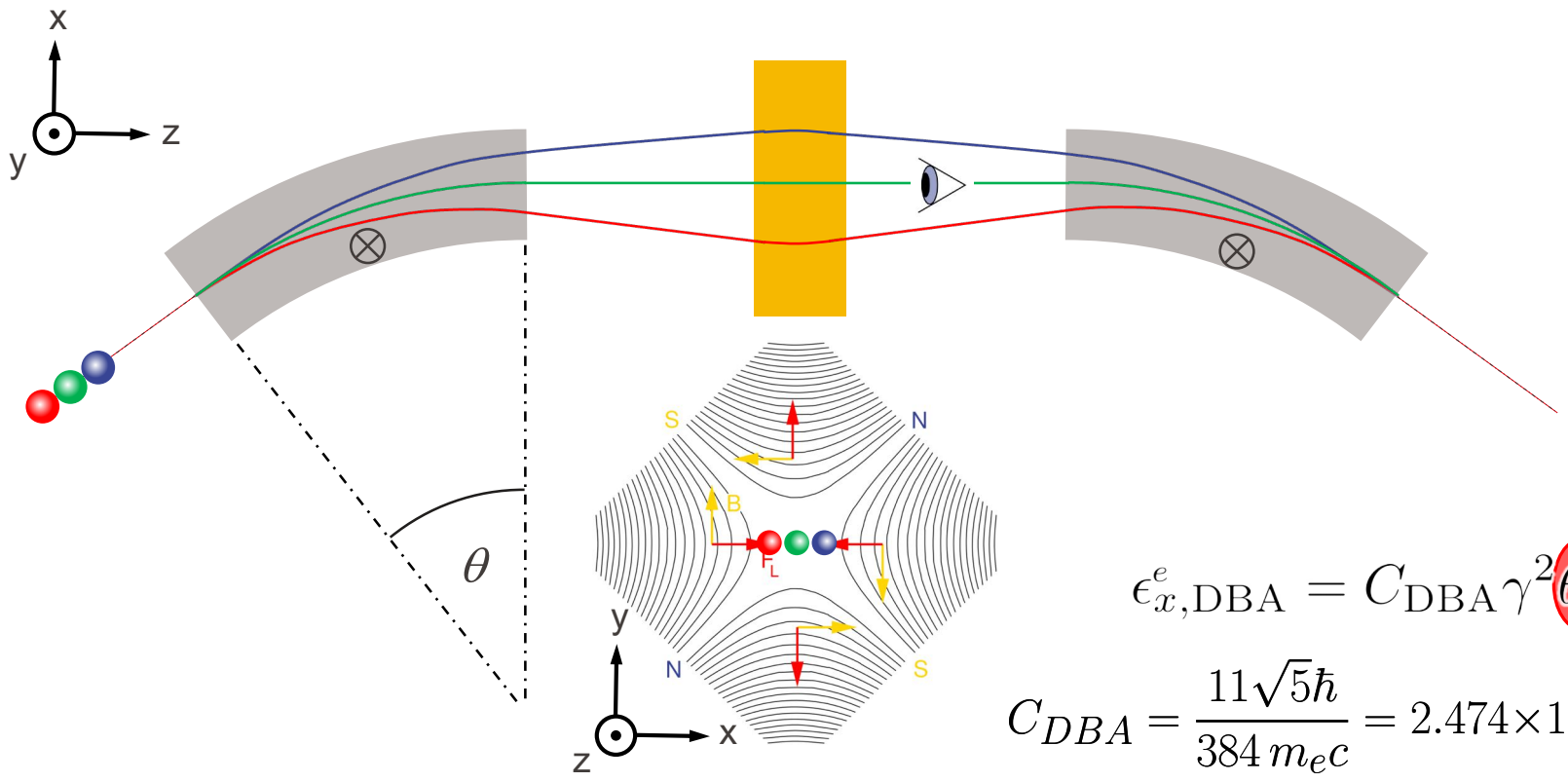
Double-bend achromats at synchrotrons

- Main limit to reducing emittance due to spread induced at bending-magnet achromats



$$\epsilon^{e}_{x,\mathrm{DBA}} = C_{\mathrm{DBA}}\gamma^2\theta^3$$

$$C_{DBA} = \frac{11\sqrt{5}\hbar}{384\, m_e c} = 2.474\times10^{-5}\,\mathrm{nm}\cdot\mathrm{rad}$$

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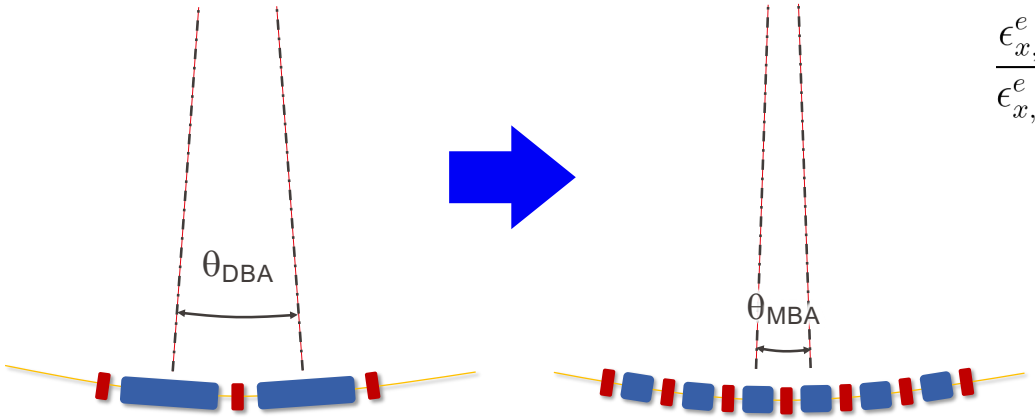
What defines a DLSR (4th generation synchrotron)?

- Increase brilliance by decreasing emittance in electrons' orbital plane (ε_x)
- How?
  - For a given arc sector, use more bending magnets (M): "multibend achromat" (MBA)

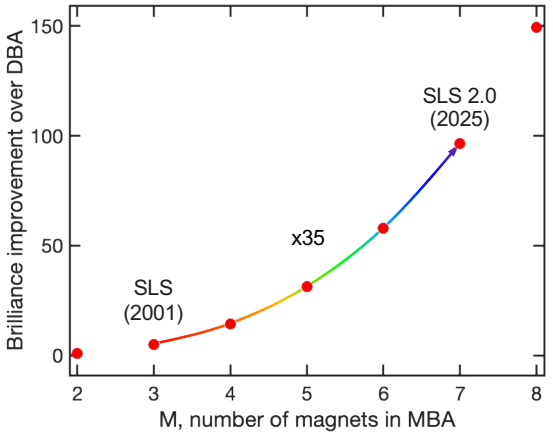


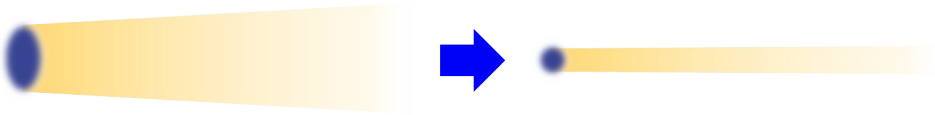
DBA @ 3rd generation synchrotrons

MBA @ DLSRs

$$\left.\frac{\epsilon^e_{x,\mathrm{DBA}}}{\epsilon^e_{x,\mathrm{MBA}}}\right|_{2\theta_{\mathrm{DBA}}=M\theta_{\mathrm{MBA}}} = 3\frac{M-1}{M+1}\left(\frac{\theta_{\mathrm{DBA}}}{\theta_{\mathrm{MBA}}}\right)^3 = \frac{3}{8}\left(\frac{M-1}{M+1}\right)M^3$$

7BA: $\dfrac{\epsilon^e_{x,\,\mathrm{DBA}}}{\epsilon^e_{x,\,7\mathrm{BA}}} = 3\dfrac{7-1}{7+1}(7/2)^3 = 96.5$





DLSRs – making the electron and photon emittances comparable

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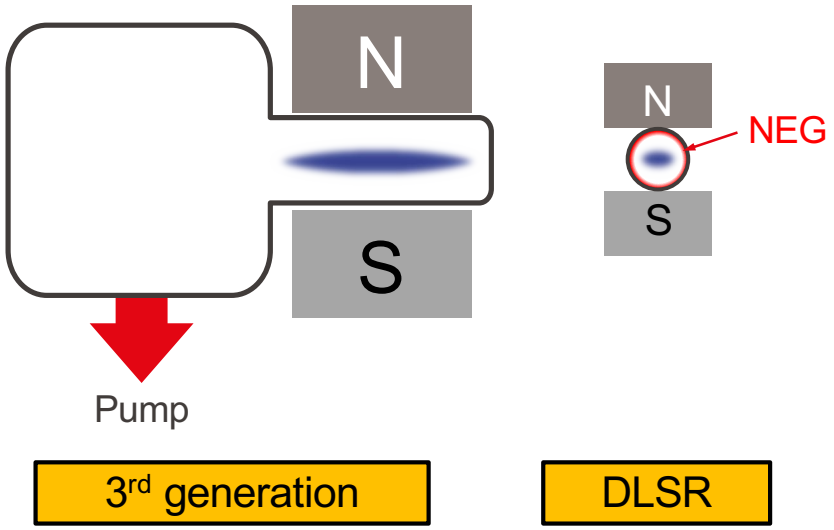
Why only now?

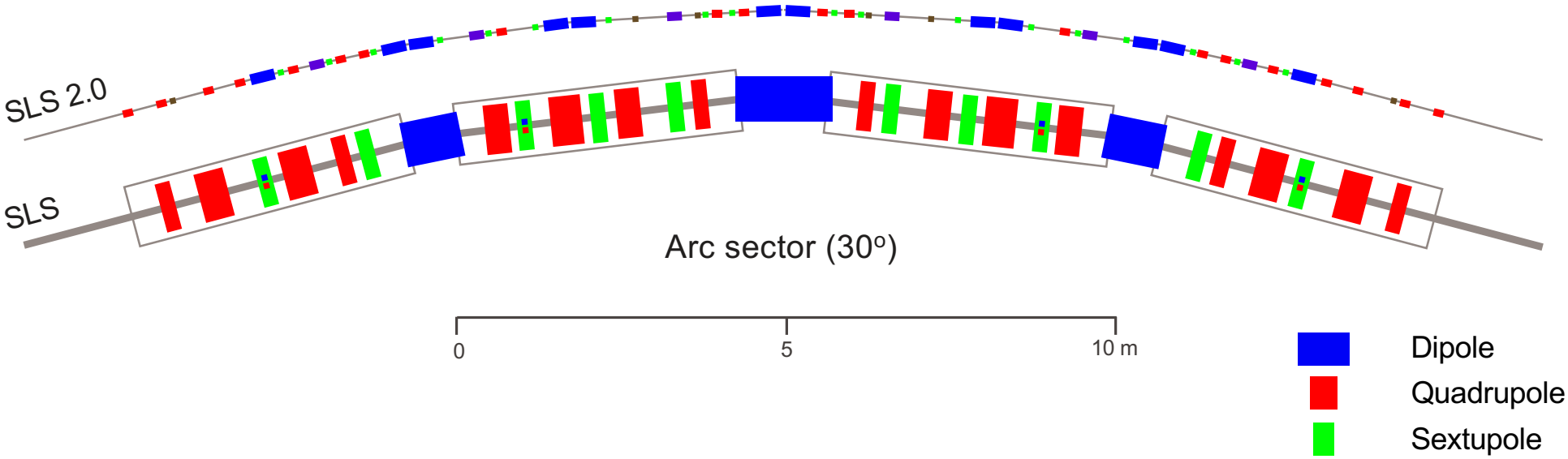
- Using large 3rd-generation magnets would result in
  - An unacceptable increase in ring circumferences
  - Unavoidable alignment errors

- Reduce
  - Magnet sizes
    - More compact 👍
    - Reduces B 👎
  - Distances between magnet poles
    - Increases again B 👍

- Small vacuum vessels
  - Difficult to pump
  - Require special “NEG” coating
    - Porous alloys of Al, Ti, Fe, V, Zr



SLS v SLS 2.0



SLS: Total # magnets = 388; ε_x = **5500 pm.rad**

SLS 2.0: Total # magnets = 1007; ε_x **= 157 pm.rad (ca. 35 x smaller)**

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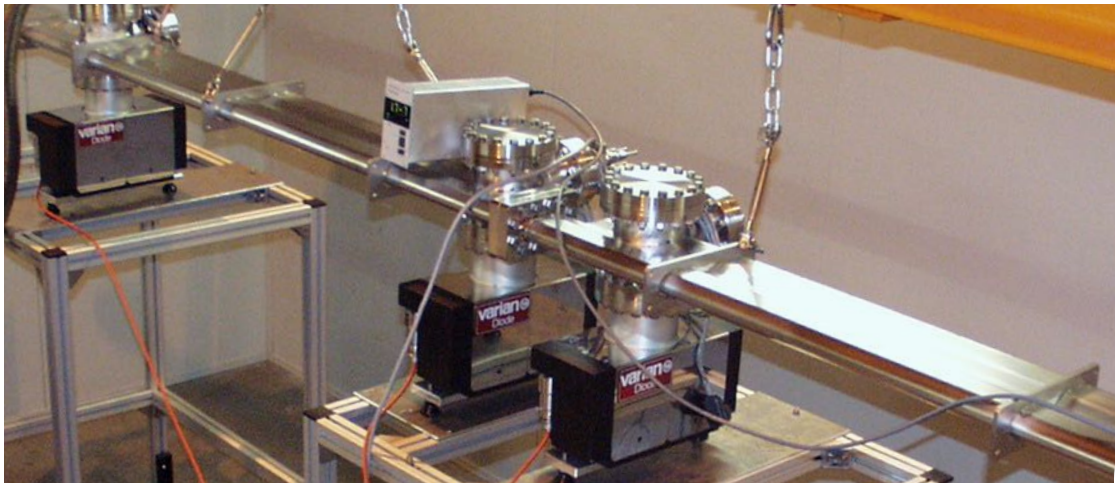
SLS v SLS 2.0



SLS

SLS 2.0

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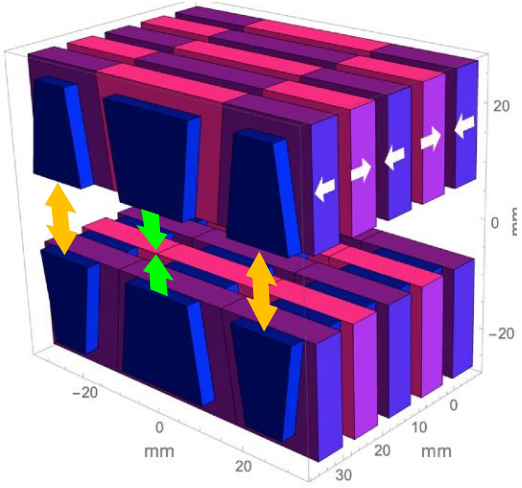
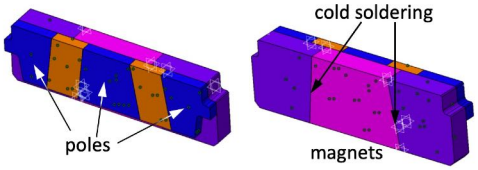
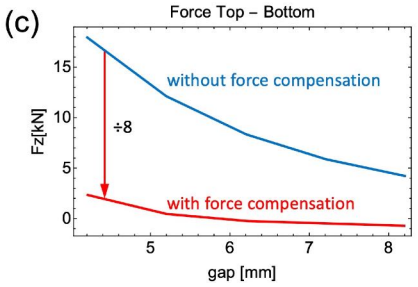
Further benefits of the small beams at DLSRs

X-ray source ("undulators")

- Smaller beam width
- ⇒ smaller magnet dimensions
- ⇒ reduced magnetic forces
  - "Force compensation" possible
- ⇒ smaller gap ⇒ **more intensity & higher hν**
- ⇒ more compact and stable designs







X-ray optics

- Smaller beam cross-sections
- ⇒ smaller dimensions of
  - x-ray mirrors
  - diffracting elements (crystals, gratings, multilayers)
- ⇒ more compact, lighter x-ray optics components
- ⇒ greater stability, less vibrations

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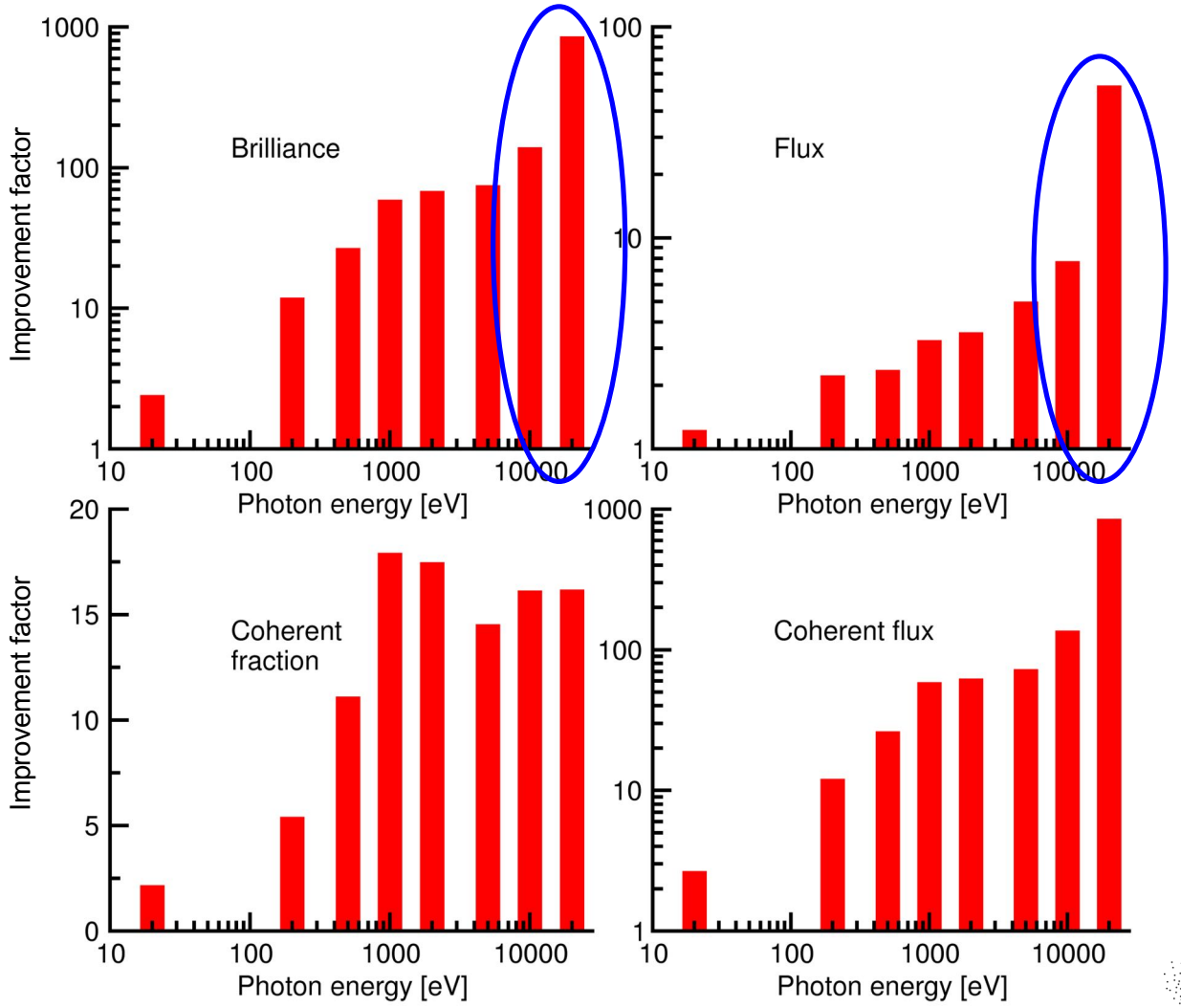
SLS v SLS 2.0 performance enhancements in numbers

Improvement factor
due to MBA and x-ray source



Modifications and improvements in x-ray optics will result in some cases to an increase in ph/s on sample of over **10'000!**

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Macromolecular structure determination at DLSRs
A bright future with complementary competition

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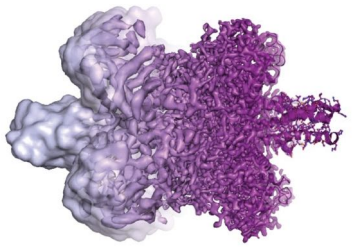
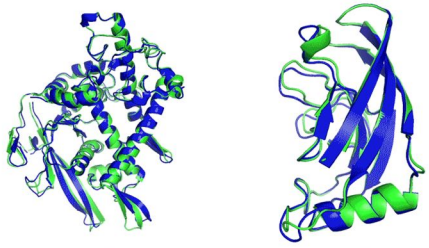
The new kids on the block

- < 2012, MX enjoyed near total dominance
- ~ 1980 – 2010: Developments in cryoEM
  - detectors
  - sample prep
  - image analysis

  } Resolution breakthrough ~ 3 Å
- CASP14 (Nov. 2020): Alphafold2
  - AI program (DeepMind, Google)
  - Predicts structure from amino-acid sequence alone
  - Based on PDB database
  - 2022: structures uploaded of ~200 million proteins from 1 million species, covering nearly every known protein on the planet



< 2012: "Blobology"



> 2012: hi-res cryoEM

Experiment
Alphafold2

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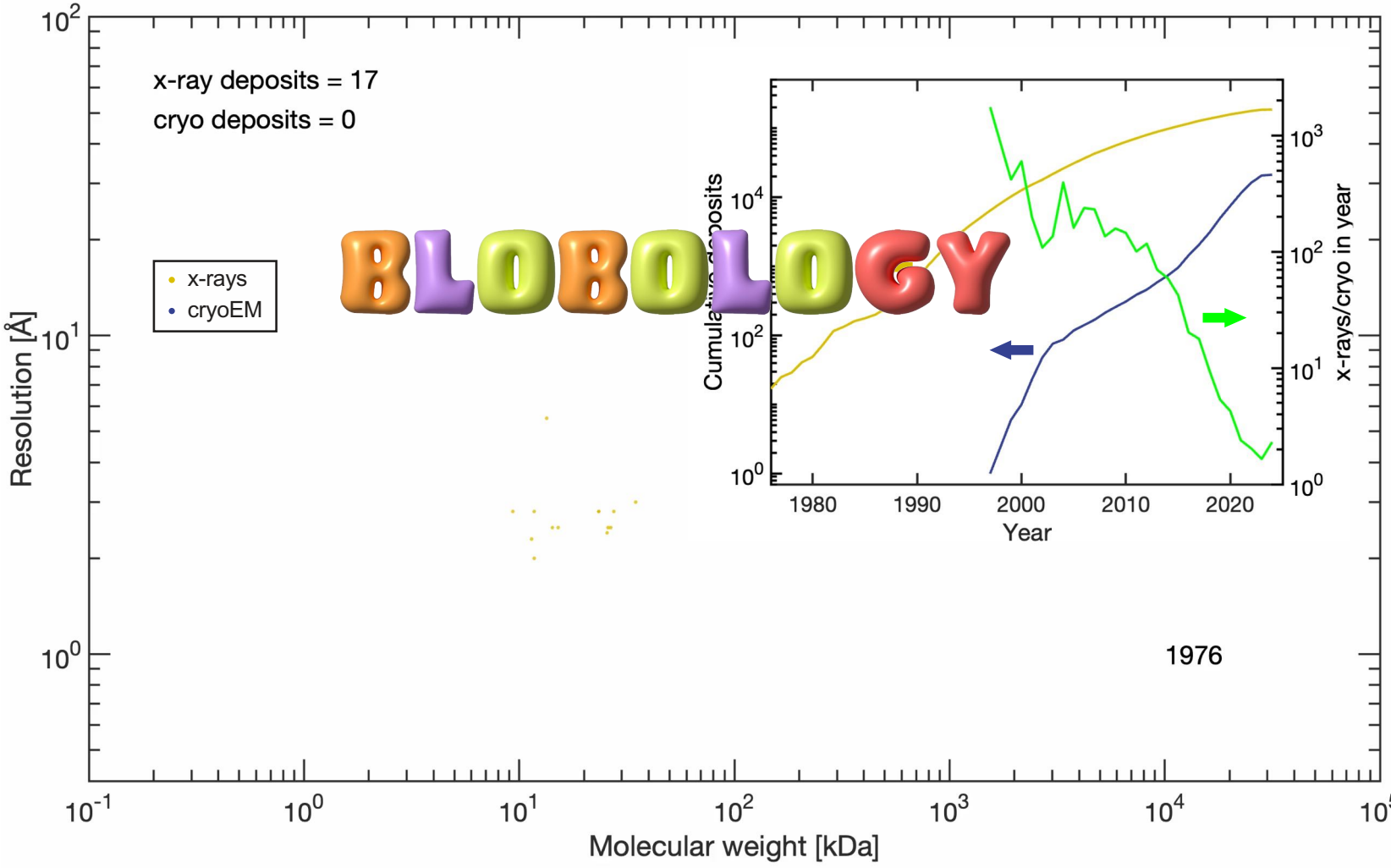
MX v cryoEM… so far (up to September 2024)



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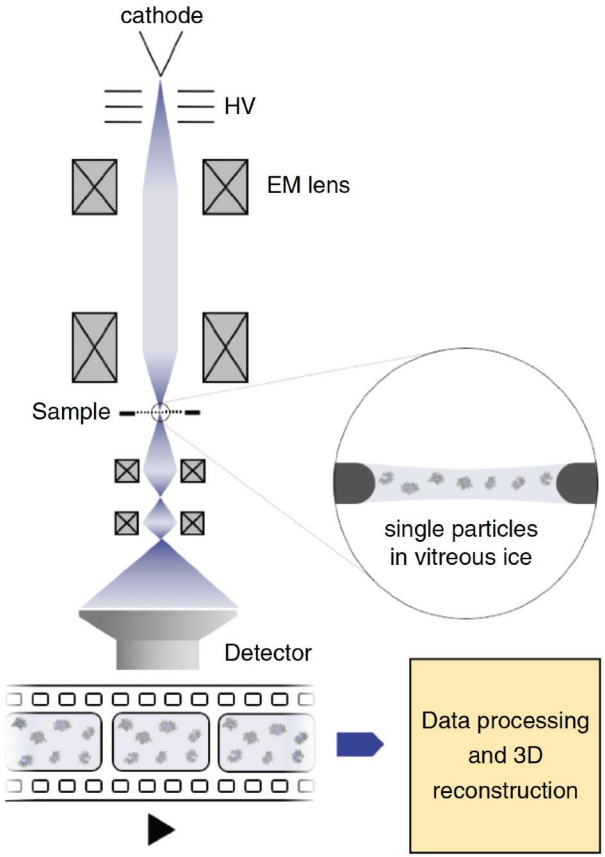
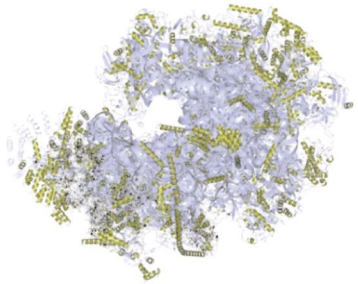
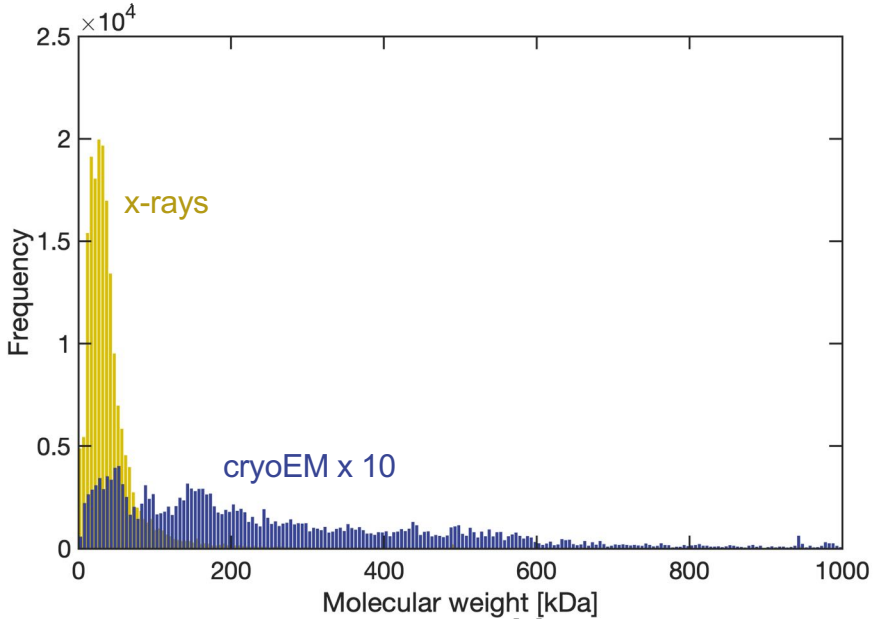
cryoEM

- Real-space imaging technique
- Single particles, no crystals needed
- Most suited for high-MW structures ~ 50 – 1000 kDa
- Resolution typically ~ 3 – 4 Å
- Highly computationally demanding (tomographic methods) ⇒ slow structural solution process
- Low-MW structures (< 30 kDa) less suited due to poor contrast/radiation dose ratio
- Free energy $G = H - TS$



Data processing and 3D reconstruction





See also SPG plenary talk, Henning Stahlberg 17:15, 09.09.2024 and Luca Rima 17:00 today, this session

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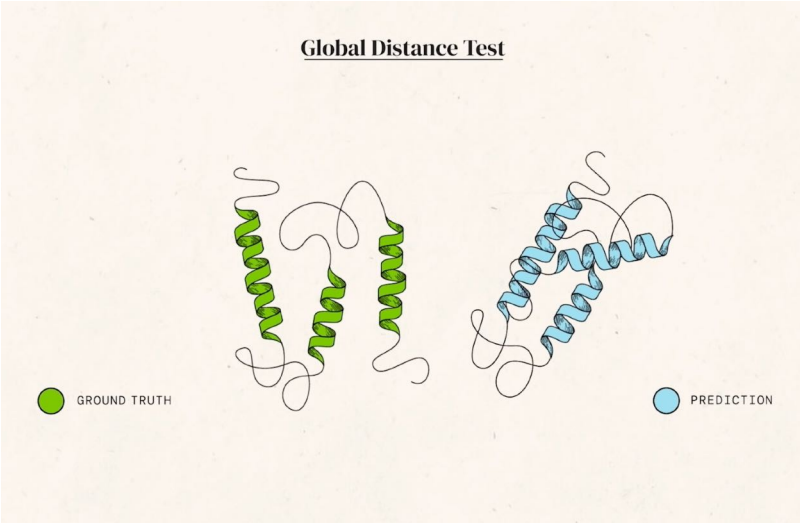
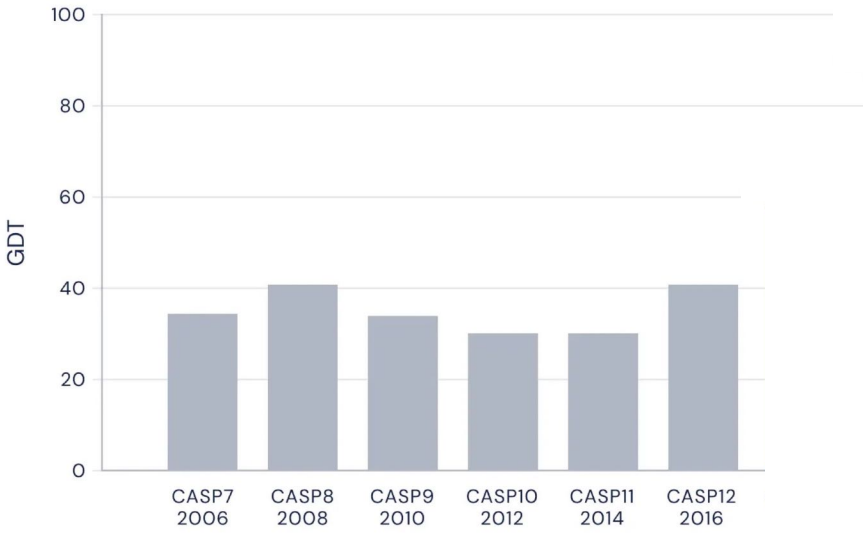
Alphafold2 *et al.*

- Critical Assessment of Protein Structure Prediction CASP14, Nov. 2020
  - Metric: Global distance test – global score (GDT-TS)
    - Percentage of well-modelled residues w.r.t. target
    - 90% is (was!) the holy grail



Extracted from https://www.youtube.com/watch?v=gg7WjuFs8F4&t=149s



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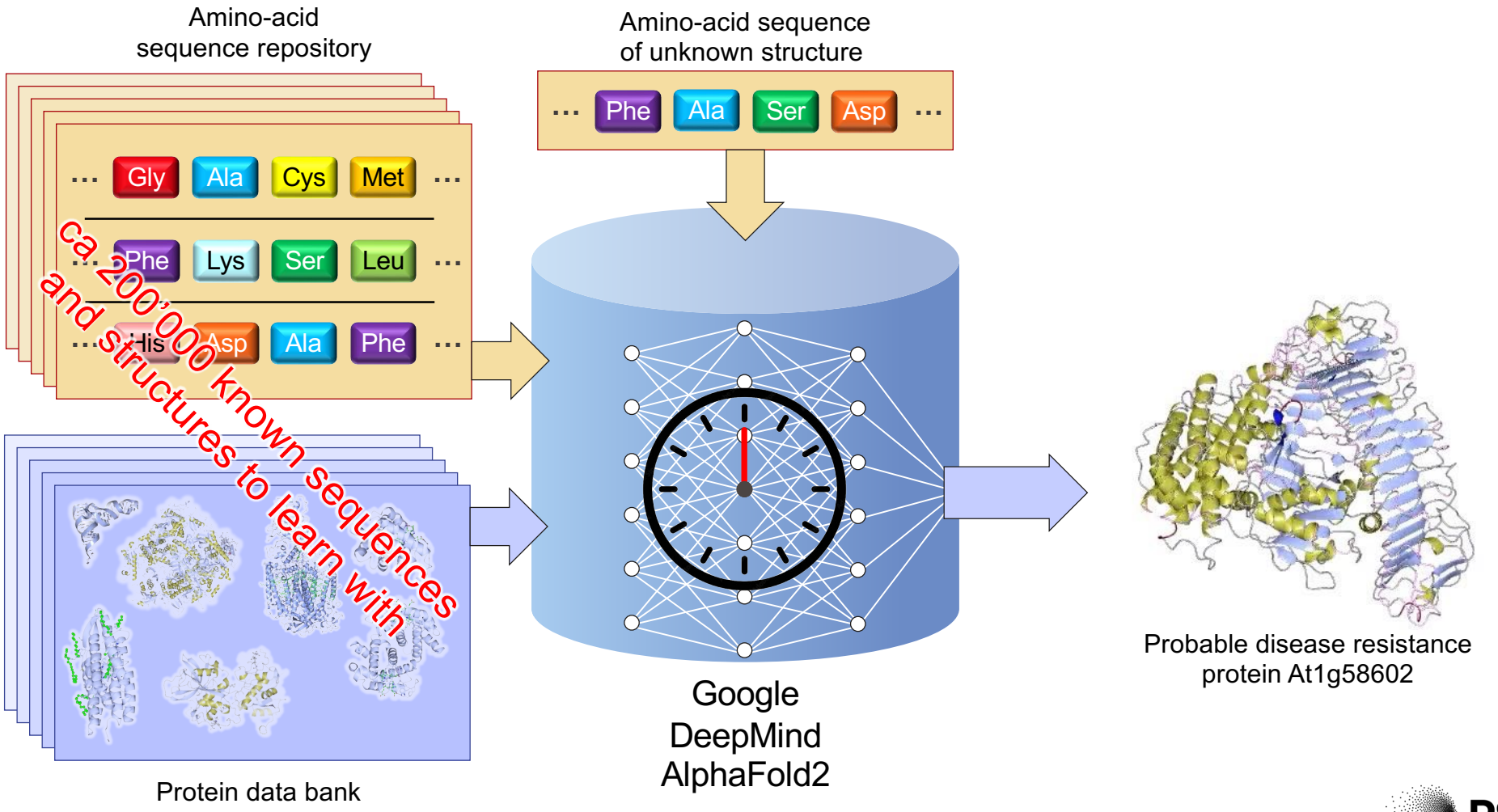
Alphafold2 in a nutshell



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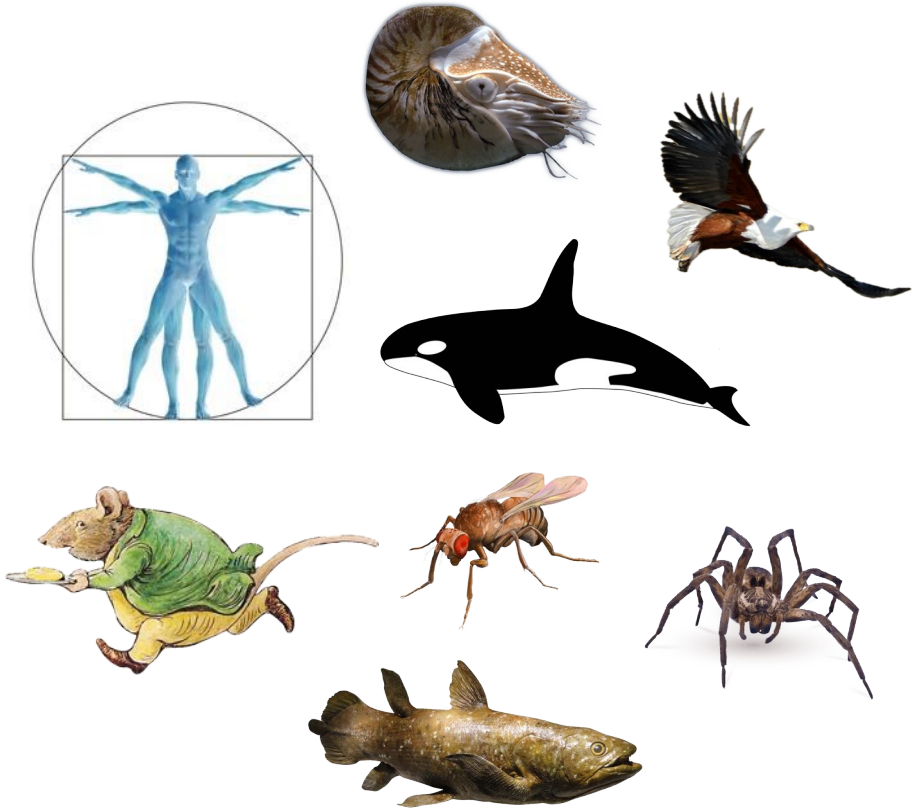
Alphafold2 in summary



1 million species

Images: creative commons and PRW

- Phase problem essentially solved!
- MIR, MAD, SAD, etc. no longer needed
- Molecular replacement (MR) still workhorse
- Refinement of Alphafold2 predictions
  - Is crystal structure = in-vivo structure?
- July 2024: Number of proteins solved by Alphafold2 since November 2020…

> 200’000’000!!

- 35% “highly accurate”
- 45% “sufficiently accurate for many applications”

https://www.nature.com/articles/d41586-022-02083-2

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Atomic resolution in structure determination

- A spatial resolution of approximately 2 Å or better is required to resolve individual atoms within a protein structure
- At this resolution:
  - Individual atoms and their positions can be distinguished
  - The electron density map is detailed enough to identify the atomic structure, including side chains of amino acids
  - Bond lengths and angles can be accurately measured
- For very high-resolution structures, resolutions better than ca. 1.5 Å are needed
  - Provides even more precise details about the atomic arrangement
  - ca. 1 Å resolution allows identification of hydrogen atoms, which are typically challenging to resolve at lower resolutions

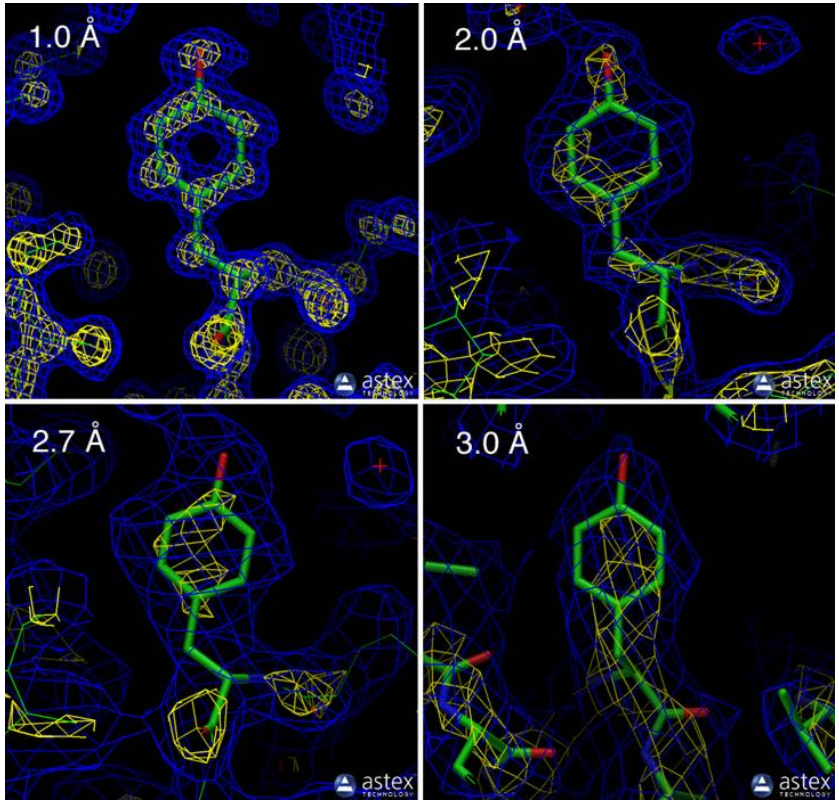
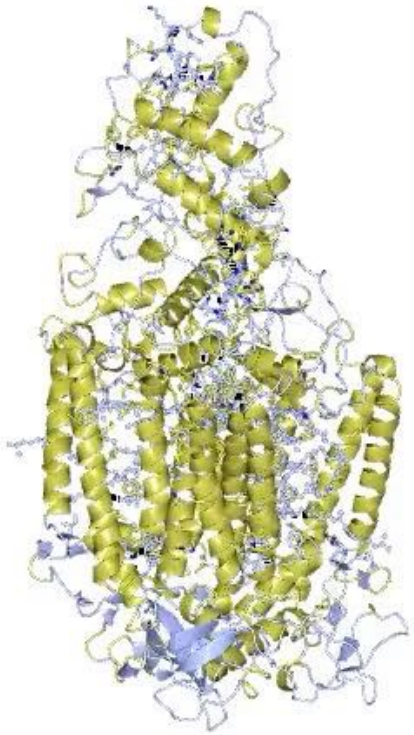


Image from:
https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/resolution

Membrane proteins and GPCRs

- Membrane proteins
  - Relay signals between cell's internal and external environments
  - Transfer chemicals across cell membrane
  - Molecular weights ~ 10 to over 200 kDa

- G-protein-coupled receptors (GPCRs)
  - Recognize a wide variety of stimuli
    - Photons, ions, proteins, neurotransmitters, hormones...
  - Activate cellular responses
  - Molecular weights ~ 40 to 100 kDa

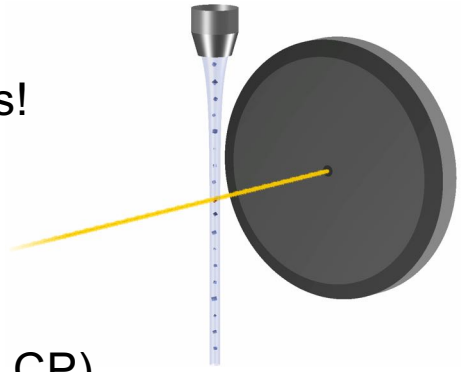


Photosynthetic Reaction Centre

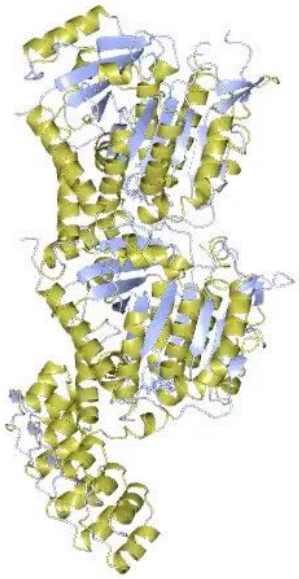
See also Michael Hennig 16:00, 11.09.2024

Serial Synchrotron Crystallography (SSX) @ DLSRs

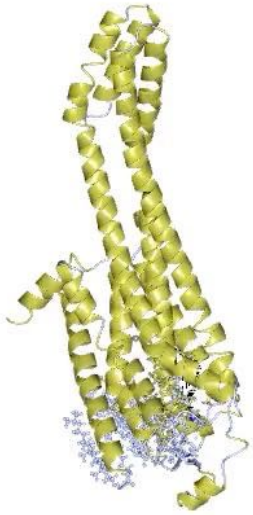
- Membrane proteins
  - 1/3 of all proteins
  - 2/3 of medicinal drug targets
  - 1 – 2% of all MX-solved structures!
- Why so under-represented?
  - Hydrophobic, hard to crystallize
  - Often micron-sized, poor quality
  - Improve using lipid cubic phase (LCP)
- Serial synchrotron crystallography (SSX)
  - **RT** or cryo
    - Conformational landscapes (3D shape)
    - Dynamics down to μs
  - Uses much less material than SFX @ XFELs



RT-SSX



$TD1_{Apo}$



$A_{2A}R$

RT-SSX @ PXI, SLS, 2017

Weinert *et al.*, Nature Comms. **8** 542 (2017)

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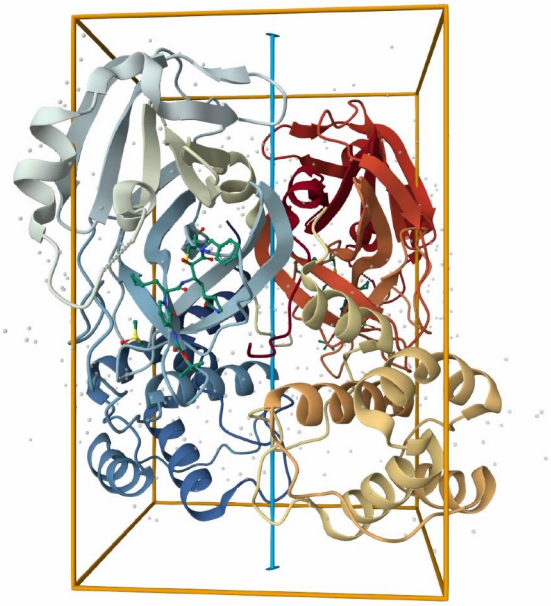
Requirements for fragment screening

- Precise location and orientation of SMALL fragment on LARGE biological target
  - High resolution ~ 1.8 Å or better
- Fast throughput (100's of samples)
  - @ SLS 2.0
    - ca. **30+ fragment samples/hr**
    - ca. **10 minutes/structural solution (local)**
      - Bottleneck – use off-site supercomputers
- Requires automation!!
  - 2025 onwards: ~ 10 – 100 TB/day!!
- Resolution and time-consuming structural solutions make cryoEM unsuited to fragment screening



α-ketoamide inhibitor with
SARS-CoV-2 main protease
1.95 Å, PDB 6Y2F
Zhang *et al*., Science 368, 409–412 (2020)

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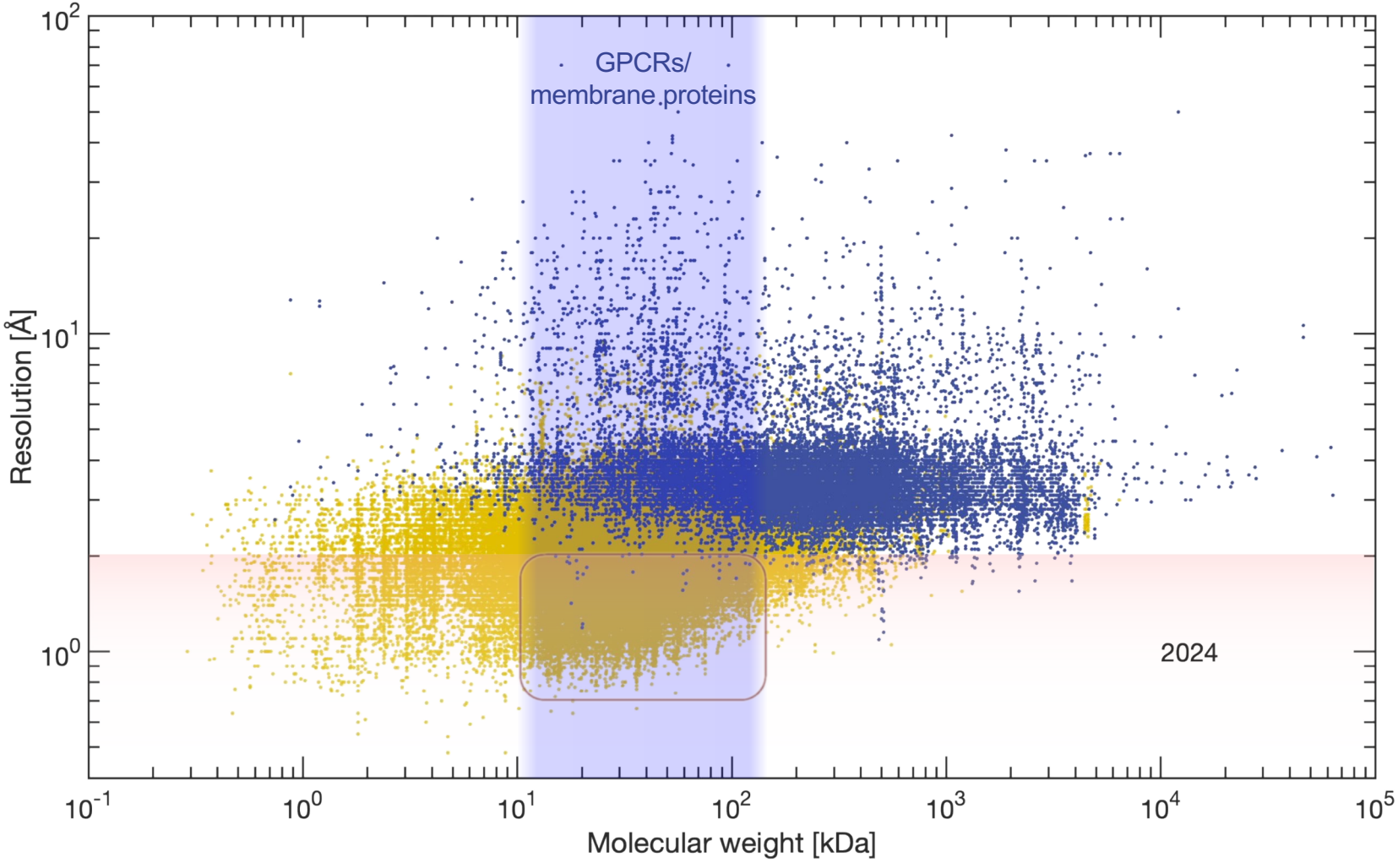
Requirements for fragment screening



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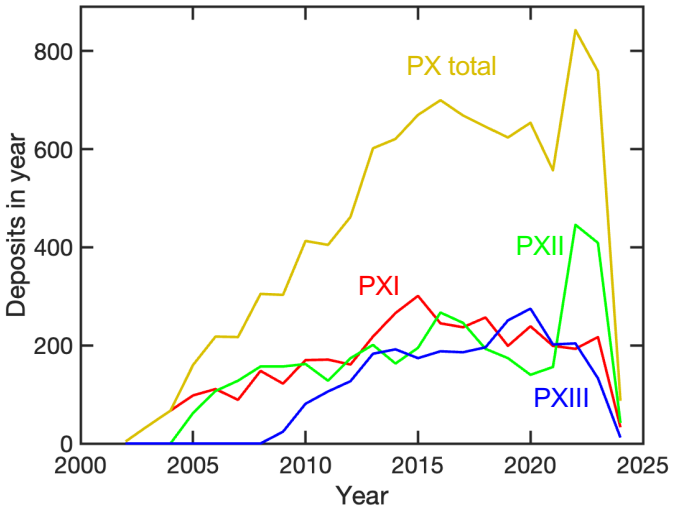
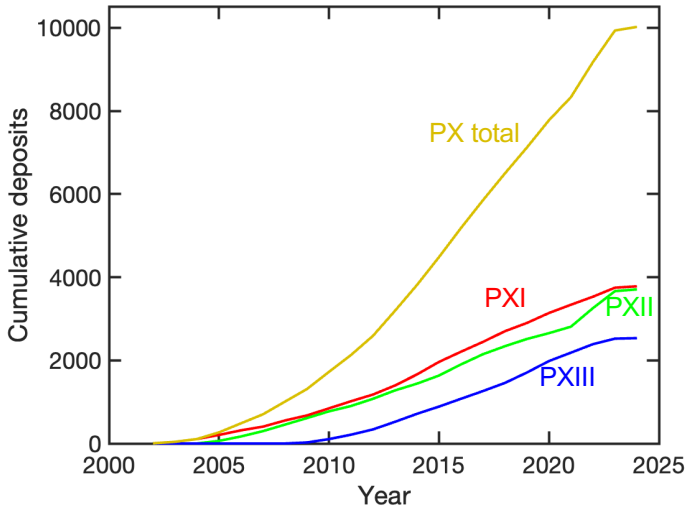
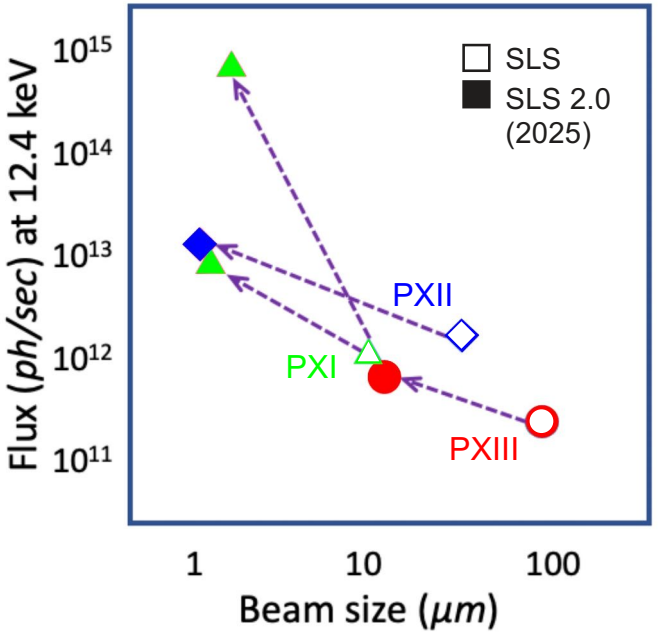
MX @ SLS and SLS 2.0

- Three beamlines
  - **2001**: PXI – ID beamline: mainly scientific research, cutting-edge developments; some industry
  - **2004**: PXII – ID beamline: exclusively proprietary and drug discovery beamtime. Funded by industry
  - **2007**: PXIII – SB beamline: partly research, diffraction screening, phasing, industry. Upgraded BL completed in 2023, first users!!
- July 31st 2024: the **10'001st** PDB entry from SLS registered!
  - Most # PDB entries/year/BL worldwide







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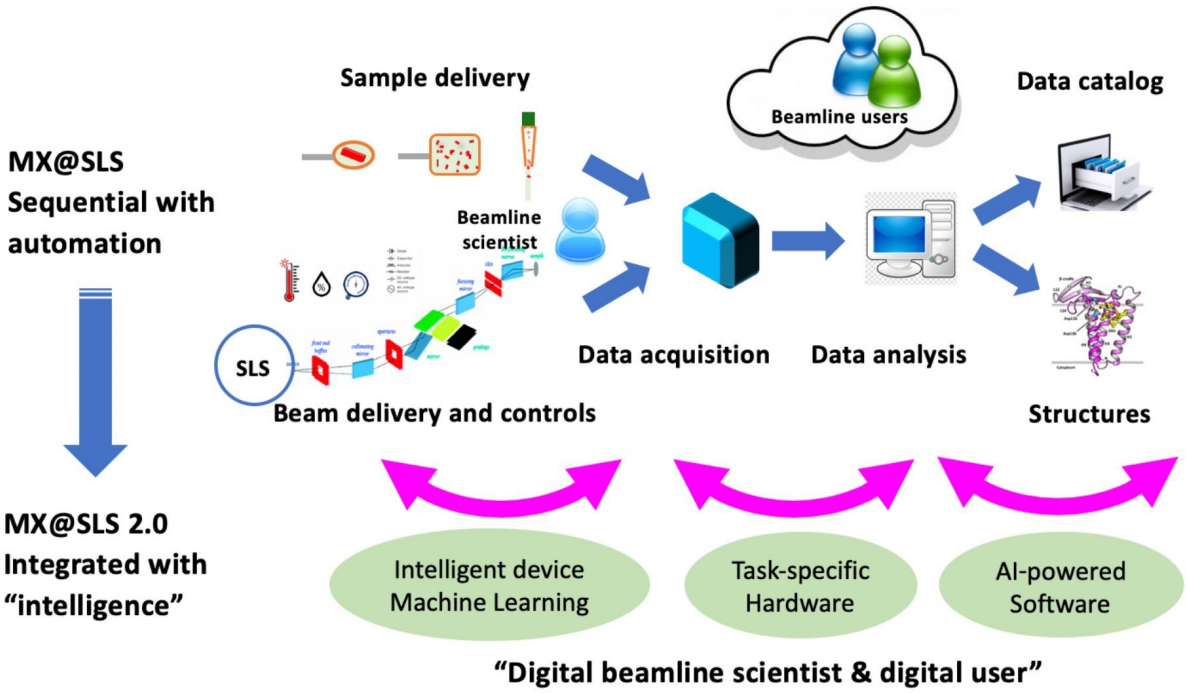
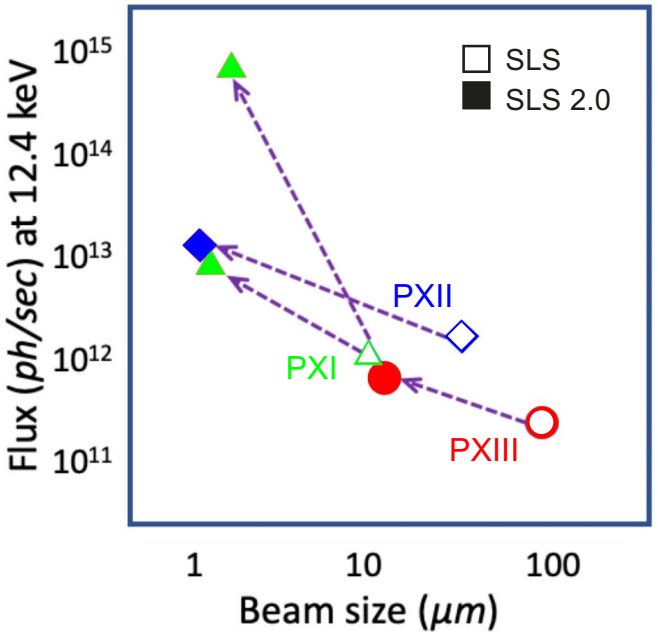
MX @ SLS and SLS 2.0

- SLS 2.0 – hi-speed, automated, intelligent learning





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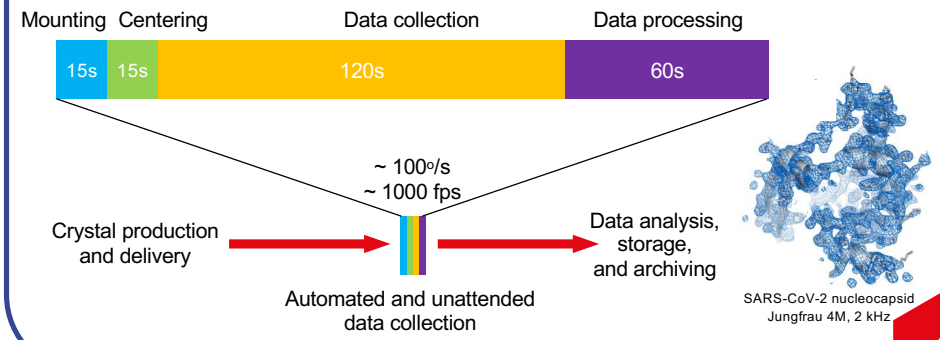
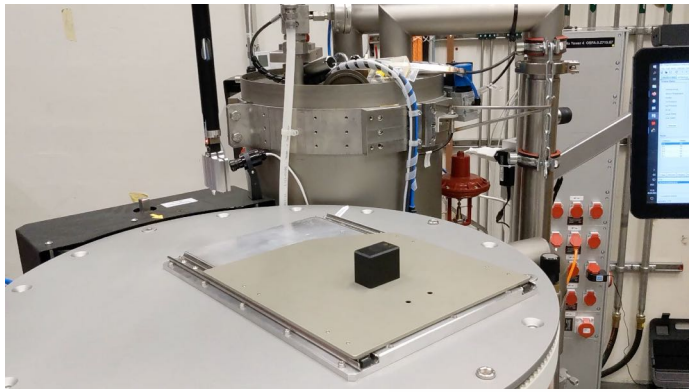
MX @ SLS 2.0 – "1^3"

High-speed hi-res crystallography, ~ 1 s/sample



Serial synchrotron crystallography of ~ 1-μm crystals



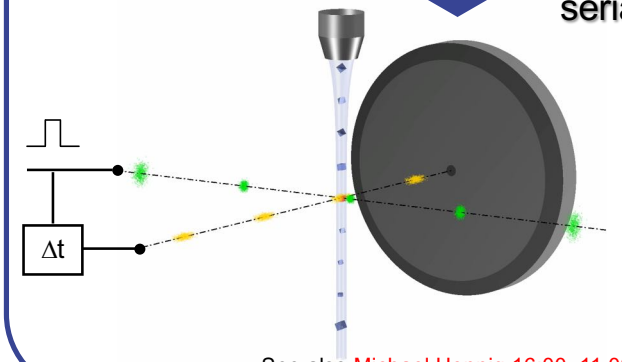
1s
1μm
1μs

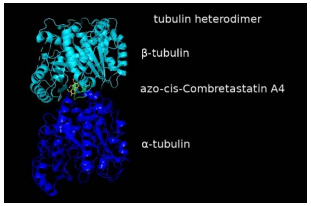
Efficient fragment screening
Drug discovery

(Exploiting Alphafold 3)

Detector developments
in-house

Time-resolved serial crystallography down to ~ 1 μs





SFX & SSX@ PSI

See also Michael Hennig 16:00, 11.09.2024

Dynamics
Conformational ensembles
and changes
RT and cryo

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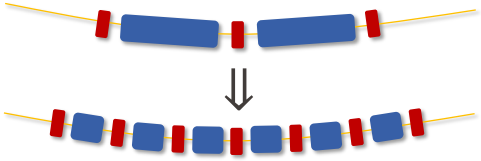
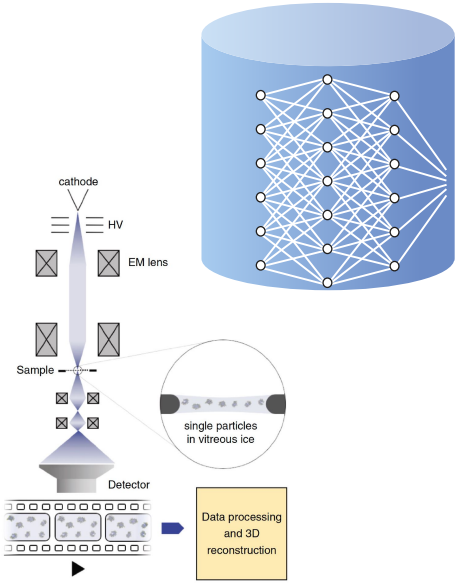
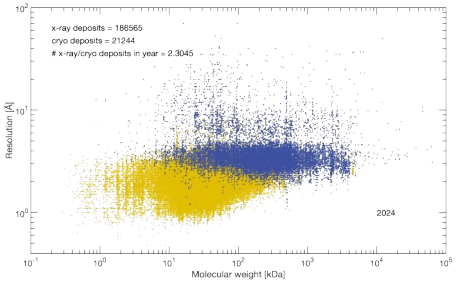
MX @ SLS 2.0 and machine learning for drug design

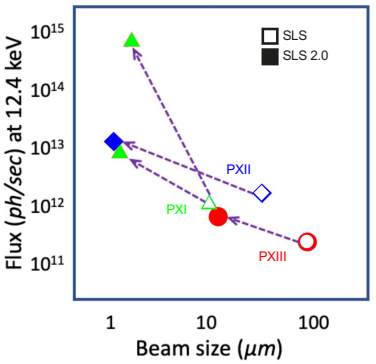
- Predictive vs. experimental validation:
  - AI/machine learning:
    - Predicts static structures with high accuracy
    - No physiological information about the dynamic nature or how proteins interact with ligands, etc.
    - Not (yet) reliable re. details of potential binding sites or how different ligands interact with these sites
    - Generates initial structure models and potential binding sites ⇒ speeds up preliminary stages of drug discovery
  - MX fragment Screening:
    - Directly observes how and where small chemical fragments bind to a target protein – crucial for drug discovery!
    - Helps identify binding sites, understand binding affinities
    - Guides the design of more potent and selective compounds
    - Can identify conformational flexibility
    - Provides insights into how binding events can induce structural changes/dynamics
    - Important for understanding the full range of a protein's functional states
    - Essential experimental validation of predictive AI models
- Summary
  - AI algorithms are transformative tools for predicting protein structures
  - Fragment screening in MX remains a vital experimental tool
  - These two methods are complementary: AI-driven predictions provide valuable initial insights that guide experimental validation and optimization in drug discovery

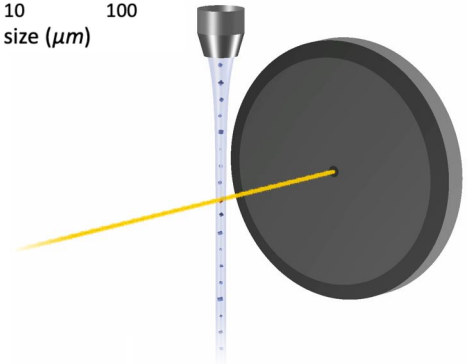
Summary

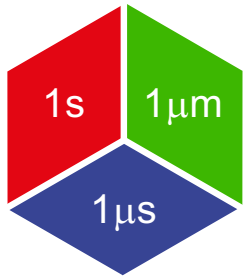












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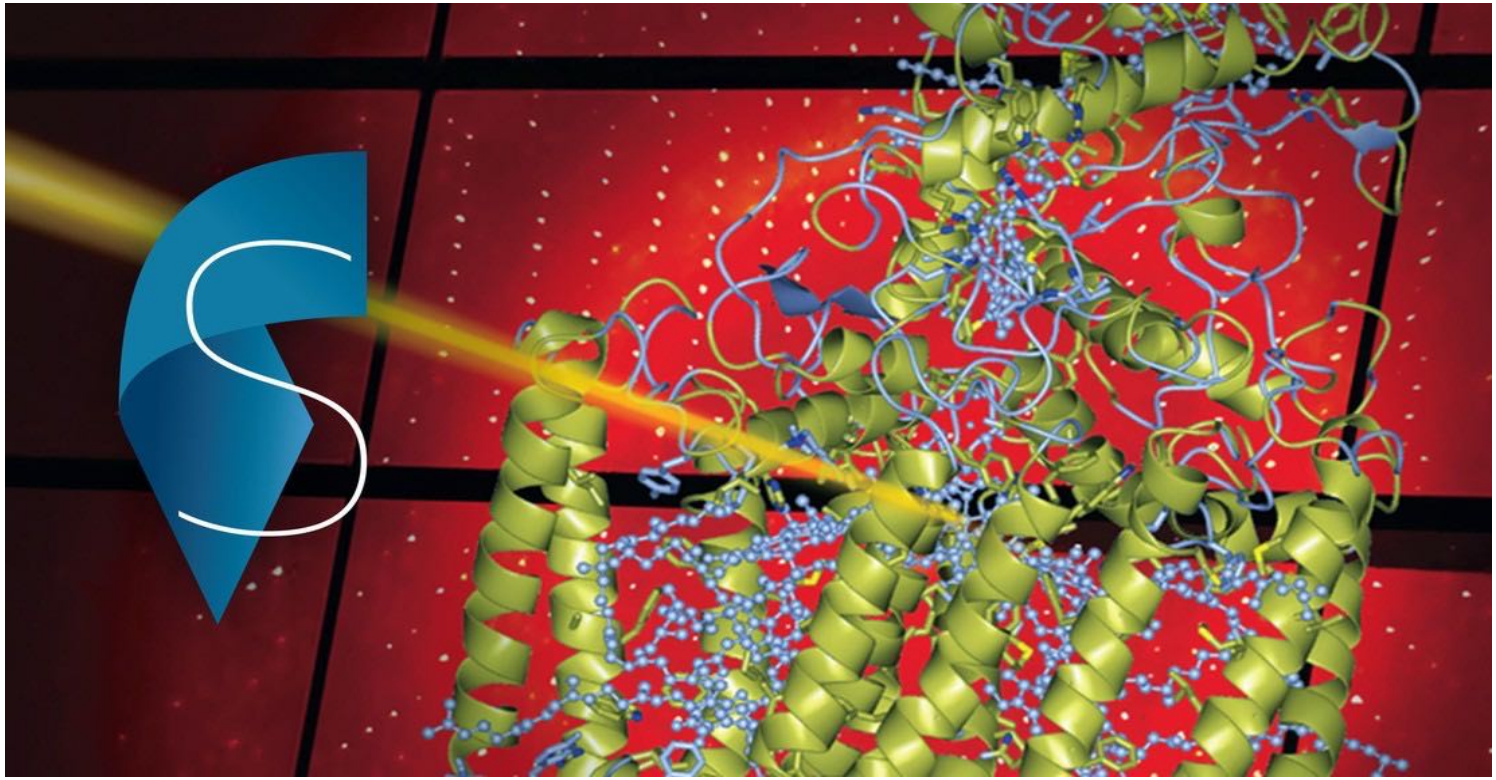
Synchrotron/XFEL massive open online courses (MOOCs)



EPFL: two six-week Massive Open Online Courses (MOOCs)

- Introduction to synchrotron and XFEL radiation – Part 1
- Introduction to synchrotron and XFEL radiation – Part 2

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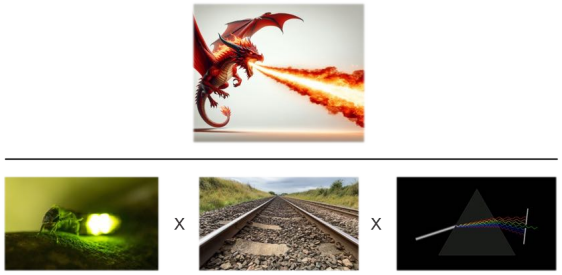
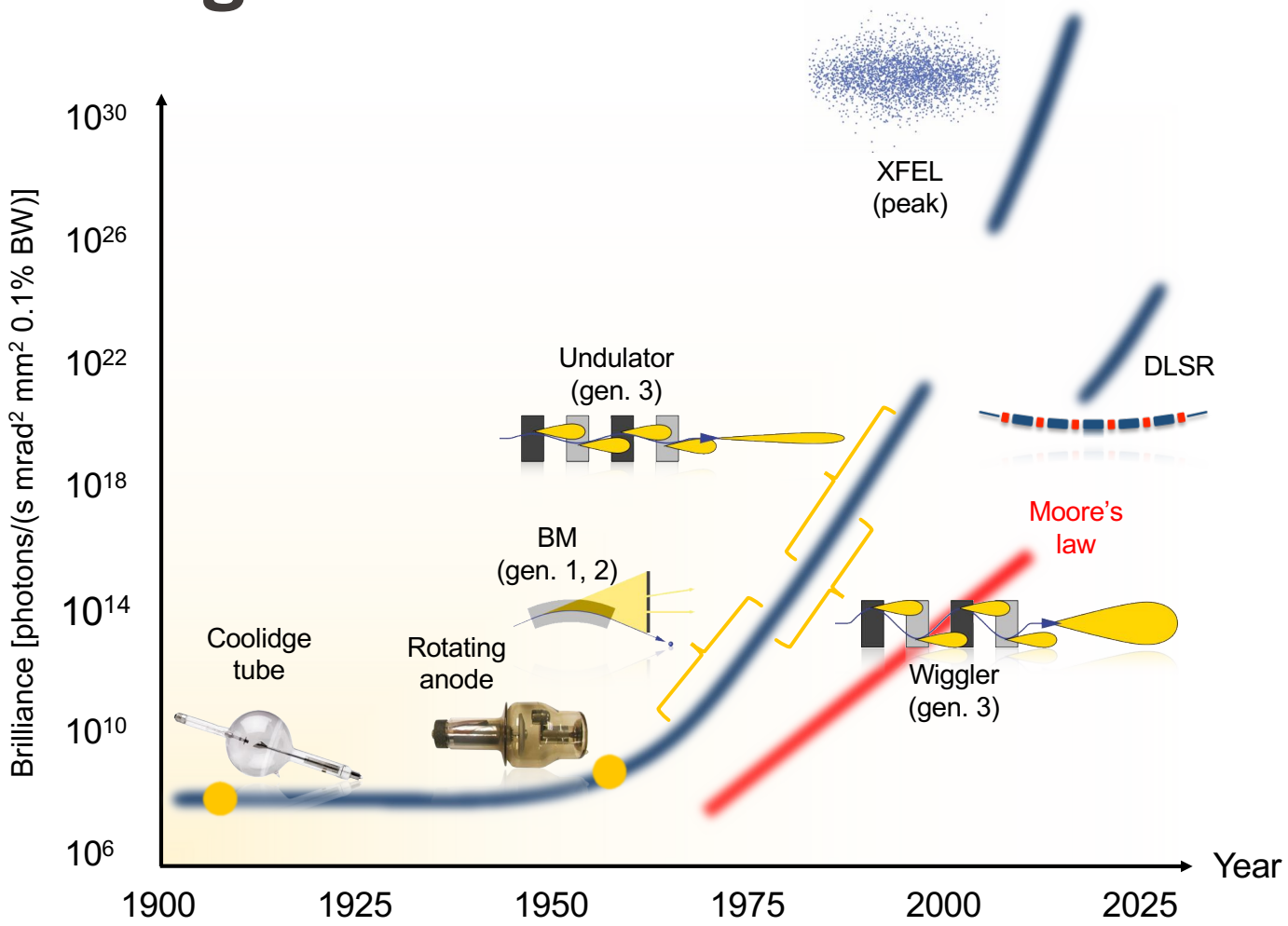
Brilliance since Röntgen





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