Structural Biology Program: Scientific Gaps and New Beamline Concepts

Sean McSweeney

September 11, 2018

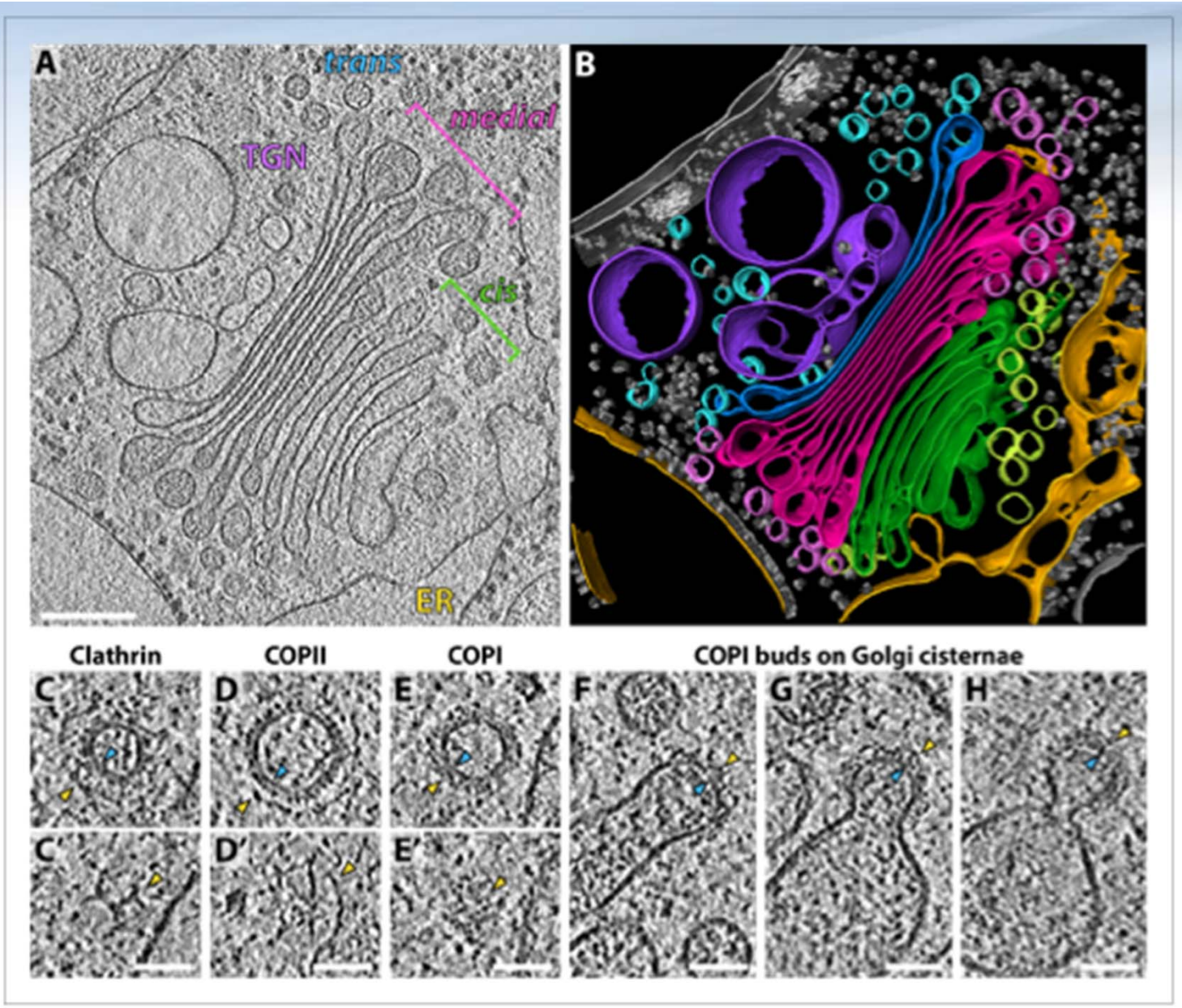
U.S. DEPARTMENT OF ENERGY | Office of Science

BROOKHAVEN NATIONAL LABORATORY | National Synchrotron Light Source II

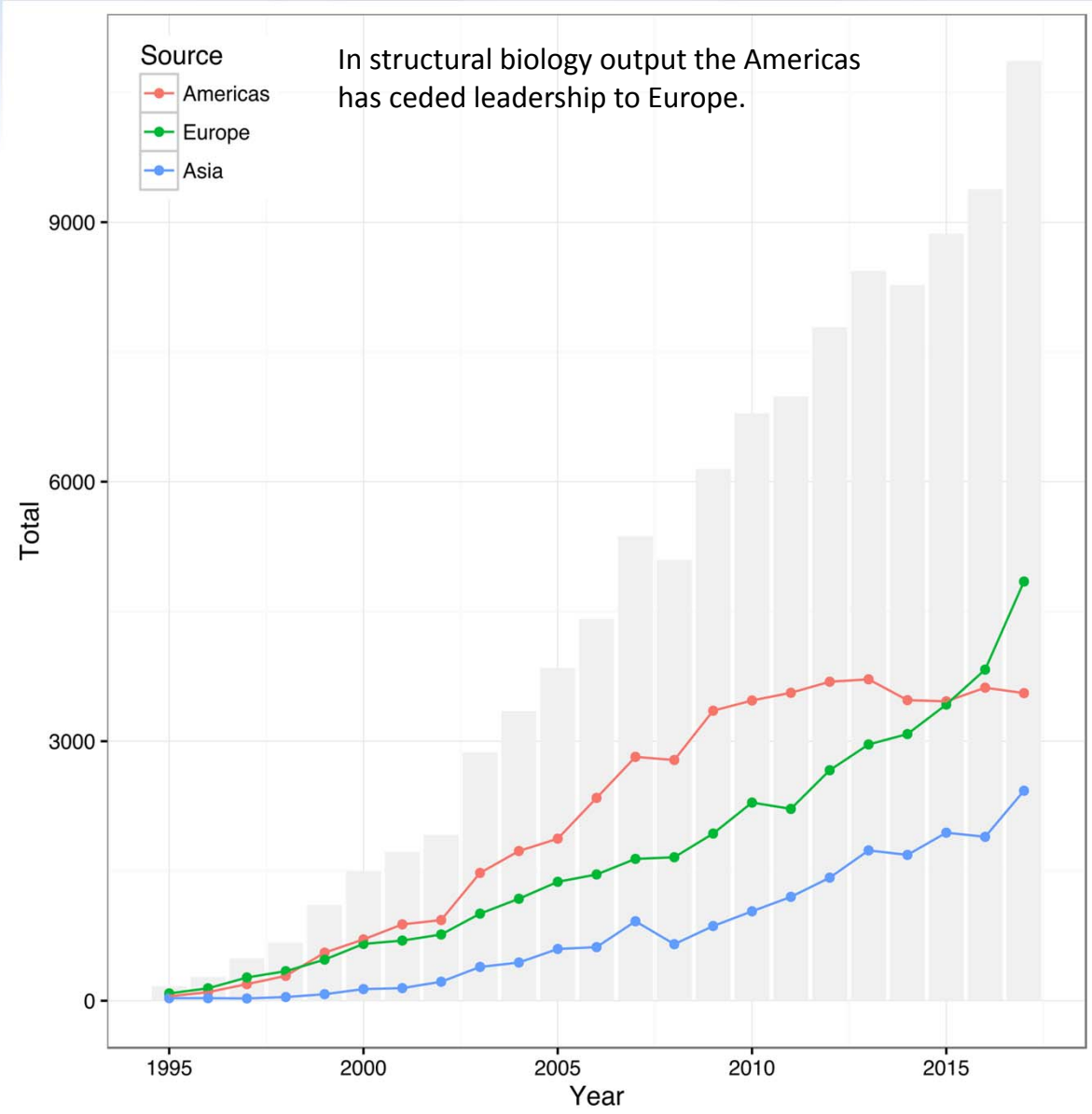
Introduction to the structural biology concepts.

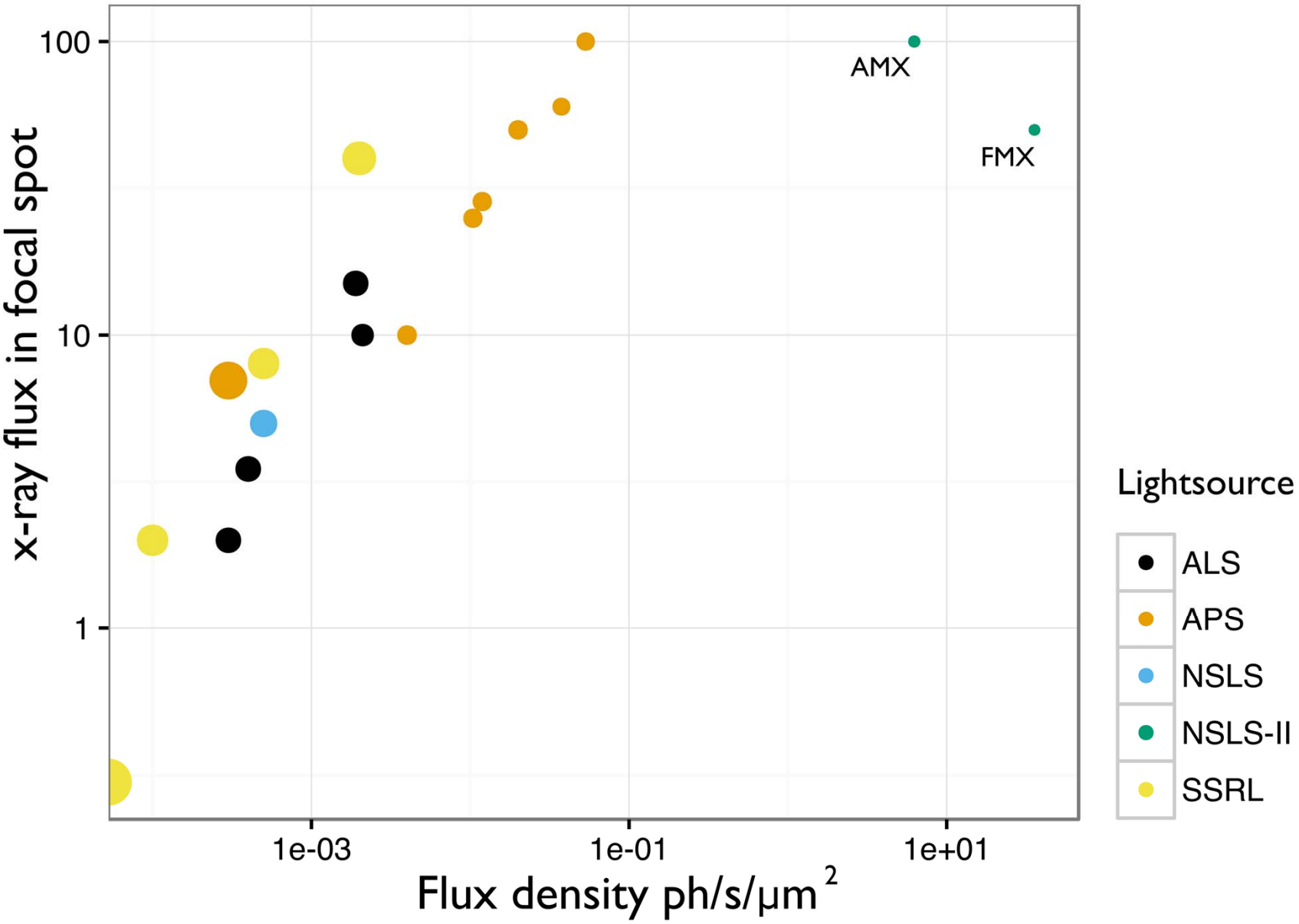

A
B
D

2017 Bykov et al eLIFE



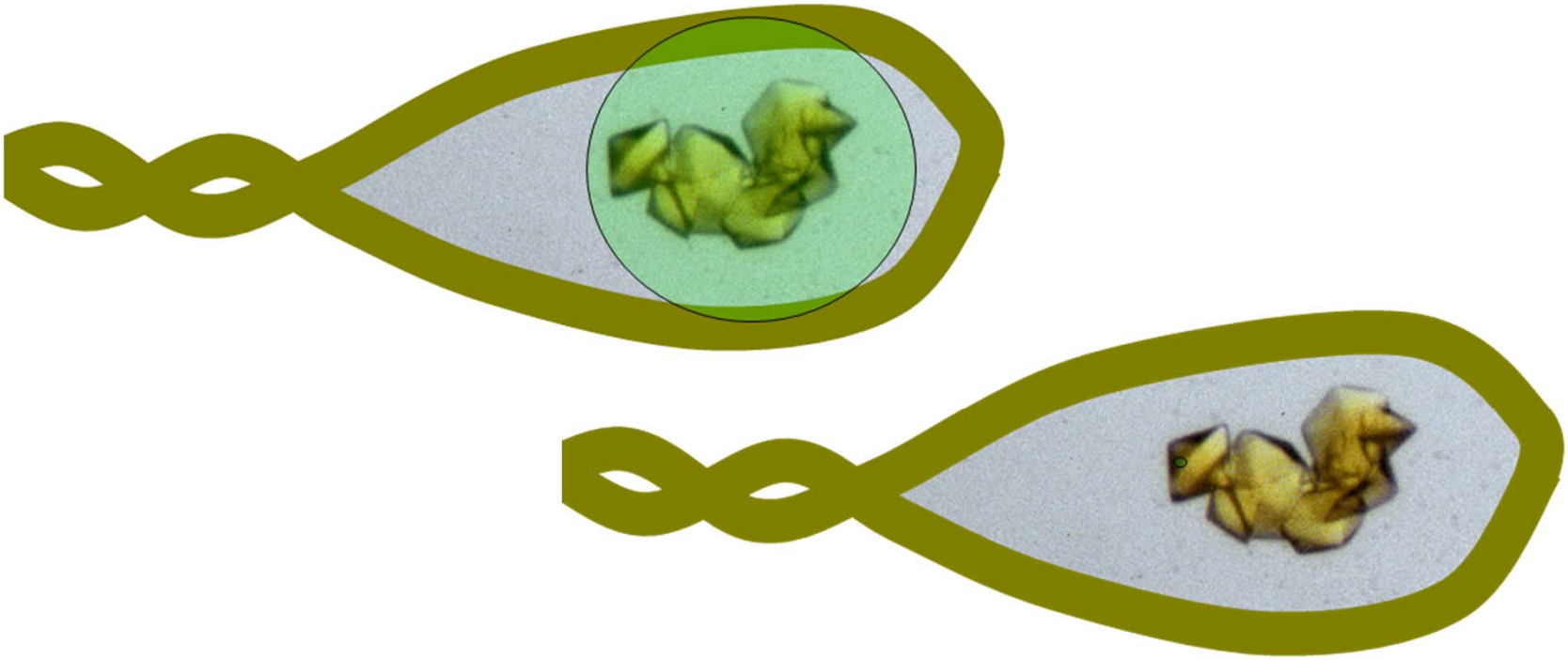
Molecular architecture of the Chlamydomonas Golgi apparatus and transport vesicles revealed by in situ cryo-ET

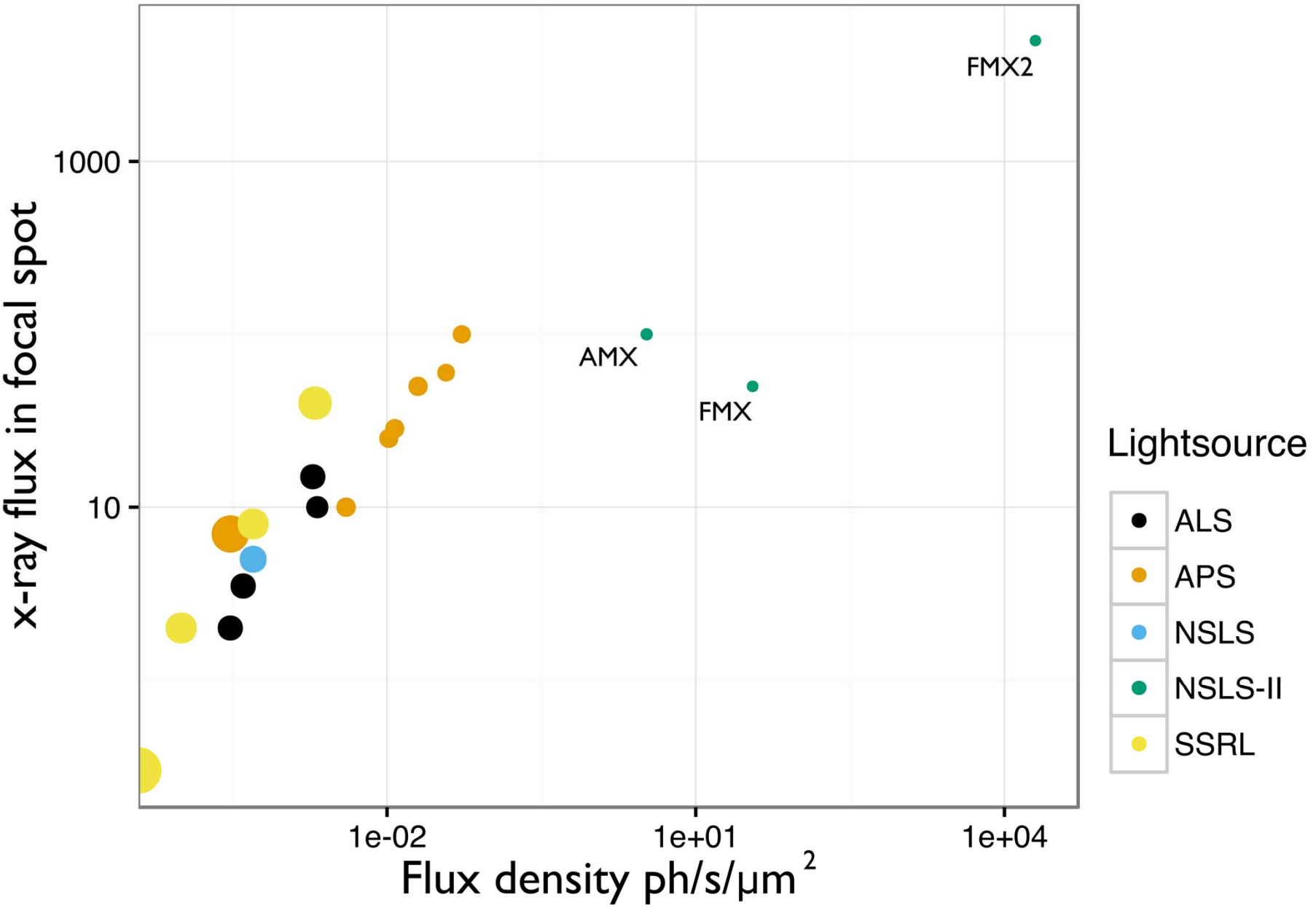

Source
Americas
Europe
Asia
In structural biology output the Americas has ceded leadership to Europe.
9000
6000
3000
0
Total
1995
2000
2005
2010
2015
Year



comparison NSLS-II beamlines wrt other US macromolecular crystallography beamlines. (both axis *10)

the old & new brightness problems





comparison NSLS-II beamlines wrt other US macromolecular crystallography beamlines. (both axis *10)

Biological Needs (IMHO)

- To serve the Bio-science communities we need:
  - **Strong structural Biology** : retain NIH community, develop DOE, increase capacity
  - **A Bio (cryo)microscopy program** : dedicated, optimized instruments, a bigger community, data analysis and visualization on-the-fly.
  - **A presence in EM and Electron Tomography**.
  - A way of joining the above together
  - Inhouse science program that joins lightsource staff to real problems.

Fixed Energy MX

Lonny Berman, Sean McSweeney, Robert Sweet

September 11, 2018

Rationale for the Proposed New Beamlines

- **What is being proposed:** Two fixed-wavelength beamlines for MX viewing an undulator source, leaving space in the sector to accommodate a second (canted) undulator and tunable beamline that can be built later.
- **Science case, including national and/or global context:**
  - Several synchrotron sources are planning to shut down shortly in order to implement major upgrades: CHESS in 2018-2019 (already started), ESRF in 2019-2020, APS in 2022-2023, ALS in 2024-2025. This will severely impact their MX user communities.
- **User interests and demand:**
  - An opportunity exists to deliver substantially higher MX beamline capacity than currently exists at NSLS-II. With the proposed beamlines, NSLS-II could support ~500,000 samples/yr and attract new communities including those displaced elsewhere.
  - There is good reason to believe that the additional user communities to be accommodated through these beamlines would become permanent additions to the NSLS-II community.

Rationale for the Proposed New Beamlines

- **NSLS-II portfolio impact:** The beamlines will be ideal for MX experiments for which wavelength tunability isn't vital – reducing load on AMX, FMX, and NYX, allowing them to focus on their unique capabilities.
- **Scope, performance, and feasibility:**
  - We propose to employ instrumentation proven at both APS and ESRF. The cost is relatively inexpensive and will result in beamlines ~20 times more intense than existing examples of this design, because of the NSLS-II source. The two beamlines can be constructed in 3 years owing to their simplicity.
  - The beamlines will be fixed wavelength (~0.98 Å) viewing an undulator source, based on a diamond monochromator (Laue geometry) and CRL focusing system for each, with automated MX endstations. The anticipated flux is ~5×10^{12} ph/sec, with a ~25 µm beam focus.

High Flux Serial Synchrotron Crystallography (HSX)

Martin Fuchs, Sean McSweeney

September 11, 2018

Rationale for the Proposed New Beamline

- *Concise one-sentence on what is being proposed*
  HSX - A beamline for high flux serial synchrotron crystallography (SSX) at highest dose rate, highest time resolution & highly flexible sample delivery. "FEL type serial crystallography experiments at a Storage Ring"
- *Science case, including national and/or global context*
  Beam size for smallest crystals (<0.5 um). Dose rate for µs enzyme kinetics (FMX does ms) and for room temperature measurements of functionally important conformations.
  Dedicated beamline & endstation for SSX at ESRF (EBSL8) & PETRA (TREXX P14.EH2). SSX at MAX IV (MicroMAX) & Diamond (I24). Fixed target FEL developments at LCLS (MFX). Micro Electron Diffraction.
- *User interests and demands*
  Membrane proteins and enzymes are still held back by MX limitations. With HSX, hard to grow small crystals become viable targets. Accommodate challenging sample conditions (cryo & room temperature, light & substrate droplet activation). Minimize time to acquire complete data from microcrystals of one project. SSX support from sample loading to structure.

Rationale for the Proposed New Beamline

- *NSLS-II portfolio impact*
  World class SSX beamline even with ESRF upgrade (EBSL8 specs 0.5 µm focus, 10^{16} ph/s). Dedicated beamline for SSX to support beamline specialization in Biovillage. MX beamlines over-subscription (APS shutdown). Sample delivery for SSX requires high flexibility (more than FMX can provide).
- *Scope, performance, and feasibility, including implementation as a new endstation or as a new branchline on an existing beamline*
  Highest flux from full-length (not-canted) undulator (10^{16} ph/s at 12.7 keV); energy range 5 - 20 keV (Si detector still efficient); multiple bandwidths DMM (0.1 – 1%); focusing to <0.5 µm with mirrors for highest flux performance; CRLs or kinoform lenses for defocusing; sample delivery with rapid scanner, high viscosity jet, conveyor belt, droplet ejection; laser excitation; goniometer with ultrafast limited range rotation; faster detector (2-10 kHz, integrating?)

Low-energy Anomalous X-ray Diffraction
Beamline LAX

Wayne A. Hendrickson, Sean McSweeney

September 11, 2018

Rationale for the LAX Beamline

- Concise one-sentence on what is being proposed

The LAX beamline is designed to optimize biomolecular diffraction analyses, emphasizing exploitation of the anomalous scattering from sulfur atoms in proteins and phosphorus atoms in nucleic acids.

- Science case, including national and/or global context

Although many crystal structures can be solved using information from known structures (molecular replacement), true unknowns require phases for the measured diffraction data. Currently over 90% of *de novo* crystal structures are determined by either multi- or single-wavelength anomalous diffraction (MAD or SAD) methods. Recent advances (Hendrickson, *Quart. Rev. Biophys.* **47**, 49, 2014) that exploit multiple crystals and lower energy x-rays have made it possible to solve structures directly and efficiently from native SAD experiments, but few beamlines in the world are optimized for these experiments.

Refinements from low-energy anomalous diffraction data also facilitate accurate elemental identifications, such as for biological ions: Na^{+}, K^{+}, Ca^{2+}, Cl-, and PO_4^{2-}, which are especially useful in studies of ion channel proteins and in environmental biology.

- User interests and demands

Recent demonstrations of the effectiveness of native SAD analyses, even at 6-7 keV, make clear that ready access to a beamline optimized for native SAD will make it an instrument of choice.

Rationale for the LAX Beamline

- NSLS-II portfolio impact

LAX will complement the existing suite of macromolecular crystallography (MX) beamlines at NSLS-II (AMX, FMX and NYX) with its specialization in the low-energy regime while it will also provide a significant gain in capacity for accommodating MX generally.

- Scope, performance and feasibility

The intended spectral coverage by LAX will be from 2 – 7 keV (≈ 6 - 1.8 Å) and energy resolution will adequate to fully exploit uranium M edges ($\Delta E/E \approx 10^{-5}$). The detector system will access $2\theta \leq 110^{o}$ (d_{min} = 2.5 Å at 3 keV). Monochromatic flux will be as focused from a 1 m undulator.

LAX will be a companion beamline to NYX, sharing the 19-ID straight section with a canted undulator having a period optimized to the spectral region. NYX was designed to accommodate LAX, including a large offset double crystal monochromator and focusing mirrors. Optics will be in vacuum, but the diffractometer system will be in a helium environment system supplied through a LN-cooled He gas used to maintain samples at 100K while also purging the diffraction environment.

The detector will be a specially designed pixel array detector (PAD). One option would be a cylindrical array similar to the Pilatus detector implemented at Diamond beamline I23. Another option is a hemispherical design based on the geometry of virus particles such as adenovirus.

MAX
Massively Automated Crystallography

Jean Jakoncic, Alexi Soares & Sean McSweeney

September 11, 2018

U.S. DEPARTMENT OF ENERGY | Office of Science

BROOKHAVEN NATIONAL LABORATORY | National Synchrotron Light Source II

Rationale for the Proposed New Beamline

- Concise one-sentence on what is being proposed

Very high throughput “hands off” beamline enabling screening/data collection of >100 000 samples per year in full operation.

- Science case, including national and/or global context

-No such a beamline worldwide, US best semi “hands-off” beamline: 20000 samples/year.

-World best beamline” hands-off”: 30000 samples/year. Will enable AMX and FMX to focus on the most challenging samples and the most challenging projects

-New Science out of the many more conditions /samples / ligands / dynamics

- User interests and demands

-Pharmaceutical companies are expressing increased need of using NSLS-II, users are requesting a hands-off beamline for standard experiments on hundreds of samples.

-APS shutdown

Rationale for the Proposed New Beamline

- NSLS-II portfolio impact

More users / samples / PDBs / articles / revenue (pharma) at NSLS-II. AMX/FMX too much in demand and facility needs one extra dedicated MX beamline: state of the art in high throughput for samples 25 microns and up.

- Scope, performance, and feasibility, including implementation as a new endstation or as a new branchline on an existing beamline

Total construction project (3PW: **BM16**) from front end to end station; using either spare detector or eiger9m . 25x25 microns beam and 10^{12}ph.s^{-1}, 25 sec sample exchange (limited raster/vector), implementing HW derived from AMX/FMX and simpler optical elements (CC mono, limited energy range 11-14 keV or fixed energy), CRLs for focusing.

Robot / Optimized Dewar following AMX/FMX design.

Simplified version of AMX / FMX end-station

Dry hutch required for open Dewar operation.

MAX2
Massively Automated Crystallography

Jean Jakoncic, Alexi Soares & Sean McSweeney

September 11, 2018

Rationale for the Proposed New Beamline

- Concise one-sentence on what is being proposed

Very high throughput “hands off” beamline enabling screening/data collection of >150 000 samples per year in full operation.

- Science case, including national and/or global context

-No such a beamline worldwide, US best semi “hands-off” beamline: 20000 samples/year.

-World best beamline” hands-off”: 30000 samples/year. Will enable AMX and FMX to focus on the most challenging samples and the most challenging projects

-New Science out of the many more conditions /samples / ligands / dynamics

- User interests and demands

-Pharmaceutical companies are expressing increased need of using NSLS-II, users are requesting a hands-off beamline for standard experiments on hundreds of samples.

-APS shutdown

Rationale for the Proposed New Beamline

- NSLS-II portfolio impact

More users / samples / PDBs / articles / revenue (pharma) at NSLS-II. AMX/FMX too much in demand and facility needs one extra dedicated MX beamline: state of the art in high throughput for samples 25 microns and up.

- Scope, performance, and feasibility, including implementation as a new endstation or as a new branchline on an existing beamline

Total construction project (ID) from front end to end station; using either existing eiger9m or Nextgen Eiger. ~25x25 microns beam and >10^{13}ph.s^{-1}, 25 sec sample exchange (excel in raster/vector), implementing HW derived from AMX/FMX and simpler optical elements (CC mono, limited energy range 11-14 keV), CRLs for focusing. 2 Branches to be built: fixed energy using Diamon beam splitter 1st anf tunable branch. >> up to 250 000 samples / year

Robot / Optimized Dewar following AMX/FMX design.

Simplified version of AMX / FMX end-station

Dry hutch required for open Dewar operation.