Context of the ALBA upgrade and your input in it

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Mission: ALBA as open facility

- Contribute to the improvement of well-being and progress of society as a whole through provision of scientific instruments dedicated to solving societal challenges such as health, environment, energy and communication.

- Act as a catalyst for regional and national collaborations addressing overarching societal challenges.

- December 2020: green light by funding agencies to ALBA-II

- Preliminary timeline
  - Now-Sep 2021: Proposal New beamlines by scientific community
  - Jan 2022: provide a Scientific Case, including non X-ray instruments (White Paper)
  - Construction in several phases 2022-2030
The Context: ALBA II at a Glance

ALBA II

- Existing IDs
- New IDs

1st plot
move injection

13 IDs BLs
8 BM BLs
1 IR BL

2 Strights for rf
1 Straight for injection

2nd plot
move MSPD

original long BL

ALBA14BL?
Life Sciences at ALBA now

From Harkiolaki et al. DOI:10.1042/ETLS20170086 ; CC BY 4.0 modified
The ambitious program requires:

Set of **state of the art X-ray tools**.

Extension of the services to **cryo-EM**.

**Laboratory infrastructure** to interface other labs techniques with synchrotron core activities.

Integration of **computational biology**

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**The Section Reviews**

- Mission verification.
- Strategy review.
- Gap Analysis.
- Service review.

**Workshops & Colloquium**

- Identification of challenges.
- Identification of needs.
- Creating awareness
- Organizing community.

**Call for New Beamlines or services**

- Identification of instruments and services
- Grouping key-players around proposed instruments.
ASTIP is in the preproposal stage, which includes AMBIC for Life Sciences

The life sciences center AMBIC centers around:

- Macromolecular Structure & Dynamics
- System & Cell Imaging
- Cellular Biology
- Computational Biology
- Sample Preparation and Support Facility
MX Future: XALOC and XAIRA
**XALOC and XAIRA**

**XALOC:**
- High throughput
- Ligand screening
- High resolution
- High energy

**XAIRA:**
- Micro beam (1x1 to 10x10 um²)
- Native phasing (down to 4 keV)
- Fixed-target SSX
- Low background (He chamber)
Future opportunities

• Near future: **Upgrades and improvements**
  • Improvements in **equipment**
  • Improvements in **workflows and automation**
  • Extension of **data processing** capabilities

• Next generation > **ALBA II**
  • Increased flux > **higher intensity**
  • Increased **coherence** > ??
  • Time signatures of the beam > pulses and timings for **time-resolved MX**

Taylor new scientific capabilities to users needs
Short/medium term actions

- Extend data processing pipelines to include phaser and ARCIMBOLDO (new data management section)

- MXCuBE improvements:
  - Incorporate BL elements in the GUI (shower, aperture)
  - Migrate to the MXCuBE3 version

- Upgrade detector to Pilatus 3 100 Hz, ~450x450 mm

- Xtal farm
MX will provide a reliable and automated tool for standard, high throughput and high resolution crystallography and scanning sample holders for suitable crystals

**Equipment:**
- Detector: speed, pixel size, sensitivity etc
- Sample changer: capacity, speed, barcode reader? etc
- .......

**Workflow:**
- Automation: full? Semi?
- Experimental techniques: expert data collection? Xtal scanning? Serial?
- ....

**Access:**
- Reduce shift length?
- Upgrade remote?
- ....

**Data processing:**
- Full pipeline needed?
- Focus on different programs???
- ....

**Missing anything?**
**Prioritize**
Future of cryoEM at ALBA
Correlation with the other Life Science programs

- Phasing of MX data
- Docking of crystal structures in higher order assemblies
- SAXS cross-validation of cryoEM complexes
- Computational modelling of missing regions
- Characterisation of cryoEM sample
- General sample visualisation
- Fitting of SPA structures into cell tomogram reconstructions
Current status

(PROGRAMED) CAPABILITIES

> **Glacios cryo-TEM** w/ 200 kV X-FEG, autoloader, Falcon IV & Ceta-D detectors, AFIS-capable
> **Vitrobot** for (semi)automated vitrification of aqueous suspensions (+ **EM GP plunger** at BioLab)
> **BioLab** w/ facilities for protein expression and purification. And a **glow discharger**.
> **Concurrent image processing** to assist data collection
> **Data storage** at ALBA, for at least 2 months

• The **MID-TERM** program goal is to ensure **successful cryoEM SPA experiments from non-expert users**
  cover from sample preparation to data analysis
  our main focus (& differential trait)
  including combination with data from other LS programs

• The **LONG-TERM** program goal is to implement a **cryoET pipeline** (from FIB-milling to data collection & analysis)
Plans for cryoEM SPA program

Sample characterisation
Sample preparation for cryoEM
Grid characterisation
Data collection
Data processing & analysis

Sample characterisation
Buffer optimisation

BioLab
FPLC

Glow discharger
Vitrobot / EMGP

Sample writing
Jet vitrification

Metal evaporator + plasma cleaner

Glacios 200 kV

300 kV cryoTEM

Higher possible resolution (SPA)

Time-resolved experiments

Test different surfaces

Training

Alternatives to blotting

PLANNED

120 kV TEM

DLS
SEC-MALS
DSF

HPC
Data processing pipelines
Data analysis pipelines

Prioritize

- Dynamics & isoforms analysis
- Combination w/
  Crystal structures (MX)
  SAXS envelopes
  Comp. modelling & dynamics
  Tomogram reconstructions
  (MISTRAL, cryoET)

Missing anything?
Cellular and Tissue Biology at ALBA
• **MISTRAL – Soft Xray Microscopy:**
  - Plunge freezing of whole cells
  - 40-50 nm in 3D volumes
  - 10 μm depth

Now developing a super-resolution microscope (SIM) working at cryogenic temperatures (Eva Pereiro)

Allows 3D correlation between cryo-SIM and cryo-SXT on the same cell to locate specific structures, molecules or events in the cellular landscape

cryo-SIM: 120nm lateral res & 240nm depth

• **MIRAS – FTIR:**
  - Studies on tissues
  - 3 μm resolution
  - Mapping of biological fingerprints

**Synchrotron X-ray Fluorescence and FTIR Signatures for Amyloid Fibrillary and Nonfibrillary Plaques**, ACS Chem. Neurosci. (2021); DOI: 10.1021/acschemneuro.1c00048.
MISTRAL, MIRAS, FAXTOR and SXF

- **FAXTOR** – Full field microscopy
  - Large 3D volumes
  - 1 μm resolution
- New in vivo capabilities?
- BSL?

- **SXF** (scanning xray fluorescence):
  - <100 nm resolution
  - Mapping of biological fingerprints
  - Sensitive to oxidation states

Representative SR-μFTIR maps Fe SR-nXRF distribution of the fibrillary plaques:

left column, μFTIR distribution of β sheet aggregates aggregation (1627 cm⁻¹/1657 cm⁻¹)

middle column, Fe distribution map of the same area;

right panel, Fe distribution along the line marked in the Fe distribution map.

Discussion and input
1. Do you identify techniques that may fill in the gap between your expertise and the use of the synchrotron?

2. Which conditions you would like for such techniques? Cryo?, biosafety level?, others?

3. Which are the beamlines you would like to be built?

4. Do you want to be involved in any initiative to propose a beamline? easy pre-proposal deadline 6 Sep, developed proposals 6 Dec, help from ALBA staff
5. Which experiments would like to do at the beamlines?
   Apart from raster / helical etc. Jet serial? Fixed target serial?

6. Which equipment upgrades would you like to see in MX beamlines?
   New detector, upgraded sample changer, peripherals (humidifier, lasers etc)? Temperature range?

7. Access: which improvements in user access would you like to have?
   Shorter shifts? Other interface? Communication with staff ok?

8. Data processing:
   Up to what point? More programs, or more capabilities? Ligand visualization on site?
9. Which experiments would like to do at the microscopes?
   Cryoelectron tomography? Time-resolved SPA? Drug development?

10. Which support would you need?
    Self service? Send-in sample? User training possibilities?

11. Data processing:
    Up to what point? Which capabilities?