STRUCTURAL BIOLOGY BAG MEETING 2023 QUESTIONNAIRE

6TH FEBRUARY 2023

https://www.esrf.fr/home/Industry/contact/questionnaire-for-bag-responsibles.html

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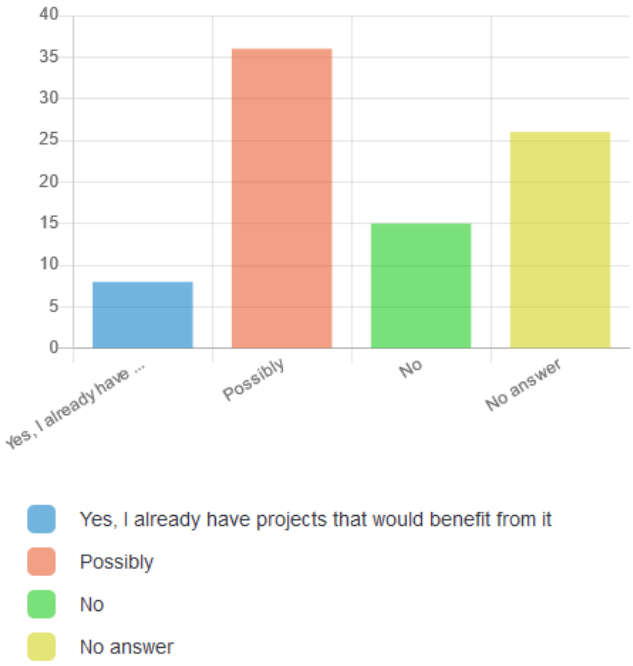
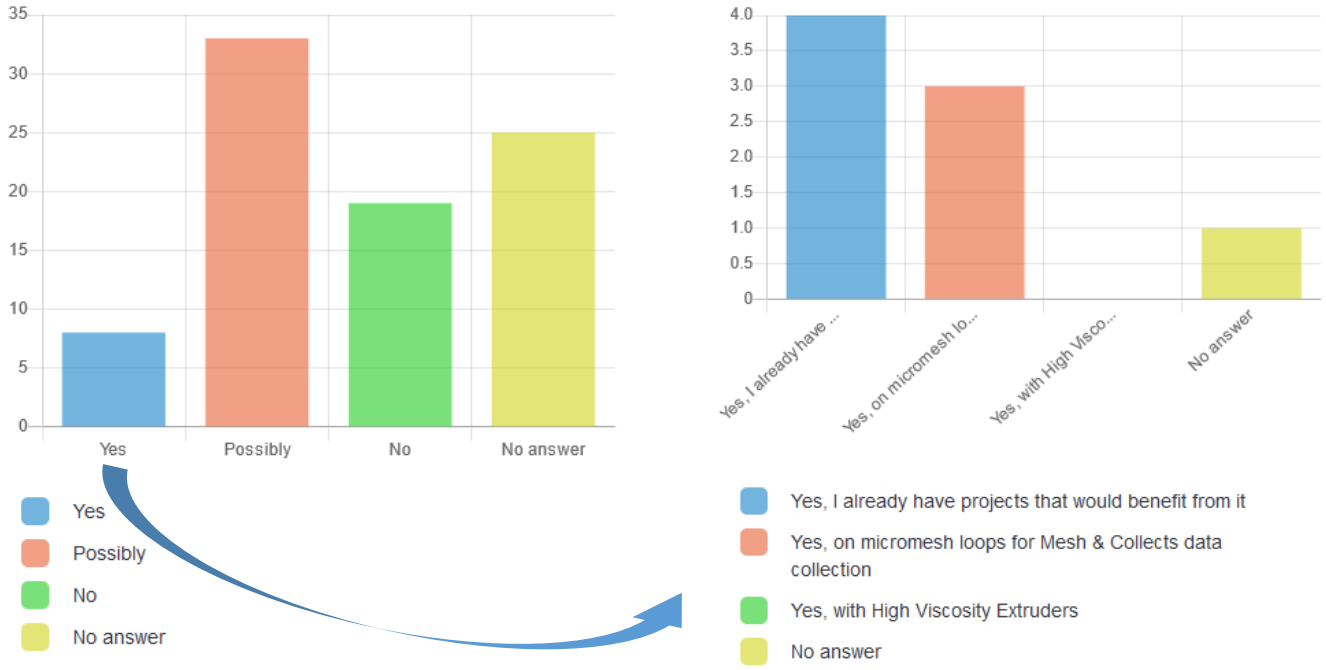
FUTURE INTERESTS

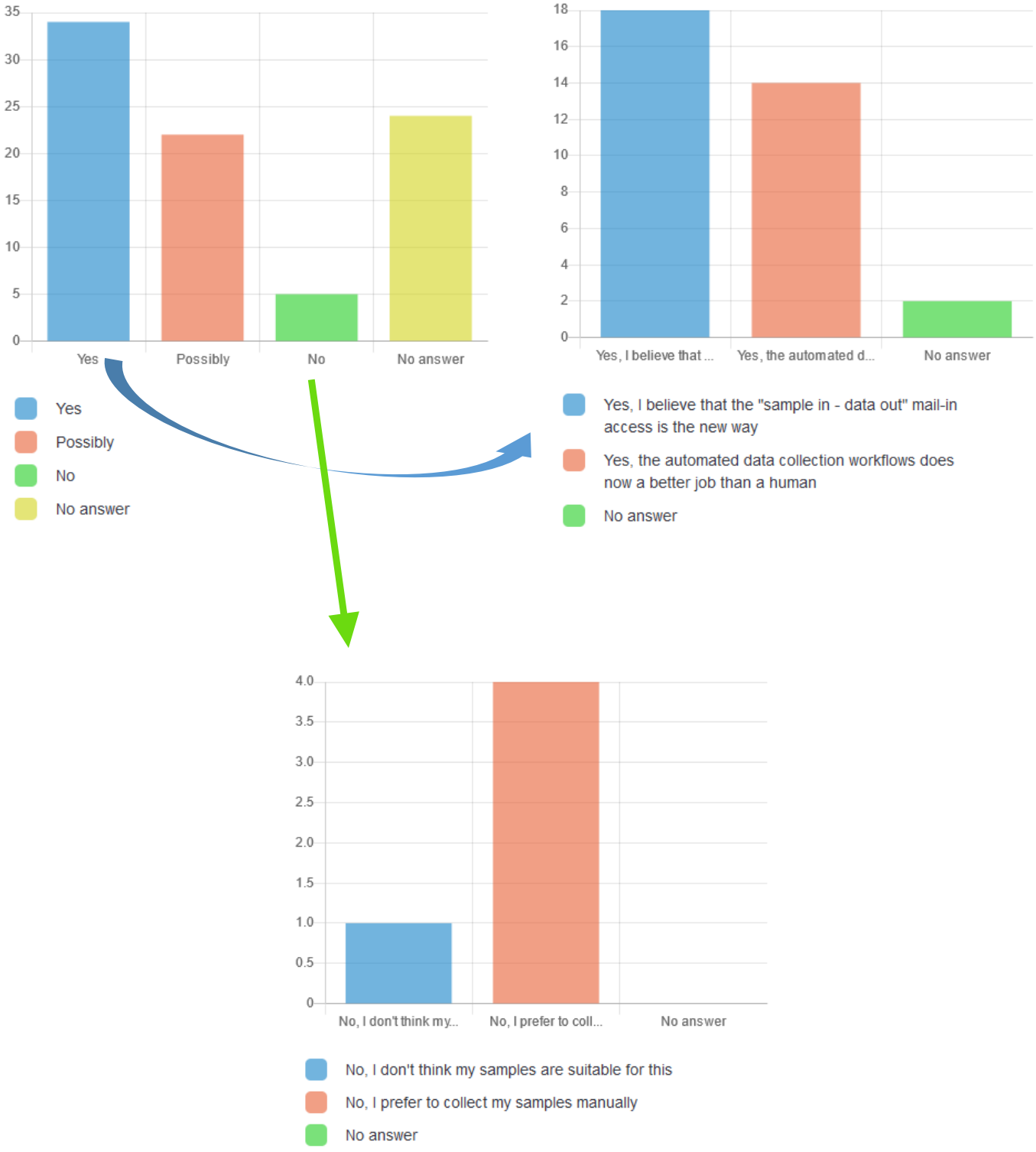
1. Would you be interested in time-resolved crystallography? *(Please also see the dedicated "tr-ssx & rt-ssx" section for more detailed questions about ssx on our id29 beamline)*



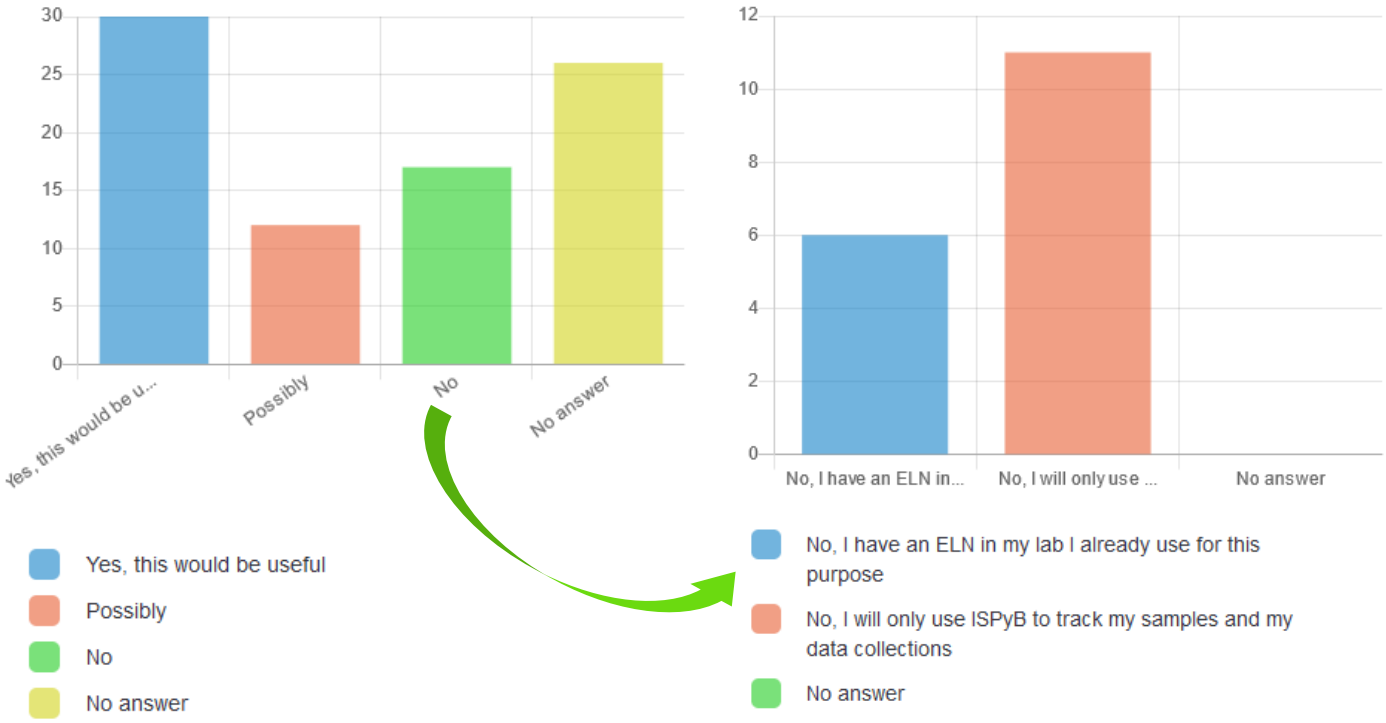
2. Would you be interested in doing more serial crystallography experiments on a non-SSX dedicated beamline? *(Please also see the dedicated "tr-ssx & rt-ssx" for more detailed questions about ssx on our id29 beamline)*



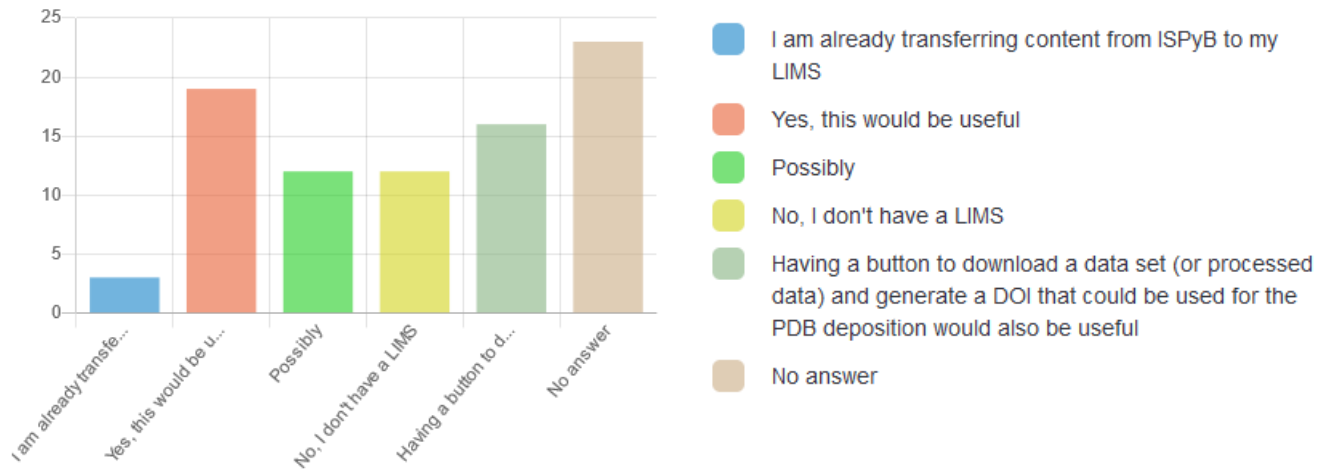
3. Would you benefit from using more often fully automated mail-in data collection in the years to come?



4. Would you use ISpyB as an electronic lab notebook (ELN) to manually store associated data to your samples or to your experiment you could then access at any time *(e.g. Crystallisation conditions, lab prep, result snapshots, etc.)*?



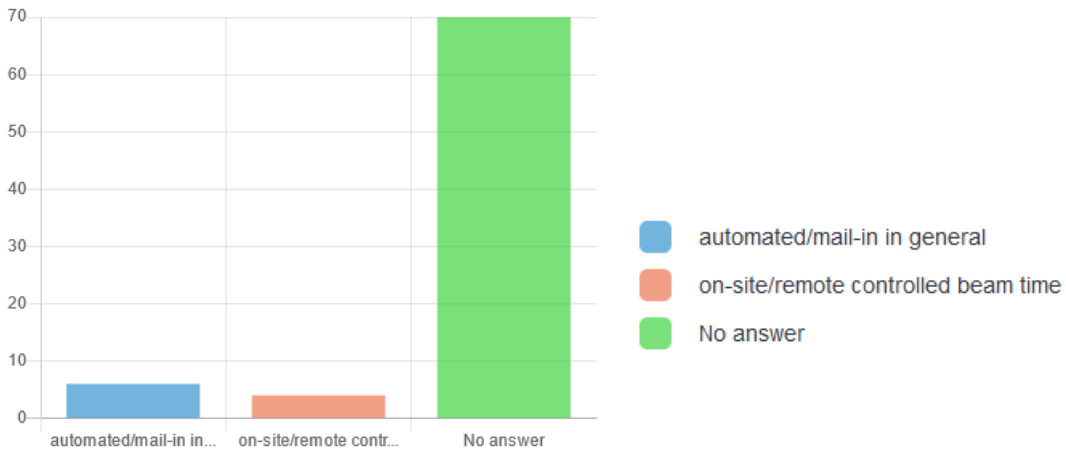
5. Would you like ISPyB to offer the possibility to link sample-associated content with your LIMS database to either prepare an experiment or transfer metadata?



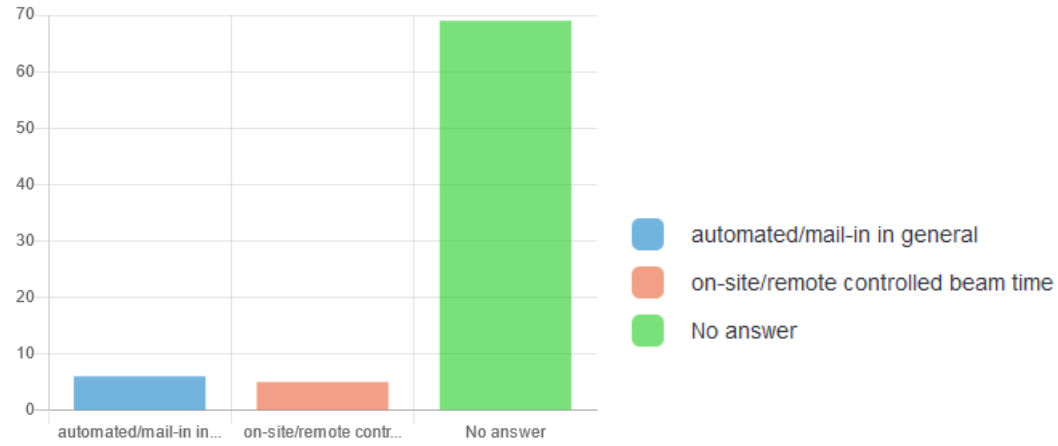
COMMENTS ABOUT YOUR FUTURE INTERESTS

- *Possibly in IceBear would be a useful tool together with ISPyB for data input/output and for shipping purposes from local labs.*
- *ISPyB and LIMS user manual will be really good to use ISPyB and link it to LIMS in local labs.*
- *More access to automatic beam line*

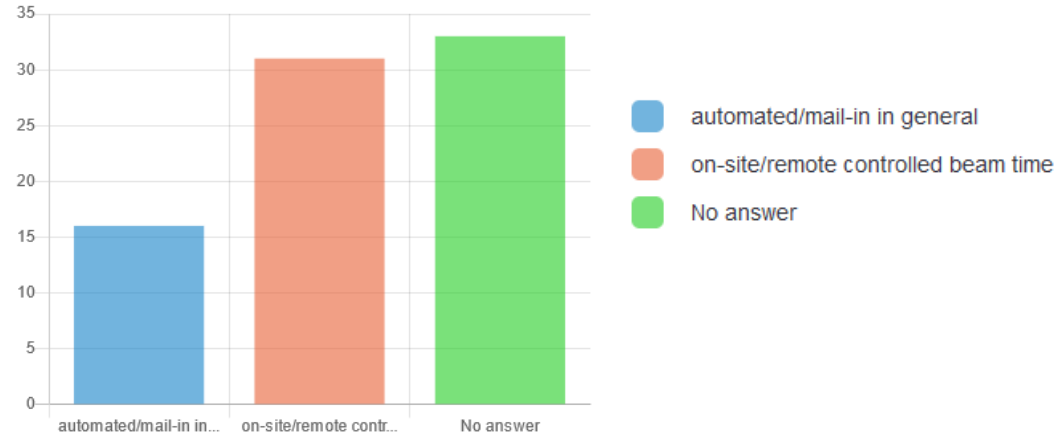
1. Are the delays in general **too long** for



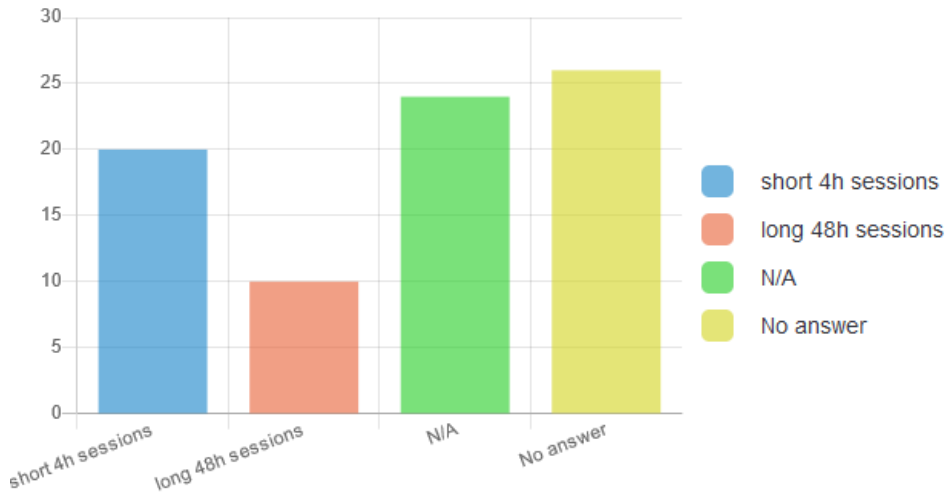
2. Are the delays in general **too short** for



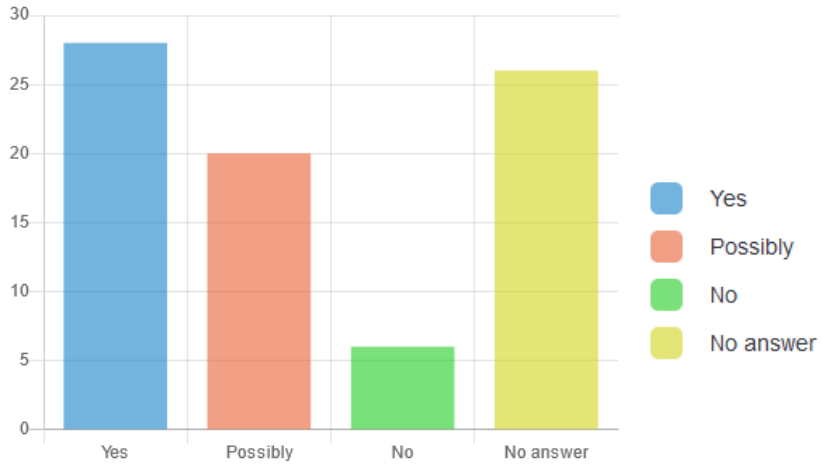
3. Are the delays in general **just right** for



4. Would you be interested in booking more



5. Would you like to have the opportunity to prebook your beam time (or part of your beam time allocation) through an online collaborative calendar as you do for MASSIF-1?

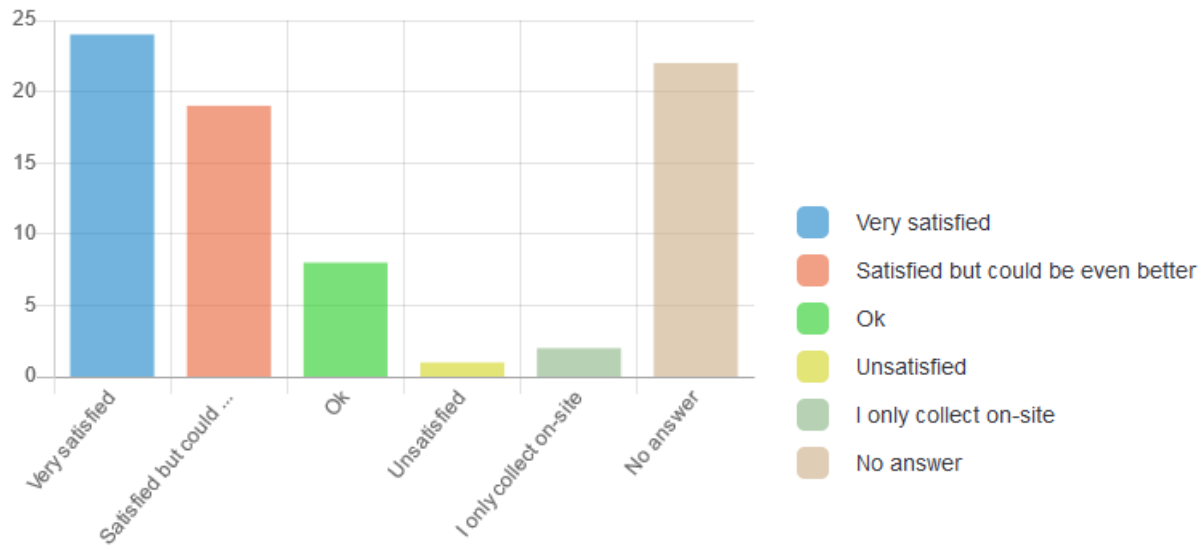


COMMENTS ABOUT THE BEAMTIME RESTERVATION

- *We haven't used MASSIF-1 much in recent years. However, we find that the ESRF beamtime bookings often are excessive for the number of samples that we can send - this leads to cancellation or wasted beamtime that is not made available to other users. We also use other sources, namely ALBA, and we find that the scheduling often overlaps, again leading to one of the periods being cancelled due to an insufficient number of samples. Should it be possible to implement, booking of short periods 1/2 to 2 shifts would be ideal, even if on automatic data collection mode.*
- *With varying need for beam time it could be useful to book ourselves.*
- *It would nice to be able to book beam time by hours (e.g 5 or 6 hours).*

CURRENT PRACTICES

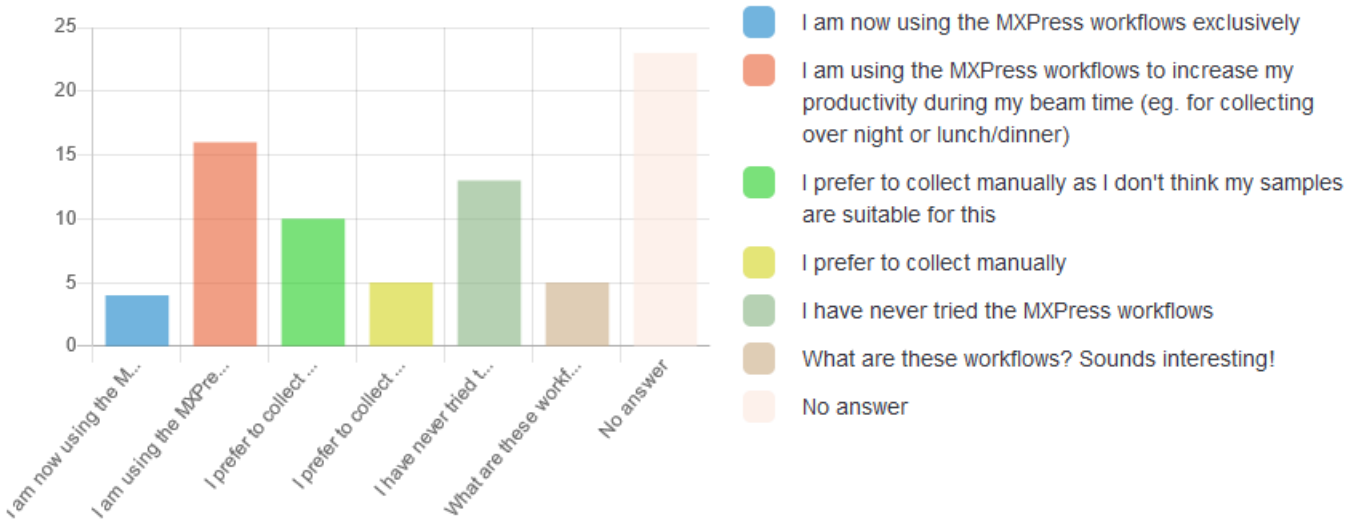
1. How would you rate your overall experience with remote MX data collection at the ESRF?



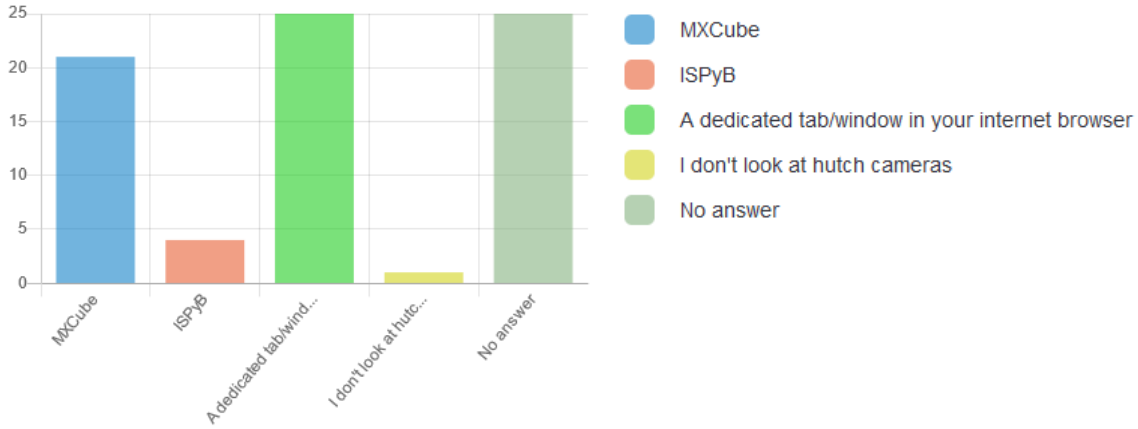
COMMENTS ON THIS QUESTION 1 - OVERALL EXPERIENCE WITH REMOTE MX DATA COLLECTION AT THE ESRF

- *The remote data collection is not adapted for a particular aspect: inspection of images and this relies completely on IspYB. Although it is useful, better inspection of images is required very often. Adxv is not working very well with the remote current set-up*
  On the tunable beamlines, we experienced difficulties in using energy scan being not usable at all
- *We would benefit from:*
  - *fast IspyB*
  - *better night support by LCs*
  - *more shorter sessions*
  - *faster remote handling*
  - *better documentation of programs/scripts on local computers*
- *It depends on the local contact. Sometimes are not very helpful.*
- *The browser based system is really user friendly*
- *In case of a technical problem, there should be a 24/7 support.*
- *Many times there are troubles with the beamline when trying to switch to SAD/MAD data collection. Delays in connecting.*
- *Throughput is still on the lower end if you compare to other synchrotrons; especially X-ray rastering is tedious*
- *Often delays at start. We often have problems with old samples appearing instead of the new ones.*

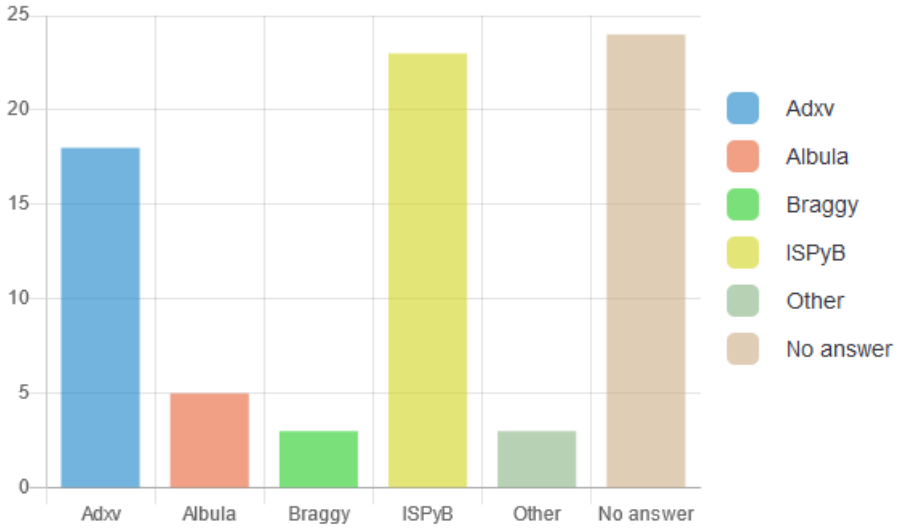
2. What is your overall experience with automated data collection?



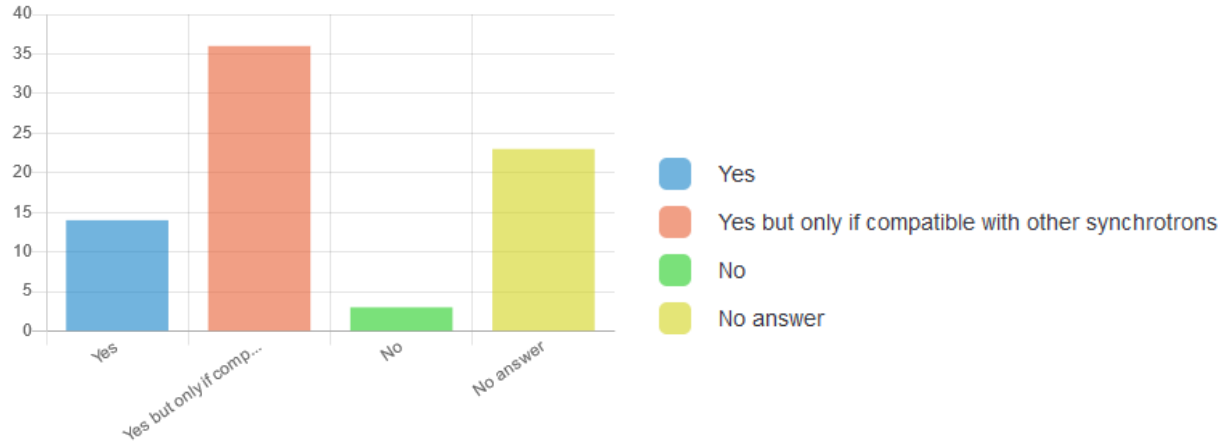
3. Would you prefer to have hutch cameras (live view of the sample changer, goniometer head, ETC.) available in:



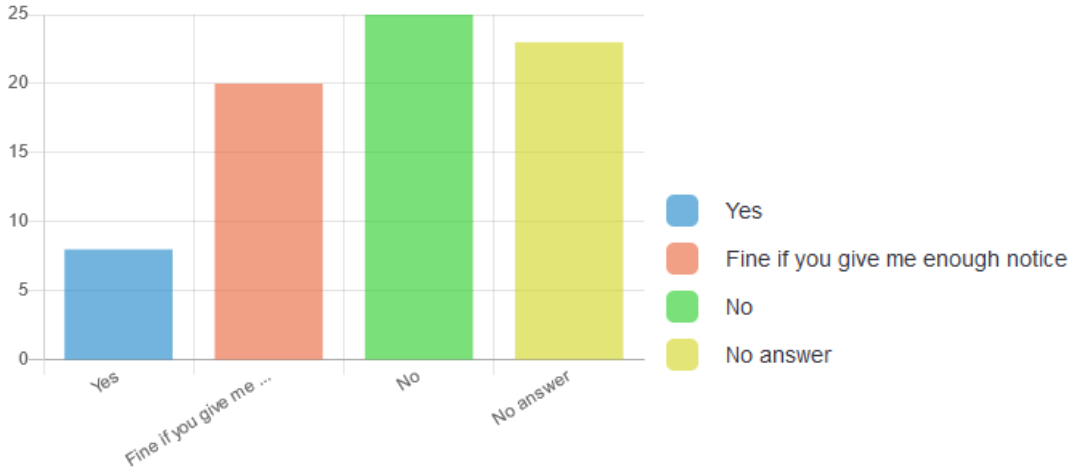
4. What are you using to visualise diffraction image remotely?



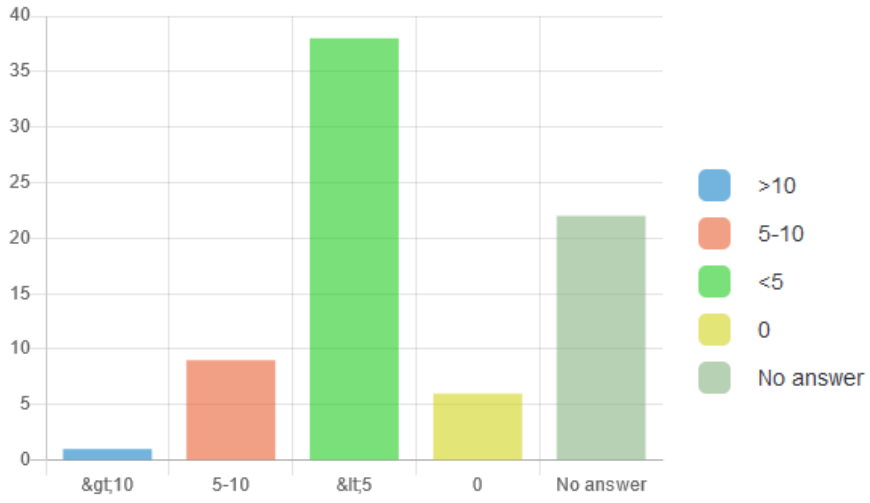
5. Would you accept the ESRF labelling your Uni-pucks for automatic identification by sample changers to decreased chances of assignment errors?



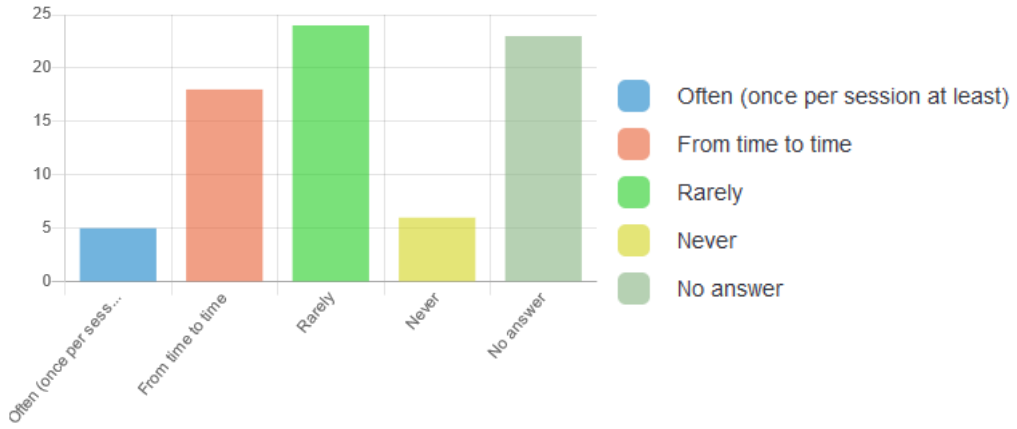
6. If we change our beamlines to only accept Uni-pucks in 2024, would this cause a problem for you (logistically and/or financially)?



7. For how many structures per year do you think you would need anomalous signal-based experimental phasing?



8. Do you still use tunable beamline to change energies in order to identify elements within your protein structures?

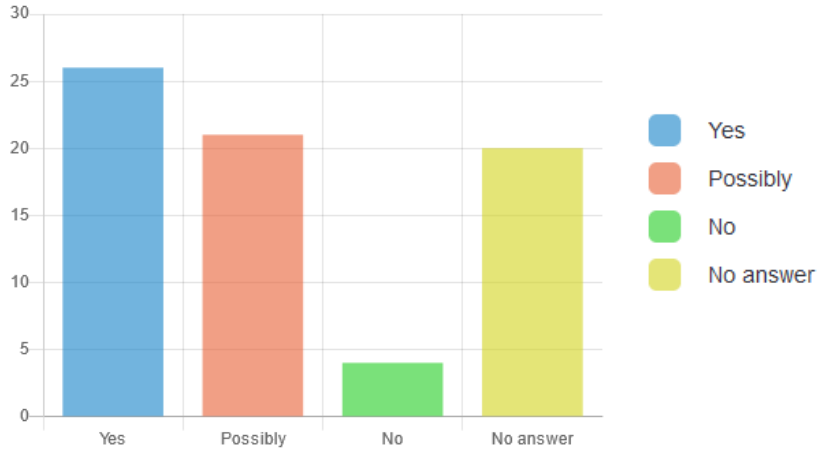


COMMENTS ABOUT YOUR CURRENT PRACTICES

- *Unipuck labeling is a good idea, but only for new sets. Ours already have been labeled by the supplier.*
  We still have a lot of SPINE pucks in stock, which represented a considerable financial investment - I would prefer that some SPINE slots are kept (in some of the beamlines at least).
- *Don't go with UNI-pucks. ESRF was instrumental to bring about the SPINE design, continue to be a part of that great tradition :).*
  Although uni-pucks can load more crystals. It is tedious and unlike the SPINE pucks, its design is overly complicated and not user friendly...
- *Ability to tune energy is important in the field of metalloproteins and metal-protein interactions (and not "just" to get experimental phases, which I understand will get obsolete with AF models)*
- *To be able to see the hutch is relevant when some problem happens. I do not see the need to expend bandwidth with cameras but it should be easy to visit the cameras tab when required.*
- *When trying to identify ions in protein structures, tunable beamlines are the only option. It is essential for my research as I work on cation-binding proteins whose biological activity heavily depends on the ions present*
- *Our samples are often heterogeneous in diffraction which makes a human operator more successful; this might change if algorithms change (i.e. simply looking for number ofo Bragg spots is not sufficient).*
  We already have 2D-barcodes on our pucks (SLS-style) so it needs to be compatible.

1. What type of training do you think would be most beneficial to you or newcomers?

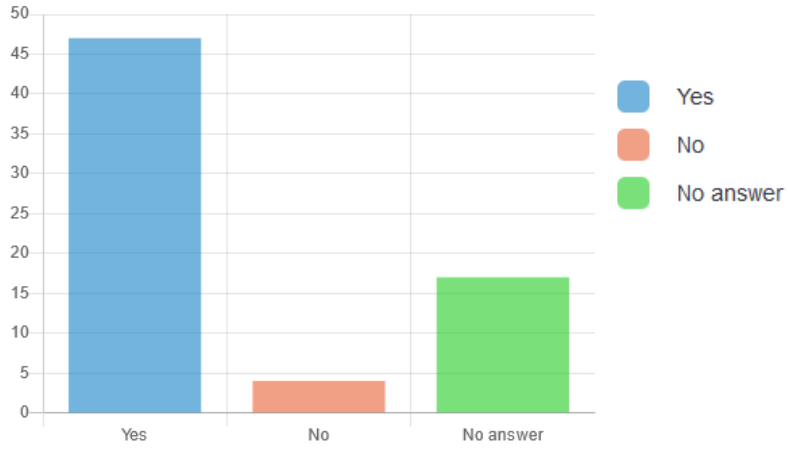
2. Would you be interested in having specific experimental sessions dedicated to the training of students (these sessions would then be more staffed than the other sessions)?



COMMENTS ABOUT TRAINING IN GENERAL

- *I think that the ideal situation for BAG training would be for smaller groups with more hands-on experience, especially for learning new features.*
- *Continuing to have days where the new generation of biophysicists and crystallographers are trained on how to operate beamline instruments is all important. Otherwise a next generation of scientists will have less and less knowledge of what to do when things go awry.*
- *These could be done virtually and could also be available afterwards on Youtube*
- *I am teaching structural biology at the University of Vienna and as our home source is broken and will not be replaced, it would be nice to do data collections with the students remotely.*
- *Hand-on training will be beneficial at-least one session before actual on-site data collection.*

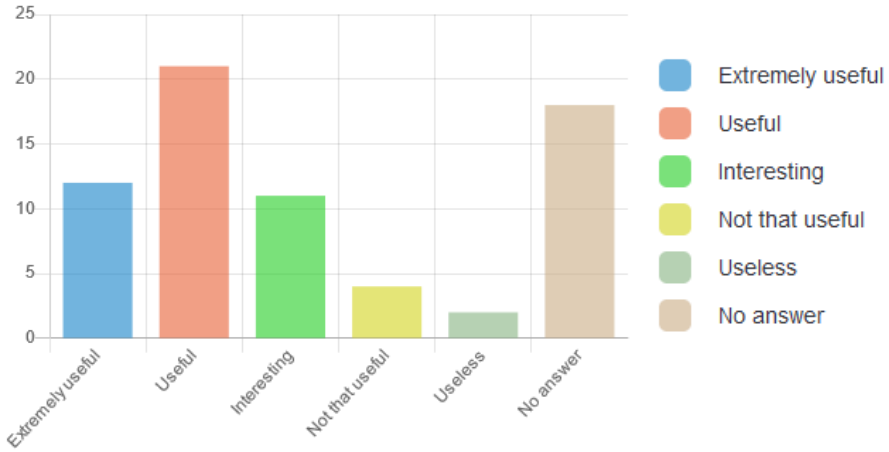
1. Does the data analysis we currently offer meet your expectations *(e.g. Auto-mr, structure refinement, etc.)*?



COMMENTS ON THIS QUESTION 1 – DATA ANALYSIS WE CURRENTLY OFFER

- *It would be great if MR can be done without specifying the space group. Meaning I give a PDB or uniprot ID for the alphafold model and the program finds the number of molecules per asymmetric unit and then starts MR. To save processing capacity it would be great if there is a column in the sample form where I can tick if MR should de done for my sample. Same goes for SAD phasing. It should not be doen on all samples but just the ones I want.*
- *If possible HKL2000*
- *I always do my structural analysis myself and only consider raw images from the synchrotron.*

2. Would a way to pre-select which processing pipelines are executed be



3. Would you have any suggestions to make alphafold2 molecular replacement more user friendly?

    - *It will be more user friendly but don't know how it will run it it different OS having GUI*
    - *As steated above it should be independant on Spacegroup.*
    - *Not really interested.*
    - *Not aware of the tools for MR with alphafold2. Need more information.*
    - *RAS*
    - *Isn't it already straightforward and efficient ?*
    - *Will not use. Will only use in-house as data is proprietary*

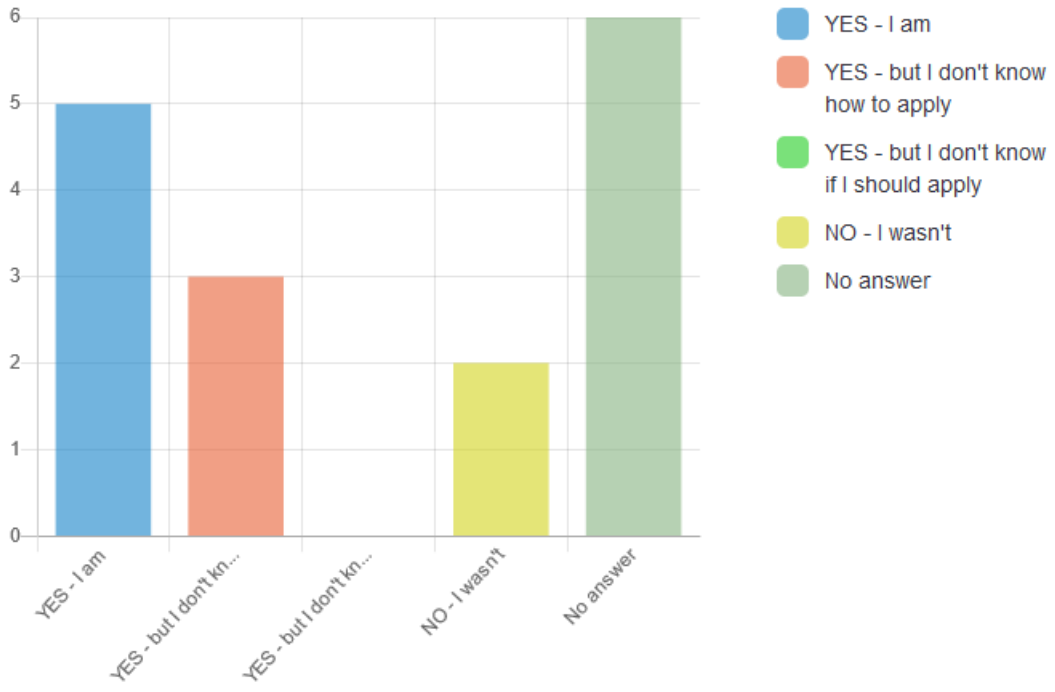
COMMENTS ABOUT DATA PROCESSING IN GENERAL

- *For several of our structures we don't need the full data processing pipeline - it is a waste of resources. In the data collection tab on MXCUBE3 perhaps check marks could be added to select the level of processing to be done, from simple data processing to MR and SAD phasing.*
- *Yes, but also other pipelines for looking at dynamic data, having FO-FO map files, etc would be useful*
- *the results display can be a bit overwhelming. I would like a more streamlined way*
- *Being able to efficiently donwload from ISPyB the full outputs from data processing pilines and not just the.mtz files would be important.*
- *Data processing for larger unit cells takes time and often doesn't complete. Some measures to resolve this will be highly beneficial.*
- *I think it's a good thing to keep sevreral different processing pipelines as they don't give the same result, depending on crystal ...*

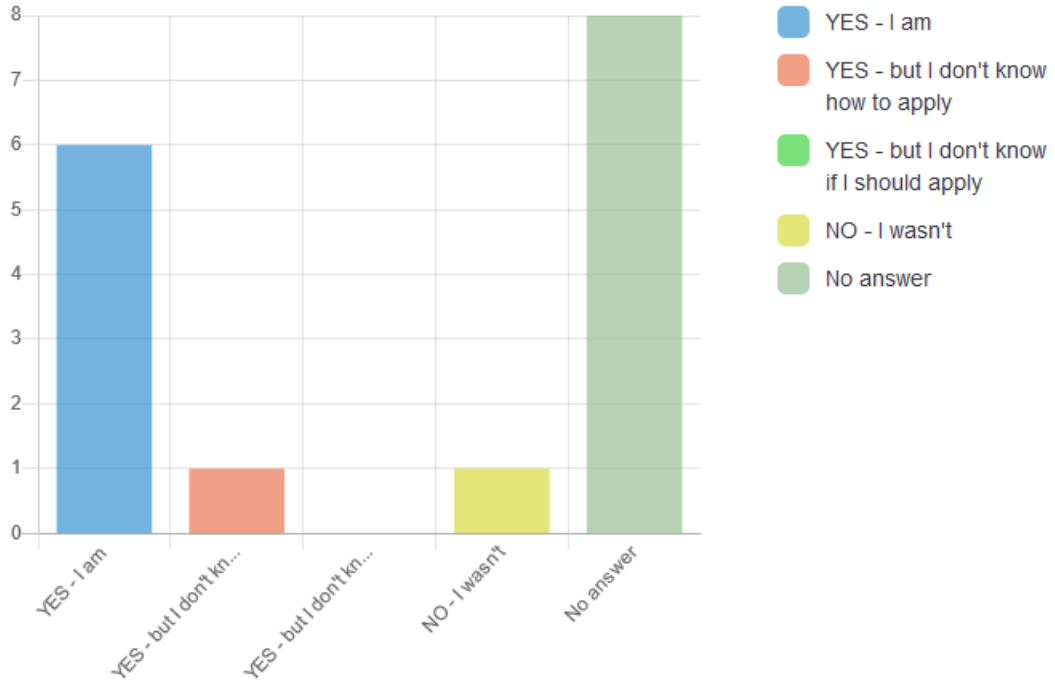
  It would be very nice to be able to reprocess the data with a given resolution. It would be more convenient for data storage and also data deposition at the PDB and publication. Especially with anisotropic processing. This a bit of a nightmare when when there is discrepancies between resolution written in Exi-ispyb and the various data files.

COMPLEMENTARY TECHNIQUES / ANCILLARY EQUIPMENT AVAILABLE

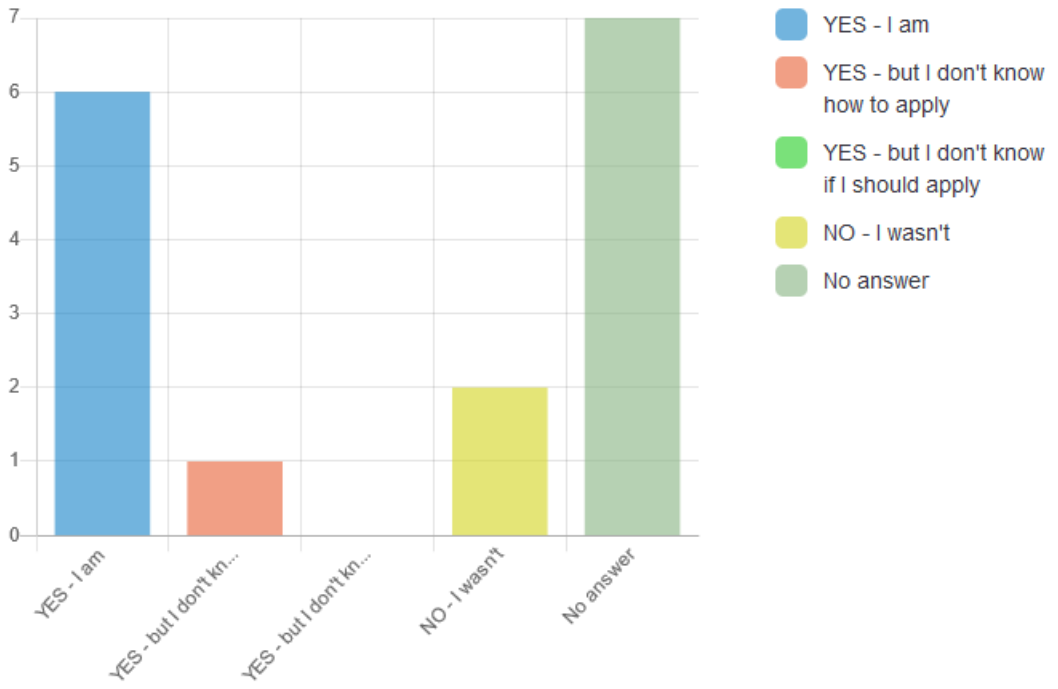
1. Are you aware of the possibility of recording optical spectroscopy data on your protein crystals (metalloproteins, coloured cofactors/ligands) to probe redox state/photoactive state/intermediate state?



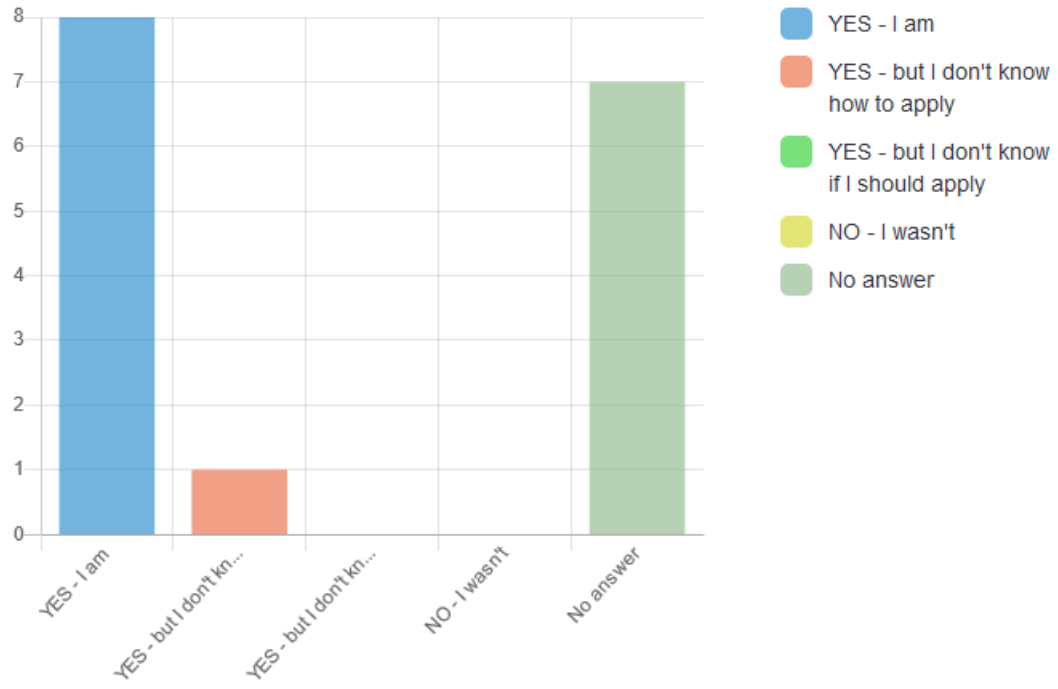
2. Are you aware of the possibility of pressurizing protein crystals with various gases to probe the location of affinity sites or explore the conformational landscape of proteins?



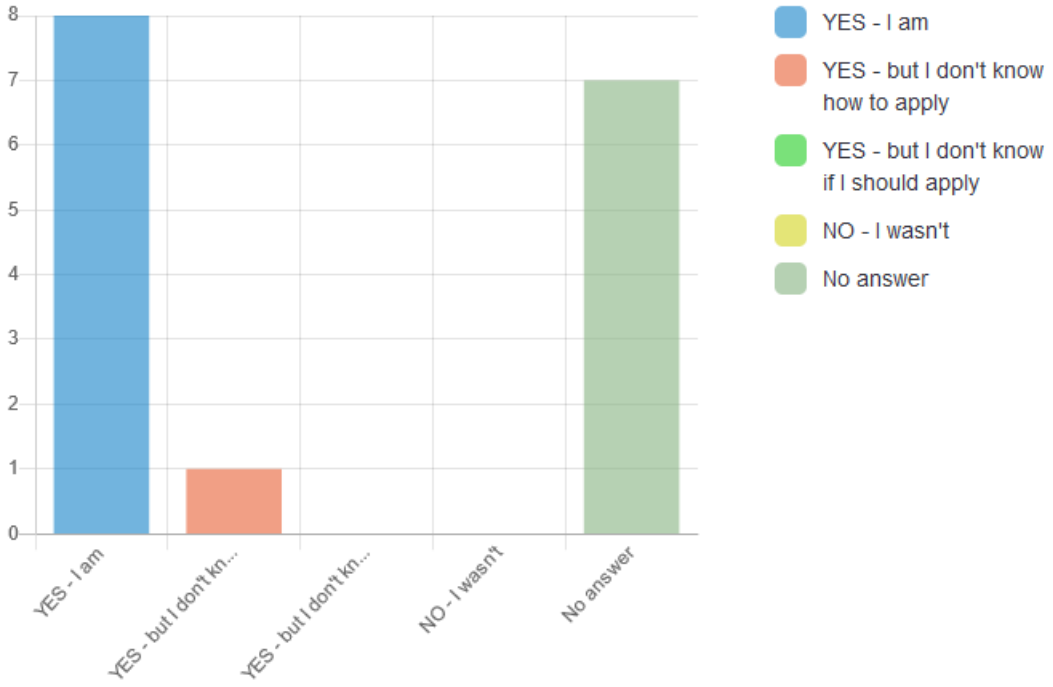
3. Are you aware that room temperature crystallography has now become easier on our beamlines thanks to the HC-Lab and the CrystalDirect Harvester on MASSIF-1, allowing more physiological protein dynamics to be probed?



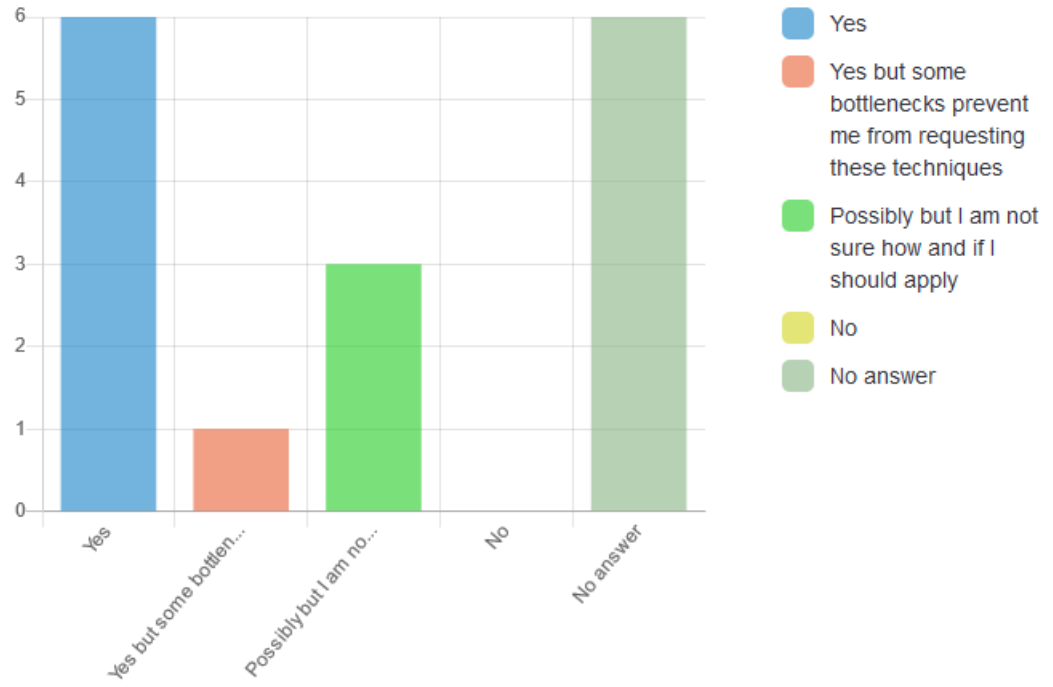
4. Are you aware of the possibility to collect data IN-SITU (directly into crystallisation plates)?



5. Are you aware of the possibility to get your crystal samples automatically harvested and collected on MASSIF-1?



6. Do you have suitable projects in your BAG for these complementary techniques?

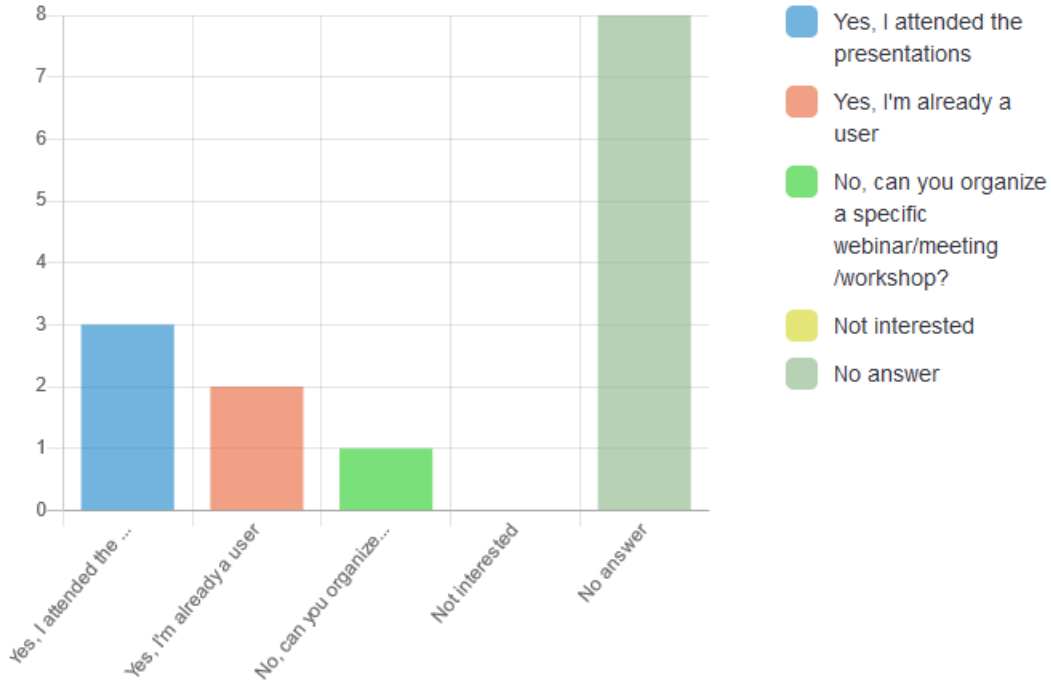


COMMENTS ABOUT THESE COMPLEMENTARY TECHNIQUES

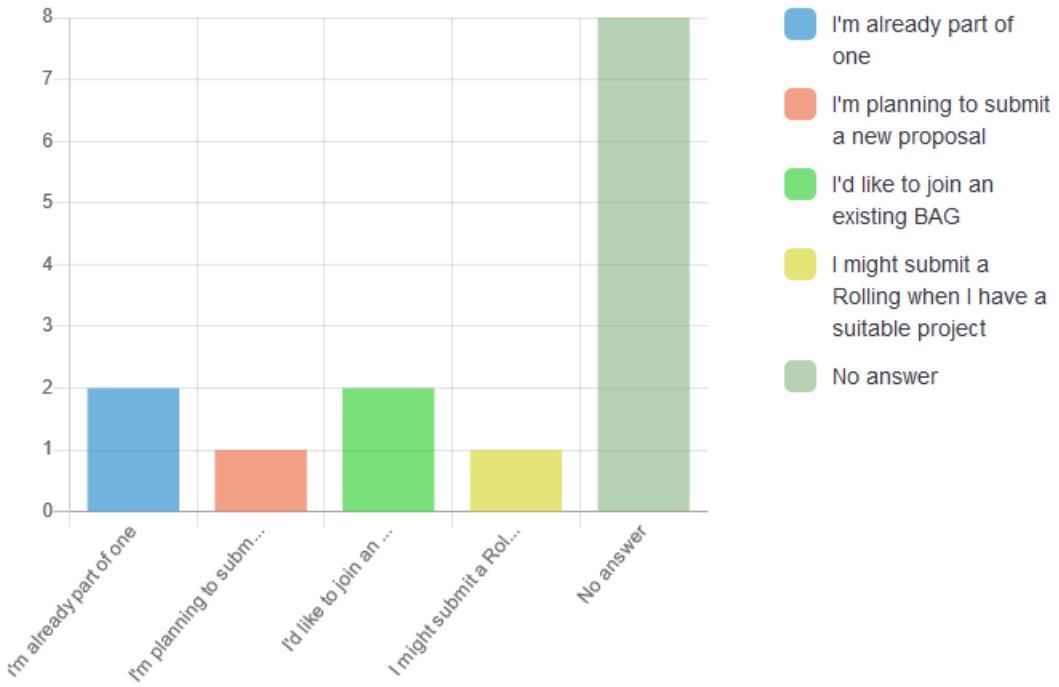
We have used ICOS and the HPX lab before. The results from the ICOS RAMAN platform were not terribly good, perhaps due to the crystals being too large. The results from the HPX lab pressurization with Kr were OK, but with O2 the location of the O2 molecules was rather tricky.

ACCESS & TRAINING

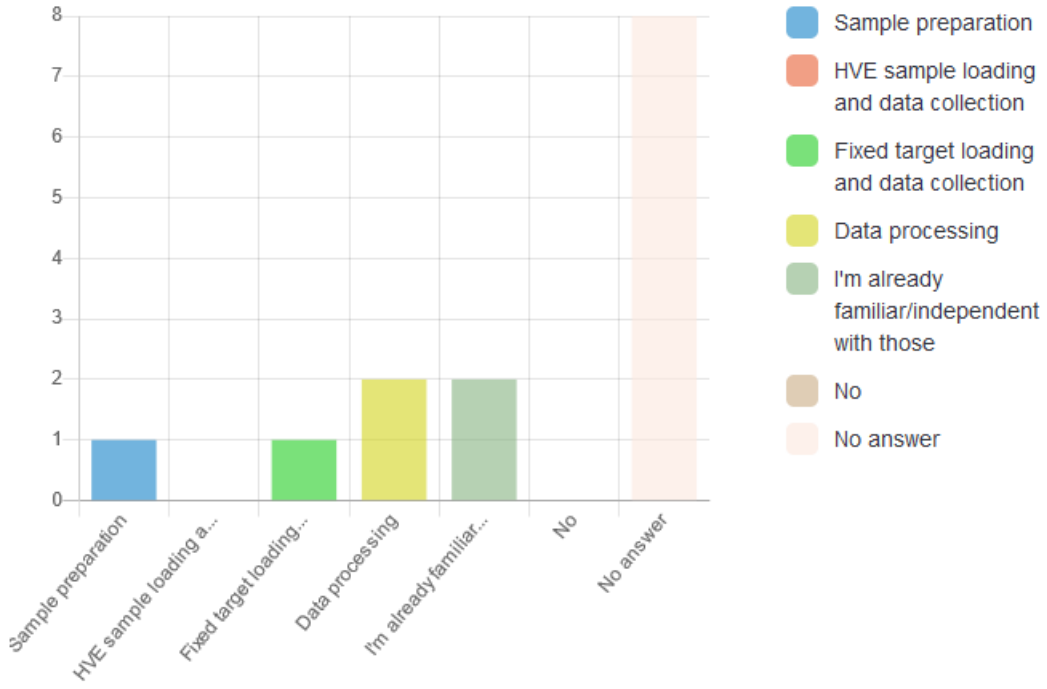
1. Are you aware of the unique beamline characteristics of the new ID29 and what can offer?



2. Access to ID29 beamtime is done via dedicated BAGs (not classic MX) or rolling



3. Would you be interested in specific training session on:

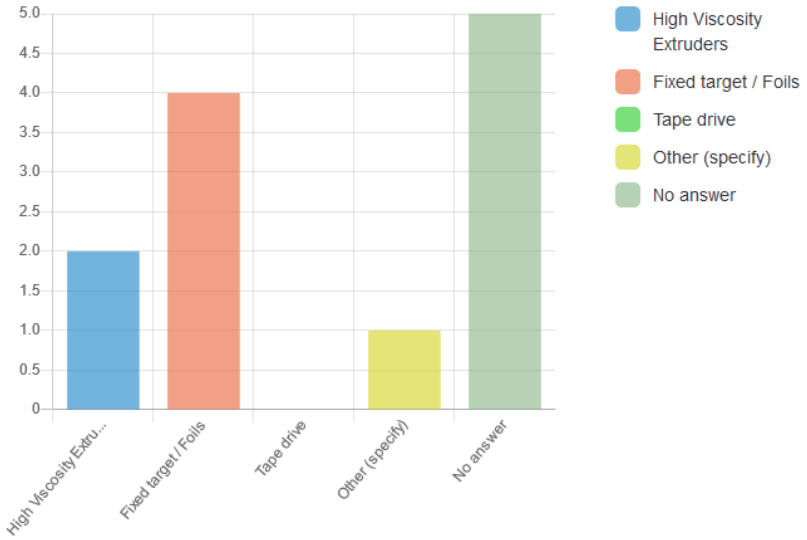


COMMENTS ON THIS ACCESS & TRAINING TOPIC

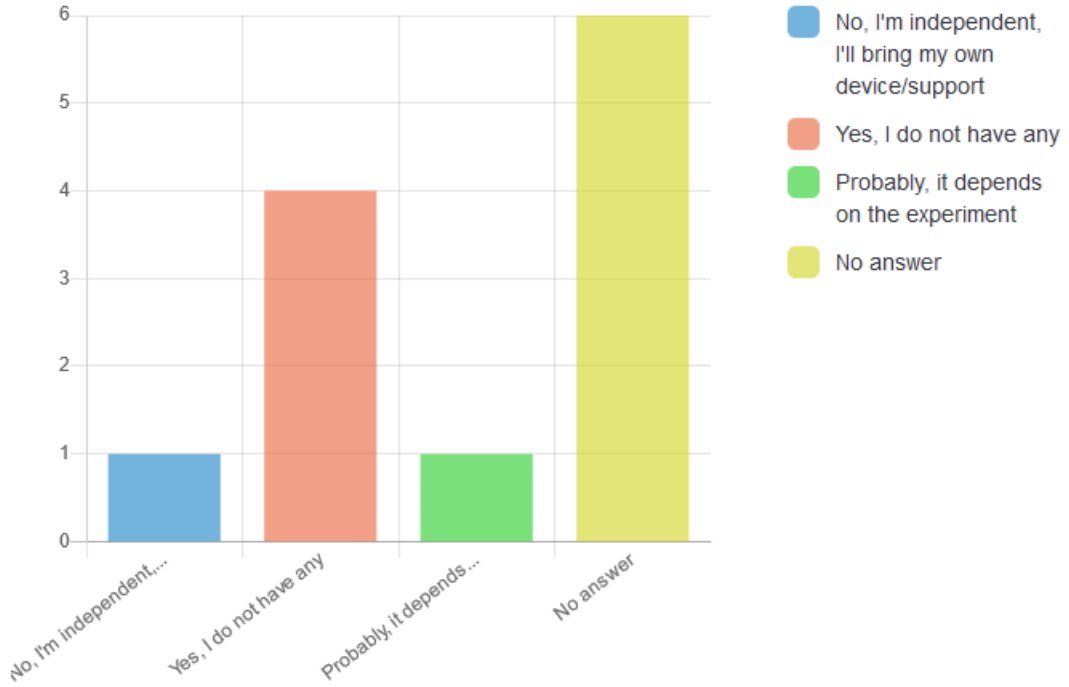
- *I am one of the developers and main users of T-REXX. Training on how to think about TRX experiments, designing experiments, loading samples, data processing etc. is all important for the success of this newly branching field. Dynamic structural analysis is where the future of structural biology, including crystallography, cryo-EM, etc is heading. To best prepare for this change, training the new generation of young scientists is paramount to its success and acceptance amongst the larger biochemistry and biophysics communities.*
- *Please can you please organize a complete session. i am interested.*

TECHNIQUE

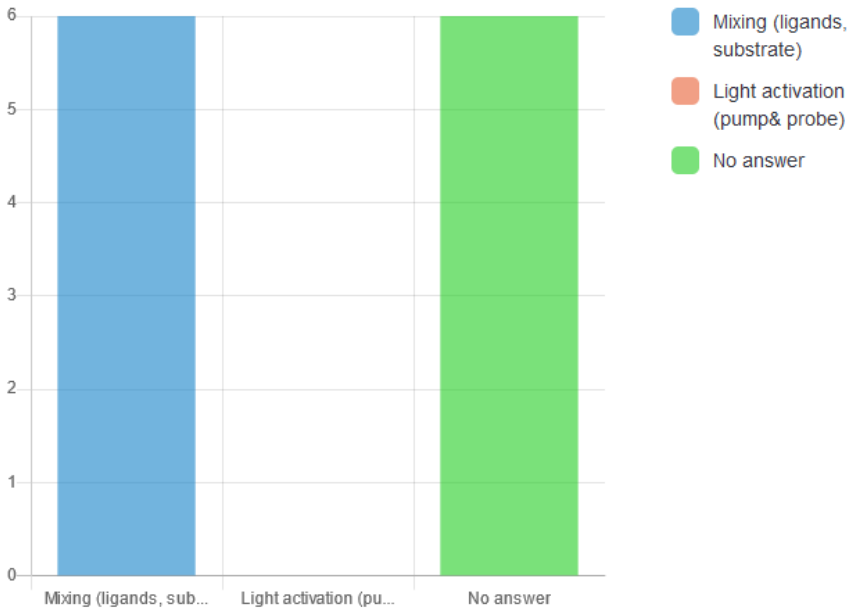
1. Which sample delivery methods would you be interest in using



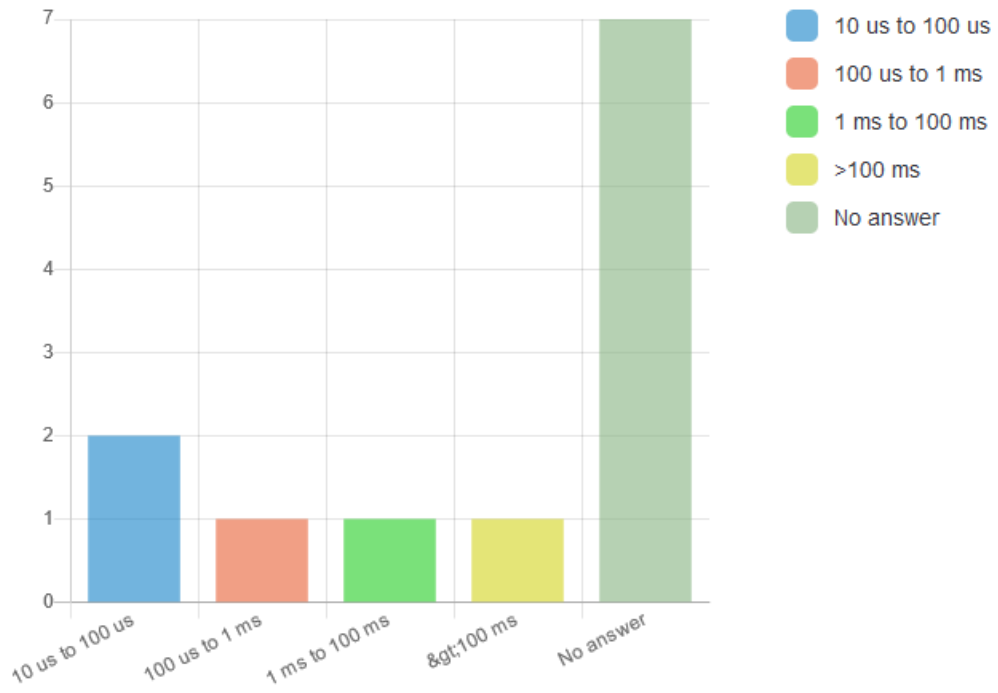
2. Would you need to access the beamline sample delivery methods to perform your experiments?



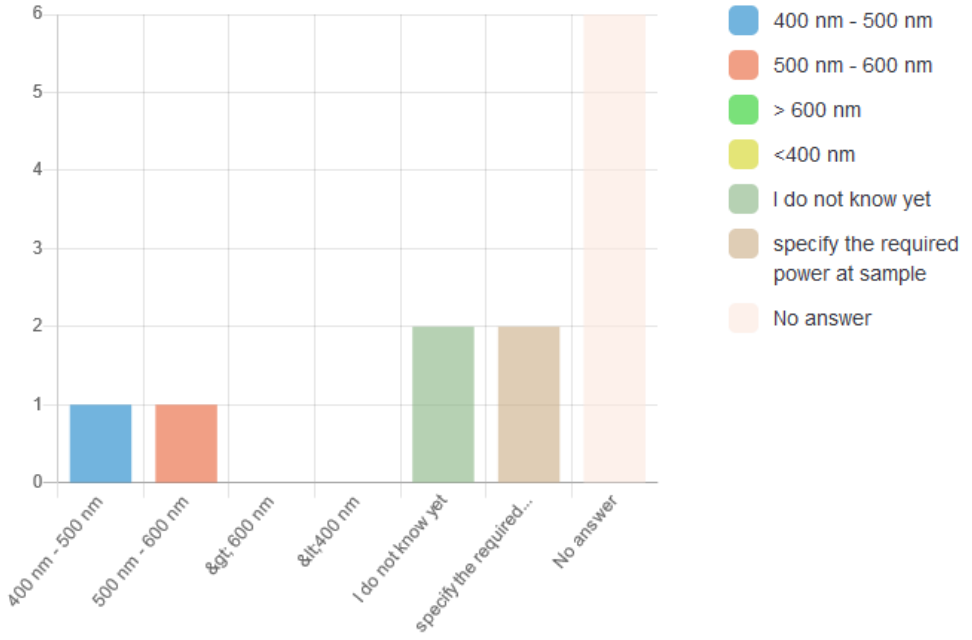
3. For time resolved studies, would be interested in:



4. Which time delays would you be interested in:



5. For pump&probe experiments which wavelengths would you be interested in using :

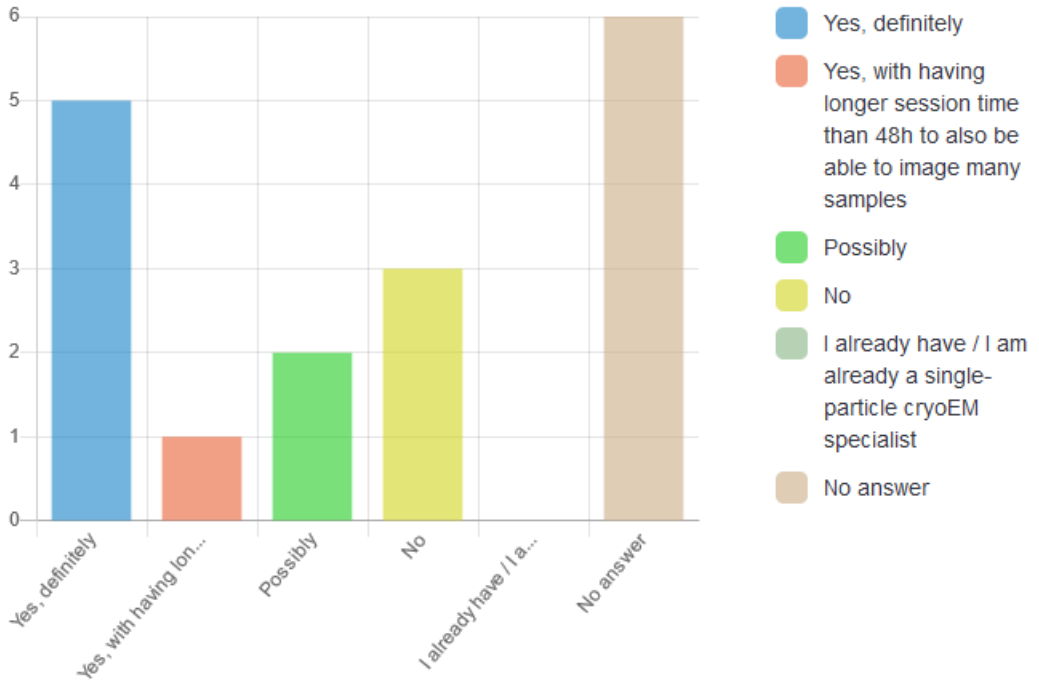


COMMENTS ABOUT THESE TR-SSX AND RT-SSX TECHNIQUES

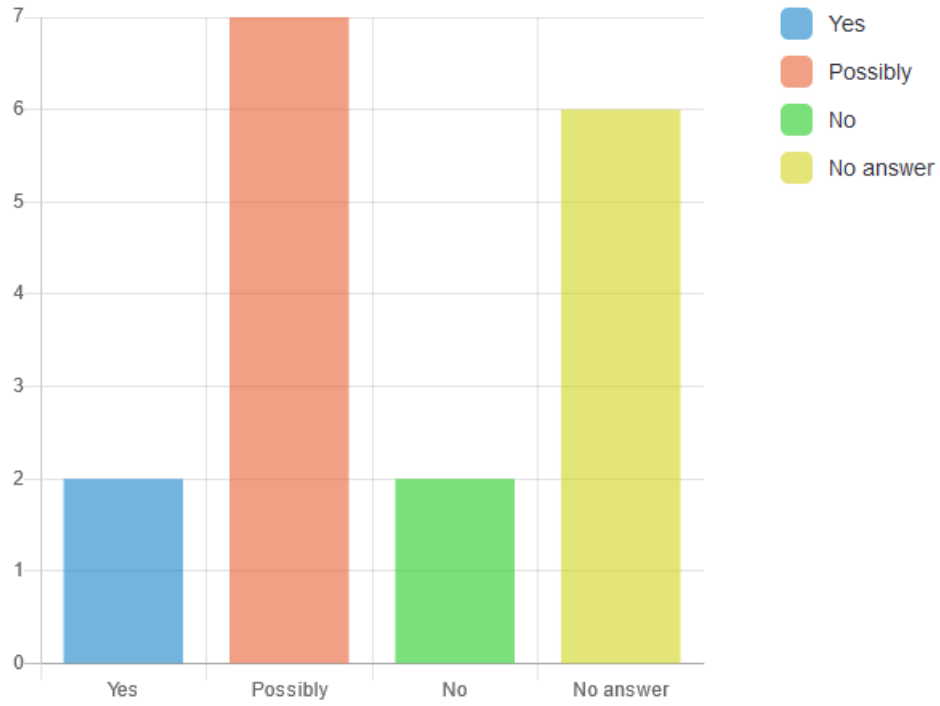
- *Some users need both mixing and light activation, it's not one or the other.*
  Importantly, for the beamline staff to make sure users understand that the Pump stage of the experiment, especially when done with light, is important to understand laser fluence and over excitation, and that power titration experiments sometimes important for making sure the final density that's observed is real and biologically relevant.
- *If we come to perform TR diffraction it would be to search for enzymatic intermediates. So mixing is important but sample consumption is a limiting issue currently. So we are still waiting for further development in sample deliveries. No hurries.*

SINGLE PARTICLE IMAGING

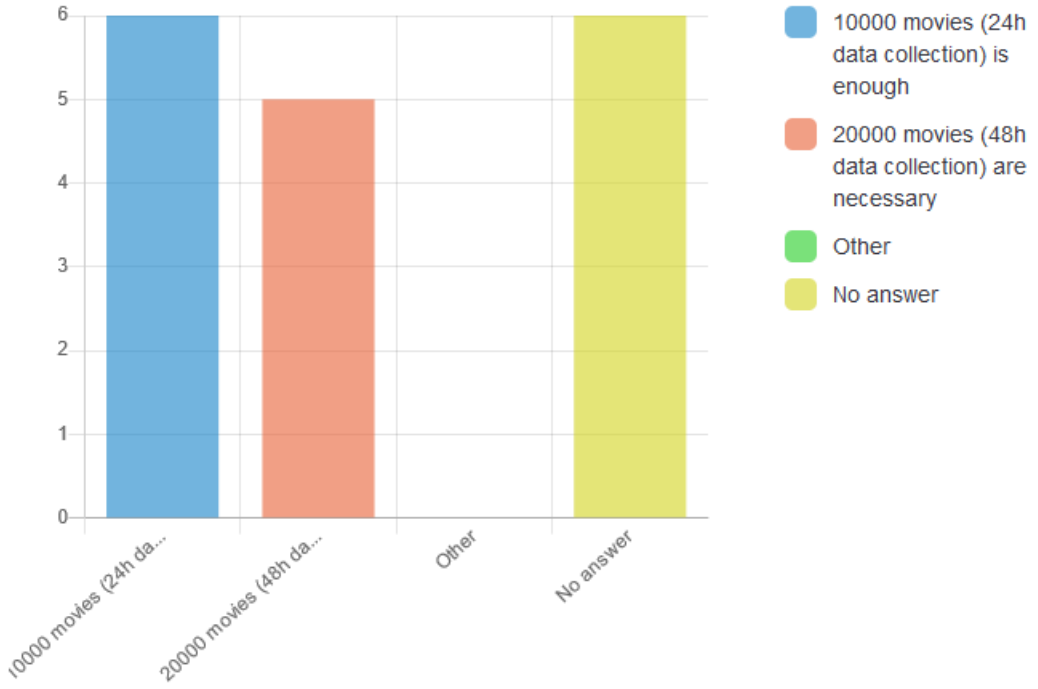
1. Would you be interested in having a super-user trained on CM01 for single-particle cryoEM for making the most of your BAG time?



2. If you already have if you are a single-particle cryoEM specialist, would she/he/you be interested to have remote control of the CM01 microscope (selecting the best grid and setting up of the data collection in EPU)?



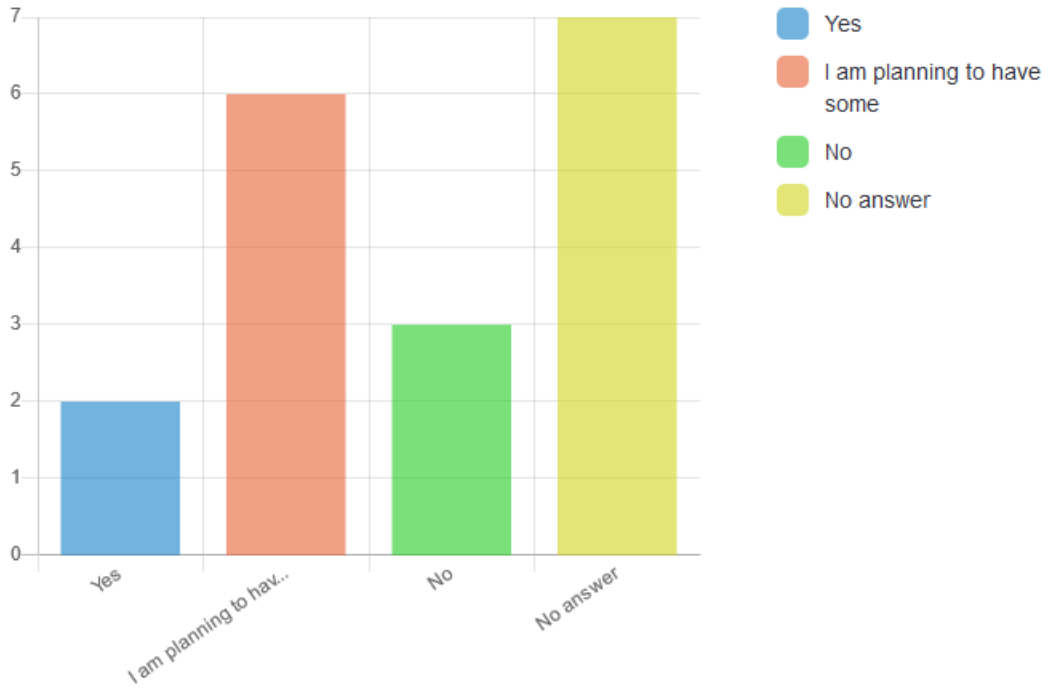
3. For an ideal data set for single particle experiments



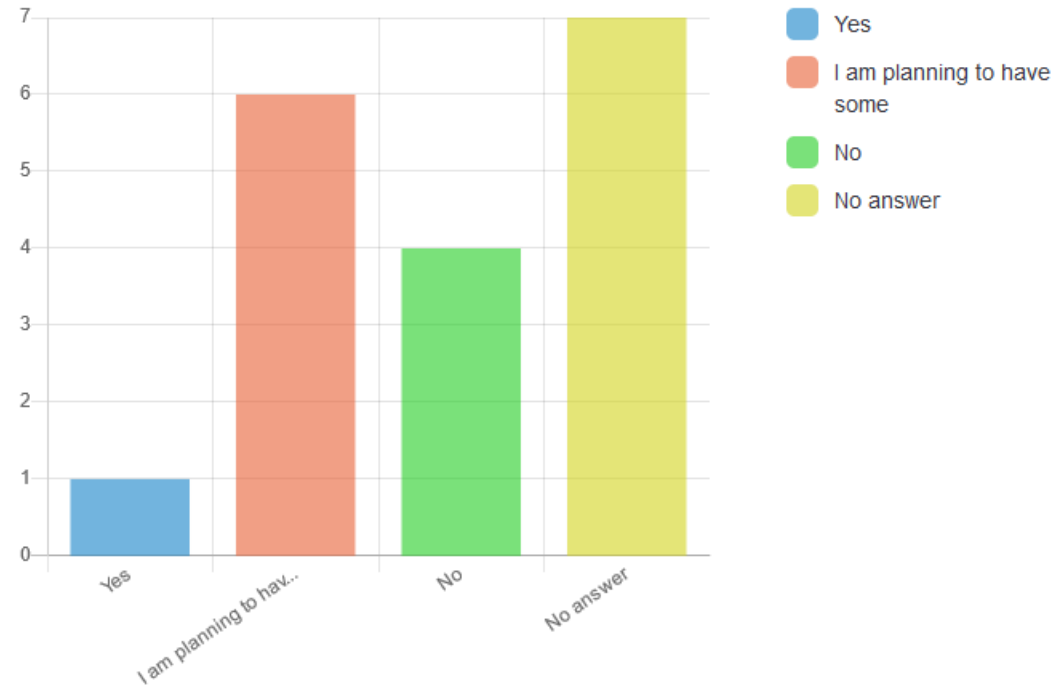
COMMENTS ABOUT THE SINGLE PARTICLE IMAGING TECHNIQUE

- *The possibilty is not offered fully for 24h data collection and this depends on the local contact. A clearer process is needed for this (and likely more people to do local contact.*
- *I have had great experiences with the SPA set up for the French BAG and the local contacts at ESRF. We are very greatful to be part of the french BAG, which has allowed us access to high end microscopes that we otherwise would not have had.*

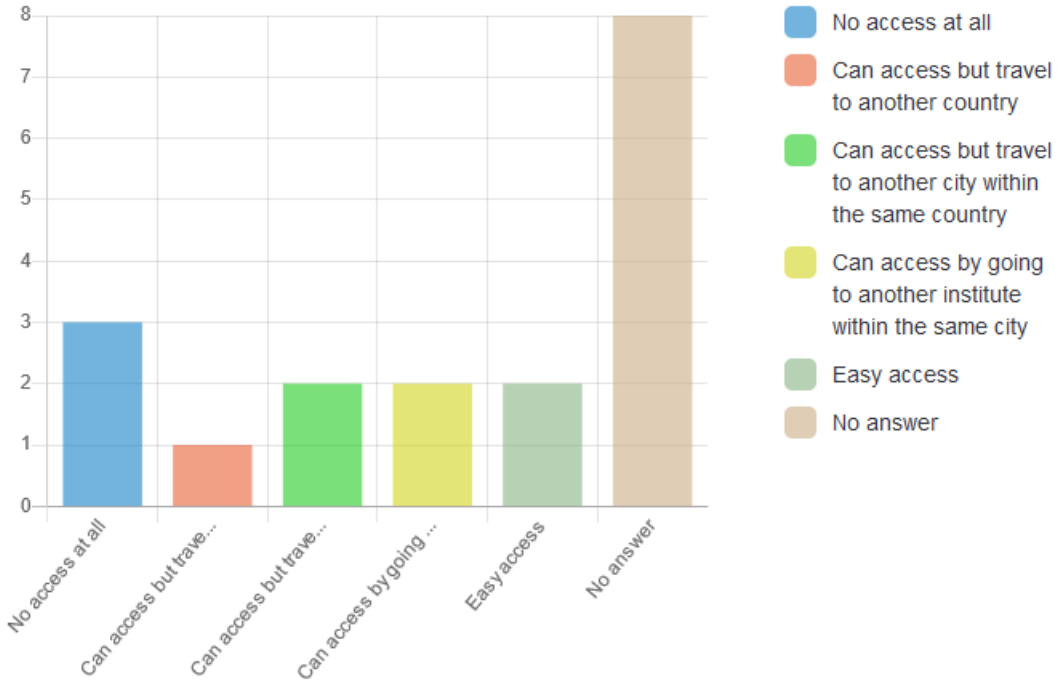
1. Would you have samples ready for "regular" (non-correlative) cryo-ET?



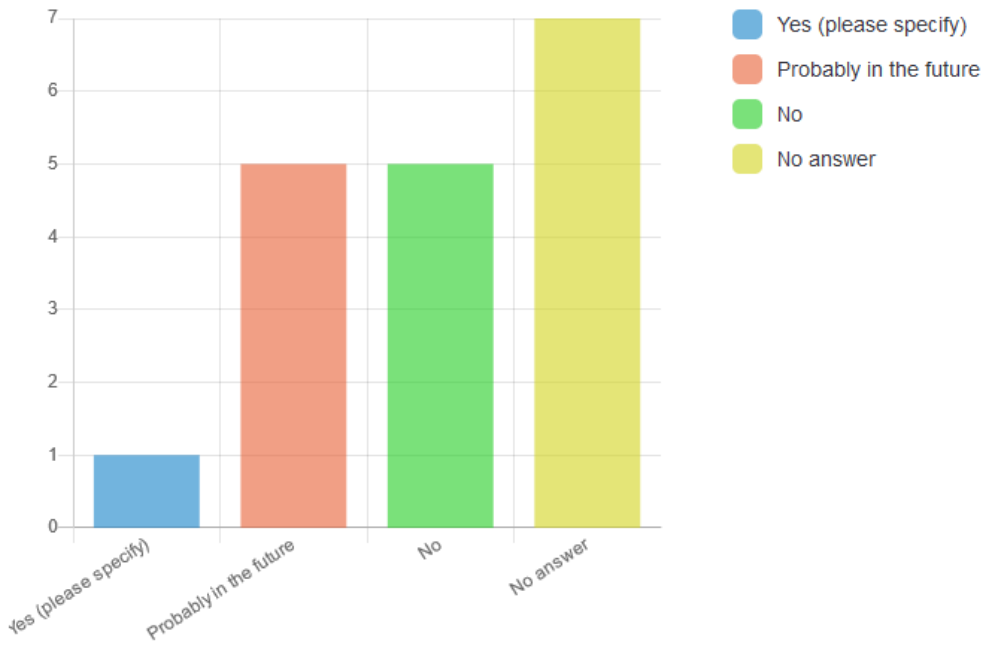
2. Would you have samples ready for cryo-Correlative Light Electron Microscopy?



3. How easy is your access for sample preparation for tomography (lamella)?



4. Do you or someone of your group already have a project that would benefit from combining data from Xray-nanotromography and cryo-ET?

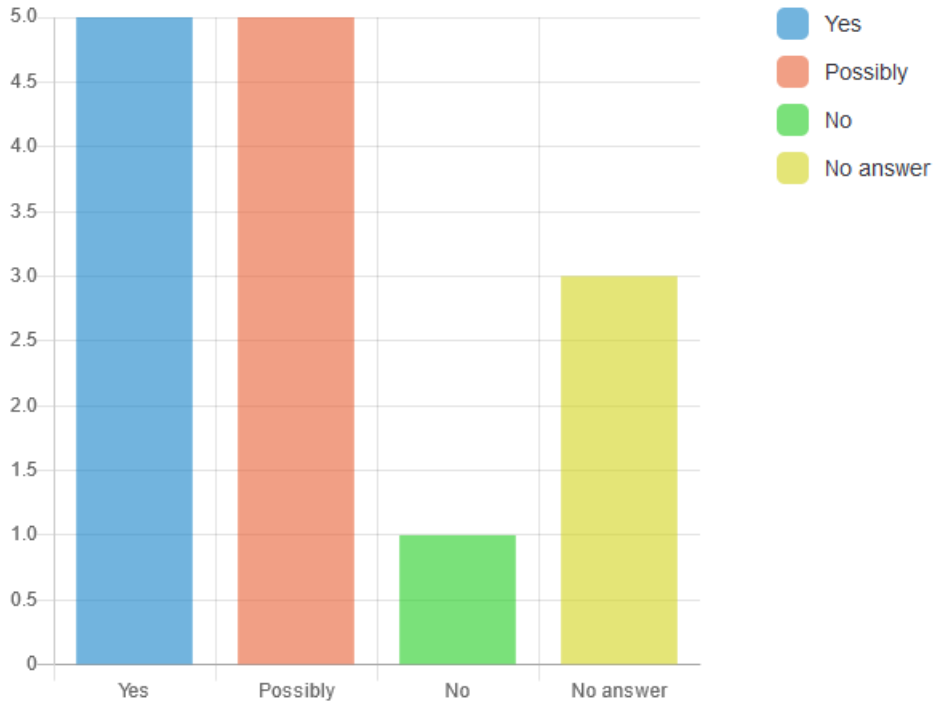


COMMENTS ABOUT THE CRYO-ET TECHNIQUE

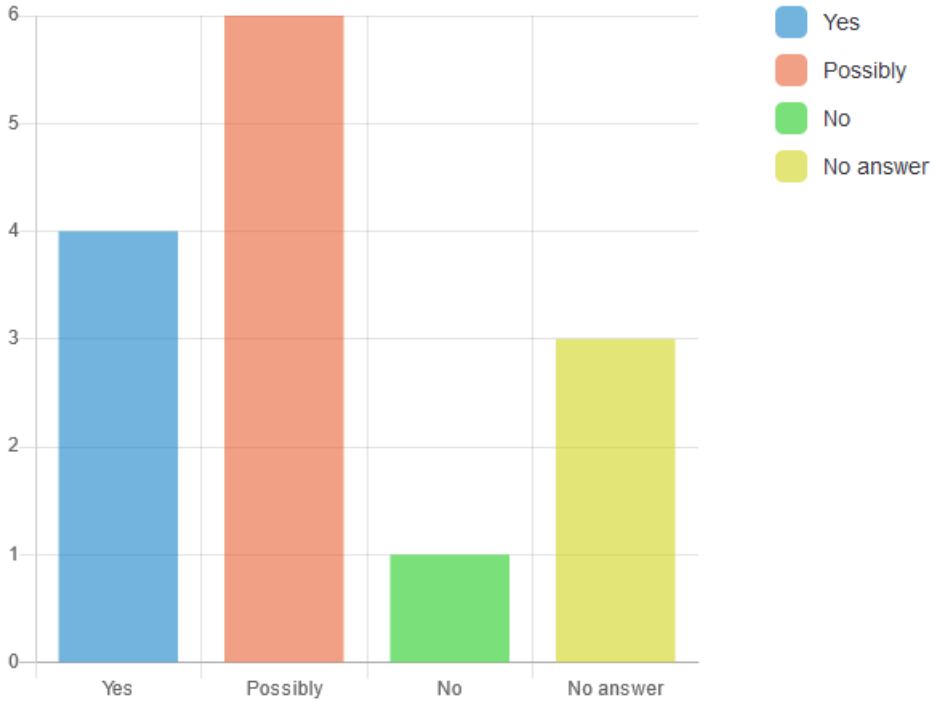
- *We are currently not doing cryo ET but would be very interested in doing so for the future.*
- *At the moment we have access in Pasteur and Madrid (CNB-CSIC) but we are planning to implement the equipment inhouse.*

BIOSAXS

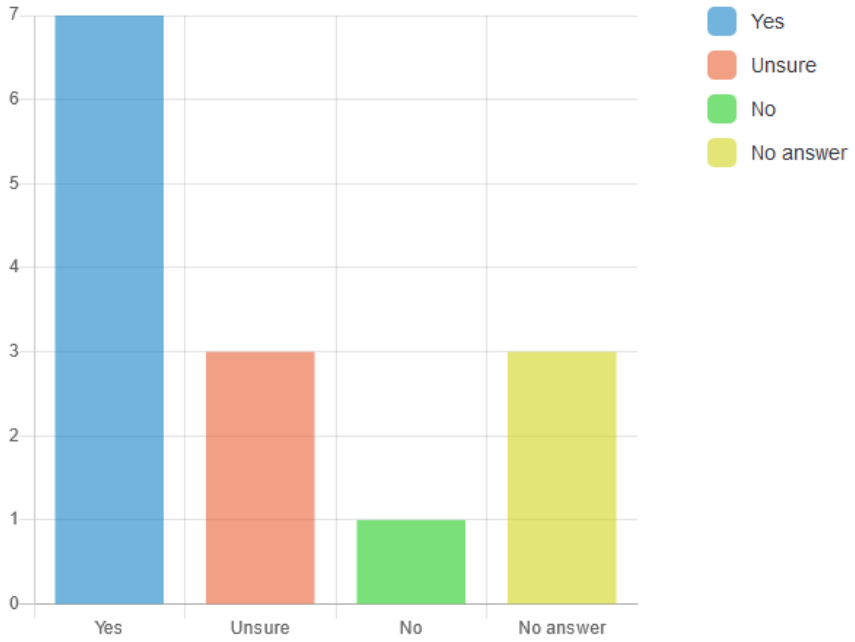
1. Would you be interested in having regular mail-in experimental sessions (ran by the ESRF staff) open once per month or so for a couple concentration series or/and sec_saxs runs to complete a measurement or do a preliminary study?



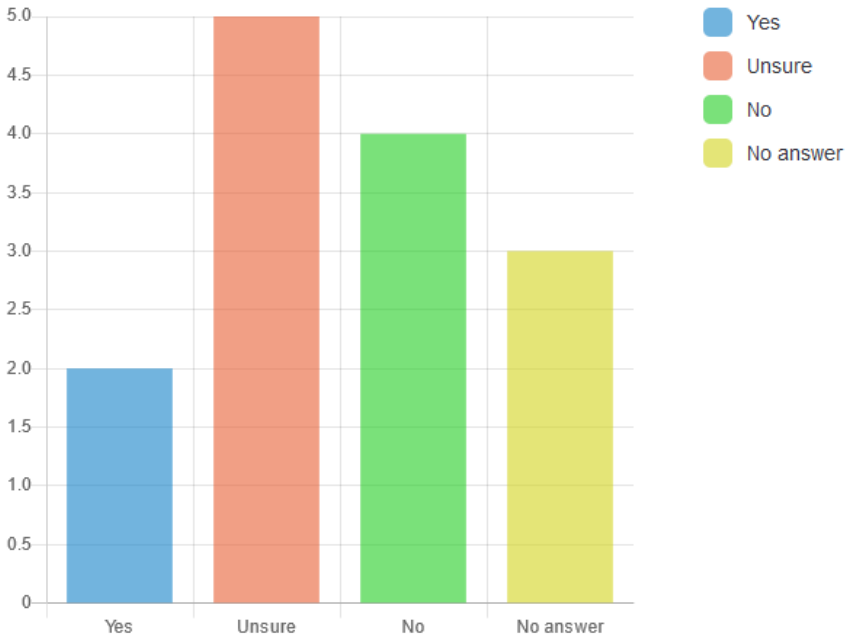
2. Would you be interested to attend on-site tutorials/trainings over a day or two to be trained on sample prep, data collection and data analysis, using your own samples?



3. Would automatic envelope calculation be a valuable addition to the processing pipeline?



4. Would you be interested in using microfluidics compatible exposure unit (i.e. no robot or sec-saxs mode possible) for experiments on gels, 3D printed chips, tissues,... in a grouped beamtime (a couple of days to one week regularly during a run)?

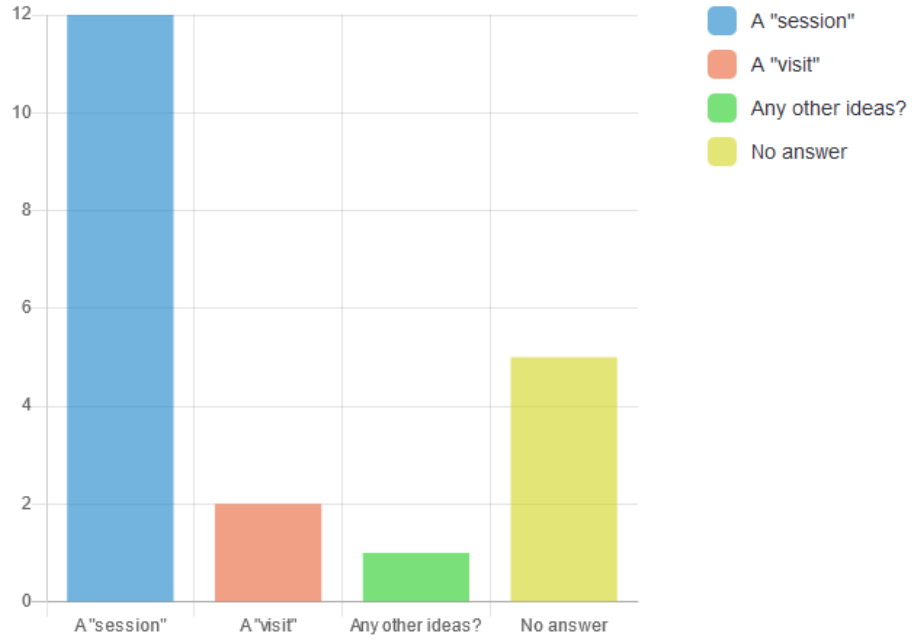


COMMENTS ABOUT BIOSAXS

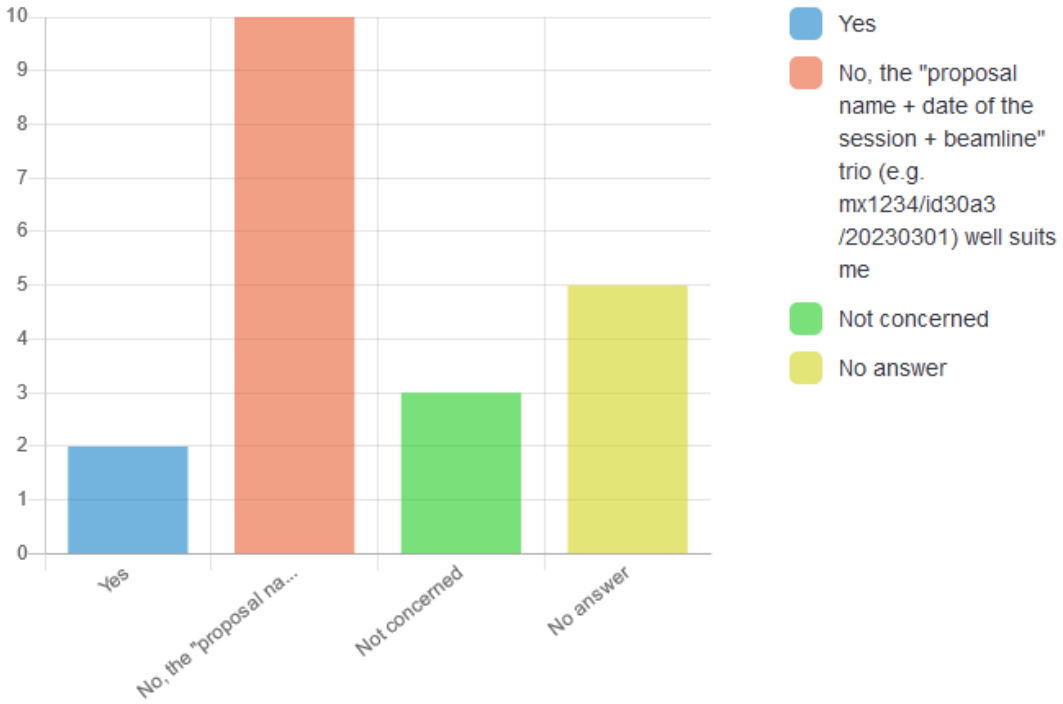
- *What we really need is that data is relability processed and with the processed files immedialtely available. These h5 files are not sufficient. Most of the time, IspyB aumatic processing does not work*
- *I always collect SEC data at SOLEIL and batch data at ESRF. I never tried SEC on BM29 but my colleagues convinced me to keep the SEC samples for SOLEIL because the data quality is better. It's really a pity and we are waiting for an improvement in this area (SEC on BM29).*

SESSION DESIGNATION

1. What do you think would be the best single-term for an "experimental session"?

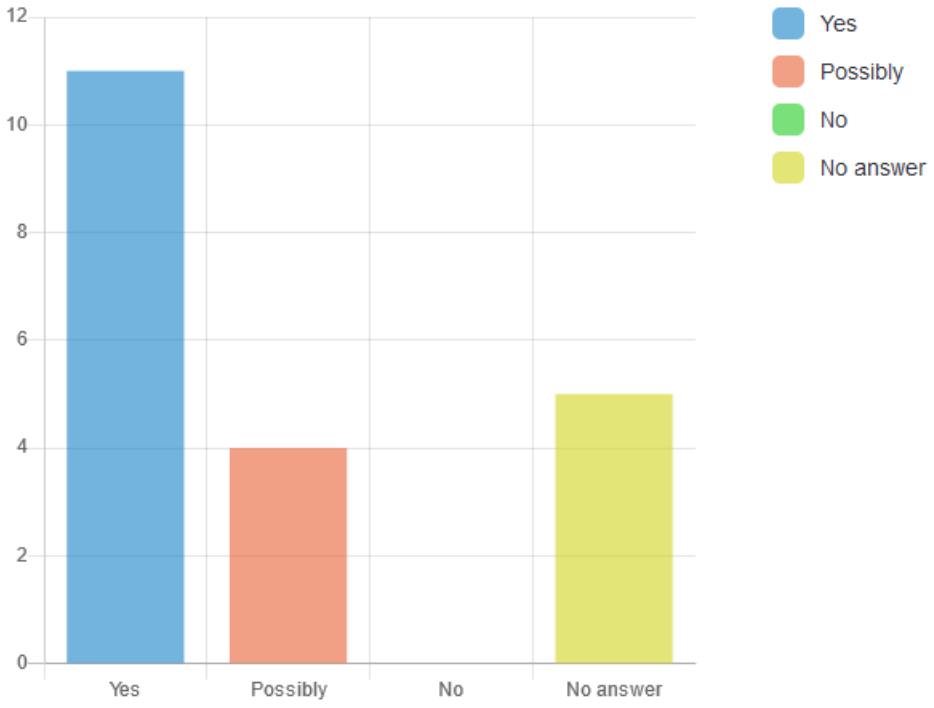


2. Would you prefer to have a unique number to identify an experimental session (*e.g. mx1234-124*)?



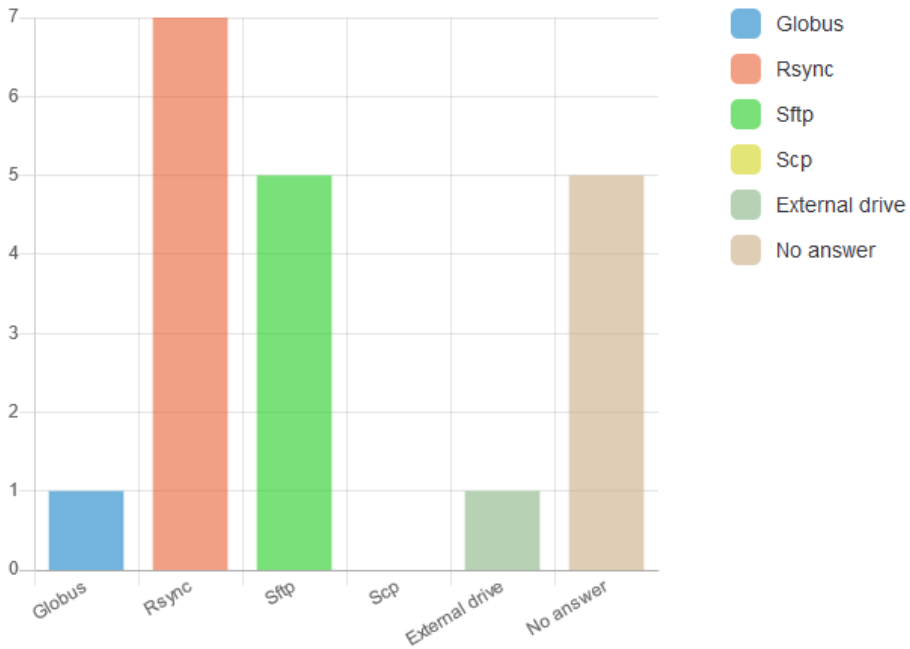
SAMPLE TRACKING

1. Would you consider a unique 2D code label to stick on your shipping dewar case as a paper-free option for the on-site tracking of your dewar through ISpyB?

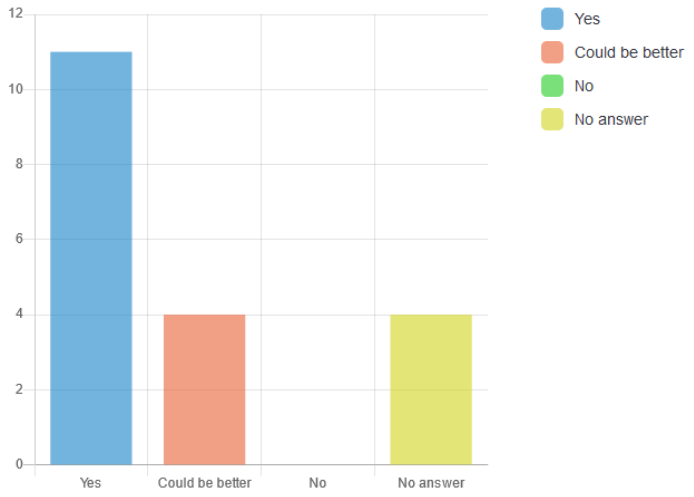


FILE TRANSFER

1. Which file transfer solution do you use the most?



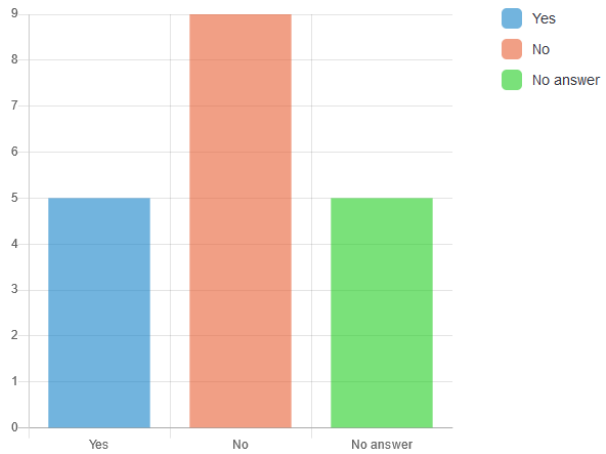
2. Are you satisfied with file transfer from the ESRF?



COMMENTS ON THE FILE TRANSFER AT THE ESRF

- *Sometimes the file transfer speed is rather slow. For cryoEM data for exemple, that can be very large (several TB/24h), the data transfer requires sometimes almost one week. Of course the transfer rate might not be the problem of ESRF infrastructure, but it can also be at my institute.*
- *In some cases we've got lost for very peculiar data. Otherwise we have an automatic procedure doing a good job. Although from time to time changes in the data directories make it useless for a while. Would be great to make all this more fluid through exchange with our BAG super-user/manager.*
- *Speed is sometimes an issue*

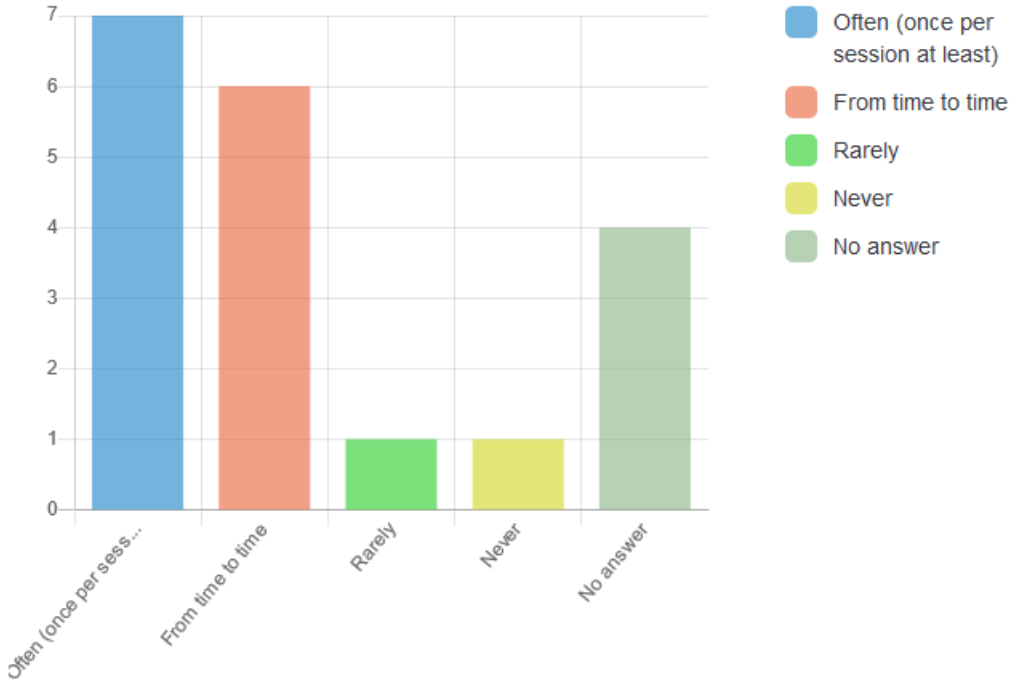
3. Would you need another file transfer solution offered by the ESRF?



COMMENTS ON THIS QUESTION 3 - WOULD YOU NEED ANOTHER FILE TRANSFER SOLUTION?

- *Aspera*
- *See comment above. This may have to be connected to reprocessing of data (for resolution issue for example). Overall, the ESRF provides an excellent procedure but there is always place for some improvements. Would need some more discussion with experts in the field on one side and BAG users on the other side.*

4. Do you use ISPyB to download autoprocessed data files?



COMMENTS ABOUT FILE TRANSFER IN GENERAL

Regarding BioSAXS, from ISPyB it is possible to download 1d averaged data but the .dat files do not contain the header info.

GENERAL COMMENTS

There was only one comment stating "*several of the questions should allow for more than one answer*".

END