How to prepare a “perfect” (?) SAXS beamtime application

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Structure-function relations of flavoenzymes

BioSAXS User since 2002
in Priorities Evaluation Committee (PEC)

EMBL Hamburg P12 Virtual User Meeting
November 17—19, 2020

Before you start writing your application for beamtime @EMBL-HH:

- read carefully the notes from this user meeting and the BioSAXS group pages
- remember that the complete application will be subjected to evaluation with respect to:

  -Scientific quality of the proposal
  -Biological relevance and expected impact
  -Track record with respect to the proposed project
  -Successful publication record of previous EMBL proposals (if applicable)
  -Importance for instrument development (if applicable)

  and also

  -Technical feasibility
  -Estimate of actual beamtime needed to carry out experiments

If needed: contact EMBL-Hamburg staff before you start for advice

The application consists of two main parts

-Application form:

-«Proposal Summary»

-Heading (Title)

-Concise information on type of proposal, proponents, expertize, experimental plan (9 tabs!!!)

- «Main Text» of the proposal

-«Detailed Sample information» (3 tabs + Save!!!)

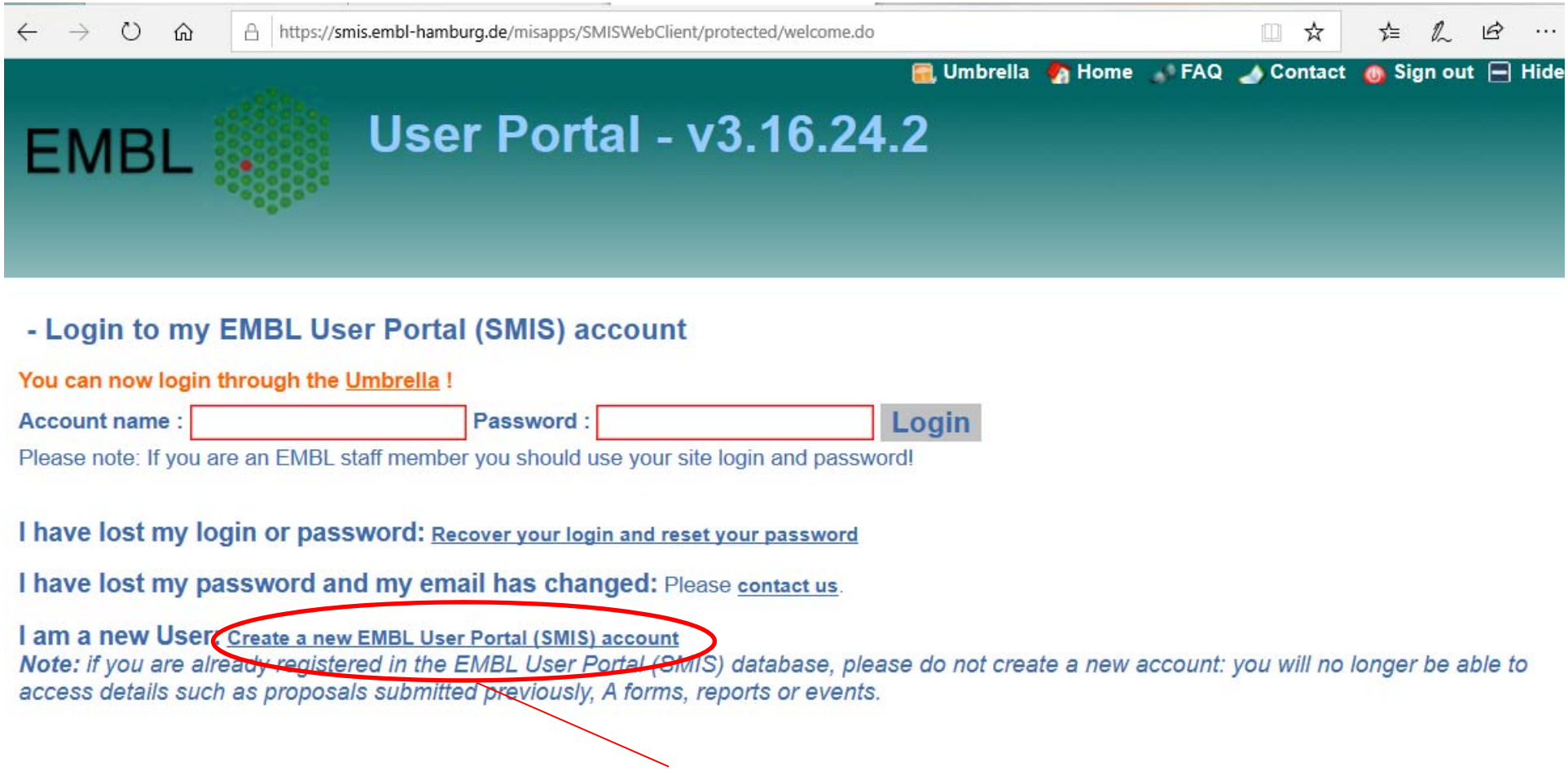
Please do balance information in different parts and sections keeping in mind that:

«Application» will be available to the Priority Evaluation Committee and EMBL Technical Staff

«Detailed Sample information» will be available to EMBL Staff only for evaluation of technical feasibility and advice on shifts allocation

Your contact at EMBL will read the complete application to prepare for your visit and to assist you during measurements

START @: https://smis.embl-hamburg.de



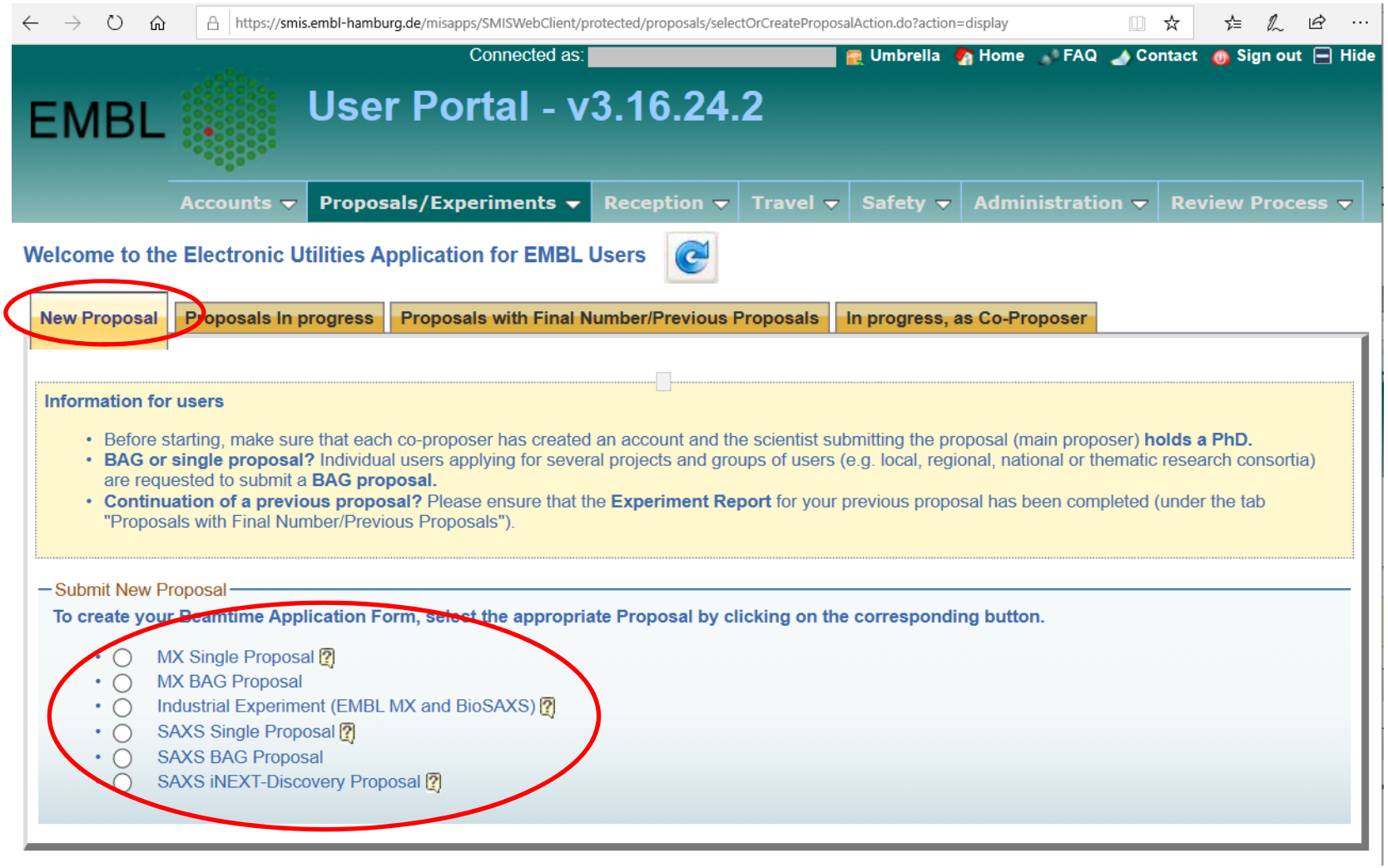
Register as new user; get login and password
or login with your (known) password

Select New Proposal and the Type of proposal

You can go back and edit «Proposal in progress»,

You can also check «Previous proposals»

You will need to access the proposal to file the experimental report after measurements



«Proposal Summary»

- You will have to answer the questions in these 9 tabs

Once this is provided you will be asked to:

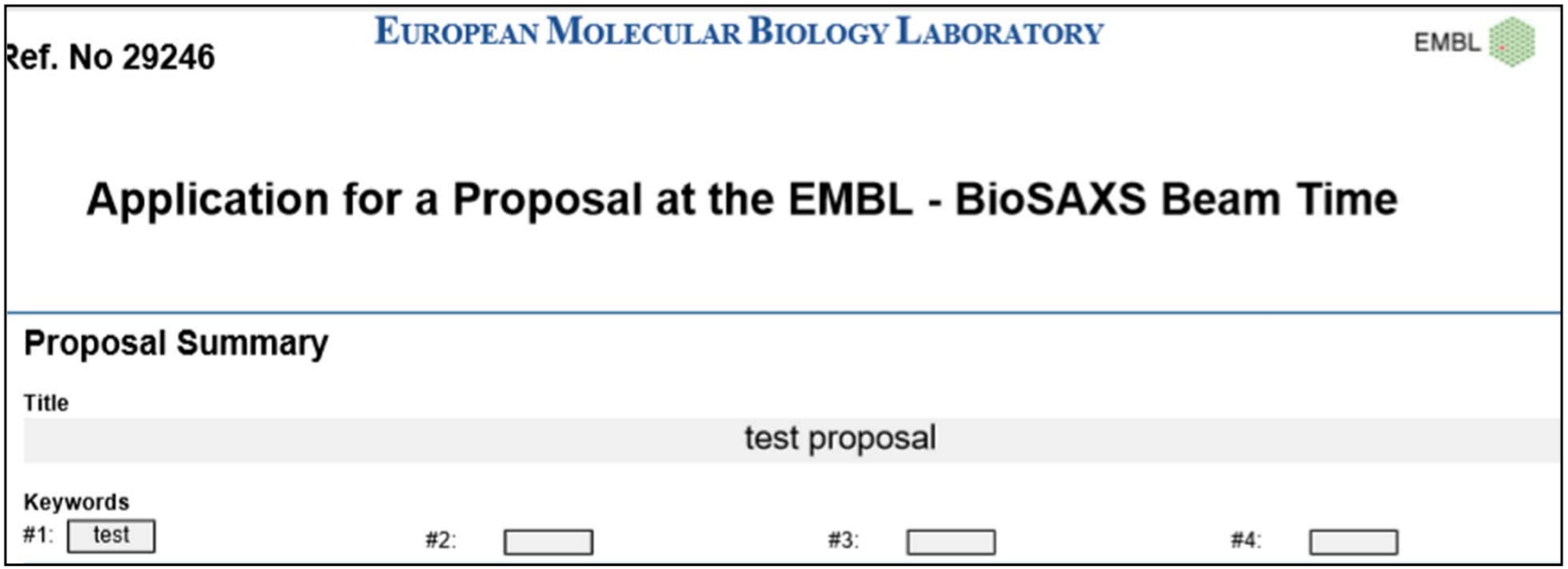
- Upload "Main text"
- Give "Detailed sample information"
- Save

DO balance information in different parts and sections
DON'T fill these fields in a hurry

The Application

- «Proposal Summary» : Information on proposal type, proponents, samples, etc (from the 9 tabs)

- «Main text» (to be uploaded as a pdf)



Ref. No 29246

EUROPEAN MOLECULAR BIOLOGY LABORATORY

EMBL

Application for a Proposal at the EMBL - BioSAXS Beam Time

Proposal Summary

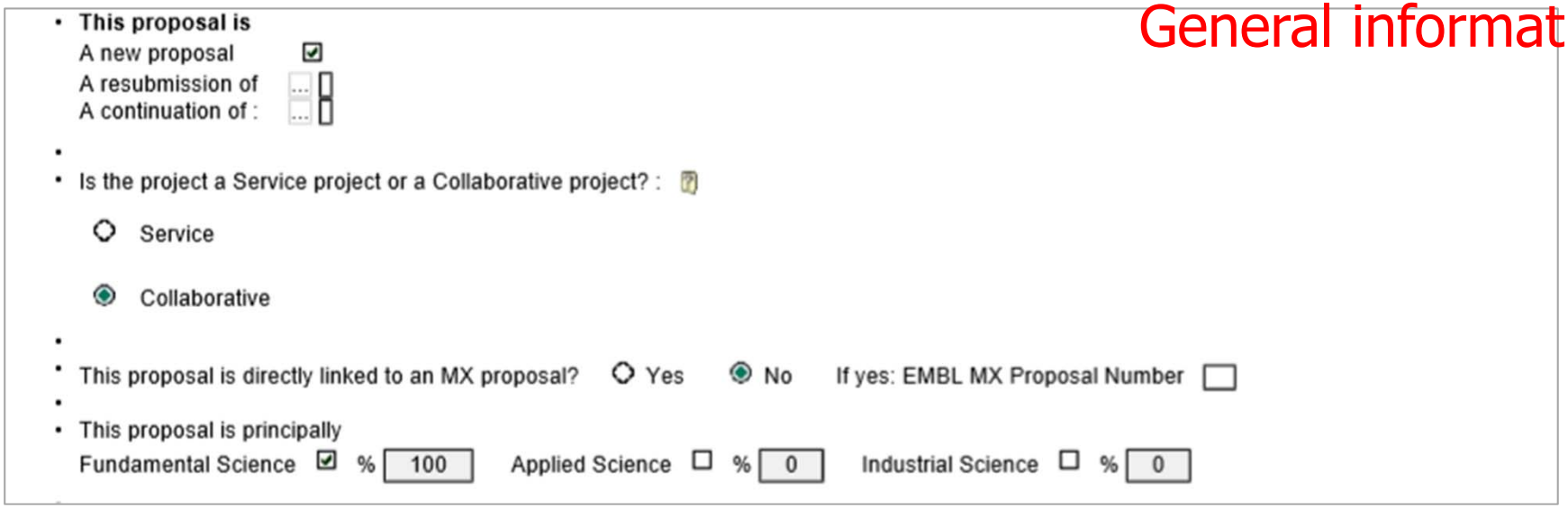
Title

test proposal

Keywords

#1: test #2: #3: #4:

General information



- New project, resubmission or continuation of previous project at EMBL-HH
- If multiple proposals are to be submitted by one group (or closely collaborating lab from, e.g., same institution, network), better to apply as a single Block Allocation Group (BAG) for more efficient management of allocated time.
- Service or collaborative:

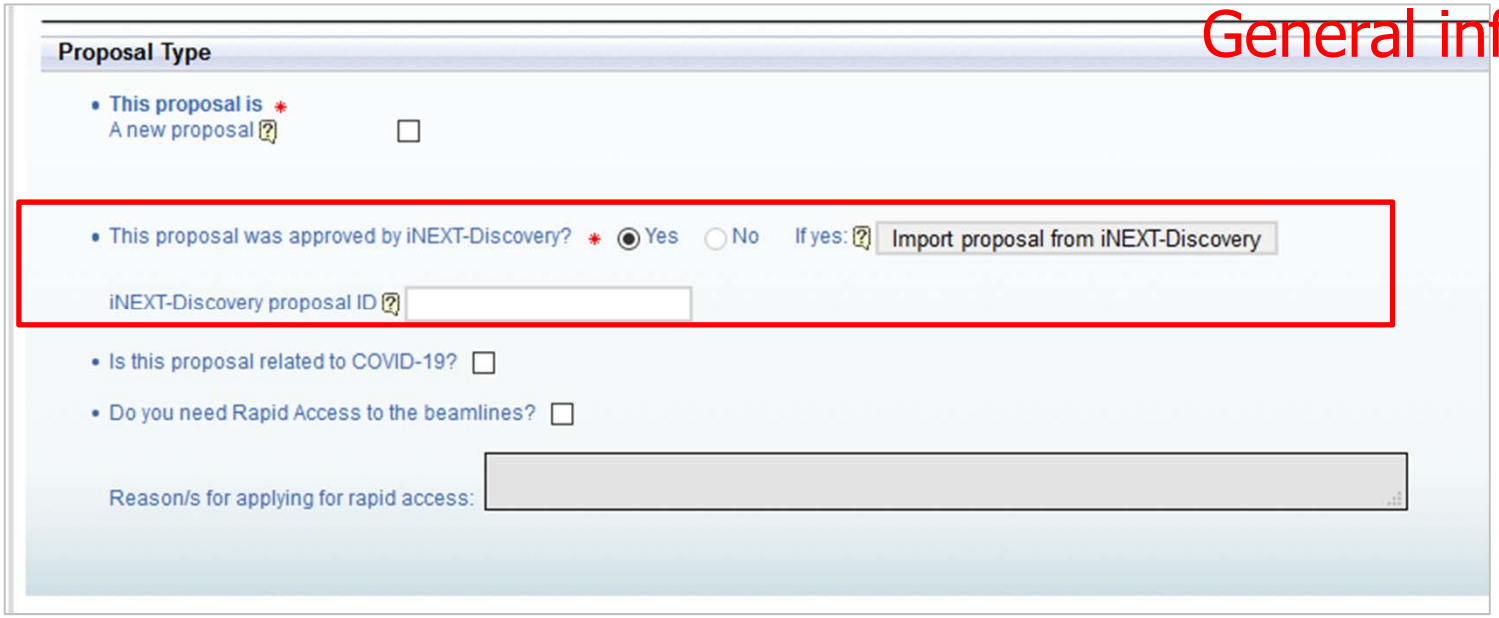
  - Service:
    - you will get training on the use of the beamline
    - you will be responsible for experimental design, data acquisition, data analysis;
    - there will be a scientist helping you with beamline-related technical issues

  - Collaborative:

    Establish contact with collaborator at EMBL-HH before filing the application

    Coordinate proposal with collaborator @ EMBL-HH (exchange drafts)
- Link to MX proposal.

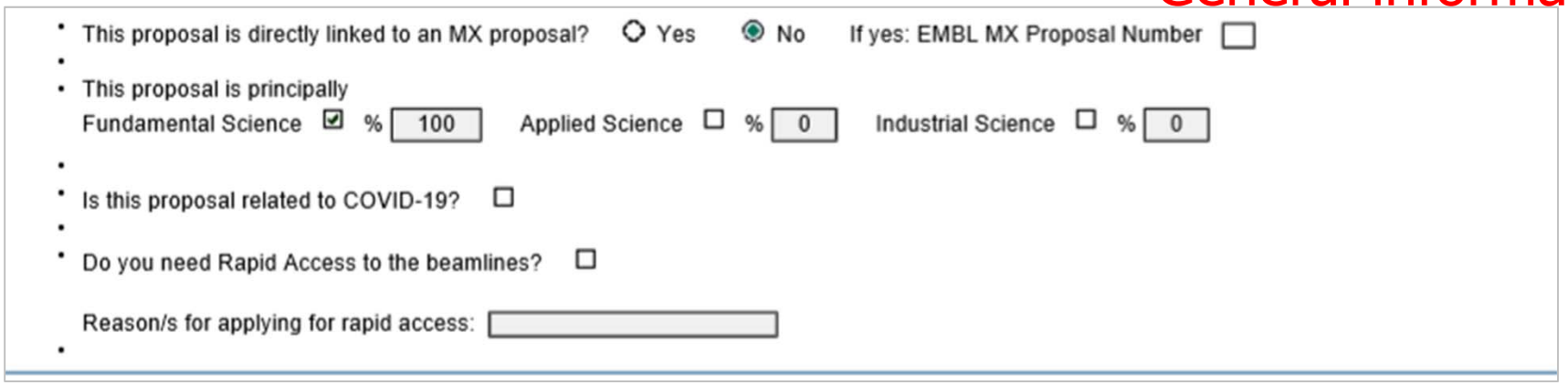
General information



- iNEXT-D:
  - do apply under iNEXT-Discovery program
  - if the proposal is approved by iNEXT:
    - (ltd) expense refund may be available (e.g.: sample shipping costs)
    - the proposal will be given high priority (unless serious problems are found with the proposal, which were missed by the iNEXT reviewers).
  - may file application to both EMBL@HH and iNEXT, but do notify EMBL@HH if your proposal has been approved by iNEXT ASAP to avoid double allocation of beamtime

covered by: Th. Nov.19, 2020 Hello from the User Office - Sarah Marshall-Bensch

General information



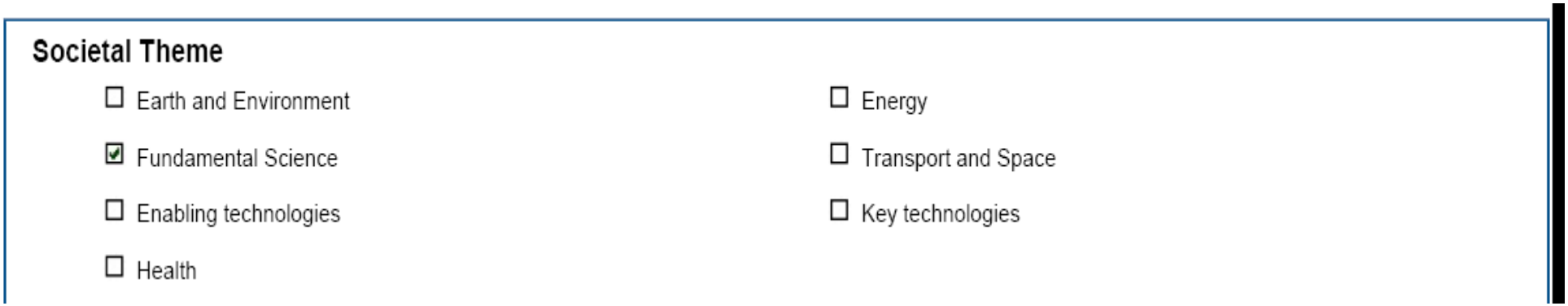
If «COVID-19» box is checked, «Covid-19» will be appended to the proposal title. Still it will be evaluated thoroughly as all other proposals.

If «Rapid Access» box is checked , please fill in the box here and remember to insert a paragraph in the proposal main body explaining the special urgency of the SAXS measurements

For special cases do contact the EMBL@HH staff

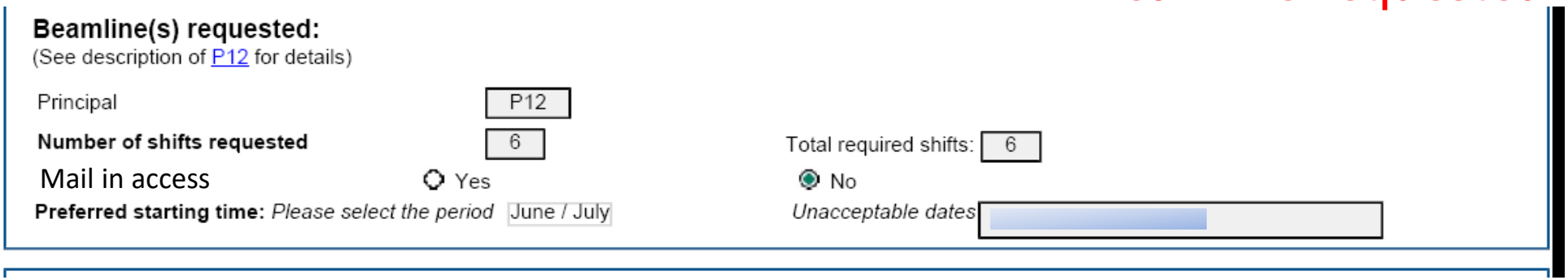
E.g.: Industrial science: beamtime allocation and fees depend on the goal of the measurements.

Societal theme



The Societal theme does not influence scoring, but remember this is BioSAXS!!!

Beamline requested



- P12 is the BioSAXS beamline

Shifts requested

- 1 shift = 8 h
- Normally, no more than 3 shifts are assigned to single projects, due to oversubscription, and one cannot split shifts in more than one visit.
- Take into account the measuring time, special setups, sample preparation time (measuring time may be much shorter than sample preparation!) ***See specific presentations***
- Applications within a BAG, may allow optimization of allocated beamtime.
- In the «Detailed Sample Description» form you will be able to further justify your beamtime request

Mail-in access: the beamline scientists will carry out the experiment for you.
Read carefully https://www.embl-hamburg.de/biosaxs/mailin.html

Proposers

Main proposer (to whom correspondence will be addressed):

Laboratory University of Milano Dept. of Biomol. Sciences and Biotechnology Via Celoria 26 IT - 20133 Milano

Name Prof **VANONI** **Maria Antonietta** Phone +390250314901 Fax +390250314895 Email maria.vanoni@unimi.it

Co-Proposers (Laboratory if different from main proposer)

Laboratory EMBL EMBL Notkestrasse, 85 DE - 22603 HAMBURG

Name Dr **SVERGUN** **Dmitri** Email svergun@embl-hamburg.de

Main proposer:

«Senior scientist» (PI) that will take responsability for the project (including on-site supervision of co-proposers)

Co-proposers:

- List the people that will participate in the experiments.
- See «Hello from the User Office - Sarah Marshall-Bensch" for registration with SMIS, safety courses for access to beamline, User lab; request DACHS card and accommodation.

If Collaborative:

include your EMBL-HH collaborator(s) after making specific arrangements with him/her and agreeing upon the collaboration and the experimental plan.

Laboratory support facilities

Laboratory Support Facility

- ☑ DLS
- ☐ FPLC
- ☐ Refractometer
- ☐ SLS

Equipment list and manuals are available at:
https://www.embl-hamburg.de/biosaxs/user_info.html

Additional equipment at the Sample purification and characterization facility (SPC) facility, but one needs to contact scientists at SPC in advance to make arrangements:
http://www.embl-hamburg.de/services/spc/index.html

*** See specific presentations***

Sample environment

Sample Environment
Items Supplied by EMBL

Solution scattering experiments

☑ BioSAXS with automated robotic sample changer. 25 ul sample; 0.2-10 mg/ml; 20 x 50 ms exposures (1 s total); 1 min turnover (loading, measurement, cleaning). Temperature 5-50C. Remote and mail-in measurement possible

☐ Standard size exclusion chromatography (SEC)-SAXS with or without UV. FPLC or HPLC SEC column connected directly to the beam line. Requires 45-80 min per experiment. Maximum 100 ul injection (recommend 50-100 ul sample at 5-10 mg/ml.). Room temperature only

☑ Size exclusion chromatography (SEC)-SAXS with with parallel MALLS, DLS, RI and UV detection for molecular weight validation and hydrodynamic radius measurements (Wyatt TREOS/QELS/T-rex system. Collaborative projects only. Please fill out section 4 of THIS FORM). Room temperature only

Non-standard ('in air') sample environments:

◉ No "Non-standard ('in air')" sample environment required

○ Temperature controlled (5-95C) capillary holder

○ Stopped flow, time resolved setup. temperature: 5-80C (on collaborative basis only)

○ Other (specify, for example your own tailored sample environment):

☐ We plan to use lasers

☐ For scattering vectors outside the standard range, s = 0.05-4 nm-1 (e.g., WAXS, please fill out section 5 of THIS FORM)

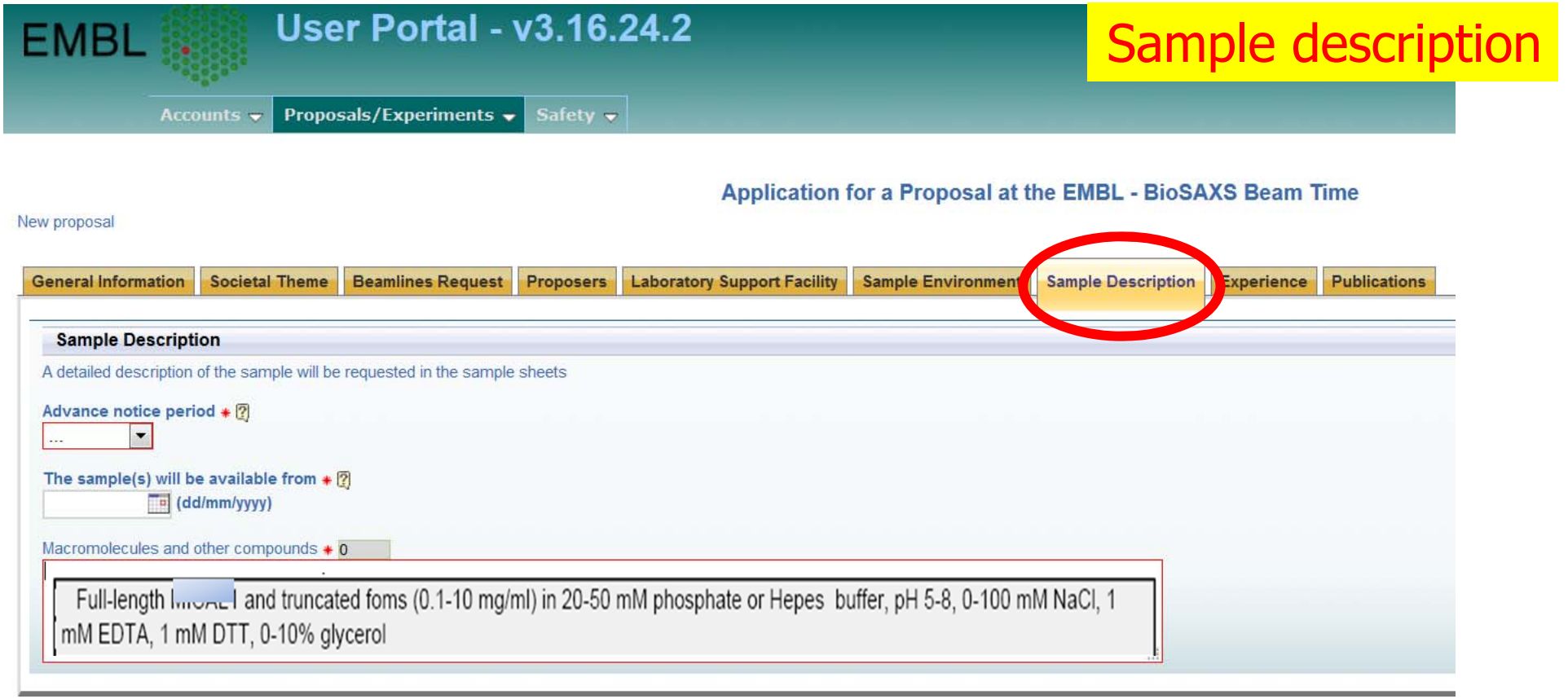
☐ Anomalous SAXS, ASAXS ((5)7-20 keV; Collaborative only)

Use of SEC – SAXS needs to be justified in the proposal.

SEC-SAXS if MALLS is required, and all other «Non standard set-ups» available on a collaborative basis only

*** See specific presentations***

Sample description

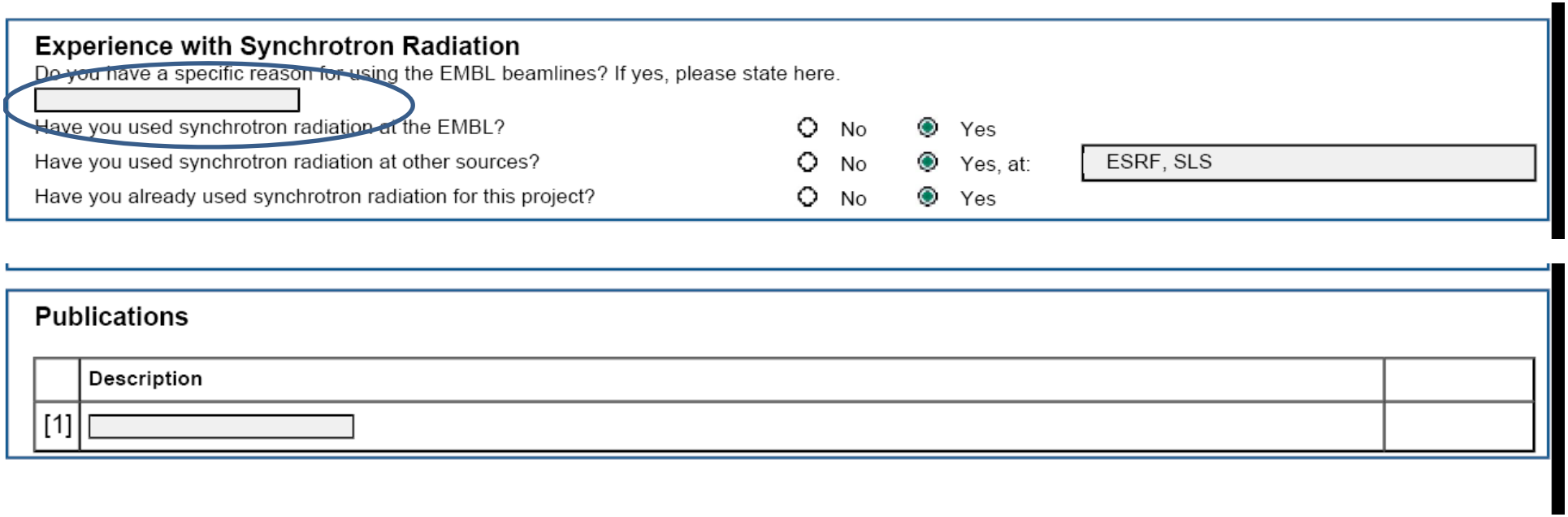


A «detailed sample description» is required in a separate section (see later)

Here, give sufficient information on the samples (quantities and quality) (and in the main text) to allow PEC to evaluate (also) feasibility.

- Make sure you have enough sample*** (also in case you need to repeat measurements if something goes wrong)!

Experience with synchrotron radiation



Experience with Synchrotron Radiation

Do you have a specific reason for using the EMBL beamlines? If yes, please state here.

Have you used synchrotron radiation at the EMBL?	○ No	◉ Yes	
Have you used synchrotron radiation at other sources?	○ No	◉ Yes, at:	ESRF, SLS
Have you already used synchrotron radiation for this project?	○ No	◉ Yes	

Publications

	Description	
[1]		

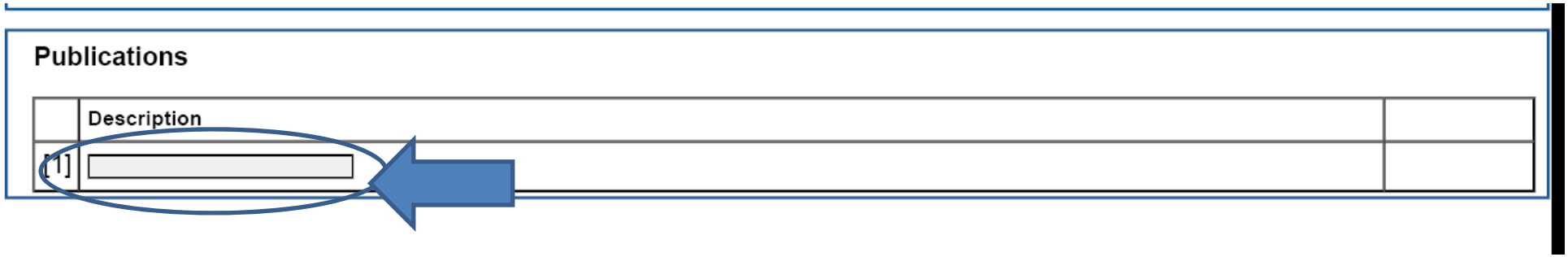
Here highlight:

- Technical reasons for applying to this beamline
- Previous experience with this and other SAXS beamlines

for proposal evaluation and also to help evaluating the level of assistance you will need from the EMBL staff.

New users are welcome!

Expertize of the proponents



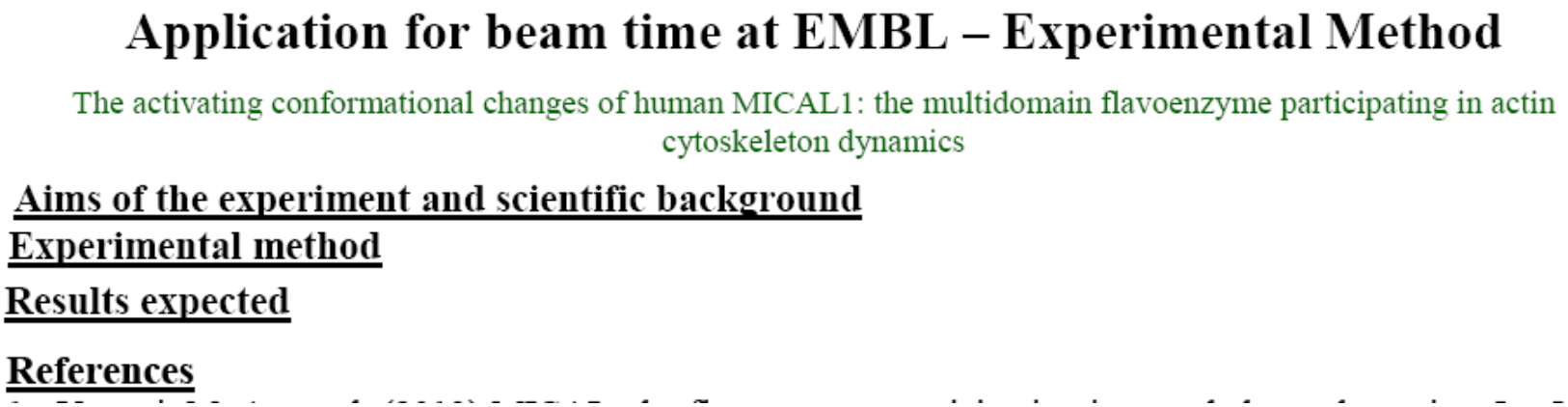
- Here list a representative selection of your publications and/or those of co-proponents (not of the EMBL staff that will assist you or you will be collaborating with, but it is OK to include publications in collaboration with BioSAXS group) to show the relevant expertise with respect to:

  - use of synchrotron radiation, especially SAXS (hands-on)
  - the specific project

  and your publication record

- Limit the list to ~5 references
- Include full reference: authors' list, full title, year, Journal, volume, pages

«Main text» of the proposal (to be uploaded as a separate file)
It will be appended to the Project summary (9 tabs) for evaluation and later use.
In the allowed space, justify your application for beamtime and propose your experiments in order to allow the proposal to be evaluated for the scientific relevance and technical feasibility by the Project Evaluation Committee and the Technical Staff



Application for beam time at EMBL – Experimental Method

The activating conformational changes of human MICAL1: the multidomain flavoenzyme participating in actin cytoskeleton dynamics

Aims of the experiment and scientific background
Experimental method
Results expected
References

Your contact at EMBL will later read and use the proposal to prepare for your visit and assist you during the visit

Aims of the experiment and scientific background

Remember to clarify:

- The biological relevance of the project.
- The specific question you will ask:
  - which relevant information you are looking for
  - why SAXS measurements will provide key information and how urgent they are (e.g.: The SAXS data are needed to complete an on-going project/paper *vs*. preliminary/ exploratory work).

- If Rapid Access box was checked, please make sure to include a paragraph that explains the extreme urgency

Experimental method and Results expected.

- Clarify the experimental plan with information that will allow to establish the feasibility *vs.* the stated aims including, e.g.:
- Amounts and quality of protein available (size, stability, tendency to aggregate, data from gel filtration, DLS, AUC, other spectroscopies, MX, kinetics...).
- Ancillary information (e.g.: X-ray, NMR strucures available on this or homolog/similar systems). If dealing with complexes: K_d and stoichiometries (if known).

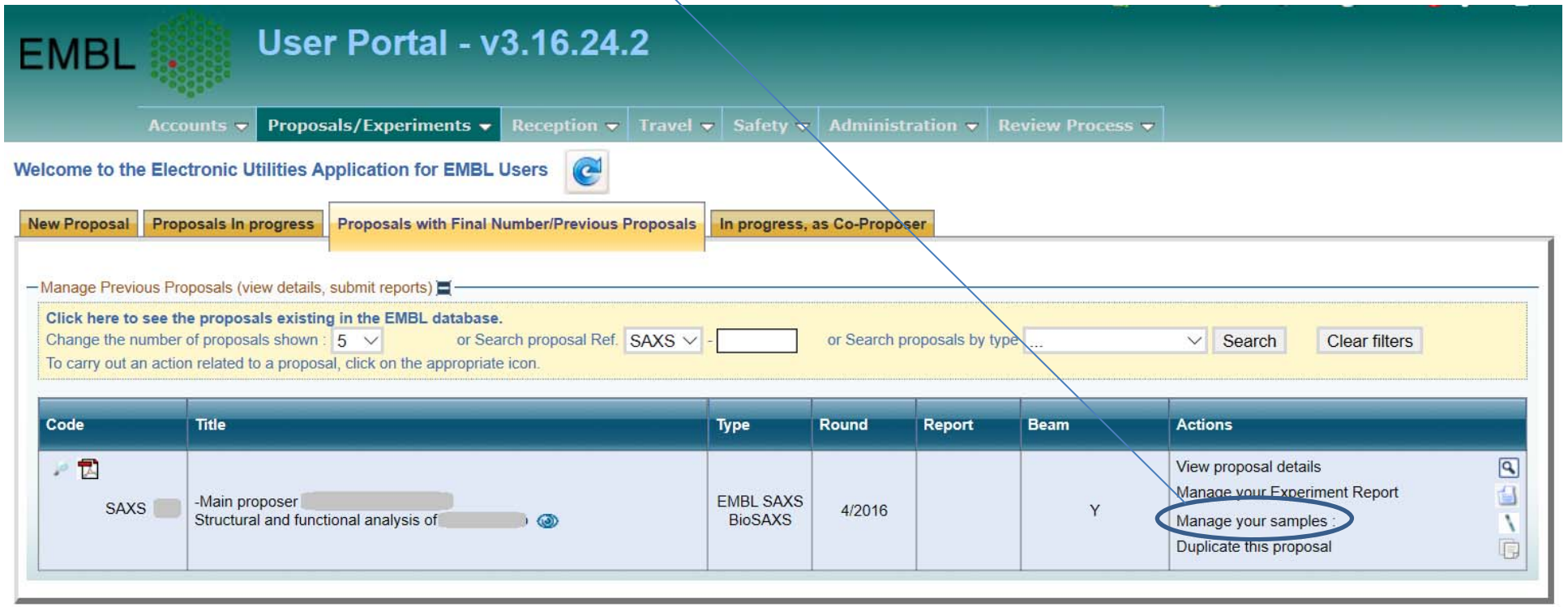
Balance information between here, «sample description» and the «Detailed sample description form»

References (relevant to the actual proposal):

- Include Title and full reference; if possible include the full list of authors;
- Make clear in the text which is the work from your lab and what comes from the literature.

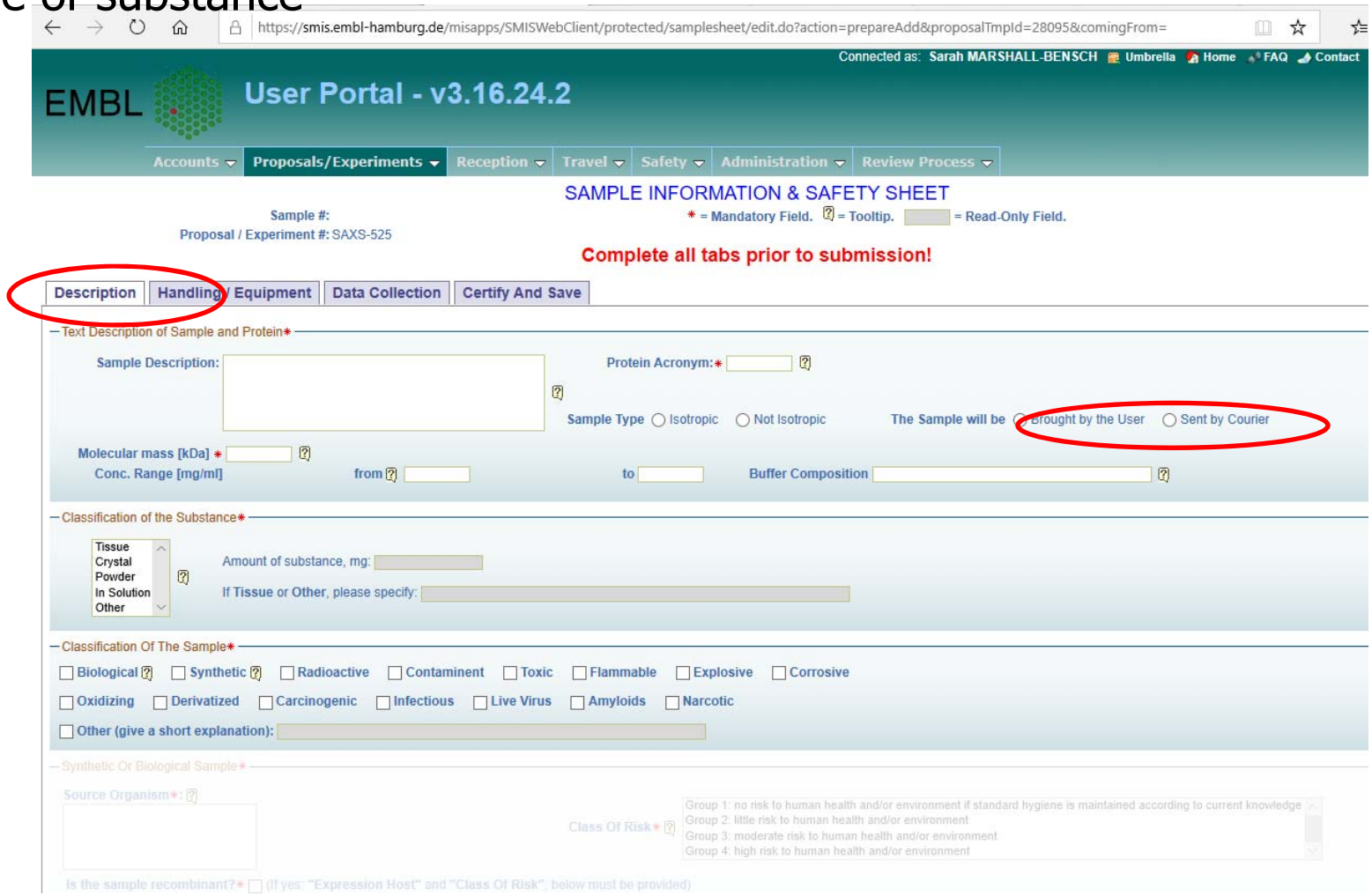
Consider to include a figure and/or a table to clarify what you know, your questions, the expected outcomes.

«Manage your samples» will lead to
«Detailed sample information» = «Sample information & safety sheet»
= «Sample sheet»



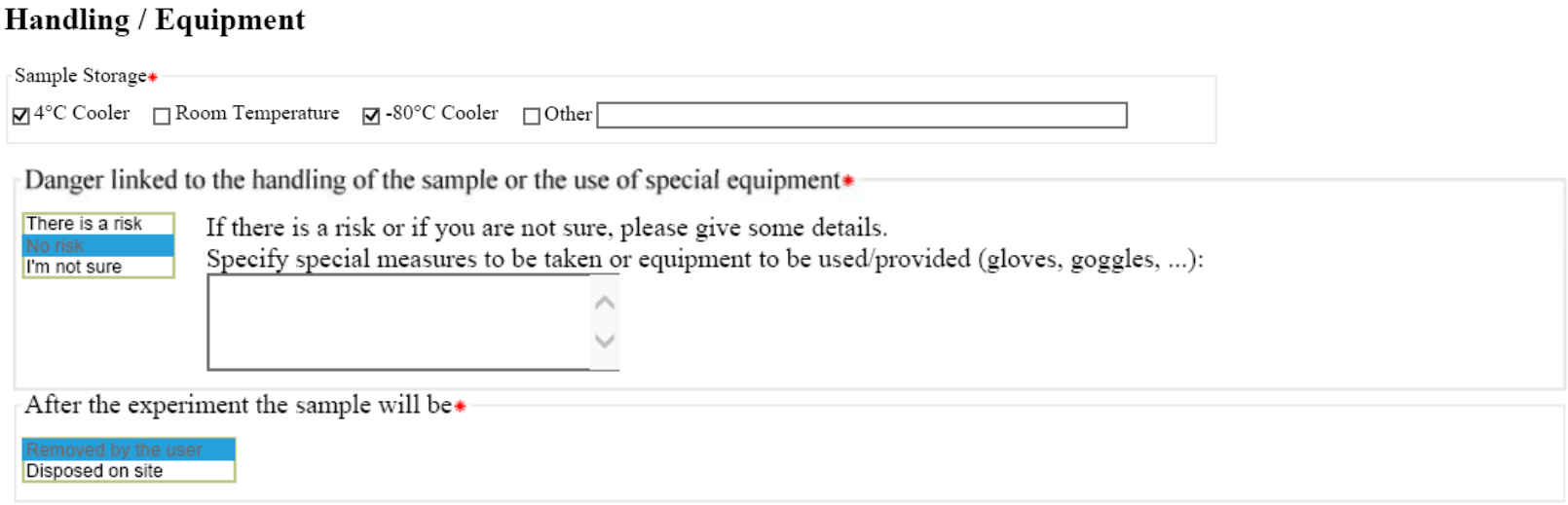
«Sample information and safety sheet» (3 tabs + Save) will be accessible to the EMBL scientists to establish technical feasibility, advice on shifts allocation and, later, to prepare for your visit and help you on site.

«Description» tab includes practical information on sample shipment and type of substance

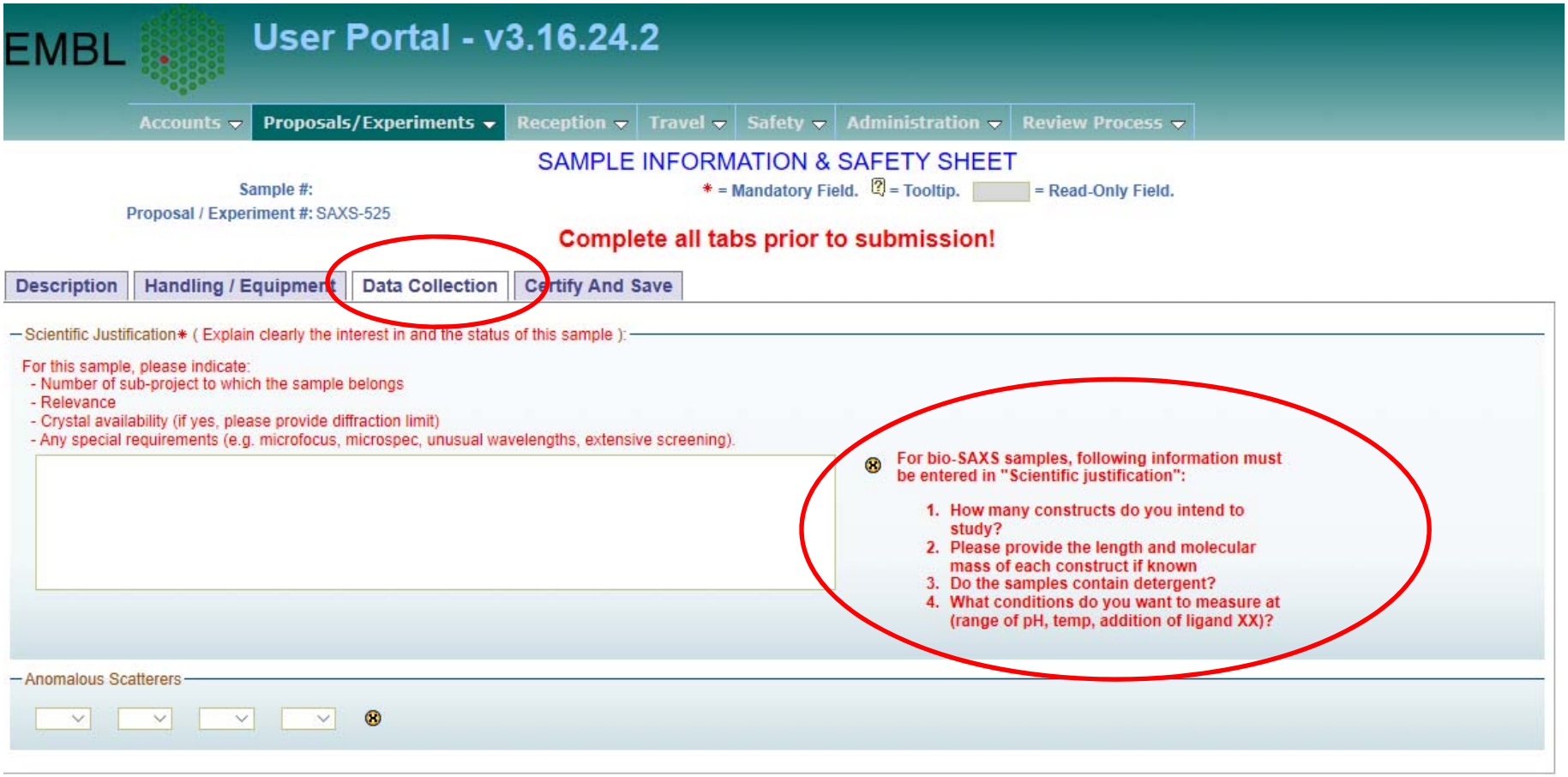


«Handling and Equipment » tab

- includes more practical information on samples ;
- details will be given here if special equipment is going to be used.



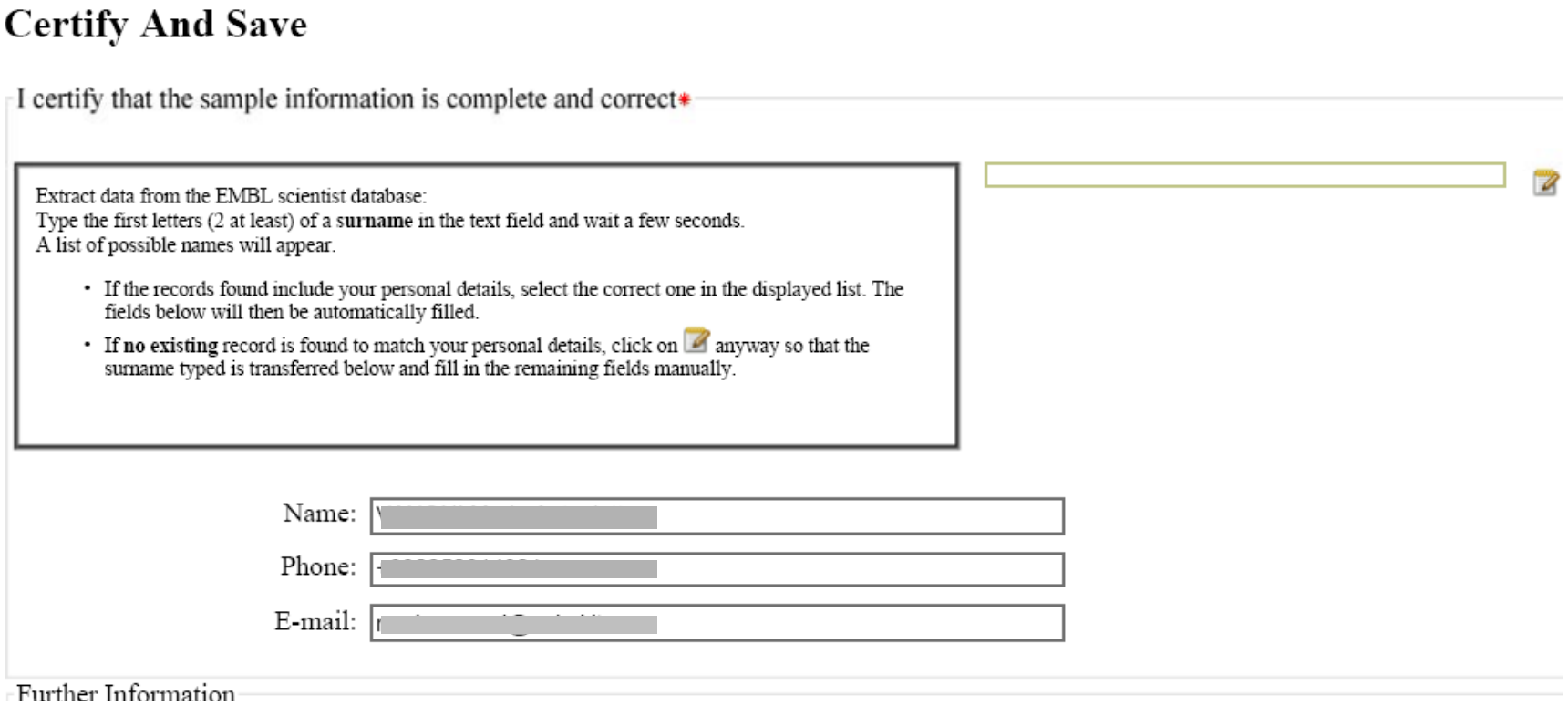
«Data Collection» tab:



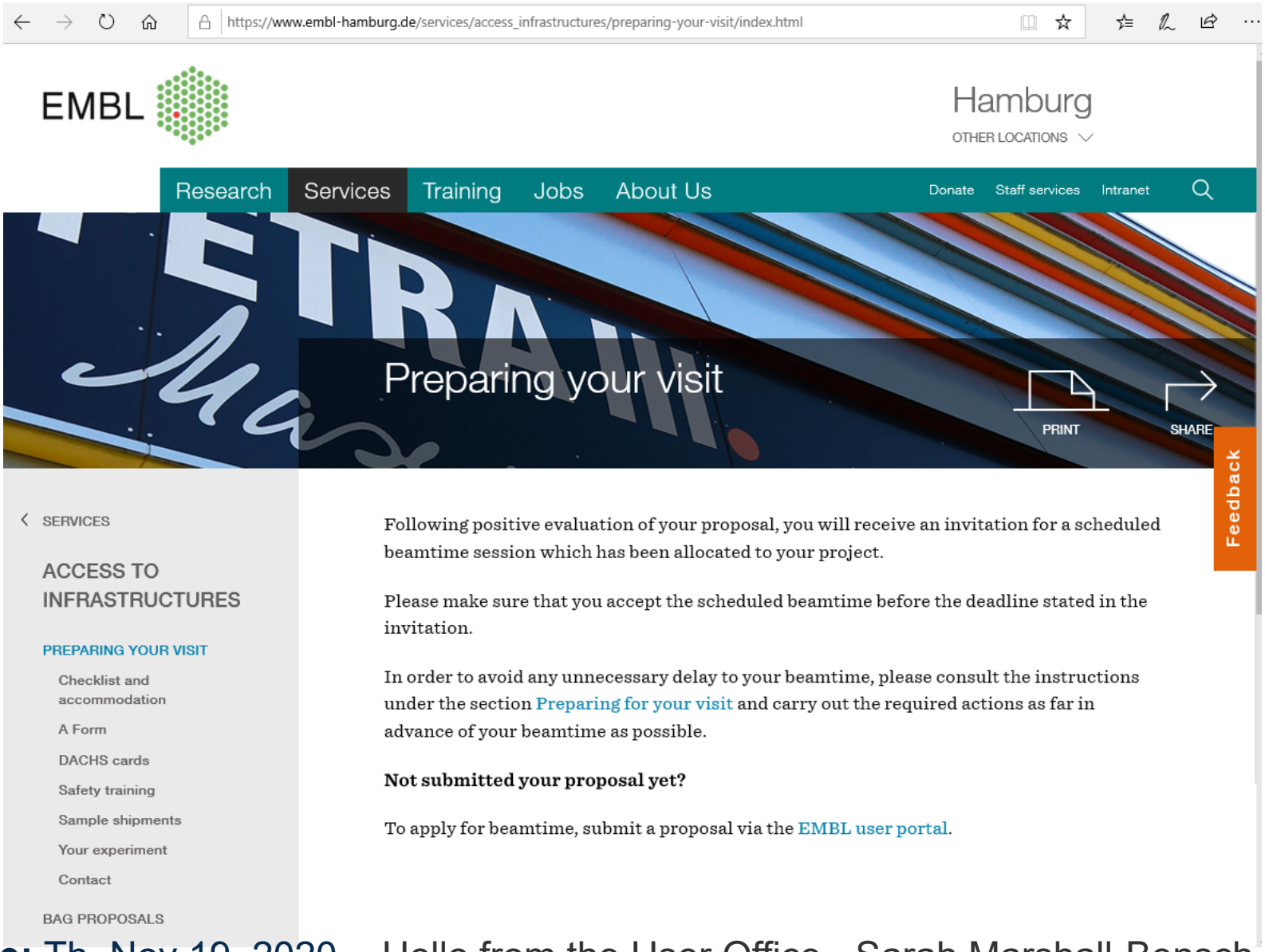
DON'T cut-and-paste from the Proposal form.

DO complete with technical/practical information (see questions in red as a guide, but add what is relevant to your system/planned experiments)

Remember to save!



«TO DO THINGS» after your application is approved: just follow the instructions!



See: Th. Nov 19, 2020 - Hello from the User Office - Sarah Marshall-Bensch

What if?

-What if my proposal was well received , but could not be granted beamtime?

- What if I find out that I badly need to perform SAXS experiments after the deadline for presentation of «regular proposals»?

Can (re)apply as «Rolling proposal» throughout the year, depending on beamtime availability. Check the EMBL website.

- What if I have been awarded beamtime, but my experimental plans change because of novel results? (e.g.: no longer need beamtime, need to change experiment design, a novel/better protein form is available, can't produce (enough) protein, ...)

- Adjustments/refinements to the original experimental plan are expected (update «sample sheet» prior to actual visit)
- Major changes: Contact SAXS people ASAP to discuss novel scenario

Good luck!

... and thanks to the Svergun Group!