Planning your experiment at BioCAT

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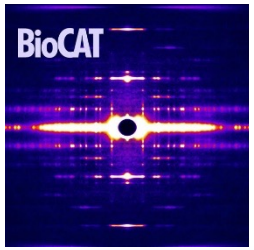
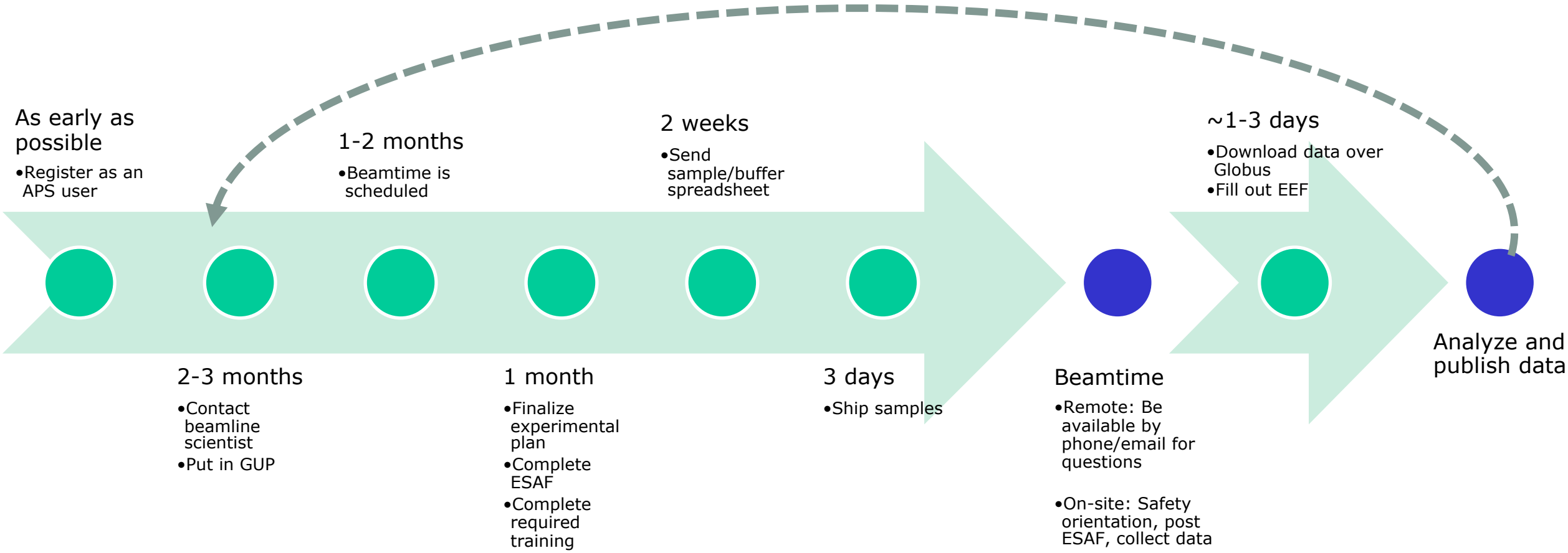
CSRRI



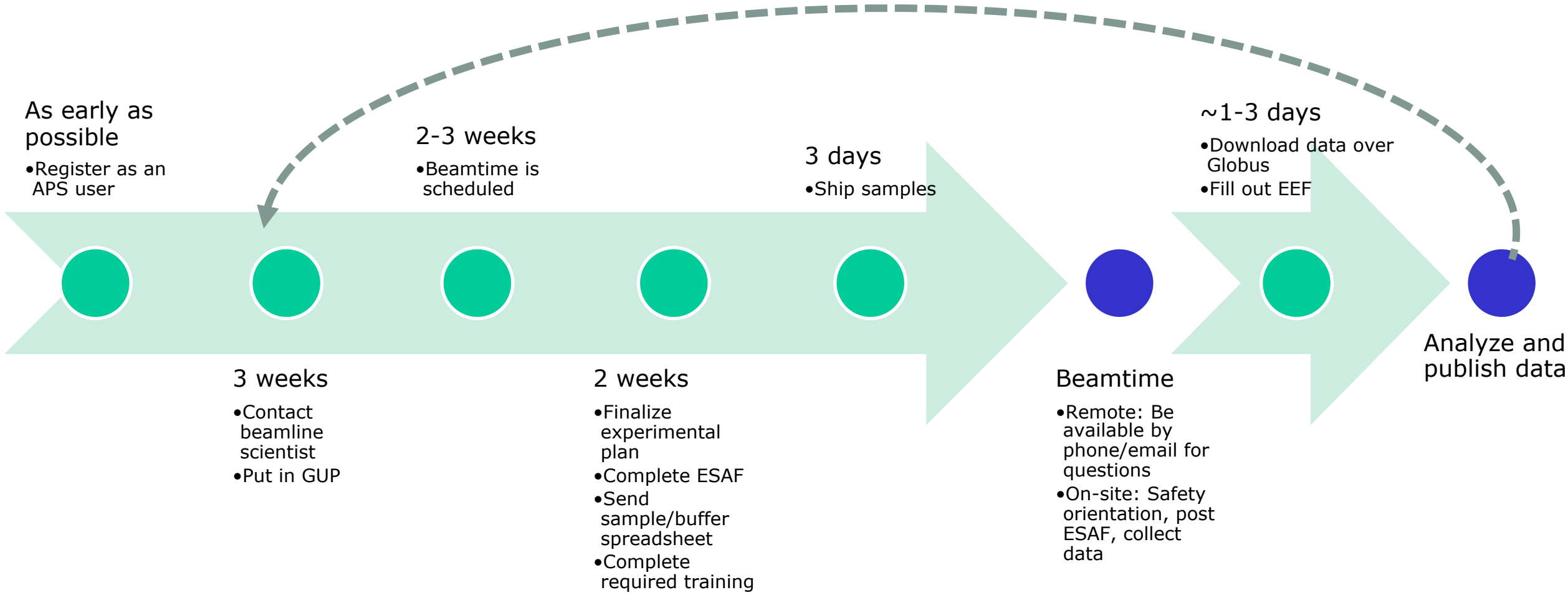
Information to get started

- Website: https://www.bio.aps.anl.gov/
- Scientific contacts:
  - Jesse Hopkins – jhopkins1@iit.edu
  - Max Watkins - mwatkins2@iit.edu
- Guide to applying for beamtime:
  - https://www.bio.aps.anl.gov/pages/applying-for-time.html
- Guide for experiment planning:
  - https://www.bio.aps.anl.gov/pages/how-to-design-saxs-exp.html
  - https://www.bio.aps.anl.gov/pages/how-to-prepare-saxs.html

Timeline - preferred



Timeline - compressed



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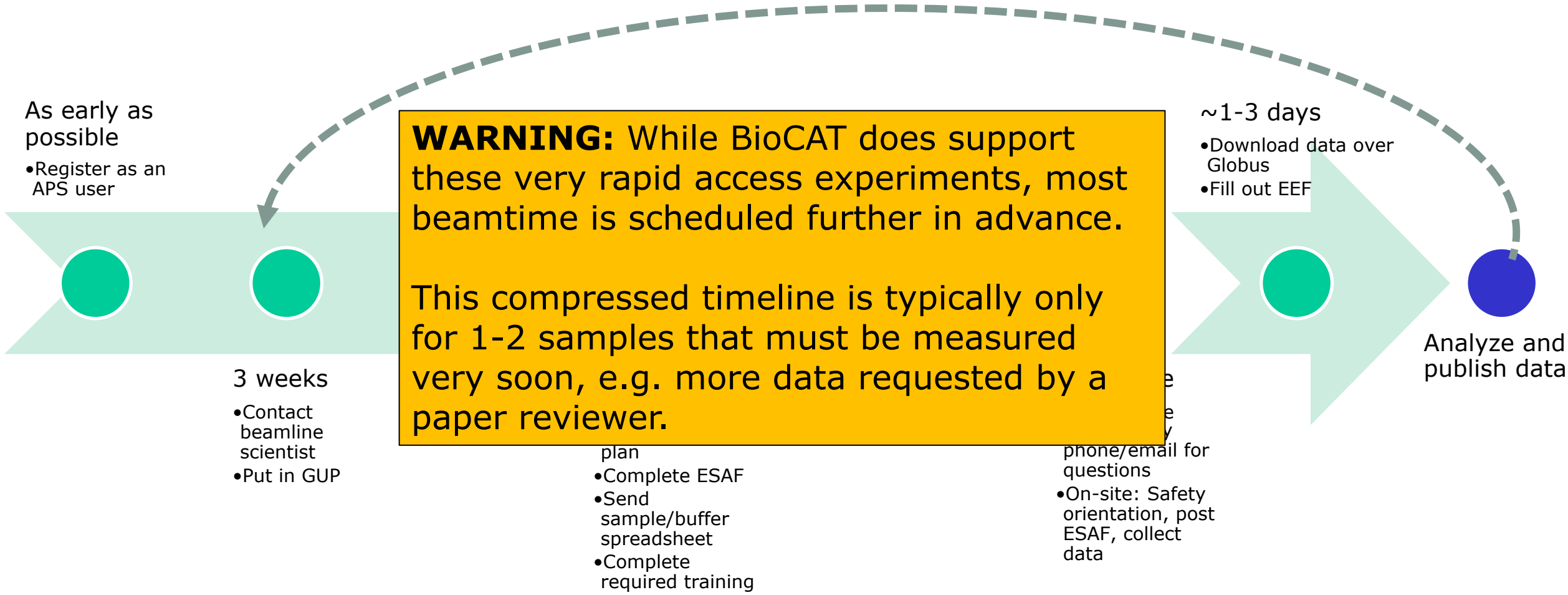
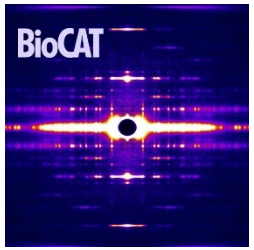
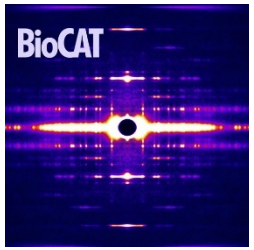




Different types of experiments

- On-site
  - Users come on-site
  - Trained at beamline in data collection and analysis by a beamline scientist
  - Work initially collaboratively then independently to collect their data
  - Optimal, as it allows best on-site preparation, active feedback on experiments, iteration of conditions (e.g. concentration, buffer components, etc) during a beamtime
  - Users analyze data after beamtime either independently or in collaboration with beamline scientists
  - Beamline scientists are co-authors on publications if appropriate, but not expected
- Remote collaboration:
  - Users mail samples to beamline
  - Beamline scientist is closely involved in experiment planning, do all the on-site prep and measurement, provide first analysis of data
  - Users analyze data after beamtime either independently or in collaboration with beamline scientists
  - Beamline scientists are expected to be co-authors on publications
- Mail-in:
  - Users mail samples to beamline
  - Beamline scientist does minimal prep, makes all measurements, provides automated first look at data analysis
  - Users analyze data after beamtime either independently or in collaboration with beamline scientists
  - Beamline scientists are co-authors on publications if appropriate, but not expected
  - Discouraged, as we find mail-in experiments often become remote collaborations in practice, if not name

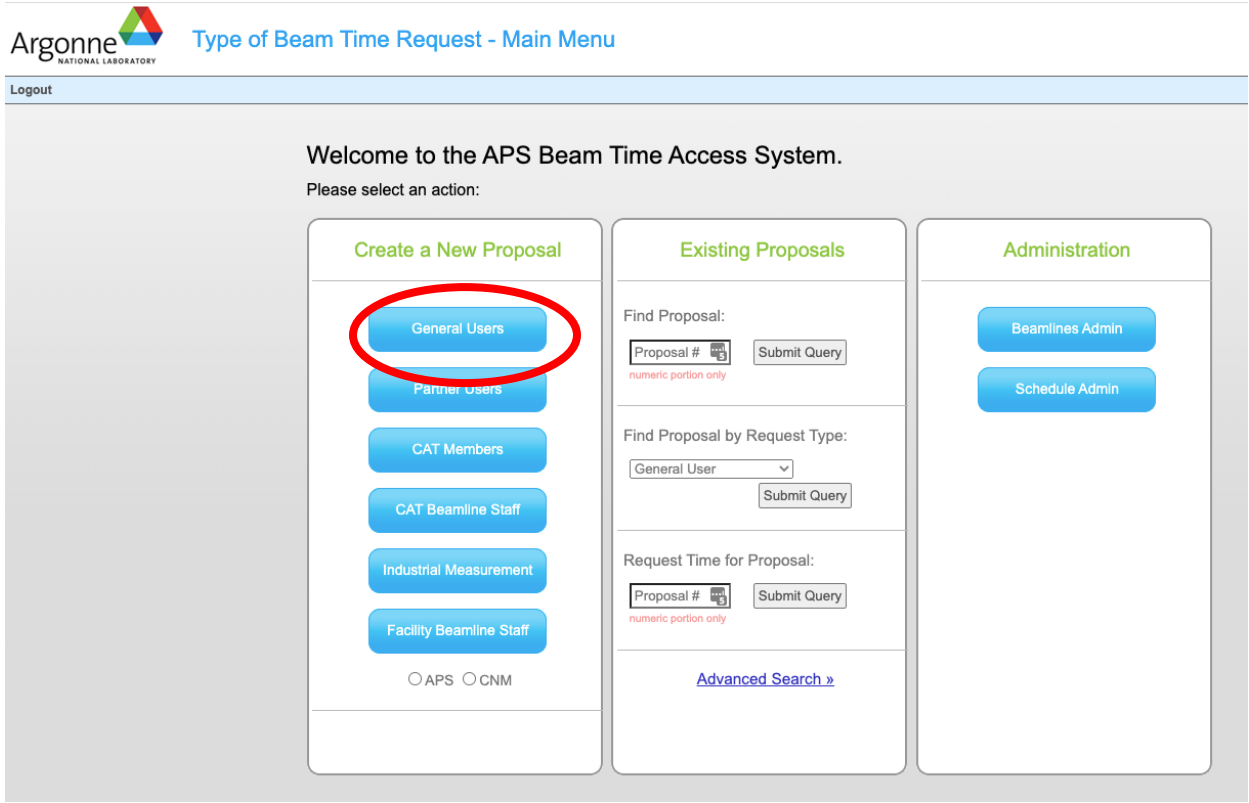


Questions to start your planning

- What scientific question do I want to answer?
  - Determines whether SAXS is a suitable technique
- Can I produce samples in sufficient volume, concentration and purity?
  - How do I know the purity?
  - How stable are they after purification?
  - Will they survive the trip to the beamline (e.g. freeze/thaw cycle)?
- How many samples and buffers do I have?
  - Can I combine any similar buffer conditions to reduce time wasted changing buffers?
  - Determines amount of time needed, suitableness of different experiments
- What types of experiments do I need/want?
  - SEC-SAXS
  - SEC-MALS-SAXS
  - Other
- Can I go in person, or do I need a remote collaboration?

Applying for beamtime

- Register as user (required to submit proposal)
  - https://beam.aps.anl.gov/pls/apsweb/ufr_main_pkg.usr_start_page
- Submit proposal
  - https://www.aps.anl.gov/Users-Information/About-Proposals/Apply-for-Time
  - Standard general user proposal (GUP) good for 2 years, multiple beamtimes. Deadlines 3 times per year
    - If you have a standard GUP, submit a beamtime request (BTR) instead
  - Rapid access GUP good for 1 beamtime, rolling submission
  - Standard GUPs are scored, allocated by APS ~1 month after deadline
  - Rapid access GUPs are scheduled at discretion of beamline
    - Given lower priority than successful standard GUPs which must be scheduled



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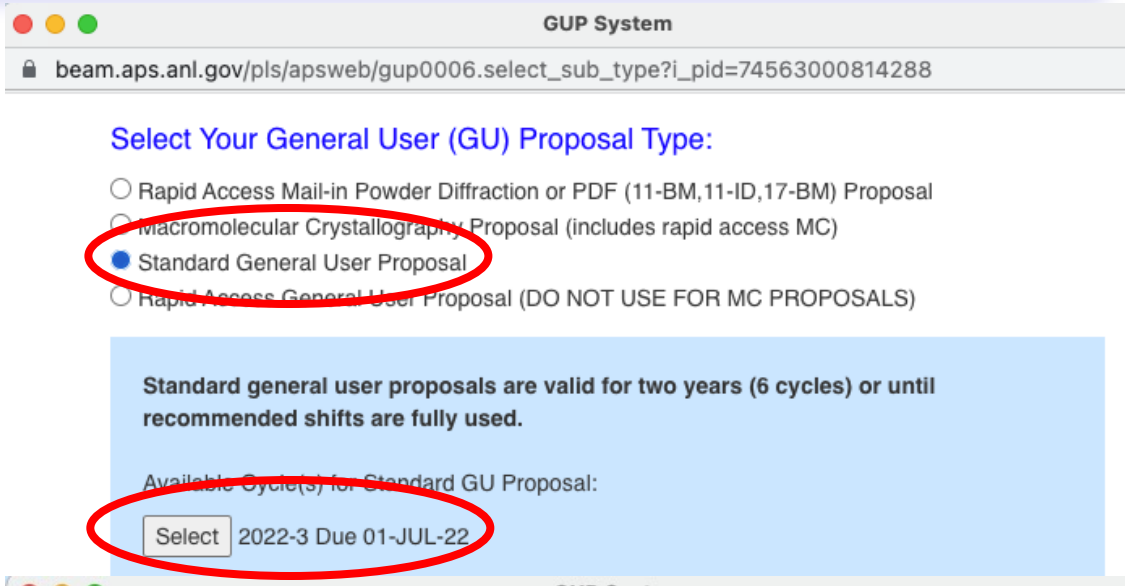

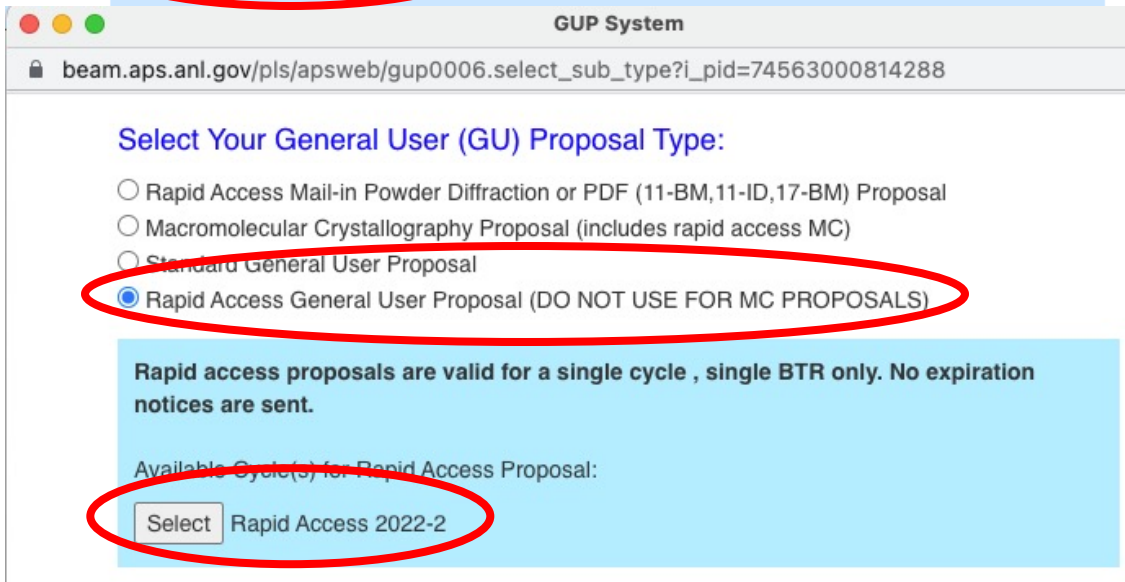

Things to discuss with beamline scientist

- What questions you're trying to answer, if SAXS is appropriate for your system
  - Beamline scientist can tell you if analysis is feasible
  - Beamline scientist may recommend other samples/constructs that you will want to run in order to carry out the desired analysis
- The types of experiments you want to do/they recommend
- If you have enough pure sample for all the planned experiments
  - What is your purification protocol, and how do you know the sample is pure enough?
  - Have you done any other biophysical or structural characterization of the sample?
- Appropriateness of buffer conditions
  - Staff may recommend changes to buffer based on our experience
- When you want to do the experiments
  - Also if you can come on-site or not

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- If you have enough
  - What is your purification protocol, and how do you know the sample is pure enough?
  - Have you done any other biophysical or structural characterization of the sample?

The more communication you have in advance with the beamline staff the more likely your experiment is to succeed

- Appropriateness of buffer conditions
  - Staff may recommend changes to buffer based on our experience

- When you want to do the experiments
  - Also if you can come on-site or not

Filling out your ESAF

- The APS requires an experimental safety assessment form (ESAF) for each experiment
  - https://beam.aps.anl.gov/pls/apsweb/esaf0001.start_page
  - https://www.bio.aps.anl.gov/pages/esafs.html
  - Without the ESAF we cannot run your samples

- ESAFs must be submitted . . .
  - On-site: 2 weeks in advance
  - Mail-in: 1 week in advance
  - If you miss this deadline the APS will not approve the ESAF and we cannot carry out the experiment

- Requires all samples, buffers, and any other equipment you will be bringing to the APS, as well as all experimenters involved in the experiment

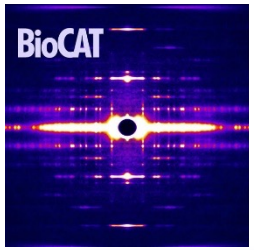
- If an experimenter is not listed on the ESAF, or the ESAF is not approved you will not be able to get on site when you arrive

- The ESAF will also list required training for each experimenter

Sample prep

- Pre-beamline characterization
  - Single band on SDS-PAGE gels and native gels
  - Symmetric single peaks on SEC
  - No visible particulates or precipitates
  - SEC-MALS or AUC
  - DLS
  - Test shipping conditions (e.g. freeze/thaw cycle)

- Make sure you can provide enough sample concentration and volume
  - Optimal concentration:
    - 240/MW in kDa for SEC-SAXS
    - 60/MW in kDa for batch mode
  - Optimal volume:
    - ~350 uL for SEC-SAXS
    - ~100 uL for batch mode SAXS
  - Useful to provide 2x volume, so that if something goes wrong, like the beam dumping in the APS ring, we can try again

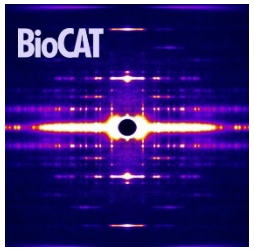


Sample prep

- Consider buffer composition
  - Suitable buffer choice
    - Consider pH range, also temperature stability
    - Non-radical scavenging buffers preferred (PBS)
  - Recommend some salt (50-150 mM typical)
  - Include glycerol, reducing agents only if needed for sample stability
    - Consider lifetime of reducing agents, TCEP lasts longer than DTT for example

- Provide sufficient buffer
  - SEC-SAXS: 4*(CV)*(# samples +1) + 100 mL
  - SEC-MALS-SAXS: 4*(exp. time)*(flow rate) + (equil. time)*(flow rate)
  - Never provide less than 500 mL for any experiment

- Most users ship buffers at 5-10x, we dilute at the beamline
  - All buffers must be 0.2 μm (SEC-SAXS) or 0.1 μm (SEC-MALS-SAXS) filtered
  - Buffers diluted at the beamline will be refiltered



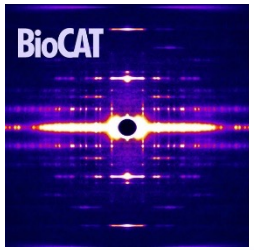
Shipping samples

- BioCAT provides short term room temperature, 4° C, -20° C, and -80° C storage for user samples and buffers
  - Any samples left at the end of a run may be discarded
- Shipping address available on BioCAT website:
  - https://www.bio.aps.anl.gov/pages/shipping.html
- You should send disposable shippers (e.g. cardboard box and styrofoam cooler, rather than a dry shipper)
- Ship via FedEx Priority Overnight if you want samples to arrive the morning after (FedEx is the preferred shipper at Argonne)
  - Other methods may not arrive until the afternoon or even the next day
- Send tracking info to your scientific contact
- We cannot receive samples on the weekend, so make sure to ship with that in mind!

Sample and buffer spreadsheet

- BioCAT provides a spreadsheet to all experimenters. We request that you fill in your sample and buffer details and return it to use two weeks before you ship your samples
  - Helps organize the experiment
  - Lets us make sure the right setup is ready for you
  - Lets us catch any obvious issues (insufficient sample volume or concentration, not enough buffer, etc) before beamtime starts

	A	B	C	D	E	F	G
1	**Experimenter**	**Phone number**	**Email**	**Experiment date (yyyy/mm/dd)**			
2	Jesse Hopkins	630-252-3062	jhopkins1@iit.edu	2021/07/30			
3							
4	**Sample Tube Label**	1	2	3	4	5	6
5	**Exp. Type**	SEC-SAXS	SEC-SAXS	SEC-MALS-SAXS	Batch	Batch	Batch
6	**Total sample vol. (uL)**	1000	500	200	100	100	100
7	**Sample conc. (mg/ml)**	16.2	2	2	8	8	8
8	**Column (SEC/IEC Exp.)**	Superdex 75 Increase 10/300	Superdex 200 Increase 10/300	Superdex 200 Increase 10/300			
9	**Loading vol. (uL)**	300	300	200	100	100	100
10	**Loading conc. (mg/ml)**	16.2	2	2	4	2	1
11	**Needs processing before experiment?**	N	N	N	Y	Y	Y
12	**Exp. Temp. (4-50 C)**	20	20	20	20	20	20
13	**Running buffer**	50 mM sodium citrate, 150 mM NaCl, pH 4.5	50 mM Tris, 150 mM NaCl, 1 mM MgCl2, pH 7.5	100 mM Tris, 150 mM NaCl, pH 8.0	50 mM Tris, 200 mM NaCl, 1mM DTT pH 7.5	50 mM Tris, 200 mM NaCl, 1mM DTT pH 7.5	50 mM Tris, 200 mM NaCl, 1mM DTT pH 7.5
14	**Storage Temp.**	-80 C	4 C	-20 C	-20 C	-20 C	-20 C
15	**Sample description**	Lysozyme	Glucose isomerase	Urate Oxidase	Urate Oxidase	Urate Oxidase	Urate Oxidase
16	**Sample MW (kDa, monomer)**	14.3	43	34.15	34.15	34.15	34.15
17	**Expected oligomer**	monomer	tetramer	tetramer	tetramer	tetramer	tetramer
18	**Sample MW (kDa, oligomer)**	14.3	172	136.6	136.6	136.6	136.6
19	**E280 (M^{-1} cm^{-1}, monomer)**	38940	45660	53581	53581	53581	53581
20	**Notes**	Should be single peak with possibly a small leading edge shoulder	Should be single peak		Serial dilutions to 4, 2, 1 mg/ml before loading	Serial dilutions to 4, 2, 1 mg/ml before loading	Serial dilutions to 4, 2, 1 mg/ml before loading



During and after your beamtime

- On-site users
  - Do sector orientation, post ESAF before you can do any work, including sample prep
    - Takes ~30 minutes
  - Beamline staff train you in data collection and analysis
  - Collect data initially collaboratively with staff, then independently

- Remote collaborators/mail-in users
  - Be available by phone and email
  - Staff will contact you if we see something unusual with the samples, or have questions about what to do next

- Transfer data
  - Data can be transferred to a user-provided external hard drive (on-site) or via Globus
  - For mail-in samples, staff will provide an initial summary of data, and notes about any issues during data collection
  - May take several days for data to be available on Globus

- Acknowledge BioCAT in any publications:
  - This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357. This project was supported by grant P30 GM138395 from the National Institute of General Medical Sciences of the National Institutes of Health.