EUROPEAN SYNCHROTRON RADIATION FACILITY
INSTALLATION EUROPÉENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Application for a Macromolecular Crystallography Rolling Proposal at the ESRF - Crystallography beam time

(Open for all users, except those involved in BAG projects)

Proposal Summary

Title

SMALL MOLECULE INHIBITORS AGAINST SERINE ACETYL TRANSFERASE FROM SALMONELLA TYPHIMURIUM

Keywords

#1: SMALL MOLECULE #2: Enzyme kinetics #3: inhibitors #4: crystallography

Abstract

Cysteine regulatory complex (CRC), isolated from Salmonella typhimurium and Planctomyces limnophilus and was characterized as a multi-enzyme assembly comprised of two oligomeric enzymes, serine acetyltransferase (SAT) and O-acetylserine sulfhyrdrylase (OASS), that drive the last two steps of cysteine synthesis. SAT is a critical enzyme in the formation of cysteine in plants and bacteria, but not in vertebrates, and as such, it might be a good target for novel antibacterial drugs. Five simulated hit compounds were acquired and evaluated against Salmonella and Planctomyces SAT. In a 96-well plate format, a screening approach employing Ellman's reagent to indirectly quantify SAT activity was established, giving five compounds with a concentration of 1µM and 10µM in the case of Salmonella.The results obtained from this study is interestingly good and need crystallography data to support our research.

- **This proposal is:**
  A new proposal ☑
  A resubmission of ... ☐
  A continuation of : ... ☐
- This proposal is:
  Fundamental Science ☑ % 100 Applied Science ☐ % 0 Industrial Science ☐ % 0

Societal Themes

- ○ Earth and Environment
- ○ Health
- ○ Information and Communication Technology (ICT)
- ○ Other Functional Materials
- ○ Energy
- ◉ Fundamental Science
- ○ Other

Scientific Area of the proposal

◉ MX - Macromolecular Crystallography

Main proposer (to whom correspondence will be addressed):

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Co-Proposers (Laboratory if different from main proposer)

No Co-proposer found

Beamline(s) requested:

Principal: BM07
OR (alternative): ID23-2

Number of shifts required 9 Total required shifts: 9

Preferred starting time: *Please select the period* August/September *Unacceptable dates*

Beam Requirements

☐ 16 Bunch Mode ☐ 4 x 40mA Mode ☐ Multi Bunch
☐ Circular polarization ☐ White beam ☐ Monochromatic beam
☐ Fixed energy [keV]: ☐ Tunable energy [keV] from: ☐ to: ☐
Beam energy resolution [meV]: Spot size on sample [µm]:
Other:

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RCS Grenoble D 338 723 919
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Postal Address: ESRF User Office - CS 40220 - 38043 Grenoble Cedex 9 - France
Location: Polygone Scientifique Louis Néel - 71, avenue des Martyrs - 38000 Grenoble - France
Telephone (Switchboard): 33-(0)4 76 88 20 00 - Telefax: 33-(0)4 76 88 20 20

Laboratory Support Facility

- ☑ Biology Lab
- ☐ PSCM Labs (Science Building)
- ☐ icOS Lab (in crystallo Optical Spectroscopy)

Sample Environment

Items Supplied by the ESRF

☐ Furnace ☐ Magnet ☑ Cryostat ☐ Cryogenic gas stream ☐ Refrigerator

☐ Laser Class ... Wavelength [nm]

☐ High pressure Pressure range [GPa] from to

☐ Fixed temperature Temperature range [K] from to
Temperature [K]

Detector system

Other equipment

Items Not Supplied by the ESRF

List all equipment that you will insert into the instrument

☐ Laser Class Wavelength [nm]

☐ Other equipment

Please indicate requirements for special equipment or facilities

Sample Description

Substance and formula

SERINE ACETYL TRANSFERASE

☑ Single crystal ☐ Powder ☐ Polycrystalline ☐ Multilayer ☐ Liquid ☐ Gas

☐ Nanoparticles ☐ Prepared at ESRF

☐ Other

Average size [mm] Volume [mm³] Surface area [mm²]

Mass [mg] Matrix or solvent Conc.of absorb.[mmol]

Molecular mass [kDa] Space group Cell dimensions at T= K:

a= Å b= Å c= Å alpha= Â° beta= Â° gamma= Â°

Container (capillary, flat plate, type of pressure cell, grids [type] for CryoEM, etc.)

Extra information required for cryoEM experiments: please add sufficient proof (raw images, 3D reconstruction, class averages etc.) to your application using the experiment method form.

Extra information required for Macromolecular Crystallography:

Origin and expression system

Previously observed diffraction (resolution, X-ray source, exposure/Â°)

Safety

Is the sample:

☐ Radioactive? ☐ Contaminant? ☐ Corrosive? ☐ Oxidizing?

☐ Explosive? ☑ Biologic? ☐ None of those

Is there any danger associated with the proposed sample, with any preparation at ESRF, or with sample equipment?

○ Yes ○ Uncertain ◉ No

If you have ticked Yes or Uncertain, you must give details of the associated risks in the box below:

Will you use live animals *on site for your experiment* ***(all kinds of animals are concerned)?*** ○ Yes ◉ No

After the experiment *, will the sample be:* ◉ Removed by user? ○ Stored at ESRF?

To be filled by ESRF	***Comments by safety Officer:***
Sample environment code:	

Experience with Synchrotron Radiation

What are the technical reasons which make ESRF necessary for your experiment?Why are other synchrotron radiation sources not appropriate?

Our lab has solved many structures related to the cysteine regulatory complex (CRC). we have collected data in our in-house x-ray facility but apparently our facility has gone down. awe need some high-resolution crystal structure to support our lab research.

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

Publications

Please give the references of papers published by the proposers during the past 3 years as a result of experiments done at the ESRF.

Origin (1): if from data from ESRF beamlines ONLY, (2) : if from data from more than one source

	Description	Origin

New ESRF User

New ESRF users may list below up to 10 publications not involving any data taken at ESRF. (Do NOT list any ESRF publications here, this MUST be done in the section above).

	Description	
[1]		

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Application for beam time at ESRF – Experimental Method

Template for ESRF Standard proposals, CRG proposals, MX Rolling Crystallography and MX Rolling BioSAXS proposals.
This document should consist of a **maximum** of **two A4 pages** (including references) with a minimal font size of **12 pt**.

Proposal Summary (should state the aims and scientific basis of the proposal):

Cysteine regulatory complex (CRC), isolated from *Salmonella typhimurium* and was characterized as a multi-enzyme assembly comprised of two oligomeric enzymes, serine acetyltransferase (SAT) and O-acetylserine sulfhyrdrylase (OASS), that drive the last two steps of cysteine synthesis. SAT is a critical enzyme in the formation of cysteine in plants and bacteria, but not in vertebrates, and as such, it is proposed to be a good target for novel antibacterial drugs. Five simulated hit compounds were acquired and evaluated against *Salmonella*. In a 96-well plate format, a screening approach employing Ellman's reagent to indirectly quantify SAT activity was established, giving few compounds with a inhibition concentration of 1µM and 10µM in *Salmonella*. The results obtained from this study is very promising and we need crystallography data to study the mechanism of inhibition and develop better inhibitors.

Scientific background: We resolved few crystal structures of the SAT from *Salmonella* and *Plantomyces* in both apo and ligated states (unpublished data). We continue to screen computationally predicted selected few compounds. Among those, we have tested five different compounds against *St*SAT (*Salmonella*). Compounds were incubated with at two different concentrations (1µM and 10.0 µM for *St*SAT). The final concentration of SAT was ~40ng/well. Control experiments was also carried out in which no SAT protein was added. Nevertheless, 10 minutes time point was selected as the optimal endpoint measurement for calculation of the percent inhibition. The most important finding was the identification of a hit chemical compounds which inhibit SAT. All five compounds exhibited better inhibitory effect on SAT activity in case of *Salmonella* at 1µM and 10µM. We have used an indirect assay (DTNB assay). with the release of CoASH, SAT catalyzes the transfer of the acetyl moiety of acetyl-CoA to serine. The dip in absorbance at 232 nm caused by the hydrolysis of the acetyl-CoA thioester bond can be used to calculate SAT activity. However, because most organic compounds have a high absorbance at 232 nm, this approach is not ideal for screening compound libraries. We employed an indirect test to determine SAT activity since the acetyl group of acetyl-CoA is transferred to serine along with the release of the free thiol CoASH. The highly oxidizing disulfide link in 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB, commonly known as Ellman's reagent) is stoichiometrically reduced by free thiols coming from a mixed disulfide and a molecule of 5-thio-2-nitrobenzoic acid (TNB).

Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc):
Experimental technique: No special experimental technique needed
Required set-up(s): need an optimised beam (low wavelength) for data collection
Measurement strategy: 100% data completeness
Sample details: ~50 crystals of SAT

Beamline(s) and beam time requested with justification :

Beamline(s):

1) **FIP2** (*French beamline for Investigation of Proteins*) is located on a Bending Magnet section 07 (*BM07*) of ESRF. It is especially dedicated to crystallography of biological macromolecules. This

beamline can be used either for standard diffraction or for multiwavelength diffraction, using anomalous dispersion. Its optics can deliver a focused beam on a fixed sample position, with an energy resolution of about $2.e^{-4}$ in a large accessible energy range (7-17 keV now, 5 to 25 keV in a near future). The beam size is around ~60×230 µm VxH at 12.6 keV.

2) **ID23-2** A fully automated beamline for the autonomous collection of data from crystals of macromolecules
3) **ID23-2** is a fixed energy beamline dedicated to MX. ID23-2 offers a standard MX sample environment, but with a focused microbeam (10x4 micron).

Beamtime requested: need 6-9 shifts (~62 hours) As we have greater number of crystals, therefore we need more beamtime.

Results expected and their significance in the respective field of research :

If we got the crystallography data, we can deduce the structural information from that and moved towards further experiments.

References

1. Kaushik, A., Ekka, M. K., & Kumaran, S. (2017). Two Distinct Assembly States of the Cysteine Regulatory Complex of Salmonella typhimurium Are Regulated by Enzyme–Substrate Cognate Pairs. Biochemistry, 56(18), 2385-2399.

2. Kaushik, A., Rahisuddin, R., Saini, N., Singh, R. P., Kaur, R., Koul, S., & Kumaran, S. (2021). Molecular mechanism of selective substrate engagement and inhibitor disengagement of cysteine synthase. Journal of Biological Chemistry, 296.

3. Singh AK, Ekka MK, Kaushik A, Pandya V, Singh RP, Banerjee S, Mittal M, Singh V, **Kumaran S** (2017) Substrate-Induced Facilitated Dissociation of the Competitive Inhibitor from the Active Site of O-Acetyl Serine Sulfhydrylase Reveals a Competitive-Allostery Mechanism. ***Biochemistr***y, 56, 5011-5025.