

EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment details & BAG Progress Report

BAG Beam time Progress Report

This represents a summary of the BAG progress in the reporting period, and is in addition to the standard ESRF report sheet for each project which will be used for the Review of the BAG.

BAG Title	Moras
Allocation Period	September 2000 – January 2001
List of publications resulting from ESRF beam time	
<ol style="list-style-type: none">1. B. Delagoutte, D. Moras, J. Cavarelli. tRNA Aminoacylation by arginyl-tRNA synthetase: induced conforma during substrate binding, (2000), <i>EMBO J.</i>, Vol 19, N°21, 5599-5610.2. B. Delagoutte, G. Keith, D. Moras, J. Cavarelli. Crystallization and X-ray crystallographic Analysis of yeast arginyl-tRNA synthetase – yeast tRNA complexes, (2000), <i>Acta Cryst.</i> D56, 492-494.3. “Crystal structure of the orphan nuclear receptor RZR ligand-binding domain”, Stehlin et al., submitted4. Gangloff, M., Ruff, M., Eiler, S., Duclaud, S., Wurtz, JM, Moras D., Crystal structure of a mutant ER LBD for the mechanism of partial agonism, <i>JBC</i>, in press5. .Eiler, S., Gangloff, M., Duclaud, S., Moras D., Ruff M., Overexpression, purification and crystal structure o ER LBD, <i>Prot. Exp. And Purif.</i>, in press6. Gall, A.L., Ruff, M., Kannan, R., Cuniasse, P., Yiotakis, A., Dive, V., Rio, M.C., Basset, P., Moras, D., Cry (MMP-11) catalytic domain complexed with a phosphinic inhibitor mimicking the transition state. <i>JMB</i>, in press	

Global overview/summary

A. IGBMC Projects and associated co-researchers

1) Aminoacyl-tRNA synthetases (aaRS):

- a) ArgRS+tRNA(Arg) + small substrates (Delagoutte, Bey, Cavarelli)
- b) GlyRS (Torres, Sankaranarayanan, Cura, Dock-Bregeon)
- c) ThrRS from E.coli with leader sequence of mRNA (Torres, Dock-Bregeon, Rees)

2) Nuclear receptors:

- a) RAR LBD / RXR LBD (Bourguet)
- b) VDR LBD + vit. D₃ and synthetic agonists (Rochel, Tocchini-Valentini)
- c) VDR LBD / RXR LBD with natural ligands 9-*cis* retinoic acid and vit. D₃ (Tocchini-Valentini, Rochel)
- d) PPARs LBD + synthetic ligands (Zeyer, Renaud)
- e) CBP + PPARs LBD + synthetic ligands (Klein, Cavarelli)
- f) ERs LBD + synthetic agonists/antagonists (Ruff, Cura, Eiler, Duclaud)
- g) EcR LBD / USP + 20-Hydroxyecdysone / synthetic agonists (Billas, Rochel)
- h) RZR LBD, ERR LBD, NURR1 LBD, and other nuclear orphan receptor LBDs (Stehlin, Greschik, Zeyer, Renaud)

3) Aldose reductase (Howard, Mitschler, Podjarny)

4) Transcription factor TFIID (Romier)

5) Topoisomerases (Lamour, Mitschler)

6) Streptococcal antigen I/II (Troffer-Charlier, Cavarelli)

8) Transcription factor TFIIF (Jawhari, Mitschler, Poterszman)

B. IBMC Applications

8) HIV-1 reverse transcription initiation complex (Dumas)

9) Complex between an RNA fragment and an aminoglycoside (Westhof)

C. LCM3B Applications

10) Supercooled lysozyme (Lecomte, Jelsch, Guillot)

11) Paraoxonase (Chabrière, Lecomte, Fontecilla-Camps, Masson)

Visits made to the ESRF

Date(s) of visits	Beamline	No. of Shifts	Short Summary of each Visit
IGBMC Group			
July 7, 2000	ID14-3	1	<p>(LS1658) RZR LBD (orphan receptor): -a data set was collected, resolution 1.9 Å, time used: 8H00 (high + low); p212121, a=52.3, b=58.5, c=106.4, Rsym=3.6% , completeness 99.9%. The structure could be solved and fully refined. Publication submitted.</p>
July 24,2000	BM14	3	<p>(LS-1658) Antigen I/II SrV+ domain MAD: 3 wavelength experiment Space group: P6322, Unit cell: a=b=124.6 c=147.7 Max res: 2.8 Å. <u>Peak. Rsym</u>:7.0 % . Completeness: 99.5% <u>Edge. Rsym</u>: 6.5% . Completeness: 99.8% <u>Remote. Rsym</u>: 6.9% . Completeness: 99.9%</p> <p>SAD : 1 wavelength experiment Max res: 2.3 Å. <u>Peak. Rsym</u>:6.6 % . Completeness: 99.5% Structure solved form MAD and refined form SAD R=21.1 Rfree=23.5 . Publication submitted.</p>

September 25-26, 2000	ID14-4	3	<p>Ligand Binding Domain (LBD) of estrogen receptor alpha (ER) has been crystallised with different ligands. Two data sets were collected: -ER+ligand 186, collected at 2 Å, R=7.1%, 91% completeness, space group P21, a=87.2 b=94.2 , c=110.8, beta=94.9° Time used 1H30.</p> <p>- ER+ligand 209, collected at 2.4 Å, R=6.9%, 96% completeness, space group P6522, a=b=58.4, c=276.3, oscillation angle 0.1°, 1s exposure. Time used 2H.</p> <p>Gyrase B (ATP binding site) has been crystallized in complex with novobiocin. Two data sets were collected:</p> <p>- one collected at 2.3 Å, R= 6,4%, completeness 96.6%, space group P21, a= 44.88, b=125.55, c=79.83, beta=96.36. Time used = 1h.</p> <p>- one collected at 3.5 Å, R= 5,8%, completeness 99,5%, space group P21, a= 44.88, b=125.55, c=79.83, beta=96.36. Time used = 20min.</p> <p>Gyrase B (entire protein) has been crystallized in complex with novobiocin. Data collection was interrupted due to crystal decay. Time used = 30min</p> <p>Topoisomerase I from <i>Thermotoga maritima</i> (TopA). Seventeen very small crystals (30x30x10µm³) were tested , some of them showed some diffraction around 7Å. Time used = 4h</p> <p>PPARγ LBD + agonist:</p> <p>-six crystals were tested, time used: 1H00;</p> <p>-two data sets were collected, resolution 2.4 Å, time used: 3H00; the data could not be processed (one twinned crystal, one crystal with a high mosaicity (1.5°)).</p> <p>PPARγ LBD + antagonist:</p> <p>-a full data set, high + low resolution, was collected, resolution 2.6 Å, time used: 2H30; sg c2, a=93.1, b=61.1, c=118.0, β=103°.</p>
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September 28-30, 2000	ID14-1	6	<p>GlyRS from <i>E. coli</i>. Complete data set at 3.2 Å. Space group I4, a=b=136.9, c=112.7. 3H used. ER+ligand 209, collected at 2.5 Å, R=11.5% (3A), 99% completeness, space group P6522, a=b=58.4, c=276.3, oscillation angle 0.2°, 1s exposure. Time used 3H30.</p> <p>Gyrase B (entire protein) has been crystallized in complex with novobiocin. Five crystals were tested. Time used = 1h</p> <p>Nurr1 LBD (orphan receptor):</p> <ul style="list-style-type: none"> -three crystals were tested, time used: 0H30; -a partial data set was collected, resolution 2.8 Å but twinned, time used: 0H45; -a data set was collected, resolution 3.0 Å, time used: 1H15; then low resolution pass, time used: 0H30. The data could not be processed successfully. <p>PPARγ LBD + agonist:</p> <ul style="list-style-type: none"> -three crystals were tested, time used: 0H30; -a full data set was collected, resolution 3.4 Å, time used: 1H00; sg p212121, a=49.2, b=63.5, c=118.0, R_{sym}=8%, completeness 100%. <p>TFIIH p34/p44 subcomplex:</p> <ul style="list-style-type: none"> -three cryocooled crystals were tested, diffraction limit 7 Å, time used: 0H30; -one crystal in a capillary was tested, no diffraction, time used: 0H15. <p>Topoisomerase I from <i>Thermotoga maritima</i> (TopA). Four small crystals were tested. Time used = 1h</p>
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November 03-04,2000	ID14-1	2	<p>TFIIH p34/p44 subcomplex: 1H30. Five crystals tested, resolution limits 4.8 Å , 20 frames collected.</p> <p>PPARγ LBD + agonist: 7H00. Ten crystals tested. - Data collection high and low resolution. Resolution limits 2.9 Å .sg p212121, a=49.2, b=63.5, c=118.0, Rsym=5,7%, completeness 80%. - Data collection high and low resolution. Resolution limits 2.7 Å .sg p212121, a=49.2, b=63.5, c=118.0, Rsym=5,9%, completeness 84%</p> <p>PPARγ LBD + agonist2: 1H30. 5 crystals tested, no collection</p> <p>PPARγ LBD + antagonist: 4H00. 5 crystals tested. Data collection : high and low resolution. Resolution limits 2.9 Å Space group :P41212 , a=b= 62.37 Å , c = 165,58 Å . Rsym = 6,1%. Completeness : 95%.</p>
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November 09-10, 2000	ID14-3	3	<p>LBD of estrogen receptor alpha (ER). Two data sets were collected:</p> <ul style="list-style-type: none"> - ER+ ligand 281, collected at 2.45 Å, R=3.6%, 96.5% completeness, space group P6522, a=b=58.4, c=275.9, Oscillation angle 0.1°, 2 passes of 10s exposure, time used 15H. - ER+ligand 186 soaked with K2PtCl4, collected at 3.4 Å, R=4.8%, 87% completeness, space group C2, a=135.5 b=94.0, c=87.7, beta=124.8°. Time used 4H.
November 30, 2000	BM30	3	<p>The TFIIF p34/p44 subcomplex .</p> <p>Various crystal were tested and for the first time spots at 3.2 Å were observed (sp p6122 or p6522, a= b = 80, c=376). As this complex contains two zinc atoms a 3 wavelength MAD dataset was collected (exposure 300s, mar 345, oscillation 1°, R=3.9% at 3.5 Å, R(3.6-3.7 13%). No phases have been obtained yet. Time used 24H</p>
December 10-12, 2000	BM30	3	<p>SAD collection on Se-Met crystals (sp C2) of TFIID TAF20/TAF135 subcomplex. Incomplete data set due to weak diffraction and long exposure time. Time used 20 H.</p>
January 27_28, 2001	ID14-1	3	<p>ERR LBD (orphan receptor) :</p> <ul style="list-style-type: none"> -a data set was collected, resolution 2.7 Å, time used: 2H30; the crystal was twinned; -a full data set was collected (high + low), resolution 2.7 Å, time used: 5H00; sg p43212, a=b=83.3, c=240.6, Rsym=5.3%, completeness 97.3%. <p>PPARγ LBD + antagonist: 4H30.</p> <p>5 crystals tested, one data collection at 2.6 Å , Rsym=4,3% 96% completeness. Space group :P41212 , a=b= 62.37 Å , c = 165,58 Å.</p> <p>PPARγ LBD + agonist: 7H00.</p> <p>Collected at 2.7 Å , Rsym=4,0% 95% completeness. Space group P212121, a=49.2, b=63.5, c=118.0</p> <p>Tests :</p> <p>CBP (Nterm) +PPAR LBD: 10 crystals have been tested. Diffraction limits 4 Å- Space group P4x2x2, a=b=192., c=61. No data collection</p> <p>E. Coli ArgRS/tRNAArg</p> <p>Weak diffraction. 5 crystals tested. No data</p>

February 15-16, 2001	BM30	3	<p>Crystals of the TFIID p34/p44 subcomplex (sp p6122 or p6522, a= b = 80, c=376) were soaked with various HA derivatives were tested. No useful derivative has been found yet. Time used 10H.</p> <p>SAD collection on Se-Met crystals (sp C2) of TFIID TAF20/TAF135 subcomplex. Data not usable due to R-sym too high (9%). Time used 12 H.</p>
February 16-17, 2001	ID14 – 1	2	<p>Crystals of the TFIID p34/p44 subcomplex (sp p6122 or p6522, a= b = 80, c=376) were soaked with various HA derivatives were tested. No useful derivative has been found yet. 3 crystals tested</p> <p>The first crystal of VDR/RXR complex was tested (sp P422 a=b=82 c=163, oscillation 1°, 3 passes of 3s, resolution 3.5)</p> <p>Crystals of RXR LBD bound to Docoso-Hexaenoic Acid and to a peptide (sp P4₃2₁2, a=b=64 Å, c= 114 Å, oscillation 0.5°, 4 passes of 2.5s and 3 passes of 0.5s, resolution 1.9 Å). Global R_{sym}=3%, 2-1.9Å R_{sym}=11.0%. Structure solved by molecular replacement.</p> <p>3 crystals used only one was correct.</p>
February 16-17, 2001	ID14 - 2	2	<p>Threonyl-tRNA synthetase from <i>E.coli</i> .</p> <p>Several crystals of the synthetase with part of the leader sequence of the mRNA were tested. Spots at 3.2 Å could be seen but there is little useful diffraction beyond 4Å. A complete data set has been collected but its quality seems not sufficient for molecular replacement to work (R = 13.7 % at 3.9 Å). The crystals are monoclinic (P2, a= 189, b=102, c=200 Å, b=114°). The conditions for obtaining the best crystals were better defined</p>

Date(s) of visits	Beamline	No. of Shifts	Short Summary of each Visit
IBMC Group			
28/09/2000	ID14-1		<p>HIV-1 DIS (Mal-isolate; UU construct) (Native) Space group: C2, Unit cell: a=119.7, b=27.3, c=91.7, β=92.7, Max res: 2.9 Å Rsym: 5.9%, Completeness: 73% Result: Structure in progress</p> <p>HIV-1 DIS (Mal-isolate) (OsCl₄ derivative) Space group: P312₁, Unit cell: a=59.1, c=64.5, Max res: 2.6 Å Rsym: 5.7%, Completeness: 98.8%. Result: Not substituted</p> <p>HIV-1 DIS (Mal-isolate) (HgCl₂ derivative) Space group: P312₁, Unit cell: a=59.1, c=64.5, Max Res: 2.8 Å Rsym: 6.2%, Completeness: 99.1%. Result: Not substituted</p> <p>Test of other crystals: Ribosomal L5-RNA complex from <i>M. jannaschii</i> (Collaboration with M. Garber, Russia) Space group : P2₁, Unit cell : a=51.9, b=99.7, c=65.8, β=100.0 Max res: 2.5 Å, Rsym: 6.7%, Completeness: 99.9%. Result: Structure in progress</p> <p>rRNA fragment-antibiotics complex 180° collected (osc. 1°). resolution range 10-2.5Å space group P2₁2₁2₁. a=33.0Å, b=45.9Å, c=95.3Å Rsym=5.5%. Completeness=95%. Averaged I/σI=24.4 R-factor=20.6%.Rfree=24.7% Submitted for publication (Vicens & Westhof, 2001)</p>

10/11/2000	ID14-3		<p>HIV-1 (DIS Mal-isolate; UU construct) (Native) Space group: C2, Unit cell: a=119.7, b=27.3, c=91.7, β=92.7, Max res: 2.9 Å Rsym: 5.9%, Completeness: 96.3%. Result: Structure in progress</p> <p>HIV-1 DIS (Lai-isolate) (Native) Space group: C222₁, Unit cell: a=27.3, b=114.7, c=95.1. Max res: 2.8 Å Rsym: 5.8%. Completeness: 86.6%. Result: Structure in progress</p> <p>HIV-1 DIS (Lai-isolate) (Native) Space group: C222₁, Unit cell: a=61.9, b=92.1, c=90.9, Max res: 2.7 Å Rsym: 4.8%, Completeness: 99.3%. Result: Structure in progress</p> <p>rRNA fragment-antibiotics complex Tests on different crystals for higher resolution. No data collected</p>
11/12/2000	BM-30		<p>HIV-1 DIS (Lai-isolate) (PtCl₄ derivative) 3 wavelength experiment Space group: C222₁, Unit cell: a=62.3, b=92.7, c=91.1 Max res: 3.00 Å. <u>Peak</u>. Rsym: 5.6%. Completeness: 98.6% <u>Edge</u>. Rsym: 5.5%. Completeness: 98.6% Remote. Rsym: 4.6%. Completeness: 98.8% Result: Pt present, but insufficient phasing</p> <p>HIV-1 DIS (Lai-isolate) (Br-Uridine derivative) 2 wavelength experiment (lack of time) Space group: C222₁ Unit cell: a=27.4, b=116.2, c=95.1, Max res: 2.7 Å <u>Peak</u>. Rsym: 7.3%. Completeness: 98.8% <u>Edge</u>. Rsym: 7.8%. Completeness: 95.3% Result: Unexpected poor phasing (one Br visible, instead of two)</p> <p>rRNA fragment-antibiotics complex Tests on different crystals for attempts at MAD solving. No data collection due to a crash of the computer network</p>

24/02/01	ID14-2		rRNA fragment-antibiotics complex 260° collected (osc. 1°). resolution range 30-2.55Å space group P2 ₁ . a=47.1Å, b=33.1Å, c=52.3Å, β=107.9° Rsym=5.9%. Completeness=95.9%. Averaged I/σI=19.5
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