Form 7／様式7

(Report 1) FY 2009

JAPAN SOCIETY FOR THE PROMOTION OF SCIENCE (JSPS)

Report on JSPS BRIDGE Fellowship Activities

by individual BRIDGE Fellows

1. Fellow's BRIDGE Fellowship ID

BR090202

2. Affiliated JSPS Alumni Association

Association des anciens boursiers francophones de la JSPS, Strasbourg France, President Prof. M. C. LETT

3. Name in Full

FAMILY	First	Middle
MILON	ALAIN	

4. Host Researcher

Name in Full	Affiliation
Shigeyuki Yokoyama	RIKEN Systems and Structural Biology Center

5. Period of BRIDGE Fellowship tenure

From	To
20 / 2 / 2010 (Day / Month / Year)	7 / 3 / 2010 (Day / Month / Year)

6. Mailing address

a. Office:	b. Home:
Pr Alain MILON IPBS 205 rte de Narbonne, 31077 Toulouse France Tel: 33 5 61 17 54 23 Fax: e-mail alain.milon@ipbs.fr	MILON Alain 28 av d'ingine, 31750 Escalquens, France Tel: 33 5 61 27 96 69 Fax: e-mail alain.milon@ipbs.fr

7. Please write on the attached form.

8. Please write on the attached form.

9. Please write on the attached form.

Date: _____

NAME (Print) : MILON Alain

Signature: _____

(Notes)

1. Please send this completed form to both JSPS's Tokyo headquarter and your affiliated alumni association within one month after finishing your tenure under the BRIDGE Fellowship.

7. Research network created, sustained and/or strengthened with Japanese researchers through your visit. (Please add lines if needed)

7-1) Research network created:

At RIKEN Systems and Structural biology center, I got to know with nine patented researchers of SSBC and discussed with them. For example, Dr. Maeda tries to build up High Temperature Supra Conductor, 1.03 GHz, 4K cryoprobe. Dr. Honma has high technology in the ligand screening and the development of in house docking. Dr. Sakamoto achieves much result in the incorporation of non natural amino acids. Dr. Hato established new isoprenoid based lipids for cubic phase crystallization. Dr. Kigawa have tried to determine the structures of THAP 1, THAP 2 (in pdb) , THAP 7, THAP 10. THAP 1 construct precipitated at 1mM, THAP 7 1-99 construct (tag C – term SGPSSG) gave good spectra. They have special two robots of cell free expression, one of it for 1000 proteins per day (20ug), another for 60 proteins per day (2mg) ; easy incorporation of any labeled mixture of a Sample of NMR : 15N/13C/2H 3,000 EUR , methyl protonated 24,000 EUR , SAIL 80,000 EUR(!). In these robots, SS bond formation control, triple labeling possible, NMR of VRKal 46 kDa.

Is there a possibility of the above network yielding an application for a JSPS program?

If yes, please state the name of the program and researchers who may participate on both sides.

7-2) Research network sustained:

I visited to Prof. Osamu Nureki's Lab., Division of Structure Biology, Department of Basic Medical Sciences, Institute of Medical Science, The University of Tokyo. Prof. O. Nureki wrote 111 articles cited 2759 times : Nature (10 times), Science (4 times), Proceedings of the National Academy of Sciences (8 times), The EMBO Jounal (7 times). He also coordinates one theme in Targeted Protein Research Program (a national project of Japan) I had a chance to discuss with three Assistant Professors and five PhD students. I took interests in there reseach themes. In particular, Dr. Tomoya Tsukazaki' work (membrane proteins, MgtE, SecYE translocon) and Dr. Ryuichiro Ishitani's work (non – coding RNA, signal transduction, tRNA interacting enzymes) are characteristic.

I had discussions with Prof. Gota Kawai, Laboratory for Structural Biology, Departent of Life and Environmental Sciences, Faculty of Engineering, Chiba Institute of Technology.

He researches localization of structures of RNA (~ 40 bases) derived from VIH by NMR, kissing loops, conformational dynamics, RDC with RNAs. Ass. Plof. Taiichi Sakamoto works development of aptamers binding to IgG, NMR characterization. Their result led to the production of labeled rNTP and dNTP and of oligo in the market(Taiyo Nippon Sanso, one 16bp oligo double atranded, doubley labelles: ~ 2,000 EUR). Their produces frozen kits for cell free synthesis are very simple ; warm, add construct, and wait.

Is there a possibility of the above network yielding an application for a JSPS program?

If yes, please state the name of the program and researchers who may participate on both sides.

7-3) Research network strengthened:

Prof.. Shigeyuki Yokoyama, director of RIKEN Systems and Structural Biology Center (SSBC) , the center for structural biology, has a characteristic such as follows ; cell free expression, 40 of NMRs, X-ray C. He led "Protein 3000 project". Protein 3000 is a national project of Japan, 3000 structures determined in 5 yesrs, 3 x 900 MHz, 10 x 800 MHz 1500 structures by NMR, 1500 by X-ray ; Half published so far < Shigeyuki Yokoyama , RIKEN, 200-2009>, in WoS ; 486 articles cited 7707 times. He presently coordinates one theme in Targeted Protein Research Program (a national project of Japan) strenuously as director of SSBC, this center seem to be the protein structure factory. I had abundant time to discuss Prof. S. Yokoyama deeply.

I visited to Prof. Ichio Shimada's Lab., Laboratory of Physical Chemistry, Graduate School of Pharmaceutical Science, The University of Tokyo. Prof. I. Shimada achieves results Cross saturation and Membrane proteins. To use of Cross saturation from one protein to another (triply labeled) is able to characterize of the interface (Nature Structural and Molecular Biology 2000, Journal of Molecular Biology 2002) It is similar to imino saturation transfer, can be used in a transferred way with large MW complexes (ex IgG of 164 kDa) if fast exchange. I forecast to be tried with perdeuterated THAP. For the result of Membrane proteins, dual gate properties of KcsA, methyl TROSY reveals two populations, assignment by mutagenesis. I wonder it is possible to collaborate on KcsA in lipososomes by ssNMR.

Is there a possibility of the above network yielding an application for a JSPS program?

If yes, please state the name of the program and researchers who may participate on both sides.

8. Results of your research and networking activities in Japan

I have visited four laboratories in Japan, the level of science and laboratories is really of extremely high level. I performed a series of seminars for my current work in their laboratories. My seminars was consisted two program;

1st Conference (3 times) **"NMR structure of the THAP - *RRM1* complex : a novel DNA - binding recognition"**

V. Gervais, S. Campagne, O. Saurel and A. Milon

Institute of Pharmacology and Structural Biology, UMR 5089 / Université de Toulouse, UPS, CNRS

This conference in Japan was made possible via the **JSPS / BRIDGE program** with which

Prof Alain Milon could be invited in Japan from February 20th to March 7th 2010

Human THAP1 is the prototype of a large family of cellular factors sharing an original THAP zinc finger motif responsible for DNA binding [1]. The DNA recognition is directed by an 11 – base - pair DNA motif (THABS) specifically recognized by the THAP domain of THAP1. Human THAP1 is an endogenous regulator of endothelial cell proliferation and G1 / S cell - cycle progression, through modulation of pRb / E2F cell - cycle target genes including *rrm1* [2].

We first solved the 3D structure of the DNA binding domain of THAP1 [3]. Deletion mutagenesis and multidimensional NMR spectroscopy revealed that the THAP domain of THAP1 is an atypical zinc finger of 80 residues, distinguished by the presence between the C2CH zinc coordinating residues of a short antiparallel β - sheet interspersed by a long loop – helix - loop insertion. Alanine scanning mutagenesis of this loop – helix - loop motif resulted in the identification of a number of critical residues for DNA recognition. NMR chemical shift perturbation analysis was used to further characterize the residues involved in DNA binding. The combination of the mutagenesis and NMR data allowed the mapping of the DNA binding interface of the THAP zinc finger to a highly positively charged area harbouring multiple lysine and arginine residues.

We then determined the three - dimensional structure of the complex formed by the DNA - binding domain of THAP1 and the THABS motif found within the *rrm1* target gene. In order to improve the quality of the NMR spectra, we constructed a THAP mutant providing better protein stability. A 16 - bp oligonucleotide containing the THABS motif identified in the natural rrm1 responsive element was chosen for further structural and biophysical characterisation of the specific DNA protein complex (20 kDa). The THAP mutant retained its *rrm1* binding activity as shown by electrophoresis mobility shift assay and a dissociation constant of 480 ± 60 nM was determined by Trp fluorescence anisotropy. After complete assignment of the protein and DNA resonances, an ensemble of 1796 NOEs (including 39 intermolecular NOEs) and 55 ^{15}N - H residual dipolar couplings allowed to determine a structure of the complex with a rms deviation of 1.22 ± 0.32 Å over all backbone atoms of both protein and DNA. A comparison was made with a random oligonucleotide in terms of affinity and chemical shift perturbation.

Our studies reveal new insights into the DNA - specific recognition mechanisms in which this atypical zinc finger participates, which will be discussed.

(1) Clouaire, T., Roussigne, M., Ecochard, V., Mathe, C., Amalric, F., and Girard, J. P. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 6907 – 6912

(2) Cayrol, C., Lacroix, C., Mathe, C., Ecochard, V., Ceribelli, M., Loreau, E., Lazar, V., Dessen, P., Mantovani, R., Aguilar, L., and Girard, J. P. (2007) *Blood* **109**, 584 – 594

(3) Bessiere, D., C. Lacroix, S. Campagne, V. Ecochard, V. Guillet, L. Mourey, F. Lopez, J. Czaplicki, P. Demange, A. Milon, J. P. Girard, and V. Gervais (2008) *J Biol Chem* **283**, 4352 - 4363.

Extended access to the EU-NMR facility in Frankfurt (6th Framework Program of the EC, contract number RII3 – 026145) is duly acknowledged

2nd Conference (3 times) : **"NMR structure and dynamics of kpOMPA in detergent micelles and in lipid bilayers"**

V. Réat, O. Saurel, M. Renault, I. Iordanov, and A. Milon

Institute of Pharmacology and Structural Biology, UMR 5089 / Université de Toulouse, UPS, CNRS

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kpOmpA is a membrane protein belonging to the outer Membrane Protein A family. Its transmembrane domain (216 aa) presents a significant homology with *E. coli* OmpA (80 %), whose three dimensional structure has been determined by X - ray crystallography and by NMR [1]. The difference is mostly concentrated in the extracellular loops which are larger in the case of kpOmpA. This protein was shown to activate macrophages and dendritic cells through the TLR2 dependent pathway, and these larger loops are supposed to play a specific role in the interactions with the immune system [2,3].

The present work is focused on structural and dynamical studies of kpOmpA transmembrane domain by high resolution liquid state NMR. This domain, in fusion with a C - terminal His - tag, was overexpressed in *E. Coli* as inclusion bodies, and subsequently purified and refolded in 3-14 zwittergent. CD and gel electrophoresis demonstrated the formation of a beta barrel type protein. It was also produced in fully ^{13}C, ^{15}N, ^{2}H labelled form for NMR studies. Preliminary experiments in a variety of detergents demonstrated that DHPC is the detergent providing the highest quality spectra. D_2O exchange experiments revealed that in the NMR sample conditions a number of resonances were non exchangeable for weeks, confirming the presence of a stable fold. This sample was subjected to a whole set of 3D experiments in their TROSY version: HSQC, HNCACB, HN (CO) CACB, HNCO, HN (CA) CO, (HNC) CCCNH - TOCSY at 700 MHz (Toulouse), 600, 800 and 900 MHz with cryoprobes (Frankfort EU - NMR facility). This set of experiments has allowed the assignment of 85 % of ^{1}H and ^{15}N resonances 90 % of C', 88 % C α and C β, and 81 % of other aliphatic side - chain ^{13}C resonances. A 4D HN - HN NOESY experiment has revealed a ensemble of H - H proximities characteristic of a β barrel, also confirmed by CSIs. A sample of methyl protonated otherwise perdeuterated protein was used to measure CH_3-NH and CH_3-CH_3 constraints. An ensemble of NMR constraints have been determined, including 528 distance restraints, 128 H - bond restraints and 264 torsion angle restraints. The structure calculation was accomplished within CNS providing a set of 20 best structures with a rmsd of 0.564 Å within the β barrel. The dynamical analysis has been performed using ^{15}N relaxation for ns loops dynamics and time dependent TROSY for interfacial slow ms time scale motions [4].

The protein has also been reconstituted in DMPC lipid bilayers [5], and preliminary solid state NMR MAS 2D ^{13}C spin diffusion spectra have been acquired in order to compare the structure and dynamics in detergent and bilayer environment. As part of a European network on "structural biology of membrane proteins", in collaboration with A. Engel (Basel), and D. Muller (Dresden), we are currently analysing kpOmpA in lipid bilayer by electron microscopy, atomic force microscopy and single molecule force microscopy.

(1) A. Pautsch et al. (2000) *J Mol Biol*, A. Arora et al. (2001) *Nat. Struct. Biol.* (2) P. Jeannin et al. (2000) *Nat. immunol.* ; (2005) Immunity 2005

(3) PhD thesis, M. Sugawara (2003) UPS Toulouse, France (4) M. Renault et al. (2009) *J. Mol. Biol.*, **385, 117**

(5) M. Renault et al. (2006) *C.R. Chimie*

Extended access to the EU-NMR facility in Frankfurt (6th Framework Program of the EC, contract number RII3 – 026145) is duly acknowledged

I had some meetings with not only research organizations but also official institutions: French Embassy (Prof. P. Destruel, Prof. P. Dauchez, Attachés scientifiques), JSPS (Mrs M. Oyama (Director), Mr H. Kato (head of overseas fellowship division)), European office (Dr B. Rhode (Director), Mr A. Kimura (Research officer)).

9. Contributions to networking between researchers in your alumni association's country and colleagues in Japan :

This visit to Japan after 23 years have been both extremely nice and fruitful. The purpose of my visit to Japan was both for scientific (i.e. directly linked to my research interest) and for scientific policy, since, as a current Vice – president of my University. I achieved both of two.

At the first purpose of me, through the JSPS / BRIDGE program, I built wide scientific network with many scientists who I have discussed in Japan. The level of science and laboratories I have seen here is really of extremely high level. I hope for a collaboration with some of them and intend to perform it in near future. The series of seminars of my current research led to deep discussion with many scientists who belong to these laboratories I visited to. To obtain network with them and to know the highest results of research of them is very precious for my research in future.

At the second purpose, I used this opportunity to the maximum. It is valuable for me that I have much time with Prof. S. Yokoyama and Prof. O. Nureki as members of coordinators in a National Project of Japan (Targeted Protein Research Program). It is also important matter that I had a meeting with Dr. Tomoya Ogawa, Director of RIKEN Yokohama Institute. RIKEN carries out high level experimental and research work in a wide range of fields, including physics, chemistry, medical science, biology, and engineering, covering the entire range from basic research to practical application. RIKEN and the Paul Sabatier University formed a strong connection at institutional level.

As well as my visits to research institutions, I spent my time to inspect official institutions.

French Embassy:

I discussed with Prof. P. Destruel, Prof. P. Dauchez about science in Japan, and I gave presentations of JSPS / BRIDGE program and of the University of Paul Sabatier.

JSPS,:

Mrs M. Oyama,Mr H. Kato and I talked to exchange our information of each other's countries. I also gave presentation of the University of Paul Sabatier.

European Office of the Marie Curie Initial Training Networks (ITN) - Structural Biology of Membrane Proteins(SBMPs):

I am managing director of the ITN at SBMPs . The European Office in Japan, I took good time with Dr. B. Rhode and Mr. A. Kimura; to give my presentation of Paul Sabatier and of JSPS / BRIDGE program, to dispence some advices on how to link the ITN - SBMPs to Japanese laboratories, and use other Marie Curie instruments such at outgoing fellowships, to project of proposing something to the EU in the field of Biology capable of creating a new call. Alain Milon, with the help of his Japanese contacts and of Prof. P. Destruel will make propositions.

In this way, the both of my purposes were achieved. Established Network of institutional level is useful for scientific and for scientific policy.

The Symposium of Ada E.Yonath in the University of Tokyo, who is a scientist of Weizmann Institute of Science and a recipient of Nobel Prize in Chemistry at 2009, was appealed to me the one example that Japan contributed to scientific research multilaterally.

Finally I mentioned the my thanks for everyone. As expected my hosts did the maximum to have me enjoy a very pleasant and intellectually stimulating stay. I am particularly grateful to all of them who took a large amount of time in their busy schedules to take care of me and exchange so many informations and ideas. I wish to congratulate sincerely the JSPS / BRIDGE program for this way of promoting exchange between France and Japan. Seeing again old friends, I could feel how strong the links were in our memory, and I am convinced that fruitful collaborations will emerge from these visits and scientific exchanges.

IPBS

Université de Toulouse

CNRS

Prof Alain Milon,

JSPS BRIDGE program, Japan, **February 20th to March 7th**

ITINERARY:

Date	Place visited and outcome (research, lectures, inspection of the research organization)
February 20	Arrival in Tokyo, discussion with Pr **I. Shimada**, and Prof **O. Nureki**
February 21	Tokyo, discussion with Dr **M. Sugawara**, Japan Science and Technology
February 22	Univ. of Tokyo, Prof O. Nureki, *1st conference*
	" NMR structure of the THAP-RRM1 complex : a novel DNA-binding recognition"
February 23	Univ. of Tokyo, Prof O. Nureki, *2nd conference*
	"NMR structure and dynamics of kpOMPA in detergent micelles and in lipid bilayers"
	Discussions with assistant prof. (3) and PhD students/Master (5) of Prof Nureki's lab
February 24	Visit of RIKEN, Prof S. Yokoyama, *2nd conference*
	Discussions with PIs of Riken: Dr **Maeda**
February 25	Visit of RIKEN, Prof S. Yokoyama, *1st conference*
	Discussions with PIs of Riken: Dr **Sakamoto**, Dr **Honma**, Dr **Tanaka**, Visit of the facilities of
	Riken: NMR, crystallization robots and protein cell free expression
February 26	Visit of RIKEN, Prof S. Yokoyama
	Discussions with PIs of Riken: Dr **Hato**, Dr **Muto**, Dr **Hayashi**, Dr **Kigawa**, Dr **Goroncy**
	Meeting with T. **Ogawa**, Director of RIKEN Yokohama institute
Feb. 27, 28	Kyoto
March 1	Chiba, Institute of Technology, Prof **G. Kawai**
March 2	Chiba, Institute of Technology, Prof G. Kawai, *1st conference*
March 3	Reporting
March 4	Meeting and plans for future collaborations with Prof. O. Nureki
March 5	Meetings with research organizations and officials in Japan
	French Embassy : Prof **P. Destruel**, Prof **P. Dauchez**, Attachés scientifiques
	JSPS : Mrs **M. Oyama** (Director), Mr **H. Kato** (head of overseas fellowship division)
	European office : Dr **B. Rhode** (Director), Mr **A. Kimura** (Research officer)
March 6	Visit of Prof I. Shimada, Univ. Tokyo, dept Pharmacy, *2nd conference*
	Conference of **Ada E. Yonath**, "ribosome structure", Nobel prize for chemistry, 2008
	Meeting, final conclusions with Prof. **S. Yokoyama**, research host of this JSPS/BRIDGE
March 7	Return to France

1st Conference (3 times)

NMR structure of the THAP-*RRM1* complex : a novel DNA-binding recognition

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We first solved the 3D structure of the DNA binding domain of THAP1 (3). Deletion mutagenesis and multidimensional NMR spectroscopy revealed that the THAP domain of THAP1 is an atypical zinc finger of 80 residues, distinguished by the presence between the C2CH zinc coordinating residues of a short antiparallel β-sheet interspersed by a long loop-helix-loop insertion. Alanine scanning mutagenesis of this loop-helix-loop motif resulted in the identification of a number of critical residues for DNA recognition. NMR chemical shift perturbation analysis was used to further characterize the residues involved in DNA binding. The combination of the mutagenesis and NMR data allowed the mapping of the DNA binding interface of the THAP zinc finger to a highly positively charged area harbouring multiple lysine and arginine residues.

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(1) Clouaire, T., Roussigne, M., Ecochard, V., Mathe, C., Amalric, F., and Girard, J. P. (2005) *Proc. Natl. Acad. Sci.*

U. S. A. **102**, 6907–6912

(2) Cayrol, C., Lacroix, C., Mathe, C., Ecochard, V., Ceribelli, M., Loreau, E., Lazar, V., Dessen, P., Mantovani, R., Aguilar, L., and Girard, J. P. (2007) *Blood* **109**, 584–594

(3) Bessiere, D., C. Lacroix, S. Campagne, V. Ecochard, V. Guillet, L. Mourey, F. Lopez, J. Czaplicki, P. Demange, A. Milon, J. P. Girard, and V. Gervais (2008) *J Biol Chem* **283**:4352-4363.

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2nd Conference (3 times):

"NMR structure and dynamics of kpOMPA in detergent micelles and in lipid bilayers"

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The present work is focused on structural and dynamical studies of kpOmpA transmembrane domain by high resolution liquid state NMR. This domain, in fusion with a C-terminal His-tag, was overexpressed in *E. Coli* as inclusion bodies, and subsequently purified and refolded in 3-14 zwittergent. CD and gel electrophoresis demonstrated the formation of a beta barrel type protein. It was also produced in fully ^{13}C, ^{15}N, ^{2}H labelled form for NMR studies. Preliminary experiments in a variety of detergents demonstrated that DHPC is the detergent providing the highest quality spectra. D_2O exchange experiments revealed that in the NMR sample conditions a number of resonances were non exchangeable for weeks, confirming the presence of a stable fold. This sample was subjected to a whole set of 3D experiments in their TROSY version: HSQC, HNCACB, HN(CO)CACB, HNCO, HN(CA)CO, (HNC)CCCNH-TOCSY at 700 MHz (Toulouse), 600, 800 and 900 MHz with cryoprobes (Frankfort EU-NMR facility). This set of experiments has allowed the assignment of 85% of ^{1}H and ^{15}N resonances 90% of C', 88% Cα and Cβ, and 81% of other aliphatic side-chain ^{13}C resonances. A 4D HN-HN NOESY experiment has revealed a ensemble of H-H proximities characteristic of a β barrel, also confirmed by CSIs. A sample of methyl protonated otherwise perdeuterated protein was used to measure CH_3-NH and CH_3-CH_3 constraints. An ensemble of NMR constraints have been determined, including 528 distance restraints, 128 H-bond restraints

and 264 torsion angle restraints. The structure calculation was accomplished within CNS providing a set of 20 best structures with a rmsd of 0.564 Å within the β barrel. The dynamical analysis has been performed using ^{15}N relaxation for ns loops dynamics and time dependent TROSY for interfacial slow ms time scale motions [4].

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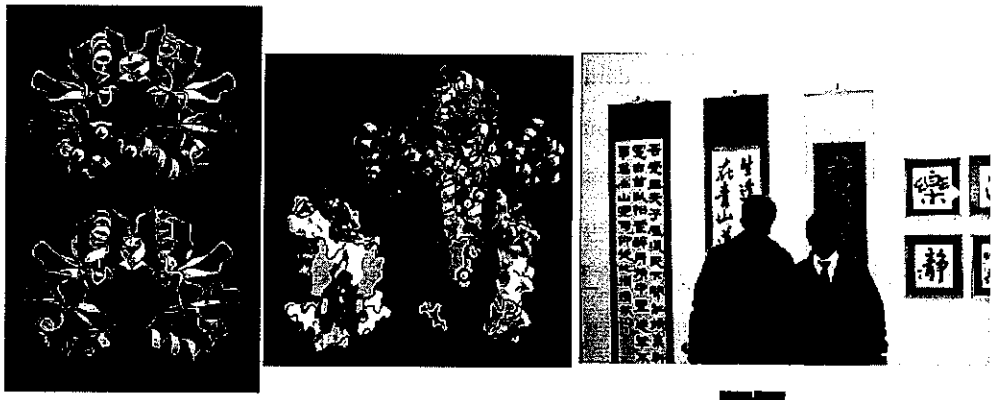
This work has been made possible via access to EUNMR (contract number RII3-026145)

EU-NMR contact : F. Löhr, Center for Biomolecular Magnetic Resonance (BMRZ), Frankfurt

[1] A. Pautsch et al. J Mol Biol. 2000, A. Arora et al., Nat. Struct. Biol., 2001 [2] P. Jeannin et al., Nat. immunol., 2000; Immunity 2005 [3] PhD thesis, M. Sugawara, UPS Toulouse, France, 2003 [4] M. Renault et al. J. Mol. Biol., 385, 117 (2009) [5] M. Renault et al., C.R. Chimie, 2006

PROF O. NUREKI, UNIVERSITY OF TOKYO

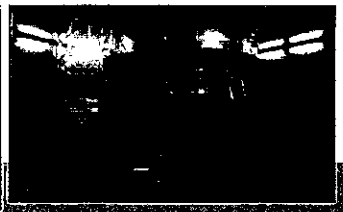
Institute of Medical Science, Division of Structure Biology
111 articles cited 2759 times: Nature (10), Science (4), PNAS (8), EMBO J (7)
Discussions with Ass. Prof (3) and PhD students (5)
T. Tsukazaki: membrane proteins, MgtE, SecYE translocon
R. Ichitani: non coding RNA, signal transduction, tRNA interacting enzymes



PROF S. YOKOYAMA, RIKEN

THE center for structural biology, cell free expression, NMR (40), X-ray, 400 M€
Protein 3000 = 3000 structures determined in 5 years, 3x900 MHz, 10x800 MHz
1500 structures by NMR, 1500 by X-ray: Half published so far
« S. Yokoyama, RIKEN, 2000-2009 », in WoS: 486 articles cited 7707 fois



SOME HIGHLIGHTS FROM THE DISCUSSIONS

Dr Yokoyama: coordination of this protein structure « factory »

Dr Maeda: High Temperature Supra Conductor, 1.03 GHz, 4K cryoprobe

Dr Honma: ligand screening, development of in house docking

Dr Sakamoto: incorporation of non natural amino acids

Dr Hato: new isoprenoid based lipids for cubic phase crystallisation

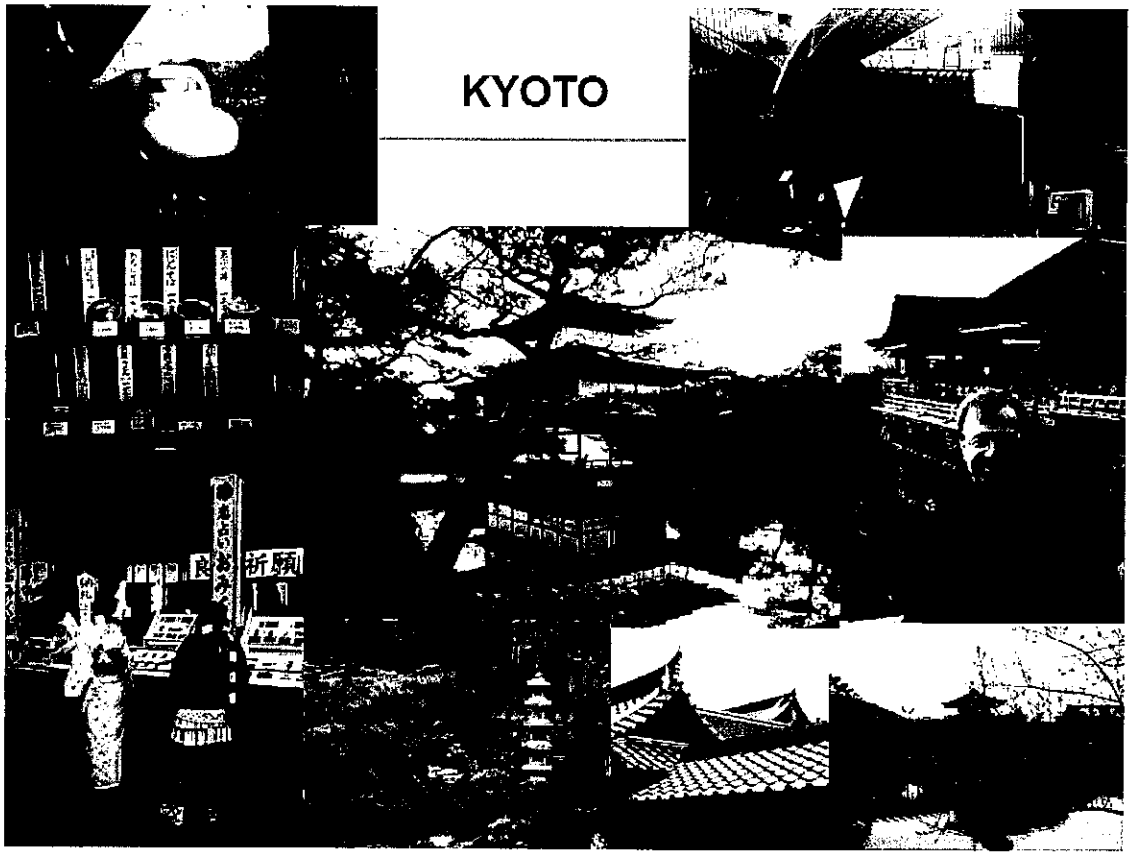
Dr Kigawa: director of the NMR core facility; have tried to determine the structures of THAP1, THAP 2 (in pdb), THAP 7, THAP 10.

THAP1 construct precipitated at 1 mM, THAP7 1-99 construct (tag C-term SGPSSG) gave good spectra.

Cell free expression: one robot for 1000 proteins per day (20 ug), one robot for 60 proteins per day (2 mg); easy incorporation of any labelled mixture of aa

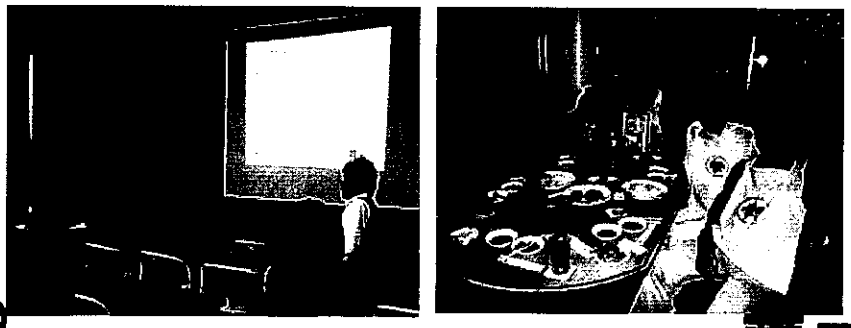
Sample for NMR: $^{15}N/^{13}C/^{2}H$ 3 k€, methyl protonated 24 k€, SAIL 80 k€ (!)

SS bond formation control, triple labelling possible, NMR of VRK1 46 kDa



PROF. G. KAWAI, CHIBA INST. OF TECHNOL.

G. Kawai: NMR structures of RNA (~40 bases) derived from VIH,
kissing loops, conformational dynamics, RDC with RNAs,
T. Sakamoto: development of aptamers binding to IgG, NMR charactization
Production of labelled rNTP and dNTP and of oligo (Taiyo Nippon Sanso)
One 16bp oligo double stranded, doubly labelled: ~2 k€
Produces frozen kits for cell free synthesis: warm, add construct, and wait...



PROF I. SHIMADA, UNIVERSITY OF TOKYO

- **Cross saturation** from one protein to another (triply labelled): characterisation of the interface: *Nat Struct Biol 2000, J Mol Biol 2002:* Similar to imino saturation transfer: can be used in a transferred way with large MW complexes (ex IgG of 164 kDa) if fast exchange (to be tried with perdeuterated THAP?)

-**Membrane protein**: dual gate properties of KcsA: methyl TROSY reveals two populations, assignment by mutagenesis, NMR sample: KcsA 0.4 mM in DDM 5 mM. (*PNAS USA, 2010*) Possible collaboration on KcsA in lipososomes by ssNMR?

Conf **Ada E. Yonath**
Nobel prize 2008
Ribosome structure

VISIT OF OFFICIAL INSTITUTIONS IN TOKYO

French Embassy: Prof **P. Destruel**, Prof **P. Dauchez**, Attachés scientifiques
Discussions about science in Japan, presentation of the JSPS/BRIDGE program, presentation of the Université Paul Sabatier

JSPS : Mrs **M. Oyama** (Director), Mr **H. Kato** (head of overseas fellowship division): exchange of information, presentation of the Université Paul Sabatier.

European office: Dr **B. Rhode** (Director), Mr **A. Kimura** (Research officer)

- Presentation of the Université Paul Sabatier
- Presentation of the JSPS/BRIDGE program
- Advices on how to link the ITN SBMPs to Japanese labs, and use other Marie Curie instruments such at outgoing fellowships
- Project of proposing something to the EU in the field of Biology capable of creating a new call. A. Milon, with the help of his japanese contacts and of P. Destruel will make propositions.

Conclusions and prospects for future collaborations

This visit to japan after 23 years have been both extremely nice and fruitful. The level of science and laboratories I have seen here is really of extremely high level. As expected my hosts did the maximum to have me enjoy a very pleasant and intellectually stimulating stay. I am particularly grateful to all of them who took a large amount of time in their busy schedules to take care of me and exchange so many informations and ideas.

I wish to congratulate sincerely the **JSPS / BRIDGE program** for this way of promoting exchange between France and Japan. Seeing again old friends, I could feel how strong the links were in our memory, and I am convinced that fruitful collaborations will emerge from these visits and scientific exchanges.

Professor **Alain MILON**
Université de Toulouse, UPS, CNRS
France