Perspectives in tRNA biology

October 28-30, 2012

Institut de Biologie Moléculaire et Cellulaire
Strasbourg

www-ibmc.u-strasbg.fr/trna-2012/
Through this event "Perspectives in tRNA biology" we wish to honor Richard Giegé and his scientific contributions to the field of tRNA biology over his fruitful career. Richard is presently an Emeritus CNRS researcher at the Institut de Biologie Moléculaire et Cellulaire (Strasbourg) and turned 70 in 2012. The Symposium is also a unique occasion to bring together all friends and colleagues who have appreciated collaborating with him over the years. Last but not least, it is a great opportunity to share the latest developments about the biology of tRNA, in particular with a new generation of scientists from the French-German University, as a "clin d'œil" to Richard's long lasting commitment to promote international scientific collaboration and friendship.

We wish you a pleasant time in Strasbourg on this special occasion!

Catherine Florentz, Joern Pütz, Claude Sauter, Eric Westhof
Perspectives in tRNA biology

Program

Sunday, October 28 2012
From 17:30 Registration, Hôtel Hannong, 15 rue du 22 Novembre, Strasbourg
From 19:00 Reception (with dinner buffet), Hôtel Hannong, 15 rue du 22 Novembre, Strasbourg

Monday, October 29 2012
8:00 Registration, IBMC 15 rue René Descartes, Strasbourg
9:00 **Eric Westhof**, Strasbourg
   *Introduction*

9:15 **Mathias Sprinzl**, Bayreuth (Chair)
   *tRNA brings together molecules and people. From aminoacylation and peptide bond formation to lasting friendship*

9:25 **Paul Schimmel**, La Jolla
   *Setting out on an endless frontier with Richard Giegé*

9:45 **Ya-Ming Hou**, Philadelphia
   *Synthesis of an essential tRNA modification by analogous enzymes*

10:00 **Sheng-Xiang Lin**, Québec
   *Physico-chemistry in biological research: from aminoacyl-tRNA synthetases to steroid-converting enzymes*

10:15 **Joe Ng**, Huntsville
   *Current reflections on the crystallogenesis of macromolecules*

10:30-11:00 Coffee Break

11:00 **Valentin Vlassov**, Novosibirsk (Chair)
   *New probes for investigation of RNA structure developed by Richard Giegé and his Siberian colleagues*

11:10 **Jacques Lapointe**, Québec
   *The major role of Professor Richard Giegé since 1976 in the Strasbourg-Québec collaborations on bacterial tRNA aminoacylation*

11:25 **Jody Puglisi**, Stanford
   *The molecular choreography of translation*
11:40  Ruslan Afasizhev, Irvine  
*Cryptogenes and RNA Editing Machines*

11:55  Sylvain Blanquet, Palaiseau  
*Richard Giegé and the French biochemical community: A view from Palaiseau*  
*Talk sponsored by Société Française de Biochimie et de Biologie Moléculaire, French Society for Biochemistry and Molecular Biology*

12:10-14:00 Lunch – Buffet at College Doctoral Européen  
(at walking distance from IBMC)  
*Statements by Sylviane Muller (CNRS Deputy-Director)*  
*Alain Beretz (President, Strasbourg University)*  
*Maria Leprévost (French-German University)*

14:00  Tom RajBhandary, Cambridge (Chair)  
*Reading of the isoleucine codon AUA in eubacteria and in archaia*

14:10  Anita Marchfelder, Ulm  
*The importance of being 3´ matured*

14:25  Henri Grosjean, Gif-sur-Yvette  
*Deciphering the genetic code in minimal organisms: the cases of Mollicutes and Insect Symbionts*

14:40  Charlie Carter, Chapel Hill  
*Class I TrpRS and Class II HisRS Urzymes exhibits comparable, full catalytic repertoires to translate the genetic code for Tryptophan and Histidine*

14:55  Hans J. Gross, Würzburg  
*From RNA research to cognitive neuroscience: The inborn numerical competence of humans and animals*

15:10  Dieter Söll, Yale (Chair)  
*Rewiring Translation for Genetic Code Expansion*

15:20  Abel Moreno, Mexico  
*Gels, Physics and Chemistry Applied to Protein Crystallogenesis*

15:35  Osamu Nureki, Tokyo  
*Dynamic and strict recognition of tRNA by proteins*

15:50  Michael Holmes, Richmond  
*Exploration of N’G37 tRNA methyltransferases*

16h15-16h45 Coffee Break
16:45 Shigeyuki Yokohama, Tokyo (Chair)
*Structural basis of aminoacyl-tRNA synthesis*

16:55 Glauco Tochini-Valentini, Rome
*Avatar pre-tRNAs help elucidate the properties of tRNA splicing endonucleases that produce tRNA from permuted genes*

17:05 Richard Giegé, Strasbourg
*Fifty Years Excitement with Science*

17:35 Franziska Pinker and Anna Meier, Strasbourg, Saarbrücken
*The student’s view*

17:45 Catherine Florentz, Strasbourg
*No conclusion*

20:00 Dinner at Maison Kammerzell, Place de la Cathédrale, Strasbourg

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**Tuesday, October 29 2012**

**9:45 – 12:00 Round Table I: “tRNA: from text-book to research”**

9:45 Joern Pütz, Introduction, Seminar Room, IBMC

10:00 **3 sessions in parallel**

Group A (at Plateforme de Biologie): **Initial bachelor students** (10 students)

Moderator: Joern Pütz

Intervenants/Referenten: Jody Puglisi (USA), Hans J. Gross (Germany), Chantal Abergel (France), Sharief Barends (The Netherlands), Youri Motorin (France) and anyone interested

Group B (at Plateforme de Biologie): **Advanced bachelor students** (16 students)

Moderator: Manfred Schmitt

Intervenants/Referenten: Joe Ng (USA), Roland Hartmann (Germany), Mathias Sprinzl (Germany), Vincent Mikol (France), Ruslan Afasizhev (USA) and anyone interested
Group C (at Seminar room, IBMC): **Final bachelor students** (16 students)

Moderator: Claude Sauter

Intervenants/Referenten: Osamu Nureki (Japan), Mark Helm (Germany), Mike Holmes (USA), Hagen Schwenzer (France), Susan Martinis (USA), Anita Marchfelder (Germany) and anyone interested

12:00 Lunch Break, IBMC

**14:00 – 15:45 Round Table II: “Perspectives on research-oriented careers for students enrolled in French-German double degrees: mobility as key issue”**

Seminar room, IBMC

Moderator: Manfred Schmitt

Participants: all students from the morning session

Intervenants/Referenten: Jody Puglisi (USA), Chantal Abergel (France), Sharief Barends (The Netherlands), Youri Motorin (France), Zendra Zhener (USA), Joe Ng (USA), Roland Hartmann (Germany), Mathias Sprinzl (Germany), Vincent Mikol (France), Ruslan Afasizhev (USA), Hagen Schwenzer (France), Osamu Nureki (Japan), Mark Helm (Germany), Mike Holmes (USA), Claude Sauter (France), Susan Martinis (USA)

15:45 Richard Giegé – Concluding remarks
Perspectives in tRNA biology

Scientific presentations
tRNA brings together molecules and people

From aminoacylation and peptide bond formation to lasting friendship

Mathias Sprinzl

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Highlights of 40 years research, side by side with Richard Giegé, will be presented. The most recent chapter on this long way concerns the peptide bond formation on ribosomes. Two tRNAs, the aminoacyl- and peptidyl –tRNA, when placed properly on moving and programmed ribosomes, give rise to a new peptide bond without additional chemical catalysis, just by virtue of their affinity to their respective binding sites.
Richard Giegé and I first met in 1972 at a conference on tRNA in Princeton, New Jersey. Subsequently, Richard came to MIT in 1973-74 to work with Alexander Rich and to collaborate with me and Richard Lord. Over the years we had many occasions to have scientific discussions in Europe, Asia, and the US, to have dinners and social events in our homes, and to design and oversee projects that resulted in joint publications spanning three decades. But perhaps the most profound influence of Richard on my work came from his seemingly obscure paper which appeared in the literature before we ever met. This paper enabled an ‘Endless Frontier’ for me and my students.
Synthesis of an essential tRNA modification by analogous enzymes

Ya-Ming Hou

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The m1G37 base modification is essential for maintaining the translational fidelity of tRNA. Elimination of this base modification leads to accumulation of frameshift errors during protein synthesis on the ribosome, leading to cell death. However, while the occurrence of the m1G37 base in tRNA is conserved in evolution, the enzymes that catalyze the synthesis of the modification are distinct among the biological domains of life. While eukaryotes and archaea employ the Trm5 enzyme, which possesses a Rossmann fold domain for binding the methyl donor S-adenosyl methionine, bacteria use the TrmD enzyme, which is unrelated to Trm5 and contains a deep trefoil-knot structure for binding the methyl donor. Structure and function analysis of Trm5 and TrmD reveals that they are also fundamentally distinct in their reaction mechanism. The separation of Trm5 from TrmD is reminiscent of the separation of the two classes of aminoacyl-tRNA synthetases, providing a parallel between reactions at the two ends of the tRNA L shape.
Physico-chemistry in biological research: from aminoacyl-tRNA synthetases to steroid-converting enzymes

Lin SX¹, Zhou M¹, Aka J¹, Mazumdar M², Wang ED², Zhang RG², Ye S²

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In E.coli, structures of most aminoacyl-tRNA synthetases have been determined except that of arginyl-tRNA synthetase, one of the small group aaRSs, which need their cognate tRNAs to proceed aminoacid activation. Here we report the first structure of E.coli ArgRS/tRNA complex. In this complex, there are two ArgRS/tRNA complexes in one asymmetric unit (ASU), associated by pseudo two-fold rotation symmetry.

At each substrate arginine-binding site of the two ArgRSs in one ASU, the enzyme can readily recognize one arginine by its characteristic triangular-shaped guanidino group. Interestingly, the two arginine-substrates share the same interaction pattern at the guanidino part, but with different orientations at the amino acid part. Comparing with the ternary complex structure of yeast ArgRS/tRNA/Arg complex, our structure shows that the arginine harbored in chain B is similar to that in the yeast complex. Additionally, structural comparison shows that the guanidino group of substrate arginine in the yeast complex is tethered more tightly than that in the E.coli complex. Superposition of tRNA-complexed ArgRS with Arg-complexed ArgRS shows no remarkable conformational change between those two structures. Due to the binding of tRNA, ArgRS undergoes a slight inward bending toward the tRNA, which can be explained as a classic induced-fit of enzyme and substrate.

As a graduate of IBMC and Shanghai Institute of Biochemistry, I have been using physico-chemistry in biology, e.g. in studying steroid-converting enzymes. In fact, 17beta-hydroxysteroid dehydrogenase (17beta-HSD1) has been believed to be the most active estrogen activating enzyme, with a turnover number reaching 9/s with purified enzyme. In the 1.7 Å resolution structure of 17beta-HSD1-DHT complex, attempt to fit the ligand density with normal binding orientation resulted in 90 Å² B factor while 47 Å² in surrounding residue, and 80 Å² B factor with the alternative binding. Only refinement with the combined occupancy of the two orientations resulted in the best B-factor, very similar to the level of surrounding residues. It is thus demonstrated that the alternative binding does occur, and this can lead to the inactivation of the most active androgen DHT, demonstrating the second catalytic function of the enzyme. In fact, cell biology study has clearly demonstrated that the dual function of 17beta-HSD1 contributes jointly to the stimulation of hormone-dependent breast cancer. In the recent differential proteomic study, the enzyme has been shown to regulate other estrogen responsive genes. All these are closely related to the early appearance of 17beta-HSD1 in the evolution of steroid-converting enzymes (Aka et al., 2012, Mol. Endocrinol. 24:832; Lin et al., 2010, Nat. Rev. Endocrinol. 6:485).
Macromolecular crystallization has been and still is the bottle-neck step to the
determination of three-dimensional structures by crystallography techniques. Much has been revealed about the nature of macromolecular crystallogenesis in the past 20 years in terms of crystal growth phenomena and its application to structural biology. The incredible journey with Richard in studying nucleic acid and protein crystal growth involving extremophiles, space and others will be reviewed highlighting what we have learned in the past and how it reflects our current thinking now.
Chemical reagents capable of cleaving RNA under physiological conditions represent useful tools for probing RNA structure. Sequence specific RNA cleaving reagents are considered as perspective therapeutics. In early 80\textsuperscript{th} importance of such reagents has been recognized Prof. J.-P. Ebel, who started contacts with Siberian researchers in order to develop new methods for investigation of RNA structure and interactions. Since that time Richard’s laboratory has been receiving chemists and biologists from Novosibirsk. I, my son and a number of Russian chemists enjoyed hospitality of Richard and creative atmosphere at IBMC. We are thankful to our French friends, who gave us a hand and provided a possibility of experimental work at IBMC during the 90\textsuperscript{th}, that were disastrous in Russia.

Our joint projects resulted in discovery of small alkylating reagents that found application as probes for studying structure of RNAs and RNA complexes. We have established mechanism of oligonucleotide binding to structured RBNAs and developed oligonucleotide conjugates equipped with catalytic RNA cleaving groups.
The major role of Professor Richard Giegé since 1976 in the Strasbourg-Québec collaborations on bacterial tRNA aminoacylation

Jacques Lapointe

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Professor Richard Giegé promoted efficiently during more than 35 years scientific exchanges between the « Institut de Biologie Moléculaire et Cellulaire du CNRS » in Strasbourg, and the Departments of Biochemistry and of Chemistry at Laval University in Québec.

Highlights of our joint discoveries will be presented, in particular: the mechanisms of aminoacyl-tRNA synthetases (aaRS), the use of stable analogues of aminoacyl adenylates synthesized in the laboratory of Professor Robert Chênevert (Chemistry, Laval) in mechanistic and structural studies of aaRSs; the identification of the 2-thio group of nucleotide 34 in the anticodon of E. coli tRNA^{Glu} as its main identity element for the GluRS, using a set of partly undermodified « modivariants »; the secondary structure of a denatured conformer of E. coli tRNA^{Glu}; the E. coli GluRS paralog Glu-Q-tRNA^{Asp} synthetase; the dynamic transamidosomes of the mesophilic bacteria Helicobacter pylori, which include a non-discriminating GluRS or AspRS, tRNA^{Gln} or tRNA^{Asn} and a trimeric aminoacyl-tRNA amidotransferase, involved together in the faithful translation of the glutamine and asparagine codons.

Several French and Canadian students who participated to these exciting collaborative and competitive projects in the field of bacterial protein synthesis, with senior investigators from Strasbourg, Québec, and sometimes Japan or USA, are now active in this or other fields of biological research.

1) Some aspects of the catalytic mechanism of E. coli glutamyl-tRNA synthetase (GluRS).
2) The use of stable analogues of aminoacyl adenylates synthesized in the laboratory of Professor Robert Chênevert (Chemistry, Laval) in mechanistic and structural studies of aaRSs.
3) the identification of the 2-thio group of nucleotide 34 in the anticodon of E. coli tRNA^{Glu} as its main identity element for the GluRS, using a set of partly undermodified tRNA^{Glu} « modivariants ».
4) the secondary structure of a denatured conformer of E. coli tRNA^{Glu}.
5) the E. coli GluRS paralog Glu-Q-tRNA^{Asp} synthetase.
6) the dynamic transamidosomes of the mesophilic bacteria Helicobacter pylori, which include a non-discriminating GluRS or AspRS, tRNA^{Glu} or tRNA^{Gln} and a trimeric aminoacyl-tRNA amidotransferase.
Biological systems evolve temporally in both composition and conformation. We have developed fluorescence methods to track biological systems in real time at the single-molecule level. We have applied these approaches to study translation, whereby the ribosome catalyzes the mRNA-directed synthesis of proteins. Our results have probed the dynamics of initiation and elongation, and revealed the mechanism of antibiotic action. It all started with tRNAs, and my love for them born in Strasbourg more than 20 years ago. Thanks Richard.
RNA editing is a collective term referring to changes in RNA sequence apart from splicing, capping or 3' extension. Our research focuses on uridine insertion/deletion mRNA editing, RNA processing and translation in mitochondria of trypanosomes. The mitochondrial genome is composed of two distinct classes of DNA molecules: few maxicircles encoding ribosomal RNAs and proteins, and thousands of minicircles bearing guide RNA genes. To produce functional mRNAs, nuclear-encoded factors mediate pre-mRNA interactions with guide RNAs leading to massive changes in RNA sequence. Editing reactions of mRNA cleavage, U-insertion or deletion, and ligation are catalyzed by protein complex-embedded enzymes while each step is directed by the secondary structure of gRNA-mRNA hybrid. Therefore, the overall fidelity is determined by intrinsic specificities of each enzyme in the pathway. Conversely, the efficiency of editing is enhanced by assembling enzymes and RNA binding proteins into stable multiprotein complexes. In addition to editing, recent studies unraveled a coupled network of pre- and post-editing processing events essential for mRNA stabilization and translation. An integrated model of mitochondrial genome expression will be discussed based on physical interactions and functional links between mRNA editing and 3' modification complexes, and ribosomes.
Richard Giegé and the French biochemical community:

A view from Palaiseau…

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…where in particular the influence of neutrons and of gratin dauphinois on tRNA and aminoacyl-tRNA synthetase research will be discussed.
Reading of the isoleucine codon AUA in bacteria and in archaea

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Of the sixteen four codon boxes in the genetic code, the AUN box is unique in that three of the four codons, AUU, AUC and AUA specify one amino acid (isoleucine) whereas the fourth codon, AUG, specifies another amino acid (methionine). This 3 to 1 distribution of codons within a four codon box is different from essentially all other four codons boxes, in which all four codons either specify one amino acid or are split 2 to 2, codons ending in pyrimidines specifying one amino acid and codons ending in purines specifying a different amino acid.

In bacteria and in archaea, the isoleucine tRNA designed to read the AUA codon is highly unusual in containing a modified cytidine in the anticodon wobble position. This raises two interesting questions: (1) How does a modified C base pair exclusively with A of the AUA isoleucine codon without also base pairing with G of the AUG methionine codon. (2) Why have bacteria and archaea evolved a mechanism utilizing a modified C to base pair with A, instead of U or a modified U? I shall describe recent results of work on the crystal structure of the archaeal isoleucine tRNA bound to the AUA codon on the ribosome (collaboration with Venki Ramakrishnan and colleagues, LMB, Cambridge, UK) and characterization of a C→U mutant of the same tRNA, including the identification of a new modified nucleoside in the anticodon wobble position and its codon recognition properties using archaeal ribosomes (collaboration with Peter Dedon and colleagues, MIT).
Aminoacyl-synthetases require a fully processed tRNA molecule as substrate. Since tRNAs are not directly transcribed as functional molecule but as precursor RNAs, they require several processing steps to generate the functional tRNA molecule. Two of these processing steps are the removal of the additional sequences 5´ and 3´ of the tRNA. While the removal of the additional 5´ sequence (the 5´ leader) has been studied in great detail, maturation of the tRNA 3´ end is not as well studied. Generation of an exact tRNA 3´ end is essential for the addition of the CCA triplet and thus for aminoacylation making the tRNA 3´ end maturation step vital for the cell. Processing at the tRNA 3´ end is catalysed by the endonuclease tRNase Z, which we first identified in Arabidopsis thaliana. To determine the substrate spectrum of this newly identified tRNA 3´ processing enzyme, Richard invited us to Strasbourg to show us how to characterise the structures of tRNA variants.
Deciphering the genetic code in minimal organisms: the cases of Mollicutes and Insect Symbionts

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Bacterial organisms like Mollicutes and Insect Symbionts have evolved in parasitic life style by massively loss of genes. These organisms however retain their full capacity to decipher accurately the genetic code and hence to synthesize all proteins required to sustain life.

Genes coding for isoacceptor tRNAs and tRNA modification enzymes have been identified in the genomes of 57 Mollicutes and 15 Insect Symbionts. In agreement with gene economization strategy, these organisms display a minimalist non-redundant set of tRNAs, each with distinct anticodons that are sufficient to decode all sense codons corresponding to 20 canonical amino acids. Moreover, only a few modification enzymes, all-acting on nucleotides of the anticodon loop of tRNA, are resistant to gene loss. The same observation applies to modification enzymes acting on helix 44 in 16S rRNA and helix 69 in 23S rRNA. These could be among the minimal sets of genes coding for tRNA and t+rRNA modification enzymes required to build up a minimalist protein synthesis machinery, a question that has been at the center of the field for many years, especially for understanding the emergence and evolution of the genetic code or for reconstructing minimal organisms from synthetic genomes.

Tryptophanyl-tRNA Synthetase Urzyme exhibits a full catalytic repertoire to translate the genetic code for Tryptophan

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The “operational RNA code” proposes a primitive translation apparatus in which a simpler recognition code used only determinants in the tRNA acceptor stem to assign amino acids to tRNA precursors. That code in turn drove the emergence of the contemporary genetic code. Charging of “mini-” and “micro-“ helices devoid of the anticodon stem-loop afforded key experimental evidence for such a code. Complementary evidence that aminoacyl-tRNA synthetase precursors acylate tRNA via interaction only with the acceptor stem has been much more elusive. We have generated and characterized active-site fragments of aaRS from Class I TrpRS, LeuRS, and Class II HisRS that contain only the conserved tertiary structural core shared by all enzymes of the same class. These Urzymes, from Ur = primitive, earliest + enzyme, are monomeric and have 120-140 amino acids.

Surprisingly, all three Urzymes accelerate cognate amino acid activation by ATP ~10^9-fold, overcoming by at least 10^4-fold the rate-limiting step for uncatalyzed protein synthesis. Active-site titration, sensitivity to genetic manipulation, and altered Michaelis constants confirm the authenticity of the activities. AARS Urzymes bind ATP tightly but amino acids weakly, and are relatively nonspecific. Their catalytic rate constants are nearly comparable to those of the native enzymes. The TrpRS Urzyme (130 aa) acylates tRNA<sub>Trp</sub> faster than it activates tryptophan in the absence of tRNA, and thus functions as a ribonucleoprotein.

The TrpRS Urzyme lacks both the CP1 insert and the C-terminal anticodon binding domain and contains only the N- and C-terminal β-α-β crossover connections and specificity-determining helix of the Rossmann fold. It’s only possible contact with tRNA<sub>Trp</sub> is via the acceptor stem. Models suggest that the discriminator base and 1-72 base pair afford sufficient binding surface to catalyze acyl-transfer and effect translation. AARS Urzyme activities therefore facilitate recapitulating operational RNA code evolution by combinatorial perturbation of complementary RNA and protein fragments of their contemporary descendants.

From RNA research to cognitive neuroscience: The inborn numerical competence of humans and animals

Hans J. Gross

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More than 140 years ago, the careful experiments of an English economist revealed the unexpected result that humans can correctly recognize and remember only up to four objects if sequential counting is rigorously prevented (1). This process has later been termed “subitizing” (from Latin “subito”). It turned out that to “subitize” is an inborn competence which cannot be improved by training, quite in contrast to counting, which we have to learn and to exercise from childhood on. During the past decades, the numerical competence of many different animals – salamanders, pigeons, crows, dolphins, chimpanzees and others has been studied, with the remarkable result that they were also able to recognize and to remember correctly up to four objects and not more. The most recent surprise was our finding that also an invertebrate, the honeybee, has numerical competence and recognizes and remembers three to four objects (2). It was completely unexpected that the honeybee, with its tiny brain of one million neurons, has a numerical competence comparable with that of the chimpanzee which has 20,000 times more, namely 100 billion brain cells. The common occurrence of the “magical number four” (3) and its importance for the inborn numerical competence of humans and animals remains an enigma (4,5) since it might possibly indicate that this ability is an ancient heritage from very early evolution.

Rewiring translation for genetic code expansion

Dieter Söll

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A central aim of Synthetic Biology is to endow organisms with the ability to produce proteins with a greater variety of amino acids than provided by the canonical twenty members. Thus, efficient formation of non-canonical aminoacyl-tRNAs and their effective delivery to the ribosome creates a major experimental and intellectual task, as it plainly challenges the natural purpose of quality control systems for protein synthesis. We focused our efforts on the ‘natural’ non-genetically encoded amino acids (e.g., phosphoamino acids) and also on selenocysteine which is refractory to protein engineering. I will discuss our progress in re-wiring translation enabling the development of methods for unlimited, site-specific insertion of O-phosphoserine and of selenocysteine into proteins expressed in Escherichia coli.

Most of the proteins already crystallized have shown different physical and chemical data about their crystallization behavior. However, the role that plays the intrinsically disorder proteins or biomacromolecular complexes in modern Biology is still a challenging issue in Protein Crystallogenesis. On this talk, it is explored from the practical point of view the Physics and Chemistry behind the protein crystallization process, the importance of having diffusing transport, the role of impurities and the basic science, which should be considered when investigating the crystallization of proteins. The author spent a year working as a sabbatical visiting professor in the lab of Richard Giegé, fruitful comments, anecdotes, and scientific achievements will be shortly shown along this talk. Finally, the impact on my scientific carrier when visiting Richard’s lab (getting a lot of inspiration) during these recent years will be highlighted and described.
Dynamic and strict recognition of tRNA by proteins

Osamu Nureki

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I started the research on tRNA-synthetase interaction at an atomic resolution when I joined Richard's lab. in my PhD student days. Footprinting analysis indicated that tRNA\textsubscript{Ile} is dynamically and specifically recognized by the cognate synthetase. Crystal structure of isoleucy-tRNA synthetase (IleRS) has a dynamic and multi-domain structure, which explains the dynamic and specific tRNA recognition mechanism. The structure also enabled us to discover the amino acid editing mechanism. On the other hand, methionyl-tRNA synthetase (MetRS) lacks the editing domain, thus compromising editing mechanism. Crystal structure of MetRS•tRNA complex revealed that the enzyme specifically recognizes the three anticodon bases by mimicking Watson-Crick base pair. As previously reported, tRNA\textsubscript{Ile}\textsubscript{2} isoacceptor is charged by MetRS in unmodified state, while upon one base modification the specificities towards amino acid as well as codon are drastically converted to Ile. Crystal structure of the modification enzyme, TilS, complexed with tRNA\textsubscript{Ile}\textsubscript{2} showed that the enzyme dynamically changes its domain arrangement so that the tRNA is specifically and sequentially recognized from the acceptor end to the anticodon. We finally established “animated crystallography” by linking the crystallographic snapshots. The example of CCA-adding polymerase showed that tRNA changes its conformation dynamically during the catalysis.
Exploration of N\(^{1}\)G\(^{37}\) tRNA methyltransferases

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The N\(^{1}\)G\(^{37}\) tRNA methyltransferases of eubacteria (trmD), are homodimeric proteins containing two S-Adenosyl Methionine (SAM) binding sites. Unlike the human trm5 enzyme, these essential enzymes require a purine at position G36. Molecular dynamic simulations of the \(E.\ coli\) enzyme suggest that two mobile loops constitute a portal for SAM binding, and that the two SAM sites might be mutually interactive. To test this hypothesis, cysteine residues were introduced forming disufhydryl bonds which might immobilize the portal loops. Mutant enzymes were active only in the presence of Dithiothreitol, suggesting that loops had indeed been coupled. Further Molecular Dynamic Simulation data suggested that the sites may not be equivalent and could possibly interact. Enzymatic Assays of Hybrid enzymes with one inactive site demonstrate that these sites are interactive. Titrating Calorimetry (ITC) has shown that a) two binding sites interact with SAM with large differences in affinity and b) only a single tRNA is bound by the enzyme at a time. Surprisingly, the product of the reaction S-Adenosyl Homocysteine was found to be more tightly bound than the substrate SAM. trmD has been subjected to a high throughput screen for inhibitors and numerous potent trmD inhibitors have been identified. The co-crystal structures of several of these inhibitors in complex with a trmD protein have been obtained. Two of these inhibitors which enter the active site will be discussed and the implications of these results for probing enzymatic mechanisms will be discussed.
The genetic code is the rule that relates codon triplets to amino acids and depends on the steps of aminoacyl-tRNA synthesis. We have been analyzing crystal structures of aminoacyl-tRNA synthetases and partner enzymes, in order to understand the “direct” and “indirect” mechanisms of aminoacyl-tRNA synthesis. I would like to present our recent structural studies on aminoacyl-tRNA synthesis, and their relations to the pioneering works by Dr. Richard Giegé.
Avatar pre-tRNAs help elucidate the properties of tRNA splicing endonucleases that produce tRNA from permuted genes

Giuseppe D. Tocchini-Valentini, Glauco P. Tocchini-Valentini

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What we are going to discuss is a mechanism responsible for the generation of a particular kind of genes, the permuted tRNA genes, originally discovered in a red alga.

Permuted tRNA genes could result from DNA rearrangement through alternative pathways that followed a duplication event. The rearranged duplicated tRNA gene must produce a single transcript capable of being cleaved by the pre-existing tRNA endonuclease to form a permuted, functional tRNA.

We developed a strategy involving the use of two tRNA splicing endonucleases characterized by two distinct mechanisms of recognition of the pre-tRNA and two avatar (av) or model pre-tRNAs. The goal is to see if the model pre-tRNAs help elucidate the features that a transcript derived from a rearranged duplicated gene must have in order to give rise to permuted tRNA.
Fifty years excitement with science

Recollections with and without tRNA

Richard Giegé

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Instead of an abstract, there will be a surprise...
Perspectives in tRNA biology

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Funding programmes for researchers –
doctoral programmes, international mobility, networking

As well as promoting and funding integrated degree programmes, the FGU supports cooperation in the fields of structured doctoral programmes and Franco-German research. It is particularly active in promoting collaboration and an intense pooling of knowledge and experience between researchers.

Structured doctoral programmes

Franco-German graduate schools (FGGS)
The aim of the Franco-German graduate schools is to foster structured doctoral programmes, in all disciplines, which require candidates to research in both France and Germany. The funding primarily helps doctoral students to research and travel abroad.

Cotutelle de thèse
A Franco-German cotutelle system allows doctoral students to write their dissertation under the supervision of at least one professor in Germany and another in France. Students are awarded a doctoral degree from both institutions after passing the joint defence.

PhD tracks
The aim of this call for applications is to develop Franco-German programmes in all disciplines that combine the two years of the master’s programme with a further three years spent on a doctoral degree to form a five-year study period in total.

Networking opportunities

Franco-German research workshops for junior researchers
The research workshops provide junior researchers (doctoral and postdoctoral candidates), professors, lecturers, students who will shortly sit their degree examinations, and advanced undergraduate students with a forum where they can work on and discuss an academic topic chosen by the organisers. These workshops often take an interdisciplinary approach.

Academic Encounters / exchanges
The Academic Encounters are used as a basis for organising and holding meetings between partner universities and for supporting the development of new degree and research programmes. The funding covers travel and accommodation expenses.
Main directions

Institut de Biologie Moléculaire et Cellulaire (IBMC)
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Collège Doctoral Européen (CDE)
46 boulevard de la Victoire

Hôtel Hannong
15 rue du 22 novembre

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