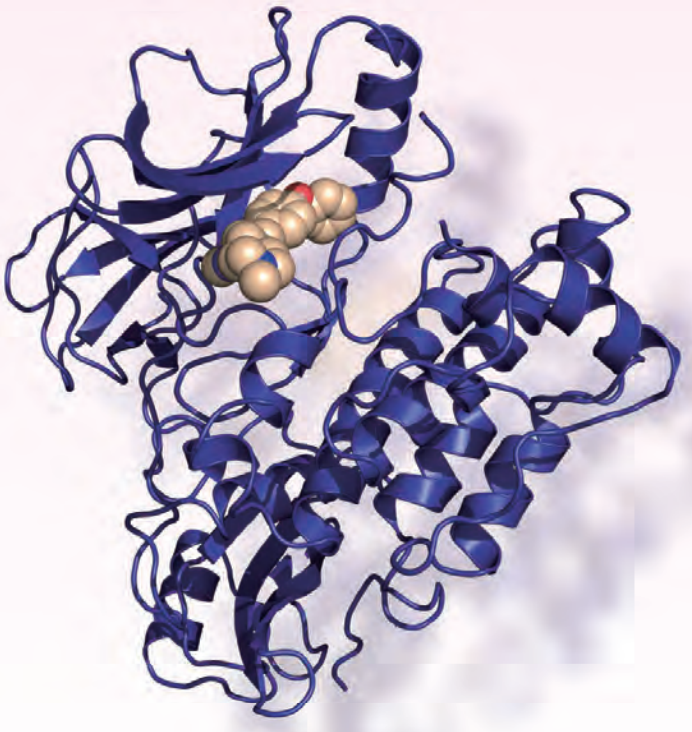
The second
RIKEN CLST – Karolinska Institutet – SciLifeLab
JOINT SYMPOSIUM

Structural Biology for Drug Discovery



November 12 (Thu) 2015

Main Office Building Hall,
RIKEN Campus in Yokohama, Japan

RIKEN CLST SciLifeLab Karolinska Institutet

This Symposium is a part of RIKEN Symposium Series.

Welcome

The collaborative relationship between Karolinska Institutet (KI) and RIKEN has delivered steady and successful results since January 2001, when KI and RIKEN Genome Science Center entered into a comprehensive collaboration agreement in the field of genomic science. Particularly strong outcomes have been achieved in the FANTOM (Functional Annotation Of Mammalian Genome) 5 Project, which is a RIKEN-led international research consortium involving over 100 institutions worldwide, and it should be emphasized that scientists from KI have played a critical role in this project and achieved excellent publications. Following the successful collaborative relationship, KI and RIKEN signed an International Program Associate agreement in 2009. Since 2010, KI and RIKEN started a joint effort to foster human resources by offering a joint doctoral course that graduate students from both institutions can attend, and this is held every other year either in Sweden or in Japan.

In the field of molecular imaging, KI and RIKEN have been working together on elucidating disease mechanisms, especially those of neuropsychiatric disorders, by using positron emission tomography (PET). Particularly, a unique PET study on monozygotic twins has been going on since 2011. The close collaborative relationship also extends to the development of new molecular probes and to PET-CT and PET-MRI studies with experimental animals.

Of course we have to continue and expand the successful collaborative relationship in the fields described above. Considering the fact that science always evolves and creates new boundaries and new disciplines however, I would like to continuously seek collaborative relationships in emerging research fields for the benefit of KI, SciLifeLab and RIKEN CLST. Furthermore, we should also promote collaboration between different areas, such as basic science and clinical research.

The title of the joint symposium this year is “Structural Biology for Drug Discovery”. In this field, by utilizing national projects since 2002, RIKEN has created infrastructure valuable to the study of structural biology. NMR facilities in Yokohama, and SPring-8 and SACLA in Harima, are the achievements of this effort. Therefore, collaborative relationships that maximize RIKEN’s infrastructure suggest new possibilities for scientific discovery. In addition, drug discovery is becoming an increasingly interdisciplinary area, emphasizing the importance of open innovation. Following this worldwide trend, RIKEN launched “RIKEN Program for Drug Discovery and Medical Technology Platforms (DMP)” in 2010. DMP is a matrix organization integrating various

RIKEN research centers. Open innovation is intrinsically difficult and there is no established formula for success. The environment or cultural background of each nation might be deeply related to its process. Thus, I am convinced that exchange of knowledge and experience between Sweden and Japan is, and will continue to be, mutually beneficial. Finally, I would like to point out that as RIKEN has several centers for life science research other than CLST, there is plenty of opportunity for new collaborative relationships with Sweden.

Your active participation in this symposium is very much appreciated.

Yasuyoshi Watanabe
Director
Center for Life Science Technologies

■ Program

9:30 - 9:40 Opening Remarks Yasuyoshi Watanabe, Director, RIKEN CLST

9:40 - 11:05 Session 1

Chair : Kazuhiko Tanzawa (RIKEN CLST)

9:40-10:10 Academic drug discovery centers - Cultural bridges over the valley of death?
Per I Arvidsson (Karolinska Institutet)

10:10-10:40 Preclinical target validation using patient-cell derived assays
Michael Sundström (Karolinska Institutet)

10:40-11:05 Structure-based development of epigenetics research tools
Takashi Umehara (RIKEN CLST)

11:05-11:25 Coffee break

11:25-12:50 Session 2

Chair : Carsten Daub (Karolinska Institutet, RIKEN CLST)

11:25-11:55 Karolinska Institutet infrastructure in protein structure and drug discovery
Karin Dahlman-Wright (Karolinska Institutet)

11:55-12:20 NMR facility and protein RNA projects
Toshio Yamazaki (RIKEN CLST)

12:20-12:50 Analysis of drug selectivity profile in their relevant cellular contexts by CETSA-MS
Janne Lehtiö (Karolinska Institutet)

12:50-14:00 Lunch

14:00-15:25 Session 3

Chair: Kam Zhang (RIKEN CLST)

14:00-14:30 Two steps forward, one step back: Successes and Failures in Structure-based Discovery of GPCR ligands
Jens Carlsson (Stockholm University)

14:30-14:55 Optimization of X-ray complex structures and binding affinity prediction by FMO
Teruki Honma (RIKEN CLST)

14:55-15:25 Tuning the allosteric modulation of ion channels through computational design
Erik Lindahl (Stockholm University)

15:25-15:45 Coffee break

15:45-17:40 Session 4

Chair: Mikako Shirouzu (RIKEN CLST)

15:45-16:15 Structural biology on membrane proteins and X-ray free electron laser
So Iwata (RIKEN SPring-8 Center/Kyoto University)

16:15-16:45 Establishing the molecular mechanisms of solute-carrier (SLC) transporters: from snapshots to movies
David Drew (Stockholm University)

16:45-17:10 Elucidate functional structure of membrane proteins in lipid environment by electron cryo-microscopy single particle analysis
Hideki Shigematsu (RIKEN CLST)

17:10-17:40 CETSA as a new strategy to understand efficacy, adverse effects and resistance development of anticancer drugs
Pär Nordlund (Karolinska Institutet)

17:40-17:50 Closing Remarks Hiroshi Matsumoto, President, RIKEN

18:10- Reception

Academic drug discovery centers
– Cultural bridges over the valley of death?

Per I. Arvidsson

Karolinska Institutet

Per I Arvidsson is Executive Director of the Drug Discovery & Development Platform at Science for Life Laboratory (SciLifeLab). Before joining SciLifeLab & Karolinska Institutet in 2013, Prof. Arvidsson held various roles at the CNS & Pain iMED unit of AstraZeneca Pharmaceuticals – the last one as Project Director within the neurodegeneration research area. Parallel to his work at AstraZeneca, Prof. Arvidsson continued to pursue academic research as Adjunct professor in bioorganic chemistry at Uppsala University (2007-2013). Since 2013, he is also affiliated to the College of Health Science, University of KwaZulu Natal, Durban, South Africa as part-time research professor. Prof. Arvidsson is named inventor on some 15+ patent applications, and co-author to some 95+ publications, two of which have won “most cited papers” awards.

Selected publication

- Borhade, S. R., et al. “Investigation of physicochemical and pharmacokinetic properties of the acyl sulfonimidamide functional group: Discovery of a potential carboxylic acid bioisostere with improved oral bioavailability”, ChemMedChem 2015, 10, 455-460.
- Farahani, M. D., et al. “Proline N-oxides: Modulators of the 3D Conformation of Linear Peptides through “NO-turns”, Org. Biomol. Chem. 2014, 12, 4479-4490.

Abstract

The former NIH Director Elias Zerhouni used the expression "valley of death" to describe the enormous challenges associated with transition drug discovery programs from laboratory success to human clinical trials. In this talk, I will outline why academic drug discovery centers with industry standard infrastructure and expertise have been build at many places through the world, and share my own experiences of establishing the national Swedish infrastructure SciLifeLab Drug Discovery & Development in 2013. Although both academic and industrial parties put much hope to that these centra will increase the likelihood of translational success,

there are cultural differences between the parties that need to be carefully balanced.

References

Arvidsson, P. I., *et al.,* "Open for collaboration – SciLifeLab Drug Discovery & Development", *Drug Discovery Today*, submitted.

Preclinical target validation using patient-cell derived assays

Michael Sundström

Karolinska Institutet

Michael Sundstrom received his PhD from Uppsala, followed by PostDoctoral studies at the Karolinska Institutet. From 1993-2000 he was at Pharmacia as Director for structure based drug design and oncology R&D portfolio management. Between 2001 and 2003 he held senior positions at the Swedish Biotechs Actar and Biovitrum. In 2003 he joined the Structural Genomics Consortium (SGC) at the University of Oxford, as Chief Scientist. In 2007 he assumed the position as Managing Director for the Novo Nordisk Foundation Center for Protein Research (Copenhagen). From end of 2011, we was VP Discovery Research at Karolinska Development. He then re-joined the SGC and is since mid-2014 Scientific Director for European Initiatives, leading an IMI consortium focused on target validation in inflammatory diseases.

Abstract

The number of first in class therapies has remained constant for decades, often less than 10 per year. For such new medicines for pioneer targets, the level of attrition in Phase 2 proof-of-concept clinical studies remains the biggest hurdle. This is in large part due to the poor correlation of results from commonly used model systems with the clinical realities.

Thus, the use of disease models based on human samples is critical to increase our understanding of pathophysiology. However, securing regular access to well-annotated samples from patients is challenging to organize, raises ethical issues and requires new organizational models, involving academic and pharmaceutical industry researchers, clinicians and disease foundations.

Within the SGC Open-Source Target Discovery Partnership, our aim is to define and validate under-explored protein targets by profiling high quality chemical and antibody probes in patient-cell derived assays in inflammation, oncology and CNS disorders, providing biomarker and phenotypic read-outs from test systems that more accurately mimic the disease itself.

References

The promise and peril of chemical probes. *Arrowsmith CH et al. Nat Chem Biol. 2015 Jul 21;11(8):536-41*

Preclinical target validation using patient-derived cells. *Edwards AM, Arrowsmith CH, Bountra C, Bunnage ME, Feldmann M, Knight JC, Patel DD, Prinos P, Taylor MD and* ***Sundström*** *M (2015). Nature Reviews Drug Discovery. 2015 Mar;14(3):149-50*

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Structure-based development of epigenetics research tools

Takashi Umehara

RIKEN CLST

Takashi Umehara currently leads Epigenetics Drug Discovery Unit in RIKEN Center for Life Science Technologies (CLST). He is concurrently one of researchers supported by PRESTO program of Japan Science and Technology Agency (JST). He received his Ph.D. in pharmaceutical sciences from the University of Tokyo in 1999, and carried out his postdoctoral research in JST until 2003. He then joined Prof. Shigeyuki Yokoyama's group in RIKEN as research scientist (until 2007) and senior scientist (until 2013). He received the young investigator award from the Japanese Society for Epigenetics (JSE) in 2011. His research interests are biochemistry, structural biology, and synthetic biology on epigenetics.

Selected publication

- Nagaoka, K. et al. Lysine-specific demethylase LSD2 suppresses lipid influx and metabolism in hepatic cells. Mol. Cell. Biol. 35, 1068-1080 (2015).
- Hino, S. et al. FAD-dependent lysine-specific demethylase-1 regulates cellular energy expenditure. Nature Commun. 3, 758 (2012).
- Ito, T. et al. Real-time imaging of histone H4K12-specific acetylation determines the modes of action of histone deacetylase and bromodomain inhibitors. Chemistry & Biology 18, 495-507 (2011).
- He, F. et al. Structural insight into the zinc finger CW domain as a histone modification reader. Structure 18, 1127-1139 (2010).
- Umehara, T. et al. Structural basis for acetylated histone H4 recognition by the human BRD2 bromodomain. J. Biol. Chem. 285, 7610-7618 (2010).

Abstract

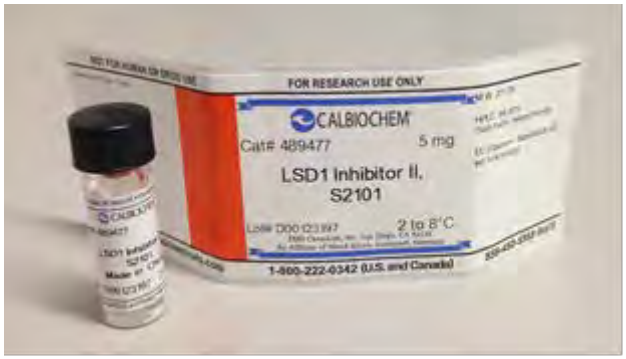
Post-translational modifications (PTMs) of histones are major sources of epigenetic information for the regulation of eukaryotic gene expression. Epigenetics-regulating compounds are potential drug candidates, since aberrant epigenetic modifications are often linked with cancer- or disease-related cells. We have been performing structural analyses of enzymes and

factors that are either substantially or potentially involved in the regulation of epigenetics. The histone demethylase LSD1/KDM1A is a prime example of these drug target proteins, since overexpression of LSD1 are often associated with cancerous cells, and LSD1 inhibition restores the expression of abnormally silenced genes in such cells. Based on the structural comparison between LSD1 and monoamine oxidase-B, both bound with a non-specific inhibitor 2-PCPA, we developed cell-permeable, LSD1-specific inhibitors such as S2101 (refs 1-2). I will talk about our structure-guided development of LSD1 inhibitors, and studies identifying biological roles of the histone demethylase activity, through the utilization of developed compounds as chemical probes.

I will also introduce our synthetic biology to reconstitute 'epi-nucleosomes' with specific PTMs at designed residues (ref 3). These epigenetics research tools, along with structurally designed chemical probes, can be applied for a variety of chromatin biology and drug discovery.

References

1) Mimasu S et al., Structurally designed trans-2-phenylcyclopropylamine derivatives potently inhibit histone demethylase LSD1/KDM1. Biochemistry 49, 6494 (2010).
2) Hino S et al., FAD-dependent lysine-specific demethylase-1 regulates cellular energy expenditure. Nature Commun. 3, 758 (2012).
3) Yanagisawa T et al., Multiple site-specific installations of Nε-monomethyl-L-lysine into histone proteins by cell-based and cell-free protein synthesis. Chembiochem 15, 1830 (2014).



(Figure) Structurally designed inhibitor for histone demethylase LSD1

Karolinska Institutet infrastructure in protein structure and drug discovery

Karin Dahlman-Wright

Karolinska Institutet

Karin Dahlman-Wright is professor in Molecular Endocrinology and since 2015 Karolinska Institutet vice dean for infrastructure. Between 2009 and Aug 2015, she held the position as head of department of Biosciences and Nutrition. Between Sept 2013 and June 2015, she was appointed KI Scientific Director, SciLifeLab, with special responsibility for the national platforms and was part of the SciLifeLab operational management team. 1994 and 2000, she held different line and project management positions at Pharmacia and Upjohn. In 2001, she founded the BEA core facility, which provides advanced services within the area of genomics and bioinformatics.

Karin Dahlman-Wright is a member of the SciLifeLab Stockholm steering board and the joint steering committee for AstraZeneca SciLifeLab. She has acted as Chairman, vice Chairman and member of evaluation panels at the Swedish Research Council.

Selected publication

- Zhu, J., Zhao, C., Zhuang, T. Jonsson, P., Williams, C., Sinha, I., Strömblad, S. and Dahlman-Wright K (2015) RING finger protein 31 (RNF31) promotes p53 degradation in breast cancer cells. Oncogene, Jul 6. doi: 10.1038/onc.2015.260. [Epub ahead of print] PMID: 26148235
- Gao H, Mejhert N, Fretz JA, Arner E, Lorente-Cebrián S, Ehrlund A, Dahlman-Wright K, Gong X, Strömblad S, Douagi I, Laurencikiene J, Dahlman I, Daub CO, Rydén M, Horowitz MC, Arner P. (2014) Early B cell factor 1 regulates adipocyte morphology and lipolysis in white adipose tissue. Cell Metab. Jun 3;19(6):981-92. doi: 10.1016/j.cmet.2014.03.032. Epub 2014 May 22
- Zhao, C., Qiao, Y., Jonsson, P., Wang, J., Xu, L., Rouhi, P., Sinha, I., Cao, Y., Williams, C. and Dahlman-Wright K. (2014) Genome-wide profiling of AP-1-regulated transcription provides insights into the invasiveness of triple-negative breast cancer. Cancer Res. Jul 15;74(14):3983-94. doi: 10.1158/0008-5472.CAN-13-3396. Epub 2014 May 15. (2014)
- Zhu, J., Zhao, C., Kharman-Biz, A., Zhuang, T., Jonsson, P., Williams, C., Qiao, Y., Zendehdel,

K., Strömblad, S., Treuter, E and Dahlman-Wright K. (2014) The Atypical Ubiquitin Ligase RNF31 Stabilizes Estrogen Receptor α and Facilitates Estrogen-dependent Breast Cancer Cell Poliferation. Oncogene. Aug 21;33(34):4340-51. doi: 10.1038/onc.2013.573. Epub 2014 Jan 20.

Abstract

In this presentation, I will give an overview of KI infrastructure in Protein Structure and Drug Discovery including relation to Swedish national efforts.

NMR facility and protein RNA projects

Toshio Yamazaki

RIKEN CLST

Toshio Yamazaki is a senior scientist and deputy director of NMR facility in the Division of Structural and Synthetic Biology in RIKEN Center for Life Science Technologies (CLST). He received a Ph.D. in chemistry from Osaka University in 1992, and carried out postdoctoral research in the area of NMR spectroscopy under Prof. Lewis Kay at University of Toronto until 1995. Dr. Yamazaki conducted protein NMR research as an assistant professor in Protein Research Institute of Osaka University, and then, as an associate professor. His research focuses on solution and solid-state NMR for biological targets. He moved to RIKEN in 2001, and is now especially interested in development of NMR methods with the use of photoisomerization and high magnetic field.

Selected publication

- Hashi, K. et al. Achievement of 1020 MHz NMR. J. Magn. Reson., 256, 30-33 (2015) Ohyama, T. et al. Structure of Musashi1 in a complex with target RNA: the role of aromatic stacking interactions. Nucleic Acids Res., 3218-31 (2012)

Abstract

In my talk, I will introduce the NMR facility as the platform for nation-wide researches in various fields. We are running more than 10 high field NMR spectrometers, and 30% of the machine time is shared with outer researchers. My group is focusing on the moving molecules by solution NMR and membrane proteins by solid-state NMR.

Recently we developed a light-irradiation NMR tube for efficient and uniform light irradiation to solution. By linking photo-isomerizing azobenzene to biomolecules of interest, we succeeded in obtaining a structure-correlation spectra, which interconnect signals from unfolded structure and those from the native structure. During data collection, repeated bidirectional photoisomerization takes place by UV and blue light synchronously illuminated by

NMR pulse sequence for more than 10000 times. This technique will be used for in-situ observation of functioning biomolecules and also for study of folding path of proteins.

High resolution solid-state NMR is becoming an important tool for structural and functional analysis of membrane proteins, because we can observe proteins in lipid bilayer. Triple resonance NMR experiments for uniformly 13C, 15N labeled proteins are universal way to get assignment of NMR signals to specific atoms in the molecule. We will present a current stage of study on Aquaporin Z (AqpZ), which is water channel of bacteria. Since AqpZ is consists of 231 amino acids, many of signals are overlapped even in 3-dimensional NMR spectra. We can achieve partial assignment for backbone atoms. The obtained chemical shifts are now used for local structure evaluation and also used for determination of ligand/drug binding site. Many structures for this protein are reported by crystallographic methods. Now we can tell that which structure is adopted in lipid bilayer environment. We strongly expect that NMR magnet with higher field is developed. Proton detection method using ultrafast sample spinning combined with higher field will make solid-state NMR more powerful, and solid-state NMR will be an easier and universal tool for biomolecular study.

Analysis of drug selectivity profile in their relevant cellular contexts by CETSA-MS

Janne Lehtiö

Karolinska Institutet

Professor Janne Lehtiö is director of the Clinical Proteomics Mass Spectrometry platform at Science for Life Laboratory and leads the cancer proteomics research group at Department of Oncology-Pathology, Karolinska Institutet. He received a MSc in biochemistry from the University of Helsinki, Finland in 1997, and PhD in Biotechnology at Royal Institute of Technology, Stockholm, Sweden. After PhD studies, he carried out research few years in biotech industry in California, USA and Scandinavia. At 2004, Dr. Lehtiö was appointed as director of the Karolinska University Hospital's clinical proteomics facility and since 2010 he is platform director of the clinical proteomics mass spectrometry facility at the national research center Science for Life Laboratory. In 2009, Lehtiö was granted an Associate Professorship in Proteomics at Karolinska Institutet, Sweden and in 2015 he received a faculty professor position in Medical Proteomics at Karolinska Institutet. His research focuses on mass spectrometry based proteome analysis including methods development and cancer proteomics.

Selected publication

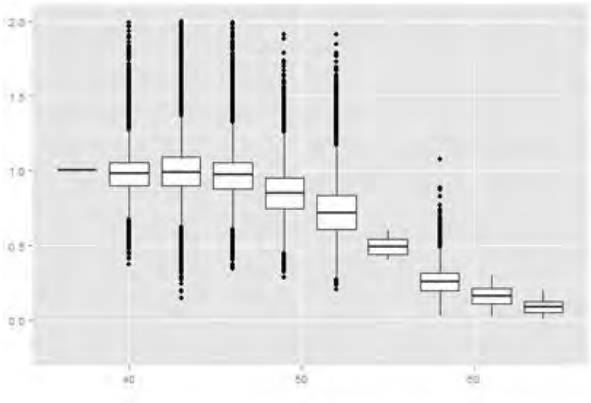
- Boekel J, Chilton JM, Cooke IR, Horvatovich PL, Jagtap PD, Käll L, Lehtiö J, Lukasse P, Moerland, Griffin TJ. Multi-omic data analysis using Galaxy. Communication, Nature Biotechnol. 2015 Feb 6;33(2):137-9.
- Zhu Y., Hultin-Rosenberg L., Forshed J., Branca R.M., Orre L.M., Lehtiö J., SpliceVista, a tool for splice variant identification and visualization in shotgun proteomics data. Mol Cell. Prot. 2014 Jun;13(6):1552-62.
- Branca R.M., Orre L-M., Johansson H.J., Granholm V., Huss M., Pérez-Bercoff Å., Forshed J., Käll L., Lehtiö J. HiRIEF LC-MS enables deep proteome coverage and unbiased proteogenomics. Nature Methods, 2014 Jan;11(1):59-62.
- Johansson H.J, Sanchez B.C., Mundt F., Forshed J., Lundgren B., Martens U., Kovacs A., Máthé G., Yakhini Z., Helou K., Einbeigi Z., Krawiec K., Kanter L., Hjerpe A., Stål O., Linderholm B.K., Lehtiö J. Retinoic acid receptor alpha has potential predictive value in tamoxifen treated breast cancer patients. Nature Commun. 2013. Jul 19;4:2175.

Abstract

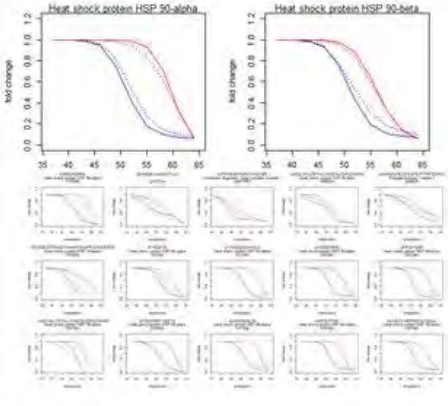
Systems biology and omics approaches have presented a number of potential druggable targets and the improved chemical biology workflows generate candidate compounds. Further, phenotypic screening is making a remarkable comeback in the drug discovery workflow due to the fact that target-based approaches have not yet been translated into greater number of approved medicines. These developments create a great demand for methods and platforms both to determine protein targets of small-molecule compounds and to perform target engagement analysis to confirm that the small molecule interacts with its intended protein target in a relevant biological system. Chemical proteomics methods are powerful mass spectrometry based approaches for identifying proteome-wide compound-target interactions.

We have in our group developed number of methods to increase proteome coverage of the analysis of complex proteomes, to improve the quantitative accuracy in proteomics and to refine the mass spectrometry (MS) data-analysis tools to allow a variant specific proteome view. A key technology to increase the information content of MS-based proteomics is the use of peptide pre-fractionation by high resolution isoelectic focusing (HiRIEF) developed at Lehtiö laboratory1. Here we describe the combination of these proteomics tools with lysate and cellular thermal shift assay to study the selectivity profiles of drugs. The HiRIEF LC-MS-CETSA method was tested to study selectivity of HSP90 inhibitors in melanoma. We quantified thermal melting curves of more than 8200 proteins in the studied melanoma cell lines with baseline and supplemented with HSP90i. Clear target binding was observed on HSP90 alpha and beta and several other interactors were discovered.

Proteome view:
"Meltome" of melanoma a cell line



Target view: Thermal shift of HSP90α and β, a protein (upper panel) and peptide centric view HSP90α (lower panel)



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1) Branca R.M. et. al., HiRIEF LC-MS enables deep proteome coverage and unbiased proteogenomics. Nature Methods, 2014 Jan;11(1):59-62.

Two steps forward, one step back : Successes and Failures in Structure-based Discovery of GPCR ligands

Jens Carlsson

Stockholm University

Jens Carlsson completed a PhD in computational chemistry at Uppsala University in 2008 under the supervision of prof. Johan Åqvist. His postdoctoral research was carried out in prof. Brian Shoichet's group at the University of California in San Francisco and focused on structure-based ligand discovery. In 2012, he established an independent research group at Stockholm University. In July 2015, his group moved to the dept. of Organic Pharmaceutical Chemistry and the Science for Life Laboratory at Uppsala University.

The goal of Jens' research is to improve understanding of receptor-ligand interactions at the atomic level using computer models. Using structure-based methods such as molecular dynamics simulations and molecular docking, his group focuses on prediction of how small molecules interact with G protein-coupled receptors and modulate their function, often in close collaboration with experimental groups.

Selected publication

- Rodríguez D, Gao ZG, Moss SM, Jacobson KA, and Carlsson J Molecular docking screening using agonist-bound GPCR structures: Probing the A_{2A} adenosine receptor. J Chem Inf Model, 55, 550-563 (2015).
- Rodríguez D, Brea J, Loza MI, and Carlsson J Structure-based discovery of selective serotonin 5-HT_{1B} receptor ligands. Structure, 8, 1140–1151 (2014).
- Chen D, Ranganathan A, Ijzerman AP, Siegal G, Carlsson J Complementarity between in silico and biophysical screening approaches in fragment-based lead discovery against the A_{2A} adenosine receptor. J Chem Inf Model, 53, 2701-2714 (2013).
- Carlsson J, Coleman RG, SetolaV, Irwin JJ, Fan H, Schlessinger A, Sali A, Roth BL, and Shoichet, BK (2011) Ligand discovery from a Dopamine D_3 receptor homology model and crystal Structure. Nat Chem Biol, 7, 769-778 (2011).
- Carlsson J, Yoo L, Gao GZ, Irwin JI, Shoichet BK, and Jacobson KA Structure-based discovery of A_{2A} adenosine receptor ligands. J Med Chem, 53,3748-3755 (2010).

Abstract

G protein–coupled receptors (GPCRs) are intensely studied as drug targets and for their role in signaling. High-resolution crystal structures of GPCRs capturing different receptor conformations are now available, which have provided insights into the mechanism of activation and ligand selectivity for this important class of drug targets.

I will present a series of structure-based screens for novel ligands of the A2A adenosine receptor (A2AAR), which is a drug target for Parkinson's disease (antagonists) and ischemia (agonists). As crystal structures for both inactive- and active-like receptor conformations of the A2AAR have now been determined, molecular docking screens for novel ligands can be performed. Virtual screens against different conformations of the A2AAR were carried out to investigate if structure-based methods can be used to identify agonists and antagonists. Our results shed light on the importance of access to crystal structures and the role of the chemical library in screens for ligands with specific pharmacological properties.

For most GPCRs, no experimental coordinates are available and structure-based screens are forced to rely on homology models. However, it is still unclear if models of GPCRs are sufficiently accurate to be used in ligand discovery. The determination of crystal structures for dopamine and serotonin receptors, and the challenges to the community to blindly predict these in the GPCR Dock competitions, have enabled us to carry out comparisons of ligand discovery from models versus crystal structures. Our results from these challenges reveal opportunities and limitations of the use of homology models in ligand discovery and design of selective lead candidates.

Optimization of X-ray complex structures and binding affinity prediction by FMO

Teruki Honma

RIKEN CLST

Teruki Honma is a Team Leader in the Division of Structural and Synthetic Biology in RIKEN Center for Life Science Technologies (CLST). After graduation of graduate school of Hokkaido university, he joined Banyu Pharma (Merck) in 1993. He received a Ph.D. in chemistry from Hokkaido University in 2001. He moved to Pfizer in 2004 as a leader of computational chemistry group. After closing of Pfizer research institute in Japan, he moved to RIKEN and was appointed as a team leader in 2008. He has been participating in drug discovery projects such as MEXT targeted protein program, AMED platform of drug discovery, AMED next cancer program and RIKEN drug discovery program by developing new in silico screening technologies.

Selected publication

- Okada-Iwabu M, Honma T et al., A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature, 503, 493-499 (2013).
- Saito Y, Honma T et al., A pyrrolo-pyrimidine derivative targets human primary AML stem cells in vivo. Sci Transl Med, 5(181), 181ra152 (2013).
- Takaya D, Honma T et al., Prediction of Ligand-Induced Structural Polymorphism of Receptor Interaction Sites Using Machine Learning. J Chem Inf Model, 53 (3), 704–716 (2013).
- Yuki H, Honma T et al., Prediction of sites of metabolism in a substrate molecule, instanced by carbamazepine oxidation by CYP3A4. Bioorg Med Chem, 20(2), 775-83 (2011).
- Sato T, Honma T et al., Combining Machine Learning and Pharmacophore-based Interaction Fingerprint for in silico Screening. J Chem Inf Model, 50(1), 170-85 (2010).

Abstract

Quantitative prediction of binding affinity based on X-ray structures or docking models has been the most important issue in structure-based drug design. Since development of the first docking program DOCK in 1982, many approaches based on various modeling theories such as molecular mechanics, statistics of PDB, molecular dynamic simulation, quantum mechanics and Jarzynski equation have been proposed. However, one almighty prediction method has not yet developed because it is difficult to accurately consider all kinds of enthalpy and entropy energy terms, dynamics of all players in water solution at the same time.

Currently, to quantitatively predict binding affinity, MM-PBSA or MD-based alchemical methods are frequently used. One of the problems of those MM/MD-based methods is insufficient accuracy of MM (molecular mechanics) to describe intermolecular interactions. On the other hand, in 2003, Dr. Kitaura et al developed new efficient calculation method of QM (quantum mechanics) called FMO (fragment molecular orbital) method. Using FMO, we can calculate a quantum mechanics calculation of a protein system with dramatically shorter computation time. Now we have been developing a new affinity prediction method using FMO together with PBSA term called FMO-PBSA1). The preliminary results using Pim1 and Estrogen receptor cases will be presented.

References

1) Fukuzawa K, Honma T *et al.,* Activity cliff prediction of Pim1 inhibitors using FMO method together with solvent term PB/SA, **in preparation**.

Tuning the allosteric modulation of ion channels through computational design

Erik Lindahl

Stockholm University

Dr. Erik Lindahl is Professor of Theoretical Chemistry at Stockholm University, with a co-appointment as Professor of Theoretical Biophysics at the Royal Institute of Technology. He received a PhD at the Royal Institute of Technology in 2001, after which he performed research work at Groningen University, Netherlands; Stanford University, USA, and the Pasteur Institute, France. He joined the faculty of Stockholm University in 2004, and was elected to the Young Academy of Sweden in 2011. The lab's research is focused on understanding structure and function in complex biological systems, in particular membrane proteins such as ion channels, through both computational and experimental methods.

Selected publication

- Johansson, A., and Lindahl, E., *Protein contents in biological membranes can explain abnormal solvation of charged and polar residues,* Proc. Natl. Acad. Sci., **106,** 15684-89 (2009)
- Murail, S., Wallner, B. Trudell, J.R, Bertaccini, E., Lindahl E., *Microsecond Simulations Indicate that Ethanol Binds between Subunits and Could Stabilize an Open-State Model of a Glycine Receptor*, Biophys J **100**, 1642-50 (2011)
- Henrion, U., Renhorn, J., Börjesson, S.I., Nelson, E.M., Schwaiger, C.S., Bjelkmar, P., Wallner, B., Lindahl, E.†, Elinder, F†, *Tracking a complete voltage-sensor cycle with metal-ion bridges*, Proc. Natl. Acad. Sci. U S A **109**, 8552-7 (2012)
- Pronk, S., Pall, S., Schulz, R., Larsson, P., Bjelkmar, P., Apostolov, R., Shirts, M.R., Smith, J.C, Kasson, P, van der Spoel, D., Hess, B., Lindahl, E., *GROMACS 4.5: A high-throughput and highly parallel open source molecular simulation toolkit,* Bioinformatics **29(7)**, 845-854 (2013)
- Nyblom, M., Poulsen, H., Gourdon, P., Reinhard, L., Andersson, M., Lindahl, E., Fedosova, N., Nissen, P., *Crystal structure of Na^+,K^+-ATPase in the NA^+-bound state*, Science **342**, 123-127 (2013)

Abstract

Ligand-gated ion channels exhibit a wide range of responses, and the very same allosteric modulator can either inhibit or potentiate channels depending on single-residue mutations. I will present our work on deciphering this complex modulation with a combination of computational modeling, simulations, free energy calculations as well as electrophysiology experiments. We have been able to show that ligand-gated ion channels likely have two separate modulatory sites – in addition to the primary agonist-binding site, and that one of these leads to channel inhibition while the second one is responsible for potentiation. By using computational design, we can design mutations that alter these properties in model channels such as GLIC (Fig. 1), and selectively target allosteric modulators to one site or another. This is one of the first examples where we can use computations not only to screen and optimize binding in a specific site, but target the efficacy of the drugs on a biological process. This leads to fascinating insights about conformational selectivity and dynamics in complex receptors, and it has potential implications for drug design particularly in anesthesia and addiction disease.

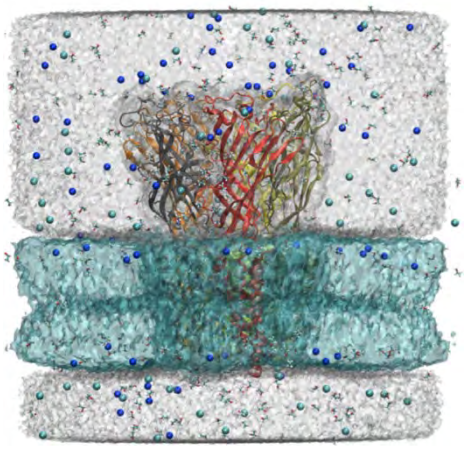

Fig.1: The transmembrane and extracellular domain of the GLIC ion channel embedded in a lipid bilayer. Agonist binding or pH changes alter the structure of the extracellular domain, which leads to a conformational wave that forces the transmembrane domain to rotate and open the ion pore. Similar ligand-gated channels are responsible for the post-synaptic signal transmission in all our cells, which makes them highly important targets for modern neuropharmacology.

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Structural biology on membrane proteins and X-ray free electron laser

So Iwata

RIKEN SPring-8 Center

So Iwata was awarded a PhD at University of Tokyo in 1991. After staying Germany as a postdoctoral fellow, he joined Uppsala University to serve as a lecture. He moved to Imperial College London in 2000 as the Chair of Membrane Protein Crystallography. He also held position as a Diamond Fellow at Diamond Light Source. Since 2007, he has undertaken a position of Professor at Graduate School of Medicine, Kyoto University. Since 2012, he also holds positions of Group Director at SACLA Science Research Group, RIKEN SPring-8 Center. His current research includes structural biology of membrane proteins and free electron laser.

Selected publication

- Nomura, N. *et al.,* Structure and mechanism of the mammalian fructose transporter GLUT5. *Nature.*
- Manolaridis, I. *et al.,* Mechanism of farnesylated CAAX protein processing by the intramembrane protease Rce1. *Nature.* 504(7479):301-305(2013)
- Lee, C. *et al.,* A two-domain elevator mechanism for sodium/proton antiport. *Nature.* 501(7468); 573-577 (2013)
- Hu, N-Jen. *et al.,* Crystal structure of a bacterial homologue of the bile acid sodium symporter ASBT. *Nature* 478(7369); 408-411 (2011)
- Shimamura, T. *et al.*, Structure of the human histamine H_1 receptor complex with doxepin. *Nature* 475(7354); 65-70 (2011)

Abstract

Radiation damage of crystals is one of the most hampering problems in the current macromolecular crystallography. The X-ray beams at the latest beamlines, including microfocus beamlines, are so intense that the crystals suffer serious radiation damage during even very-short exposure-time. The problem is particularly severe for crystals of membrane proteins or macromolecular assemblies, which are extremely radiation sensitive. X-ray Free Electron Laser (XFEL) could provide a solution to this problem. Very high dose rates delivered by the intense femtosecond pulses of XFELs reduce the amount of damage suffered by a crystal during its irradiation. Single shot diffraction patterns are collected from a series of small crystals and by combining them, we could swiftly complete the dataset without any serious radiation damage. At the Japanese XFEL facility, SACLA, we are currently developing a data collection system focusing on drug-target protein crystals including those from membrane proteins and flexible multi-modular proteins. The system is composed of a diffraction chamber with a sample injector and a fast readout multiport CCD (mpCCD) detector. The sample injector is optimized for the data collection from crystals in the lipidic cubic phase (LCP), which are common for membrane proteins. The injector is also capable to handle the crystals obtained from solutions by making the solution viscous using additives including gels and grease. The system requires only several 100 micrograms of proteins to complete the dataset. The system can dramatically accelerate the structure determination of membrane proteins. In my talk, I will present the recent result on phasing and pump-probe experiments using our SFX setup.

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Establishing the molecular mechanisms of solute-carrier (SLC) transporters: from snapshots to movies

David Drew

Stockholm University

David Drew is a current EMBO Young Investigator and holds a Wallenberg Academy Fellowship at the Centre for Biomembrane Research at the Department of Biochemistry and Biophysics at Stockholm University. He received a Ph.D. in biophysics and biochemistry from Stockholm University in 2005, and thereafter carried out an EMBO postdoctoral fellowship in the area of X-ray Crystallography under Prof. So Iwata at Imperial College London. From 2009 Dr. Drew started as a University Royal Society Fellow at Imperial College London. From 2012 he obtained his current position at Stockholm University. He aims to understand how solute carrier (SLC) transporters function at the atomic level, as their dysfunction is associated with human diseases, such as cancer and cardiovascular heart disease. These goals are facilitated by the development of novel methods in membrane protein overexpression, purification and crystallization.

Selected publication

- Nomura N et al. Structure and mechanism of the mammalian fructose transporter GLUT5 Nature. (2015). in press
- Lee C et al. A two-domain elevator mechanism for sodium/proton antiport. Nature. (2013) 501(7468):573-7.
- Hu NJ, Iwata S, Cameron AD, Drew D. Crystal structure of a bacterial homologue of the bile acid sodium symporter ASBT. Nature. (2011). 478:408-11.
- Newstead S, Kim H, von Heijne G, Iwata S, Drew D. High-throughput fluorescent-based optimization of eukaryotic membrane protein overexpression and purification in S. cerevisiae. Proc Natl Acad Sci U S A. (2007).104(35):13936-41.
- Drew D, Lerch M, Slotboom D, Kunji, E, de Gier JW. Optimizing membrane protein overexpression and purification using GFP-fusions. Nature Methods. (2006). (4):303-313.

Abstract

Crystal structures of small-molecule transporters have shed light on the conformational changes that take place during structural isomerization from outward to inward-facing states. Rather than a simple “rocking” movement of two bundles around a central substrate-binding site, it has become clear that even the most simplistic transporters utilize non rigid-body rearrangements. Here I will present two examples of SLC transporters and their homologues that revel novel refinements to the basic alternating access model currently shown in most textbooks.

References

1) Nomura N et al. Structure and mechanism of the mammalian fructose transporter GLUT5. Nature. (2015). in press
2) Lee C et al. A two-domain elevator mechanism for sodium/proton antiport. Nature. (2013) 501(7468):573-7.

Elucidate functional structure of membrane proteins in lipid environment by electron cryo-microscopy single particle analysis

Hideki Shigematsu

RIKEN CLST

Hideki Shigematsu is a Senior Scientist in the Protein Functional and Structural Biology Team in the Division of Structural and Synthetic Biology in RIKEN Center for Life Science Technologies (CLST). He received a Ph.D. in Biotechnology from Tokyo Institute of Technology in 1999. His research interest has been in the function and structure relationship of membrane proteins since he started postdoctoral research at Pharmaceutical Research Laboratory, Kirin-Brewery Co., Ltd in 2000. And he continued the research as an assistant professor in Tokyo Institute of Technology, a research scientist at National Institute for Physiological Sciences and an associate research scientist and manager of cryo-EM facility at Yale University School of Medicine. He started working with current title since September 2014 for projects in structural biology with cryo-EM in general in collaborations with other researchers in RIKEN CLST and also in unique technique for membrane proteins in lipid environment.

Selected publication

- Shigematsu, H. *et al.* Structural Characterization of the Mechanosensitive Channel Candidate MCA2 from Arabidopsis thaliana. *PLoS ONE*, *9*(1), e87724 (2014).
- Taylor, D. W., Ma, E., Shigematsu, H., *et al.* Substrate-specific structural rearrangements of human Dicer. *Nature Structural & Molecular Biology*, 20(6), 662–670 (2013).
- Shigematsu, H. and Sigworth, F. J. Noise models and cryo-EM drift correction with a direct-electron camera. *Ultramicroscopy*, *131C*, 61–69 (2013).
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Abstract

The structural study of membrane protein is getting more and more popular as for the importance in its function especially for drug discovery. Recent progress in studies of structure and function relationship of membrane proteins is based on the near atomic structures obtained by X-ray crystallography. Recently, cryo-EM single particle reconstruction of membrane proteins has been reported in such a resolution with the benefit of direct electron detector and high performance computation[1,2]. Even though those are useful to understand the structure and function relationship, it would be important to remind that those are in detergent solubilized state. The final goal of structural study of membrane protein is to utilize the membrane proteins in lipid environment.

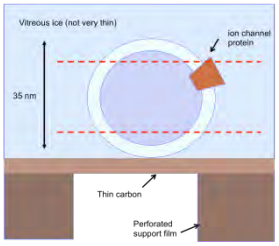

Fig. 1 Schematic illustration of vertical cross-section of specimen.

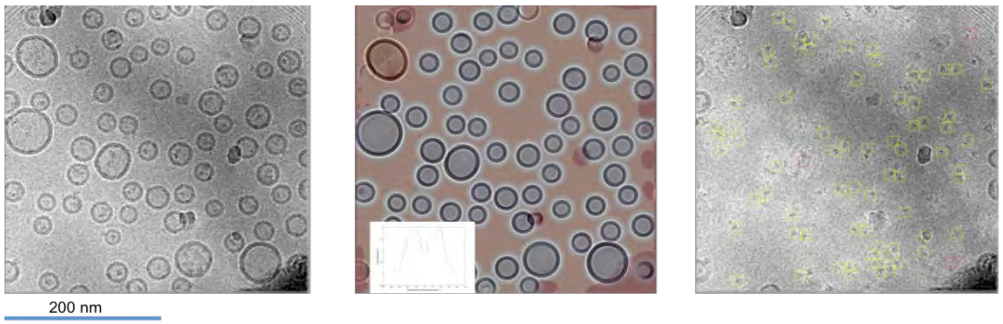

Fig. 2 Image processing pipeline for RSC reconstruction. The density of vesicles are found and computed to subtract from the micrograph to pick particles.

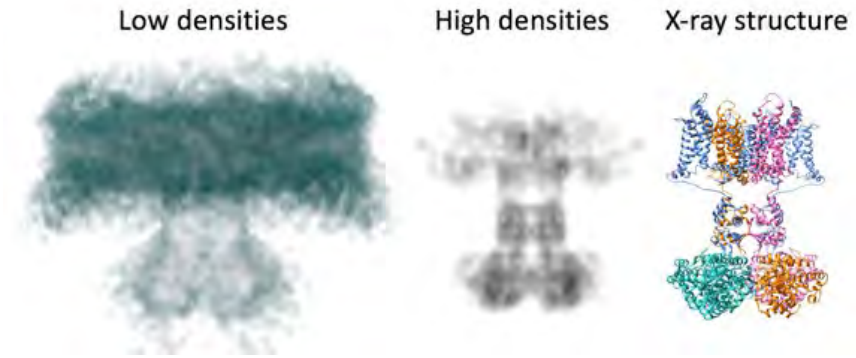

Fig. 3 The resulting 3D map from RSC reconstruction displayed in the low-density range (primarily lipid) and high-density range (protein). The X-ray structure (PDB: 2A79) is shown for comparison. The map was computed from 7000 particles.

References

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3) Wang, L., and Sigworth, F.J. Structure of the BK potassium channel in a lipid membrane from electron cryomicroscopy. Nature *461*, 292–295(2009).

CETSA as a new strategy to understand efficacy, adverse effects and resistance development of anticancer drugs

Pär Nordlund

Karolinska Institutet

Pär Nordlund is a structural biologist now working in the area of molecular and cellular cancer research. At the Karolinska Institute he funded the Swedish node of the Structural Genomics Consortia that solved 140 human protein structures during the period 2005-2010. His groups have developed expertise in membrane proteins and did in 2007 solve the first structure of a human integral membrane protein. He is a member of the Nobel Assembly and the Swedish Academy of Science, as well as a reviewing editor for Science Magazine. He is the co-funder of the biotech companies Sprint Bioscience, Pelago Bioscience and Evitra Proteoma.

Abstract

A key step of the action of most drugs is their binding (engagement) of the target protein(s). However, limitations in the available methods for directly accessing this critical step have added uncertainties in many stages of drug development.

We have developed a generic method for evaluating drug binding to target proteins in cells and tissues (Martinez Molina, 2013). The technique is based on the physical phenomenon of ligand-induced thermal stabilization of target proteins; the method is therefore called the cellular thermal shift assay (CETSA). The technique allows for the first time to directly measure the biophysical interactions between a drug and protein target in non- engineered cells and tissues. We show that using CETSA a range of critical factors for drug development can be addressed at the target engagement level, including drug transport and activation, off-target effects, drug resistance as well as drug distribution in cells, patient and animal tissues. Using quantitative mass-spectrometry, proteome-wide CETSA has been established which allows for off-target effects as well as downstream biochemistry to be discovered (Savitsk, 2014). Together the data supports that CETSA is likely to become a valuable novel tool for biomedical research in the future.

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■ Organizers of the symposium

RIKEN

RIKEN is Japan's largest research institute for basic and applied research. Over 2500 papers by RIKEN researchers are published every year in leading scientific and technology journals covering a broad spectrum of disciplines including physics, chemistry, biology, engineering, and medical science. RIKEN's research environment and strong emphasis on interdisciplinary collaboration and globalization has earned a worldwide reputation for scientific excellence.
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About the Center for Life Science Technologies (CLST)

The RIKEN Center for Life Science Technologies aims at the development of key technologies for breakthroughs in medical and pharmaceutical applications by conducting ground-breaking research and development programs for next-generation life sciences. CLST comprises the Division of Structural and Synthetic Biology, the Division of Genomic Technologies, and the Division of Bio-function Dynamics Imaging, which will work together in this endeavor. Research and development programs are carried out in collaboration with companies, universities, and international consortia, in order to disseminate the center's achievements to the global community.
Website: www.clst.riken.jp/en/index.html

Karolinska Institutet

Karolinska Institutet is one of the world's leading medical universities. Our mission is to contribute to the improvement of human health through research and education.

Karolinska Institutet accounts for over 40 per cent of the medical academic research conducted in Sweden and offers the country´s broadest range of education in medicine and health sciences. Since 1901 the Nobel Assembly at Karolinska Institutet has selected the Nobel laureates in Physiology or Medicine.

Karolinska Institutet was founded by King Karl XIII in 1810 as an "academy for the training of skilled army surgeons". Today, Karolinska Institutet is a modern medical university and one of the foremost in the world.
With our close relationship to the clinical milieu, a well established infrastructure and a stable financial situation, Karolinska Institutet has excellent prerequisites for sustaining high quality research and education.
Website: http://ki.se

SciLifeLab

SciLifeLab, Science for Life Laboratory, is a Swedish national center for molecular biosciences, with the mission to develop, use and provide advanced technologies for applications in health and environmental research. The center was established in 2010 and became a national resource in 2013, making technologies and expertise available to researchers in all of Sweden and beyond.

Today the center comprises more than 1 200 researchers and personnel.
We offer a cross-disciplinary research setting that interacts with healthcare, authorities and industry to meet the need for new clinical methods and a better environment. In addition, SciLifeLab provides education for students and researchers at all levels.

SciLifeLab is hosted by four universities; Karolinska Institutet, KTH Royal Institute of Technology, Stockholm University and Uppsala University. The infrastructure is mainly located in Stockholm and Uppsala but we also offer services at other Swedish universities.

Website: http://www.scilifelab.se/

RIKEN

centers and facilities across Japan and around the world

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Center for Advanced Photonics

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Brain Science Institute
Nishina Center for Accelerator-Based Science
Radioactive Isotope Beam Factory
Advanced Center for Computing and Communication
Cluster for Industry Partnerships
Chief Scientist Laboratories
Associate Chief Scientist Laboratories
Distinguished Senior Scientist Laboratories
Initiative Research Units
Special Research Units
Research Groups
Global Research Cluster

Yokohama
Center for Sustainable Resource Science
Center for Integrative Medical Sciences
Center for Life Science Technologies

Nagoya

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HPCI Program for Computational Life Sciences
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RIKEN-SJTU Joint Research Center (China)
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NCTU-RIKEN Joint Research Laboratory (Taiwan)
USM-RIKEN International Center for Aging Science (Malaysia)
RIKEN-NCBS Joint Research Center (India)
RIKEN-JNCASR-IISc Joint Research Center (India)
RIKEN-KRIBB Joint Research Center (South Korea)

RIKEN Center for Life Science Technologies Researchers at the Division of Structural and Synthetic Biology in Yokohama are advancing technologies for the fundamental structural analysis of macromolecular complexes using electron cryomicroscopy (left), x-ray crystallography and nuclear magnetic resonance spectroscopy.

About RIKEN

RIKEN is Japan's largest and most comprehensive research organization for basic and applied science and a world leader in a diverse array of scientific disciplines.

For nearly a century since its foundation in 1917, RIKEN has fostered pioneering, innovative research in fields spanning the entire range of the natural sciences, from developmental biology and neuroscience to quantum physics and computer science.

Today, RIKEN encompasses a network of world-class research centers across Japan, with main campuses in Wako, Tsukuba, Yokohama, Kobe and Harima offering state-of-the-art facilities that rank among the best in the world. This high-quality, high-performance research environment, combined with a uniquely bottom-up approach to scientific innovation, has enabled RIKEN to foster an environment in which researchers are able to thrive.

About the Center for Life Science Technologies (CLST)

The RIKEN Center for Life Science Technologies was founded in 2013, aiming at the development of key technologies for breakthroughs in medical and pharmaceutical applications by conducting ground-breaking research and development programs for next-generation life sciences. CLST comprises the Division of Structural and Synthetic Biology, the Division of Genomic Technologies, and the Division of Bio-Function Dynamics Imaging, which will work together in this endeavor. Research and development programs are carried out in collaboration with companies, universities, and international consortia, in order to disseminate the center's achievements to the global community.

Life Science Technology Platform

RIKEN Center for Life Science Technologies has a rich set of advanced facilities used for research in medicine and other areas of life sciences.

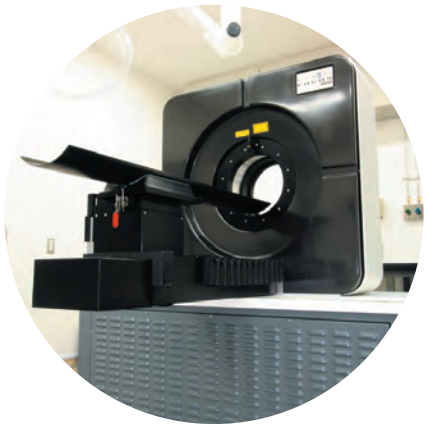
The NMR facility in Yokohama—one of the world's largest—operates ten nuclear magnetic resonance spectrometers, which are used for three-dimensional structural analysis of proteins and other molecules. In addition to medicine, these tools are being used to promote technological innovation.

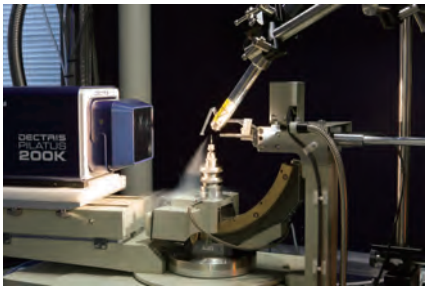
The Genome Network Analysis Service (GeNAS), also in Yokohama, offers gene expression analysis and genomic sequencing using high-throughput next-generation sequencers.

And the molecular imaging facility in Kobe, equipped with microPET scanners and cyclotrons for producing PET scanner tracers, as well as MRI and CT facilities, provides human resource development program for analyzing the dynamics of various molecules in the body.

900 MHz NMR spectrometer


Next-generation sequencers


microPET scanner for animals



X-ray diffraction device


A robot to conduct comprehensive analysis of ncRNA


A three-tesla magnetic resonance imaging scanner

Support and services based on advanced technologies

CLST promotes innovation through collaborations with other sectors such as industries and universities by providing and transferring a broad platform including protein structure analysis, gene regulation network and drug disposition.

NMR Facility


NMR structure analysis pipeline with high-field NMR

GeNAS
(Genome Network Analysis Support)

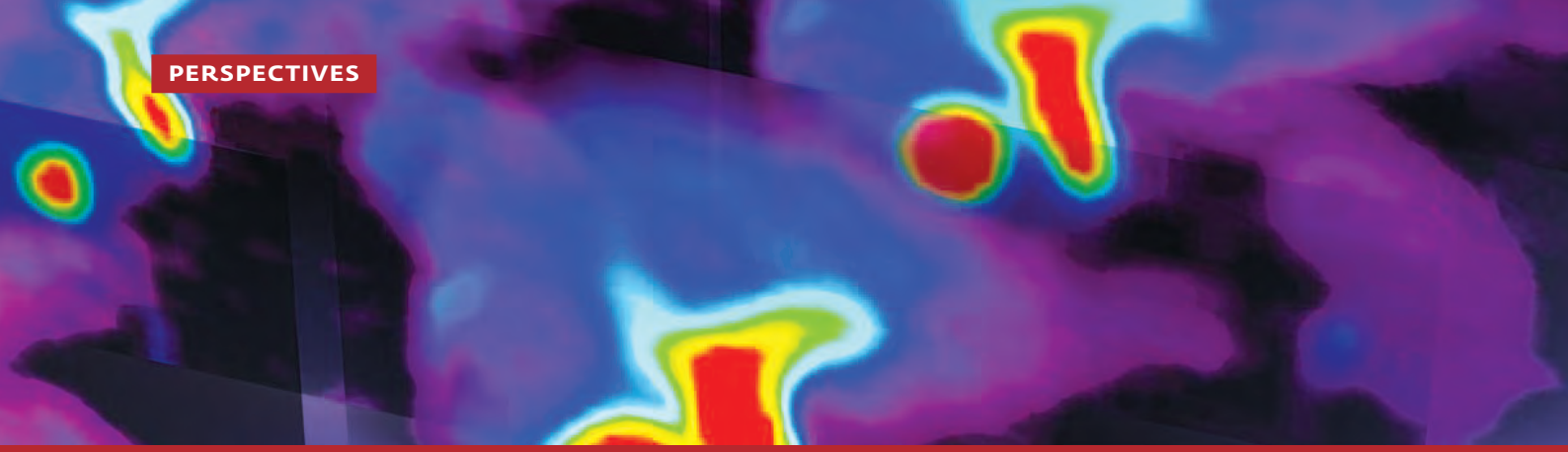

Next-generation sequencing services and training course

PET Academy


Training of graduate students and industry researchers

Technology transfer ⌄ ⌃ Collaboration

Research institutes | Universities | Companies | Hospitals



Drug discovery

Innovation in drug discovery technology

YASUYOSHI WATANABE
Director
RIKEN Center for Life Science Technologies

Technology has been a major driver of progress in drug discovery. However, further improvements are needed to bring about new advances in the medical and pharmaceutical sciences. Tools and technologies derived from synthetic biology, genomics, functional imaging and other disciplines promise a more dynamic and integrated approach to biomedical research and will make a new era of drug discovery possible.

Drug discovery in the life sciences has historically taken two broad approaches. In one approach, researchers consider the human body as a black box in which they evaluate potential therapies against observable symptoms, with little regard for the mechanism of drug activity or specific biological effects. Alternatively, researchers reduce the body to a series of genes and signal transduction pathways and then seek drugs that interact with these targets to selectively treat the deficit responsible for a disease with minimal adverse effects.

Yet both approaches are limited: the physiology-based approach is too top-down, and the target-based approach too bottom-up. Neither captures the complexity of biological systems and disease mechanisms while simultaneously taking into account the physiological consequences of modulating particular targets. Furthermore, these approaches are limited by the tendency of academic research institutes in the life sciences to focus on only one disease discipline, such as cancer, or one sub-field of biological investigation, for example genomics.

Drug-related research and development must therefore reach across traditional disciplines and levels of study, from basic research at the level of genes and proteins all the way up to the broadest conceptualization of disease progression across multiple systems and entire organisms.

Innovation at the heart of drug discovery

Technology and innovation have always been central to drug discovery. Hence, advancing the current suite of tools available to biomedical researchers will be essential to establishing the linkages necessary to speed up the discovery process.

In the early days, natural products derived from plants and microorganisms served as the predominant source of bioactive compounds and drug candidates. Advances in chemistry made it possible to synthesize large families of compounds with a high degree of structural diversity that could be screened to identify molecules with potential biological activity. With the dawn of the biotechnology industry and human genome sequencing, chemistry has given way, in part, to biology. By using molecular biology, genomics and computation, researchers can now design drugs with desired properties and create medicines by manipulating basic biological building blocks, such as proteins and nucleic acids.

Still, productivity in the drug industry has languished in recent years as the classic approaches have begun to reach the limits of their effectiveness. New and more powerful technologies are critical to tackling the persisting fundamental questions in the life sciences.

RIKEN is committed to the realization of innovation for science and improved human health, leading its efforts with an integrated, non-traditional approach. The recently established RIKEN Center for Life Science Technologies brings together systems and structural biologists, omics scientists, molecular imaging specialists and engineers to collaborate in the pursuit of technological foundations for all areas of medical and pharmaceutical science.

A new therapeutic paradigm

To date, most drugs have been designed to inhibit or activate particular molecules with a very narrow specificity. These conventionally designed drugs usually follow the ‘one drug–one

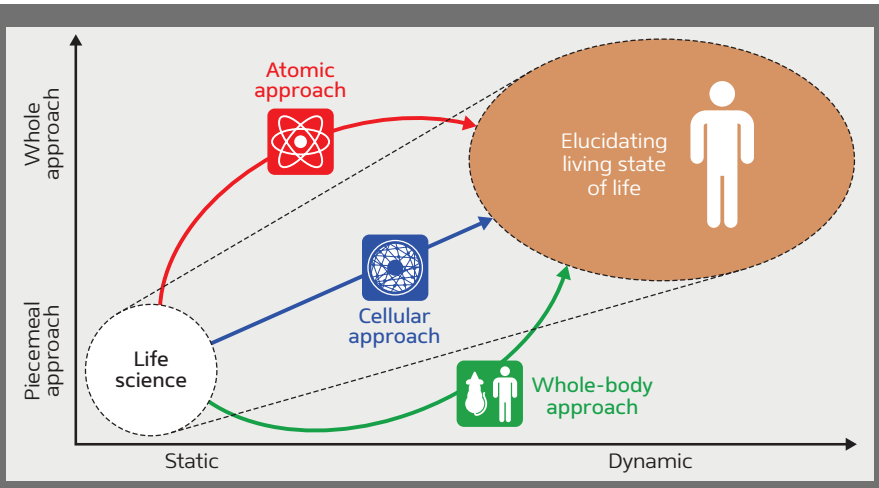


Figure 1: A 'live science' approach

New technologies that reveal whole-system dynamics will enable the next generation of drug discovery.

target-one disease' approach, which rarely captures the intricacies of true biology. Complex diseases have multiple 'causes' and drug discovery for such diseases requires a multi-target line of attack.

A new therapeutic paradigm is needed to effectively combat complex diseases—one that is based on 'live science'. Live science is a methodology that develops and harnesses technology to go beyond static snapshots to clarify the dynamic processes that must be understood in order to achieve higher sensitivity and specificity in drug discovery (Fig. 1).

Among other advancements, live science methodologies could help to realize medical 'regulatory molecules'. These molecules are drugs that rather than inhibiting or activating a single target would modulate an entire pathway implicated in a disease to yield the desired therapeutic benefits. Constructed with insights from structural and synthetic biology, regulatory molecules would be made using the same building blocks of conventional drugs. Ultimately, they would be cheap to manufacture, easy to deliver and have wide-reaching effects across several targets of interest.

The development of new technologies will be necessary to make efficient and economical regulatory molecules a reality. For instance, current projects at RIKEN and elsewhere are combining engineering principles with molecular biology to build networks of molecules that can be tweaked and perturbed to better understand whole systems.

Integration and innovation

Genomics also holds great promise for drug discovery in the near future. However, the insights gleaned from genome sequencing projects must also be connected to gene function. At RIKEN, teams are developing methods to decode the genomes of single cells to reveal biological heterogeneity at greater resolution in cancer cells, reprogrammed stem cells, neurons and other types of tissue.

RNA molecules in the cell that do not code for proteins—known as non-coding RNA—are another area to be explored. Also referred to as genomic 'dark matter' and only partially understood, non-coding RNA performs a regulatory role in many biological events and could explain the origin of complex disease traits such as obesity and heart disease. Efforts by large consortia, such as FANTOM (Functional Annotation of the Mammalian Genome) spearheaded by RIKEN, will continue to be instrumental in illuminating the non-coding RNA world and the roles it may play in complex diseases.

Improved drug discovery will also require advanced imaging modalities to better observe emergent symptoms and drug metabolism in real time at unprecedented resolution. Such modalities can be achieved both through novel innovations and by upgrading and combining existing approaches, such as positron emission tomography (PET), magnetic resonance imaging (MRI), optical imaging and electron microscopy. Work at RIKEN has created new labeling methods to visualize drug candidate molecules and track their efficacy, pharmacokinetics and pharmacodynamics in animal models and human study subjects including, for example, ways to follow drug absorption in the intestine and trace its subsequent distribution in tissue and ultimate excretion through the liver and related organs[1].

The future of drug discovery

Older models of research and development must be changed and refined, not only to enhance the process of drug discovery but also to reduce costs. Bringing a new drug to market from scratch typically takes 10 to 15 years and costs more than 100 billion yen. A fusion of techniques and the technology to support these new methods will drive the drug discovery field forward, thus reducing direct costs and increasing the overall efficiency of therapies.

Integrating disparate disciplines and emergent technologies will be challenging but can be done. For decades, the biomedical sciences have been held back by technical limitations, but now the time is ripe to develop live science and move beyond existing partial and static approaches toward whole-system and dynamic ones. Live science will enable researchers to answer some of the most interesting questions in the biomedical sciences today.

1. Takashima, T., Kitamura, S., Wada, Y., Tanaka, M., Shigihara, Y., Ishii, H., Ijuin, R., Shiomi, S., Nakae, T., Watanabe, Y., *et al.* PET imaging-based evaluation of hepatobiliary transport in humans with (15*R*)-^{11}C-TIC-Me. *The Journal of Nuclear Medicine* **53,** 741–748 (2012).

News

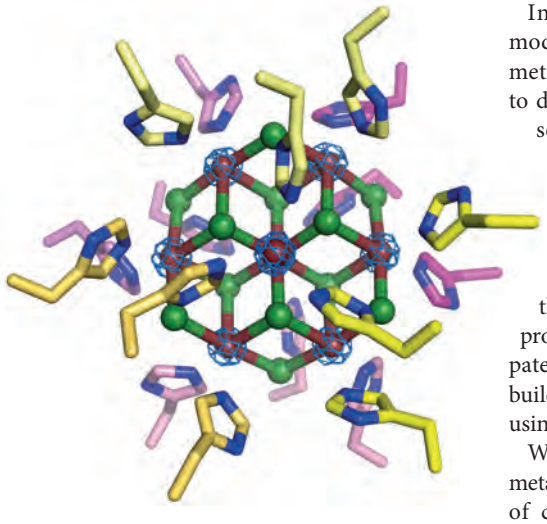
Waiter, there's a nanocrystal in my pizza!

A tiny crystal between two artificially engineered proteins could provide valuable insights into how proteins in nature incorporate metals into their structures

The smallest crystal reported so far has been created by RIKEN researchers. The cadmium chloride nanocrystal consists of just 19 atoms and is sandwiched between two copies of an artificially designed protein.

In the natural world, proteins use the process of biomineralization to incorporate metallic elements into tissues, creating diverse materials such as seashells, teeth and bones. However, this process is not well understood.

Previously, scientists at the RIKEN Center for Life Science Technologies and collaborators at Yokohama City University in Japan developed the artificial protein Pizza6, so called because it resembles a pizza cut into six identical slices. Their goal was to design novel proteins that do not exist in nature and that could be used in various applications. Proteins like Pizza with its high degree of symmetry are attractive scaffolds for creating new hybrid biomaterials suitable for applications such as drug packaging and delivery to cells, or even bioremediation of hazardous metals in the environment.



In the current study, the researchers modified the Pizza protein by introducing a metal-binding site. "Our initial impetus was to design metal-binding sites to control the self-assembly of our designed symmetric proteins," says first author Arnout Voet. "We used computational methods to find a rational way to incorporate a metal-binding site into the Pizza protein we had previously designed, based on the idea that this could allow us to control protein assembly easily." The scientists anticipated that this would provide a new tool for building novel proteins from the ground up by using very cheap metal reagents.

When the proteins were modified to have a metal-binding site and then placed in a solution of cadmium chloride, multiple subunits spontaneously bound together to form one copy of Pizza. Using RIKEN's SPring-8 synchrotron and other facilities, the researchers analyzed the structure at the atomic level and discovered that the atoms of cadmium and chloride had formed a tiny lattice—a crystal structure—sandwiched between two Pizza proteins (see image).

"We were very excited to see the formation of the crystal, as it provides insights into the process of biomineralization," says Kam Zhang, who led the RIKEN team. "Our results indicate the feasibility of using rationally designed symmetric proteins to biomineralize nanocrystals. Achieving this could allow us to make a wide range of nanodevices such as biopharmaceuticals, biosensors, light-driven switches and synthetic enzymes from the bottom up."

"We have many ideas about how this might be put to further use," Zhang continues, "and will continue to experiment to find novel properties in these artificially designed proteins."

Reference

Voet, A. R. D., Noguchi, H., Addy, C., Zhang, K. Y. J. & Tame, J. R. H. Biomineralization of a cadmium chloride nanocrystal by a designed symmetrical protein. Angewandte Chemie International Edition 54, 9857–9860 (2015).

The world's most powerful NMR

The most powerful nuclear magnetic resonance (NMR) system in the world, equipped with magnets that can generate a 1,020-megahertz magnetic field, has been developed by a team of researchers at the RIKEN Center for Life Science Technologies, Japan's National Institute for Materials Science, Kobe Steel, Ltd. and JEOL RESONANCE Inc., with support from the Japan Science and Technology Agency. The work took 20 years to complete, including recovering from damage caused by the 2011 Great East Japan Earthquake and a critical shortage in the global supply of helium, which is essential for cooling.

NMR is a technique used to determine the structural and chemical properties of compounds based on the nuclear spin of atoms under an external magnetic field—the stronger the magnetic field, the easier it is to detect these properties. The research team used high-temperature superconducting technology to produce the powerful magnetic field in their system, which will support drug development and research into structural biology, analytical chemistry and materials engineering. It could also translate into advances in similar technologies that rely on precise magnetic fields, such as magnetic resonance imaging.

www.nims.go.jp/eng/news/press/2015/07/201507010.html

Hideaki Maeda (second from right), director of the NMR Facility at the RIKEN Center for Life Science Technologies.

News

A dictionary of the language of cells

We rely on a complex network of communication between cells

In their struggle to survive and prosper, multicellular organisms rely on a complex network of communication between cells, which in humans are believed to number about 40 trillion. Now, in a study published in Nature Communications, a research group led by scientists from the RIKEN Center for Life Science Technologies (CLST) has published an overall map of how the cells in the human body communicate by systematically analyzing the relationship between ligands—substances such as insulin and interferon that embody messages between cells, and receptors—the proteins on cell surfaces that receive these messages when bound by the ligands.

The group looked at the expression of 1,894 ligand-receptor pairs—based on 642 ligands and 589 receptors—that have been reported in the literature so far, and drew up a large-scale map of cell-to-cell communication between 144 primary human cell types. The survey found that most cells express tens or even hundreds of ligands and receptors, creating a highly connected signaling network made up of cell types that can communicate with each other through multiple ligand-receptor paths.

The development of multicellular organisms from unicellular ancestors is one of the most profound evolutionary events in the history of life on Earth. During this transition, the cells of multicellular organisms began to communicate with each another in many ways, playing specific roles in the development and then control of their cellular functions. This process is particularly critical during early embryonic development when a cell's differentiation and ultimate fate are controlled by communication with neighboring cells. In the developed organism, intercellular communication coordinates the activities between multiple cell types and makes organism-wide processes such as immune response, growth, and homeostasis. Defects in cell-cell communication, including the dysregulation of autocrine signaling, are also implied in the development of cancer, and autoimmune and metabolic diseases.

Despite the importance of this process, studies in intercellular communication between specialized cells in higher organisms such as mammals have generally focused on communication between just a few cell types and have been limited in scope to small numbers of ligand-receptor pairs. Currently there are no reports based on systematic studies attempting to elucidate and quantify the repertoire of signaling routes between different cell types.

To address this, the group looked at gene-expression data measured by the CAGE method in the RIKEN-led FANTOM5 project, using information from existing databases and large numbers of past publications, to generate the first large-scale draft map of primary cell-to-cell interactions.

Based on the analysis, the authors gained new insights into how cells communicate. According to Jordan Ramilowski, the first author of the study, "One intriguing conclusion is that signals between cells of the same type are surprisingly common, accounting for approximately two-thirds of cell-cell partners. We also discovered that receptors in general seem to have evolved earlier than ligands."

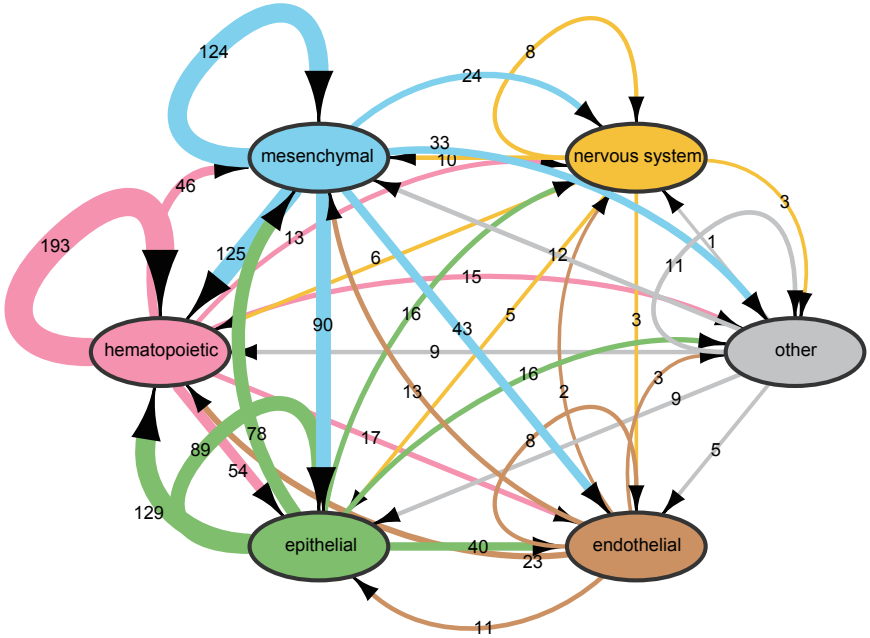
According to Alistair Forrest, who led the project, "Elucidating how cells communicate in an organism can contribute to the development of medical treatments. In particular, this data can be a key to discovering receptors that can be targets for therapies in various diseases."

Considering the importance of these findings for the development of new medical therapeutics, the team has decided to publish the data online. The database is available online here.

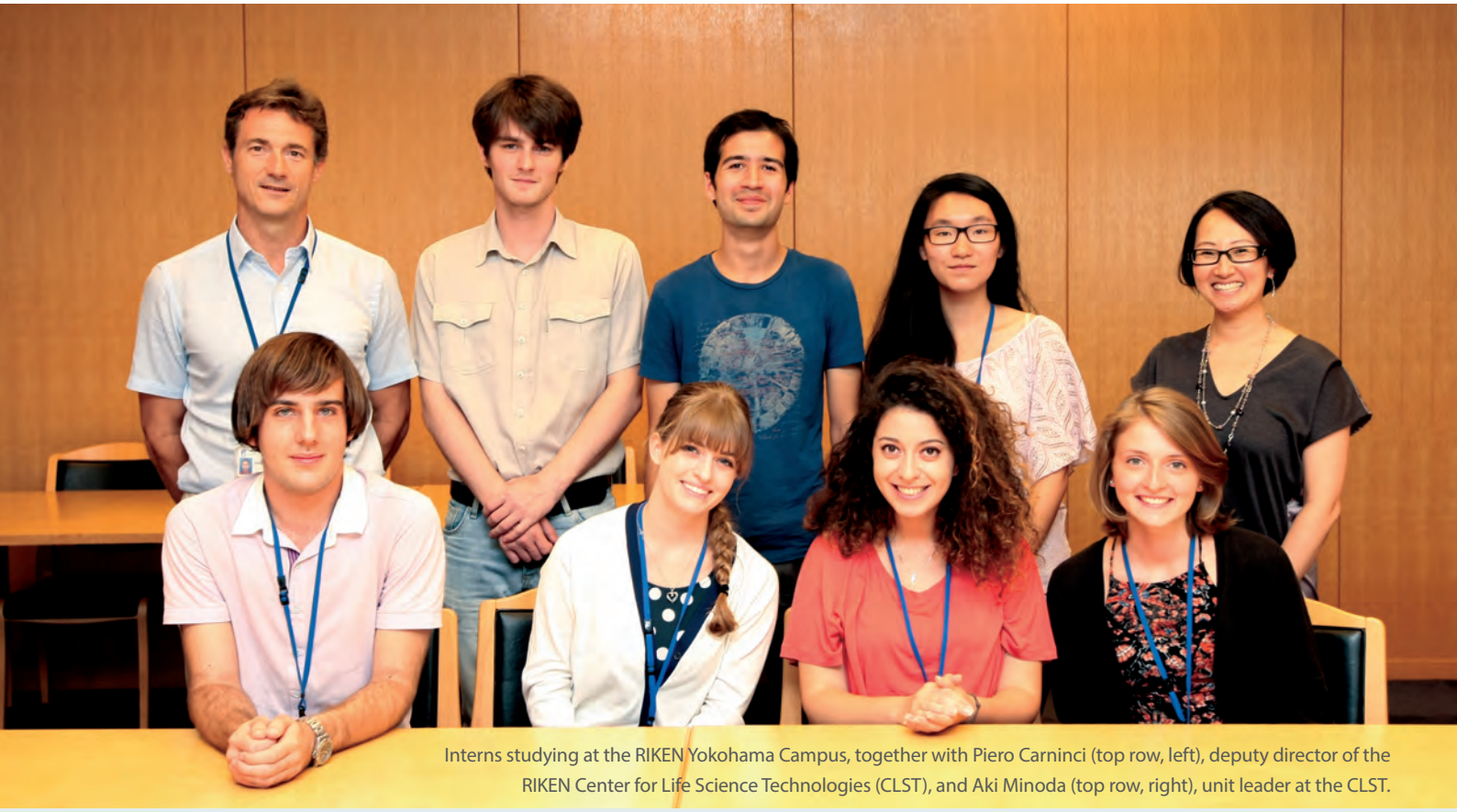
The FANTOM project (for Functional Annotation of the Mammalian genome) is a RIKEN initiative launched in 2000 to build a complete library of human genes using the capabilities offered by new, state-of-the-art cDNA technologies. Over 250 experts in primary cell biology and bioinformatics from 114 institutions based in more than 20 countries and regions are working as part of FANTOM 5, the 5th edition of the project.

Reference

Ramilowski J, Goldberg T, Harshbarger J, Kloppman E, Lizio M, Satagopam V, Itoh M, Kawaji H, Carninci P, Rost B, Forrest A. A draft network of ligand-receptor-mediated multicellular signaling in human. *Nature Communications* (2015)



Schematic of the ligand-receptor network. The figure shows how cells of different biological functions and origins (lineages) interact with each other. The thick lines emphasize the importance of intra-lineage signaling (signaling between cells of the same lineage).



Interns studying at the RIKEN Yokohama Campus, together with Piero Carninci (top row, left), deputy director of the RIKEN Center for Life Science Technologies (CLST), and Aki Minoda (top row, right), unit leader at the CLST.

Programs for Junior Scientists

RIKEN strives to provide the best and most exciting opportunities for young scientists from all over the world in the crucially important early stages of their careers. The Special Postdoctoral Researcher Program offers creative young scientists the chance to participate in autonomous and independent research under the direction of a RIKEN laboratory head. The Junior Research Associate program provides part-time positions for enthusiastic and open-minded young researchers enrolled in doctorate programs at Japanese graduate schools. RIKEN also accepts non-Japanese PhD candidates as International Program Associates through a cooperative undertaking of students between RIKEN and collaborating universities.

Internship and summer schools

"Should I get a job or should I go to graduate school and get a PhD?" This is one of the biggest life-changing questions that young scientists face. We strongly believe that even undergraduate students should be given a chance to experience a research environment.

The RIKEN Brain Science Institute gives young researchers a stimulating opportunity to study brain science. At the Nishina School, students from selected universities can learn about theoretical and experimental nuclear physics at the RIKEN Nishina Center for Accelerator-Based Science. The RIKEN Center for Integrative Medical Sciences offers students the chance to learn about recent research in immunology. And with the help of the Asia–Oceania Forum for Synchrotron Radiation Research, RIKEN conducts the Cheiron School, where participants can learn about synchrotron radiation science at SPring-8, the world's largest third-generation synchrotron facility.

Also, the RIKEN Center for Life Science Technologies hosted many undergraduate interns from various countries this summer. These interns contacted us directly or through their supervisors to ask for internship opportunities. From our experience launching the international FANTOM (Functional Annotation of the Mammalian Genome) consortium, we have learned that promoting friendship among scientists all over the world, including upcoming young researchers, is critical for conducting mutually fruitful research.

Even if you do not find a suitable program at RIKEN, don't give up—we are always open to new ideas. Please contact us and ask us for a chance to become an intern at RIKEN.

RIKEN CLST

Karolinska Institutet

SciLifeLab

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