

2002 Regular KOLIS Meeting

**UC San Francisco
April 20, 2002**

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Welcome

Dear Colleagues,

It is my pleasure to welcome you to an exciting and timely meeting at UCSF, organized by KOLIS (Korean Life Scientist). In this meeting, we are going to have two sessions with title of *Drug Discovery & Delivery* and *Signaling & Clinical Applications* receptively. It is our honor to host Dr. Joong Myung Cho as a plenary lecturer. Dr. Cho, is one of senior KOLIS member who will present very elegant approaches of drug development techniques based on academic research. Furthermore, Samsung will present SAIT (Samsung Advanced Institute of Technology)'s current visions of biotech area in special presentation section.

I am sure that this one day meeting is not only enlarge your friendship but also help your current work significantly. This is a good chance to share about our vision and exchange new information. It is also good opportunity to contact many responsible persons from university and biotech industry for your future career.

As you know very well, bioscience is one of most shifting science and its outcome often impact highly on our socio-economical environment as well as medical improvement. During last decade, huge amounts of technical advance in many areas create new concepts like, post-genomic research, proteomics, HTS, gene-therapy, stem cell research, DNA array, gene-chip, SNP, tissue-specific knockout, bio-informatics etc. Consequently, many industries are focusing on developing new profitable drugs or products using these advanced techniques.

As a similar aged Korean scientist who educated his/her undergraduate at 80' and early 90' for most members, we have enough reasons to share our experiences. I understand well, there are some negative opinions on Korean scientist meeting because of personal attitude or less impressive experiences from previous participation. However, I eagerly recommend participating KOLIS meeting with fresh mind since we are reorganizing to provide best opportunities based on active scientific communication.

Thanks to financial contribution from several sponsors listed in front page, KOLIS can organize this meeting and annual meeting for August 24. I am sure we can make better KOLIS meeting if you participate. KOLIS is preparing wonderful party with delicious Korean food in attractive lounge in UCSF. I truly appreciate your full participation in this regular meeting.

Sincerely

Doo-Sup Choi, Ph.D.
KOLIS President

2002 KOLIS Regular Meeting Program

12:00 - 1:00 Registration & Greeting (Name tag and sign)

Plenary Lecture:

Chair: Dr. Jae-Beom Kim

1:00 - 2:00 Speaker: Joong Myung Cho, Ph.D., Crystal Genomics

Session I: Drug Discovery and Delivery

Chair: Dr. Kwang Ho Cheong

2:00 - 2:30 Speaker 1: Insil Kim, M.D., Stanford

2:30 - 3:00 Speaker 2: Kwang Ho Cheong, Ph.D., Stanford

3:00 - 3:30 Speaker 3: Joon-Sig Choi, Ph.D., UC San Francisco

3:30 - 4:00 Recess: Snack and Drink

Session II: Signaling and Clinical Application

Chair: Dr. Jinh Kim

4:00 - 4:30 Speaker 4: Sunghoon Kwon, Ph.D. student. UC Berkeley

4:30 - 5:00 Speaker 5: Kichoon Lee, Ph.D., UC Berkeley

5:00 - 5:30 Speaker 6: Jeongwon Sohn, M.D., Ph.D., UC San Francisco

Special Presentation :

5:30 - 6:00: Samsung Advanced Institute of Technology (SAIT)

6:00 - 9:00 Dinner and Party at UCSF Hall

Plenary Lecture: 1:00 – 2:00, Chair: Dr. Jae-Beom Kim

Title: Structural Genomics and Chemogenomics : Drug Discovery

Speaker: Joong Myung Cho, CrystalGenomics, Inc

Abstract:

Drug targets are massively identified through various genomic studies in these post-genomic days. Consequently, the acceleration of lead discovery that is the next step of target identification in the drug discovery process is required. Thus, CrystalGenomics has developed structural chemogenomic libraries that are chemical libraries targeting the active site of specific protein families.

Most drugs interact with the active site of their target proteins through the recognition based on the 3-dimensional arrangements of pharmacophoric moieties. Moreover, since active sites of a protein family are similar to each other, they can recognize same chemical moieties (chemotypes). Thus, for the construction of structural chemogenomic libraries, we classify protein structures according to 3D structures of their active sites and construct chemical libraries to recognize the active site of each protein family.

The procedures for the lead generation through the structural chemogenomic libraries consist of five steps. The 1st step is to generate folding dictionary of active sites. In this step, we classify all the available protein structures (obtained from our company's structural genomic studies and PDB) according to their active site structures. The 2nd step is to identify chemotypes that can recognize the protein active site of each family. For this step, CrystalGenomics has employed virtual screening methods using proprietary technologies including chemotype analysis system, drug-likeness filtering system, virtual chemical libraries and scoring functions. The 3rd step is to construct structural chemogenomic libraries. In this step, we synthesize and collect chemicals containing the chemotypes identified in the 2nd step. The 4th step is biological assays of structural chemogenomic libraries for the drug targets included in a protein family. The 5th step is to confirm that the active ligand binds to the active site by elucidating the structure of target protein / inhibitor complex. The resulting structure becomes the starting point of lead optimization.

CrystalGenomics has applied the structural chemogenomic libraries for the lead generation of several enzyme families including phosphatases, kinases and proteases and successfully obtained drug lead chemicals. In this presentation, we describe the build of structural chemogenomic libraries for phosphatases and the generation of initial lead inhibitors for several phosphatases.

CrystalGenomics, Inc.: Company for the High-Speed Discovery of Drug Candidates

Many new technologies to speed up the drug discovery process are required in these post-genomic days because drug targets are massively identified through genomic studies.

Thus, CrystalGenomics, Inc. has developed Crystal_Tech's based on structural genomics and chemogenomics. These technologies support 5 steps of research process for drug candidate discovery: target evaluation, protein preparation, structure determination of target proteins, lead generation and lead optimization.

For the target evaluation, Crystal_E_Tech (CET) has been used. The CET evaluates many targets suggested from academic laboratories and functional genomics companies. In the protein preparation step, CrystalGenomics has employed Crystal_Gene_Tech (CGT) and Crystal_Pro_Tech (CPT). These includes unique cloning of target gene into a variety of expression vectors to increase the solubility of target protein, followed by efficient purification. Crystal_X_Tech (CXT) and Crystal_NMR_Tech (CNT) represent our technologies to determine 3D-structure of target proteins using X-ray crystallography and NMR spectroscopy, respectively. We have obtained many synergistic effects from the simultaneous usage of both techniques. For the lead generation on the basis of protein 3D-structures, CrystalGenomics has innovated database-docking methods (Crystal_Virtual_Tech: CVT) and combined them with experimental confirmation using NMR spectroscopy (Crystal_HTSNMR_Tech: CHT). Moreover, CrystalGenomics has constructed structural chemogenomic libraries (Crystal_Library_Tech: CLT) that are focused chemical libraries targeting specific protein families. For the last step, lead optimization, we have developed an integrated drug-discovery-informatics technology (Crystal_Info_Tech: CIT). The Crystal_Info_Tech includes virtual medicinal chemistry, virtual bioassays, virtual ADMET and virtual patent office. The ultimate goal of the Crystal_Info_Tech is to propose sets of synthetic target chemicals for the discovery of drug candidates.

CrystalGenomics has applied these techniques to various known and unknown drug targets. Then, we obtained several new protein structures and lead compounds that are now optimized to discover drug candidates. In this presentation, the concepts of these techniques and the results of their application will be discussed.

Session I : Drug Discovery and Delivery, Chair: Dr. Kwang Ho Cheong

Speaker 1: 2:00 – 2:30

Title: Structural investigation of Hepatitis C virus internal ribosomal entry site (HCV IRES)

Speaker: Insil Kim, Joseph D. Puglisi, Stanford

Abstract:

Hepatitis C is a major world health problem, caused Hepatitis C virus (HCV). Therapeutic intervention against HCV has been hindered by a lack of understanding of the fundamental processes in the viral life cycle. HCV uses a novel mechanism of translation initiation of its polyprotein mRNA. Instead of normal cap-mediated initiation, the 40S ribosomal subunit binds directly to an internal ribosome entry site (IRES) that is formed by the mRNA structure. This binding event tethers the 40S subunit near the start codon for translation. We have used a combined biochemical and biophysical approach to determine the structural basis for IRES-40S interaction and subsequent initiation.

We are currently investigating the structure of the 100 kDa IRES RNA by NMR. Although NMR has proven a powerful tool to determine structural and dynamic features of RNA molecules, the application of NMR to RNA has been limited to small molecules because of resonance overlap and broadening. To study the structure of the huge IRES by NMR, we are building on assignments made from isolated oligonucleotide fragments of the IRES, and also we investigated the structure of domain in the intact IRES RNA by using segmental labeled RNA. Our data indicate that the isolated domain II form similar structures in the intact IRES and segmental labeling of RNA can have a wide range of potential applications in determination of global conformation in NMR. Moreover, we show how NMR, used in conjunction with biochemical and physical methods can provide powerful insights into RNA function.

Session I: Drug Discovery and Delivery, Chair: Dr. Kwang Ho Cheong

Speaker 2: 2:30 – 3:00

Title: Development of a new assay system to test novel therapeutic molecules that block the membrane association of HCV NS5A

Speaker: Kwang Ho Cheong, Harry B. Greenberg, Jeffrey S. Glenn, Stanford

Abstract:

Hepatitis C virus (HCV) is an important worldwide cause of liver disease. Current therapies are inadequate for the majority of patients which underscores the need for novel anti-HCV strategies. We recently demonstrated that molecular genetic disruption of amphipathic helix(AH)-mediated membrane association of NS5A abolishes the replication of HCV replicons. We hypothesize that *pharmacologic* disruption of amphipathic helix-mediated membrane association of NS5A may similarly inhibit HCV RNA replication. The purpose of this study was to develop a new biochemical assay system for studying the mechanistic details of AH-mediated NS5A membrane association, and to screen novel therapeutic molecules designed to block the membrane association of NS5A. Methods: By using Opti-Prep density gradients, we established a membrane floatation assay to monitor the membrane association of NS5A or a fusion protein composed of the amphipathic helix of NS5A fused to the N-terminus of green fluorescent protein. Results: We used this membrane floatation assay to confirm that the same genetic mutations in the amphipathic helix that impaired the replication of HCV subgenomic replicons disrupted the posttranslational membrane association of NS5A. We next synthesized various competitive peptides homologous to the NS5A N-terminal amphipathic helix to evaluate whether these peptides are capable of inhibiting the amphipathic helix-mediated membrane association of NS5A. The membrane association of NS5A was inhibited in a dose-dependent manner by synthetic peptides homologous to the wild-type amphipathic helix, but not by mutant peptides with a disrupted AH. Conclusion: These results suggest that the amphipathic helix-mediated association of NS5A with host cell membranes is amenable to pharmacologic disruption. Moreover, this membrane floatation assay can be a useful tool for ongoing development for new therapeutic strategies against Hepatitis C.

Session I: Drug Discovery and Delivery, Chair: Dr. Kwang Ho Cheong

Speaker 3: 3:00 – 3:30

Title: Research and development of synthetic nonviral vectors for gene therapy

Speaker: Joon Sig Choi and F. Szoka, UC San Francisco

Abstract:

Gene therapy is generally considered as a promising approach not only in the treatment of diseases with genetic defects but also in the development of strategies for treatment and prevention of chronic diseases such as cancer, cardiovascular diseases and rheumatoid arthritis. Intensive investigation has been conducted during the past decade into methods for transferring genes to cells. The methods used so far make use of either viral systems (retroviruses or adenoviruses) or nonviral systems (polymers, electroporation, calcium-phosphate precipitation, polypeptides and liposomes). Even though some viral systems showed remarkable levels of expression, they are limited for general use *in vivo* due to short and long-term risks such as generation of host immune responses as well as their low capacity for large sized therapeutic genes and the possibility of inserted genes combining with endogenous viruses or activation of oncogenes.

Consequently, there has been increasing attention focused on the development of safe and efficient nonviral gene transfer vector systems, which are either polycationic polymers or cationic lipids. Researches related to nonviral gene carriers comprising chemically synthesized molecules have increased enormously to overcome and eventually, to replace the viral vectors. Polycationic polymers and cationic lipids have constituted the main subjects of the studies. Various polymers from synthetic to naturally occurring ones have been introduced and tested for their suitability in the field of gene therapy. Several cationic polymers were found to be promising but their intrinsic drawbacks, such as solubility, cytotoxicity and low transfection efficiency, limited their use as *in vivo* gene carriers. Among them, however, dendrimers are still very attractive to many scientists for the design of gene carriers because of their well-defined structure and ease of control of surface functionality. Already, both polyamidoamine dendrimer and polyethylenimine dendrimer were reported for their potential utility and have exhibited high transfection efficiency *in vitro* and *in vivo*. However, these dendrimers have not yet overcome the

problems of solubility of the complex with DNA and cytotoxicity. In addition, cationic lipids are attractive and widely used because it is possible to design and synthesize various kinds of derivatives that are outstanding in the aspects of transfection efficiency, biodegradability and low toxicity. Some cationic lipids are commercially available and several lipids have already been used in the clinical setting. Among them, cationic cholesterol derivatives are known to be very useful for their high transfection efficiency and low toxicity. However, there are still many issues to be tackled such as the gene transfer potency, the half-life in the blood stream, and the biocompatibility deserving less cytotoxicity.

Block copolymers containing poly(ethylene glycol) (PEG) have been used for many drug carriers owing to their high solubility in water, non-immunogenicity and improved biocompatibility. PEG has also been coupled to numerous polycationic polymers, such as poly(L-lysine), polyspermine, polyethylenimine or liposomes. Therefore, the conjugated PEG helps the reagents to improve the half-life in the blood stream, to increase the solubility, and to reduce the immune reaction of complexes with DNA.

In this talk, it will be presented the characteristics of some nonviral vectors developed and some recent methods reported. It was tried to couple linear PEG with the globular macromolecule, poly(L-lysine) dendrimer (PLLD), to form an AB type or ABA type linear-dendritic block copolymer. It was performed that the characteristics of polyionic complex formation of the copolymers with plasmid DNA *via* supramolecular self-assembly and the application to practical *in vitro* tests. Also, new cationic lipids composed of L-lysineamide, L-ornithineamide and cholesterol as gene delivery agents will be introduced and presented. In addition, some trials for preparation of hyaluronic acids oligomers as targeting ligands and preparation of stabilized plasmid-lipid particles (SPLP) using novel pH-sensitive PEG-lipids will be presented and discussed.

Session II: Signaling & Clinical Application, Chair: Dr. Jinoh Kim

Speaker 4: 4:00 – 4:30

Title: Fluorescent cellular imaging via micro confocal imaging array (microCIA)

Speaker: Sunghoon Kwon, and Luke P. Lee, Bioengineering Graduate Group Berkeley
Sensor and Actuator Center, UC Berkeley

Abstract:

A research on development of micro scale confocal microscope for lab-on-a-chip system is described. The confocal scanning microscope is a powerful tool for imaging of semitransparent biological samples like cells and tissues because of its non-invasive high resolving power and 3D reconstruction capability. The miniaturization of this confocal microscope will be an enabling technology for quantitative high resolution imaging in many applications such as in-vivo endoscopy, and handheld hypodermic microscopy. Various micromachined optical components such as scanning mirrors, microlenses, and beam splitters are promising candidates for this purpose. Mass productive integration of a whole confocal microscope including laser sources, intermediate optics, scanners and detectors within a millimeter cubic will enable compact handheld biological imaging systems. Furthermore, an array type integration of multiple MEMS confocal microscopes will play an important role in high-throughput handheld lab-on-a-chip systems as well as in microvision for autonomous microrobots and low mass micro optical imagers for space investigation.

In this talk, the design, fabrication, and characterization of the stacked microlens scanner, a key component of micro confocal imaging array are described. The first fluorescent cellular imaging via microconfocal imaging array proves the feasibility of the micro confocal imaging array.

Session II: Session II: Signaling & Clinical Application, Chair: Dr. Jinoh Kim

Speaker 5: 4:30 – 5:00

Title: Paternally imprinted Pref-1/Dlk1 is the key determinant of human uniparental disomy (UPD)14 syndromes

Speaker: Kichoon Lee, Yang Soo Moon, Cynthia M. Smas, Josep A. Villena, Kee-Hong Kim, Sunjoo Lee, Chulho Kang, Eun Jun Yun and Hei Sook Sul, UC Berkeley

Abstract:

Preadipocyte factor-1 (Pref-1/Dlk1), encoding a transmembrane protein containing EGF-repeats homologous to the Notch/Delta/Serrate family, is a paternally expressed gene localized at homologous imprinted regions of syntenic chromosomes 12, 14, and 18 in mouse, human, and sheep, respectively. Perturbance of imprinted gene expression in human paternal uniparental disomy (UPD)14 causes growth retardation and bone malformation, whereas maternal UPD14 leads to growth retardation, obesity, scoliosis, blepharophimosis, early puberty, and short stature. The exact gene responsible for such disorders is yet to be identified. Here we have generated both Pref-1 overexpression and knockout mice to examine whether Pref-1 is responsible for these symptoms. Pref-1 overexpression results in most of the phenotypes of human pUPD14, including growth retardation, perinatal lethality, and bone malformation. Moreover, transgenic mice had less fat mass due to decreased adipogenesis. Similar to type II diabetes, these mice exhibited hypertriglyceridemia and impaired insulin homeostasis. Pref-1 null mice display growth retardation, obesity, blepharophimosis, and high levels of serum lipid metabolites. In addition, the phenotypes observed in Pref-1 null mice are also present in heterozygotes harboring a paternally-inherited, but not maternally-inherited, pref-1 null allele. Our results demonstrate that Pref-1 is indeed paternally expressed, is essential for normal development and homeostasis of adipose tissue. These phenotypes establish Pref-1 as the key determinant of human UPD14 syndromes.

Session II: Session II: Signaling & Clinical Application, Chair: Dr. Jinoh Kim

Speaker 6: 5:00 – 5:30

Title: Increased expression of pro-inflammatory cytokines and metalloproteinase-1 by transforming growth factor- β 1 in rheumatoid arthritis, but not normal synovial fibroblasts

Speaker: Hyeon-Ju. Cheon and Jeongwon. Sohn, Department of Biochemistry, Korea University College of Medicine and Dept. of Growth and Development, UC San Francisco

Abstract:

TGF- β is a cytokine with diverse biological activities ranging from regulation of cell differentiation, apoptosis, tumor generation and metastasis to modulation of inflammation and immunity. Moreover, the effect of TGF- β can often be contradictory depending on cell types and conditions. One such example can be found in its effect on immune and inflammatory responses. TGF- β 1 is mostly known as immune-suppressive and anti-inflammatory cytokine, but it is also capable of promoting inflammation. In the rheumatoid arthritis (RA) synovium, both active and inactive forms of TGF- β 1 as well as its receptors are expressed at high levels suggesting that TGF- β 1, per se or in combination with other cytokines plays an important role in the progression of RA. We have investigated the role of TGF- β 1 in the inflammation of RA using cultured fibroblast-like synoviocytes (FLS) from RA, osteoarthritis (OA) patients and non-arthritic individuals. mRNA expressions of IL-1 β , tumor necrosis factor (TNF)- α , IL-8, Macrophage inflammatory protein (MIP)-1 α , and metalloproteinase (MMP)-1 were increased in RA and OA FLS by TGF- β 1 treatment, but not in non-arthritic FLS. Enhanced protein expression of IL-1 β , IL-8, and MMP-1 was also observed in RA FLS. Moreover, TGF- β 1 showed a synergistic effect in increasing protein expression of IL-1 β and matrix metalloproteinase (MMP)-1 with TNF- α and IL-1 β , respectively. Biological activity of IL-1 determined by mouse thymocyte proliferation assay was also enhanced by 50% in response to TGF- β 1 in the culture supernatant of RA FLS. DNA binding activities of nuclear factor (NF)- κ B and activator protein (AP)-1 were shown to increase by TGF- β 1 as well. These results suggest that TGF- β 1 contributes for the progression of inflammation and joint destruction in RA, which is specific for the arthritic synovial fibroblasts. The possibility that TGF- β may enhance IFN- β expression upon viral infection will also be discussed.