The Chemistry of Cellular Communication

ICS Symposium Honoring Wolf Prize Laureates
Bonnie L. Bassler, Carolyn R. Bertozzi, and Benjamin F. Cravatt

June 15, 2022

The Open University of Israel, Ra'anana
Chais Auditorium

08:30-09:00	Gathering and Registration
09:00-09:30	Opening session
	Prof. Ehud Keinan, President, Israel Chemical Society and IUPAC President-elect Prof. Dan Shechtman, Wolf Foundation, Council Acting Chairperson Prof. Mimi Ajzenstadt, President, The Open University of Israel
Session 1	Chair: Prof. Anat Milo, Ben-Gurion University of the Negev
10:00	Prof. Bonnie Bassler, Princeton University and HHMI, USA *Quorum Sensing Across Domains: from Viruses to Bacteria to Eukaryotes*
10:30	Prof. Michael M. Meijler, Ben-Gurion University of the Negev *Chemical Information Exchange Between Species: Living Together by the Grace of Good Chemistry*
11:00	Prof. Irit Sagi, Weizmann Institute of Science *Tumor Reactive Human Antibodies Evolve to Target Matrix Remodeling Enzymes*
11:30-12:00	Coffee Break
Session 2	Chair: Prof. Sharon Ruthstein, Bar Ilan University
12:00	Prof. Carolyn Bertozzi, Stanford University and HHMI, USA *Therapeutic Opportunities in Glycoscience*
12:30	Prof. Ashraf Brik, Technion - Israel Institute of Technology *Advances in the Synthesis, Activation and Delivery of Uniquely Modified Peptides and Proteins*
13:00	Prof. Nir London, Weizmann Institute of Science Covalent Ligand Directed Release Chemistry
13:30-14:20	Lunch Break
Session 3	Chair: Prof. Galia Blum, The Hebrew University of Jerusalem
14:20	Prof. Benjamin F. Cravatt, The Scripps Research Institute, La Jolla, CA, USA *Activity-Based Proteomics – Target and Ligand Discovery on a Global Scale*
14:50	Prof. Micha Fridman, Tel Aviv University *Chemical Biology Approaches for Deciphering the Biological Activities of Antifungal Drugs and Improving their Performance*
15:20	Dr. Jacqueline Blankman, Lundbeck La Jolla Research Center, CA, USA *Development of Monoacyglycerol Lipase Inhibitors for the Treatment of Neurological and Neuropsychiatric Disorders*
15:50	Concluding remarks


Bonnie Bassler


Michael Meijler


Irit Sagi


Carolyn Bertozzi


Ashraf brik


Nir London


Benjamin Cravatt


Micha Fridman


Jacqueline Blankman

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Abstracts

Quorum Sensing Across Domains: from Viruses to Bacteria to Eukaryotes

Bonnie Bassler

Department of Molecular Biology, and Howard Hughes Medical Institute,
Princeton University, NJ, USA

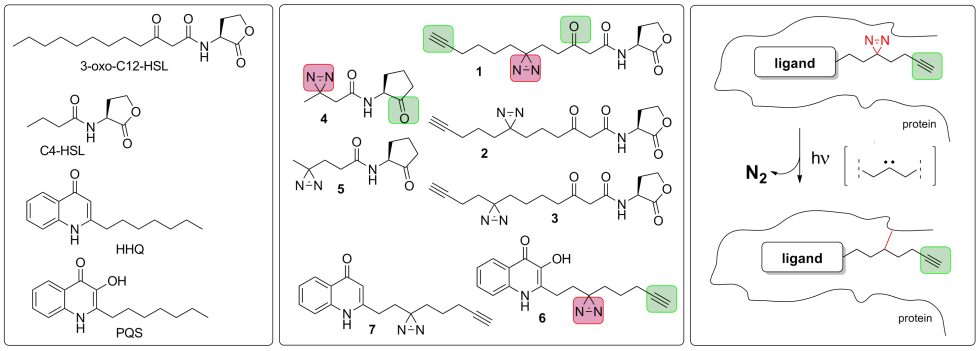
Bacteria communicate with one another via the production and detection of secreted signal molecules called autoinducers. This cell-to-cell communication process, called “Quorum Sensing”, allows bacteria to synchronize behavior on a population-wide scale. We showed that behaviors controlled by quorum sensing are ones that are unproductive when undertaken by an individual bacterium acting alone but become effective when undertaken in unison by the group. For example, quorum sensing controls virulence factor production and biofilm formation. We discovered that eukaryotes that harbor quorum-sensing bacteria participate in these chemical conversations by providing the substrates bacteria need to make autoinducers. We found that quorum-sensing autoinducer information can be hijacked by viruses that infect and kill bacteria. Thus, interactions across the eukaryotic, bacterial, and viral domains all rely on quorum sensing. Using what we have learned, we built quorum-sensing disruption strategies for development into new anti-microbials. We have also engineered viruses to respond to user-defined inputs, rather than the bacterial autoinducers, to make phage therapies that kill particular bacterial pathogens on demand.

Chemical Information Exchange Between Species: Living Together by the Grace of Good Chemistry

Michael M. Meijler

Department of Chemistry, Ben-Gurion University of the Negev, Be'er Sheva, Israel

Life on earth is heavily based on chemical communication between cells. Quorum sensing enables unicellular organisms to coordinate their behavior and function in such a way that they can adapt to changing environments and compete, as well as coexist, with multicellular organisms. Prime examples of this phenomenon are displayed by the opportunistic pathogens *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens*, which cause disease in humans and plants, respectively – but most often don't. Quorum sensing in these pathogens is mediated by small amphiphilic signaling molecules such as 3-oxo-C_{12}-HSL and 3-oxo-C_8-HSL, leading to biofilm formation and secretion of virulence factors. The Meijler group is targeting QS in various pathogens with several chemical tools, such as a set of electrophilic and photoactivatable 'tag-free' probes that are designed to bind QS receptors covalently. These probes are used as molecular tools to obtain new insights into the mechanisms of activation, deactivation, and recognition of bacterial quorum sensing – using bioorthogonal chemistry. Diverse eukaryotes have been found to react strongly to the presence of these compounds, and the recognition of QSMs is mediated by mostly unknown receptors. Recently we identified and validated the role of a human receptor for HSLs, called the Major Vault Protein (MVP), as an important immunomodulator.



J. Rayo, R. Gregor, N. T. Jacob, et al, V. V. Kravchenko, M. M. Meijler: Immunoediting Role for Major Vault Protein in Apoptotic Signaling Induced by bacterial N-Acyl Homoserine Lactones *Proc. Natl. Acad. Sci. USA*, 118(12), 2021

A. Yashkin, J. Rayo, L. Grimes, M. Welch, M. M. Meijler: Short-Chain Reactive Probes to Unravel the *Pseudomonas Aeruginosa* Quorum Sensing Regulon *Chem. Sci.,* 12 (12), 4570-4581, 2021.

Tumor reactive human antibodies evolve to target matrix remodeling enzymes

Irit Sagi

Department of Immunology and regenerative biology. The Weizmann Institute of Science

Proteolytic processes in the extracellular matrix (ECM) and on cell surfaces dictate cell behavior and tissue integrity under normal homeostasis and in diseased tissues. The tumor microenvironment hosts antibody-secreting cells (ASCs) associated with a favorable prognosis in several types of cancer. Patient-derived antibodies have diagnostic and therapeutic potential; yet, it remains unclear how antibodies gain autoreactivity and target tumors. Recently we reported (Mazor et al, Cell 2022) that somatic hypermutations (SHMs) promote antibody antitumor reactivity against surface autoantigens in high-grade serous ovarian carcinoma (HGSOC). Patient-derived tumor cells were frequently coated with IgGs. Intratumoral ASCs in HGSOC were both mutated and clonally expanded and produced tumor-reactive antibodies that targeted self-enzymes (matrix metalloproteases, MMPs) among which, MMP14 (MT1-MMP), which is abundantly expressed on the tumor cell surface. We found that these newly derived tumor-reactive autoantibodies are either naturally occurring or evolve through an antigen-driven selection process. Recent advancement in the field highlights the potential of decoy receptors and inhibitory antibodies to target the structurally homologous enzymes belonging to the MMPs/ADAMs super family. Our recent findings outline the potential applicability of patient derived human autoantibodies directed at surface antigens for tumor targeting in cancer patients.

Therapeutic opportunities in glycoscience

Carolyn R. Bertozzi

Department of Chemistry and Howard Hughes Medical Institute, Stanford University, CA, USA

Cell surface glycans constitute a rich biomolecular dataset that drives both normal and pathological processes. Their “readers” are glycan-binding receptors that can engage in cell-cell interactions and cell signaling. Our research focuses on mechanistic studies of glycan/receptor biology and applications of this knowledge to new therapeutic strategies. Chemical technologies are central to our research, including bioorthogonal chemistries, synthetic glycopolymers and mass spectrometry-based glycoproteomics. Our recent efforts center on immune suppressive glycans in the tumor microenvironment and new therapeutic modalities based on the concept of targeted degradation.

Advances in the Synthesis, Activation and Delivery of Uniquely Modified Peptides and Proteins

Ashraf Brik

Schulich Faculty of Chemistry, Technion - Israel Institute of Technology

Chemical Protein synthesis offers great opportunities to synthesize uniquely modified proteins with high homogeneity and workable quantities. The field relies on the synthesis of peptide fragments and the ligation of these peptides in their unprotected forms employing different ligation strategies such as native chemical ligation (NCL). We have recently reported that transition metals such as palladium and gold complexes can be used remove multiple Cys protecting groups within minutes in a fully aqueous medium, which could be coupled in-situ with NCL to provide excellent yields of the desired product. We have also demonstrated unprecedented gold mediated depropargylation from an amide bond to facilitate the synthesis of difficult peptides and proteins. We further showed the use of the propargyl group to mediate amide bond cleavage at different sites. This chemistry was further extended for the cyclization of a wide range of peptides bearing a propargyl group. Furthermore, we extend our chemistry for the removal of protecting groups from Cys residues for the rapid and one-pot disulfide bond formation in various bioactive peptides. Finally, using this chemistry we developed a strategy for the cellular delivery and on demand activation of synthetic proteins.

Covalent Ligand Directed Release Chemistry

Nir London

Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel

Targeted covalent inhibitors are an important class of drugs and chemical probes, the majority of which specifically target cysteine residues. However, relatively few electrophiles meet the criteria for successful covalent inhibitor design. We recently discovered α-substituted methacrylamides as a new class of electrophiles suitable for targeted covalent inhibitors. While typically α-substitutions inactivate acrylamides, we show that hetero α-substituted methacrylamides have higher thiol reactivity and undergo a conjugated addition–elimination reaction ultimately releasing the substituent. Their reactivity toward thiols is tunable and correlates with the pKa/pKb of the leaving group. In the context of the BTK inhibitor ibrutinib, these electrophiles showed lower intrinsic thiol reactivity than the unsubstituted ibrutinib acrylamide. This translated to comparable potency in protein labeling, in vitro kinase assays, and functional cellular assays, with improved selectivity. The conjugate addition–elimination reaction upon covalent binding to their target cysteine allows functionalizing α-substituted methacrylamides as turn-on probes. To demonstrate this, we prepared covalent ligand directed release (CoLDR) turn-on fluorescent and chemiluminescent probes. In the reverse direction we can also use this chemistry for site-specific irreversible labeling of target cysteine, where the directing ligand leaves, allowing us to tag enzymatically active proteins with various functionalities. We used this tactic to label BTK with a variety of functional tags in vitro and in cells. Altogether this is a very versatile chemistry that unlocks many new applications in chemical biology.

Activity-based proteomics – target and ligand discovery on a global scale

Benjamin F. Cravatt

Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037

Advances in DNA sequencing have radically accelerated our understanding of the genetic basis of human disease. However, many of human genes encode proteins that remain uncharacterized and lack selective small-molecule probes. The functional annotation of these proteins should enrich our knowledge of the biochemical pathways that support human physiology and disease, as well as lead to the discovery of new therapeutic targets. To address these problems, we have introduced chemical proteomic technologies that globally profile the functional state of proteins in native biological systems. Prominent among these methods is activity-based protein profiling (ABPP), which utilizes chemical probes to map the activity state of large numbers of proteins in parallel. In this lecture, I will describe the application of ABPP to discover and functionally annotate proteins that contribute to human diseases, such as cancer and autoimmunity. I will also discuss the generation and implementation of advanced ABPP platforms for proteome-wide ligand discovery and how the integration of these global 'ligandability' maps with phenotypic screening and function-first assays can expand the druggable fraction of the human proteome for basic and translational research objectives.

Chemical Biology Approaches for Deciphering the Biological Activities of Antifungal Drugs and Improving their Performance

Micha Fridman

School of Chemistry, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv, 6997801, Israel.

Research from our group in the past several years using derivatives of antifungal drugs and chemical probes based on these agents has unraveled important details about their modes of action that have been translated into new drug design concepts. Azole and echinocandin antifungals are amongst the very few classes of antifungal drugs used as first line treatment of fungal infections. We developed inherently fluorescent antifungal azole probes that localize either to the fungal cell mitochondria or to the endoplasmic reticulum (ER). The ER harbors the target of the azoles, lanosterol 14α-demethylase (also known as CYP51). Demonstrating the importance of drug localization, the antifungal activity of ER-localized azoles against a panel of fungal pathogens was up to two orders of magnitude more potent than that of the mitochondria-localized azoles. We also developed fluorescent probes of drugs of the echinocandin class. These drugs non-competitively inhibit β-(1→3)-glucan synthase, the membrane-bound protein complex that catalyzes the formation of an essential component of the fungal cell wall. A rapid and simple assay that measures the intracellular uptake of the fluorescently labeled drug enabled the prediction of echinocandin resistance. Moreover, live-cell imaging of different fluorescently labeled echinocandin drugs revealed a correlation between antifungal potency and localization on the cell surface that harbors the target glucan synthase complex. Selective removal of the single benzylic alcohol from echinocandins anidulafungin and rezafungin restored efficacy against echinocandin-resistant fungal pathogens. The dehydroxylated derivatives provided clues to how the echinocandins bind to the catalytic subunit of the glucan synthase complex. Our work demonstrates that targeting of an antifungal drug to a particular region of the cell can significantly improve its therapeutic properties and also highlights the pivotal role of chemical biology approaches in resolving key biological questions.

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Development of Monoacyglycerol Lipase Inhibitors for the Treatment of Neurological and Neuropsychiatric Disorders

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Lu AG06466 is a small molecule inhibitor of monoacylglycerol lipase (MAGL), a serine hydrolase enzyme that regulates metabolic flux of the endocannabinoid 2-arachidonoylglycerol (2-AG). Inhibition of MAGL enhances 2-AG concentrations in the central nervous system (CNS) and potentiates 2-AG signaling through the cannabinoid receptors CB1 and CB2, while also reducing brain concentrations of arachidonic acid and downstream pro-inflammatory prostanoid lipids, resulting in beneficial effects in preclinical models of pain, anxiety, and epilepsy.

Lu AG06466 was identified using activity-based protein profiling (ABPP) as a core screening technology to simultaneously evaluate and optimize on-target potency and off-target selectivity. Lu AG06466 is a potent inhibitor of MAGL activity in mouse, rat, dog, and human brain tissue by ABPP and also inhibits 2-AG hydrolysis in human brain and peripheral blood mononuclear cell (PBMC) preparations. Lu AG06466 was extensively profiled by gel- and mass spectrometry-based ABPP in human tissue and cells and found to be highly selective. Oral administration of Lu AG06466 to rodents resulted in potent, selective and sustained inhibition of MAGL and elevation of 2-AG in the brain and periphery. A pharmacokinetic/pharmacodynamic (PK/PD) relationship was defined in the rat formalin model of pain and used to predict the clinical dose.

In Phase 1 clinical trials, Lu AG06466 was tolerated following single- and multiple-ascending doses to healthy human volunteers. ABPP and a substrate biomarker assay were used to confirm dose- and time-dependent selective target engagement of MAGL in PBMC and a novel, MAGL-specific PET probe was used to confirm MAGL occupancy in the brain. Currently Lu AG06466 is being explored in several Phase 1b studies in patients with neurologic and psychiatric disorders.