(Biochemistry and Chemical Biology)
Oral Abstract

Coumaplatin, a photocaged and nucleolus-targeted Pt(IV) anticancer prodrug to reduce drug resistance

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Targeted anticancer prodrugs that can be controllably activated are highly desired for cancer therapy. Herein, we report coumaplatin, an oxaliplatin-based and photocaged Pt(IV) prodrug, to realize nuclear accumulation along with "on-demand" activation. This prodrug is based on a Pt(IV) complex that can be efficiently photoactivated via water oxidation without the requirement of reducing agents. Coumaplatin exhibits up to two orders of magnitude increased photocytotoxicity than that of oxaliplatin, presents strikingly enhanced tumor penetration ability and utilizes a distinct action mode to overcome drug resistance. Our findings provide a novel strategy for the rational design of controllably-activated and nucleolus-targeted Pt(IV) prodrugs.[1]

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(Biochemistry and Chemical Biology)
Oral Abstract

Rapid detection of SARS-CoV-2 by mass spectrometry

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Abstract

The recent outbreak of COVID-19 has caused a serious worldwide health threat. To combat this pandemic, prompt detection of SARS-CoV-2 is essential. Currently, two main streams of detection approaches, i.e., nucleic acid and serological detection, are used. Real-time reverse transcription polymerase chain reaction is the golden method which aims to detect the viral RNA of SARS-CoV-2. Although this method possesses advantages such as high sensitivity and specificity, the accuracies of the results may be significantly lowered with the samples of low quality, which cannot provide viral RNA of good quality for the detection. The serological method detects the antibodies induced by SARS-CoV-2 and allows fast and on-site detection. However, it suffers from the high chance of false negative results especially during the onset of infection stage, and also the cross reactivity to other similar viruses. Here we propose a rapid and simple mass spectrometric detection method which is targeting the specific peptides of the SARS-CoV-2 proteins. Pretreatment steps of samples, e.g., saliva samples, include only a fast buffer exchange process and 3-minute trypsin digestion. Digestion patterns of the viral proteins were studied and peptides that are specific to SARS-CoV-2 were chosen as our targets. Multiple reaction monitoring (MRM) is utilized to detect the specific peptides because of its high specificity and sensitivity. Positive detection of the specific viral peptides indicates the presence of SARS-CoV-2. A semi-quantitative analysis could also be conducted to trace the changes of viral load in patients during the medical treatment.

(Biochemistry and Chemical Biology)
Oral Abstract

Development of NAD tagSeq for identification and characterization of NAD^+-capped RNA

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Abstract

Eukaryotic mRNA generally forms m^7G cap to protect the capped mRNA from degradation by exonucleases. Recent studies found NAD^+ (nicotinamide adenine dinucleotide) capping occurs in both prokaryotic and eukaryotic RNA. Several strategies have been developed to identify the NAD^+-capped RNA (NAD-RNA), including NAD captureSeq, however, the strategies are based second-generation sequencing and cannot directly quantify ratio of NAD^+ capping and characterize structure of NAD-RNA.[1]

Herein, we modify NAD captureSeq and develop a strategy called NAD tagSeq that utilizes third-generation sequencing platform to enable direct RNA sequencing of NAD-RNA.[2] NAD tagSeq firstly employs an enzyme called ADP-ribosyl cyclase to modify NAD^+ cap with a clickable group, then a click reaction is applied to graft an RNA tag onto the cap. The RNA tag could be used to discriminate NAD^+-capped RNA from noncapped RNA, it also serves the purpose if isolation of NAD-RNA is required. The RNA undergoes library preparation, followed by direct sequencing by Oxford Nanopore technology.

Using NAD tagSeq, we found that NAD-RNA in *Arabidopsis thaliana* were mostly produced by about 200 genes.[3] For some genes, NAD^+ capping accounted for higher than 5% of their transcripts. Comparison of mRNA with NAD^+ capping and m^7G capping showed the same sequence structure. Compared with NAD captureSeq, NAD tagSeq affords a simpler procedure for identification of NAD-RNA and quantification of NAD^+ capping ratio. NAD tagSeq allows for further analysis of the involvement of NAD^+ capping in regulation of RNA activity.

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(Biochemistry and Chemical Biology)
Oral Abstract

Point-of-care Pathogen Detection Based on Isothermal Amplification Methods

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The identification of pathogenic organism is essential to the prevention and distinguish the problems connected with health and safety. The growing public concern over the spread of the disease and the tough legislation in food industry made the failure to detect an infection might cause dreadful consequences. People are looking forward to obtaining the analytical results as soon as possible, the traditional pathogen detection protocol spend up to 6 to 10 days to yield an answer. Such long period is dissatisfactory, and many experts have geared their efforts towards the rapid, sensitivity methods. The availability of modern detection platforms plays a key role in the speed and accuracy of monitoring, surveillance, and quantitative infectious biological agent, and has a major influence on enacts regulations to promote the best practices to prevent pathophoresis.

This presentation is basically focused on developing convenient and effective isothermal amplification based methods for rapid pathogen detection and combining different techniques for nucleic acid quantitative analysis. On the basis of developed isothermal amplification detection technique, I explored its applications in POCT device, which exhibited great potentials in the electricity-free and equipment–free pathogen detection in field. Also, microfluidic technology minimized the POCT device with the help of novel valve design.

In the first part, an overview of POCT device for nucleic acid analysis devices including their significance, current advances and challenges are present. In the rest part, I present the research projects completed during past few years, including POCT in the field: a hand-powered and sample-in-answer-out device (HASDE) for nucleic acid detection without the requirement of electrical supply and extra equipment and a new paradigm for valve design in microfluidic chip for the facile isothermal sample-in-answer-out nucleic acid identification.

(Biochemistry and Chemical Biology)
Oral Abstract

Development of Multifunctional Synthetic Nucleosomes to Interrogate Chromatin-Mediated protein interactions

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Abstract

Genomic information of eukaryotic cells is stored in a DNA-protein complex termed chromatin. The basic repeating unit of chromatin is nucleosome, which consists of ~147 base pairs of DNA wrapping around a histone octamer containing two copies of histone H2A, H2B, H3 and H4 respectively. The composition and organization of chromatin are orchestrated by many chromatin-associated proteins, such as the enzymes that add or remove histone posttranslational modifications and chromatin remodelers that modulate nucleosome assembly. Malfunction of chromatin-associated proteins often results in dysregulation of DNA-templated processes such as gene transcription, DNA replication and damage repair, thus leading to human diseases such as cancer. Therefore, it is essential to carefully interrogate chromatin-associated proteins. Current methods to study chromatin-mediated protein interactions either have difficulty in characterizing the specific elements that mediate the interaction (e.g. chromatin immunoprecipitation) or fail to profile the context-dependent interactions using fragments of histones or DNA as 'bait'. Furthermore, the interactions mediated by dynamic chromatins are usually weak and transient, which are easily lost during biochemical pulldown processes. To address these problems, we developed the multifunctional synthetic nucleosomes that are site-specifically equipped with i) a photo-reactive group to establish covalent linkage to trap weak interactions upon UV irradiation; ii) a biorthogonal handle facilitating the isolation of crosslinked proteins; iii) a disulfide moiety that can rapidly release crosslinked peptide for mass spectrometry (MS) analysis. When coupled with state-of-art MS, the designer nucleosomes can not only identify specific chromatinassociated proteins from complex proteomes, but also characterize the binding regions of proteins that bind to chromatin in a nucleosomal context.

(Biochemistry and Chemical Biology)
Oral Abstract

Catalytic artificial organelles, multi-enzyme assemblies in *Escherichia coli* for terpene biosynthesis

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Abstract:
Multienzyme complexes, or metabolons, are natural assemblies or clusters of sequential enzymes in biosynthesis. Spatial proximity of the enzyme active sites results in substrate channeling effect, streamlines the cascade reaction, and increases the overall efficiency of the metabolic pathway[1-4]. In our lab, we are inspired to mimic the design of nature, and devise the synthetic multienzymes complexes as the catalytic nanomachineries inside lab workhorses such as *Escherichia coli* to attain a higher control of the metabolic flux and maximize the production of valuable natural compounds, such as beta-carotenoids, amopha-4,11-diene and a-farnesene.

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(Biochemistry and Chemical Biology)
Oral Abstract

Facile One-Pot Synthesis of Cyclic Peptide-Conjugated Photosensitizers for Targeted Antitumoral and Antibacterial Photodynamic Therapy

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Abstract

A novel synthetic strategy for in situ cyclization of peptides and conjugation with photosensitizers based on boron dipyrromethene (BODIPY) or zinc(II) phthalocyanine through a bifunctional linker has been developed. The bifunctional linkers are incorporated with a bis(bromomethyl)benzene unit and an azide or a cyclopentadiene moiety, which can facilitate the cyclization of peptides with two cysteine residues through site-selective alkylation, followed by coupling with a series of bicyclo[6.1.0]non-4-yne-functionalized BODIPYs via strain-promoted azide-alkyne cycloaddition or a maleimide-substituted phthalocyanine via Diels-Alder reaction, respectively. With this methodology, a series of cyclic peptide-conjugated photosensitizers were prepared readily. One of the cyclic RGD peptide-conjugated BODIPYs exhibited high and selective affinity toward the $\alpha_v\beta_3$ integrin-overexpressed cell lines and induced high photocytotoxicity. The phthalocyanine conjugated with an epidermal growth factor receptor (EGFR)-targeted cyclic peptide displayed superior features as an advanced photosensitizer for killing EGFR-overexpressed colorectal carcinoma cells, both in vitro and in vivo.[2] In addition, a phthalocyanine conjugated with a cyclic antimicrobial peptide was also prepared which exhibited a synergistic chemo-photodynamic cytotoxic effect against a spectrum of Gram-positive and Gram-negative bacterial strains, including ATCC-type and clinical isolates of multidrug-resistant bacterial strains.[3]

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(Biochemistry and Chemical Biology)
Oral Abstract

Discovery of Antimicrobials Targeting RNAP-Sigma Factor Interaction

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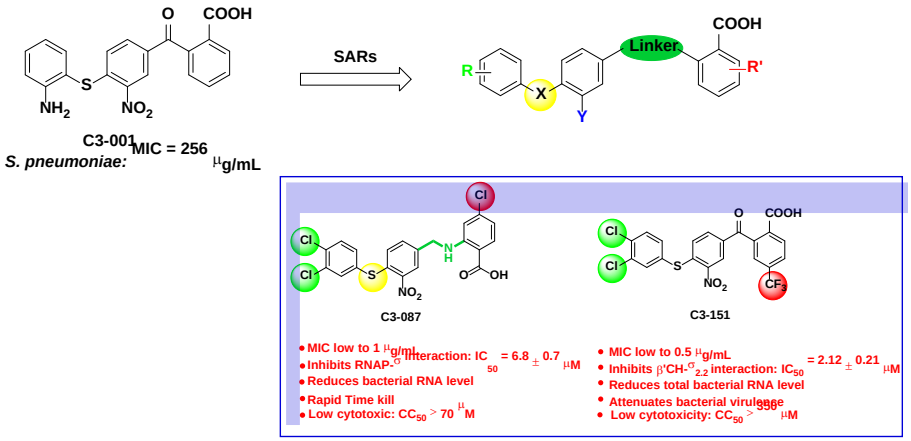
Transcription, which is regulated by RNA polymerase (RNAP) and a set of factors, is a critical process for bacterial viability. Our recent advances in the study of the interaction between RNAP and the initiation factor σ afforded a pharmacophore model for the development of novel antimicrobials. Some hits have been identified. Amongst, compound **C3-001** showed good inhibitory activity in disrupting the binding of σ to RNAP.

We thoroughly explored the structure-activity relationship (SAR) of compound **C3-001**. Several derivatives with significantly improved antibacterial activity were identified. The representative derivatives exhibited antibacterial activity against a panel of pathogens with minimum inhibitory concentrations (MICs) below 1 μg/mL, comparable to conventional antibiotics. Preliminary pharmacokinetic and safety studies also demonstrated the drug-like properties of compounds for further development.



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(Biochemistry and Chemical Biology)
Oral Abstract

NucleicNet and FragFeatureNet: Predicting RNA Binding Preferences on Proteins and Protein Fragment Inhibitors using Deep-Learning

HKUST - Michael Alexander Suarez Vasquez

Protein-RNA interaction plays important roles in post-transcriptional regulation, but the task of predicting these interactions computationally given a protein structure is difficult. Our deep-learning based NucleicNet model can thus serve to provide quantitative fitness of RNA sequences for given binding pockets or to predict potential binding pockets and binding RNAs for previously unknown RNA binding proteins. This was tested on three RNA-binding proteins; Fem-3-binding-factor, Argonaute 2 and Ribonuclease III successfully as experimentally observed interactions modes were recovered.

Fragment based drug design plays an important role in the drug-discovery process as a way to reduce the complex small molecule space into a more manageable fragment space. We developed a deep learning based model FragFeatureNet that incorporates both the protein drug target and inhibitor information and learns from the thousands of protein co-crystal structures in the PDB Database. We use it on the protease protein family with particular focus on the well-studied HIV-1 Protease for validation and study potential inhibitors of the newly discovered Sars-CoV-19 Main Protease. Preliminary results show FragFeatureNet's ability to recover a significant amount of inhibitor fragments for those two proteins tested against experimental literature.

(Biochemistry and Chemical Biology)
Oral Abstract

Title

Exploration of potential protein *R*-2-hydroxyglutarylation with synthetic probes

Authors

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Abstract

R-2-Hydroxyglutarate (*R*2HG), an important oncometabolite, is produced by mutated isocitrate dehydrogenase 1/2 (IDH1/2) in most reported cancers[1]. Current studies on its oncogenicity focus on IDH1/2 mutations and inhibition of α-ketoglutarate (αKG)-dependent dioxygenases[2-3]. However, according to published models, there remain unexplained regulatory differences between the effects of *R*2HG accumulation and IDH1/2 mutations on cellular fates[4-5]. The controversial roles of *R*2HG and its enantiomer *S*2HG in regulating αKG-dependent enzymes are still unclear[3, 6]. Encouraged by recently reported protein post-translational modifications (PTMs) such as succinylation[7], glutarylation[8-9] and lactylation[10], we hypothesized that *R*2HG also functions as a novel PTM, which may shed light on the relation between *R*2HG accumulation and its pathology.

In a MS/MS analysis of cell lysates, we identified a modified peptide with lysine (K) *R*2HGylation. A comparison of tandem MS spectra with those of synthetic peptides confirmed the modification and LC-MS co-elution revealed a carbonyl-selectivity of K-*R*2HGylation. Preliminary biological tests on 2HGylation targets suggested some unreported effects of *R*2HG pretreatment on multiple cellular fates including autophagy and phosphoprotein signaling pathways. Meanwhile, several alkyne probes were prepared based on their structural similarity to *R*2HG and metabolic labelling performance. With functionalized azide probes, we have identified multiple candidate *R*2HGylation protein targets that were enriched from total cell lysates and mapped with MS-based proteomics. Those synthetic molecular probes should accelerate our investigation on the 2HGylation-regulated process in human cancers.

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