2nd International Conference of RNA Nanotechnology and Therapeutics

April 3-5, 2013

Crowne Plaza-The Campbell House
Lexington, KY
Welcome

Dear Meeting Participants,

We want to thank everyone in attendance for convening in Lexington, KY at the Crowne Plaza Hotel to bring together the top RNA nanotechnologists in the world. The 2nd International Conference of RNA Nanotechnology & Therapeutics is the second series of a highly successful meeting first organized in 2010 in Cleveland, OH. We are elated to bring together more than 45 invited speakers and more than 200 participants from around the world (USA, UK, France, Denmark, Poland, Saudi Arabia, India, China, Japan, and Australia) to disseminate their knowledge involving RNA nanotechnology and its applications. This meeting aims to provide a platform for researchers from academia, government, and the pharmaceutical industry to share existing knowledge, vision, technology, and challenges in the field and promote collaborations among researchers interested in advancing this fascinating scientific discipline.

RNA is unique in nanoscale fabrication due to its amazing diversity of function and structure. A variety of single stranded loops are suitable for inter- and intra-molecular interactions serving as mounting dovetails in self-assembly without the need for external linking dowels. Computer modeling and simulation has allowed researchers to explore the folding properties and unique designs of a multitude of RNA nanoparticles. Furthermore, the novel properties of RNA nanoparticles have been explored for treatment and detection of diseases and various other realms. This meeting hopes to provide insight into many of the valuable applications that RNA nanotechnology has to offer to the scientific community.

Although we aim to dive deep into the vast world of RNA nanotechnology with a plethora of scientific presentations, we also want to promote collaborations throughout this broad field. In this regard, we have set forth time throughout the conference targeted to foster communication. On Tuesday night, a pre-meeting social will be held at the University of Kentucky’s College of Pharmacy. For Wednesday night, over 50 poster presentations will be sprawled across the banquet room of the Crowne Plaza where current and future stars of the field strive to provide an in-depth one on one interaction discussing their research. Thursday night will provide a mixture of events in which an international performing arts presentation will lead into an optional poster presentation or visit to the NIH/NCI Cancer Platform at the University. Finally, on Friday, immediately following the concluding remarks of the conference, shuttles will take participants to Keeneland to celebrate the rich horse culture that Lexington has to offer.

We have tried to eliminate as many hiccups as possible, but if there is anything we can do to facilitate your stay, please ask either the friendly staff here at Crowne Plaza or one of our amazing members of the local organizing committee. We will provide conference assistance throughout the meeting for any types of questions or concerns located at the registration desk. It is the goal of this conference to establish a relationship with you and your group, and we want to continue to foster this conference in the future, so please, any suggestions are greatly appreciated. Conference chairs, scientific committee, and all invited speakers will have a short discussion at dinner on Wednesday night to discuss how to build this group both in number and strength, including how to collaborate to build one cohesive RNA Nanotech community.

We ensure that you will find the meeting presentations pioneering, awe-inspiring, and insightful!

Thanks again,

Peixuan Guo (Chair)
William Farish Endowed Chair in Nanobiotechnology
Markey Cancer Center
College of Pharmacy, University of Kentucky

Neocles Leontis (Co-Chair)
Professor
Chemistry Department
Bowling Green State University

Bruce Shapiro (Co-Chair)
Head, Computational RNA Structure Group
Senior Investigator
Center for Cancer Research, National Cancer Institute

John Rossi (Co-Chair)
Morgan and Helen Chu Dean’s Chair
Professor, Dept. of Molecular and Cellular Biology
City of Hope
**Keynote speakers**

**Eric Westhof, Ph.D**
University Professor,
Head of the UPR 9002-ARN CNRS
“Architecture et Réactivité de l’ARN”
Institut de biologie moléculaire et cellulaire du CNRS, Strasbourg, France

April 4, 2013 10:40 AM
“Unusual” RNA Base Pairs in Recognition and Decoding

---

**David Lilley, Ph.D**
Professor of Molecular Biology
Director of the Cancer Research UK Nucleic Acid Structure Research Group
College of Life Sciences, University of Dundee, Dundee, Scotland, UK

April 3, 2013 10:40 AM
The k-turn: a key architectural element in RNA
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2nd International Conference of RNA Nanotechnology and Therapeutics

Conference Committee

Peixuan Guo (Chair)
University of Kentucky

Neocles Leontis (Co-Chair)
Bowling Green State University

Bruce Shapiro (Co-Chair)
National Cancer Institute

John Rossi (Co-Chair)
City of Hope
2nd International Conference of RNA Nanotechnology and Therapeutics

Local Host Committee

University of Kentucky

Chairs
Chad Schwartz
Farzin Haque

Members
Patrick McNamara
Daniel Binzel
Hui Li
Terry Keys
Peixuan Guo

Financial Committee
Judy Pistilli
Jonathan Burns
Janice Butner
# Meeting Overview

## Tuesday, April 2\textsuperscript{nd}

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:30 p.m. – 10:00 p.m.</td>
<td>Pre-Meeting Social</td>
<td>Univ. of Kentucky, Biopharm Complex</td>
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## Wednesday, April 3\textsuperscript{rd}

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>8:00 a.m. – 12:00 p.m.</td>
<td>Conference Sessions</td>
<td>Colonial Ballroom</td>
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<tr>
<td>12:00 p.m. – 1:00 p.m.</td>
<td>Lunch</td>
<td>Summit Room*</td>
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<tr>
<td>1:40 p.m. – 5:30 p.m.</td>
<td>Conference Sessions</td>
<td>Colonial Ballroom</td>
</tr>
<tr>
<td>5:30 p.m. – 6:30 p.m.</td>
<td>Dinner / Business Meeting</td>
<td>Paddock Room*</td>
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<tr>
<td>7:00 p.m. – 10:00 p.m.</td>
<td>Poster Session</td>
<td>Colonial Ballroom</td>
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## Thursday, April 4\textsuperscript{th}

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<tr>
<td>7:00 p.m. – 8:30 p.m.</td>
<td>Performance Arts</td>
<td>Colonial Ballroom</td>
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<tr>
<td>8:30 p.m. – 10:00 p.m.</td>
<td>Optional Guided Tour</td>
<td>Univ. of Kentucky, Biopharm Complex</td>
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<tr>
<td>8:30 p.m. – 10:00 p.m.</td>
<td>Optional Posters Session</td>
<td>Colonial Ballroom</td>
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## Friday, April 5\textsuperscript{th}

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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:00 a.m.  – 11:15 a.m</td>
<td>Conference Sessions</td>
<td>Colonial Ballroom</td>
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<tr>
<td>11:15 a.m. – 11:30 a.m.</td>
<td>Board Vans to Keeneland</td>
<td>Front Hotel Entrance</td>
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<tr>
<td>12:00 p.m. – 5:30 p.m.</td>
<td>Horse Racing</td>
<td>Keeneland Track</td>
</tr>
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* Lunches and dinners are reserved for invited speakers and paid registrants with food option.
Hotel and Conference Location:

Crowne Plaza Hotel
1375 South Broadway, Lexington, KY 40504, USA
Tel: 859-255-4281
(Note: A shuttle will be provided from the Airport to the Crowne Plaza Hotel. Please contact the hotel prior to your arrival for arrangements (859) 255-4281.)

Registration, Badge Pick-Up, and Conference Assistance:

Tuesday, April 2, 2013 7:00 p.m – 10:30 p.m.
Wednesday, April 3, 2013 7:30 a.m. – 5:00 p.m.
Thursday, April 4, 2013 8:00 a.m. – 5:00 p.m.
Friday, April 5, 2013 7:30 a.m. – 12:00 p.m.

Location: Pre-function Lobby (directly outside Colonial Ballroom)

General Information:

- **Badge**: Admission to all scientific sessions, poster areas, exhibits and social functions is by badge only.
- **Business Center**: Located in the hotel first floor.
- **Internet access**: Free wireless internet throughout the hotel and conference rooms.
- **Parking**: Free parking in the lot surrounding the hotel.

Tuesday, April 2, 2013

7:30 – 10:00 p.m.  *Pre-Meeting Social*
University of Kentucky, College of Pharmacy
Room: 589, BioPharm Complex
789 South Limestone Ave. Lexington, KY, 40536

(Note: A shuttle will be provided to and from the Crowne Plaza Hotel and the University of Kentucky College of Pharmacy; 10 min walking distance from the hotel)
Wednesday, April 3, 2013

6:30 – 7:45 a.m.  Breakfast  
*Kilbern’s Restaurant*  
(Provided for all hotel guests)

8:00 – 8:15 a.m.  Opening Remarks  
*Patrick McNamara*, University of Kentucky  
*Dean of College of Pharmacy*

8:20 – 8:35 a.m.  Introduction and Welcome  
*Peixuan Guo*, University of Kentucky  
*Oral Abstract # 1*

**Session 1: Design, Construction, and Assembly of RNA Nanoparticles - I**

**Session Chairs:**  
*Neocles Leontis*, Bowling Green State University  
*Daxiang Cui*, Shanghai Jiao Tong University, China

8:40 – 9:00 a.m.  Aptamer-enabled synthetic immune response  
*Hua Shi*, University at Albany, SUNY  
*Oral Abstract # 2*

9:00 – 9:20 a.m.  RNA Design Rules from a Massive Open Online Laboratory  
*Rhiju Das*, Stanford University  
*Oral Abstract # 3*

9:20 – 9:40 a.m.  Single-molecule Imaging to Study the Structure and Assembly of RNA Nanoparticles  
*Hui Zhang*, University of Kentucky  
*Oral Abstract # 4*

9:40 – 10:00 a.m.  Next Generation X-Aptamers for Personalized Nanomedicine  
*David Gorenstein*, The University of Texas Health Center at Houston  
*Oral Abstract # 5*

10:00 – 10:20 a.m.  Non-Coding RNAs as New Theranostic Tools  
*Shufeng Zhou*, University of South Florida  
*Oral Abstract # 6*

10:20 – 10:35 a.m.  *Coffee Break*

**Keynote Address - I**

10:40 – 11:15 a.m.  The k-turn: a key architectural element in RNA  
*David Lilley*, University of Dundee, United Kingdom  
*Oral Abstract # 7*
Session 2: RNA Dynamics and Intermolecular RNA Interactions in RNA Nanoparticles

Session Chairs: Bruce Shapiro, National Cancer Institute  
Donglu Shi, University of Cincinnati

11:20 – 11:40 a.m. Synthetic RNP-based nanostructures and genetic switches: Possible therapeutic applications  
Hirohide Saito, Kyoto University, Japan  
Oral Abstract # 8

11:40 – 12:00 p.m. RNA 3D Hub: Web resources for RNA Structure prediction, comparison, and design  
Neocles Leontis, Bowling Green State University  
Oral Abstract # 9

12:00 – 1:00 p.m. Lunch  
Summit Room  
(Provided for invited speakers and paid registrants with included meals)

1:40 – 2:00 p.m. Characterization of trans-acting RNA-based switches for gene expression control  
Carla Theimer, University at Albany, SUNY  
Oral Abstract # 10

2:00 – 2:20 p.m. Nanoimaging of Nucleic Acids and Nucleoprotein Complexes  
Yuri Lyubchenko, University of Nebraska Medical Center  
Oral Abstract # 11

2:20 – 2:40 p.m. Predicting 3D RNA structure and dynamics using Discrete Molecular Dynamics  
Nikolay Dokholyan, University of North Carolina at Chapel Hill  
Oral Abstract # 12

Session 3: Synthetic carriers, Biological, and Exosomal Delivery of RNA Nanoparticles to Cells - I

Session Chairs: Huang-Ge Zhang, University of Louisville  
Shufeng Zhou, University of South Florida

2:45 – 3:05 p.m. DNA repair, modification and engineering by transcript RNA  
Francesca Storici, Georgia Institute of Technology  
Oral Abstract # 13

3:05 – 3:25 p.m. Nanosized Structures as siRNA Carriers Improved for Gene Therapy  
Xing-Jie Liang, National Center for Nanoscience and Technology, China  
Oral Abstract # 14
Simultaneous activation of multiple split functionalities inside cells through anagnoristic R/DNA hybrids: a novel approach in therapeutic RNA nanotechnology
**Kirill Afonin**, National Cancer Institute
*Oral Abstract # 15*

**3:45 – 4:00 p.m.**  
*Coffee Break*

Microfluidic capture of tumor-derived extracellular vesicles from serum
**Kristan Van der Vos**, Harvard University
*Oral Abstract # 16*

Hepatitis C virus subverts cellular RNA granules and the liver-specific microRNA miR-122 to promote viral gene expression
**Cara Pager**, University at Albany, SUNY
*Oral Abstract # 17*

Studying the therapeutic neural stem cell plasticity at nanoscale
**Stefano Pluchino**, University of Cambridge, United Kingdom
*Oral Abstract # 18*

Synthetic carriers of siRNA by rational design
**Tuo Jin**, Shanghai Jiao Tong University, China
*Oral Abstract # 19*

**5:30 – 6:30 p.m.**  
*Dinner and Business Meeting*  
*Paddock Room*  
(Provided for invited speakers and paid registrants with included meals)

**7:00 – 10:00 p.m.**  
Posters  
*Colonial Ballroom A, D and F*
Thursday, April 4, 2013

6:30 – 7:45 a.m.  Breakfast  
*Kilbern’s Restaurant*  
(Provided for all hotel guests)

**Session 4:  Design, Construction, and Assembly of RNA Nanoparticles - II**

**Session Chairs:**  Douglas Taylor, University of Louisville  
David Gorenstein, The University of Texas Health Center at Houston

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8:00 – 8:20 a.m.  Computational Prediction and Experimental Self-assembly of Multi-Sequence Nucleic Acid-Based NanoStructures  
*Bruce Shapiro*, National Cancer Institute  
*Oral Abstract # 20*

8:20 – 8:40 a.m.  Specific RNA-capsid contacts in the virions of a positive-strand RNA virus.  
*C. Cheng Kao*, Indiana University  
*Oral Abstract # 21*

8:40 – 9:00 a.m.  Approaches in constructing RNA nanoparticles with applications in nanotechnology and nanomedicine  
*Peixuan Guo*, University of Kentucky  
*Oral Abstract # 22*

9:00 – 9:20 a.m.  Beyond Fluorescence: Small and Bright Upconversion Nanoparticles for Biological Imaging  
*Gang Han*, University of Massachusetts Medical School  
*Oral Abstract # 23*

9:20 – 9:40 a.m.  Toward the Rational Pre-Clinical Application of Nanomaterial RNA and Protein Conjugates  
*Robert DeLong*, Missouri State University  
*Oral Abstract # 24*

9:40 – 10:00 a.m.  Global structure of a three-way junction in a phi29 packaging RNA dimer determined using site-directed spin labeling  
*Peter Qin*, University of Southern California  
*Oral Abstract # 25*

10:00 – 10:20 a.m.  Nanopore-facilitated single molecule detection of circulating microRNAs in lung cancer patients  
*Liqun (Andrew) Gu*, University of Missouri  
*Oral Abstract # 26*

10:20 – 10:35 a.m.  Coffee Break
**Keynote Address - II**

10:40 – 11:15 a.m. “Unusual” RNA Base Pairs in Recognition and Decoding  
*Eric Westhof*, CNRS Strasbourg, France  
*Oral Abstract # 27*

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**Session 5: Synthetic, Biological, and Exosomal Delivery of RNA Nanoparticles to Cells - II**

**Session Chairs:**  
*Liqun (Andrew) Gu*, University of Missouri  
*Robert Batey*, University of Colorado Boulder

11:20 – 11:40 a.m. Exosomes that Target Antigen Specifically and Deliver dsRNA of Choice for Physiologic for Exquisitely Specific siRNA Therapy  
*Philip Askenase*, Yale University  
*Oral Abstract # 28*

11:40 – 12:00 p.m. Polymeric/Viral Nanobiovectors for Efficient Gene Delivery  
*Dan Pack*, University of Kentucky  
*Oral Abstract # 29*

12:00 – 1:00 p.m. **Lunch**  
*Paddock Room*  
(Provided for invited speakers and paid registrants with included meals)

1:40 – 2:00 p.m. Exosome RNA cargoes serve as a “liquid biopsy” for diagnosis of cancer  
*Douglas Taylor*, University of Louisville  
*Oral Abstract # 30*

2:00 – 2:20 p.m. Dual function therapeutic Aptamer-siRNA nanoparticles  
*Kato Shum*, City of Hope  
*Oral Abstract # 31*

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**Session 6: Design, Construction, and Assembly of RNA Nanoparticles - III**

**Session Chairs:**  
*Stefano Pluchino*, University of Cambridge, United Kingdom  
*Robert DeLong*, Missouri State University

2:25 – 2:45 p.m. Fluorescent RNA Biosensors for Bacterial Signaling Molecules  
*Ming Hammond*, University of California Berkeley  
*Oral Abstract # 32*

2:45 – 3:05 p.m. Assembly of Thermodynamically and Chemically Stable RNA Nanoparticles Escorting siRNA, Ribozyme and miRNA with independent folding for Cancer targeting  
*Farzin Haque*, University of Kentucky  
*Oral Abstract # 33*
3:05 – 3:25 p.m.  Clicks and Tricks with RNA for Nanobiotechnology
Subha Das, Carnegie Mellon University
Oral Abstract # 34

3:25 – 3:45 p.m.  Towards facile engineering of novel riboswitches
Robert Batey, University of Colorado Boulder
Oral Abstract # 35

3:45 – 4:00 p.m.  Coffee Break

4:05 - 4:25 p.m.  Selenium-Nucleic Acids (SeNA) for Structure, Function, and Drug Discovery Studies
Zhen Huang, Georgia State University
Oral Abstract # 36

4:25 – 4:45 p.m.  Nanoengineering new siRNA delivery platforms
Jorgen Kjems, Aarhus University, Denmark
Oral Abstract # 37

4:45 – 5:05 p.m.  Nanoparticle-based Artificial RNA Silencing Machinery for Antiviral Therapy
Y. Charles Cao, University of Florida
Oral Abstract # 38

5:05 – 5:25 p.m.  RNA nanotechnology for next generation computer design
Meikang Qiu, University of Kentucky
Oral Abstract # 39

5:30 – 6:30 p.m.  Dinner
Paddock Room
(Provided for invited speakers and paid registrants with included meals)

7:00 – 8:30 p.m.  Presentation of International Performing Arts
Colonial Ballroom

8:30 – 10:00 p.m.  Option # 1
Guided Tour of NCI- NIH/NCI Cancer Nanotechnology Platform Partnership
Program: "RNA Nanotechnology for Cancer Therapy"
Location: University of Kentucky, College of Pharmacy
Room: 576, BioPharm Complex
789 South Limestone Ave. Lexington, KY, 40536

For any questions, contact: 859-218-0139 (Lab);
(See maps in the back of abstract book for directions to the facility; 10 min walk from hotel; shuttle will be available 8:30 - 10:30 p.m.)

8:30 – 10:00 p.m.  Option # 2
Viewing of posters
Colonial Ballroom A, D and F.
**Friday, April 5, 2013**

6:30 – 7:45 a.m.  Breakfast  
*Kilbern’s Restaurant*  
(Provided for all hotel guests)

<table>
<thead>
<tr>
<th>Session Chairs:</th>
<th>Synthetic, Biological and Exosomal Delivery of RNA Nanoparticles to Cells - III</th>
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**Session Chairs:**  
**Hirohide Saito,** Kyoto University, Japan  
**Zhijie Liu,** Institute of Biophysics, CAS, China

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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</table>
| 8:00 – 8:20 a.m.| The Utilization of Macrophage-Derived Extracellular Vesicles to Deliver RNA Molecules to Target Cells  
**Melissa Piper,** The Ohio State University  
*Oral Abstract # 40* |
| 8:20 – 8:40 a.m.| Exosomes as mediators of intercellular transport of oncogenic cargo  
**Suresh Mathivanan,** La Trobe University, Australia  
*Oral Abstract # 41* |
| 8:40 – 9:00 a.m.| Transcriptional and Translational control in the Germ line  
**Prashant Rangan,** University at Albany, SUNY  
*Oral Abstract # 42* |
| 9:00 – 9:20 a.m.| Targeting colorectal cancer liver metastases with folate-conjugated ultrastable RNA nanoparticles  
**Piotr Rychahou,** University of Kentucky  
*Oral Abstract # 43* |
| 9:20 – 9:40 a.m.| Intestine mucus-derived exosomes provide a bridge between the gut and the liver that suppresses liver inflammation through prostaglandin E₂ induction of NKT cell anergy  
**Huang-Ge Zhang,** University of Louisville  
*Oral Abstract # 44* |
| 9:40 – 10:00 a.m.| Engineered “restriction RNases” for sequence-specific cleavage of dsRNA and RNA in DNA-RNA hybrids  
**Janusz Bujnicki,** Int'l Institute of Molecular and Cell Biology In Warsaw, Poland  
*Oral Abstract # 45* |
| 10:00 – 10:20 a.m.| Valproic Acid causes proteasomal degradation of DICER, changes miRNA expression and miRNA composition in exosomes  
**Stefan Stamm,** University of Kentucky  
*Oral Abstract # 46* |
| 10:25 – 10:40 a.m.| Coffee Break |
10:45 – 11:15 a.m.  *Closing Remarks and Awards Ceremony*
*Peixuan Guo*, University of Kentucky
*Bruce Shapiro*, National Cancer Institute
*Neocles Leontis*, Bowling Green State University

11:30 a.m.  Board Shuttles to Keeneland

12:00 – 5:30 p.m.  Keeneland Horse Track
(Note: A shuttle will be provided from hotel to airport and from horse track to airport.)
Oral Abstracts
In order of presentation

Crowne Plaza – The Campbell House
Lexington, Kentucky, USA
April 3-5, 2013
Welcome and introduction to RNA nanotechnology

Peixuan Guo

Nanobiotechnology Center, Markey Cancer Center, and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY, USA. Email: peixuan.guo@uky.edu

I am glad to see that so many outstanding scientists from different countries have come to this meeting to discuss common interests in RNA nanotechnology and RNA therapeutics.

RNA nanotechnology is a unique field that is distinct from the classical studies on RNA structure and function. RNA nanotechnology focuses on construction and assembly of RNA architectures composed purely of RNA in nanometer scale. This conference includes a heavy volume of presentations on RNA computation and folding. The computation and folding in RNA nanotechnology focuses on inter-RNA interactions for assembling quaternary RNA nanoparticles, while conventional approaches focus on intra-RNA folding.

RNA has recently emerged as an important nanotechnology platform due to its diversity in structure and functions. RNA nanoparticles can be fabricated with a level of simplicity characteristic of DNA, while possessing versatile tertiary structure and catalytic functions that can mimic some proteins. RNA is unique in comparison to DNA by virtue of its higher thermodynamic stability, the formation of both canonical and noncanonical base pairings, the capability in base stacking, and distinctive \textit{in vivo} attributes. A large variety of single stranded loops are suitable for inter- and intra-molecular interactions, serving as a mounting dovetail in quaternary structure assembly. Controlling the ratio of 2’-modified ribonucleotide enables regulation of the stability for the desired \textit{in vivo} circulation time frame. Unique features in transcription, termination, self-assembly, and self-processing enable \textit{in vivo} production of nanoparticles harboring aptamer, siRNA, ribozyme, riboswitch, or miRNA for therapy, detection, regulation, and intracellular computation. I will provide some solutions and perspectives on the chemical and thermodynamic stability, \textit{in vivo} half-life and biodistribution, yield and production costs, \textit{in vivo} toxicity and side effects, specific delivery and targeting, as well as endosomal trapping and escape.

References:
Oral abstract: 2

Aptamer-enabled synthetic immune response

Hua Shi

Department of Biological Sciences and the RNA Institute, University at Albany, SUNY

Keywords: aptamer, synthetic biology, cancer

As a branch of synthetic biology, synthetic immunology seeks to modulate existing immunity or generate new immune response in living organisms. This forward engineering approach provides an exciting perspective for the rational and predictive control of the immune system to fulfill experimental or therapeutic purposes. At the center of the complex nature of both innate and adaptive immune systems is the dynamic relationship between pathogens and the immuno-effector mechanisms that destroy and eliminate them. Consequently, modifying or creating the pathogen-effector interaction is a promising strategy to transform our capability of treating recalcitrant diseases such as cancer. A nano-scale synthetic molecular linkage forged between a disease-causing molecule or cell and an immune effector could bring about the damage or removal of the target, especially when the target is not recognizable by the immune system as “foreign.” We have been implementing this scheme through the design and construction of multi-valent aptamers. We have successfully elicited multiple effector mechanisms enacted by the complement system to annihilate both molecular and cellular targets.
Oral abstract: 3

RNA design rules from a massive open online laboratory

Jeehyung Lee¹, Wipapat Kladwang², Minjae Lee¹, Daniel Cantu², Martin Azizyan¹, Hanjoo Kim³, Alex Limpaecher³, Sungroh Yoon³, Adrien Treuille¹, Rhiju Das²,⁴, EteRNA players

¹Computer Science Department, Carnegie Mellon University, Pittsburgh, PA 15206, USA; ²Biochemistry Department, Stanford University, Stanford, CA 94305, USA; ³Department of Electrical and Computer Engineering, Seoul National University, 151-741, Korea; ⁴Physics Department, Stanford University, Stanford, CA 94305, USA

Keywords: cloud biochemistry, crowd sourcing, chemical mapping, internet-scale science, videogame

Self-assembling RNA molecules present compelling substrates for the rational interrogation and control of living systems. However, imperfect in silico folding models – even at the basic secondary structure level – hinder efforts to design new RNAs that function properly when synthesized. Here, we describe EteRNA, a 37,000-participant on-line discovery project focused on RNA design. Unique among internet-scale projects, EteRNA connects participants with high-throughput experiments in which designs are synthesized and structurally probed. Through this continuous experimental feedback, EteRNA players now substantially outperform existing algorithms for designing new RNA secondary structures, including complex dendrimer folds and scaffolds for small-molecule sensors. Furthermore, we have formalized community-proposed rules using machine learning into an automated design algorithm EteRNABot, which also significantly outperforms prior algorithms in rigorous tests. These results show that experimentally driven hypothesis generation – the scientific method itself – can be successfully carried out by a “massive open on-line laboratory”.

Oral abstract: 4

Single-molecule imaging to study the structure and assembly of RNA nanoparticles

Hui Zhang, Dan Shu, Peixuan Guo

Nanobiotechnology Center, Markey Cancer Center and Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40536, USA

The dimension of RNA nanoparticles is often beyond the limit of resolution power of conventional optical imaging system. Special imaging system is desirable to elucidate the structure and assembly of RNA nanoparticles. A customized dual-channel single molecule imaging system has been constructed to study the phi29 DNA-packaging motor that is geared by a hexameric pRNA ring. The motor pRNA molecules have been reported to serve as building blocks in RNA nanotechnology, and as vehicles for specific delivery of therapeutics to treat cancers and viral infections. The understanding of the stoichiometry and the 3D structure of the pRNA is both fundamentally and practically important. The imaging system with single fluorophore sensitivity and multicolor detection ability is the combination of a low-temperature (-80°C) sensitive electron multiplied CCD camera with both objective- and prism-type TIRF (Total Internal Reflection Fluorescence) mechanism. A laser combiner was introduced to facilitate simultaneous multi-color imaging. Two lasers with different wavelengths were delivery synchronically via an optic fiber to the sample chamber with TIRF capability. Single molecule photobleaching combined with binomial distribution analysis revealed the stoichiometry of the pRNA on active motors. Crystallography and biophysical studies further confirmed that the pRNA forms a hexamer ring on the motor. The ability of using single molecule FRET in distance determination within the nanomotor was tested. Single molecule FRET study was carried out to investigate the structure of the pRNA dimer. Ten pRNA monomers labeled with single donor/acceptor fluorophore pairs at various locations were constructed, and eight partner pairs were assembled into dimers. FRET signals were detected for six dimers and utilized to assess the distance between each donor/acceptor pair. The results provided the distance constraints to refine the previously reported 3D model of the pRNA dimer. The analysis of the pRNA structure will help to improve the designs of therapeutic pRNA-based nanoparticles.

References:

Oral abstract: 5

Next Generation X-aptamers for personalized nanomedicine

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Enhanced nuclease stability, affinity and specificity of oligonucleotide siRNA and aptamer ligands can be accomplished by substituting sugar phosphate backbones with either monothiophosphates or dithiophosphates and other chemical modifications. We can also enhance the affinities and specificity of next-generation “X”-aptamers by substituting 5-X-dU’s with a wide range of small organic drugs and amino-acid like sidechains into the bases. A unique automated “split-pool” bead-based combinatorial library and bead selection technology allows substitutions of both bases, sugars and phosphates in any position along the oligonucleotide chain. These X-aptamers thus represent a hybrid of aptamer backbone, protein amino acid-like sidechains, and small drug molecules in a self-folding scaffold that can be readily identified by oligonucleotide sequencing. We show that dithioate modified siRNA’s have enhanced silencing properties and will discuss targeted nanoparticle delivery of drugs with X-aptamer targeting reagents. Examples of application of the bead-based X-aptamer selection are demonstrated for targeting and imaging of cancer tumors expressing CD44, E-Selectin and patient-specific surface receptors for personalized medicine.

*Disclosure: Financial interest in AM Biotechnologies, Inc.
Oral abstract: 6

Non-coding RNAs as new theranostic tools

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A non-coding RNA (ncRNA) is a functional RNA molecule that is not translated into a protein. ncRNAs include highly abundant and functionally important RNAs such as transfer RNA (tRNA) and ribosomal RNA (rRNA), as well as RNAs such as snoRNAs, miRNAs, siRNAs, snRNAs, exRNAs, and piRNAs and the long ncRNAs. miRNAs are the most well studied ncRNAs and their pivotal role in orchestrating tumor initiation and progression has been confirmed in all types of cancers. Hence, these small ncRNAs have emerged as attractive therapeutic targets and diagnostic tool. However, the delivery of ncRNAs into target sites is a challenge given that ncRNAs are often unstable and negatively charged. Naked siRNA is rapidly degraded by enzymatic degradation, and cellular uptake of siRNA is poor in vivo. Additionally, several intracellular barriers, such as unpacking of siRNA/polycation complex, must be circumvented when developing of new, safe and efficient siRNA delivery vectors. Our laboratory has designed different methods to improve the delivery of siRNAs. For example, a redox-responsive hyperbranched poly(amido amine) (named PCD) was synthesized and used to form ternary complex with siRNA and hyaluronic acid (HA) for target-specific siRNA delivery. A targeting amphiphilic cyclodextrin-fullerene based supramolecule serving as the siRNA delivery vehicle have been constructed, and the multifunctional nano-system is aimed to elicit synergistic effect and deliver siRNA to disease target preferentially. We are developing a traceable, specific and stable quantum dot-based delivery approach to deliver siRNA for cancer therapy. An ideal delivery system should be able to deliver the ncRNAs into target cells and the behavior of ncRNAs can be easily traced.
The k-turn: a key architectural element in RNA

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The kink-turn (k-turn) is a widespread and important element in the architecture of RNA molecules. It generates a local kink in an RNA helix with an included angle of 60°, thus mediating long-range interactions in folded RNA. k-turns occur in both ribosomal subunits, in box C/D and H/ACA snoRNA, the U4 snRNA and some riboswitches. They are therefore important in translation, RNA modification and splicing, and control of gene expression. In the absence of metal ions, the k-turn adopts a less-tightly kinked structure, but can be induced to fold into its kinked conformation by one or more of three processes.

(1) By the addition of metal ions in a two-state folding process.
(2) The binding of specific proteins can induce the folding of k-turns. This can occur with very high affinity; for example A. fulgidus L7Ae induces the folding of Kt-7 with an apparent affinity of $K_d = 10$ pM. Other ribosomal proteins including L24, S17 and S11 also induce the formation of k-turn structure. We have analyzed the process of protein-induced folding of the k-turn using single-molecule experiments, from which we conclude that it occurs by conformational selection.
(3) Tertiary contacts in species such as the SAM-I riboswitch can stabilize the folded form of the k-turn, and indeed stabilize k-turns that are unable to fold by addition of metal ions. Thus potentially k-turns can play a key role in RNA folding in a delicate interplay involving sequence variation, protein binding and tertiary contacts.

We have dissected the role of hydrogen bonding in stabilizing the folded conformation of the k-turn in detail. We show that the k-turns exist in two structural classes that differ in key hydrogen bonding patterns. Moreover, the same sequence can adopt either structure depending upon its environment. We have determined the crystal structure of Kt-7 in a variety of situations, showing the variation in geometry as a function of protein binding and tertiary contacts. We have created a web-based database of k-turn structures, see: http://www.dundee.ac.uk/biocentre/nasg/kturn/

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Synthetic RNP-based nanostructures and genetic switches: possible therapeutic applications

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We have recently succeeded in designing nanostructures by using RNA-protein complexes (RNP). Synthetic nanostructures consisting of biomacromolecules such as nucleic acids have been constructed using bottom-up approaches. In particular, Watson-Crick base pairing has been used to construct a variety of two- and three-dimensional DNA nanostructures. We show that RNA and the ribosomal protein L7Ae can form a nanostructure shaped like an equilateral triangle that consists of three proteins bound to an RNA scaffold. The construction of the complex relies on the proteins binding to kink-turn (K-turn) motifs in the RNA, which allows the RNA to bend by ∼ 60° at three positions to form a triangle. Functional RNA-protein complexes constructed with this approach could have applications in nanomedicine and synthetic biology. Design and construction of functional RNP nanostructures that detect and kill specific cancer cells will be discussed.

Moreover, we have developed synthetic RNP translational switches to control protein production level depending on intracellular environment. We use three components to construct a system: an intracellular protein as a trigger factor, the RNA aptamer module that binds to the trigger factor, and the designed mRNA or short hairpin RNA (shRNA) as a gene expression controller: The aptamer module was successfully incorporated into the synthetic mRNA or shRNA to generate functional RNP switches. The designed ON/OFF translational switches detect the expression of the trigger factor and repress or activate the expression of a desired protein (e.g.; apoptosis regulator) in the target mammalian cells. The method has potential for curing cellular defects or improving the intracellular production of useful molecules by bypassing or rewiring intrinsic signal networks.

Taken together, we would like to emphasize that RNA-protein interacting motifs could be employed for constructing both designer genetic switches and functional nanoarchitectures to regulate cellular functions. these approaches could have applications in nanomedicine and synthetic biology.
Oral abstract: 9

RNA 3D hub: web resources for RNA structure prediction, comparison, and design

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I will discuss the conceptual tools we have developed to analyze, classify, and annotate RNA 3D structures (Hoehndorf et al. 2011). Using these tools, we have written software to automatically annotate all RNA-containing PDB structures for key interactions and structural features (see link 1 below) and to search RNA 3D structures to find, identify and cluster 3D motifs (WebFR3D application, link 2) and to align 3D structures (WebR3DAlign application, link3) (Rahrig et al. 2010; Petrov et al. 2011). We have created an automatic data pipeline to maintain updated Non-Redundant lists of RNA-containing PDB files for searching and motif extraction (link 4). PDB files are grouped in equivalence classes (for example, all structures containing E.coli 16S rRNA belong to the same class). All hairpin, internal and junction motifs are extracted from the NR dataset and clustered by structural similarity on a regularly updated basis and are made available as the RNA 3D Motif Atlas (link 5). The motif families are used to construct SCFG-MRF grammars for prediction of 3D motifs starting with sequence data (JAR3D application, link 6). All these resources are being integrated into a dynamic suite of software and databases called RNA 3D Hub (link 7) that will also be accessible through the NDB (link 8). Use cases to illustrate the utility of these resources will be presented.

References:


Links:
1. http://rna.bgsu.edu/rna3dhub/pdb
5. http://rna.bgsu.edu/rna3dhub/motifs
7. http://rna.bgsu.edu/rna3dhub/
8. http://ndbserver.rutgers.edu/
Oral abstract: 10

Characterization of trans-acting RNA-based switches for gene expression control

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Keywords: ncRNA, microRNA, gene expression control, RNA-protein interactions

We are characterizing a novel RNA-based nanoswitch technology termed structurally interacting RNAs (sxRNAs), which utilize post-transcriptional gene regulation as a reporter of non-coding RNA expression, such as miRNAs. Unlike other reporter systems that decrease gene expression upon miRNA induction, our system results in an increase in reporter gene expression upon miRNA induction. The sxRNA platform is based on transacting RNA complexes that can enhance or disrupt RNA binding protein (RBP) interactions. RBPs frequently bind to a stem-loop structure within the non-coding portion of the mRNA, such as the downstream-untranslated region of a message. Some RBPs increase the translation of an mRNA by an order of magnitude when they bind. In addition to characterizing artificially designed sequences, we are also characterizing natural switch sequences which demonstrate gene expression control in vivo.

It is possible to modify the mRNA to modulate translation of the reporter gene by controlling the binding of the RBP. This is accomplished by strategically altering the natural stem-loop structural target of an RBP. We have demonstrated that we can custom design a sxRNA in which the natural RBP-binding structure is altered so that it only correctly forms when a second RNA, such as a miRNA, binds in trans and stabilizes it, by base-pairing to the flanking region. In this study, we have biophysically characterized in vitro a switch that demonstrated increased binding (by as much as 5X) for the histone stem-loop binding protein (HSLBP). These findings were validated in vivo successfully. These biophysical studies examine the interactions of this sxRNA platform and how that interaction can be modulated to alter protein binding.
Nanoimaging of nucleic acids and nucleoprotein complexes

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Keywords: Atomic Force Microscopy, AFM, nanoimaging, RNA nanostructures, chromatin dynamics, DNA, single stranded DNA binding complexes

AFM is the nanoimaging technique with a great potential for molecular biophysics of nucleic acids. Advances in the sample preparation techniques for AFM coupled with emerging of high-speed AFM (HS AFM) instrumentation led to the progress in the high-resolution study of a number of nucleic acids and nucleoprotein complexes complexes. This advances are demonstrated by images of self-assembled RNA nanoassemblies of different types. The dynamics of these structures was characterized by the time-lapse HS AFM data. The time-lapse AFM technique was also instrumental in understanding dynamics of various nucleoprotein complexes including nucleosomes. We demonstrated that nucleosomes are quite dynamic rather than static systems and answered a number of important questions related to nucleosome dynamics. Single-stranded DNA binding proteins are ubiquitous systems involved in DNA replication, recombination, transcription and various modifications of DNA. We have developed a novel approach for studies of ssDNA binding proteins with AFM in which a hybrid DNA substrate with desired structure and sequence is used. In such designs, ssDNA of a specific length is either attached to one of the ends of dsDNA or placed between the two DNA duplexes. Time-lapse HS AFM was applied to look at the DNA-protein interaction dynamics including dissociation and association steps for both systems and A3G sliding along the DNA substrate.
**Oral abstract: 12**

**Predicting 3D RNA structure and dynamics using discrete molecular dynamics**

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RNA function depends on its structure, yet three-dimensional structures of most biologically important RNAs are unknown. Many RNAs contain both highly structured and also functionally important flexible elements, limiting high-resolution structure determination using either x-ray crystallography or NMR. Therefore, it is important to computationally model RNA structures as an alternative. The vast conformational space makes the direct computational modeling of RNA folding difficult. We develop a multiscale approach for RNA folding using discrete molecular dynamics (DMD). DMD simulations feature rapid sampling of conformational space, and we use a coarse-grained representation to effectively model RNA structures. Benchmark study suggests that the DMD-based RNA model is able to accurately fold small RNA molecules. However, the large conformational space and force field inaccuracies make it difficult to computationally identify the native states of large RNA molecules. We devised an automated modeling approach for determination of large and complex RNA structures using experimentally derived structural information. We tested the structure determination method on several RNA molecules with known experimental structures. In all cases, we were able to find native states of these RNA molecules. Therefore, a combination of experimental and computational approaches has the potential to yield native-like models for the diverse universe of functionally important RNAs, whose structures cannot be characterized by conventional structural methods.
Oral abstract: 13

DNA repair, modification and engineering by transcript RNA

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Keywords: RNA, DNA repair, double-strand break repair, transcript RNA, gene targeting, in vivo genome engineering

We found that RNA transcripts, generated inside cells, can have a direct role in maintaining genomic stability of cells serving as templates in the repair of damaged DNA, and can directly transfer genetic information to chromosomal DNA. A DNA double-strand break (DSB) is a highly dangerous DNA lesion, causing cell death and mutations at the greatest rate, unless properly repaired. On the other hand, a DSB is also the most potent stimulus for gene targeting and genome engineering. Working in the model system yeast Saccharomyces cerevisiae, we have uncovered that absence of Ribonucleases H1 and H2 (RNase H1 and H2) strongly enhances transcript RNA-initiated repair of a DSB occurring either in the same transcript-generating locus (in cis), or in a homologous but remote locus (in trans), >680,000-fold and 1,000-fold, respectively. Transcript RNA-directed DNA modification remained high in different conditions that are known to inactivate the reverse transcription function of the yeast transposon Ty. These results suggest that the transcript RNAs are not converted into cDNA, but rather directly modify the genomic DNA. The mechanism of transcript RNA-directed DNA modification depends on transcription of the repairing transcript RNA and requires the function of the homologous recombination protein Rad52. Our data reveal a novel mechanism of DNA repair and modification mediated by RNA transcripts and open up a new direction for gene targeting and in vivo genome engineering by RNA. (NSF-MCB-1021763; Georgia Cancer Coalition-R9028)
Nanosized structures as siRNA carriers improved for gene therapy

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Keywords: Small interfering RNAs (siRNA), siRNA delivery, disease therapy, nanomaterials

Nanotechnology is to utilize matters smaller than 100 nanometers and takes advantages of properties that are only presented at this nano level. Recent advances in nanotechnology have provided various new tools for biomedical research and clinical applications. Nanopharmaceutics is one of the disciplines that will benefit from this technology the most. Small interfering RNAs (siRNA), a new generation of biodrugs, can be utilized for silencing a wide range of target genes to treat a variety of diseases, including cancers. One obvious strategy for nucleic acid delivery is through viral vectors, but the issues of immunogenicity, carcinogenicity, and inflammation that are associated with viral vectors have inspired the parallel development of nonviral carriers based on cationic lipids and polymers. Nanomaterials, with their unique size-dependent physical and chemical properties such as high stability and easy manipulation, have showed promising advantages as gene delivery vehicles, ultra-sensitive and controllable intracellular payload release, and precisely silence of targeted genes. However, the delivery of naked siRNA to the appropriate site remains a considerable hurdle owing to rapid enzymatic digestion, limited translocation through cellular membranes, and inefficient release from endosomes. Therefore, one of the major challenges in siRNA gene therapy is to find a suitable gene delivery vehicle, the main features of which for clinical application are low toxicity and high transfection efficiency. Recently, innovative nanomaterials developed for pharmaceutics have been discovered as potential candidates with negligible cytotoxicity in tissue culture and without detectable side effects in vivo. This research might pave the way to prepare efficient gene nanocarriers and may be further exploited for clinical application.

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Simultaneous activation of multiple split functionalities inside cells through anagnoristic R/DNA hybrids: a novel approach in therapeutic RNA nanotechnology

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Keywords: RNAi, split functionalities, RNA nanotechnology, RNA-DNA hybrids

Using RNA interference (RNAi) as a therapeutic agent it is routinely possible to knock down the expression of target genes in diseased cells. We developed a new strategy based on therapeutic RNA/DNA hybrids which can be generally used for triggering the RNAi pathway as well as other functionalities inside the diseased cells. Individually, each of the hybrids is functionally inactive and the therapeutic siRNA representation can only be activated by the recombination of at least two cognate hybrids simultaneously present in the same cell. This approach allows (i) the triggered release of siRNAs inside cells, (ii) activation of different functionalities intracellularly, (iii) higher control over targeting specificity, (iv) tracking of the delivery and recombination of these hybrids in real-time inside cells, (v) introduction of additional functionalities without direct interference with therapeutic siRNA processivity, (vi) increasing the retention time in biological fluids by fine-tuning chemical stability by having or substituting the DNA strands with chemical analogs, (vi) conditional release of therapeutic functionalities. We comprehensively analyzed several pairs of cognate R/DNA hybrids in vitro and ex vivo using human breast cancer cells as a model system. We demonstrated significantly higher chemical stabilities in human blood plasma for hybrids compared to pure siRNAs together with inability of RNA/DNA hybrids to be processed by human Dicer. We examined the kinetics and affinities of hybrid recombinations and determined the lowest concentration of hybrids to recombine to be at least as low as 1 nM. Several different ex vivo experiments allowed the tracking in real time of several split functionalities (e.g. activation or deactivation of FRET) inside the target cells. The successful release of therapeutic moieties (siRNAs) was confirmed by the significant level of suppression (>95%) of the target gene expression. Interestingly, for siRNA release, cognate hybrids can be co-delivered to the cell either on the same or even on two different days and the extent of silencing efficiency will depend on structural characteristics of individual hybrids. Moreover, in vivo studies demonstrate a significant uptake of the hybrids by tumors and specific gene silencing. This split functionality approach presents a new route in the development of “smart” nucleic acids based nanoparticles and switches for a range of applications in biomedical RNA nanotechnology.
Microfluidic capture of tumor-derived extracellular vesicles from serum

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Cancer cells release extracellular vesicles (EVs), 50 nm - 1 um in diameter, containing genetic information from the tumor into the bloodstream. However, human serum also contains a variety of MVs released from normal cells that may hamper mutational analysis of tumor-derived MVs. Selective isolation of tumor-derived extracellular vesicles (EVs) from body fluids will yield a window into the genetic profile of tumors to serve as diagnostic markers for disease status.

We isolated tumor EVs from serum using antibody-mediated capture in microfluidic chambers, coated with antibodies directed against tumor antigens. The device contains eight microchannels with a herring-bone pattern on their upper surface to maximize interactions of EVs with the walls of the chip. Avidin was bound covalently to the surface of the chamber, allowing functionalization with biotinylated antibodies.

Efficient EV capture was validated using tumor EVs from various glioma cell lines spiked into healthy human serum. Samples were passed through microfluidic chambers coated with antibodies directed against tumor antigens. Captured EVs were lysed inside the chip and RNA was isolated and compared with input and output RNA. Taqman assays for tumor-specific messages were performed to analyze the tumor EV capture.

Initial experiments indicated that high-quality RNA can be isolated from EVs captured inside our microfluidic device. Subsequent Taqman analysis of tumor-specific messages showed that tumor EVs were enriched in devices coated with an EGFR antibody. This enrichment was illustrated visually by fluorescent imaging of captured EVs from glioma cells expressing palmitoylated GFP. Next, we designed a cocktail of antibodies that can potentially capture tumor EVs from multiple glioma subtypes. Devices coated with this cocktail showed efficient capture of EVs from different human glioma cell lines.

In conclusion, tumor-derived EVs in serum can be selectively captured in microfluidic chambers providing information about the genetic status of tumors. In future experiments serum samples from glioma patients will be tested to investigate if this innovative isolation method can increase sensitivity of detection of oncogenic mutations.

- Nominate by Xandra O. Breakefield and Peixuan Guo
Oral abstract: 17

Hepatitis C virus subverts cellular RNA granules and the liver-specific microRNA miR-122 to promote viral gene expression

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**Keywords:** Hepatitis C virus, microRNA, helicase, RCK/p54

Components of cytoplasmic Processing bodies (P-bodies) and stress granules can be subverted during viral infections to modulate viral gene expression. Because hepatitis C virus (HCV) RNA abundance is regulated by P-body components, microRNA miR-122, Argonaute 2 and the RNA helicase RCK/p54, we examined whether HCV modulated P-bodies and stress granules during viral infection. We discovered that HCV infection decreased the number of P-bodies, but induced the formation of stress granules. Immunofluorescence studies revealed that both P-body and stress granule components co-localized with viral Core protein at lipid droplets, the sites for viral RNA packaging. Depletion of selected P-body residents decreased overall HCV RNA and virion abundance. Furthermore we found that the P-body protein and RNA DEAD-box helicase RCK/p54 putatively modulated the interaction of miR-122 with the HCV genome. Depletion of stress granule components also decreased overall HCV RNA abundance, but surprisingly enhanced the accumulation of infectious, extracellular virus. Last, using sucrose density gradients we found that replicating HCV genomes specifically altered the subcellular distribution of miR-122. These data show that HCV modulates miR-122 distribution, and subverts P-body and stress granule components to aid in viral gene expression at particular sites in the cytoplasm.
Studying the therapeutic neural stem cell plasticity at nanoscale

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Compelling evidence exists that somatic stem cell-based therapies protect the central nervous system (CNS) from chronic inflammation-driven degeneration, such as that occurring in experimental autoimmune encephalomyelitis (EAE), multiple sclerosis (MS) and cerebral ischemic/hemorrhagic stroke. However, while it was first assumed that stem cells may act through direct replacement of lost/damaged cells, it has now become clear that they are able to protect the damaged nervous system through a number of ‘bystander’ mechanisms other than cell replacement. In immune-mediated experimental demyelination and stroke – both in rodents and non-human primates – others and we have shown that transplanted neural stem/precursor cells (NPCs) possess a constitutive and inducible ability to mediate efficient ‘bystander’ myelin repair and axonal rescue. Yet, a comprehensive understanding of the multiple mechanisms by which NPCs exert their therapeutic impact is lacking. We envisage that the remarkable therapeutic plasticity of NPCs results from their capacity to engage highly sophisticated programmes of horizontal cell-to-cell communication at the level of the (micro)environment and we attribute a key role to the transfer of secreted membrane vesicles (MVs) from (donor) NPCs to (recipient) neighbouring cells. We are starting to define whether this form of communication is biologically relevant for NPCs, and look forward to establishing whether it is associated to cell-to-cell trafficking of non-coding RNAs (ncRNAs), and indeed on elucidating its molecular signature and therapeutic significance for MS.

We believe that the true innovation of this approach relies in its unique peculiarity to look into an innate cellular mechanism with the visionary focus of translating the knowledge of basal stem cell functions into innovative high-impact clinical therapeutics for MS.
Oral abstract: 19

Synthetic carriers of siRNA by rational design

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Keywords: siRNA delivery, Synthetic carriers, Cationic polymer, Surface assembly

A therapeutically feasible synthetic carrier for siRNA must accomplish all the essential tasks, including (A) packing siRNA into nano-particulates to avoid pre-phagocytosis degradation; (B) adsorbing onto diseased cells selectively; (C) facilitating endosomal escape of siRNA; (D) releasing siRNA in the cytoplasm of the target cells in time; (E) metabolizing itself into nontoxic and eliminable species. For practical usage, this carrier system must be structurally simple and easy to formulate by clinic technicians. To meet these criteria, we divided the carrier assembly to two steps, (1) using a cationic polymer able to degrade to endogenous monomer and safety-known metabolite in response to endosomal environment to pack siRNA into a polyplex, (2) assembling a surface membrane of rationally designed block-copolymer around the polyplex to endow the particulate with inter-cellular recognition.

The cationic polymer involved a spermine, an endogenous amino-bearing monomer condensing genes in sperms, and an imidazole ring possessing a pKa of 5.9 polymerized through conjugated imine linkages. This structural feature enables the polymer to be stable under pH 7.4, but degrade to spermine and bisamidyzole formate (a safety known metabolite) by absorbing a proton under endosomal pH. The newly formed spermine may enhance ensomal escape by absorbing two additional protons by its two free primary amines. The block copolymer for self-assembling the functional surface membrane consists a surface guiding groups, a hydrophobic block (PCL) and an steric stabilization block (PEG). The negatively charged guiding group guided the block copolymer to the polyplex surface through electrostatic force, the hydrophobic block aligned to hydrophobic layer to isolate siRNA, and the PEG block formed steric stabilization out layer to ensure prolonged in vivo circulation. Cell targeting moieties may be conjugated to the distal end of the PEG lock of the copolymer and immobilized on the polyplex with optimized surface population by mixing the targeting copolymer with non-conjugated ones in fine-tuned ratio.

Chemical characterizations as well as in vitro and in vivo biological assays involving anti-luciferase siRNA and tumor bearing mice were carried out to examine the designed properties of this synthetic carrier. The positive experimental results demonstrate that therapeutically feasible synthetic carriers of siRNA can be rationally designed and assembled.
Oral abstract: 20

**Computational prediction and experimental self-assembly of multi-Sequence nucleic acid-based nanostructures**

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**Keywords:** multisequence nucleic acid self-assembly, computational prediction, experimental self-assembly, DNA, RNA, DNA/RNA

Nucleic acid-based nanostructures can self-assemble from specifically determined sequences of DNA, RNA or DNA/RNA hybrids. The latter case is especially interesting since by combining the two molecular species, properties that are inherently part of each molecule type can be taken advantage of. For example, the relative chemical stability and inexpensiveness of synthesis and chemical modification of DNA can be employed, while the significant spectrum of functionality and complexity of folding of RNA can also be utilized. We have developed programs that enable the prediction of the self-assembly of nucleic acid nanostructures starting from combinations of RNA and DNA strands or pre-assembled moieties present at given concentrations. The assembly process is driven by the kinetic and thermodynamic properties of the given segments. A description of the algorithms used and examples of such self-assembly predictions will be discussed. In addition, these methods have been experimentally verified by the self-assembly of RNA nanostructures from DNA templates and the assembly of functional reassociating RNA/DNA hybrids.
**Oral abstract: 21**

**Specific RNA-capsid contacts in the virions of a positive-strand RNA virus.**

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**Keywords:** RNA virus, capsid-RNA interaction, Brome mosaic virus

While the protein shells of a large number of RNA viruses have been resolved to atomic resolution, little information exists on the structure of the encapsidated RNAs. The coat protein (CP) of the brome mosaic virus (BMV), a multipartite positive-strand RNA virus, has been demonstrated to bind to and regulate the translation and replication of the RNA genome. We sought to determine whether the CP-RNA contacts are observed in the BMV virions. The implication of such an interaction could be that regulatory interactions are loaded within the virion before the entry of the virus into cells. A method to precipitate portions of the encapsidated RNAs that are in contact with the capsid, followed by cDNA synthesis and next-generation DNA sequencing, was used to identify the RNA residues that are in contact with the BMV capsid protein. Within each of the BMV RNAs, several distinct regions were found to contact the CP when the virions were at pH 5.5 and in a contracted conformation. Upon an increase in pH that induced swelling of the virions, the RNA contacts were lost. The RNA sequences that contacted the capsid were enriched in pyrimidines and could bind dissociated coat protein at pH 5 in vitro with nanomolar affinity. Virions with mutations in the CP that affected the encapsidation of one or more of the BMV RNAs were also examined for effects on capsid-RNA contact in virions. These results demonstrate that the contracted form of the BMV virion preferentially binds specific RNA sequences. The interactions between the CP and RNA motifs that regulate BMV gene expression will also be presented.
Oral abstract: 22

Approaches in constructing RNA nanoparticles with applications in nanotechnology and nanomedicine

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RNA can fold into well-defined tertiary structures with specialized functionalities. The structural motifs and tertiary interactions has been examined in many RNA molecules and the information gleaned has been used to rationally design the building blocks that self-assemble into RNA nanoparticles. Various techniques are available for the construction of therapeutic RNA nanoparticles: (1) Hand-in-hand interactions via interlocking loops; (2) Foot-to-foot interactions via palindrome sequence mediated formation of RNA dimers; (3) Robust RNA motif to drive the assembly of nanoparticles with branches; (4) RNA 2WJ, 3WJ, 4WJ or other multi-junctions as building blocks to generate quaternary structures; (5) RNA binding proteins to serve as platform for the formation of arrays; and (6) formation of RNA nanoparticles by polymerase chain reaction. We have developed “toolkits” utilizing some of the aforementioned principles to construct RNA architectures with diverse shapes and angles. RNA loops, cores, junction motifs, and palindrome sequences from the phi29 motor pRNA were gathered in a “toolkit” to demonstrate their utility for fabrication of dimers, twins, trimers, tetraplets, pentamers, hexamers, heptamers, and other higher order oligomers, as well as branched diverse architectures via hand-in-hand, foot-to-foot, and arm-on-arm interactions. These RNA nanoparticles are thermodynamically and chemically stable, resistant to denaturation in 8M urea, remains intact at ultra-low concentration, and can harbor resourceful functionalities. All incorporated functional modules, such as siRNA, miRNA, ribozymes, and aptamers folded correctly and functioned independently within the nanoparticles. The incorporation of all functionalities was achieved prior, but not subsequent, to the assembly of the RNA nanoparticles; thus ensuring the production of homogeneous therapeutic nanoparticles. Upon systemic injection, these RNA nanoparticles targeted cancer exclusively in vivo without trapping in normal organs and tissues. Furthermore, the pRNA nanoparticles displayed favorable pharmacological profiles in vivo. These findings open a new territory for cancer targeting and treatment. The versatility demonstrated by one biological RNA molecule implies immense potential concealed within the RNA nanotechnology field.

References:
Oral abstract: 23

Beyond fluorescence: small and bright upconversion nanoparticles for biological imaging

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**Keywords:** Single-molecule, optical imaging, in vivo, RNAi

Lanthanide-doped upconversion luminescent nanoparticles (UCNPs) are promising materials for in vitro and in vivo optical imaging due to their unique optical and chemical properties. UCNPs absorb low energy near-infrared (NIR) light and emit high-energy shorter wavelength photons. Their special features allow them to overcome various problems associated with conventional imaging probe at both single molecule and ensemble levels. In this talk, I will present a new type of biocompatible UCNP. They are free of autofluorescence, non-blinking and non-bleaching for in vitro single molecule cell imaging, and exhibit significantly improved signal-to-noise-ratio (i.e., 300 for Balb-c mice) and outstanding tissue penetration depth (>3cm), and minimal light scattering, all highly desired for in vivo whole animal imaging. I will also present a new development regarding about engineering UCNPs towards in vivo photounacging and some recent RNAi relating studies.
Oral abstract: 24

Toward the rational pre-clinical application of nanomaterial RNA and protein conjugates


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Keywords: Gold, zinc oxide and manganese oxide nanomaterials, RNA structure-function, splice switching oligomer (SSO), aptamer, siRNA

Our research is funded by the National Cancer Institute on a grant entitled, "Anti-Cancer RNA Nanoconjugates" (1R15CA139390-01). In the project we are exploring the beautiful nexus between cell and molecular biology and nanomaterial (NM). Our focus has been a comparative approach, studying the association of RNA to gold nanoparticle (GNP) versus NM derived of the bio-elements zinc or manganese, known to be important in regulating protein:nucleic acid interactions. We have prepared and purified controlled size and shape zinc oxide (ZnO) or manganese oxide (MnO) NM, carefully investigating several new nano-synthetic strategies. We have studied these NMs’ association to RNA by a variety of advanced spectroscopic techniques available to us via NSF MRI instrumentation grants. We are particularly interested in the pre-clinical RNA macromolecule, polyinosinic:poly cytidilic acid (poly I:C), and are studying the NMs’ effects on its structure-function and RNA bio-activity, in comparison to designed and engineered splice switching oligomer (SSO), aptamer and siRNA against several other important cancer protein targets.
Oral abstract: 25

Global structure of a three-way junction in a phi29 packaging RNA dimer determined using site-directed spin labeling

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Keywords: Phi29 Packaging RNA, RNA structure, Site-directed spin labeling, EPR spectroscopy

The condensation of bacteriophage phi29 genomic DNA into its preformed procapsid requires the DNA packaging motor, which is the strongest known biological motor. The packaging motor is an intricate ring-shaped protein/RNA complex, and its function requires an RNA component called packaging RNA (pRNA). Current structural information on pRNA is limited, which hinders studies of motor function. Here, we used site-directed spin labeling to map the conformation of a pRNA three-way junction that bridges binding sites for the motor ATPase and the procapsid. The studies were carried out on a pRNA dimer, which is the simplest ring-shaped pRNA complex and serves as a functional intermediate during motor assembly. Using a nucleotide-independent labeling scheme, stable nitroxide radicals were attached to eight specific pRNA sites without perturbing RNA folding and dimer formation, and a total of 17 inter-nitroxide distances spanning the three-way junction were measured using Double Electron-Electron Resonance spectroscopy. The measured distances, together with steric chemical constraints, were used to select 3,662 viable three-way junction models from a pool of 65 billion. The results reveal a similar conformation among the viable models, with two of the helices (HT and HL) adopting an acute bend. This is in contrast to a recently reported pRNA tetramer crystal structure, in which HT and HL stack onto each other linearly. The studies establish a new method for mapping global structures of complex RNA molecules, and provide information on pRNA conformation that aids investigations of phi29 packaging motor and developments of pRNA-based nanomedicine and nanomaterial.
Nanopore-facilitated single molecule detection of circulating microRNAs in lung cancer patients

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Developing new methods for lung cancer screening and early diagnosis is a critical issue for saving lung cancer patients’ lives. MicroRNAs (miRNAs) are small regulating RNA molecules that have been recognized as cancer biomarkers. We developed a nanopore sensor that is combined with a programmable oligonucleotide probe for selective and sensitive single molecule detection of miRNAs in lung cancer patient plasma samples. The sensor also demonstrated ability to discriminate miRNAs containing single nucleotide differences. This simple, sensitive, label-free technique requiring no amplification for miRNA detection, has the potential for noninvasive and cost-effective early diagnosis of lung cancer.
Oral abstract: 27

Keynote address - II

“Unusual” RNA base pairs in recognition and decoding

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Keywords: RNA Structure, Molecular recognition

RNA molecules are characterized by the formation of hydrogen-bonded pairs between the bases along the polymer. All base-base interactions present in nucleic acids, involving at least two “standard” H-bonds, can be classified in twelve families where each family is a 4x4 matrix of the usual bases. The common Watson-Crick pairs belong to one of these families and the other eleven families gather the non-Watson-Crick pairs. The Watson-Crick pairs form the secondary structure and all the other families are critical for the tertiary structure. In several of those twelve families, the 4x4 matrix is partially filled because only some base-base oppositions are able to lead to the formation of two “standard” H-bonds with proper geometry and distances. Some of the missing base-base oppositions are, however, observed with “unusual” features in an increasing number of crystallographically defined crystal structures. Such “unusual” base pairs involve protonation of one of the base, bifurcated H-bonds, water-mediated base pairing, and especially tautomeric forms of the bases. The origins of those observations can be ascribed partly to the free energy of crystal formation and packing together with the law of mass action in the absence of ligand competition. But, most importantly, the observations stress the dominant role of active site tightness in fidelity: the ribosome binds most favorably base pairs with Watson-Crick geometry and selects accordingly the correct codon-anticodon complex. Various environmental (ions, antibiotics) or structural factors (rRNA or tRNA mutations) can contribute positively or negatively to the overall free energy of selective binding leading to permissive or hyper-accurate ribosomes.

References:
Oral abstract: 28

Exosomes that target antigen specifically and deliver dsRNA of choice for physiologic for exquisitely specific siRNA therapy

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We have discovered a new form of T cell regulation relevant to therapy of diseases involving tailor made therapeutic exosomes. Suppressive CD8+ T cells from high dose antigen (Ag) tolerized mice release physiologic nanovesicle exosomes containing inhibitory miRNA. The suppressive exosomes are present in the plasma of the tolerized donors and thus disperse in a systemic endocrine manner. Standard CD25+ CD4+ Foxp3+ Treg are not involved. Like their cell source, the exosomes are CD8+ CD3+ and abTCR+; and also CD9+ a tetraspanin of internal cell membranes. They target and then suppress distant effector T cells by delivering inhibitory miRNA we have identified. They likely act in our system by binding to antigen bearing dendritic cells that suppress companion dendritic cells that then suppress the antigen specific effector CD4+ T cells. In mice deficient in the involved miRNA, that had no suppressive function, transfection with the particular miRNA and not controls or mimics, reconstituted suppression.

We have discovered that the suppressive miRNA exosomes act antigen-specifically. This is via a coating of antigen- specific immunoglobulin light chains (LC) demonstrated by flow cytometry, and likely derived from a B cell subpopulation activated during the tolerizing protocol. Consequently, tolerized pan immunoglobulin deficient mice cannot generate suppressor exosomes, but if antigen-specific antibody chains are added their suppression is reconstituted. The exososomes protect the miRNA in transit and act Ag-specifically to deliver the inhibitory miRNA to target effector T cells antigen-specifically. This is the first example of T cell regulation via inhibitory miRNA passing from cell to cell via exosomes and acting antigen-specifically in vivo in a systemic endocrine manner.

This work offers the new and novel approaches of Ag-specific T cell regulation via transfer of genetic instructions to achieve entirely unique new translational possibilities for the therapy of immunological and other diseases, like cancers. Exosommes can be activated for surface coating with antibody of choice and easily transfected with dsRNA of choice. The result is the construction of therapeutic exosomes as natural physiologic nanoparticles carrying siRNA of choice, delivered antigen specifically to targeted cells via an Ag-specific antibody coating. These findings have great translational possibilities for treatment of various human diseases.
Oral abstract: 29

Polymeric/viral nanobio vectors for efficient gene delivery

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Keywords: gene delivery, retrovirus, lentivirus, polymers

Human gene therapy holds the potential to revolutionize treatment of diseases ranging from cystic fibrosis to cardiovascular disease to cancer. The primary bottleneck hindering the technology, however, is delivery of the therapeutic genetic material. Recombinant viruses are highly efficient and are used in the majority of gene therapy clinical trials, but face serious safety concerns, are difficult to target to specific cells and tissues, and are expensive to produce. Polymer-based vectors are generally safer, less expensive and more versatile, but lack the efficiency needed for clinical relevance. The success of human gene therapy awaits development of safe and efficient methods for gene delivery. We are pioneering a top-down approach to construction of novel vectors assembled from viral and synthetic components that are designed to exhibit advantages of both types of vectors. Thus, we have prepared these nanobio vectors through complexation of retro- or lentivirus-like particles with polycations and cationic liposomes. These hybrid vectors are internalized by cells via endocytosis, the synthetic component provides escape from endocytic vesicles into the cytosol, and the viral components provide highly efficient trafficking of their genetic cargo into the nucleus. Further, targeting moieties may be conjugated to the polymer to provide cell-specific delivery. This new class of nanobio vectors provides gene delivery efficiency similar to recombinant viruses, provides genomic integration for sustained transgene expression, and is capable of transfecting quiescent cells. Nanobio vectors, therefore, represent a new and promising approach for safe and efficiency gene delivery.
Oral abstract: 30

Exosome RNA cargoes serve as a “liquid biopsy” for diagnosis of cancer

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Keywords: Exosomes, Transcriptome, Ovarian cancer

In 1979, we discovered and published the initial observation of circulating extracellular vesicles (EVs) and proposed their diagnostic potential (Anal. Biochem. 98:53-59, 1979). Over the past three decades, we have continued to develop technologies to define these stable, disease-specific vesicular markers for detection, disease characterization, and predicting prognosis. These temporal changes in EV profiles have been demonstrated to accurately predict disease recurrence and overall patient survival. The transcriptome of circulating EVs provide a real-time monitor of therapeutic response, serving as a companion diagnostic. In 2008, we made the initial demonstration of circulating exosomal RNA. In order to make significant strides in the successful management of ovarian cancer, a paradigm shift to reflecting disease state has to be developed. We report the development of specific RNA biomarkers for ovarian cancer:

- Markers to screen high risk populations – Within populations with specific genetic risks for developing ovarian cancer, it is essential to provide a non-invasive method for screening the early development of cancer.
- Markers to identify patients with early stage ovarian cancer – Since early stage ovarian cancer patients exhibit high response rates and better overall survival, identification of early stage disease is the ultimate goal. Current serum biomarkers exhibit low or no expression in early stage disease. Our current findings demonstrate EV transcriptomes characteristic of ovarian cancer, even within patients with Stage I disease.
- Markers to differentiate benign versus malignant ovarian masses – Ovarian masses can be often identified by ultrasound or CT imaging; however, since these approaches generally use morphological criteria and lesion size, they have limited value for detecting microscopic disease or to distinguish benign postoperative changes from tumor recurrence. Our findings show EV RNA profiles characteristic of ovarian cancer are distinct from those associated with benign ovarian disease.

By correlating these circulating markers with the molecular characteristics and real-time clinical parameters, we have established the use of circulating exosomes as a “liquid biopsy.”
A goal of our research is the application of small RNA based therapeutics for the treatment of disease. We have developed dual inhibitory RNA aptamers that function as specific inhibitory agents and delivery vehicles for therapeutic siRNAs. The first aptamer targets the HIV envelope protein gp 120. This aptamer neutralizes the virus and blocks infection. The same aptamer can deliver an attached siRNA into HIV infected cells via binding to envelope expressed on the cell surface, resulting in internalization of the aptamer and delivery of a dicer substrate siRNA to RISC. In vivo delivery of the aptamer and aptamer-siRNA conjugates into a humanized mouse model for HIV infection resulted in one million fold inhibition of the virus, demonstrated siRNA directed cleavage of the target mRNA and complete protection of T-cells from HIV mediated T-cell killing. These results represent the first such small RNA applications for the successful treatment of HIV-1 infection, and could potentially be used in HIV-1 eradication strategies.

The second aptamer we have evolved binds with high affinity to the BAFFR1 receptor expressed on B-cells. This receptor is a target for therapeutic treatment of autoimmune diseases and lymphomas since binding of the Baff ligand to the receptor initiates a B-cell proliferative response. The evolved BAFFR1 aptamer blocks Baff ligand mediated proliferation of lymphoma cells in culture as well as blocking intracellular signaling pathways. Binding to BAFFR1 results in internalization of the aptamer, allowing the delivery of therapeutic siRNAs. The specificity of this aptamer for B-cells and its ability to serve as a delivery vehicle for siRNAs opens up many therapeutic possibilities. The application of this aptamer in targeting human tumors in humanized mice will be described.
Fluorescent RNA biosensors for bacterial signaling molecules

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UC Berkeley

Keywords: RNA Engineering, Riboswitch, Biosensor

Cyclic di-nucleotides are an emerging class of signaling molecules that regulate pathogenic processes in bacteria and stimulate the innate immune response in mammals. Here we describe the design of fluorescent RNA biosensors for cyclic di-GMP and cyclic AMP-GMP. These biosensors exhibit fluorescence turn-on in response to cyclic di-nucleotides in live bacterial cells. Furthermore, the biosensors were used to demonstrate the in vivo production of cyclic AMP-GMP by the enzyme DncV.
Oral abstract: 33

Assembly of thermodynamically and chemically stable RNA nanoparticles escorting siRNA, ribozyme and miRNA with independent folding for specific cancer targeting

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One of the advantages of RNA Nanotechnology is the feasibility to construct therapeutic particles carrying multiple therapeutics with defined structure and stoichiometry. Herein, we report the construction of thermodynamically and chemically stable X-shaped and tri-star shaped RNA nanoparticles to carry one, two three or four siRNA, miRNA, RNA aptamer, or miRNA using reengineered RNA fragments derived from the central domain of the motor pRNA of bacteriophage phi29 DNA packaging motor. The nanoparticles self-assemble very efficiently in the absence of divalent salts, resistant to denaturation by 8 Molar urea, and remains intact at ultra-low concentrations. The delta G of the nanoparticles is extremely low and the slope of the temperature melting curve is close to 90°. We proved that each arm of the helices in the X-motif or tripods can harbor one siRNA, ribozyme, or aptamer without affecting the folding of the central X or 3WJ core, and each daughter RNA molecule within the nanoparticle fold into respective authentic structure and retain their biological and structural function independently. Gene silencing effects were progressively enhanced as the number of the siRNA in each pRNA-X nanoparticles gradually increased from one to two, three, and four. More importantly, systemic injection for bio distribution assay of the ligand-containing nanoparticles into the tail-vein of mice revealed that the RNA nanoparticles remained intact without showing any signs of dissociation or degradation; and strongly bound to cancers without entering liver, lung or any other organs or tissues. Pharmacokinetic analysis revealed that its half-life was extended 100-fold compared to the siRNA counterpart. Particles tested in vivo revealed that the they did not induce cytokines, interferon-I, antibody, and toxicity while retaining favorable pharmacokinetics profiles.

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Oral abstract: 34

Clicks and tricks with RNA for nanobiotechnology

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Keywords: labeling, transcription, click chemistry, siRNA, transfection

Our group has recently applied the copper-catalyzed azide-alkyne cycloaddition (CuAAC or click-chemistry) to naïve RNA (with free hydroxyl groups) as a much higher efficiency reaction that other conjugation methods. Click-compatible groups can be incorporated into RNA through chemical synthesis or biochemical means and these groups can be used to tag or label the RNA. A universal transcriptional initiator nucleoside enables post-transcriptional functionalization of RNA or even direct co-transcriptional labeling. Among other advances, this ability to rapidly label RNA has freed our lab from use of radionucleotides. Our optimized and “ligandless” click method is useful in functionalizing polymers with nucleic acids. These hybrids include novel RNA polymer architectures that can auto-internalize into cells to deliver potential therapeutic payloads. These represent a powerful class of highly customizable nanomaterials that could find applications in smart or responsive therapeutic delivery agents, a hallmark of nanomedicine.
Towards facile engineering of novel riboswitches

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Keywords: RNA regulation, transcription, riboswitch

Riboswitches are structured elements found in the 5'-untranslated regions of mRNAs that directly bind a small molecule to regulate gene expression in a cis-fashion. These riboregulatory elements typically consist of two domains: an aptamer domain that acts as the small molecule receptor and an expression platform that directs expression of the mRNA through a secondary structural switch. While many RNA devices analogous to natural riboswitches have been devised, we seek to directly employ modular parts of riboswitches to facilely create novel tools. To reverse engineer these RNAs, we asked two questions. First, are expression platforms modular? To demonstrate this, we have synthesized and tested a series of chimeric riboswitches in which non-native aptamers (both biological and synthetic) have been fused to three different expression platforms and tested for activity in vitro and in vivo. These experiments showed that the secondary structural switch of these riboswitches can be directed by diverse aptamers, and most importantly, by those derived from in vitro selection. Second, can riboswitch aptamer domains serve as structural scaffolds for in vitro selection? Using the purine riboswitch as a starting point, we designed a library that only randomized only nucleotides in the three-way junction while preserving secondary and tertiary structure. We have successfully raised a novel aptamer that conserves the overall structure of the purine riboswitch aptamer but hosts a structurally distinct binding pocket. This suggests a new strategy for raising small molecule aptamers through in vitro selection for RNA-based devices.
Oral abstract: 36

Selenium-nucleic acids (SeNA) for structure, function, and drug discovery studies

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Our laboratory has pioneered and developed atom-specific substitution of nucleic acid oxygen with selenium, which can be used as an atomic probe for structure and function studies of RNAs and RNA-protein complexes. 3D structure studies of nucleic acids provide novel insights into RNA and DNA. As oxygen and selenium are in the same elemental family, the atom-specific replacement by replacing nucleotide oxygen with selenium or tellurium has revealed novel chemistry, structure, function and mechanism of nucleic acids. The Se derivatization leads to a novel paradigm of nucleic acids and facilitates drug discovery. Furthermore, X-ray crystallography is a powerful tool for structure determination of DNA and RNA structures, RNA-protein and DNA-protein complexes with high resolution. However, besides crystallization problem, derivatization with heavy atoms for phase determination has largely slowed down structural determination of nucleic acids with novel structures and folds. Our selenium nucleic acid strategy has demonstrated great potentials as a general methodology for structure and function studies of nucleic acids as well as their protein complexes. Furthermore, we find that the Se-derivatized nucleic acids have virtually identical structures to the corresponding natives, while the conventional Br-derivatization caused severe perturbations on the local backbone and hydration. Furthermore, we found that the Se-derivatization can facilitate crystallization and increase the diffraction quality. This Se derivatization strategy via the atom-specific substitution will significantly facilitate crystal structure studies of nucleic acids as well as their protein complexes for drug discovery. Excitingly, we have recently determined the first nucleic acid-protein complex via the nucleic acid Se-derivatization and the MAD phasing. This work is supported by NIH (R01GM095881 and GM095086) and NSF (MCB-0824837 and CHE-0750235).

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Oral abstract: 37

Nanoengineering new siRNA delivery platforms

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Non-viral delivery of small interfering RNA (siRNA) in vivo is a challenging task that can be significantly improved by engineered nanocarrier systems. We have constructed a number of different cationic polymer systems and chemically modified nucleic acids optimized for in vivo by oral, intratracheal, intraperitoneal, intravenous and local administration. Effective in vivo RNA interference has been achieved in bronchiole epithelial cells, kidney and several types of immune cells in mice highlighting their potential application in RNA-mediated therapy. Other delivery reagents that contain cell targeting ligands and controlled release mechanisms are also being developed. We have also exploit is the complementary binding properties of DNA and RNA to self-assemble pre-designed nanostructures in a bottom up approach. This enables nanoscale control of ligand recognition, PEGylation and drug loading in well-defined particles. This strategy enables a rapid screening platform for siRNA delivery where the pharmacokinetic profile, immune stimulation, cell targeting and functional gene knock is addressed.
RNA interference is a fundamental gene regulatory mechanism that is mediated by the RNA-induced silencing complex (RISC). Here we report that an artificial nanoparticle complex can effectively mimic the function of the cellular RISC machinery for inducing target RNA cleavage. Our results show that a specifically designed nanozyme for the treatment of hepatitis C virus (HCV) can actively cleave HCV RNA in a sequence specific manner. This nanozyme is less susceptible to degradation by proteinase activity, can be effectively taken up by cultured human hepatoma cells, is nontoxic to the cultured cells and a xenotransplantation mouse model under the conditions studied, does not trigger detectable cellular interferon response, but shows potent antiviral activity against HCV in cultured cells and in the mouse model. We have observed a 99.6% decrease in HCV RNA levels in mice treated with the nanozyme. These results show that this nanozyme approach has the potential to become a useful tool for functional genomics, as well as for combating protein expression-related diseases such as viral infections and cancers.
Oral abstract: 39

RNA nanotechnology for next generation computer design

Meikang Qiu

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For silicon-based computer, scientists and engineers are wondering whether the Moore’s Law can be continued in the next ten years. Molecular-scale computing has been explored since 1994 due to the predictable ending of Moore’s Law for silicon-based computation devices. By exploring the potential massive parallelism, low energy consumption, and working in vivo, molecular-scale computing becomes a new computational paradigm. Inspired by the concepts borrowed from electronic computer, RNA is arising as promising possible building material for molecular-scale computer to realize basic Boolean functions and scale up into multilayered circuits. RNA can be designed and manipulated with the advantages of both DNA and protein, i.e., simple comparable to DNA and flexible comparable to protein. Varieties of small RNA can work cooperatively, synergistically, or antagonistically to produce computational logic circuits. RNA computer design provides a promising possibility for next generation computer design and will get predictable better rewards.
Extracellular vesicles (EVs) are small membrane-bound particles comprised of exosomes and various sized EVs, also known as microvesicles and microparticles. EVs are released by numerous cell types and have a variety of cellular functions from communication to mediating growth and differentiation. Previously, we showed that the majority of peripheral blood EVs are derived from platelets while mononuclear phagocytes, including macrophages, are the second most abundant population. Since EVs contain proteins and nucleic acids, we previously characterized the microRNA (miRNA) content of both platelet- and macrophage-derived EVs and found miR-223 to be the highest expressed. In preliminary studies examining the protein content of the EVs, we have found that many proteins are membrane associated and cytoskeletal. However, we find a class of chaperone proteins that may package RNA molecules to the EVs. Several of these chaperone proteins are bound to miR-223. More recently, we found that RNA molecules including miR-223 contained in the macrophage-derived EVs were transported to target cells, including monocytes, endothelial cells, epithelial cells and fibroblasts. In inflammatory diseases, we hypothesize that the RNA content is altered in the plasma EVs. Current investigation from our laboratory involves indirect or direct manipulation of the content of the EVs to use as a therapeutic means.
Exosomes as mediators of intercellular transport of oncogenic cargo

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Keywords: Exosomes, microvesicles, intercellular transfer

Exosomes are 40-100-nm-diameter nanovesicles of endocytic origin that are released from diverse cell types. Depending on the originating tissue, exosomes contain a subset of the host cell-specific molecular cargo including proteins, lipids and RNA. The affordability of accessing exosomes in bodily fluids and the presence of host-cell specific cargo have created immense interest in utilizing exosomes for biomarker analysis. Here, we describe an immunoaffinity capture method using the colon epithelial cell-specific A33 antibody to purify colorectal cancer cell (LIM1215)-derived exosomes. Mass spectrometry-based proteomic analysis revealed a significant enrichment of proteins containing coiled coil, RAS, and MIRO domains in the exosomes. We also performed RNA-sequencing to profile the small RNA content of LIM1215 exosomes. A diverse range of RNA species were identified in the exosomes which have the potential to regulate the signaling events in the target cell. With all the collated data, we performed an integrative systems biology analysis which revealed the presence of oncogenic cargo in exosomes. Overall, the oncogenic molecular signatures identified in exosomes have immense potential for their use as biomarkers and provides possible clues on the intercellular spread of oncogenic cargo.
Transcriptional and translational control in the germ line

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Setting germ cells apart from other somatic cells in the body is crucial for all sexually reproducing organisms. This process of germ cell specification occurs early in embryogenesis and requires the conserved process of transcriptional silencing. In *Drosophila* germ cells global transcriptional silencing is mediated by the gene *polar granule component (pgc)*, which codes for a small 7 kDa protein. *pgc* mutant germ cells show precocious active chromatin marks that result in the transcription of somatic genes that lead to their death. Thus transcriptional silencing mediated by *pgc* feeds into the epigenetic pathway and plays a critical role in specifying a germ cell fate by suppressing a somatic one. We have found that in *Drosophila* repressive transcriptional mechanisms also play an important role at a different stage of germ cell development, namely GSC differentiation. Repressive marks consistent with heterochromatin, namely H3K9me3, H4K20me3 and HP1, are enriched in the nuclei of the differentiating GSC daughter. Interestingly, H3K9me3 enriched chromosomal sites correlate with the location of piRNA production. The piRNA pathway is a small RNA-based mechanism that is a major component of the defense against transposable elements (TE) in the germ line. In mutants defective in heterochromatin formation, differentiation of GSC is blocked, piRNA levels are reduced, and TE activity is increased. While we thus know that heterochromatin protects the germ line by activating the piRNA pathway and repressing transposable elements, we do not understand what controls heterochromatin formation in the GSC daughter. Surprisingly, we have observed that the transcriptional silencer *pgc* that has a known role in germ cell specification is also expressed during GSC differentiation. We hypothesize that *pgc* causes transient transcriptional silencing that allows heterochromatin formation on the piRNA producing clusters and thus permits GSC differentiation.
Oral abstract: 43

Targeting colorectal cancer liver metastases with folate-conjugated ultrastable RNA nanoparticles

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. Liver metastases develop in nearly 50% of patients with advanced CRC and represent the major cause of death during the course of the disease. Emerging RNA nanotechnologies promise new approaches to early detection and treatment of metastatic CRC. Here, we designed thermodynamically stable RNA nanoparticles to carry functional siRNA sequences, and evaluated nanoparticle targeting in an experimental model of CRC liver metastasis.

METHODS. Functional X-shaped branched packaging RNA (pRNA) nanoparticles were assembled by mixing four RNA oligos; each nanoparticle carried 4 siRNA sequences against firefly luciferase. pRNA nanoparticles knockdown efficiency was evaluated in vitro in HT29 GFP-Luc and KM20 GFP-Luc CRC cell lines. RNA nanoparticle specific binding to CRC cells in vitro was confirmed with confocal microscopy. CRC liver metastases were established by intrasplenic injection of HT29LM3 and KM20 cell lines. Folate conjugated pRNA nanoparticle and self-assembling RNAi nanoparticle targeting was evaluated in a clinically relevant model of CRC liver metastasis. RNA nanoparticle specific binding to CRC cells in vivo was confirmed with macroscopic imaging and confocal microscopy of frozen tissue sections.

RESULTS. Our results show that folate conjugated pRNA nanoparticles can be taken up by CRC cells via receptor-mediated endocytosis. pRNA nanoparticles with four siRNA modules achieved a similar silencing effect as siRNA at 1 nM concentration compared with 100 nM of siRNA. Synthetic RNAi nanoparticles retained in normal liver parenchyma and did not penetrate CRC liver metastases after systemic injection. Folate conjugated pRNA nanoparticles, after systemic injection, remained intact and strongly bound to liver metastases without retention in normal liver, lung or any other organs or tissues.

CONCLUSIONS. We determined that effective siRNA therapy for metastatic CRC is currently not possible with nanocarriers that depend on enhanced permeability and retention effect. Our results demonstrate, for the first time, selective targeting of the pRNA nanoparticles to CRC liver metastases and therapeutic potential of pRNA as a delivery system for future clinical applications.
Intestine mucus-derived exosomes provide a bridge between the gut and the liver that suppresses liver inflammation through prostaglandin E\textsubscript{2} induction of NKT cell anergy

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Keywords: Exosomes, Wnt and PGE2 signaling, liver NKT anergy

The \textit{in vivo} regulation/induction of NKT cell anergy has been associated with inhibition of autoimmune and anti-tumor responses, but the underlying mechanisms are poorly understood. The mechanisms underlying NKT cell anergy are poorly understood. Here, we demonstrate that intestine mucus-derived exosomes (IDE) migrate to the liver where they induce NKT cell anergy. These effects were mediated by an exosomal PGE\textsubscript{2}. Blocking PGE\textsubscript{2} synthesis attenuated IDE inhibition of induction of IFN-g and IL-4 by \textalpha-GalCer stimulated liver NKT cells in a PGE\textsubscript{2} EP2/EP4 receptor mediated manner. Pro-inflammatory conditions enhanced the migration of IDE to the liver where both \textalpha-GalCer and PGE\textsubscript{2} induced activation of the Wnt pathway thereby inducing NKT anergy in response to subsequent \textalpha-GalCer stimulation. These findings demonstrate that IDE serves as immune modulators between the liver and intestine and maintain liver NKT homeostasis and have implications for development of NKT cell--based immunotherapies.
Engineered “restriction RNases” for sequence-specific cleavage of dsRNA and RNA in DNA-RNA hybrids

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Ribonucleases (RNases) are valuable tools applied in the analysis of RNA sequence, structure and function. Their substrate specificity is limited to recognition of single bases or distinct secondary structures in the substrate. Thus far, there have been no RNases available for purely sequence-dependent fragmentation of RNA, analogous to restriction enzymes for DNA. We have therefore searched for existing RNases that could be engineered to become sequence-specific. Using a combination of bioinformatics methods and experimental protein engineering we have obtained prototypes of two sequence-specific “restriction RNases” (RRNases): one that cleaves both strands of dsRNA within a target sequence, and one that cleaves RNA within DNA-RNA hybrids at a particular distance from the target sequence.

Based on structural analysis of enzymes from the RNase III superfamily we identified loops that could be extended to make specific contacts with bases in the dsRNA substrate. Biochemical characterization of selected members that possess extended versions of such loops revealed that some of them indeed exhibit sequence specificity. For one of such enzymes we constructed a structural model of a protein-RNA complex, and used it to guide site-directed mutagenesis aimed at elucidating the molecular basis of specificity and to increase the selectivity of cleavage. The obtained prototype RRNase recognizes a partially degenerated hexanucleotide target sequence and is capable of cleaving individual sites in long dsRNA molecules.

A prototype RRNase that cleaves the RNA strand in DNA-RNA hybrids 5 nucleotides from a nonanucleotide recognition sequence was constructed by fusing two functionally distinct domains: a non-specific RNase HI and a zinc finger that recognizes a sequence in DNA-RNA hybrids. The optimization of the fusion enzyme’s specificity was guided by a structural model of the protein-substrate complex and involved a number of steps, including site-directed mutagenesis of the RNase moiety and optimization of the interdomain linker length.

For both types of RRNases we implemented methods of specificity engineering, to enable generation of variants specific for other target sequences, making it feasible to acquire a library of enzymes that recognize and cleave a variety of sequences, much like the commercially available assortment of restriction enzymes. Potentially, RRNases may be used in vitro for production of RNA molecules with defined length and termini, which may be a cheaper alternative to chemical synthesis; they may be also used in vivo for targeted RNA degradation.
Valproic Acid (VPA) is a commonly used drug to treat epilepsy and bipolar disorders. Known properties of VPA are inhibitions of histone deacetylases and activation of extracellular signal regulated kinases (ERK), which cannot explain VPA’s clinical features fully. We found that VPA causes loss of DICER protein by inducing its proteasomal degradation. In agreement with a loss of DICER, VPA changes the miRNA composition of the cell. Unexpectedly, the concentration of several miRNAs increases after VPA treatment, caused by the upregulation of their hosting genes prior to DICER degradation. VPA changes the miRNA content of exosomes, which are membrane-covered vesicles released by cells. VPA induced exosomes can be taken up by naïve cells where they influence reporter gene expression. The data suggest that a change in miRNAs contributes to the clinical properties of VPA. We identified VPA as a substance that can be used to globally manipulate miRNA concentrations and that changes exosomal miRNA composition.
Poster Abstracts
In numerical order

Crowne Plaza – The Campbell House
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April 3-5, 2013
Poster abstract: 1

Effects of nanomaterials on nucleic acids and the structure-activity of proteins

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Keywords: RNA nanoconjugates, protein nanoconjugates. Zinc oxide nanomaterials, fluorescence, luminescence, absorbance, protamine, albumin, λ-phage DNA, Diethylaminoethyl RNA, luciferase, β-galactosidase

The primary objective of this study is to investigate the interaction of nanomaterials with biomacromolecules, specifically model RNA, DNA and proteins. Here we present spectroscopic evidence of zinc oxide nanomaterial (ZnO) interaction with diethylaminoethyl (DEAE) RNA and λ-phage DNA. A shift in ZnO fluorescence was also shown to occur in the presence of RNA, again suggesting nanomaterial interaction with RNA. Previous studies have shown that nanomaterials can interact with and affect proteins.1, 2 In this research, such interactions were further studied and analyzed. The protein interaction between ZnO and luciferase was further studied by examining the effect of protamine, previously shown to interact with ZnO1, on the binding of luciferase to ZnO. Kinetic studies were also performed on luciferase in the presence of different morphologies of ZnO, showing consistent increased activity over time. The effect of ZnO on β-galactosidase, a common and well-characterized protein in biotechnology, was also studied. It was shown that ZnO caused activation of the β-galactosidase enzyme. Nickel and silicon nitride nanomaterials were shown to cause an increase in β-galactosidase activity and a decrease in luciferase enzyme activity, respectively. 2-D Fluorescence was used to further attempt to characterize and measure the interaction between different types of ZnO and protein (protamine and albumin). These findings ultimately provide more evidence for the interaction between nanomaterials and proteins, as well as the possible use of these interactions in biotechnology, such as screening and detection.

References:
Poster abstract: 2

Assembly of biomimetic viral DNA packaging nanomotor geared by hexameric RNA toward bioreactors, single molecule sensing, and high throughput RNA/DNA sequencing

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Microscopic biomotors are as important to nanotechnology as macroscopic motors are to society. The bacterial virus phi29 DNA packaging biomimetic motor is one of the strongest motors to date that is assembled from chemically synthesized or purified recombinant components. The ingenious motor design, with its elegant and elaborate channel, has inspired its application in nanotechnology and nanomedicine. The motor is geared by a hexameric RNA ring and is driven by one ATPase. It facilitates dsDNA translocation through a dodecameric channel that forms a ring containing a central 3.6-nm channel that acts as a path for dsDNA to enter during packaging and exit during infection. The observed, single-direction dsDNA transportation mechanism provides a novel system with a natural valve that controls dsDNA loading and gene delivery in bioreactors and liposomes, or as a high throughput DNA sequencing apparatus. Insertion of the motor into the membrane of liposomes has created bioreactors with pores that can easily be turned on and off. We propose a liposome-based drug delivery system capable of concentrated drug release multivalent in nature.

We elucidated the sequential action of the motor ATPase and additional motor components used in motor action. The contact between ATPase and ATP results in a conformational change to a state with a higher binding affinity towards dsDNA. We have found that ATP hydrolysis leads to the departure of dsDNA from the ATPase/dsDNA complex, an action that pushed dsDNA to pass the connector channel. DsDNA packaging occurs through a combined effort of both the gp16 ATPase that pushes and the channel that acts as a one-way valve to control the dsDNA translocation direction. The combination of the two distinct roles of gp16 and the connector renews the perception of previous dsDNA packaging energy calculations, and provides insight into the discrepancy of the number of base-pairs translocated per ATP.

References:
Predicting the folding properties of RNA nanoscale structures is challenging, because such complexes typically consist of multiple strands that are, from an algorithmic point of view, highly pseudoknotted structures. Also, multi-pot assembly experiments call for simulating the kinetic behavior of RNA complexes. A new algorithm for simulating the assembly of multistrand nucleotide complexes is presented. This approach simulates the step-wise assembly of RNA and DNA strands. The approach is not restricted in terms of pseudoknot complexity; it also takes steric constraints into account. We show that the algorithm successfully predicts the RNA secondary structure of several RNA complexes such as the RNA tectosquare as well as RNA/DNA hybrid complexes.

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Poster abstract: 4

Thermodynamic analysis of the ultra-stable phi29 pRNA nanoparticles

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RNA nanoparticles have been shown to possess the ability to specifically target and therapeutically treat cancers and viral infections, yet the instability of the particles has left the field of RNA nanotechnology underdeveloped. Recently, novel nanoparticles developed from the core structure of the pRNA of the bacteriophage Φ29 DNA-packaging motor has resulted in the thermodynamically ultra-stable three-way junction (3WJ) and X-way motifs. Each motif is assembled from individual RNA oligo strands with high efficiency, and displays remarkably low ΔGs, as shown in novel temperature profiles with slopes near 90° angles during the assembly of the motifs. Furthermore, these nanoparticles remain intact at extremely low concentrations in vitro and in vivo, giving it a long half-life in vivo. Conjugation of functional RNA moieties, such as receptor-binding aptamer, siRNA, ribozyme, miRNA, or riboswitch, resulted in RNA nanoparticles harboring different functional modules. The inserted RNA molecules retained their folding and independent functionalities for specific cell binding, gene silencing, catalytic function, and cancer targeting both in vitro and in vivo. Here we examine the thermodynamic characteristics of the pRNA-3WJ and X-way motifs to elucidate the stable structure of these unique RNAs. The ease of folding and high stability of the pRNA nanoparticles enhances the field of RNA nanotechnology, and provides an answer to the instability issues that have previously hindered the application of RNA nanotechnology in the treatment of cancers and viral infections.

References:
New tools for RNA structure prediction, modeling, and analysis

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Keywords: RNases, RNA cleavage, RNA nanotechnology, enzymes acting on RNA, RNA-protein interaction, sequence-specific recognition

In analogy to proteins, the function of non-coding RNA molecules depends on their structure and dynamics, which are encoded in their linear sequences. A few successful strategies have been identified for practically useful predictions of protein 3D structures from their sequences. There have been, however, very few automated computational methods for 3D modeling of RNA. We propose that the same principles as those used to model protein structures are applicable to model RNAs and we have recently developed tools for RNA 3D modeling based on algorithms successfully used for protein structure prediction.

ModeRNA is a comparative modeling method. It requires a 3D structure of a template RNA molecule, and a sequence alignment between the target to be modeled and the template. It can model posttranscriptional modifications. ModeRNA can model the structures of RNAs of essentially any length, provided a homologous template structure exists. Recently, we have extended this method to use multiple templates, which improves modeling in conserved as well as variable regions.

SimRNA can fold RNA 3D structure starting from sequence alone. It is based on a reduced representation of the polynucleotide chains (5 atoms per nucleotide in the most recent version), uses a Monte Carlo sampling scheme, and a statistical potential to estimate the free energy. The SimRNA energy reproduces a characteristic funnel-like shape for energy vs model quality. The current implementation is capable of finding a native-like conformation for RNAs <100 nt in length without any restraints, and much longer molecules if additional data are available such as secondary structure and long-range tertiary contacts.

CompaRNA is an online system for continuous evaluation of RNA structure prediction methods, which allows for objective testing and head-to-head comparison of web servers and standalone programs for prediction of secondary and tertiary RNA structure. Structures of RNAs deposited in PDB and those in the RNASTRAND database are used as the benchmark, revealing strengths and shortcomings of different algorithms.

We have also developed a new method for the identification of tertiary contacts in RNA structures, including nucleotide-nucleotide pairing and stacking and base-phosphate interactions. The method has been trained on binary contacts that are classified similarly by at least two of the commonly used methods: RNAView, FR3D, and MC-Annotate. Its advantage lies in the ability to handle structures that are imperfect (e.g. unoptimized theoretical models) or coarse-grained models that have only a few atoms per residue.

Our methods are available at http://genesilico.pl
Effects of Ag2Se nanoparticles on bioactivity of RNAse A and RNA

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Keywords: RNAse A, RNA, Ag2Se, synthesis, bioactivity, interaction

With the rapid development of nanotechnology, interaction between nanoparticles and nucleic acids such as DNA, RNA and protein has become our concerns. We used Rapid Translation System (RTS) to investigate the influence of gold nanoparticles on DNA transcription and RNA translation, and then investigated the effects of RNA enzyme as template to synthesize Ag2Se nanoparticles, and then investigated the effects of RNase A-Ag2Se nanoparticles on RNA bioactivity. We also used RNA as template to synthesize Ag2Se nanoparticles, and then investigated the influence of RNA enzyme on RNA-Ag2Se nanoparticles. Results showed that gold nanoparticles within less than 10nm in diameter could improve the efficiency of RNA translation, increasing final yields. RNAse A-Ag2Se nanoparticles own enhanced damaging RNA properties. Conversely, RNA-Ag2Se nanoparticles can enhance the ability of protect RNA from being degraded by RNAse A enzyme. In conclusion, Ag2Se nanoparticles can enhance the bioactivity of RNase A enzyme, and protect RNA from being damaged by RNAse A. This phenomena may have great potential in applications such as designing of targeted drugs, or imaging contrast reagents, and preparing drug peptides in near future.

Acknowledgements:
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Fabrication of stable and RNase-resistant RNA nanoparticles active in gearing the nanomotors for viral DNA packaging

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Both DNA and RNA can serve as powerful building blocks for bottom-up fabrication of nanostructures. A pioneering concept proposed by Ned Seeman 30 years ago has led to an explosion of knowledge in DNA nanotechnology. RNA can be manipulated with simplicity characteristic of DNA, while possessing noncanonical base-pairing, versatile function, and catalytic activity similar to proteins. However, standing in awe of the sensitivity of RNA to RNase degradation has made many scientists flinch away from RNA nanotechnology. Here we report the construction of stable RNA nanoparticles resistant to RNase digestion. The 2-F (2-fluoro) RNA retained its property for correct folding in dimer formation, appropriate structure in procapsid binding, and biological activity in gearing the phi29 nanomotor to package viral DNA and producing infectious viral particles. Our results demonstrate that it is practical to produce RNase-resistant, biologically active, and stable RNA for application in nanotechnology.

References:

Poster abstract: 8

Enhanced tumor accumulation and magnetic resonance imaging using cross-linked nanoassemblies loaded superparamagnetic iron oxide nanoparticles in a glioma rat model

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Keywords: cross-linked nanoassemblies loaded superparamagnetic iron oxide nanoparticles, tumor accumulation, magnetic resonance imaging, glioma

Purpose: To develop cross-linked nanoassemblies loaded superparamagnetic iron oxide nanoparticles (CNA-IONPs) accumulating in a glioma rat model and imaging magnetic resonance (MR).

Methods: CNA-IONPs were synthesized and characterized in house. Gd-DTPA was used to monitor the glioma tumor growth as T1 contrast agent. CNA-IONPs were injected through tail vein to a glioma xenograft model. T2 weighted MRI was taken at 10 min and 2 h after the injection. T2* was used to predict the CNA-IONPs concentration increases over time. Glioma tumor, contralateral brain tissue, liver, spleen, lung, heart, intestine, bone marrow and blood were collected at 2 h or 6 h and the iron concentrations were analyzed by ICP-MS.

Results: CNA-IONPs have neutral zeta potential, hydrodynamic size around 30 nm and highly stable in serum containing medium at 37 °C over 30 h. CNA-IONPs showed significant T2 contrast enhancement 2 h after injection. However, Gd-DTPA as a typical T1 contrast agent was almost all cleared 2h after injection. \( R2^* (1/T2^*) \) at 2 h increased 48\% then \( R2^* \) at 10 min which is proportion to iron concentration. The iron concentration in the glioma tissue was 6 times higher compared with contralateral brain tissue at 2 h analyzed by ICP-MS. The blood concentration of iron did not decrease significantly from 2 h to 6 h. The iron concentrations in liver, lung, spleen and other organs were not significantly increased from 2 to 6 h.

Conclusions: CNA-IONPs had desirable physicochemical properties for \textit{in vivo} application. CNA-IONPs preferential accumulated in glioma and the tumor accumulation increased over time consistent with their long circulation time. CNA-IONPs have the potential for MRI diagnosis and provide a good platform for glioma therapeutic application.
Ant-cancer splice-switching oligonucleotide nanoconjugates

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Keywords: Splicing, Oligonucleotide, BRAF, nanoparticles

Splice switching oligonucleotides (SSOs) may hold an advantage over siRNA therapeutics. SSOs have the ability to target upstream of mRNA at the pre-mRNA level and increase or decrease the expression of their protein target. Nanomaterial:SSO conjugates show promise to penetrate the cell and act as a novel delivery system. Previously, we have shown the formation of conjugates and the functional delivery of luciferase pDNA using our gold:protamine and manganese oxide:PAMAM dendrimer nanoconjugates. The purpose of this research is to develop a suitable delivery system for SSOs. Here, we designed a series of SSOs to target the exon-intron junctions in various functionally important domains of B-Raf, a common oncogene in human cancer. The ideal nanomaterial delivery vehicle would be able to pass through the cell membrane, yet not be cytotoxic to the cell. We screened a series of metal oxides and composite nanomaterials for these qualities using a high throughput liposome interaction assay and a trypan blue cytotoxicity assay. Nanomaterials were also assayed for functional SSO delivery using the HeLa pLuc model system. This study examines the potential of these nanomaterials to functionally deliver SSOs in the HeLa pLuc system.
Poster abstract: 10

CLIP-based prediction of mammalian microRNA binding sites

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Keywords: microRNA target prediction, siRNA off-target effects

Prediction and validation of microRNA (miRNA) targets are essential for understanding functions of miRNAs in gene regulation. Crosslinking immunoprecipitation (CLIP) allows direct identification of a huge number of Argonaute-bound target sequences that contain miRNA binding sites. By analyzing data from CLIP studies, we identified a comprehensive list of sequence, thermodynamic and target structure features that are essential for target binding by miRNAs in the 3′ untranslated region (3′ UTR), coding sequence (CDS) region, and 5′ untranslated region (5′ UTR) of target messenger RNA (mRNA). The total energy of miRNA:target hybridization, a measure of target structural accessibility, is the only essential feature for both seed and seedless sites in all three target regions. Furthermore, evolutionary conservation is an important discriminating feature for both seed and seedless sites.

These features enabled us to develop novel statistical models for the predictions of both seed sites and broad classes of seedless sites. Through both intra-dataset validation and inter-dataset validation, our approach showed major improvements over established algorithms for predicting seed sites and a class of seedless sites. Furthermore, we observed good performance from cross-species validation, suggesting that our prediction framework can be valuable for broad application to other mammalian species and beyond. Transcriptome-wide binding site predictions enabled by our approach will greatly complement the available CLIP data, which only cover small fractions of transcriptomes and known miRNAs due to non-detectable levels of expression. Software and database tools based on the prediction models have been developed and are available through Sfold web server at http://sfold.wadsworth.org.

These tools can be used to address the issue of targeting specificity by short interfering RNAs (siRNAs). siRNAs are known to cause off-target effects via the microRNA pathway triggered by nonspecific binding of the siRNAs to unintended targets with mismatches. Target specificity is important for the development of a siRNA based therapeutic agent that can be delivered through nanoparticles. Our tools for miRNA binding site prediction and previously developed tools for RNAi design can be integrated for the rational design of genome-scale library of highly potent and highly specific siRNAs.

This work was supported in part by NSF grant DBI-0650991 and by NIH grant GM099811.
Determinaton of the secondary structure of Group II bulges using the fluorescent probe 2-aminopurine

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Keywords: RNA, RNA Hairpin, Group II bulge loop, 2-aminopurine, fluorescence, thermodynamics

The fluorescent analogue of adenine, 2-aminopurine, can be used to determine structural changes of proteins and nucleic acids. In this study, optical melting in 1M NaCl and fluorescence spectroscopy of RNA hairpins containing 2-aminopurine were used to study conformational changes. Control RNA hairpins were designed with 2-aminopurine substituted in base paired or unpaired (bulged) positions. The steady-state fluorescence of the control hairpins was investigated as the RNA hairpin underwent conformational changes using thermal denaturation. As expected, the 2-aminopurine in a base paired position resulted in an increase in fluorescence while 2-aminopurine in an unpaired position resulted in a decrease in fluorescence as the RNA hairpin denatured (Ballin et al. 2007). Because of the site-specific fluorescence 2-aminopurine shows, the secondary structure of ambiguous RNA hairpins can be determined. RNA hairpins containing group II bulge loops where the bulged nucleotide is identical to one of its nearest neighbors leads to ambiguity as to the exact position of the bulge. RNA hairpins were designed with 2-aminopurine substituted at one of the ambiguous nucleotides in the 5' or 3' sides of the hairpin stem. Based on the fluorescence change upon heating, it can be determined whether 2-aminopurine is the base-paired or bulged nucleotide. Previous structural probing of hairpins containing group II bulge loops indicated that the bulged nucleotide was the one farthest from the hairpin loop (McCann et al. 2011). The results of this study show that when 2-aminopurine is in the position farther from the hairpin loop it shows similar fluorescent behavior to an unpaired nucleotide suggesting that the nucleotide farther from the hairpin loop is bulged. Additionally, optical melting results of the RNA hairpins containing 2-aminopurine showed similar thermodynamics to the analogous hairpins containing adenine suggesting that the 2-aminopurine substitution did not alter the hairpins natural stability or secondary structure. The results of this study can be used to improve the accuracy of RNA secondary structure predictions for group II hairpins.

References:
Ocular delivery of RNA nanoparticles and their distribution

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Aim: The purpose was to investigate the distribution of RNA nanoparticles in the eye after subconjunctival administration.

Method: Alexa647-labeled RNA nanoparticles (pRNA-X and pRNA-3WJ) and double-stranded RNA (dsRNA) were injected via the subconjunctival route in mice. The distributions of the nanoparticles and dsRNA were assessed using fluorescence microscopy, and the number of cells in different sections of the eye (conjunctiva, cornea, retina, and sclera) with nanoparticle internalization was determined.

Results: pRNA-X, pRNA-3WJ, and dsRNA were found in the cornea, conjunctiva, and sclera after subconjunctival injection. Among these RNA systems, only pRNA-X was efficiently delivered to the cells in the retina; pRNA-3WJ and dsRNA were not observed in the retina. Compared with pRNA-3WJ and dsRNA, pRNA-X showed faster distribution to the cells in the tissues and higher clearance rates.

Conclusion: RNA nanoparticles can be effectively delivered to the cells in cornea and retina via subconjunctival injections and thus can serve as an efficient drug delivery system to treat ocular diseases.
Enhanced stability and RNA protection via the formation of mPEG-PAMAM-G4 dendrimer and nucleic acid complexes


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Keywords: Dendrimer, nucleic acid, nanocomplex, mPEG-PAMAM

Background: PAMAM dendrimers are unique compounds that are being tested as prospective gene delivery agents due to their ability to bind nucleic acid. Two common complications are associated with nucleic acid delivery: aggregation and instability. Thus, there is a need for novel and more effective polymers that can be used as prospective gene delivery agents with little to no cytotoxicity. PEGylation of the PAMAM polymer has been reported to decrease cytotoxicity and increase biocompatibility of the polymer. The interactions of mPEG-PAMAM-G4 with nucleic acid were considered here at various N/P ratios, demonstrating nanoplexes were capable of independently forming (range=240-430 nm) while maintaining stable, non-aggregate structures over time. AFM imaging established DNA compaction through dendrimer encapsulation. RNase digestion experiments clearly demonstrate protection of RNA when complexed with G4 dendrimer.

Objectives: A) To examine the interactions of mPEG-PAMAM dendrimer generation 4 with RNA at various N/P ratios. B) To determine the average size of the complexes formed at various N/P ratios. C) To analyze stability over time using the DLLS. D) To analyze the protective properties of the dendrimer against RNase. E) To examine cytotoxicity and delivery mechanisms.

Results: The study demonstrates that at different N/P ratios on an agarose gel, interactions between RNA and mPEG-PAMAM occur. As the N/P ratio decreases below one, the RNA was able to move freely through the agarose gel compared to when it was retained at an N/P ratio >1. Dynamic laser light scattering (DLLS) was used to analyze the aggregation of the mPEG-PAMAM:RNA nanocomplexes at various N/P ratios. The timeframe of the DLLS experiments was from 0-72 hours, with sizes remaining relatively constant (range=240-430 nm). Aggregation did not occur, as seen in the size data over time. AFM imaging established DNA compaction through dendrimer encapsulation. RNase digestion experiments clearly demonstrate protection of the RNA when complexed with G4 dendrimer. Cytotoxicity of the mPEG-PAMAM G4 at various concentrations indicated some cytotoxicity at higher levels. mPEG-PAMAM also proved an efficient transfection agent, as shown in the transfection of luciferase gene-containing plasmids.
RNA interference (RNAi) is an incredible endogenous regulatory pathway that can cause sequence-specific gene silencing with implications towards treatments of diseases such as viral infections and cancer. Naked, unmodified, small interfering RNA molecules (siRNAs), though avid candidates, cannot be used directly for such intervention due to their short half-lives in the blood stream and their negative charge which poses barriers in crossing biological membranes. Therefore, in vivo siRNA delivery platforms are being examined. Our group is developing a new class of lipid-based delivery platform that uses bolaamphiphiles, for the efficient delivery of siRNAs to diseased cells. Bolaamphiphiles consist of a hydrophobic chain with one or more positively charged head groups at each end. Low toxicities, long half-lives in the blood stream, ability to form poly-cationic micelles in aqueous conditions and to form complexes with negatively charged siRNAs make them preferred siRNA delivery carriers. Recently, we have reported that micelles of bolaamphiphiles GLH-19 and GLH-20 efficiently deliver siRNA with relatively low toxicities in vitro and in vivo. Our in vitro, in vivo and in silico studies validated that, bolaamphiphiles can be designed to vary the magnitude of siRNA shielding, its delivery, and further release. On this basis, we have designed and synthesized a new series of bolaamphiphiles, (GLH-45, GLH-46, GLH-58 and GLH-60), and characterized their transfection efficiency, silencing of eGFP by siRNA in a GFP expressing breast cancer cell line (MDA-MB-231). Among all the constructs tested, the GLH-58 exhibited superior transfection and eGFP silencing efficiency. These newly fabricated bolaamphiphiles will be examined further to determine their potential capability for transfection, silencing the oncogenic pathways and toxicity in the tumor model system.
Poster abstract: 15

Biophysical analyses of self-assembling packaging RNAs from the phi29 family of bacteriophages

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Keywords: phi29 packaging RNA, biophysical analyses

Self-assembling RNAs show great potential as building blocks for nanodevice design. The packaging RNA (pRNA) that is produced by members of the bacteriophage phi29 family is an example of such a self-assembling RNA. The pRNA is an important component of a powerful motor that the phage uses to package its DNA genome into the capsid. The pRNA forms a higher-order multimer by intermolecular “kissing” interactions between identical molecules, and formation of the multimer within the motor is required for DNA packaging. To gain insight into the rules that govern pRNA multimer formation and thus may enable rational engineering of the pRNA, we are studying the pRNAs from different members of the phi29 family. These different pRNAs have different sequences and kissing interactions, and thus could teach us how these differences relate to self-association. Using a combination of biochemical and biophysical methods to include native gel electrophoresis, dimethyl sulfate (DMS) probing, and analytical ultracentrifugation (AUC), we are exploring the conformational dynamics within the pRNAs, how these dynamics relate to multimer formation, and the different abilities of these pRNAs to self-associate. We have discovered that while the length and sequence of the kissing interaction plays a role in multimer formation and stability, there are important contributions from other structural elements in the pRNA. Using this information, we are developing new testable hypotheses for how these RNAs self-associate and are attempting to engineer the pRNA to create more stable multimers. Ultimately, this information may help generate engineered phi29-derived nanomotors whose function is modulated through their RNA component.
Mapping the structural change caused by tetracycline binding to the ykkCD antibiotic sensor RNA

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Keywords: Riboswitch, Structural Change, Nucleic Acid Footprinting, Gene Expression Regulations

Riboswitches are RNA aptamers that form a precise three-dimensional structure and selectively bind to a target molecule. Binding of the best molecule initiates an allosteric structural change that in turn regulates expression of the target gene. Most riboswitches specifically recognize the metabolic product of the gene that is being regulated. Expression may be regulated at transcription or translation stage of gene expression. Most riboswitches are off switches meaning they turn off expression of metabolite producing gene when metabolite concentration is high enough. The ykkCD putative riboswitch appears to bind to the antibiotic tetracycline and increases production of an efflux pump that expels toxic drugs from the cell. Based off of previous data collected the ykkCD putative riboswitch seems to be regulated at transcription. To confirm this hypothesis we want to map the structural change that takes place upon binding of the antibiotic tetracycline to the mRNA. Nucleic acid footprinting studies will be used to map the binding site of tetracycline and the allosteric change that takes place upon tetracycline binding.
Specific delivery of siRNA into brain derived endothelial cell using aptamer-pRNA nanoparticle

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Small interfering (si)RNAs show significant potential to inhibit expression of the complementary RNA transcripts and specific proteins. However, the effective delivery of siRNA remains a challenge in siRNA therapeutics. Currently, aptamers targeting specific cell surface receptors have been successfully used for the targeted delivery of siRNA. In this study, packaging RNA (pRNA) serves as the carrier to deliver siRNA into brain derived endothelial cells (bEND5 cells) by targeting transferrin receptor (TfR) via TfR aptamer (FB4). Aptamer-siRNA chimeras (FRS-NPs) were constructed via dimerization of two pRNA monomers, one of which contained TfR aptamer and the other harbored anti-ICAM-1 (an inflammatory molecule) siRNA. Our data supported the uptake of the RNA chimeras by bEND5 cells and the release of siRNA from the RNA complex in the cells. FRS-NPs reduced ICAM-1 expression in bEND5 cells in a dose dependent manner. In inflammatory model, FRS-NPs reversed the upregulation of ICAM-1 level induced by TNF-α. In oxygen glucose deprivation/reoxygenation (OGD/R) model, FRS-NPs inhibited the OGD/R-induced increase of ICAM-1 expression on bEND5 cells. These results are consistent with the data of adhesion assay. FRS-NPs inhibited adherent of monocyte to bEND5 cells under inflammatory and OGD/R conditions. FRS-NPs, as the novel brain-targeted nanoparticles, can be delivered into brain endothelial cells and knockdown ICAM-1 expression. The RNA chimeras have inhibitory effect on inflammation and may become a promising approach to treat brain disorders associated with inflammation, including cerebral ischemia and Parkinson’s disease.
Poster abstract: 18

Thermodynamically stable RNA three-way junction for constructing multifunctional nanoparticles for delivery of therapeutics

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RNA nanoparticles have applications in the treatment of cancers and viral infection; however, the instability of RNA nanoparticles has hindered their development for therapeutic applications. The lack of covalent linkage or crosslinking in nanoparticles causes dissociation in vivo. Here we show that the packaging RNA of bacteriophage phi29 DNA packaging motor can be assembled from 3–6 pieces of RNA oligomers without the use of metal salts. Each RNA oligomer contains a functional module that can be a receptor-binding ligand, aptamer, short interfering RNA or ribozyme. When mixed together, they self-assemble into thermodynamically stable tri-star nanoparticles with a three-way junction core. These nanoparticles are resistant to 8 M urea denaturation, are stable in serum and remain intact at extremely low concentrations. The modules remain functional in vitro and in vivo, suggesting that the three-way junction core can be used as a platform for building a variety of multifunctional nanoparticles. We studied 25 different three-way junction motifs in biological RNA and found only one other motif that shares characteristics similar to the three-way junction of phi29 pRNA.

References:

High specificity of RNA base pairing with 2-Se-uridine

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Keywords: RNA, U/G Wobble, Base-Pairing Specificity, Selenium-Modified Nucleic Acids, 2-Se-Uridine

The Watson-Crick base pairing system is essential for genetic information storage, replication, transcription and translation. However, the wobble base pairs, where U in RNA (or T in DNA) pairs with G instead of A, compromise the specificity of base pairing. The U/G wobble pairs are ubiquitous in RNA, especially in non-coding RNA. On the basis of the four-letter genetic code of RNA (A, C, G and U), recently Huang laboratory has developed 2-Se-uridine by replacing the 2-exo oxygen on uridine with selenium to improve the accuracy of RNA base pairing. Our biophysical and crystallographic studies of 2-Se-U-containing RNAs indicate that this single-atom replacement can largely improve base pairing fidelity against U/G wobble pair, without significant impact on U/A pair. Our discovery provides a unique chemical strategy to enhance RNA base pair recognition, minimize the off-target effects in RNA-based diagnostics and therapeutics, and design RNA nanostructures. Furthermore, 2-Se-U-RNA can serve as a powerful tool for crystallographic studies of RNA nanostructures and RNA/drug and RNA/protein complexes through crystallization facilitation and phase determination.

References:

RNA nanocube characterization with anisotropic network model complements experimental data

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Keywords: nanocubes, coarse-grained dynamics, computational characterization

We have been developing computational methods for the design and characterization of RNA nanoparticles. The ultimately goal is to produce a qualitatively new generation of therapeutics. We have developed the design process in which computer tools are used to design nano-scale structures in 3D and to optimize multiple sequence chains that are to self-assemble into the designed particles in a one-pot experimental procedure. Our RNAJunction database of n-way junctions and kissing-loops can be a source of building blocks for our programs, such as NanoTiler and RNA2D3D. These programs are used to build 3D models of user-specified or novel, computer-generated shapes. Our programs allow for structural deformations to be applied to the building blocks in order to produce fully assembled models, just as the natural RNA is deformed by larger structural contexts. The flexibility limits of the building blocks allowed in the modeling process are based on the data available in the form of alternative experimentally obtained structures (NMR, cryoEM and X-ray crystallography), results of Molecular Dynamics (MD) simulations and coarse-grained simulation methods. We present an example of the design and modeling process for three variants of a nanocube, with emphasis put on the importance of the cube flexibility characterization. Theoretical predictions of different efficiency of assembly for three nanocube variants provided by the exploratory NanoTiler modeling were confirmed experimentally. A coarse-grained Anisotropic Network Model (ANM) was used to characterize the cube variants. The ANM simulations capturing the collective low frequency motions of the cubes provided an explanation of the differences between the sizes of the initial (static) models and those of the experimentally measured nanoparticles. The ANM simulations also offered an additional insight into experimental assembly yield differences for the three cube variants and the differences in their melting temperatures. This novel application of a coarse-grained method showed that the dynamics of full nanostructures has to be considered in order to bring the computational and experimental results into agreement. The cube with the best characteristics was used as a scaffold in the delivery of multiple RNA therapeutic functionalities for biomedical applications.

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A structural and thermodynamic analysis of group III single nucleotide bulge loops in RNA hairpins

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Keywords: RNA, Hairpin Loop, Thermodynamics, Bulge Loop, In-Line Structure Probing

Fifteen RNA hairpins containing Group III single nucleotide bulge loops on either the 3’ or the 5’ side of the hairpin loop were optically melted in 1M NaCl in order to determine the thermodynamic parameters ΔH°, ΔS°, ΔG°37, and TM for each hairpin. Group III bulges are characterized by the ambiguity in the position of the bulged nucleotide of the type GA/U; where either the guanine pairs with uracil and forms a wobble base pair with the adenine bulged, or the adenine pairs with the uracil and forms a Watson-Crick base pair with the guanine bulged. Bulged loops inserted into RNA hairpin stems or in duplex motifs have been shown to be destabilizing (Znosko et. al, 2002; Lim et. al, 2012). The thermodynamics of both Group I and Group II bulges embedded in hairpins and duplexes have previously been studied and a non-nearest neighbor model has been proposed to predict the effect of the bulge loop on either duplex or hairpin stability (McCann et. al, 2011). The model suggests that the destabilization of the bulge is related to the least-stable stem. In Group II bulges, the model predicts that the position of the bulged nucleotide is also related to the stability of the second least-stable stem (Lim et. al, 2012). The thermodynamic data for the Group III bulges in duplexes has found that the effect of the bulged nucleotide is related to the second least-stable stem, consistent with the results of the studies done on Group II bulges in hairpins. This study investigated the Group III bulges embedded into RNA hairpins in order to develop a more complete picture of how bulged nucleotides influence the overall stability of the sequence in which they are embedded. Of the fifteen sequences that were melted, seven of them had the bulge on the 5’ side of the hairpin loop, and the remaining eight sequences had the bulge on the 3’ side of the hairpin loop. The thermodynamic data complements the previous Group II bulge results, in that the destabilization of the bulge is related to the stem opposite the hairpin loop. In addition to the optical melting, the bulged nucleotide was localized using in-line structure probing. The sequences used in the probing experiment were the same as the optical melting sequences; seven with the Group III bulge on the 5’ side of the hairpin loop and eight with the bulge on the 3’ side of the hairpin loop. The in-line structure probing experiments found a correlation between the bulged nucleotide being the one farther from the hairpin loop irrespective of the identity of the bulged nucleotide (A or G). This result is consistent with the results found for the Group II bulges in hairpins. The results of this study will improve our ability to predict the structure of RNA.
Poster abstract: 22

Crystal structure of 3WJ core revealing divalent ion-promoted thermostability and assembly of the phi29 hexameric motor pRNA

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The bacteriophage phi29 DNA packaging motor, one of the strongest biological motors constructed to date, is geared by a packaging RNA (pRNA) ring. When assembled from three RNA fragments, its 3-way junction (3WJ) motif, which displays a low ΔG and a Tm curve with a slope close to 90°, is highly thermostable, resistant to 8 Molar urea, and remains associated at extremely low concentrations in vitro and in vivo. It has a long half-life in vivo and can serve as a platform for the construction of RNA nanoparticles for specific targeting of cancer cells without accumulating in normal tissue or organs. To elucidate the structural basis for its novel stability, we solved the crystal structure of this pRNA 3WJ motif at 3.05 Å. The structure revealed two divalent metal ions that coordinate four nucleotides of the RNA fragments. Single molecule fluorescence resonance energy transfer (smFRET) analysis confirmed a structural change of 3WJ upon addition of Mg2+. The reported pRNA 3WJ conformation is different from a previously published construct that lacks the metal coordination sites. Subsequent modeling of a connector/pRNA complex indicates that the pRNA of the phi29 DNA packaging motor exists as a hexameric complex serving as a sheath over the dodecameric connector portal proteins. The reconstructed model of hexameric pRNA on the connector agrees with the AFM images of the phi29 pRNA hexamer and distance parameters obtained from cross-linking, complementary modification, and chemical modification interference.

References:
Poster abstract: 23

A self-transfecting siRNA-polymer hybrid nanomaterial

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Keywords: siRNA, polymer conjugate, self-transfecting

Small interfering RNAs (siRNA) offer silencing of particular target gene with high efficiency. But their application in therapeutics still remains challenging due to their poor cellular uptake. In order to facilitate the cell permeability of polyanionic siRNA, transfection methods use a self-assembly process in which a carrier such as cationic lipids or polymers form polyplexes by non-covalent interaction. These polyplexes have several limitations due to the labile and non-specific nature of the self-assembly in the presence of nucleases and polyanions (i.e. heparin). We devised a covalent polymer escort architecture for siRNA which does not require any transfection reagent for cellular uptake. In this scheme, the passenger strand of siRNA was synthesized with both 5' and 3' terminal alkynes that can be post-synthetically “click” conjugated to mono-azido functional polymers prepared by atom transfer radical polymerization (ATRP). The click-conjugation is highly efficient and purification is a simple filtration step. Subsequently, the guide strand (g-RNA) is annealed to the polymer conjugated passenger strand to yield the polymer escorted passenger siRNA (PEP-siRNA). These nanomaterials are stable against nuclease (RNaseA). In addition, the PEP-siRNA can self-transfect due to the cationic nature of the polymer. The efficacy of these constructs in RNAi was studied using a dual-luciferase reporter system in Drosophila S2 cells. We observed dose dependent knockdown of the reporter gene without the use of any transfection reagent. We then tested these PEP-siRNAs in Hela cells to knockdown an endogenous gene Glolgin160 which is required for assembly of the Golgi apparatus. Our positive results suggest that these hybrid nanomaterials could be highly promising for therapeutic application of siRNA.
Poster abstract: 24

Interference (RANi)-based strategies for diagnosis & treating viral infection

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Keywords: RNAi, Diagnosis, Viral Infection

RNA interference (RNAi) is a natural process that cells use to turn down the activity of specific genes. It has been discovered in 1998 and developed into a powerful research tool used in thousands of labs worldwide.

The RNAi evolved as a cellular defense mechanism against invaders such as RNA viruses. When they replicate, RNA viruses temporarily exist in a double-stranded form. This double-stranded intermediate would trigger RNAi and inactivate the virus’ genes, preventing an infection.

It is necessary to understand RNA’s role in normal and diseased cells, and harness the mechanism for use in medical therapies.

My research interest will be involved in developing novel RNA interference (RNAi)-based strategies for treating viral infections. RNAi is a gene silencing mechanism in which 19-21 nucleotide double stranded RNAs called small interfering RNAs (siRNAs) can guide the destruction of complementary cellular mRNAs with a high degree of specificity. A major challenge in using RNAi-based therapies in vivo is the targeted delivery of siRNAs to specific tissues/cells of interest. Targeted delivery would not only improve efficacy but also reduce potential side effects of RNAi therapy.

This goal will be achieved by recently developed a novel method for the specific delivery of siRNAs into human T cells and tested in vivo efficacy using “humanized mice”: (immunodeficient mice transplanted with human hematopoietic stem cells and consequently a human immune system). Antiviral siRNAs delivered by this approach could control HIV in these mice demonstrating the feasibility of RNAi therapy for HIV infection.

The global mission of RNAi therapeutics delivered with lipid nanoparticles technology will accelerate and facilitate basic biological and medical discovery.

Reference:
In-gel probing of individual conformers within a mixed population reveals a dimerization structural switch in the HIV-1 leader RNA.

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Definitive secondary structural mapping of large, multi-domain RNAs in vitro can be complicated by the presence of more than one structural conformer or by the multimerization of some of the molecules. Until now, probing a single structure of conformationally flexible RNA molecules has relied on mutation of the RNA or adjustment of buffer conditions or RNA concentration. In order to avoid these manipulations, we will present a technique designated in-gel SHAPE (selective 2’OH acylation analysed by primer extension), whereby a mixed structural population of RNA molecules is initially separated by native polyacrylamide gel electrophoresis and the conformers are individually probed by chemical modification within the gel matrix. Validation of the in-gel technique using a well characterized RNA stem-loop structure, the HIV-1 TAR (trans-activation response element) showed that authentic structure was maintained and that it was accurate and highly reproducible. To further demonstrate its utility, in-gel SHAPE analysis was next used to examine separated monomeric and dimeric species of the human immunodeficiency virus, type 1 (HIV-1) packaging signal RNA. Extensive SHAPE reactivity differences were observed between monomer and dimer. The results to be presented support a recently proposed structural switch model of RNA genomic dimerization and packaging and furthermore demonstrate the discriminatory power of in-gel SHAPE. Deciphering the structure of individual RNA conformers will provide novel therapeutic avenues for their disruption by RNA-based therapeutics such as steric blocking oligonucleotides.
Pharmacological characterization of chemically synthesized monomeric phi29 pRNA nanoparticles for systemic delivery

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Previous studies have shown that the packaging RNA (pRNA) of bacteriophage phi29 DNA packaging motor folds into a compact structure, constituting a RNA nanoparticle that can be modularized with functional groups as a nano-delivery system. pRNA nanoparticles can also be self-assembled by the bipartite approach without altering folding property. The present study demonstrated that 2′-F-modified pRNA nanoparticles were readily manufactured through this scalable bipartite strategy, featuring total chemical synthesis and permitting diverse functional modularizations. The RNA nanoparticles were chemically and metabolically stable and demonstrated a favorable pharmacokinetic (PK) profile in mice (half-life ($T_{1/2}$): 5–10 hours, clearance (Cl): <0.13 L/kg/hour, volume of distribution ($V_d$): 1.2 L/kg). It did not induce an interferon (IFN) response nor did it induce cytokine production in mice. Repeated intravenous administrations in mice up to 30 mg/kg did not result in any toxicity. Fluorescent folate-pRNA nanoparticles efficiently and specifically bound and internalized to folate receptor (FR)-bearing cancer cells in vitro. It also specifically and dose-dependently targeted to FR+ xenograft tumor in mice with minimal accumulation in normal tissues. This first comprehensive pharmacological study suggests that the pRNA nanoparticle had all the preferred pharmacological features to serve as an efficient nanodelivery platform for broad medical applications.

References:
microR-561 exaggerates acetaminophen-induced hepatotoxicity through down-regulation of DAX-1

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Keywords: miRNA-561; acetaminophen; DAX-1; hepatotoxicity; HepG2 cells

One of the major mechanisms involved in acetaminophen (APAP)-induced hepatotoxicity is the hepatocyte nuclear factor 4 (HNF4)-mediated activation of PXR- and CAR and subsequently transcriptional activation of the cytochrome P450 3A enzymes. A panel of miRNAs has been shown to be involved in APAP-induced hepatotoxicity in humans, however, it is unclear how these miRNAs regulate the occurrence of toxicity. In the present study, we have investigated the role of microRNA-561 and its target gene DAX-1 encoding a co-repressor of HNF4 in the process of APAP-induced hepatotoxicity. The human hepatoma HepG2 cells were used in this study. RT-PCR and Western blot assays were used to determine the expression levels of DAX-1, PXR and CAR. The levels of reactive oxygen species (ROS), LDH, and glutathione (GSH) were monitored. Treatment of HepG2 cells with APAP caused reduced expression of DAX-1 in a dose-dependent manner. Subsequent microRNA expression analysis showed the miRNA-561 was specifically induced by APAP treatment in HepG2 cells. Treatment of HepG2 cells with a miRNA-561 mimic resulted in enhanced APAP-induced hepatotoxicity. Decreased protein level of DAX-1 by APAP was also enhanced by miRNA-561 mimic treatment. Increased protein levels of PXR and CAR by APAP were also enhanced by miRNA-561 mimic treatment. On the contrary, treatment of HepG2 cells with miRNA-561 inhibitors nearly completely blocked the APAP-induced cytotoxicity. These results indicate that miRNA-561 worsens APAP-induced hepatotoxicity probably by inhibition of DAX-1 and consequent increased activation of nuclear receptors.
Automated comparative analysis of LC-MS datasets from isotopically labeled RNA

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Keywords: comparative analysis, RNA, sequencing, modifications, software development

Recently we developed CARD, a method for the Comparative Analysis of RNA Digests. By differential labeling of two samples using 16O and 18O, any RNA digests that are identical in the two samples will appear as doublets in mass spectral analysis separated by 2 Da. More significantly, differences between the two samples are represented by singlet peaks. For many applications, the differences between two samples will be minor meaning that much of the mass spectral data will be doublets with only a few singlets. The challenge in data analysis is to rapidly identify and characterize these singlets. To address this challenge, we introduce an Excel interface program based on Visual Basic for Application (VBA) for analysis of CARD data. Liquid chromatography-mass spectrometry (LC-MS) test data were obtained from previous studies. The automated processing steps in this program drastically reduce the need for manual processing of LC-MS data by applying multiple filters to remove hundreds of doublets and noise in the mass spectra. Three example CARD datasets have been used in program development, and results from the application of this program to these datasets will be presented. Further algorithm development and optimization is on-going.
MicroRNA signatures and the miR-c-Kit-ETV1 axis in the development of gastrointestinal stromal tumors

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Keywords: MicroRNA Signature, gastrointestinal stromal tumor, c-Kit, ETV1

While altered microRNA (miR) expression was found in many malignancies, what functional roles of miRs could play in cancer development remains largely unclear. Unlike most of the solid tumors, gastrointestinal stromal tumors (GISTS) are the most common mesenchymal tumors of the GI tract, potentially arising from the interstitial cells of Cajal. Both the gain-of-function mutants of receptor tyrosine kinase (RTK) c-KIT (~85-90% positive in GIST) and the elevated expression of ETS family member ETV1 (>90%) were tightly linked to tumorigenic progression of GIST. We proposed to address the importance that miR transcriptome was being altered in instructive ways in the distinctive stages of tumorigenesis, namely very low (VL), low (L), intermediate (I) and high (H) metastatic potential, to reveal the miR signatures according to the hallmark capabilities of GIST. Specifically, we performed miR profiling to audit expression levels of miRs in premalignant (VL), tumor (L & I) and metastatic (H) stages. The clustering analyses reveal a differential miR expression at each of the stages, that is, each stage bore with a distinctive miR signature. For example, comparing miR profiling of L to VL defined a L-stage miR signature including 46 down- and 19 up-regulated miRs. To gain further insights of role(s) of miRs in GIST tumorigenesis, we reasoned that the miR profiling ascribed to the c-KIT-ETV1 axis, the hallmark tumorigenic capability of GIST, should be altered during GIST progression. In this regard, only 17 miRs were altered and clustered in the tumorigenic stage miR signature, which we named as the ETV-1 miR signature. Our data further revealed that ETV1 expression were affected by 4 miRs (miR-296, miR-330, miR-627, miR-1237) of the ETV-1 miR signature. A xenograft mouse model with molecular manipulations of the miR-c-Kit-ETV1 axis was now under construction to examine the functional importance of above 4 miRs in GIST progression. Moreover, we also studied the functional and genetic interactions between c-KIT and ETV1 and found genetic modulations of c-KIT gene dosage, by RNA interference or ectopic expression, affect ETV1 expression. In sum, our study reveals the importance of the miR-c-Kit-ETV1 axis in GIST development thereby implicating a potential miR-targeting therapeutic intervention for GIST.
Poster abstract: 30

Quantitating RNA chemical footprinting experiments using an internal standard

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Keywords: Chemical mapping, RNA structure, sequencing

Chemical footprinting is a powerful technique for characterizing secondary structure, but it has been limited by ad hoc or qualitative data processing procedures. Here, we present a proposal for quantitatively measuring the reactivity of single nucleotides to a range of chemical probes using internal standards and dilution series. By adding a non-interfering hairpin to the terminus of an RNA, one can normalize the reactivity at any position to a reference that may be applied to different RNAs or with different reaction conditions. This approach is robust across a range of reagent concentrations. We use our improved workflow to revisit a challenging benchmark of six noncoding RNAs previously investigated by our lab and others. Finally, we demonstrate that our reactivity values agree within error between capillary electrophoresis and deep sequencing protocols. Our reactivity quantitation method holds promise to improve precision in chemical mapping experiments while providing accurate constraints for structure modeling.
Detection of pRNA nanoparticles in mouse retina

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Keywords: pRNA nanoparticles, retina, ocular

Phi29 bacteriophage RNA (pRNA) subunits from its DNA packaging motor can be utilized for therapeutic applications. Progress has been made in administering small interfering RNA (siRNA) by intravitreal injections in the eye, however free siRNA are unable to target specific ocular cells. pRNA domains comprise a three-way junction (3WJ) or four-way junction (4WJ) and allow for the ability to deliver different combinations of compounds to specific targeted sites. We characterized the ability of pRNA nanoparticle domains to penetrate the mouse retina and hypothesized that pRNA nanoparticles will be an efficient system for therapeutic delivery. In order to accomplish this, we performed intravitreal injections of AlexaFluor 647-labeled pRNA nanoparticles into the mouse eye. The eyes were then fixed in 4% paraformaldehyde, cryosectioned, and analyzed using laser confocal microscopy. Confocal imaging showed detection of pRNA nanoparticles within the posterior retinal layers. Robust migration of pRNA nanoparticles suggests that their structural characteristics allow them to penetrate the retinal layers easily. pRNA nanoparticles may represent a valuable platform for siRNA delivery into the retina in the treatment of ocular complications.
Regulation of specific RNA encapsidation by the N-terminal arm of the coat protein of brome mosaic virus

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Keywords: Virus, capsid, RNA, sequence-specific recognition

The coat protein of positive-stranded RNA viruses often contains a positively-charged N-terminal arm that is intrinsically disordered and reaches the inside of the virion to interact with the viral genome. We seek to understand the interplay between the viral RNA and the N-terminal arm in the encapsidation of the multipartite brome mosaic virus (BMV). The nonspecific electrostatic interaction between the viral RNAs and the N-terminal arm was found to be a major force in encapsidation. A larger amount of viral RNAs was packaged by BMV capsids with increased positive charges while a lower amount by capsids with decreased positive charges without perturbing the capsid conformation. Specific residues on the N-terminal arm were also demonstrated to regulate the selective encapsidation of the four BMV RNAs. To elucidate the residues in the N-terminal arm required for encapsidation specific RNA, the particles containing distinct RNAs were separated by density gradients and the contacting region in the capsid mapped by reversible crosslinking and peptide fingerprinting (RCAP). To map the RNA sequences that contact the capsid, a method involving crosslinking, immunoprecipitation of the CP and deep sequencing (CLIP-Seq) was used. These results will be presented and they offer rules for RNA encapsidation in the icosahedral virions of a positive-strand RNA virus.
Assembly of therapeutic pRNA-siRNA nanoparticles using bipartite approach

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The 117-nucleotide (nt) RNA, called the packaging RNA (pRNA) of bacteriophage phi29 DNA packaging motor, has been shown to be an efficient vector for the construction of RNA nanoparticles for the delivery of small interfering RNA (siRNA) into specific cancer or viral infected cells. Currently, chemical synthesis of 117-nt RNA is not feasible commercially. In addition, labeling at specific locations on pRNA requires the understanding of its modular organization. Here, we report multiple approaches for the construction of a functional 117-base pRNA using two synthetic RNA fragments with variable modifications. The resulting bipartite pRNA was fully competent in associating with other interacting pRNAs to form dimers, as demonstrated by the packaging of DNA via the nanomotor and the assembly of phi29 viruses in vitro. The pRNA subunit assembled from bipartite fragments harboring siRNA or receptor-binding ligands were equally competent in assembling into dimers. The subunits carrying different functionalities were able to bind cancer cells specifically, enter the cell, and silence specific genes of interest. The pRNA nanoparticles were subsequently processed by Dicer to release the siRNA embedded within the nanoparticles. The results will pave the way toward the treatment of diseases using synthetic pRNA/siRNA chimeric nanoparticles.

References:
Mapping protein contact sites of viral RNAs within intact virions using CLIP-Seq

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In an effort to better understand the packaging of positive-strand RNA viruses and structures of the virions, we developed methods to map the sequences within viral RNA that contact the viral capsid within intact virions. The method uses a combination of cross-linking and immunoprecipitation (CLIP) and next-generation sequencing (Seq). Virions were first purified and cross-linked by UV at 254 nm. Cross-linked virions were then dissociated to expose the capsid-protein complex. The RNAs were then fragmented and the capsid protein was immunoprecipitated. Free RNAs not bound to protein were washed away whereas RNAs bound to capsid protein were purified after protein digestion and prepared into a cDNA library for next-generation sequencing, using either 454 or Illumina platform. Fragment sequences obtained were then aligned to viral genomes. Sites of contacts were then identified using bioinformatic tools. Lastly, contact sites were compared to identify sequence or structural similarities that result in the high binding affinity to capsid protein, which is then confirmed using biochemical assays including fluorescent anisotropy.

Variations of the fragmentation step and generation of the cDNA libraries were found to be critical in ensuring high efficiency of RNA recovery. Design of primers and adaptors for cDNA library generation are important to improve sensitivity for peak identification. Different viruses, including brome mosaic virus (BMV), bacteriophage MS2 were analyzed using this method. This work will provide new insights into RNA virus structure and packaging.
Poster abstract: 35

siRNA therapy of breast cancer by targeted and traceable graphene quantum dot-based delivery system

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Small interfering RNAs (siRNAs) represent a promising anticancer therapy which works by knocking down or silencing oncogene expression in tumor cells. However, this strategy is limited by low intracellular uptake and poor blood stability of siRNAs, and non-specific immune stimulation by siRNAs, therefor requiring safe and efficient delivery to specific cells. Although some success has been reported on siRNA delivery by various methods, tracking their delivery and monitoring their transfection efficiency still remains a challenge. Here, we are developing a traceable, specific and stable quantum dot-based delivery approach to deliver siRNA for cancer therapy. Graphene quantum dots (GQDs) are highly luminescent, water soluble, and non-cytotoxic nanocrystals, which enable both delivery and tracing of the siRNA in vivo. In this study, we will conjugate GQDs with targeted-ligand, a fibronectin-mimetic peptide to integrin α5β1, and therapeutic-ligand, CXCR4 siRNA, to form this traceable gene delivery system, and test its delivering and tracing ability in α5β1-overexpressing breast cancer cell line. We expect the graphene quantum dot-based gene delivery system to be a successful platform for siRNA delivery and real-time monitor to treat breast cancer.
Poster abstract: 36

Crosslinked nanoassemblies for in vivo siRNA delivery to solid tumors

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Keywords: Crosslinked Nanoassemblies, siRNA Therapy, Delivery, Nanoparticles

siRNA therapy has the potential to reduce the expression of proteins including those deemed “un-drugable” by small molecule drugs. This therapy uses the endogenous RNAi pathway by introducing a small 19-21 nucleotide strand of RNA, siRNA, into the cytoplasm of the cell where it is complementary to a strand of mRNA. The sequence of the siRNA can be coded for virtually any strand of mRNA, allowing customization for multiple types of disease. Currently, the success of this therapy depends on the carrier used to deliver the siRNA. Several types of carriers have been evaluated but most have a much higher efficacy in Vitro compared with in Vivo results due to decreased stability and increased toxicity. Cross-linked nanoassemblies (CNAs) with a cationic core are expected to improve in vivo siRNA therapy, compared to commercially available agents, by enhancing siRNA stability, reducing toxicity, and avoiding non-specific interactions. Our CNAs use biocompatible poly(ethylene glycol)-poly(amine acid) di-block-co-polymers that have been crosslinked at the amino acid block and modified to include cationic moieties. The cationic moieties are used to allow ionic interactions between the core of the CNA and the siRNA to form complexes surrounded by a poly(ethylene glycol) (PEG) shell. The PEG shell enhances stability by shielding the particle’s charge and decreasing non-specific interactions in the blood stream. The crosslinking decreases toxicity by blocking direct interaction between the cationic moieties and cell membranes. To test this system, experiments were conducted to characterize the physical chemical properties and the biological properties of the siRNA/CNA complexes. CNAs were prepared from either poly(ethylene glycol)-poly(aspartate)(PAsp), poly(ethylene glycol)-poly(aspartate-diylenetriamine)(PDet), or poly(ethylene glycol)-poly(lysine)(PLys) block copolymers. These polymers are biocompatible and offer cationic moieties (PLys or PDet) or are able to react with a crosslinker containing a cationic portion (PAsp) to create a cationic core. The CNAs were formed via an amidation reaction with diylenetriamine or adipic acid, combining approximately thirty block copolymer chains into a single particle. CNAs were characterized using 1H-NMR, dynamic light scattering and gel permeation chromatography. Characterization confirmed our polymer compositions and showed CNAs ranging from 15 to 40 nm in size and. Complexation between CNAs and siRNA was done with a 360 nM concentration of siRNA for 2 hours at room temperature. The polymer PLys complexed with siRNA at an N/P ratio of 10 while PDet was able to complex at a ratio around 1. The CNAs made using PAsp and PLys have not shown any complexation with siRNA. Transfection experiments were conducted by regulating the luciferase expression in a human colon cancer HT-29 cells in comparison to commercially available transfection agents (Lipofectamine 2000). Future experiments will be conducted characterizing CNAs made with PDet, complexation studies, and testing in vitro. Based on preliminary data, it is expected that CNAs made with PDet will complex with siRNA and enable gene silencing.
Poster abstract: 37

Poly(lactide-co-glycolide) nanoparticles layer by layer engineered for the sustainable delivery of therapeutic proteins

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Keywords: Poly(lactide-co-glycolide), nanoparticles, antiTNF-α, RNA, GFP, polyelectrolyte, Layer by Layer

In recent years biomedical research has virtually developed in an explosive rate. Highly effective and specific therapeutic agents have been discovered. Among these so-called biologics, small interfering RNAs have been developed as innovative nucleic acid based medicines for treatment of diseases such as cancers and persistent viral infections. Another example of modern biologics is the use of therapeutic monoclonal antibodies directed against specific signalling molecules playing a key role in autoimmune diseases.

Although several therapeutic proteins for the treatment of a variety of diseases are currently undergoing clinical trials, the biggest challenge remains the intracellular delivery into the target cells. Cell membranes represent a formidable barrier against extracellular nucleic acids. In the other hand, the administration routes used nowadays include non-specific off-target binding resulting in adverse effects, caused by the required high doses of the therapeutics. Any reduction in the doses by controlling the release on the targeted site would decrease adverse effects and also the costs of these comparatively high priced medications. In addition, it is necessary to find ways of protecting the therapeutics from enzymatic degradation.

As an alternative of the encapsulation of therapeutic proteins inside nanoparticles (NPs), it is possible to carry the therapeutic on the surface of the NPs. Therapeutic proteins can be assembled on top of NPs on the basis of the Layer by Layer technique (LbL). LbL involves the electrostatic interaction between oppositely charged polyelectrolytes stepwise assembled on top of a charged surface to form a thin polymer film with nanoscale thickness. There has indeed been a significant amount of research devoted to the templation of capsules or the modification of NPs for drug delivery based on polyelectrolyte multilayers (PEMs) assembled by means of the LbL technique. PEMs can be used to coat different cargos a means of protection or to control release or targeting. Materials for delivery i.e. DNA or proteins, can be as well assembled between the layers in the PEMs. The loaded material will be released from the PEM, or the PEMs will be eventually peeled off liberating the entrapped material. In addition, an important advantage of the LbL protocol is that the doses per particle, the amount of encapsulated material, can be controlled by the number of layers assembled.

In this work, we will show the use of PEMs for the encapsulation of RNA, plasmid GFP and antiTNF-α. A strategy based on complexation with natural polyelectrolytes and LbL assembly will be developed for the loading of NPs with the therapeutics. The LbL assembly of the PEMs and then the subsequent release of the proteins from the PEMs, was first studied on planar surfaces by means of the quartz crystal microbalance with dissipation. The LbL assembly of the complex onto NPs was characterized via ζ-potential measurements. Therapeutic proteins release from NPs coating in PBS and in cell suspension was studied by specific biochemical assays, Confocal Laser Scanning Microscopy and by Confocal Raman Microscopy.
Nanoconjugates formed between gold nanoparticle, mPEG-PAMAM dendrimer, and RNA - including anti-cancer chimeric aptamer against Ras binding domain of Raf protein

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**Keywords:** Aptamer, RNA nanoconjugates, characterization

In recent years it has been discovered that nanomaterials can have profound effects on the function of biological molecules. This discovery has helped birth the emerging field of “biomolecular nanotechnology”. Our interest here is centered on the effects of conjugates formed between gold nanoparticles (GNPs) and mPEG-PAMAM dendrimer on the structure and function of various types of RNA. If biological nanoconjugates are to be applied to human medicine, it is necessary to establish well-studied characterization techniques that demonstrate conjugate properties. These techniques may be divided into three main categories: characterization of conjugate formation, biocompatibility, and effects on bio-activity. This research utilizes the aforementioned techniques for GNPs, mPEG-PAMAM dendrimer, and different types of RNA, as a way to demonstrate a model system for such characterizations. Here we have shown that shifts in the absorbance spectrum and zeta potential occur upon formation of these conjugates. Biocompatibility is demonstrated by MTT assay, as well as cell growth and morphology studies. We have investigated the effect of this conjugate system on RNA biological activity using a model aptamer system. We have designed a DNA/RNA chimeric aptamer against the Ras Binding Domain (RBD) peptide of Raf protein.
MAP-seq: massively parallel RNA chemical mapping with reduced bias

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Keywords: RNA structure, chemical mapping, high-throughput sequencing, footprinting, and multiplexing

Chemical mapping techniques have long been used to probe RNA structure, helping to infer accessibility and flexibility of the nucleotides due to correlations of reactivities with these structural properties. Prior work by Lucks and colleagues has coupled this approach with the Illumina sequencing platform, enabling the study of hundreds of molecules at once and eliminating the need for gel or capillary electrophoresis. Here we present a new optimization, dubbed MAP-seq (Multiplexed Accessibility Probing read out through sequencing), and a new MAPseeker software package for data analysis. We reduce multiple sources of bias, including eliminating PCR steps, improving adapter ligation efficiency, and avoiding problematic heuristics in prior analysis algorithms. This approach enables quantitative probing of hundreds of RNAs simultaneously using multiple chemical probes on the time scale of a day using a table-top Illumina machine. While work for improving upon this protocol remains ongoing, we hope that other RNA labs may use this to transition from electrophoretic methods and increase the throughput of RNA structural studies.
Fabrication of versatile RNA nanoparticles for specific tumor targeting without accumulation in normal organs

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Due to structural flexibility, RNase sensitivity, and serum instability, the construction of RNA nanoparticles with concrete shapes for in vivo application remains challenging. Here we report the construction of versatile RNA nanoparticles with solid shapes for targeting cancers specifically. These RNA nanoparticles were resistant to RNase degradation, stable in serum for more than 36 hr, and stable in vivo after systemic injection. By applying RNA nanotechnology and exemplifying it with these RNA nanoparticles, we have established the technology and developed “toolkits” that utilize a variety of principles with which to construct RNA architectures with diverse shapes and angles. RNA loops, cores, junction motifs, and palindrome sequences from the Φ29 motor pRNA were gathered in a “toolkit” in order to demonstrate their utility for fabrication of dimers, twins, trimers, triplets, tetramers, quadruplets, pentamers, hexamers, heptamers, and other higher order oligomers, as well as branched diverse architectures via hand-in-hand, foot-to-foot, and arm-on-arm interactions. These novel RNA nanostructures harbor resourceful functionalities for numerous applications in nanotechnology and medicine. It was also found that all incorporated functional modules, such as siRNA, ribozymes, aptamers, and other functionalities, folded correctly and functioned independently within the nanoparticles. The incorporation of all functionalities was achieved prior, but not subsequent, to the assembly of the RNA nanoparticles; thus ensuring the production of homogeneous therapeutic nanoparticles. More importantly, upon systemic injection, these RNA nanoparticles targeted cancer exclusively in vivo without accumulating in normal organs and tissues. These findings open up a new territory for cancer targeting and treatment. The versatility and diversity in structure and function derived from one biological RNA molecule implies immense potential concealed within the RNA nanotechnology field.

References:

Facile delivery of dicer substrate siRNAs via a cell targeting aptamer with a sticky bridge

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Keywords: Aptamers, dicer substrate siRNA, Sticky-bridge, HIV-1, non-Hodgkin’s lymphoma

One of the most formidable impediments to clinical translation of RNA interference (RNAi) is safe and effective delivery of siRNAs to the desired target tissue at therapeutic doses. Nucleic acid aptamers that target specific cell surface proteins have been used as delivery vehicles to target a distinct cell type, hence reducing off-target effects or other unwanted side effects. Here, we demonstrate a non-covalent strategy to conjugate the cell-targeting aptamer and the dicer-substrate siRNA via a “sticky bridge”, which is composed of a 16 base 2' OMe, 2' F modified GC-rich sequence attached to the aptamer and one of the two strands of siRNAs. As a proof-of-concept, we designed two aptamer-stick-siRNA constructs (BAFF-R aptamer-STAT3 siRNA and gp120 aptamer-tat/rev siRNA) that were shown to be specifically delivered to BAFF-R-expressing cells and gp120-expressing cells for treatment of non-Hodgkin’s lymphoma (NHL) and HIV-1 respectively. The sticky bridge facilitates the non-covalent binding and interchange of various siRNAs with the same aptamer that is more feasible for the clinical adaptation of aptamer-mediated siRNA delivery system. In both studies, the aptamer-stick-siRNA constructs were successfully internalized into target cells, resulting in specific knockdown of target mRNAs and potent inhibition of disease progression. Furthermore, in vivo delivery of the conjugates did not induce the innate type 1 interferon response and remained stable in serum for extended time. Collectively, these data demonstrate a facile, targeted approach for combinatorial delivery of dsiRNAs for treatment of various diseases.
Poster abstract: 42

DNA repair, modification and engineering by transcript RNA

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Keywords: RNA, DNA repair, double-strand break repair, transcript RNA, gene targeting, in vivo genome engineering

We found that RNA transcripts, generated inside cells, can have a direct role in maintaining genomic stability of cells serving as templates in the repair of damaged DNA, and can directly transfer genetic information to chromosomal DNA. A DNA double-strand break (DSB) is a highly dangerous DNA lesion, causing cell death and mutations at the greatest rate, unless properly repaired. On the other hand, a DSB is also the most potent stimulus for gene targeting and genome engineering. Working in the model system yeast Saccharomyces cerevisiae, we have uncovered that absence of Ribonucleases H1 and H2 (RNase H1 and H2) strongly enhances transcript RNA-initiated repair of a DSB occurring either in the same transcript-generating locus (in cis), or in a homologous but remote locus (in trans), >680,000-fold and 1,000-fold, respectively. Transcript RNA-directed DNA modification remained high in different conditions that are known to inactivate the reverse transcription function of the yeast transposon Ty. These results suggest that the transcript RNAs are not converted into cDNA, but rather directly modify the genomic DNA. The mechanism of transcript RNA-directed DNA modification depends on transcription of the repairing transcript RNA and requires the function of the homologous recombination protein Rad52. Our data reveal a novel mechanism of DNA repair and modification mediated by RNA transcripts and open up a new direction for gene targeting and in vivo genome engineering by RNA. (NSF-MCB-1021763; Georgia Cancer Coalition-R9028)
RNA 3D hub: a resource for RNA nanotechnology

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Keywords: RNA 3D Motifs, 3D structure, Databases

RNA is used to build sophisticated molecular machines in nature. Researchers have solved the 3D structures of several machines, such as the ribosome, from several different organisms at high resolution. This wealth of information can be used to design novel and useful nanoscale devices if harnessed properly. To aid in understanding RNA machines we have recently created the RNA 3D Hub (http://rna.bgsu.edu/rna3dhub), an automatically updated resource for RNA structures.

The RNA 3D Hub comprises three major components, which aim to supplement the Protein Data Bank by providing additional structural annotations. First, to address the inherent redundancy of the structural data in the PDB, RNA 3D Hub hosts Non-redundant Lists of RNA-containing 3D structures. Since many of the PDB files represent the same molecule from the same organism, we group the files into equivalence classes based on sequence similarity, 3D superpositions, and structure quality considerations. A representative structure is selected for each equivalence class.

Secondly, the Structure Atlas which has per structure annotations of base pairing, base-stacking and base-phosphate pairwise interactions, as well as information about all internal, hairpin, and three-way junction loops in the structure.

And finally, the Motif Atlas which contains groupings of loops extracted from the representative atomic resolution structures into geometrically similar motif groups. There are about 250 internal loop and 250 hairpin loop motif groups in the Motif Atlas. Similar groups are linked together based upon geometric similarity, and changes in the composition of the motif groups are tracked through time. These motif groups represent many of the building blocks used in nature. Some of them are well known such as kink-turns, however there are many examples of recurrent but unstudied motifs. Scientists interested in constructing nanomachines will benefit from studying and using these building blocks.

We have also begun a project to extend the RNA 3D Hub with secondary structure information. We have included automatically generated circular diagrams and some manually curated secondary structure diagrams of RNA structures in the Structure Atlas. The diagrams are dynamic and can be interacted with to show 3D structure and base pairing information. These diagrams allow users to easily explore structures using a familiar representation to find motifs and interactions between motifs. Potential users interested in particular visualizations or data sets are encouraged to discuss this with us. We wish to make the RNA 3D Hub a powerful resource to the RNA nanotechnology community and are seeking community feedback.

All RNA 3D Hub components are conveniently linked to each other in order to make it easy to explore RNA structural data. RNA 3D Hub is automatically updated on a regular basis and is available at http://rna.bgsu.edu/rna3dhub.
HIV-2 Rev response element (RRE) structure as a potential therapeutic target for nanoparticle-based antiviral therapy

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Keywords: RNA secondary structure, tertiary interactions, chemical probing, nanoparticle

Similar to its’ HIV-1 counterpart, the Rev response element of HIV-2 (RRE2) is responsible for transporting unspliced and partially spliced viral RNA from the nucleus to the cytoplasm. We applied complementary chemical and biochemical probing techniques to determine the structure of the RRE2 RNA. SHAPE analysis indicates that the low-energy form of RRE2 contains five peripheral stem loops linked by adjacent 4-way and 3-way junctions. Interestingly, electrophoretic fractionation of RRE2 RNA under non-denaturing conditions revealed additional conformers (open and intermediate) that undergo conversion to the more stable, low energy (closed) form in a time-dependent manner. This transition was verified by kinetic analysis, and the structures of the three forms were determined using a novel method for mathematically dissecting an ensemble of SHAPE profiles. Site-directed hydroxyl radical footprinting was used to examine the relative positioning of select motifs within these structures in three-dimensional space. Overall, our study provided detailed insight into the RRE2 conformation, and as such will aid in the development and application of nanoparticle-based antiviral drugs targeting cis-acting RNA regulatory signals. The methodology described also has direct applicability to characterizing RNA-based nanoparticle assemblies.
A new pRNA dimmer drug delivery system for tuberculosis therapy

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Keywords: Tuberculosis, pRNA drug delivery system, mce4A siRNA

Objective: Tuberculosis (TB) still exists in developing countries and also in some industrialized countries such as U.S. due to world wide transportation. It represents a severe health problem since the penetration of anti-TB drugs to granulomas and drug resistance are still a major challenge. This project aims to develop and characterize new RNA nanotechnology drug delivery systems for TB treatment. Mammalian cell entry protein (mce4A) plays a crucial role to keep Mycobacterium tuberculosis (Mtb) surviving. In the designed treatment, a small interfering RNA (siRNA) as a molecular beacon which is designed to silence mammalian cell entry protein (mce4A) mRNA and anti-TB drugs will be conjugated with our pRNA dimmer.

Methods: Using pRNA dimmer to combine approved anti-TB drugs with designed siRNA to form a pRNA dimmer product, which is intended to deliver anti-TB drugs to the targets. The chemical features and targeting potential will be assessed and the pharmacokinetics, pharmacodynamics and safety profiles will be measured.

Results: Literature search has been performed and the design of the targeted nanoparticles will be optimized. In vitro assays will be started shortly.

Conclusion: The new pRNA dimmer drug delivery system is expected to manage TB with higher efficacy and less toxicity.
Poster abstract: 46

Novel impedimetric biosensor based on enzyme catalysis-induced ion strength increase

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Keywords: Impedimetric biosensor, enzyme-catalysis-induced ion-strength increase, virus detection

Electrochemical biosensor has drawn increasing attention due to its high sensitivity, selectivity, facility, and low cost for clinic diagnosis/therapy, food safety, and environment monitoring applications. Although many approaches have been developed based on the charge change, electron-transfer and/or mass-transfer of the redox probes/labels, the strategy based on enzyme-catalysis-induced ion-strength increase has not been reported. In this study, we propose the exploitation of the enzymatic catalysis in ultra-low ion strength media to induce ion strength increase for developing a novel impedance biosensing method. Avian Influenza virus H5N1, a serious worldwide threat to poultry and human, was used as the target. Magnetic beads were modified with H5N1-specific aptamer to capture H5N1 virus, followed by binding a Concanavalin A (ConA)/Glucose oxidase (GOx)/Au nanoparticles (AuNPs) bionanocomposites through ConA-glycan interaction. The yielded sandwich complex was transferred to a glucose solution to trigger an enzymatic reaction to produce gluconic acid, which ionized to increase the ion strength of the solution, thus decreasing the impedance on a screen-printed interdigitated array electrode. This method took advantage of the high efficiency of enzymatic catalysis and the high susceptibility of electrochemical impedance on the ion strength, and endowed the biosensor with high sensitivity and a detection limit of $8 \times 10^{-4}$ HAU, which was better than that of most analogues. Furthermore, the proposed method required only a bare electrode for measurements of ion strength change and had no change on the surficial properties of the electrode. This helped to avoid the drawbacks of commonly used electrode-immobilization methods. The merit for this method makes it highly useful and promising for applications. The proposed method may create new possibilities in the broad and well-developed enzymatic catalysis fields and find applications in developing sensitive, rapid, low-cost, easy-to-operate biosensing and biocatalysis devices.
Channel Size Conversion of Phi29 DNA-Packaging Nanomotor for Sensing of Single-Stranded RNA

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Keywords: Nanotechnolgy; Nanomotor DNA packaging motor; bacteriophages phi29; Nanopore; Sequencing; Sensing

Nanopores have been utilized to detect the morphology of polymers, including DNA and RNA. The uniform channel size formed by the assembly of homogeneous biological nanoparticles is one of the advantages of biological channels. The channel of the bacterial virus phi29 DNA packaging motor is a natural conduit for the transportation of double stranded DNA, and has the largest diameter among the well-investigated biological channels. The larger channel facilitates translocation of double-stranded DNA (dsDNA), and offers more space for further channel modification and conjugation. Interestingly, the relatively large wild type channel, which translocates dsDNA, could not detect single-stranded nucleic acids (ssDNA or ssRNA) at the current experimental conditions. Herein, we reengineered this motor channel by removing the internal loop segment of the channel. The modification resulted in two classes of channels, each exhibiting a difference size. One class was the same size as the wild type channel, and the other class, the smaller of the two channels, had the cross-sectional area of about 60\% of the wild type. This smaller channel was able to translocate ssRNA and ssDNA. This finding of the size alternations in the reengineered motor channel expands the potential application of the phi29 DNA packaging motor in nanomedicine, Nanobiotechnology, and high-throughput single pore dsDNA sequencing.

References:

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Targeted tRNA identification by tandem mass spectrometry

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Keywords: tRNA, Mass Spectrometry, Global RNA detection

The global identification of individual transfer ribonucleic acids (tRNAs) can prove to be very difficult, requiring rigorous sample preparation prior to analysis. Here, we illustrate a highly optimized MS/MS assay for rapid global identification of tRNAs, building on techniques previously introduced by our group. The goal is to identify sequences of oligonucleotides that are unique to a certain isoacceptor tRNA by detecting unique fragmentation channels. This approach enables accurate tRNA identification in a manner more conducive to high-throughput analyses. Our results show 44 out of the 47 isoaccepting tRNAs predicted to be present in E. coli are routinely detected by targeting 22 precursor ions in less than 15 minutes. Validation experiments included the examination of tRNAs from a tRNA over-expression system. The approach is general enough that it could be applied to archaeal and eukaryotic organisms, and this approach should also be feasible for the targeted analysis of other small RNAs in the cell.
Poster abstract: 49

pRNA nanoparticles as tools to improve human pluripotent stem cell-based transplantation strategies for retinal degenerative disease

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Keywords: Human pluripotent stem cells, EGFR, retina, transplantation

Transplantation of human stem cell-derived progeny holds potential as a treatment for retinal degenerative diseases. However, results in animal models thus far have been modest. The development of clinically viable tools to label, sort, and/or monitor cells post-transplantation, as well as to improve donor retinal cell viability and function, could significantly aid retinal transplantation efforts. pRNA nanoparticles are thermostable, nontoxic, and nonimmunogenic, and can be manufactured to carry up to 4 functional moieties, including fluorophores, siRNA, miRNA, ribozymes, and/or pharmacologic agents, in addition to receptor ligands or aptamers for cell specific targeting (Shu D et al. (2011) Nature Nanotech, Haque F et al. (2012) Nano Today). As a first step to examine the utility of pRNA in differentiating human stem cell cultures, pRNA nanoparticles were manufactured which contained a Cy3 fluorophore and either an EGF receptor (EGFR) or control aptamer. Previous reports showed that EGFR aptamers can cause cell-specific anti-proliferative effects in cancer cell lines (Esposito CL et al. (2011) PLoS One, Li N et al. (2011) Plos One). In this study, we tested the efficacy of these custom pRNA constructs in 1) human neural progenitors (hNPCs), which highly express EGFR, and 2) neuroretinal cultures derived from differentiating human pluripotent stem cells (hPSCs).
Poster abstract: 50

**Novel amphiphilic cyclodextrin-fullerene supramolecules for the site-specific delivery of siRNA**

*Juanjuan Yin¹, Shu-Feng Zhou”*

¹Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, Florida 33612

**Keywords:** siRNA, fullerene, cyclodextrin, anti-cancer

Small interfering RNA (siRNA) is a naturally occurring endogenous regulatory process where short double stranded RNA causes sequence-specific posttranscriptional gene silencing. siRNA represents a promising therapeutic strategy, while systemic siRNA therapy is hampered by the barriers for siRNA to reach their intended targets in the cytoplasm and to exert their gene silencing activity. Problems hindering their effective application fundamentally lie in their delivery, stability, and off-target effects. Delivery systems provide solutions to many of the challenges facing siRNA therapeutics. Due to some fatal disadvantages of viral vectors, nonviral carriers have been studied extensively. Here we report the synthesis of a delivery vehicle that combines carrier, cargo as well as ligands. A targeting amphiphilic cyclodextrin-fullerene based supramolecule serving as the siRNA delivery vehicle have been constructed, and the multifunctional nano-system is aimed to elicit synergistic effect and deliver siRNA to disease target preferentially. They may represent a promising strategy for siRNA-based therapies, especially as targeting nanomaterials.
Poster abstract: 51

**In situ structure and dynamics of DNA origami revealed through all-atom molecular dynamics simulations**

Jejoong Yoo

University of Illinois at Urbana-Champaign

**Keywords:** DNA origami, molecular dynamics

Self-assembly of DNA into complex three-dimensional structures have emerged as a new paradigm for practical nanotechnology. Among many methods that have been put forward to utilize the self-assembly of DNA for practical uses, DNA origami stands out by its conceptual simplicity and the infinite range of possible applications. Among many advantages of the DNA origami technique is the ability to build complex 3D structures with sub-nanometer precision, which is still difficult to achieve using the most advanced conventional nanofabrication strategies. Despite the dramatic development of the DNA origami field, the actual structure and conformational dynamics of DNA origami in its native environment—physiological solution—has remained poorly characterized. Here, we report the first all-atom molecular dynamics (MD) simulations of several complete three-dimensional DNA origami structures. First, we demonstrate an automated protocol for conversion of popular caDNAno designs into all-atom structures of DNA origami amenable to MD simulations. Next, we report the results of our MD simulations of several rod-like DNA origami designs, characterizing their structural and dynamic properties in unprecedented detail. Our comparative analysis of DNA designs based on honeycomb and square lattices have shown the honeycomb DNA structures to be about twice more rigid than the square-lattice ones. We anticipate the MD method to become a reliable partner in future development of the DNA origami field.
Poster abstract: 52

Fluorogenic RNA nanoparticles for monitoring RNA folding and degradation in real time in living cells

Randall Reif, Farzin Haque, Le Zhang, and Peixuan Guo*

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Due to the discovery of more and more roles of cellular noncoding RNAs, the approaches for introducing RNAs including small interfering RNA (siRNA), micro RNA (miRNA), ribozyme, and riboswitch into cells for regulating cell life cycle and for the treatment of diseases have become routine practice. The understanding of RNA folding, degradation, and intracellular half-life after entering the cell is an intriguing question in biology and pharmacology. Currently, methods to detect RNA folding, degradation, and half-life in real time within the cell is extremely challenging. The common assay method to measure RNA half-life and degradation in vivo is the use of radioactive markers or fluorescence RNA labeling. The challenge is, after RNA becomes degraded or misfolded, the isotope or the fluorescence is still present in the cell, thus the signals are not a true indication of the presence of the RNA in the cell. The alternate method commonly used to measure RNA life is to isolate RNA from cells and distinguish between intact and degraded RNA by gel, chromatography, or capillary electrophoresis. However, when a cell is breaking down, ribonucleases (RNases) will be released from cell compartments, and degradation of small RNA in cell lysates occurs immediately after cell lysis. Here we report a method to monitor RNA degradation in real time in living cells using fluorogenic RNA in combination with RNA nanotechnology. The RNA aptamer that binds malachite green (MG), the ribozyme that cleaves the hepatitis virus genome, and a siRNA for firefly luciferase were all fused to the bacteriophage phi29 packaging RNA (pRNA) 3-way junction (3WJ) motif to generate RNA nanoparticles. The MG aptamer, the hepatitis B virus ribozyme, and the luciferase siRNA all retained their function independently after fusion into the nanoparticles. When the RNA nanoparticle is degraded, denatured, or misfolded, the fluorescence disappears. MG, which is not fluorescent by itself, is capable of binding to its aptamer and emitting fluorescent light only if the RNA remains folded in the correct conformation. Therefore, the MG aptamer fluorescence (in the presence of MG dye) can be used as a measure of the degradation and folding of RNA nanoparticles, the siRNA, the aptamer, and the ribozyme in the cell in real time using epifluorescence microscopy and fluorescence spectroscopy without lysing the cells. We show that the half-life ($t_{1/2}$) of the electroporated MG aptamer containing RNA nanoparticle was 4.3 hours after electroporation into cells.

References:
DNA and RNA nanostructures investigated by selenium functionalization

Wen Zhang
Chemistry Department, Georgia State University

The studies on self-assembling nucleic acid nano-architectures have been quite extensive due to their potential applications in nanotechnology and cancer treatment. DNA has been an important material for the nanostructure construction for over a decade. Likewise, design of novel RNA architecture with highly predictable and programmable self-assembly properties is possible. Due to RNA structure and function diversities, however, research in RNA nanotechnology and nano-therapeutics is much more challenging than DNA nanotechnology. Our studies indicate that selenium atom can contribute significantly in the nucleic acid nanostructure construction. Its high electron density, heavy mass, strong dehydration effect and large atomic size may facilitate the self-assembling processes of DNA bundle and RNA junction nanostructures. Plus, the Se-derivatized nucleic acids (SeNA) have remarkable advantages in nucleic acid X-ray crystallography, by providing rational phase determination, facilitating nucleic acid crystallization, and promoting high-quality structure determination. Our unique Se atom-specific mutagenesis presents a novel paradigm for the development of nucleic acid nanostructures and potential therapeutics.

References:
• Lina Lin, Jia Sheng, Zhen Huang*, "Nucleic Acid X-ray Crystallography via Direct Selenium Derivatization", Chemical Society Reviews (invited and peer reviewed), 2011, 40, 4591-4602.
Estrogen receptor coactivator MED1 in endocrine resistance and as a therapeutic target for human breast cancer

Kristine Bauer-Nilsen¹, Marissa Leonard¹, Jaijun Cui¹, Tianying Wu², Jiang Wang³, Chaoping Chen⁴, Peixuan Guo⁵, Xiaoting Zhang¹

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Keywords: Estrogen Receptor, MED1, Transcriptional Coactivator, Endocrine Resistance, RNA Nanotechnology

Despite the fact that most breast cancer patients have ERα-positive tumors, up to 50% of the patients are or soon become resistant to endocrine (tamoxifen) therapy. It has been recognized that amplification of receptor tyrosine kinase HER2 is one of the major mechanisms contributing to the tamoxifen resistance. However, although blockage of HER2 with the monoclonal antibody trastuzumab (Herceptin) has been successfully used as a second-line treatment resistance to this therapy, again, is quite high. Hence, further development of novel strategies to selectively block the activities of these pathways remains a major challenge for the treatment of human breast cancer.

Our previous studies indicated that transcriptional coactivator MED1, which co-amplifies with HER2, exists in only a subpopulation of the Mediator complex and is required for ERα-mediated transcription and breast cancer cell growth [Mol Cell (2005) 19:89]. Interestingly, our in vivo studies further demonstrated that MED1 plays rather tissue-specific roles in mediating ERα functions during the pubertal mammary gland development [PNAS (2010) 107:6765]. More recently, we have established MED1 as a novel key crosstalk point for HER2 and Estrogen receptor signaling pathways in tamoxifen resistance [Cancer Research (2012) 72:5625]. We found that HER2 over-expression led to MED1 phosphorylation and activation, while knockdown of MED1 greatly sensitized otherwise tamoxifen-resistant breast cancer cells to tamoxifen treatment. Further mechanistic studies showed that HER2 activation of MED1 resulted in the recruitment of co-activators instead of co-repressors by tamoxifen-bound estrogen receptor. Significantly, recently published data also indicated that higher MED1 expression levels correlate with poorer treatment outcome and reduced disease-free survival of patients treated with endocrine therapy.

Taken together, these findings support a key role for MED1 in HER2-mediated tamoxifen resistance and suggest its potential usage as a therapeutic target to simultaneously block both ERα and HER2 pathways for the treatment of this type of endocrine resistant breast cancer. We are currently developing RNA aptamers to disrupt the interactions between ER and MED1, as well as pRNA nanotechnology-based siRNA delivery systems to specifically diminish MED1 protein expression, in an effort to achieve the inhibition of these pathways and overcome endocrine resistance of human breast cancer.
Poster abstract: 55

Dual-channel single-molecule imaging of pRNA on phi29 DNA-packaging motor

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Bacteriophage phi29 DNA-packaging motor is geared by six packaging RNAs (pRNA). The pRNA molecules have been reported to serve as building blocks in RNA nanotechnology, and as vehicles for specific delivery of therapeutics to treat cancers and viral infections. The understanding of the 3D structure of pRNA and its location and positioning on the motor are both fundamentally and practically important. A customized single-molecule dual-color imaging system has been constructed to study the structures of pRNA molecules. The system is the combination of a low-temperature (-80 °C) sensitive electron multiplied CCD camera and the prism-type total internal reflection mechanism. A laser combiner was introduced to facilitate simultaneous dual-channel imaging. It has been applied to study the structure, stoichiometry, distance and function of the phi29 DNA packaging motor. Single molecule photobleaching combined with binomial distribution analysis clarified the stoichiometry of pRNA on the motor and elucidated the mechanism of pRNA hexamer assembly. The feasibility of the single-molecule imaging system was demonstrated in studies of single-molecule FRET. Distance rulers made of dual-labeled dsDNA and RNA/DNA hybrids were used to evaluate the system by determining the distance between one FRET pair. The single-molecule FRET was also applied to the reconstructed the 3D structure of phi29 motor pRNA monomers and pRNA dimers. Ten pRNA monomers labeled with single donor or acceptor fluorophore at various locations were constructed, and eight partner pairs were assembled into dimers. FRET signals were detected for six dimers and utilized to assess the distance between each donor/acceptor pair. The results provide the distance constraints for 3D computer modeling of phi29 DNA packaging motor. We have also re-engineered the energy conversion protein, gp16, of phi29 motor for single fluorophore labeling to facilitate the single molecule studies of motor mechanism. The potential applications of single-molecule high-resolution imaging with photobleaching (SHRImP) and single molecule high resolution with co-localization (SHREC) approaches to the study of the phi29 nanomotor were also investigated.

References:
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Dining Options

Crowne Plaza – The Campbell House
Lexington, Kentucky, USA
April 3-5, 2013
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<td>Phone: (859)-255-5972</td>
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<td><strong>7. Penn Station East Coast Subs</strong></td>
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<td>#101, 1080 South Broadway Rd</td>
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<td>Phone: (859)-254-7366</td>
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<td>Phones: (859)-259-1693</td>
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<td><strong>10. Yama To Go Japanese Grill</strong></td>
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<tr>
<td>Phone: (859)-455-3335</td>
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**Sit-Down Restaurants**

11. **Back Yard Burgers**
   - $$
   - 397 Waller Ave
   - Lexington, KY
   - Phone: (859)-226-0115
   - 0.2 mi from Crowne Plaza

13. **Casanova Italian Restaurant**
   - $$
   - 855 South Broadway Rd
   - Lexington, KY
   - Phone: (859)-309-3313
   - 0.7 mi from Crowne Plaza

12. **Buffalo Wild Wings Grill & Bar**
   - $104, 1080 South Broadway Rd
   - Lexington, KY
   - Phone: (859)-233-2999
   - 0.4 mi from Crowne Plaza

14. **Jalapenos Mexican Restaurant**
   - $$
   - 1030 South Broadway Rd
   - Lexington, KY
   - Phone: (859)-281-5171
   - 0.5 mi from Crowne Plaza

15. **Logan’s Roadhouse**
   - $$
   - 1250 South Broadway Rd
   - Lexington, KY
   - Phone: (859)-252-4307
   - 0.2 mi from Crowne Plaza

18. **Sierra Fria**
   - $$
   - 861 South Broadway Rd
   - Lexington, KY
   - Phone: (859)-554-5297

16. **O’Charley’s**
   - $$
   - 2099 Harrodsburg Rd
   - Lexington, KY
   - Phone: (859)-278-6984
   - 1.0 mi from the Crowne Plaza

19. **Sir Pizza**
   - $1064 South Broadway Rd
   - Lexington, KY
   - Phone: (859)-254-0303
   - .04 mi from Crowne Plaza

17. **Seki Restaurant**
   - $$
   - 1093 South Broadway Rd.
   - Lexington, KY
   - Phone: (859)-254-5289
   - 0.4 mi from Crowne Plaza

20. **Thai Orchid Cafe**
   - $$
   - 1030 South Broadway Rd
   - Lexington, KY
   - Phone: (859)-288-2170
Restaurant Locations

Walking directions from the Crowne Plaza to University of Kentucky College of Pharmacy
* Breakfast provided to all hotel guests
** Lunches and dinners provided to all paid registrants with food option and invited speakers
• Centers of Cancer Nanotechnology Excellence (CCNEs)
• Cancer Nanotechnology Platform Partnerships (CNPP)
• Cancer Nanotechnology Training Centers (CNTC)

Post-doc and Graduate Students Positions are available for the following program

NCI Cancer Nanotechnology Platform Partnerships (CNPP) Program:
RNA Nanotechnology in Cancer Therapy

University of Kentucky

Peixuan Guo, Ph.D.
Director
University of Kentucky,
Lexington, KY

John J Rossi, Ph.D.
Co-Director
Beckman Research Institute of City of Hope, Duarte, CA
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Professor Wei Duan, School of Medicine, Deakin University, Victoria, Australia

Ask for your RNA Test-scale at our exhibition booth at the RNA Nanotechnology and Therapeutics Conference 2013 in Lexington or at oligo@iba-lifesciences.com.
Aims and Scope

Nano LIFE is a quarterly international journal publishing peer-reviewed research in all fields of nano and biomedical sciences. The emphasis of this journal is based on its originality, importance, and interdisciplinary nature between nano and life sciences. Nano LIFE also provides current news and interpretations of critical issues in nanomedicine that caters to scientific communities and the general public.
Master of Ceremony: Lilly Xie, President of Chinese Dance Troupe in Kentucky
Artistic Director: Cheryl Pan, Kentucky Chinese American Association

April 4, 2013   at 7:00pm       Crowne Plaza, Lexington, Kentucky

1. Chinese Luogu (Drum): Harvest Celebration
   Player: UK Luogu Group, Director: K. H. Han

2. Jasmine Flower Dance
   Performer: Chinese Dance Troupe in Kentucky

3. Dizi and Erhu Duet: Flower and boy
   Player: Peixuan Guo and Wei Luo

4. Yellow River Solo Dance
   Dancer: Lilly Xie

5. Duet Singing: Tibetan beautiful
   Performer: Vicki Deng and Amy Wang

6. Erhu Solo: Horse Racing
   Player: Xiaowan Chu

7. Man group singing: Girl from Daban
   Performer: Lexington 老爷们合唱队

8. Water Lilly Dance
   Performer: Chinese Dance Troupe in Kentucky

9. Duet Singing: All I ask of you
   Player: Chong Huang and Mingjun Zhao

10. Mongolian Solo Dance
    Dancer: Becky Wang

11. Piano Solo
    Player: Amy Wang

12. Modern Dance
    Performer: Chinese Dance Troupe in Kentucky

13. Singing and dance
    Player: Xiaowan Chu

    Player: Bluegrass Chinese Music Ensemble

15. Dragon and Phoenix dance
    Performer: Chinese Dance Troupe in Kentucky

16. Chinese Waist Drum Dance
    Performer: Kentucky Chinese American Association
Name: ________________________________
Affiliation: ________________________________
Title: ________________________________
Email: ________________________________
Phone: ________________________________

- I am interested to contribute an article as part of the conference proceedings.
- It will be beneficial to setup a "RNA Nanotechnology and Therapeutics" society.
- I am interested to be a member of the "RNA Nanotechnology and Therapeutics" society.
- I would like to take a leadership position in the "RNA Nanotechnology and Therapeutics" society.
- I am not interested. Please remove me from all mailing lists.

- Please provide any additional comments:

Please return this form to the Conference Registration desk. Thanks for your response.

- Meeting Organizing Committee