



中山大學 孫逸仙紀念醫院

SUN YAT-SEN MEMORIAL HOSPITAL, SUN YAT-SEN UNIVERSITY

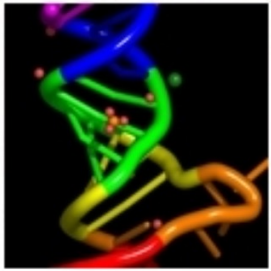
Beyond Prediction: Experimental Strategies to Expand the RNA Structure Universe

Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University
Lin Huang

Benasque 2026. 7. 8



Benasque 2018



Computational Approaches to RNA Function

2018, Jul 15 -- Jul 27

Organizers:

E. Rivas (Harvard University), E.

<https://benasque.org/2018rna/cgi-bin/talks/>

RNA Modifications

Chair: Stadler - Westhof

N6 - methylation of adenine in box C/D snoRNA : regulation of RNP assembly

Experimental methods (chemical probing)

Chair: Dmitri Pervouchine

Strategies in RNA X-ray crystallography

Applications of RNA design

Chair: Will – Gorodkin

From sequence to nanostructure - The kink turn as a building block in nano-engineering



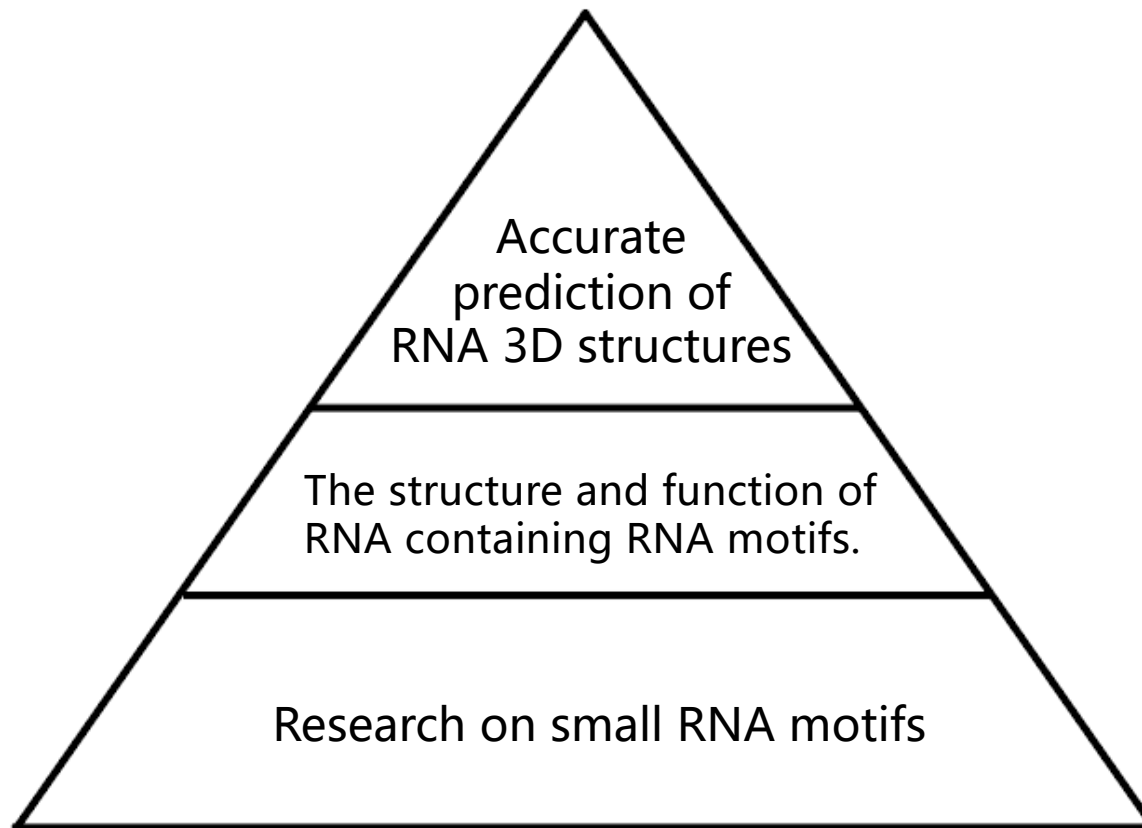
Benasque 2018

No.	Year	Title	Journal	Autors
1	2026	Structural assemblies for an RNA world	bioRxiv	Yangyi Ren, Zhenglai Zhang, Ke Chen, ..., Eric Westhof , Jia Wang*, Zhichao Miao* , Xuepeng Wei*, Lin Huang*
2	2026	Structural Basis of DNA Aptamer A58 Targeting the N-terminal Domain of Sarbecoviruses Nucleocapsid Protein	Journal of Biological Chemistry	Xiaoxue Chen, Suhua He, Shaojie Xue, ..., Zhichao Miao* , Shoudeng Chen*, Lin Huang*
3	2026	Ribocentre-aptamer: an integrative, structure-focused database for RNA aptamers	Nucleic Acids Research	Zhizhong Lu, Hao Sun, Bo Fu, ..., Baowei Huang, Shuang Zhu*, Lin Huang* , Zhichao Miao*
			Proteins: Structure, Function, and Bioinformatics	Bowen Xiao* , Yaohuang Shi , Lin Huang*
			Nucleic Acids Research	Yangyi Ren, Xiaowei Lin, Wenjian Liao, Xuemei Peng, Jie Deng, Zhe Zhang, Jian Zhan, Yaoqi Zhou, Eric Westhof , David MJ Lilley, Jia Wang*, Lin Huang*
			Angewandte Chemie	Xiaowei Lin, Yuanyin Huang, Jinchao Huang, ..., Shuo-Bin Chen*, Zhichao Miao* , Lin Huang*
			Nucleic Acids Research	Fan Bu, Xiaowei Lin, Wenjian Liao, ..., Jian Huang, Tianxin Lin, Zhichao Miao* , Lin Huang*
			Nucleic acids research	Jie Deng, Yaohuang Shi, Xuemei Peng..., Yuanyin Huang, Taijiao Jiang, David MJ Lilley*, Zhichao Miao* , Lin Huang*
			Nucleic Acids Research	Lin Huang , Jia Wang, Andrew M Watkins , Rhiju Das , David M J Lilley

The main research interests in my lab



Lab website



Riboswitch and ribozyme

Kink-turn, tRNA like RNAs

RNA structure prediction is still a big challenge

Great progress has been made in protein structure prediction and design

The Nobel Prize in Physics 2024

John Hopfield

“for foundational discoveries and inventions that enable machine learning with artificial neural networks”



John Hopfield. Ill. Niklas Elmehed © Nobel Prize Outreach

Geoffrey Hinton

“for foundational discoveries and inventions that enable machine learning with artificial neural networks”



Geoffrey Hinton. Ill. Niklas Elmehed © Nobel Prize Outreach

The Nobel Prize in Chemistry 2024

David Baker

“for computational protein design”



David Baker. Ill. Niklas Elmehed © Nobel Prize Outreach

Demis Hassabis

“for protein structure prediction”



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John Jumper

“for protein structure prediction”

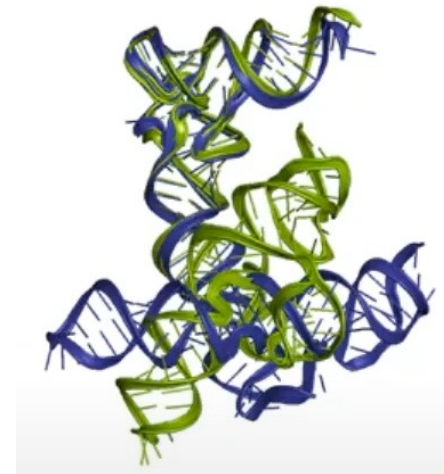
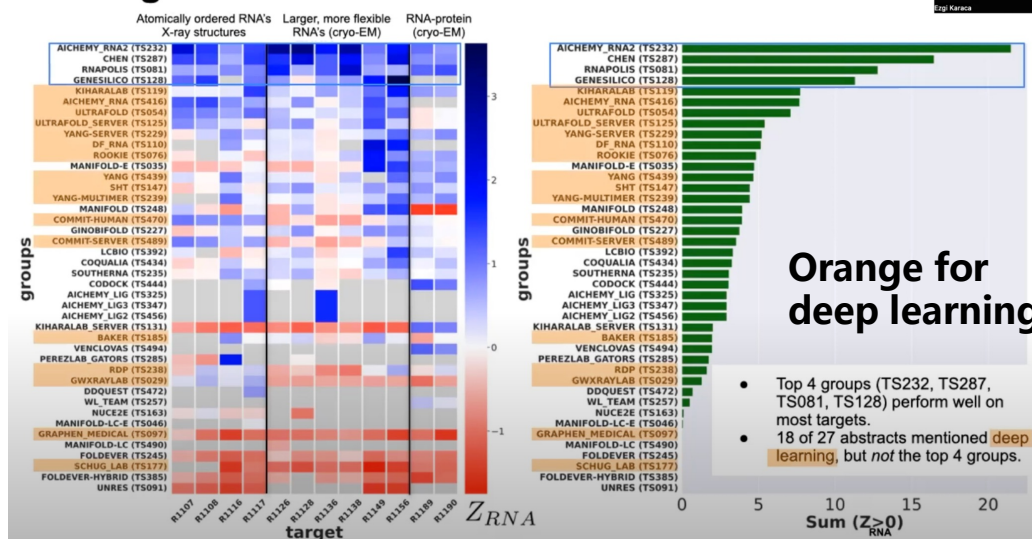


John Jumper. Ill. Niklas Elmehed © Nobel Prize Outreach

RNA structure prediction is still a big challenge

2022 CASP15: Critical Assessment of Structure Prediction

Rankings from CASP15 RNA metric

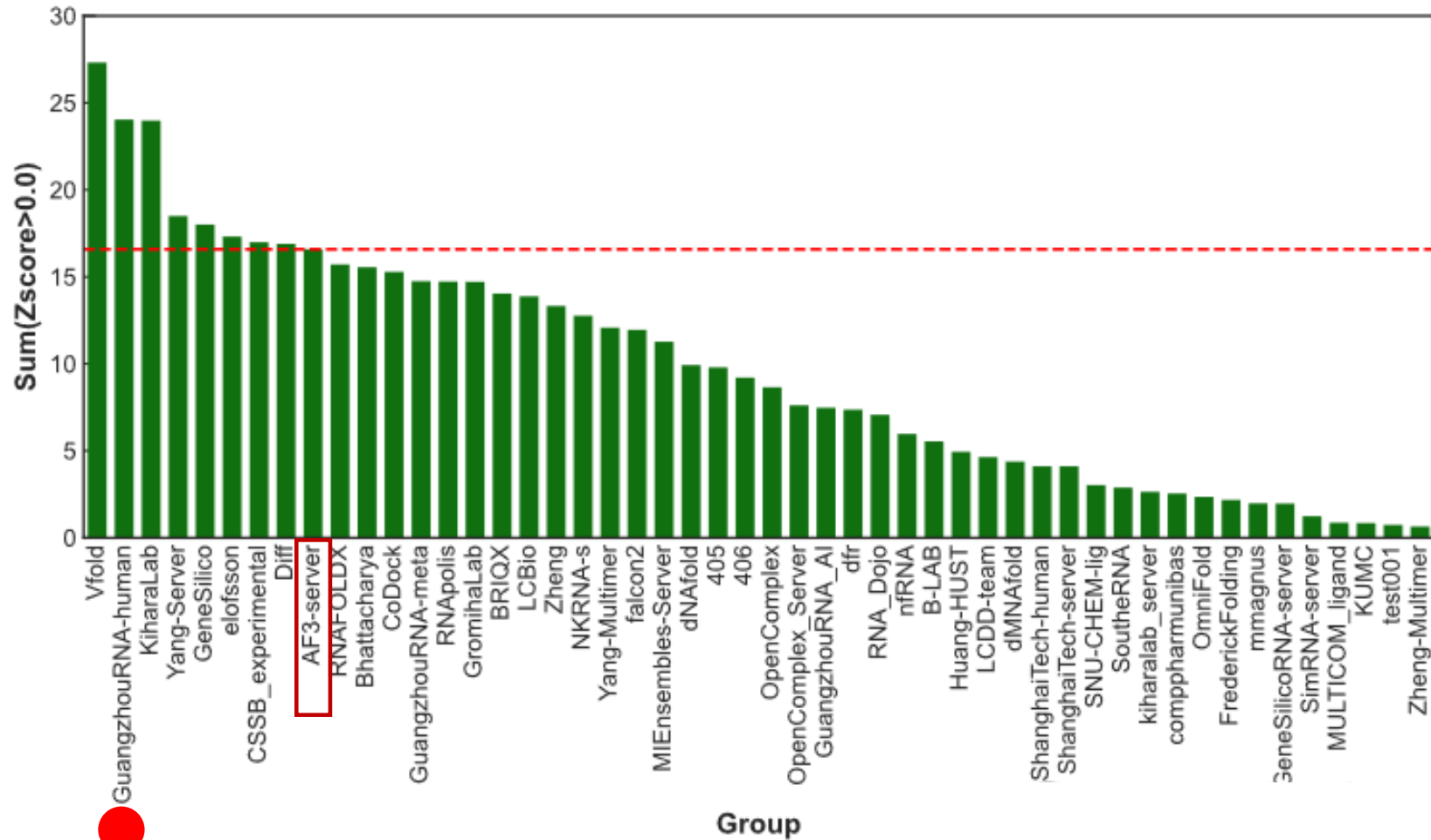


Deep learning has not provided significant assistance in RNA structure prediction.

Low accuracy in RNA structure prediction

RNA structure prediction is still a big challenge

- 2024 CASP16: Led the team to achieve second place globally



RNA structure prediction is still a big challenge

➤ 2024 CASP16: Led the team to achieve second place globally

Proteins: Structure, Function, and Bioinformatics

WILEY



RESEARCH ARTICLE

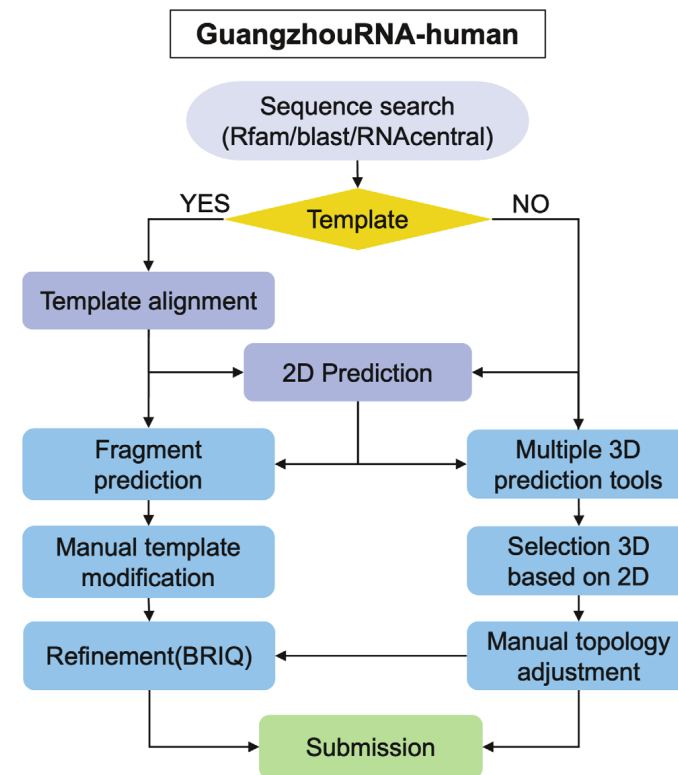
Enhancing RNA 3D Structure Prediction: A Hybrid Approach Combining Expert Knowledge and Computational Tools in CASP16

Bowen Xiao^{1,2} | Yaohuang Shi^{1,3} | Lin Huang⁴

¹Guangzhou National Laboratory, Guangzhou, China | ²Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China | ³School of Life Science and Technology, ShanghaiTech University, Shanghai, China | ⁴Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Guangdong-Hong Kong Joint Laboratory for RNA Medicine, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China

Correspondence: Bowen Xiao (xiao_bowen@gzlab.ac.cn) | Lin Huang (huanglin36@mail.sysu.edu.cn)

Invited Article



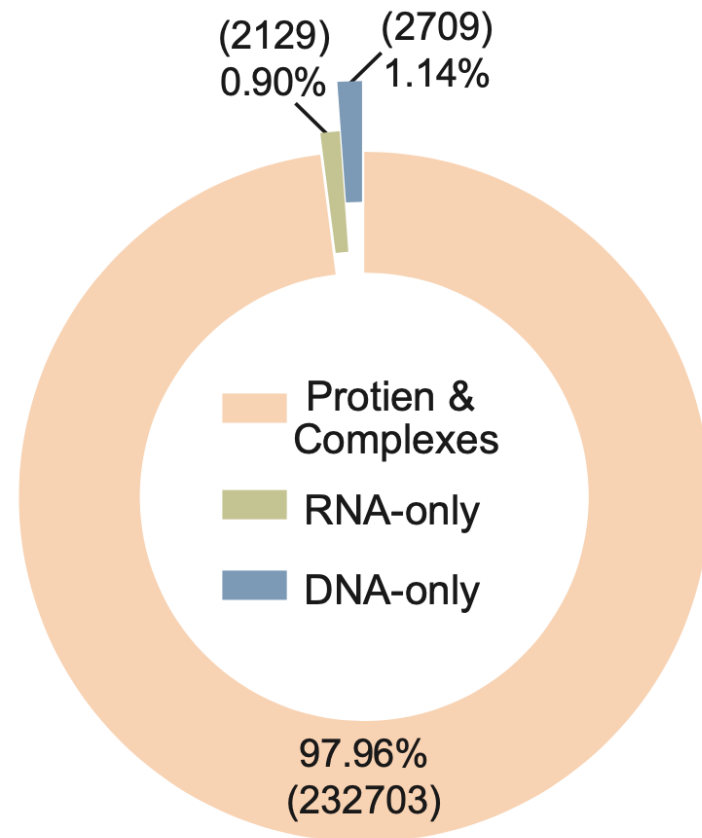
RNA structure prediction is still a big challenge



254,978
Structures from the
PDB archive

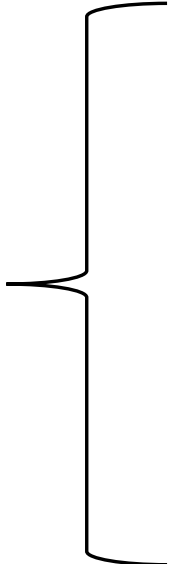


1,062,058
Computed Structure
Models (CSM)



Main reasons for low accuracy

1. Insufficient number of independently functional RNA structures, fewer than 200



16S and 23S rRNA,
18S and 28S rRNA,
group I and group II introns,
RNase P ribozymes,
tmRNAs,
tRNAs,
...

Ribozymes > 10

Riboswitches > 50

Aptamers > 50

2. Research on RNA motifs is severely lacking

The problem: Inadequate learning content for artificial intelligence and deep learning.

Summarize the existing determined RNA structures

Insufficient number of independently functional RNA structures, fewer than 300

16S and 23S rRNA,
18S and 28S rRNA,
group I and group II introns,
RNase P ribozymes,
Ribozymes > 10
Riboswitches > 50
Aptamers > 50
tmRNAs,
tRNAs,
...

Deng...Huang, *Nucleic Acids Research* 2023

Bu...Huang, *Nucleic Acids Research* 2024

Lu...Huang, Miao, *Nucleic Acids Research* 2026

D262–D268 *Nucleic Acids Research*, 2023, Vol. 51, Database issue
<https://doi.org/10.1093/nar/gkac840>

Published online 30 September 2022

Ribocentre: a database of ribozymes

Jie Deng^{1,†}, Yaohuang Shi^{2,3,†}, Xuemei Peng^{1,†}, Yuanlin He¹, Xiaoxue Chen^{1,4}, Mengxiao Li¹, Xiaowei Lin^{1,5}, Wenjian Liao^{①1,5}, Yuanyin Huang¹, Taijiao Jiang³, David M.J. Lilley^{6,*}, Zhichao Miao^{③3,7,*} and Lin Huang^{①1,*}

Nucleic Acids Research, 2024, 52, D265–D272
<https://doi.org/10.1093/nar/gkad891>
Advance access publication date: 19 October 2023
Database issue



Ribocentre-switch: a database of riboswitches

Fan Bu^{①1,2,†}, Xiaowei Lin^{③3,4,†}, Wenjian Liao^{③3,4,†}, Zhizhong Lu⁵, Yuanlin He⁴, Yuhang Luo⁴, Xuemei Peng⁴, Mengxiao Li⁴, Yuanyin Huang⁴, Xiaoxue Chen⁴, Bowen Xiao², Jiahong Jiang², Jie Deng⁴, Jian Huang^{3,4}, Tianxin Lin^{③3,4}, Zhichao Miao^{⑥6,2,*} and Lin Huang^{④4,*}

Ribocentre-aptamer: A database of RNA aptamers

Zhizhong Lu^{1,3}, Ying Ao², Bo Fu¹, Shuang Zhu^{3,*}, Lin Huang^{1,*}, Zhichao Miao^{2,*}



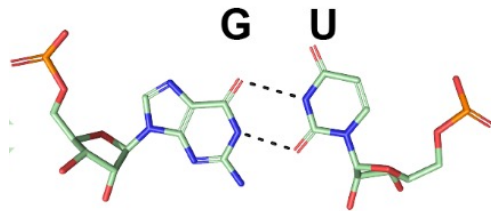
1. Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Guangdong-Hong Kong Joint Laboratory for RNA Medicine, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, China.
2. GMU-GIBH Joint School of Life Sciences, The Guangdong-Hong Kong-Macau Joint Laboratory for Cell Fate Regulation and Diseases, Guangzhou National Laboratory, Medical University, Guangzhou 510180, China.
3. School of Life Sciences and Biopharmaceutics, Guangdong Pharmaceutical University, Guangzhou 510006, China.
Email to : Lin Huang (huanglin36@mail.sysu.edu.cn), Zhichao Miao (miao_zhichao@gzlab.ac.cn)

Methods Development

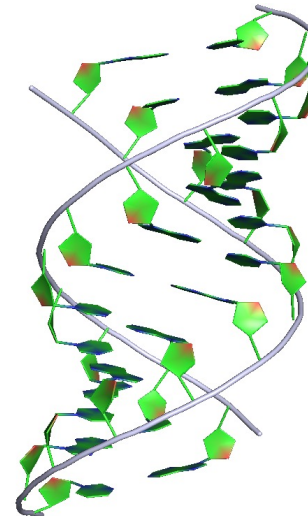
- Developing "efficient" methods for nucleic acid structures determination

Two new methods

GU base pairs engineering



The counter-intuitive "elongation of non-functional stems" method



A general strategy for engineering GU base pairs to facilitate RNA crystallization

Yangyi Ren ^{1,†}, Xiaowei Lin ^{1,2,3,†}, Wenjian Liao ^{1,2}, Xuemei Peng¹, Jie Deng¹, Zhe Zhang ⁴, Jian Zhan^{4,5}, Yaoqi Zhou ⁴, Eric Westhof^{6,7}, David M.J. Lilley ⁸, Jia Wang^{9,*} and Lin Huang^{1,*}

¹Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Guangdong-Hong Kong Joint Laboratory for RNA Medicine, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, China

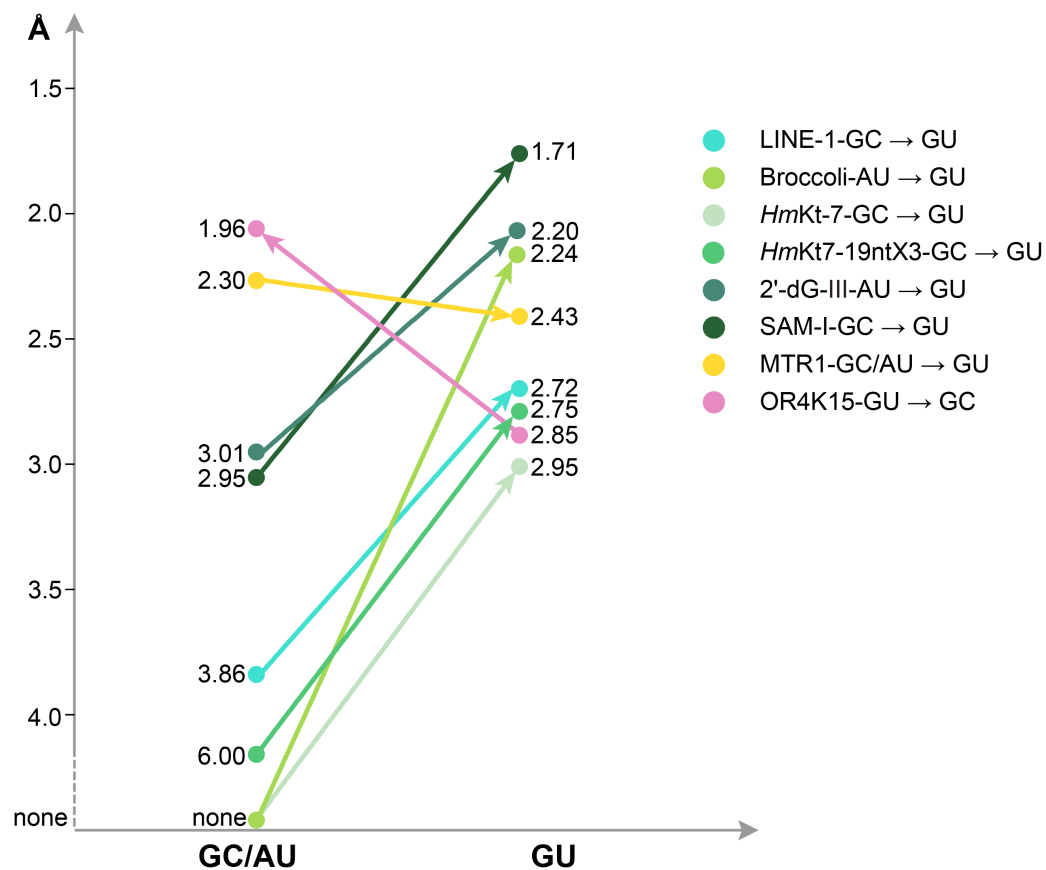


Shenzhen Technology University

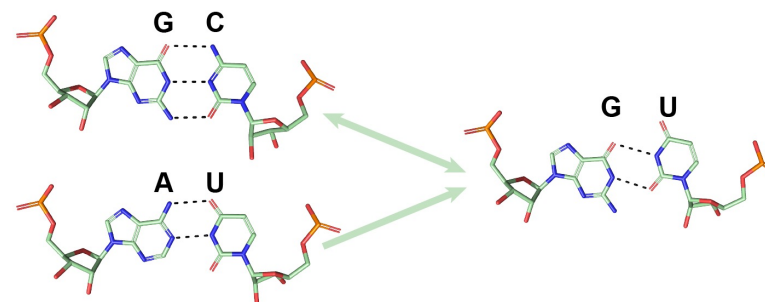
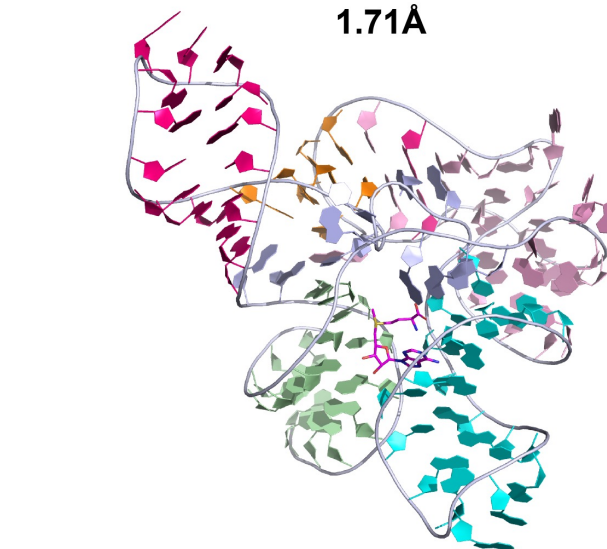
Jia WANG

Simple and effective approach for high-resolution RNA structure determination

GU base pairs engineering



SAM-I-GU 1.71Å



Simple and effective approach for high-resolution RNA structure determination

GU base pairs engineering

1. K Chen ... L Huang *Nucleic Acids Research* 53 (14) 2025
2. X Lin ... L Huang *Angewandte Chemie* 137 (22) 2025
3. Y He ... L Huang *Cell Discovery* 10 (1), 128 2025
4. Y Ren ... L Huang *Nucleic Acids Research* 53 (3) 2025
5. J Deng ... L Huang *Nature Chemical Biology* 18 (5), 556-564 2022
6. L Huang ... DMJ Lilley *Nucleic acids research* 44 (11), 5390-5398 2016
7. L Huang, DMJ Lilley *Nanoscale* 8 (33), 15189-15195 2016
8. L Huang, DMJ Lilley *Nucleic acids research* 42 (7), 4734-4740 2014



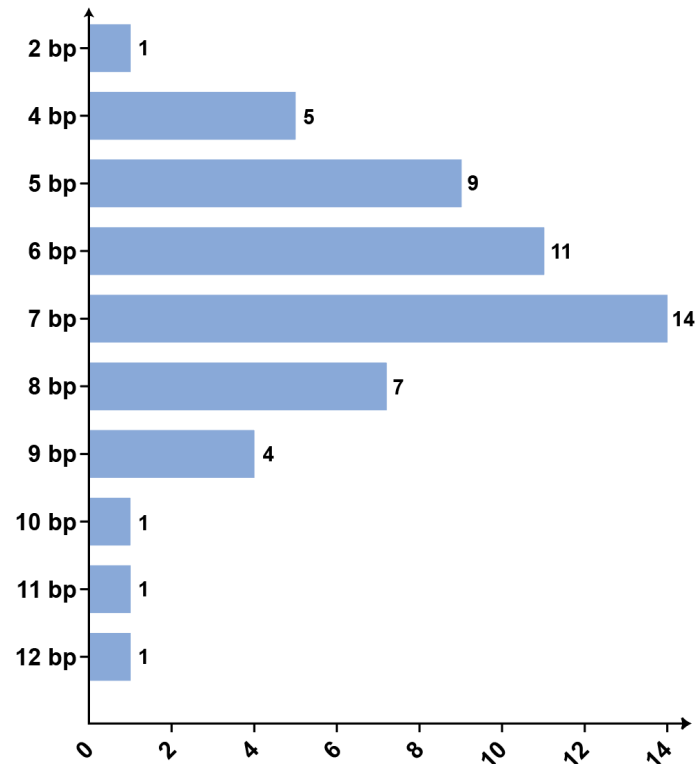
“以柔（GU）克刚”
The soft overcomes the hard

GU base-pair engineering relies on distilling prior successful examples and combining it with creative thinking. However, determine only 10 new RNA structures over 10 years is not efficient enough!

More efficient strategies that break conventional thinking are needed.

The counter-intuitive "elongation of non-functional stems" method

➤ Statistics of the length of the P1 stem in known nucleic acid structures



mostly falls within the range of 4 to 8 base pairs

Nucleic Acids Research, 2024, **52**, D265–D272
<https://doi.org/10.1093/nar/gkad891>
Advance access publication date: 19 October 2023
Database issue



Ribocentre-switch: a database of riboswitches

Fan Bu^{1,2,†}, Xiaowei Lin^{3,4,†}, Wenjian Liao^{3,4,†}, Zhizhong Lu⁵, Yuanlin He⁴, Yuhang Luo⁴, Xuemei Peng⁴, Mengxiao Li⁴, Yuanyin Huang⁴, Xiaoxue Chen⁴, Bowen Xiao², Jiahong Jiang², Jie Deng⁴, Jian Huang^{3,4}, Tianxin Lin^{3,4}, Zhichao Miao^{6,2,*} and Lin Huang^{4,*}

Secondary structure

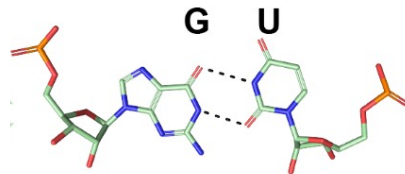


Method Development

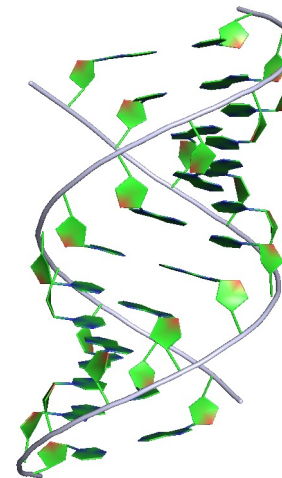
- Developing "efficient" methods for nucleic acid structures determination

Two new methods

GU base pairs engineering



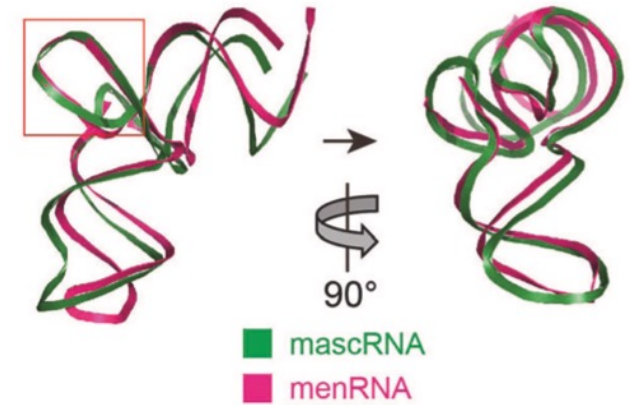
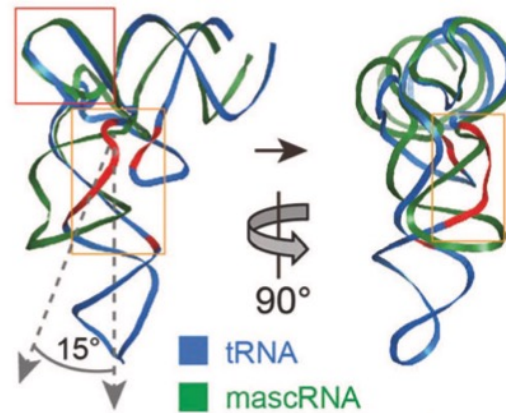
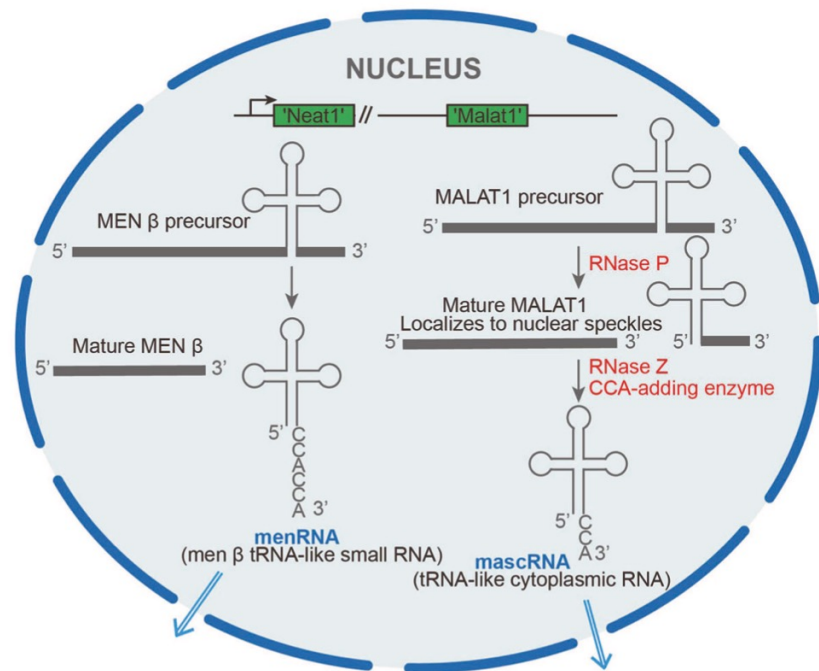
The counter-intuitive "elongation of non-functional stems" method



In the field of nucleic acid crystallography, there is rarely a method that can help to determine the structures of at least 10 RNA molecules.

Both of these methods can achieve this.

The significance of
efficiently determine
the structure of the new RNA/DNA
in “large quantities”

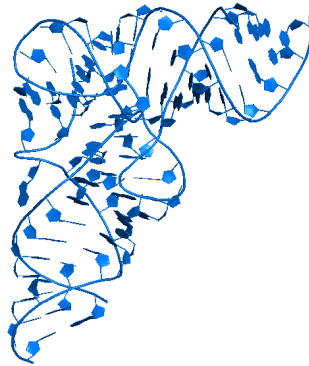


"Extensively" determine novel RNA structures

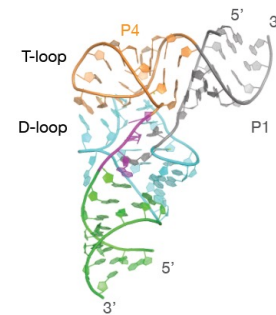
Salivarius



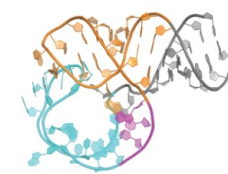
Skipping-rope



The discovery of bacterial tRNA-like RNAs



tLRNA-1
skipping-rope (RF02924)



tLRNA-2
DUF2693-FD (RF02926)



tLRNA-3
salivarius-1 (RF03030)



tRNA
(RF00005)

■ Experimental structure

■ Prediction structure

■ Experimental structure

■ Prediction structure

Unpublished

The Nobel Prize in Chemistry 2024

David Baker

“for computational protein design”



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Demis Hassabis

“for protein structure prediction”



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John Jumper

“for protein structure prediction”



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2024

The Nobel Prize in Chemistry 2024

David Baker

“for computational protein design”



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nature nanotechnology



Article

<https://doi.org/10.1038/s41565-024-01641-1>

De novo design of pH-responsive self-assembling helical protein filaments

Received: 24 October 2023

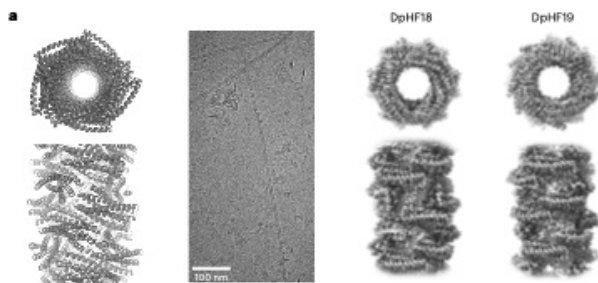
Accepted: 26 February 2024

Published online: 3 April 2024

Check for updates

Hao Shen^{1,2}✉, Eric M. Lynch¹, Susrut Akkineni^{3,4}, Joseph L. Watson^{1,2,5}, Justin Decarreau^{1,2}, Neville P. Bethel^{1,2}, Issa Benna^{2,6}, William Sheffler^{1,2}, Daniel Farrell^{1,3}, Frank DiMaio^{1,3}, Emmanuel Derivery⁸, James J. De Yoreo^{3,4}, Justin Kollman¹ & David Baker^{1,2,7}✉

Biological evolution has led to precise and dynamic nanostructures that reconfigure in response to pH and other environmental conditions. However, designing micrometre-scale protein nanostructures that are environmentally responsive remains a challenge. Here we describe the de novo design of pH-responsive protein filaments built from subunits containing six or nine buried histidine residues that assemble into micrometre-scale, well-ordered fibres at neutral pH. The cryogenic electron microscopy structure of an optimized design is nearly identical to the computational design model for both the subunit internal geometry and the subunit packing into the fibre. Electron, fluorescent and atomic force microscopy characterization reveal a sharp and reversible transition from assembled to disassembled fibres over 0.3 pH units, and rapid fibre disassembly in less than 1 s following a drop in pH. The midpoint of the transition can be tuned by modulating buried histidine-containing hydrogen bond networks. Computational protein design thus provides a route to creating unbound nanomaterials that rapidly respond to small pH changes.



2024

nature

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Article

Hierarchical design of pseudosymmetric protein nanocages

<https://doi.org/10.1038/s41586-024-08360-6>

Received: 17 June 2023

Accepted: 6 November 2024

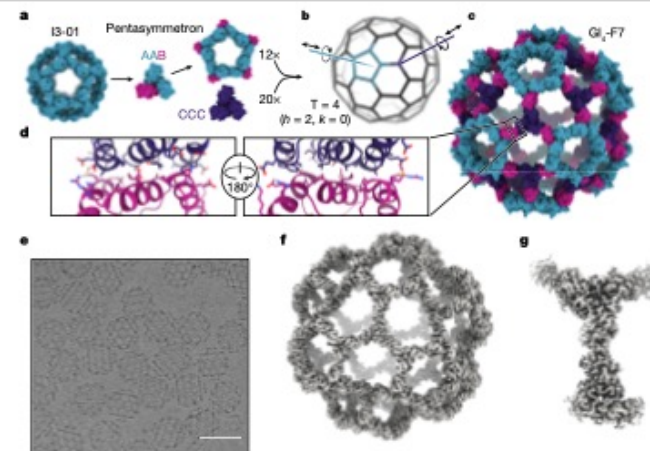
Published online: 18 December 2024

Open access

Check for updates

Quinton M. Dowling^{1,2}, Young-Jun Park⁴, Chelsea N. Fries^{2,3}, Neil C. Gerstenmaier^{2,3}, Sebastian Ols^{2,3}, Erin C. Yang^{2,3}, Adam J. Wargacki^{2,3}, Annie Dosey^{2,3}, Yang Hsia^{2,3}, Rashmi Ravichandran^{2,3}, Carl D. Walkey^{2,3}, Anika L. Burrell⁵, David Veester^{3,4}, David Baker^{2,3,4} & Neil P. King^{2,3,10}✉

Discrete protein assemblies ranging from hundreds of kilodaltons to hundreds of megadaltons in size are a ubiquitous feature of biological systems and perform highly specialized functions^{1,2}. Despite remarkable recent progress in accurately designing new self-assembling proteins, the size and complexity of these assemblies has been limited by a reliance on strict symmetry³. Here, inspired by the pseudosymmetry observed in bacterial microcompartments and viral capsids, we developed a hierarchical computational method for designing large pseudosymmetric self-assembling protein nanomaterials. We computationally designed pseudosymmetric heterooligomeric components and used them to create discrete, cage-like protein assemblies with icosahedral symmetry containing 240, 540 and 960 subunits. At 49, 71 and 96 nm diameter, these nanocages are the largest bounded computationally designed protein assemblies generated to date. More broadly, by moving beyond strict symmetry, our



David Baker

“for computational protein design”



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Demis H.

“for prot



Demis H. Outreach

2026

nature

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Article

Design of one-component quasisymmetric protein nanocages

<https://doi.org/10.1038/s41586-026-10554-z>

Received: 2 June 2025

Accepted: 15 April 2026

Published online: 20 May 2026

Check for updates

Sangmin Lee^{1,2,3,4,6,8,9}, David Chmielewski^{1,2,6}, Shunzhi Wang^{1,2,5,6}, Ryan D. Kibler^{1,2}, Jisu Shin⁴, Ann Carr^{1,2}, Young-Jun Park^{1,3}, David Veester^{1,2} & David Baker^{1,2,3,8,9}

Although the largest completely symmetric closed assembly that can be built from a single building block is the 60-subunit icosahedron¹, viruses can form capsid assemblies with hundreds to thousands of identical subunits through quasisymmetry—using the same subunit in symmetrically non-equivalent locations in the assembly^{2–5}. Quasisymmetric one-component assemblies could have considerable advantages for delivery of biologics because of the large internal volume achieved using only a single building block, but the design of these structures is challenging because of the inherent complexity of designing chemically identical subunits to both adopt different conformations and make different interactions in the distinct

2026

nature

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Article

De novo design of quasisymmetric two-component protein cages

<https://doi.org/10.1038/s41586-026-10464-0>

Received: 1 June 2025

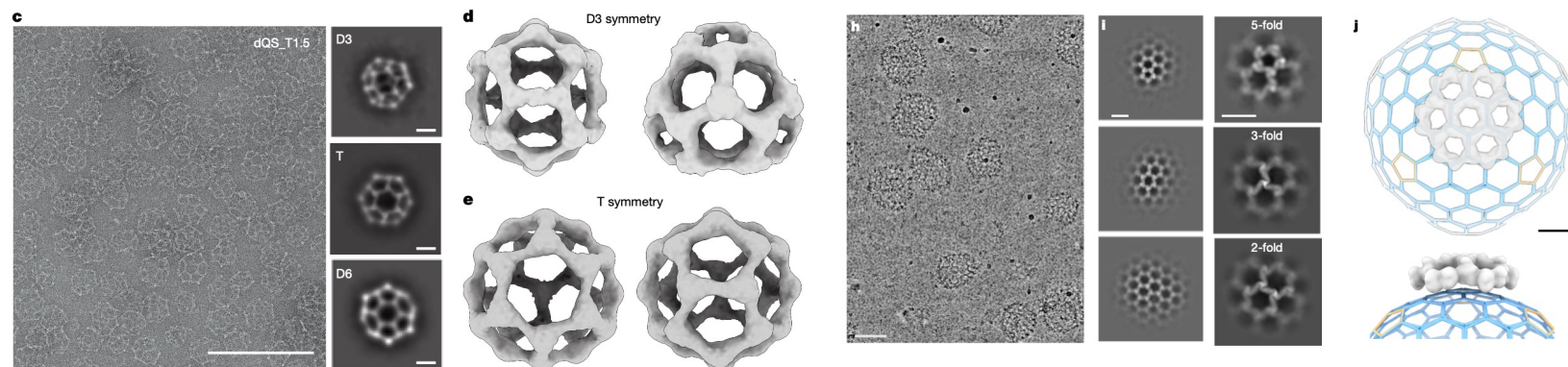
Accepted: 26 March 2026

Published online: 20 May 2026

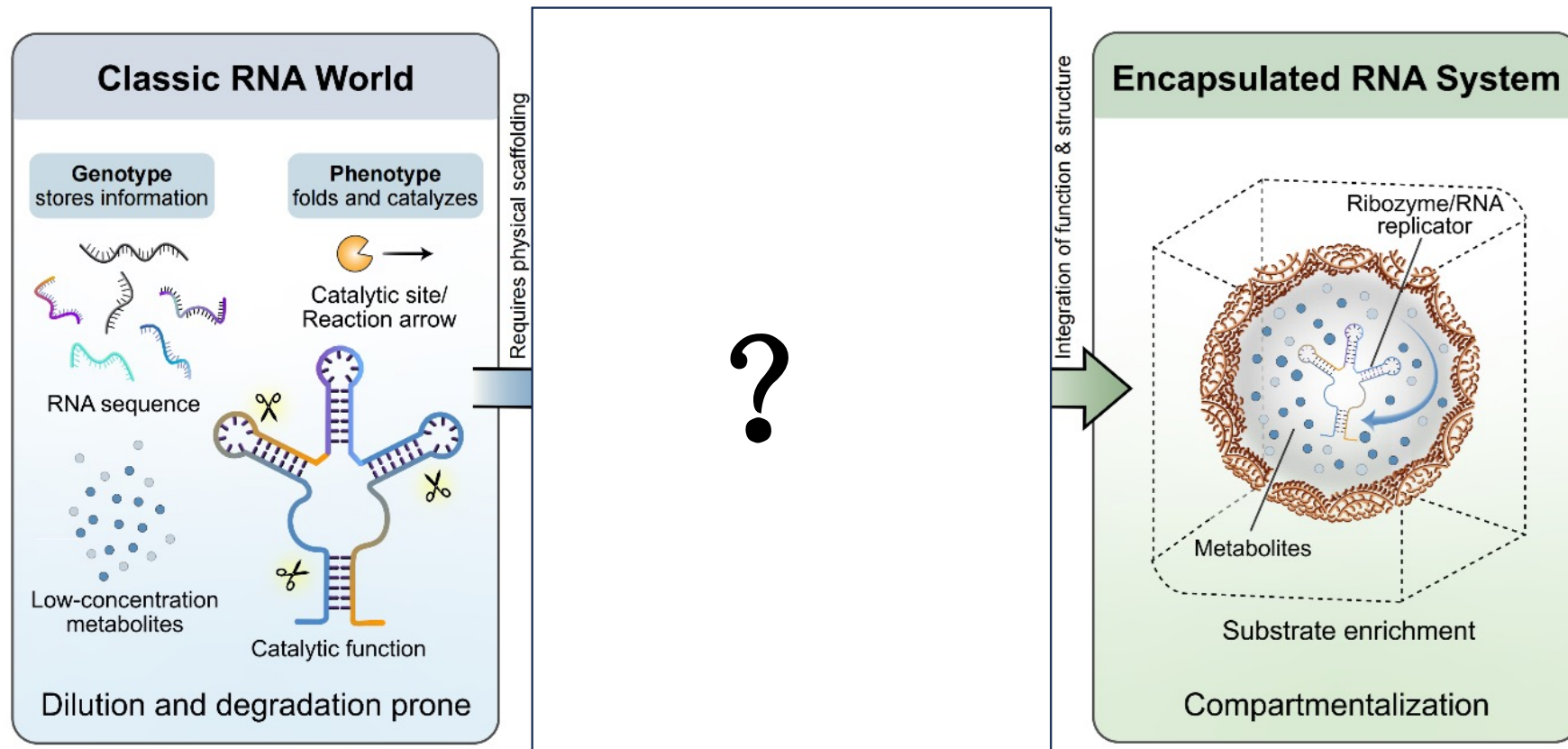
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Shunzhi Wang^{1,2,3,8,9}, Ying Xie³, David Chmielewski^{1,2}, Connor Weidle^{1,2}, Tong Shu³, Green Ahn^{1,2}, Ryan D. Kibler^{1,2}, Cindy Hernandez², Wei Chen^{1,2}, David Camilo Duran³, Ann Carr^{1,2}, Asim K. Bera^{1,2}, Sangmin Lee^{1,2,4}, Justin Decarreau^{1,2}, Alex Kang^{1,2}, Evans Brackenbrough^{1,2}, Emily Joyce^{1,2}, Kejia Wu^{1,2}, Andrew J. Borst^{1,2}, Andrew Favor^{2,5}, Buwei Huang^{2,5}, Frank DiMaio^{1,2}, Liam J. Holt³ & David Baker^{1,2,6,8,9}

Quasisymmetric icosahedral viral capsids achieve larger sizes than possible with strictly symmetric icosahedra by tessellating pentagons and hexagons using a single subunit that adopts different conformations in symmetrically non-equivalent locations^{1,2}. Recapitulating such quasisymmetric architectures through computational design is a considerable challenge in nanomaterials engineering. Here we introduce a computational design strategy based on geometric frustration to generate two-component, quasisymmetric protein cages with customizable

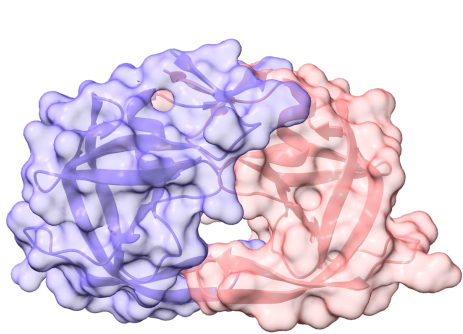


RNA World

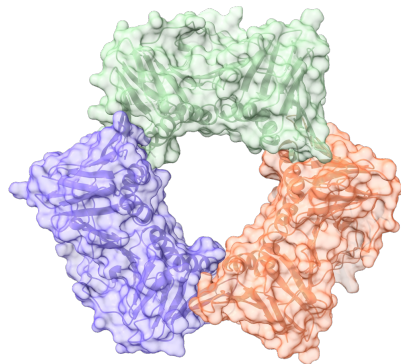


Proteins

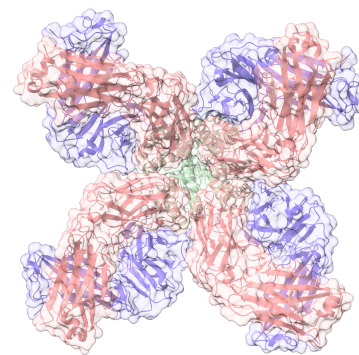
Oligomeric States of Proteins



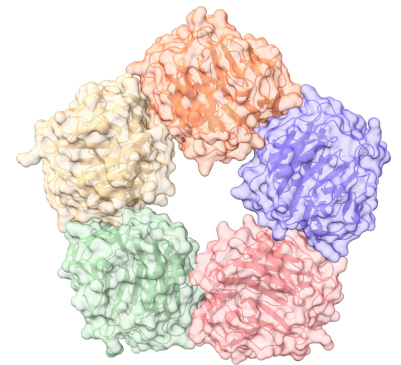
C2: HIV Protease (1HIV)



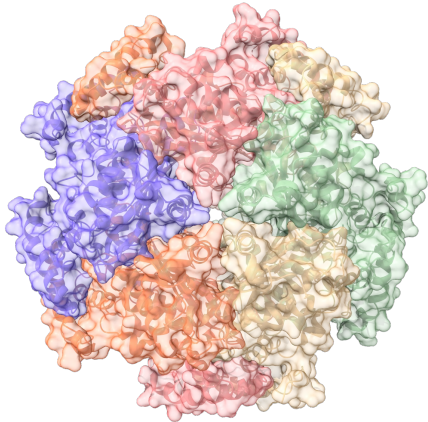
C3: PCNA (1AXC)



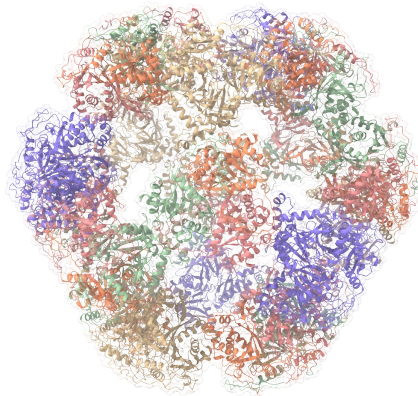
C4: KcsA (1K4C)



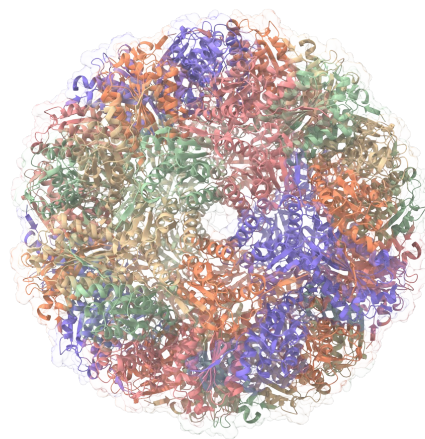
C5: C Protein (1B09)



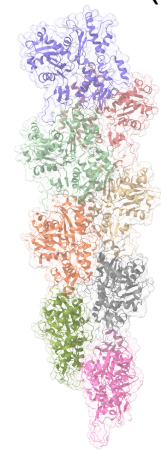
D5: Cyclohydrolase (1FB1)



O: Dehydrogenase (1AEW)

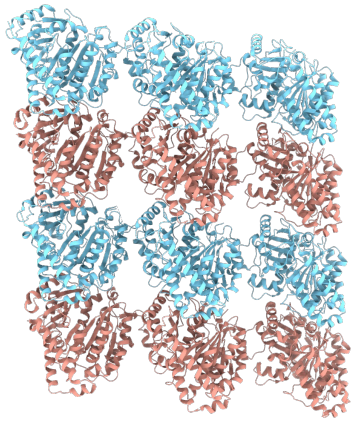


I: Synthase (1HQK)

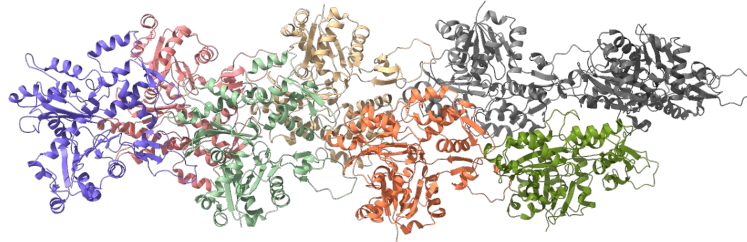


H: F-Actin (6BNO)

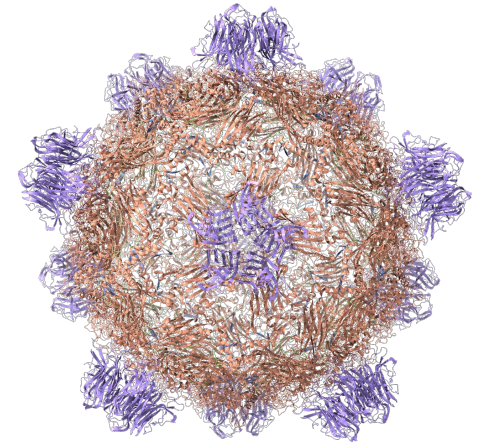
Proteins



PDB:6DPU

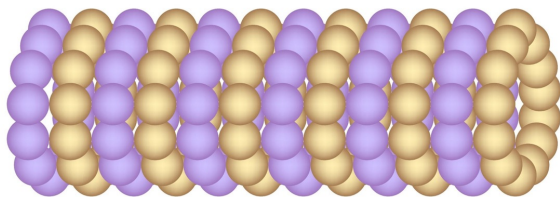


PDB:6BNO

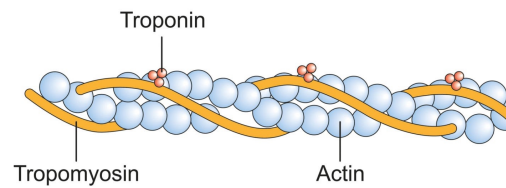


PDB:2BPA

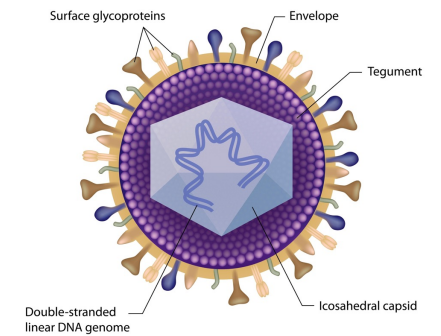
Microtubule



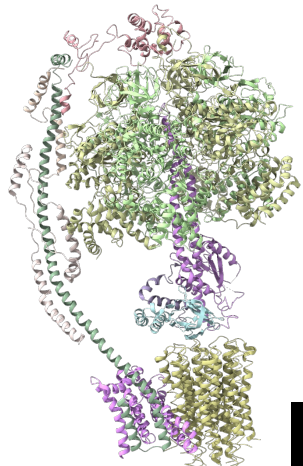
Actin Filament Structure



Herpes Simplex Virus

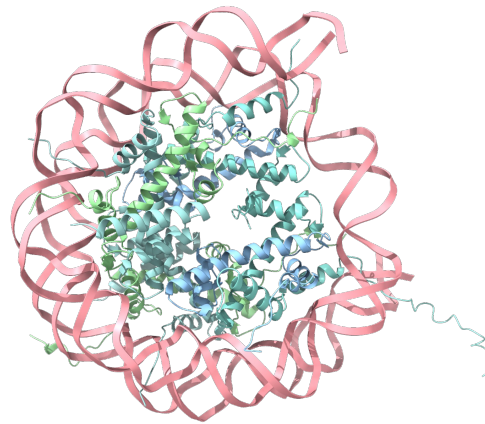
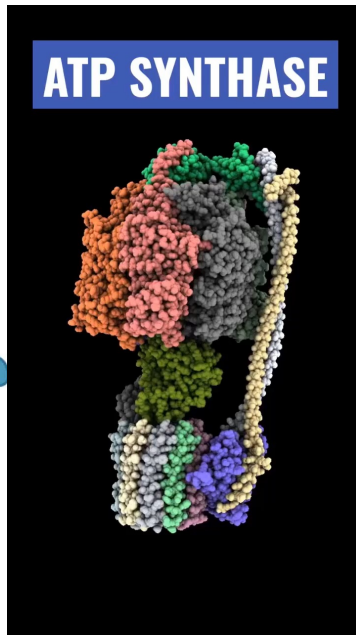


Proteins



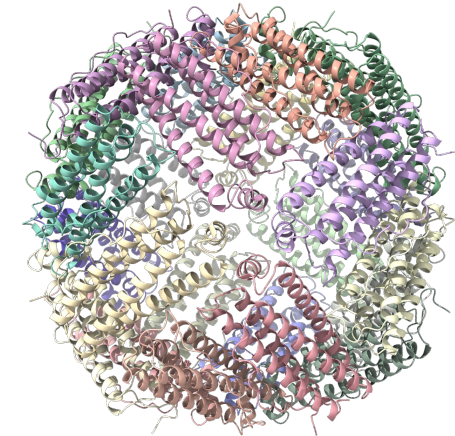
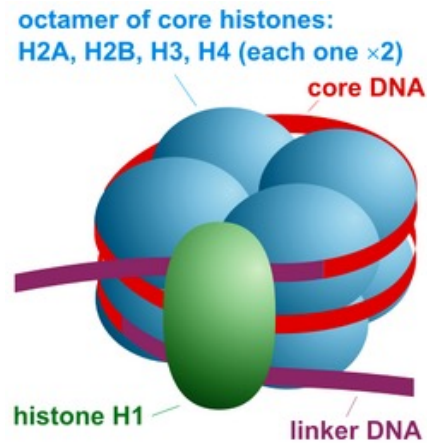
PDB:5FIK

ATP Synthase



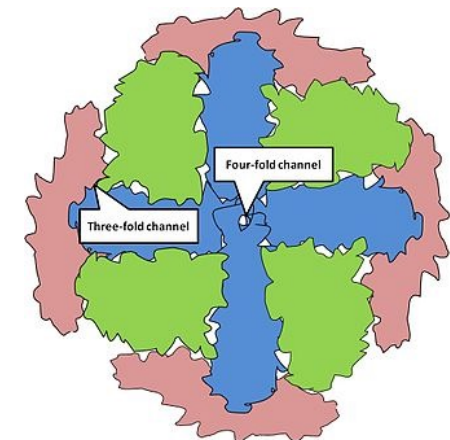
PDB:1AOI

Nucleosome Core Particle

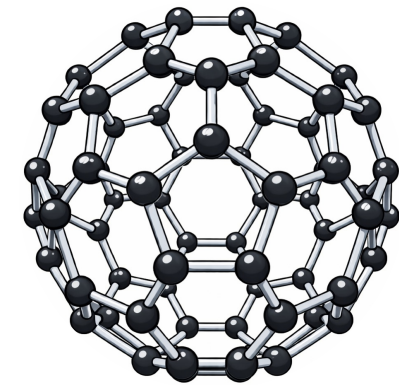
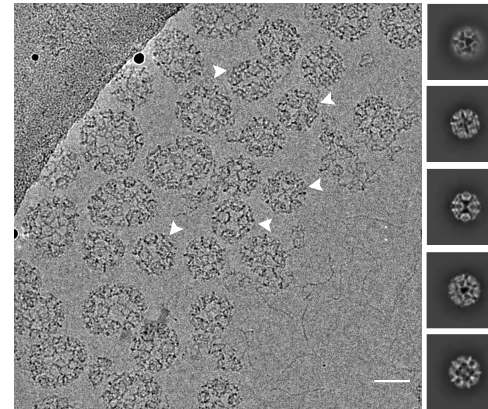
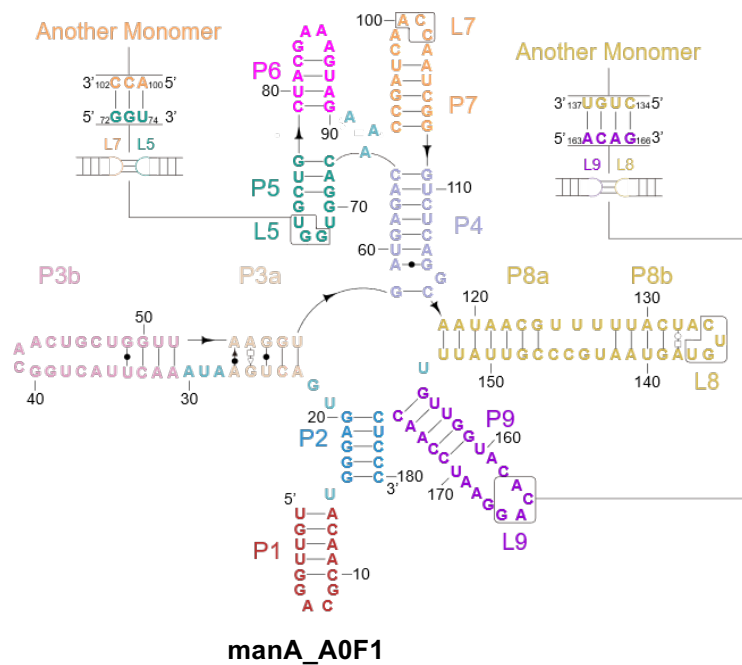


PDB:1FHA

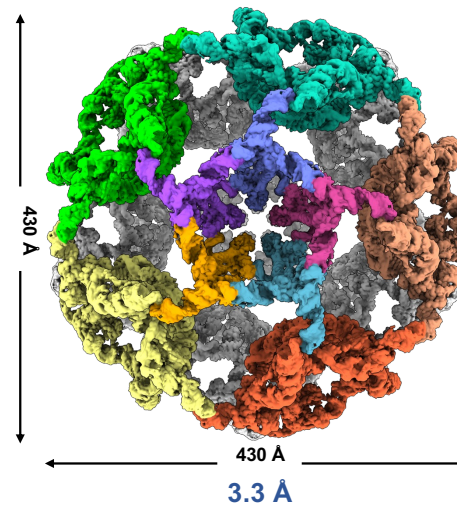
Ferritin



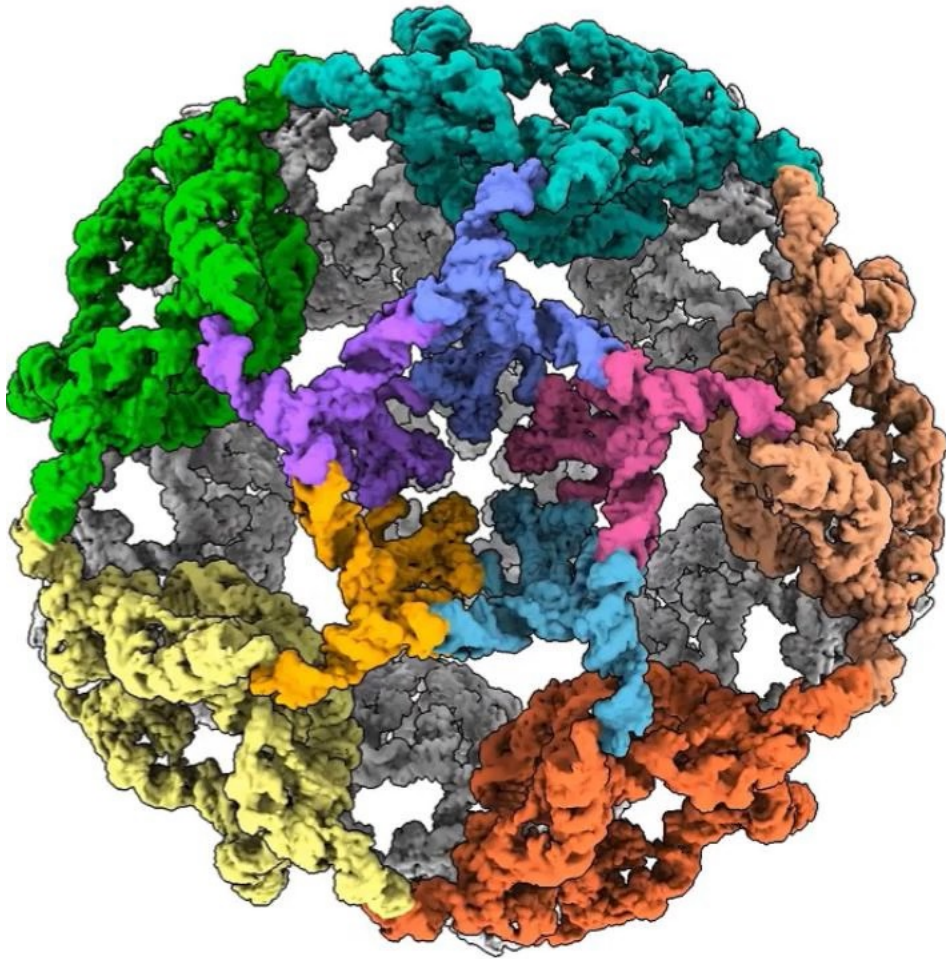
A fullerene-like RNA hexacontamer



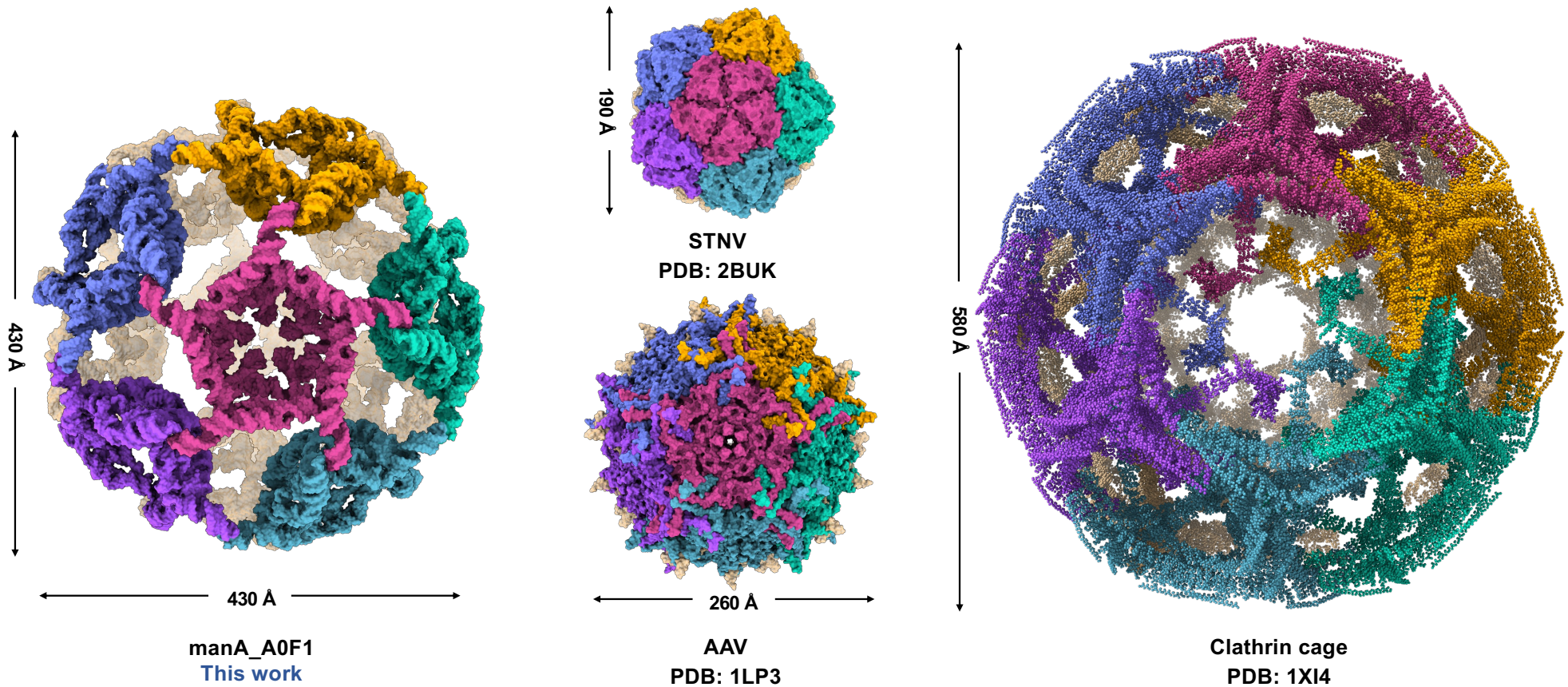
Fullerene



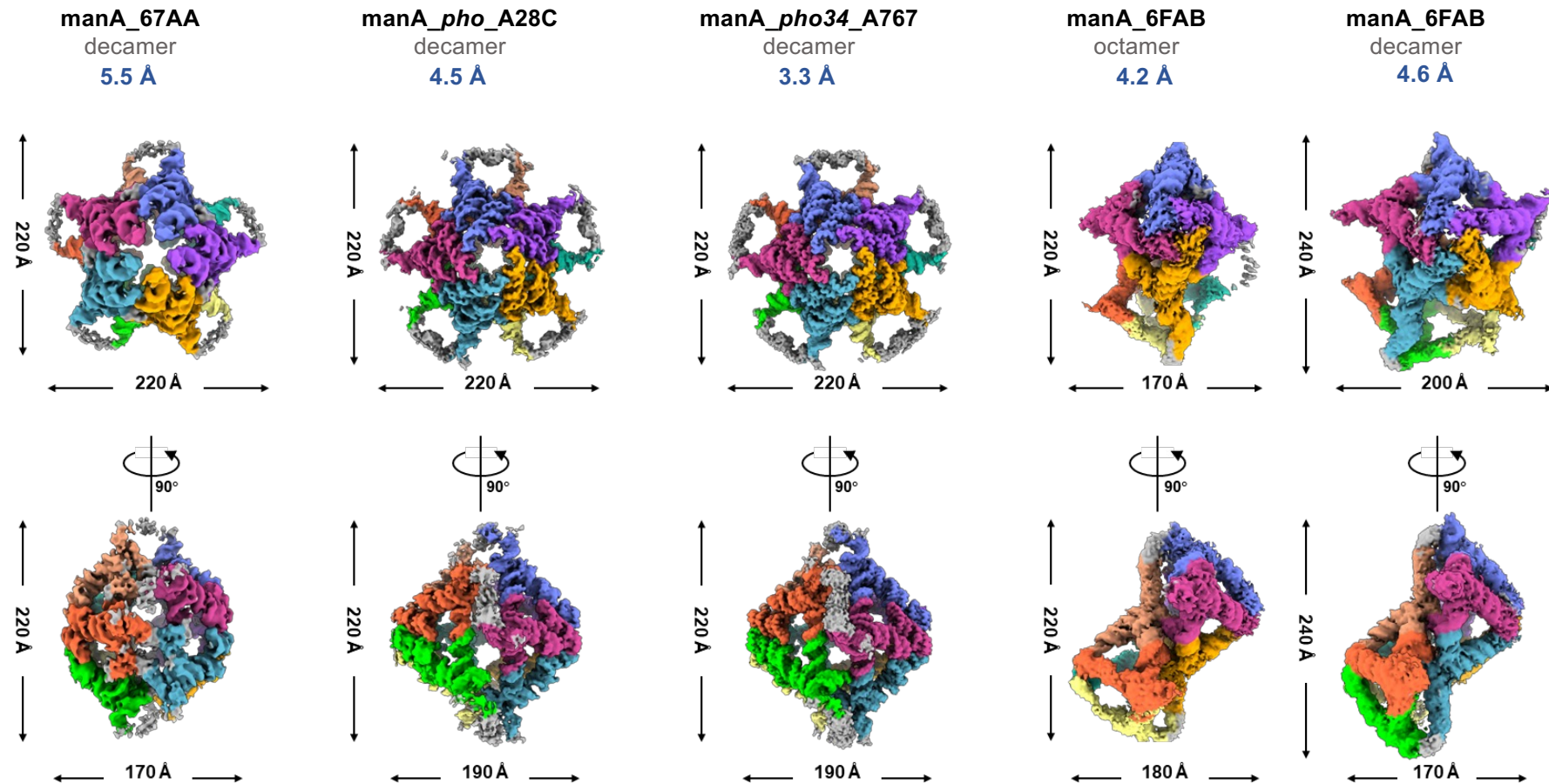
Structural assemblies for an RNA world



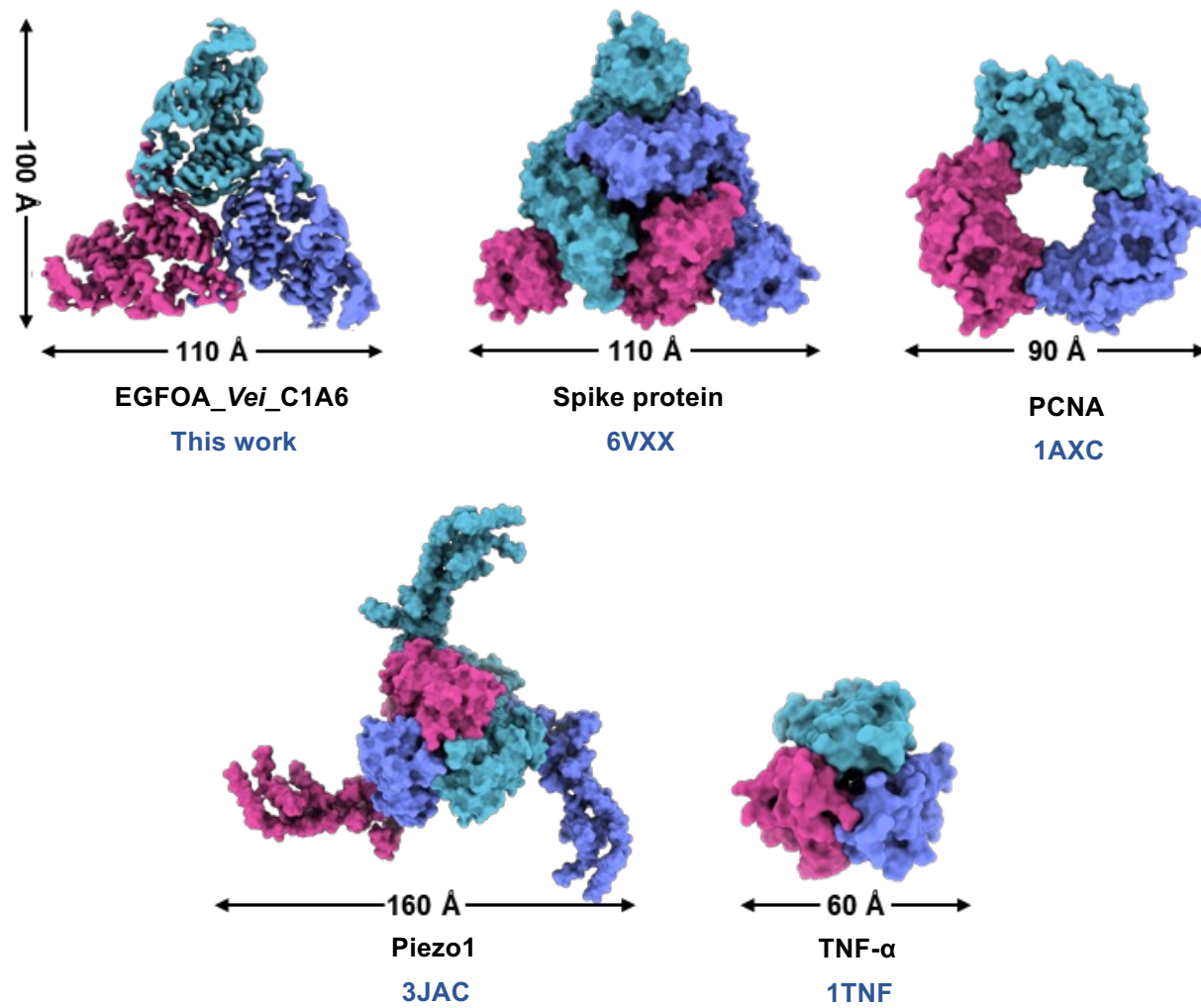
T=1 icosahedral geometry



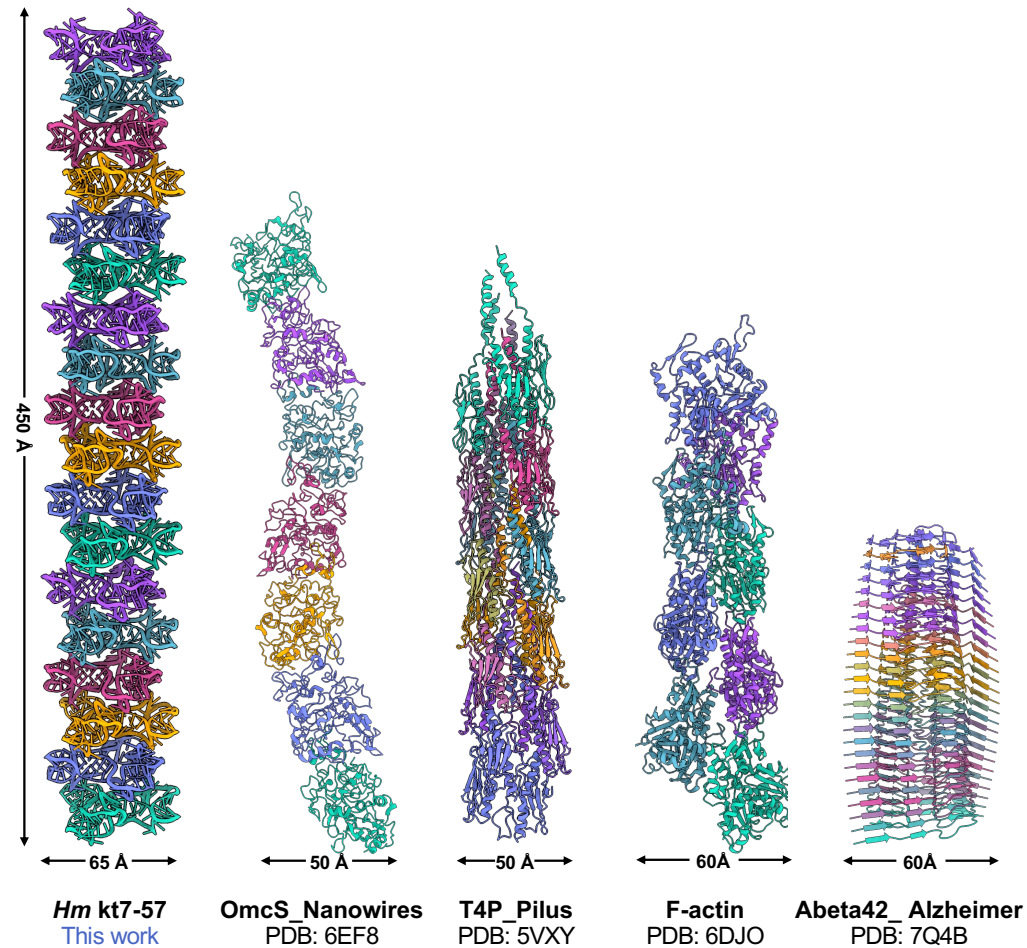
manAs smaller closed oligomers



C3 symmetric complexes

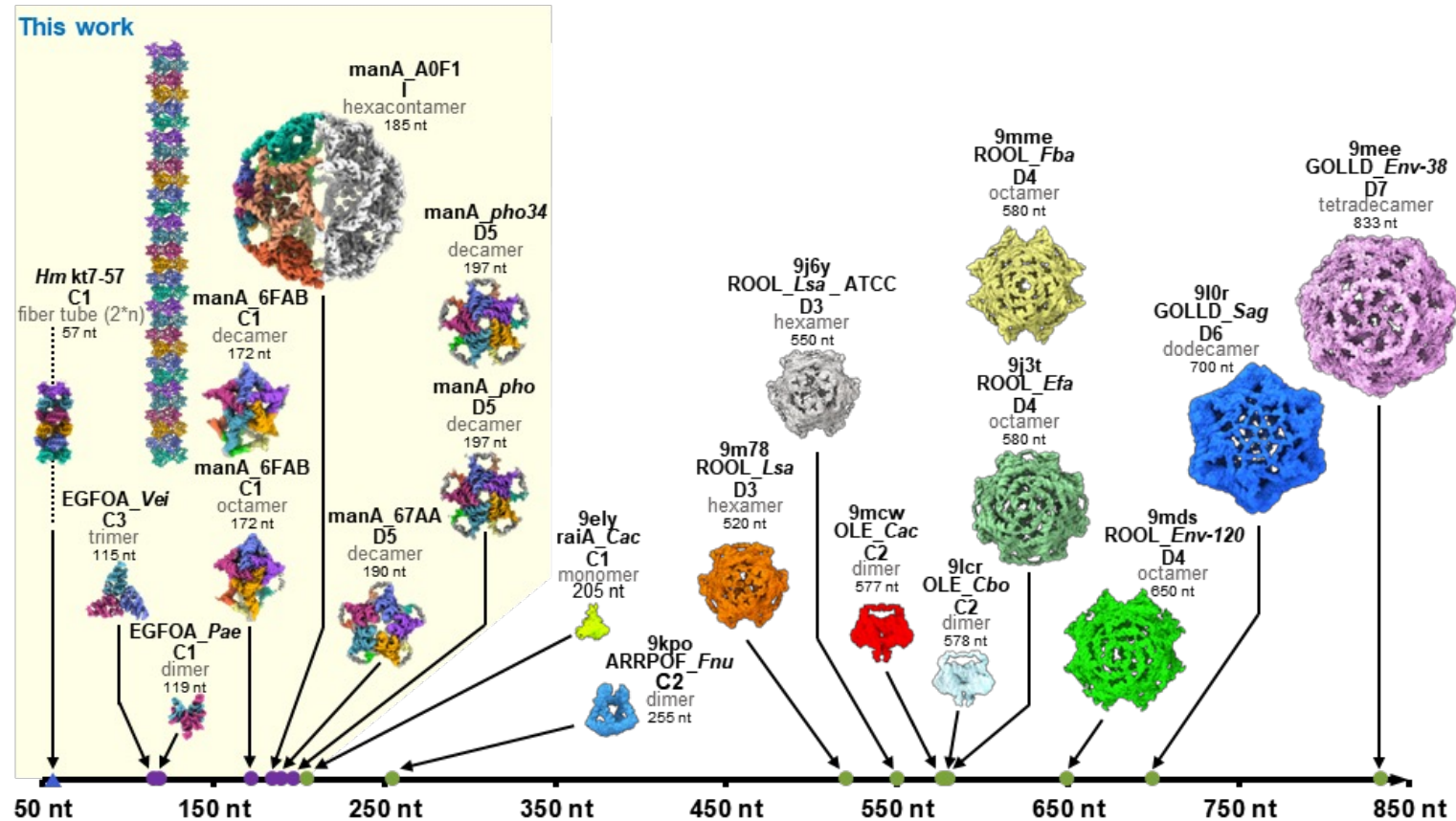


Hm kt7-57 with biological protein filaments

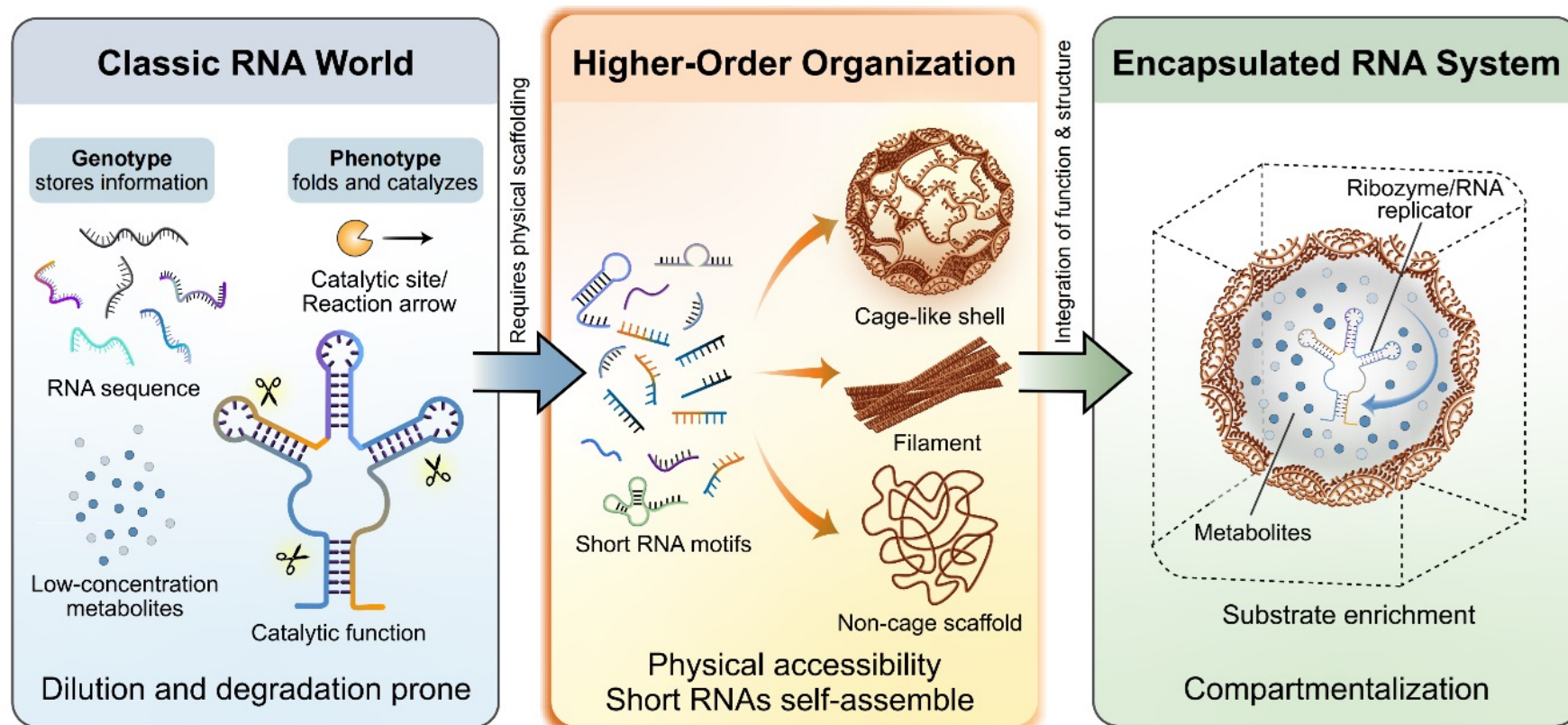


<https://www.biorxiv.org/content/10.64898/2026.07.01.735769v1>

RNAs access diverse architectural frameworks of higher-order assembly



RNA World





Collaborators



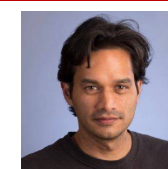
RNA Catalysis

David Lilley



Structure prediction

Eric Westhof



Structure prediction

Rhiju Das



RNA function

Yin ZHANG



Single molecular

HHMI
Taekjip Ha



Small molecule
virtual screening

Shi-Jie CHEN



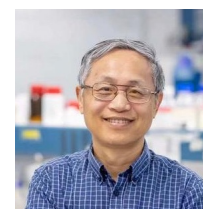
bacteria

Olga Soutourina



RNA chemistry

Liang XU



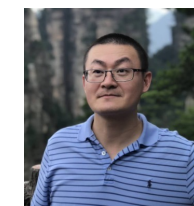
RNA function

Yaoqi ZHOU



Structure prediction

Zhichao MIAO



RNA function

Xiaorong ZHANG



Single molecular

Jie MA



"Extensively" determine novel RNA structures

3NO7

4C40 4BW0 4C4W 4CS1

5FJ1 5FK1 5FK2 5FK3 5FK4 5FK5 5FK6 5FKD

5FKF 5FKH 5FJC 5FKE 5FKG 5FJ4 5FJ0 5G4T

5G4V 5G4U 5NDI 5NEX 5NEO 5NEP 5NOM 5NDH

5NEQ 5NEF 5LQO 5LR4 5LQT 5LR3 5LR5 5O62

5NZ3 5NZ6 5NZD 5NWQ 5NY8 5O69

6FZ0 6HCT 6HBT 6HBX 6HC5 6QN3 6Q8V 6Q8U

6YLB 6YMK 6YMM 6YL5 6YMJ 6YMI 6YML 6TF1

6TF3 6TFE 6TFG 6TFF 6TFH 6TF2 6TF0 6TB7

7EAG 7EAF 7V9E

8HB8 8HBA 8HB3 8HB1 8I3Z 8ITS

Released 76 PDB

15 novel RNA folds

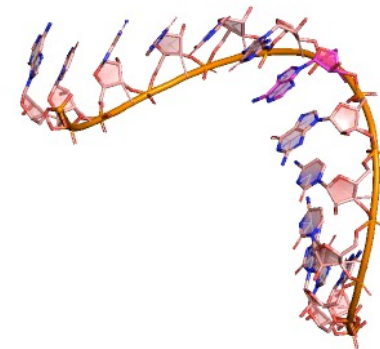
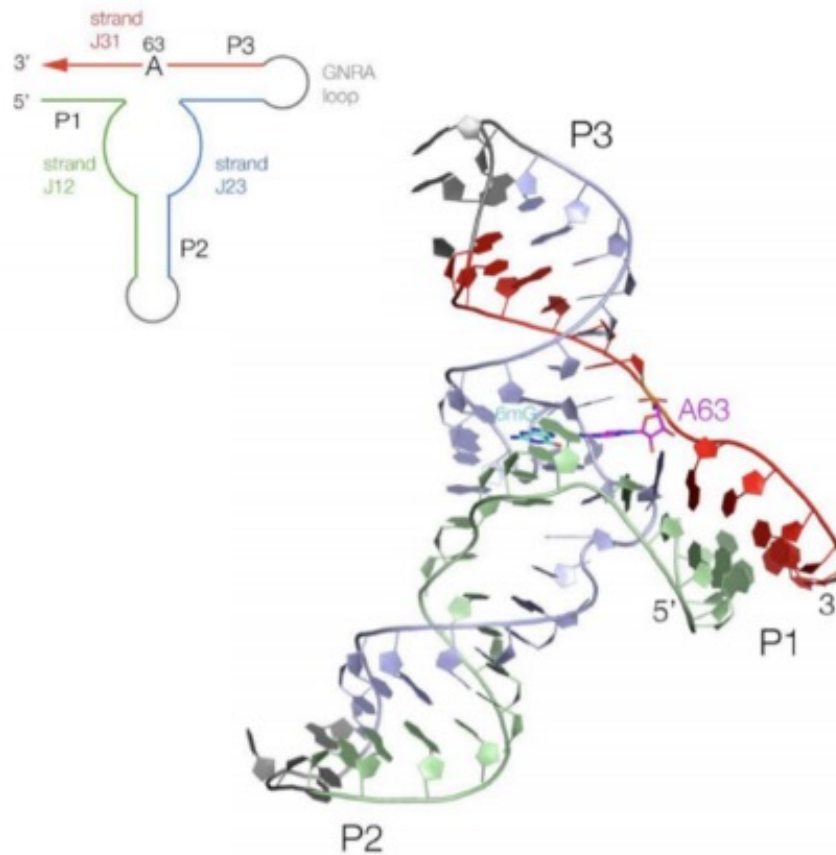
8Z9K 8Z8U 8Z8Q 8KEB 8ZA0 8ZA4 8ZAU 8K7W

9G4R 9G4Q 9G4P

Deposited 11 PDB

7 novel RNA folds

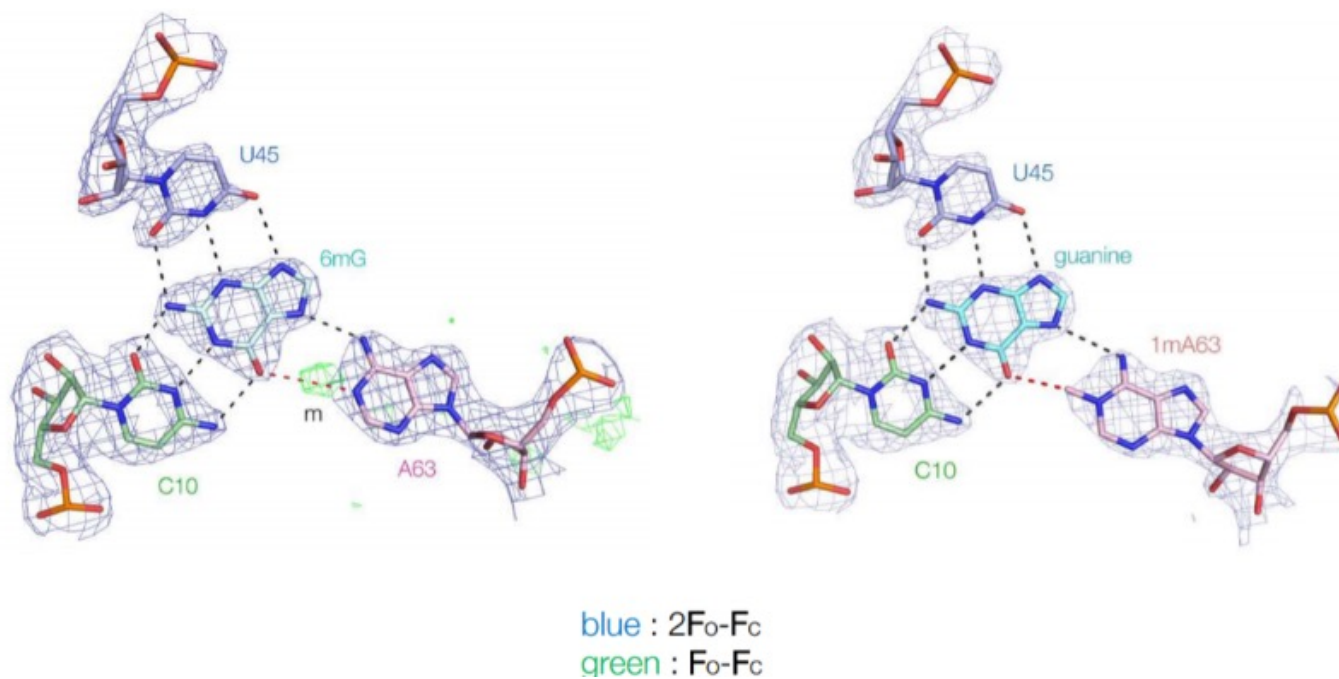
A crystal structure of MTR1 at 2.3 Å resolution



Deng...Lilley, Huang, *Nature Chemical Biology* 2022

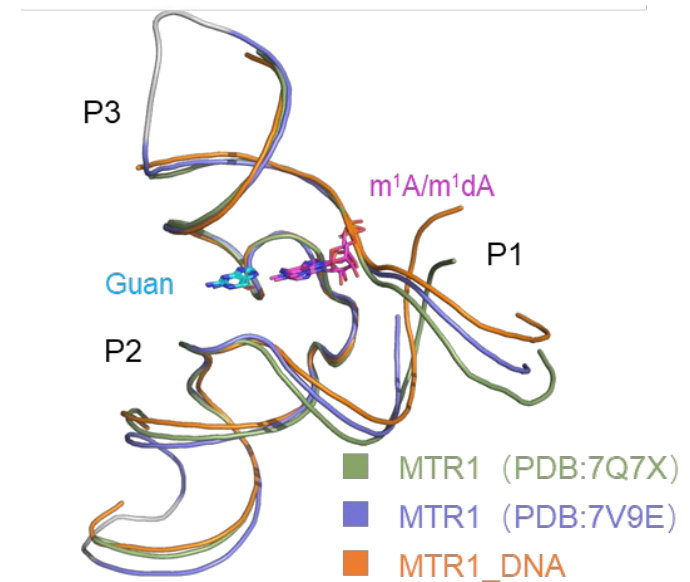
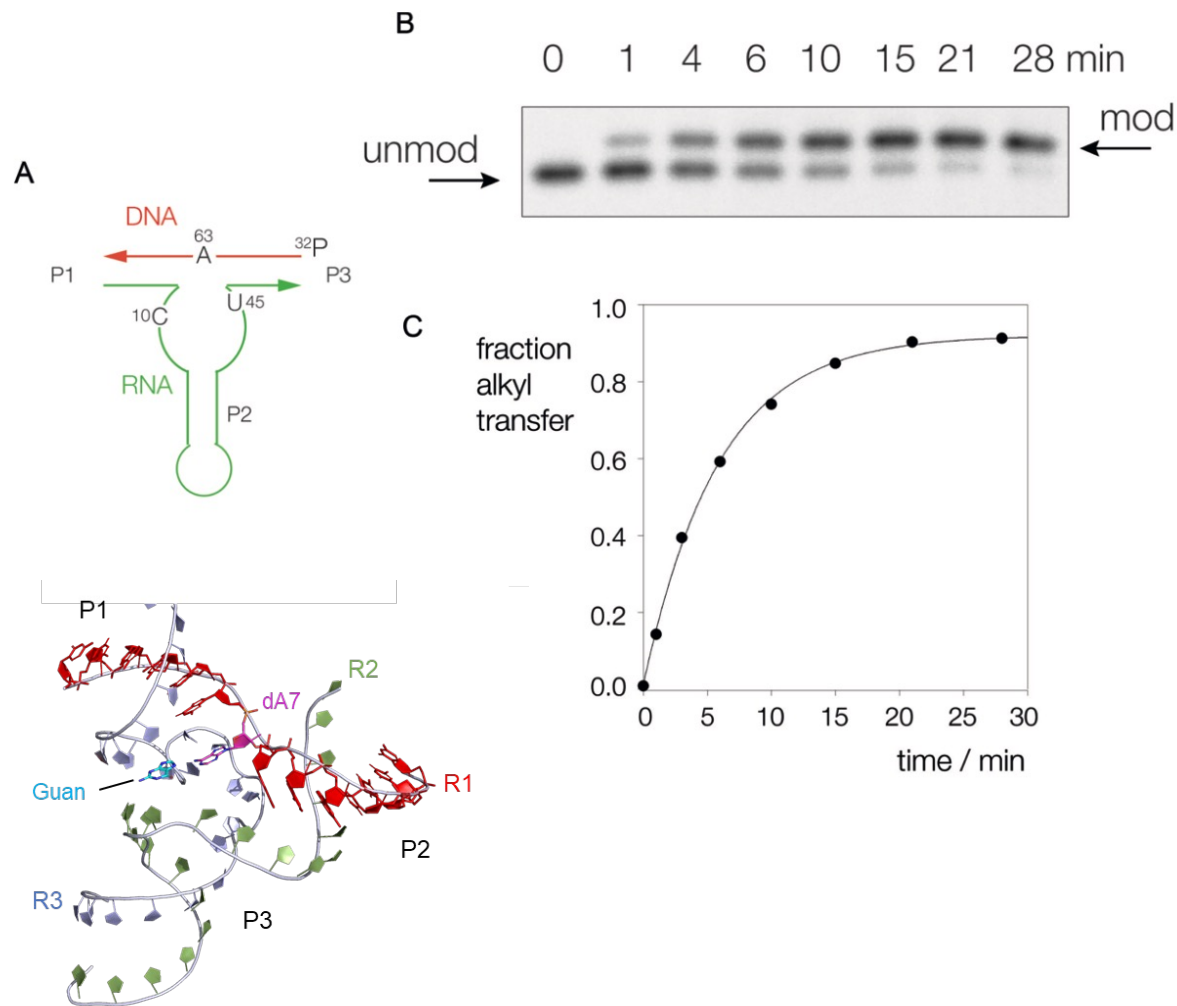
The methyl has transferred to adenine N1

2022



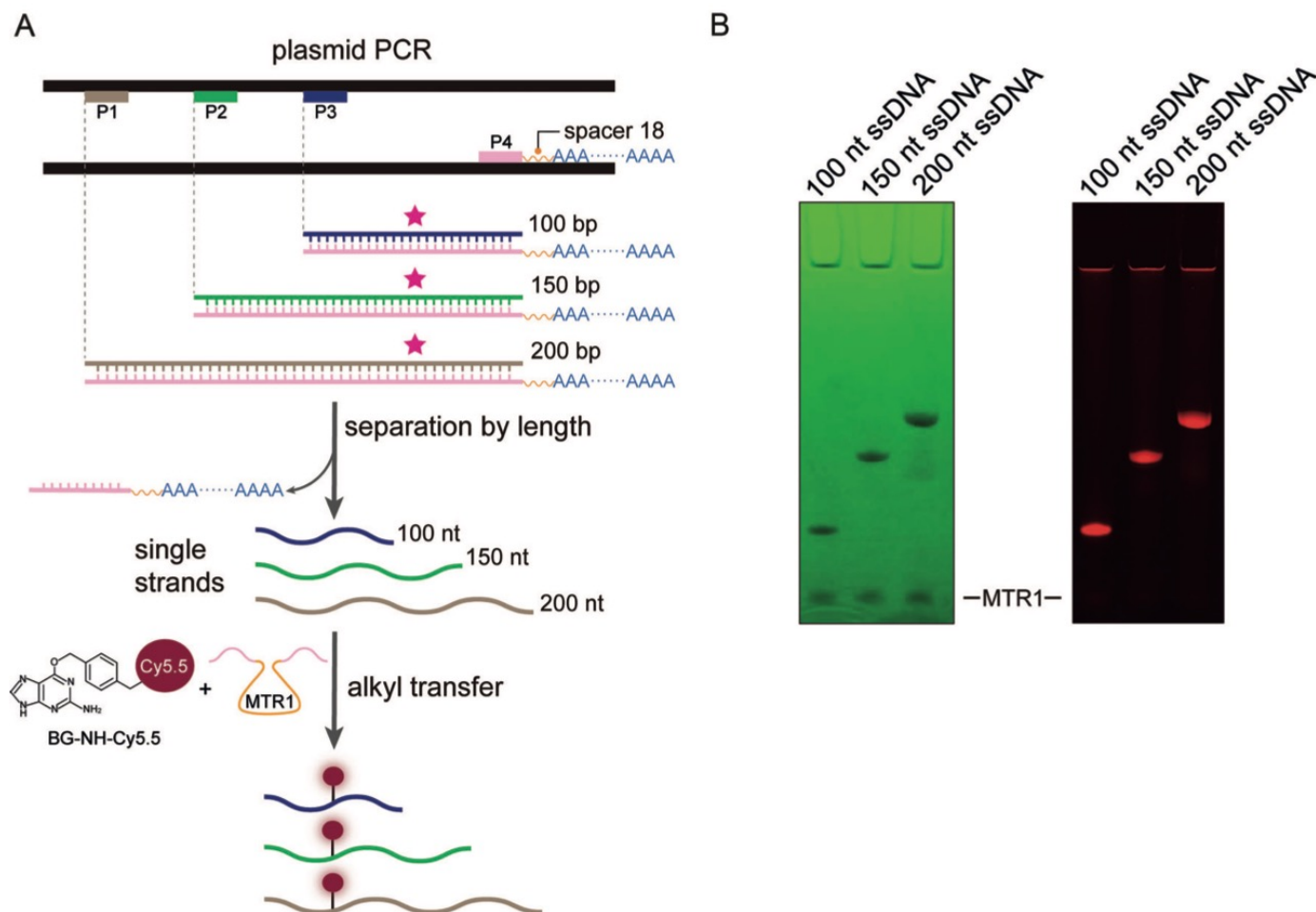
MTR1 application-- Site-specific modification For DNA

2026

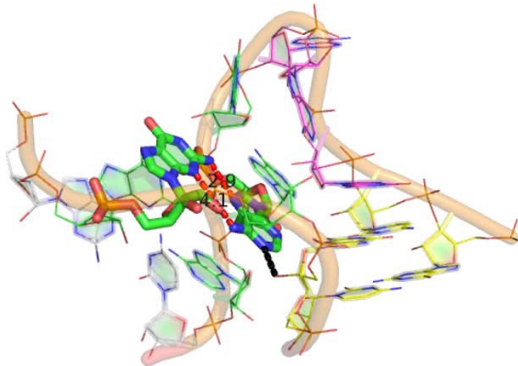
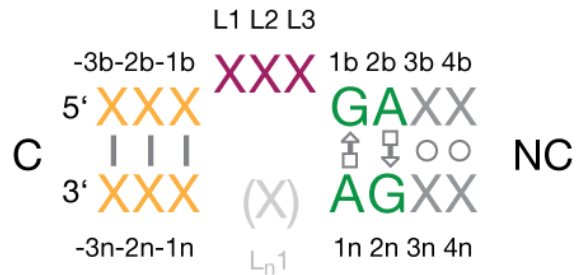


MTR1 application--Site-specific fluorescent modification of long ssDNA

2026



RNA structure modeling : k-turns



RNA 2013

Nucleic Acids Research 2014

Nucleic Acids Research 2016

Nanoscale 2016

EMBO reports 2017

Quarterly reviews of Biophysics 2018

RNA 2019

Nucleic Acids Research 2021

RNA 2023

Accurate prediction of k-turn folding, and precise 3D structure solely based on sequence

Summarize the existing determined DNA structures

Year	Ligand	Title	Journal	Method
1997	AMP	Structural basis of DNA folding and recognition in an AMP-DNA aptamer complex: distinct architectures but common recognition motifs for DNA and RNA aptamers complexed to AMP	Chem Biol	NMR
1999	Argininamide	Structure determination and binding kinetics of a DNA aptamer-argininamide complex	Biochemistry	NMR
2019	Ochratoxin A	Structure-guided post-SELEX optimization of an ochratoxin A aptamer	Nucleic Acids Res	NMR
2020	Pb ²⁺	Structure-guided development of Pb ²⁺ -binding DNA aptamers	Sci Rep	X-ray
2021	Ochratoxin A	Structural insights in to the Mechanism of High-Affinity Binding of Ochratoxin A by a DNA Aptamer.	JACS	NMR
2022	Lanthanide	Solution Structure of a Lanthanide-binding DNA Aptamer Determined Using Hiah Quality pseudocontact shift restraints	Chemistry	NMR
2023	aflatoxin B1	Structural basis for high-affinity recognition of aflatoxin B1 by a DNA aptamer	Nucleic Acids Res	NMR
2023	DFHBI-1T	Intricate 3D architecture of a DNA mimic of GFP	Nature	X-ray
2023	Cd ²⁺	Crystal structures and identification of novel Cd ²⁺ -specific DNA aptamer	Nucleic Acids Res	X-ray
2025	Theophylline	From Theophylline to Adenine or preQ1: Repumosing a DNA Aptamer Revealed by Crystal Structure Analysis	Angew Chem Int Ed Engl	X-ray

How to cite: *Angew. Chem. Int. Ed.* **2025**, e202504107
doi.org/10.1002/anie.202504107

Aptamer

From Theophylline to Adenine or preQ₁: Repurposing a DNA Aptamer Revealed by Crystal Structure Analysis

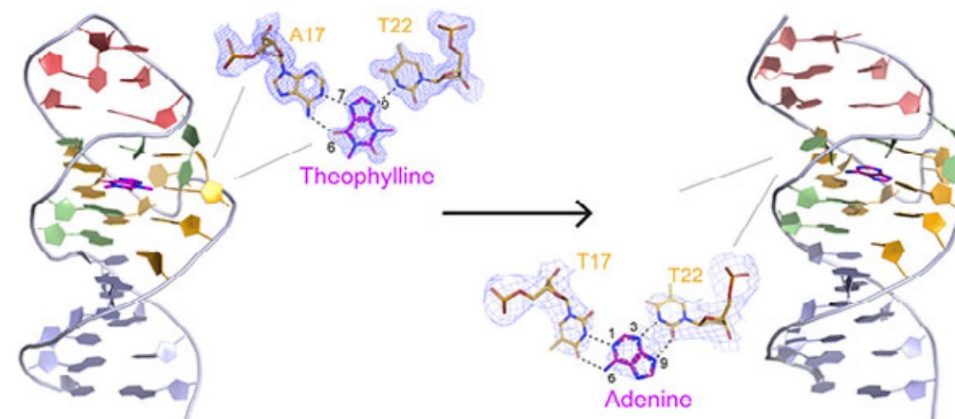
Xiaowei Lin⁺, Yuanyin Huang⁺, Jinchao Huang⁺, Hao Yuan, Yuhang Luo, Zhizhong Lu, Ying Ao, Jian Huang,* Shuo-Bin Chen,* Zhichao Miao,* and Lin Huang*

Abstract: Aptamers, which are short, single-stranded DNA or RNA, are capable of binding to a wide array of targets with exceptional selectivity. Those with high affinity for theophylline have the potential to serve as biosensors, crucial for tracking theophylline levels in the treatment of respiratory conditions. Despite the extensive structural characterization of the RNA theophylline aptamer, the DNA counterpart's ligand-recognition mechanism has remained unclear. Here, we elucidate the DNA theophylline aptamer's ligand-binding mechanism through high-resolution crystal structures of its complexes with theophylline, 3-methylxanthine, and hypoxanthine. Guided by these structural insights, we computationally redesigned the theophylline-binding pocket via rational mutagenesis of key nucleotides, generating novel aptamers selective for adenine and prequeosine (preQ₁) ligands. These engineered aptamers were validated through biochemical and crystallographic analyses. By integrating structural biology with computational design, our work provides a relatively simple and effective method for developing new aptamers. While this strategy does not supplant SELEX, it serves as a beneficial complement to it.

Introduction

Over 130 years ago, the German biologist Albrecht Kossel first extracted theophylline from tea leaves.^[1] Theophylline has since been used as a bronchodilator to treat

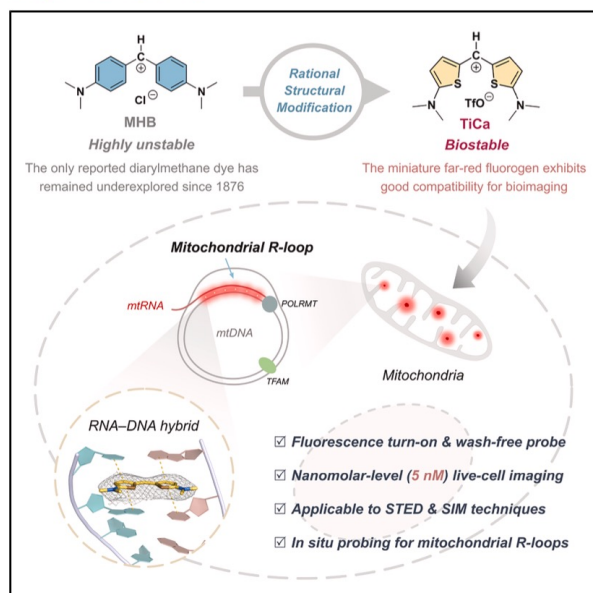
airway diseases, including chronic obstructive pulmonary disease, asthma, and infant apnea, as it relaxes bronchial muscles, reduces airway responsiveness, and exhibits anti-inflammatory properties.^[2–4] However, its narrow therapeutic index necessitates careful concentration monitoring, as elevated levels can lead to side effects such as nausea and arrhythmias.^[5,6] Theophylline-specific aptamers, short single-stranded DNA or RNA sequences, can target theophylline with high specificity, thus serving as biosensors.^[7–9] Aptamers have been increasingly used in biosensing, environmental monitoring, and clinical applications because of their easy synthesis, cost-effectiveness, and low immunogenicity.^[10–13]



[*] X. Lin⁺, Y. Huang⁺, Y. Luo, Z. Lu, J. Huang, L. Huang
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Epigenetics and Gene Regulation, Guangdong-Hong Kong Joint
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Unlocking the potential of diarylmethane dyes for living systems

Graphical abstract



Highlights

- Rational structure engineering yields the biostable diarylmethane dye TiCa
- TiCa enables ultrasensitive and high-resolution imaging of mitochondrial R-loops
- TiCa detects mitochondrial R-loops via selective recognition of RNA-DNA hybrids
- Co-crystal structure defines the binding mode of TiCa in RNA-DNA hybrid helices

Authors

Jian Wang, Yang-Yi Ren,
Ye-Ying Huang, ..., Shuo-Bin Chen,
Lin Huang, Jia-Heng Tan

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In brief

Many historically known dye scaffolds remain underexplored due to intrinsic chemical limitations. Through rational structural modification of diarylmethane dyes, this study uncovers their hidden bioanalytical potential and yields TiCa, a miniature far-red fluorogen. TiCa selectively recognizes RNA-DNA hybrids, enables live-cell imaging of mitochondrial R-loops, and its co-crystal structure with a natural RNA-DNA hybrid elucidates the molecular basis of this recognition, highlighting how revisiting long-overlooked dye frameworks can advance future probe development.

Chem Article

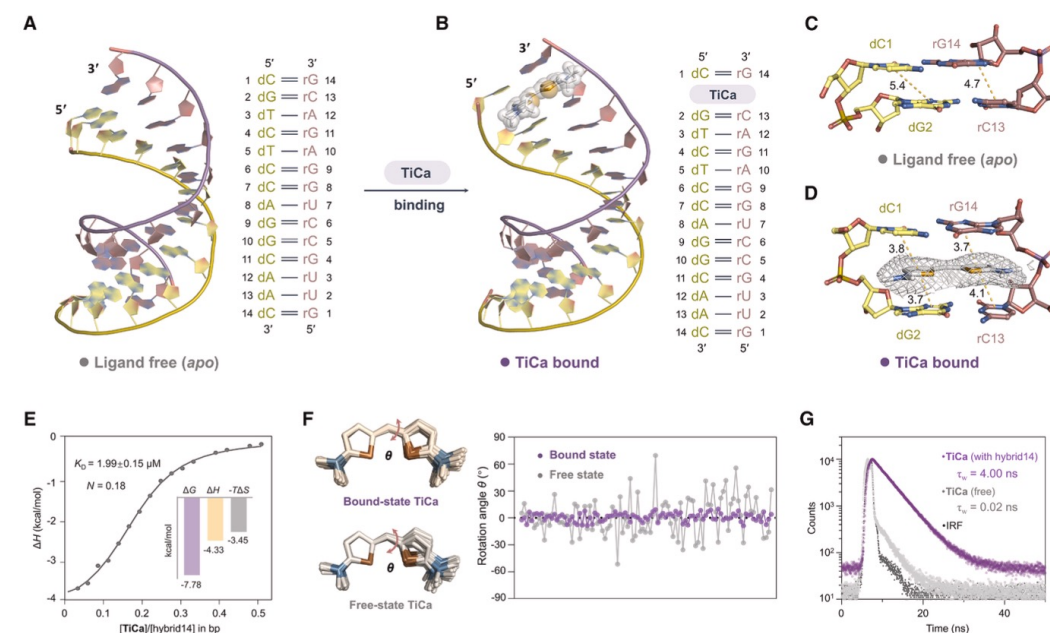
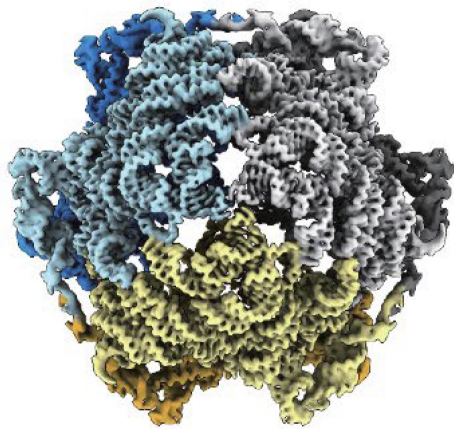
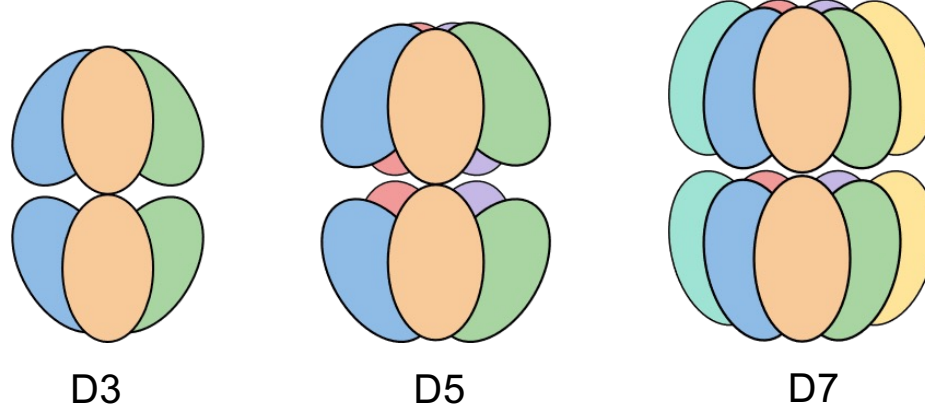


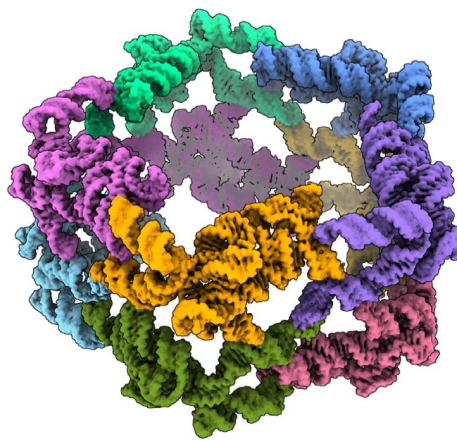
Figure 6. The binding mode and photoluminescence mechanism of TiCa

Assembly of RNA Structures

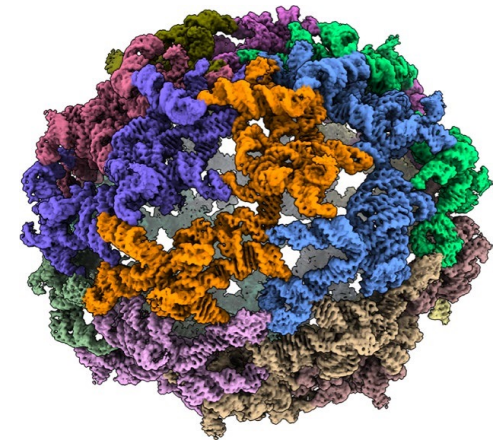
Currently Known Higher-Order Architectures
are Limited to Dihedral Symmetry



D3: *Lsa* ROOL hexamer



D5: *Sag* Golld decamer



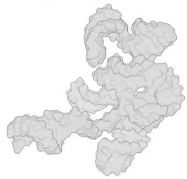
D7: *Env38* Golld tetradecamer

A small polymerase ribozyme

De novo evolution of a small polymerase ribozyme

State of the art

Class I polymerase ribozymes (>150 nt)



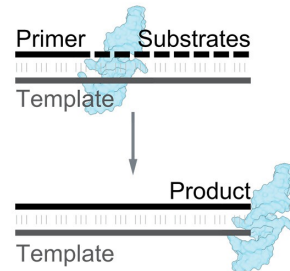
This work

Random RNA pools
QT45 polymerase ribozyme (45 nt)

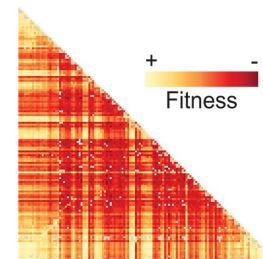


Complex RNA-polymerase activity in a small RNA motif

Polymerase activity

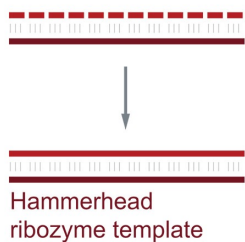


Mutational landscape

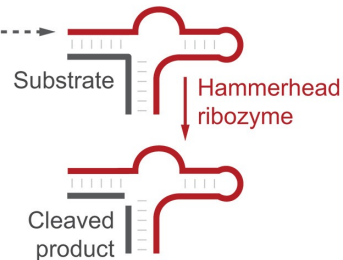


Synthesis of an active ribozyme

Hammerhead synthesis

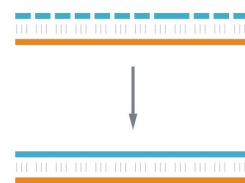


Activity

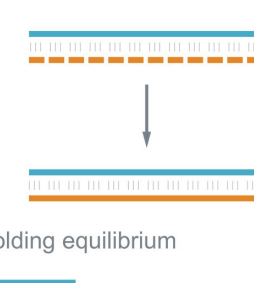


Synthesis of itself and its template

QT45 self synthesis



QT45 template synthesis



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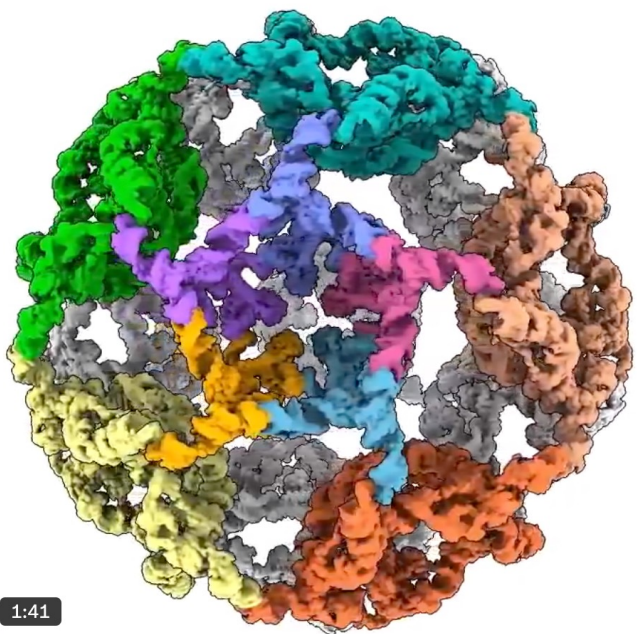
Lin Huang 黄林
@LinHuang2008

Excited to share our new preprint: Structural assemblies for an RNA world.

We show that short RNAs can self-assemble into protein-like architectures, including closed cages, finite oligomers, and filaments.

[biorxiv.org/content/10.648...](https://doi.org/10.648/biorxiv.content/10.648...)

manA_A0F1 forms a 60-mer icosahedral-like cage



8:15 AM · Jul 2, 2026 · 13.3K Views

10

61

221

59



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Lin Huang

Principal Investigator at Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University
Dundee, Scotland

Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University

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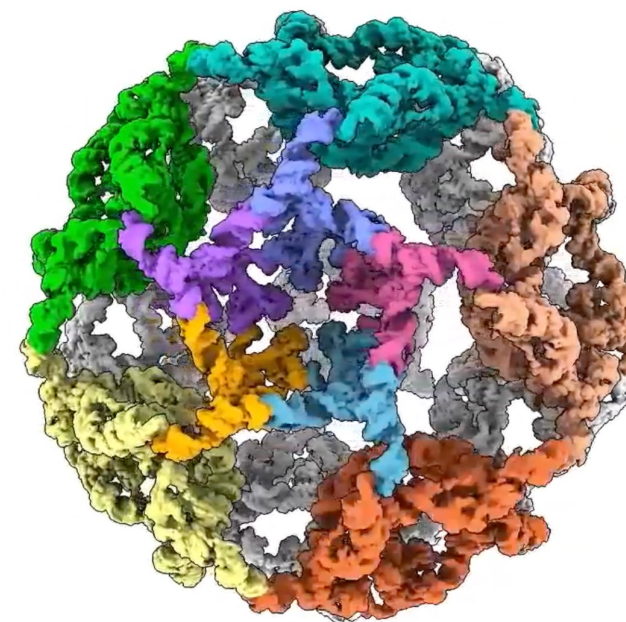
Alexey Amunts · 1st

PI at SMART and University of Münster | ERC grantee
4d ·

A short RNA self-assembles into a 60-subunit icosahedral cage like a viral capsid, but built entirely from RNA. RNA is not only a carrier of information or a catalyst, now we know that it can also build. The striking preprint also reveals a 57-nt RNA filament resolved at ~2.7 Å. Congratulations [Lin Huang](#) and the team!

<https://lnkd.in/ddrMBuKD>

manA_A0F1 forms a 60-mer icosahedral-like cage



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