RNA NanoBiotics

RNA Nanotechnology for Targeted Delivery of RNAi, Radioisotopes, and Chemotherapeutics for Oncology

October | 2024

ABOUT RNA NANOBIOTICS

RNA NanoBiotics

Focused on the Development of Nanotechnology platforms and drugs for the Targeted Delivery of RNAi, Radioisotopes, and Chemotherapeutics for cancer therapy

- Deep understanding of how to deliver small RNA and Chemo Therapeutics Into cancer cells in an effective and safe manner
- Our several preclinical drug candidates are designed to co-deliver tumor suppressor miRNAs antagonists of oncogenic miRNAs &siRNAs to stop Its cancer proliferation and migration as well as Chemo-therapeutics and potentially Radiation Therapy

Our Vision

To improve cancer patient outcomes through innovative Nanotechnology mediated Targeted Drug Delivery

THE CURRENT DIFFICULTIES IN CANCER TREATMENT

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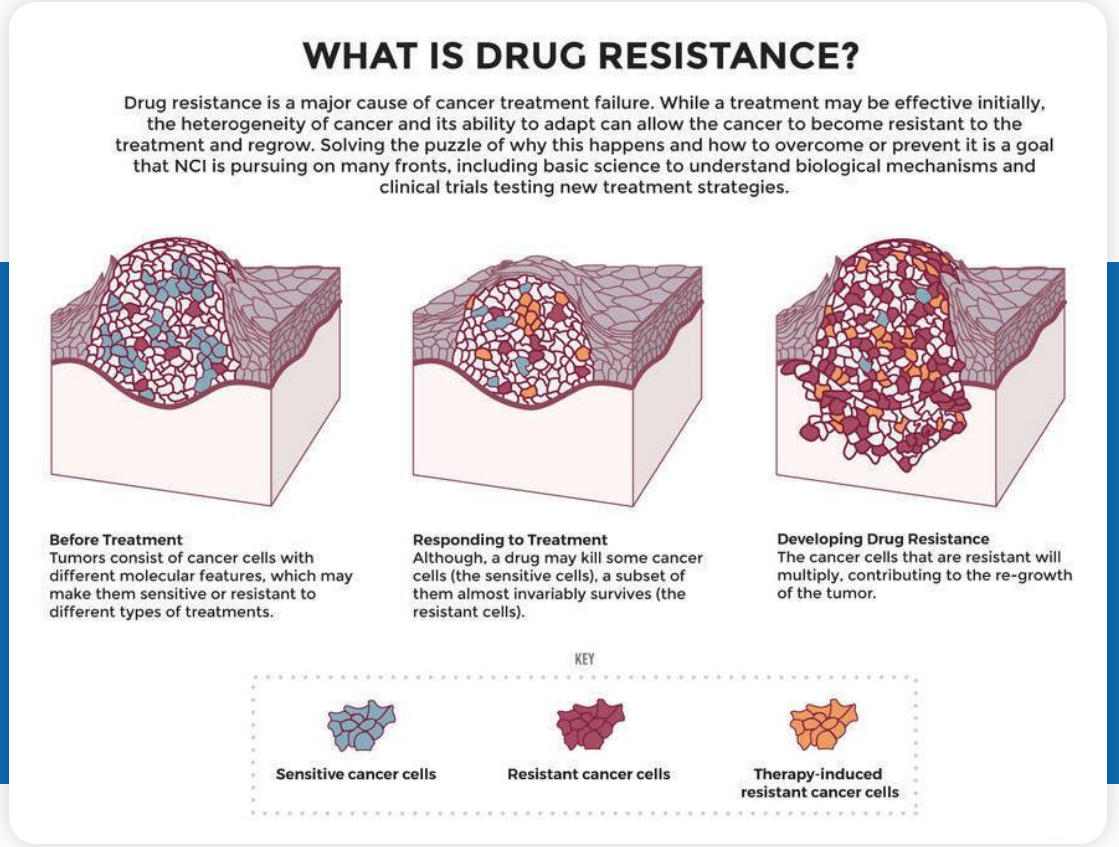
Cancer is clearly the deadliest disease in the developed world as one in three people develop cancer during their lifetime

The cure for cancer is like the Holy Grail since most of the existing treatments are not effective enough to provide full protection from the disease

- Cancer arises from our own cells, so each cancer can be as different and diverse as people are
- Cancer cells within the same tumor are not identical so some cells can survive treatments
- Cancer cells may adapt to the drug while it is being administered thus allowing them to escape its effect.
- Chemotherapeutic agents are successful at killing cancer cells but can have significant toxic side effects on various organ systems



Targeted RNA Nanoparticles Offers A Solution

Therapy directed only at the cancer cells avoiding damaging other cells or organs

Multiple therapies can be delivered to the cancer cells at once thus overcoming resistance:

- Multiple chemotherapeutic can be delivered at a higher dose per cancer cell via one RNA Nanoparticle
- RNAi drug that inhibits tumor growth via different mechanism (critical in KRAS) are engineered into RNA Nanoparticles sequence
- Targeted Radiation Therapy to kill via high energy Alpha Particles

FOUNDER AND THE INVENTOR

RNA NanoBiotics

James Carroll

President and CEO

25+ years in Executive Management, strategy, corporate and business development, and investments

- ExonanoRNA, COO
- Remedium Bio, Edulis, Bionostics, BioRad, Waters, Millipore
- Wharton Alumni Angels, Managing Director, Life Sciences
- Wharton, MIT/Sloan, Harvard, Northeastern

Peixuan Guo, PhD

Inventor

Professor, Sylvan G. Frank Endowed Chair

Pharmaceutics and Pharmacology, Ohio State University

- 2021 Innovator Of The Year Ohio State University
- Fellow of the National Academy of Inventors (NAI)
- Director of Center for RNA Nanobiotechnology and Nanomedicine
- President of International Society of RNA Nanotechnology and Nanomedicine
- International Society of RNA Nanotechnology and Nanomedicine
- Fellow of the National Academy of Inventors (NAI), 2022

RNA NANOBIOTICS' TECHNOLOGY IS LIKE "LEGO" BUILDING BLOCKS ....IDEAL MATERIAL FOR NANO TARGETED DELIVERY SYSTEMS

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A great delivery platform should have the following properties:

01 Have variety

02 Build structures with controllable shape, size, and stoichiometry

03 Automatically assemble when mixed together

04 Are thermodynamically, chemically, and enzymatically stable with a long shelf-life

05 Can be Manufactured at a Clinical Scale*

RNA Nanobiotics Technologies exhibits all of the above ideal properties

*Recent Discussions with GensScript and other CMO's for Clinical Material Production Of Nanoparticles and No major technical obstacles seen as Oligos can be synthesized and assembled. Determination on site of final derivatization work case by case

RNA NANOPARTICLES POTENTIAL
CONFIGURATIONS

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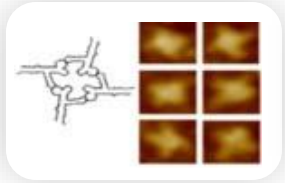
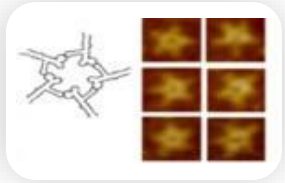
1. Trimer

2. Tetramer

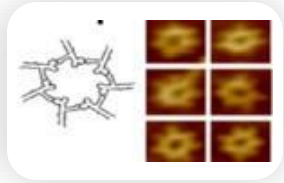


3. Pentamer

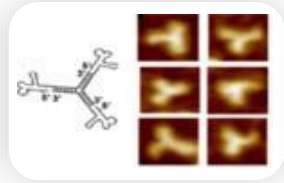


4. Hexamer

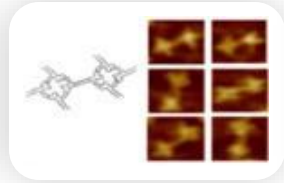
5. Heptamer



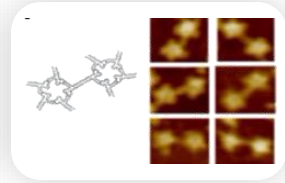
6. 3WJ- pRNA



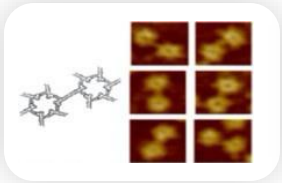
7. Foot-to-foot Tetramer



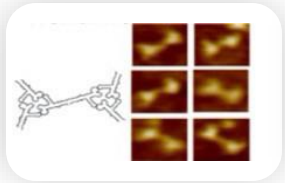
8. Foot-to-foot Pentamer



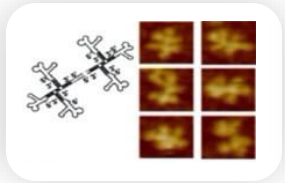
9. Foot- to - foot Hexamer



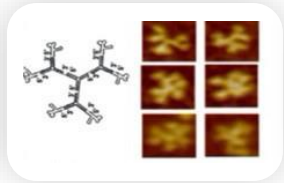
10. Foot- to-foot Trimer



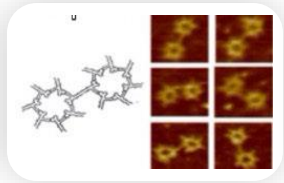
11. Foot- to-foot Tetramer



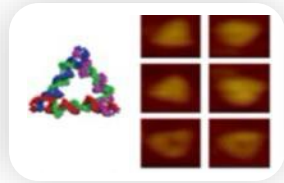
12. Arm - to - arm Dendrimer



13. Foot- to-foot Pentamer



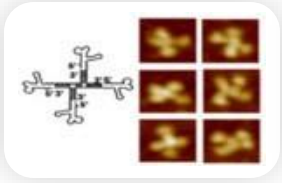
14. Triangle



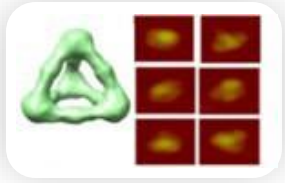
15. Square

16. Pentagon

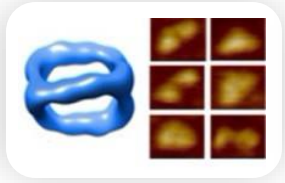
17. X- pRNA



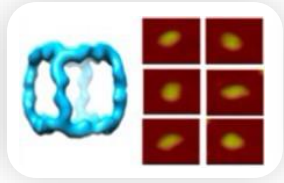
18. Tetrahedron



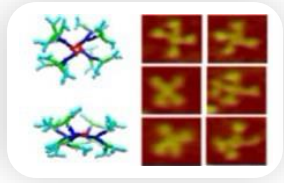
19. Prims



20. Cube



21. Dendrimer



22. Micelle

Shu Y, et al, RNA, 2013; Khisamutdinov E, et al, Nucleic Acid Res, 2.14,42:9996-100004; Xu C. et al, Nano Research, 2019: 12:41; Yin H, et al, ACS Nano; 13(1): 706-717

FOUR RNA NANO PARTICLE PLATFORMS OF RNA NANOBIOTICS

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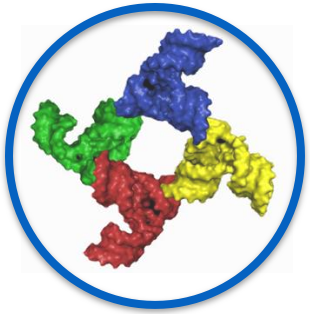
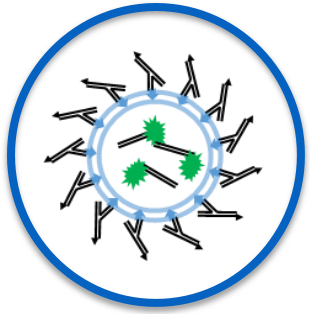


Platform 1

RNA Nanoparticles Platform

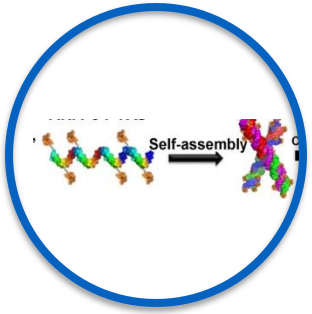
- Enhance its cancer cell targeted delivery efficiency to > 5% , vs traditional nanotechnology <0.7%
- Extraordinary PK profiles and low accumulation to VITAL ORGANS
- Increased solubility of chemical drugs, e.g., Increased Paclitaxal solubility by 32,000 folds



Platform 2

RNA- ligand Displaying Exosomes Platfom

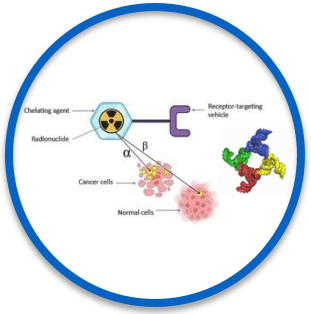
- Display RNA on exosome to make it negatively charged, minimize nonspecific binding
- Display aptamer or chemical ligand onto exosomes for efficient targeting
- Delivers siRNA to cell's cytoplasm to escape from endosome trapping



Platform 3

RNA/Drug Complex

- Conjugate chemical drugs to RNA nanoparticles to enhance the solubility and reduce the toxicity, include RNAi drug in complex for enhanced cytotoxicity to cancer cells



Platform 4

RNA/Drug/ RadioIsotope Complex

- Conjugate chemical drugs to RNA nanoparticles to enhance the solubility and reduce the toxicity, include RNAi drug and an alpha emiting radioisotope in the complex for complete cytotoxicity to cancer cells

RNA NANOPARTICLES DECORATED EXOSOMES ARE USED TO TREAT PROSTATE CANCER AND LUNG CANCER

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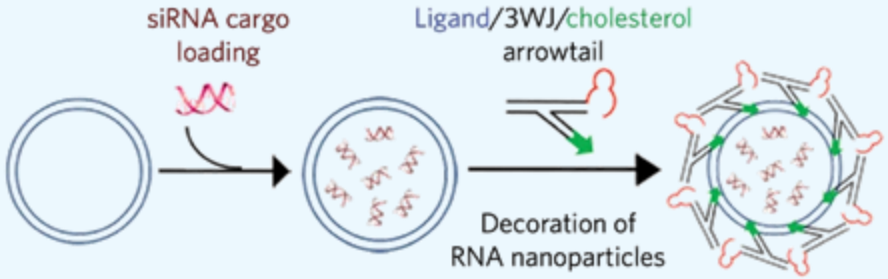
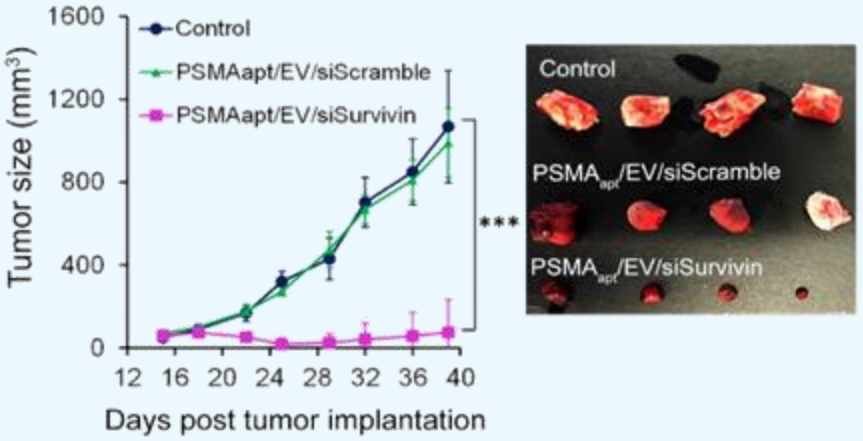
PSMA/Evs Target Prostate Cancer

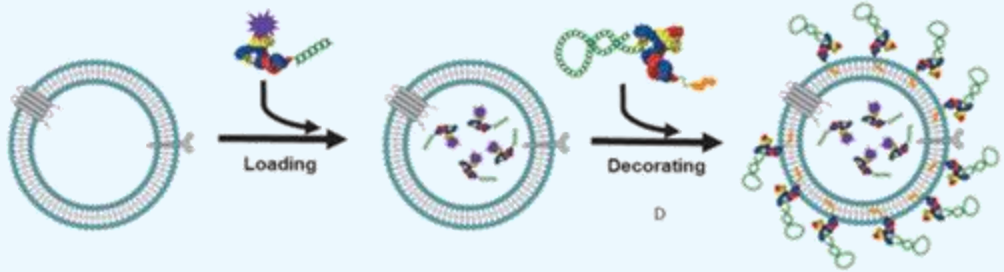


Prostate Cancer Trial

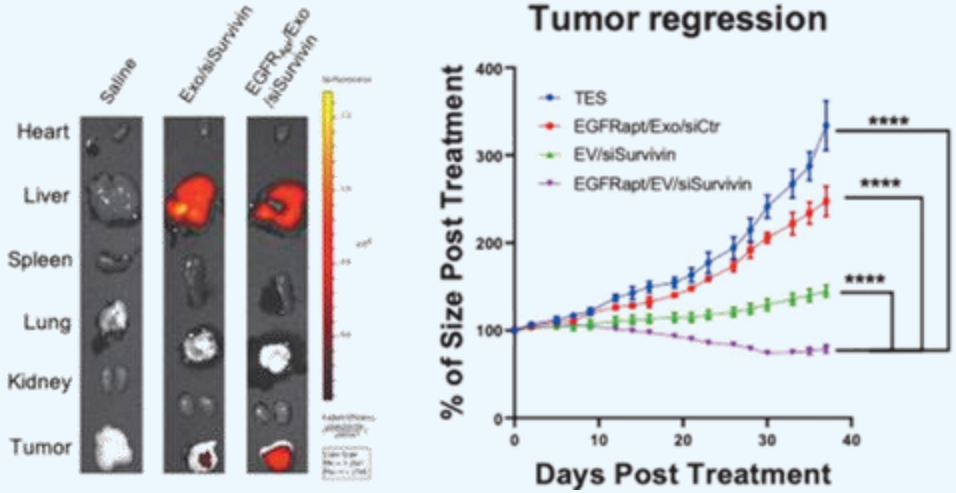


Pi F, et. al. Guo P. Nature Nanotech. 2018.
Li Z, et .al. & Guo P. Nucleic Acid Therapeutics. 2021.

EGFR/EVs for Lung Cancer



Lung Cancer Trial



4WJ RNPs-miRNA FOR CANCER TREATMENT IN MICE MODELS...The miRNA is Engineered Into RNA Nanoparticle

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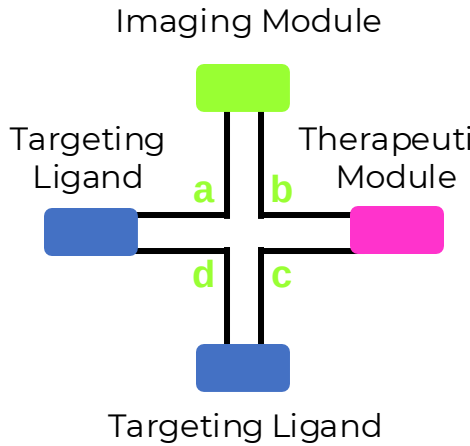
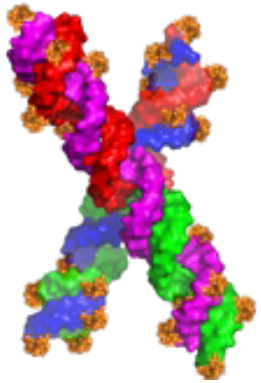
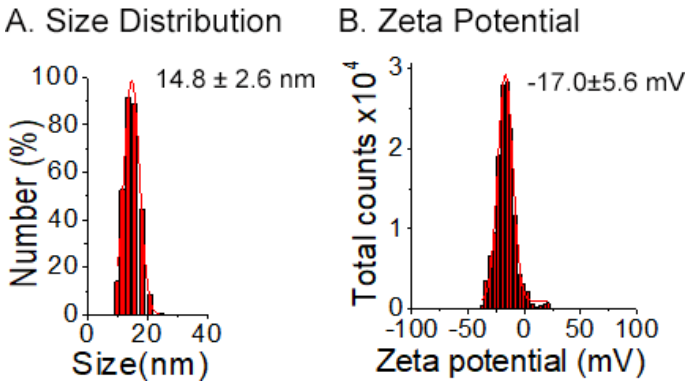
Thermodynamically stable 4WJ Scaffold



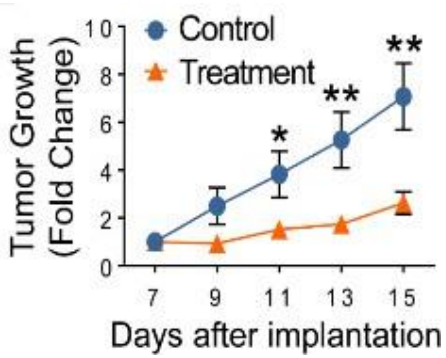
The Thermodynamically Stable 4WJ Is The Core Structure, With a Tm Higher Than 80°C

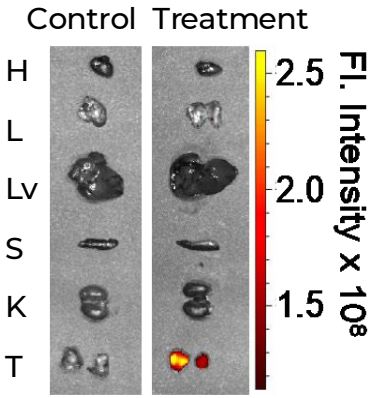
RNA NPs





Breast Cancer





ACS Nano. 2015 , 24 ;11 (1):335 - 346

4WJ LEAD CANDIDATE RNA NANOPARTICLES

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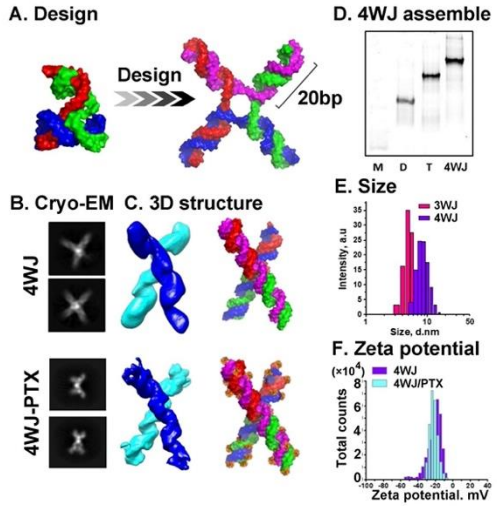


Preliminary data showing the construction of 4WJ lead candidate RNA nanoparticles.

A. The pRNA-3WJ was stretched and extended into a 4WJ.

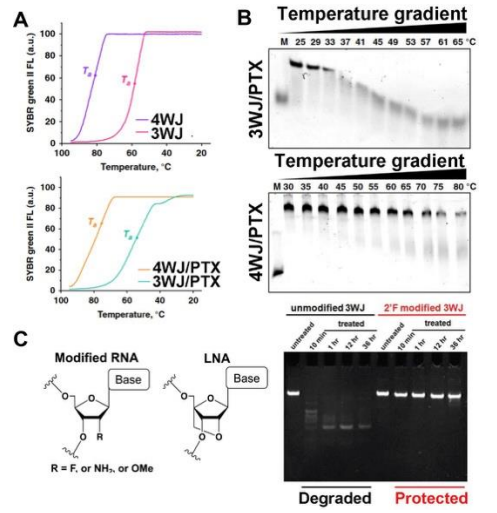
B. Cryo-EM images of 4WJ and 4WJ-24 paclitaxel (PTX).

C. 3D reconstructed Cryo-EM maps of 4WJ and 4WJ-24 paclitaxel compared to predicted structures (right).

D. Assembly stepwise gel of 4WJ.

E. DLS data characterizing size of 3WJ to 4.9 nm and 4WJ to 8.7 nm.

F. Zeta potential measured of the developed 4WJ with and without paclitaxel conjugation.



Preliminary data showing thermo and enzymatic stability of RNA nanoparticles.

A. The 4WJ shows higher Tm over the previous pRNA-3WJ without (upper) or carrying high payloads of paclitaxel (PTX).

B. Comparison of the thermostability of 4WJ with 24 copies of PTX and pRNA-3WJ with 10 PTX).

C. Serum stability of pRNA nanoparticles at 36 hrs.

RNA NANOPARTICLES TARGETED DELIVER COMBINATION THERAPEUTICS FOR LIVER CANCER (HCC) TREATMENT

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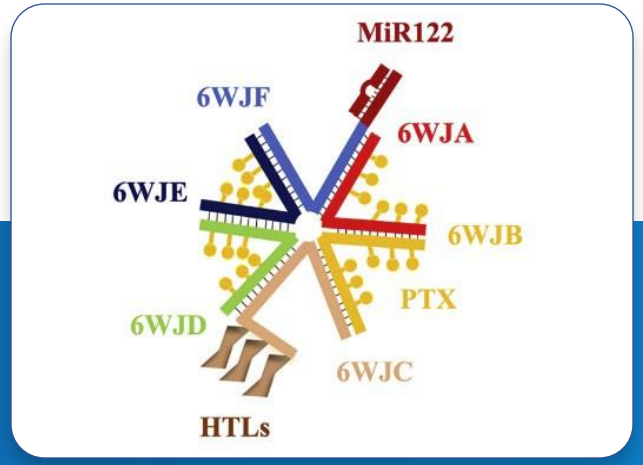
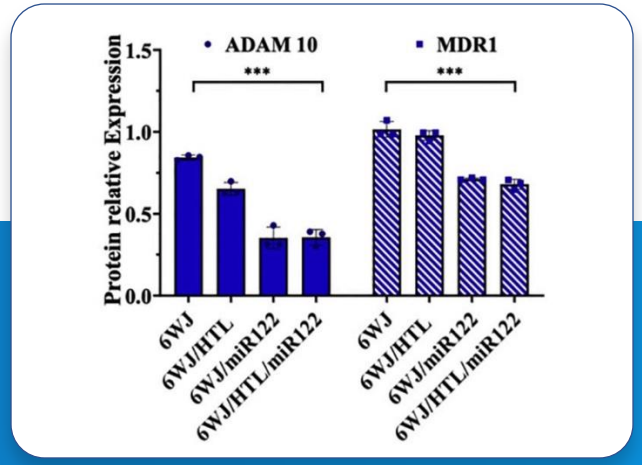
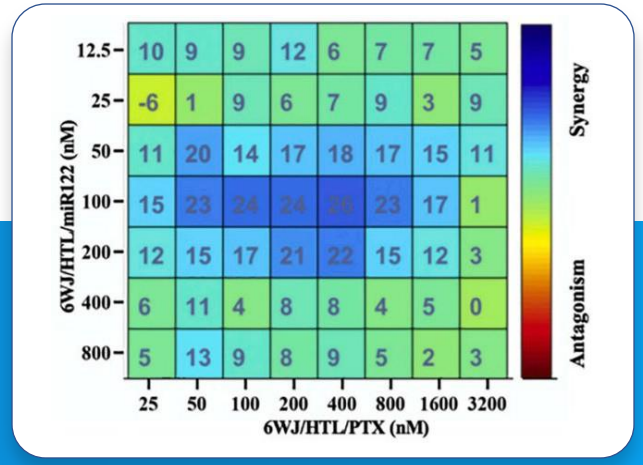
Multifunctional 6WJ



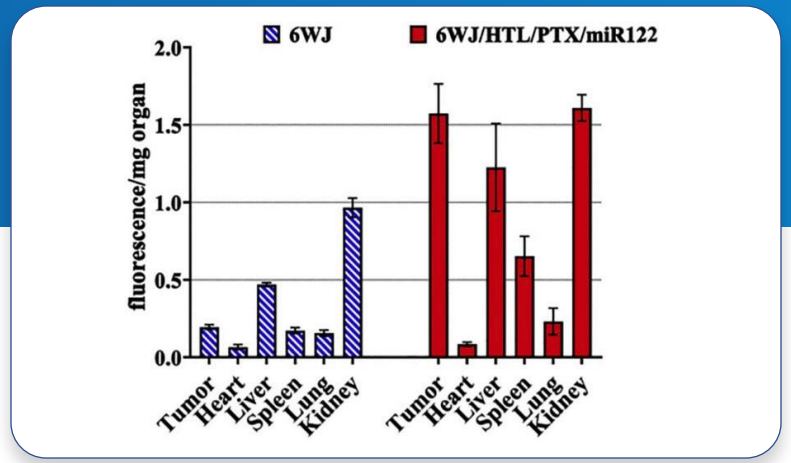
Protein knockdown by miR122



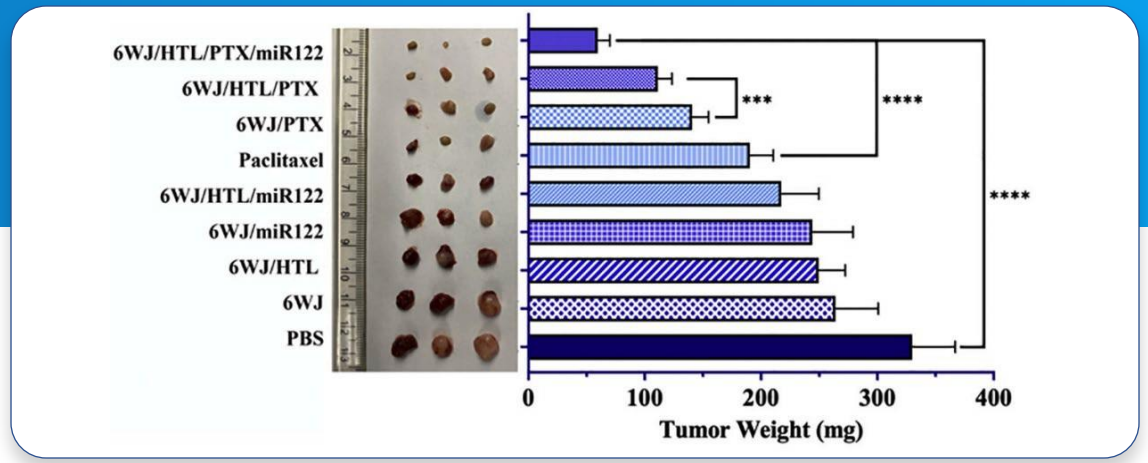
Synergistic effect



Tumor accumulation



Tumor inhibition



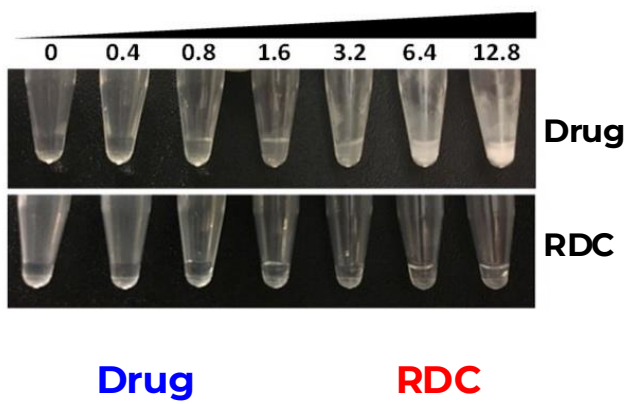
Wang H, et. al. & Guo P. JCR. 2021

RNA NANOPARTICLE CONJUGATES

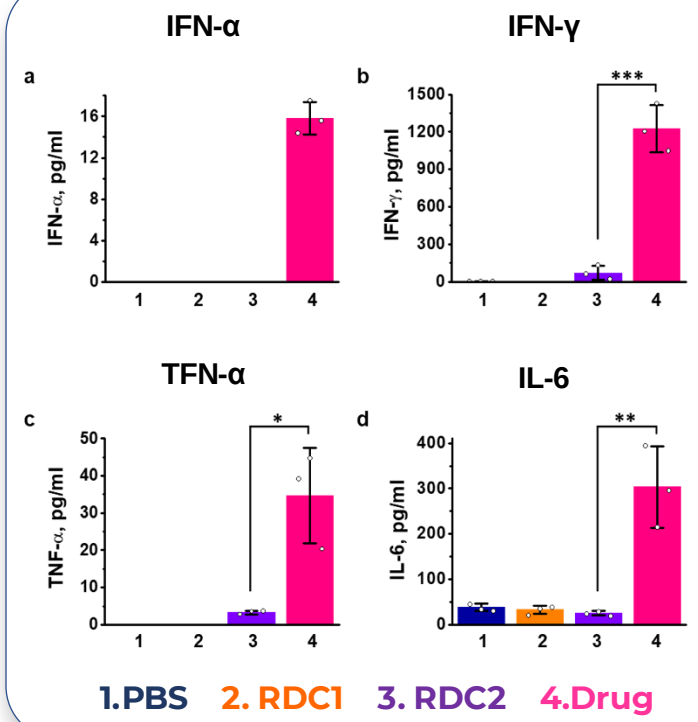
RNA NanoBiotics

Anticancer drug is conjugated to RNA Nanoparticles to target and kill cancer cells while sparing healthy cells.

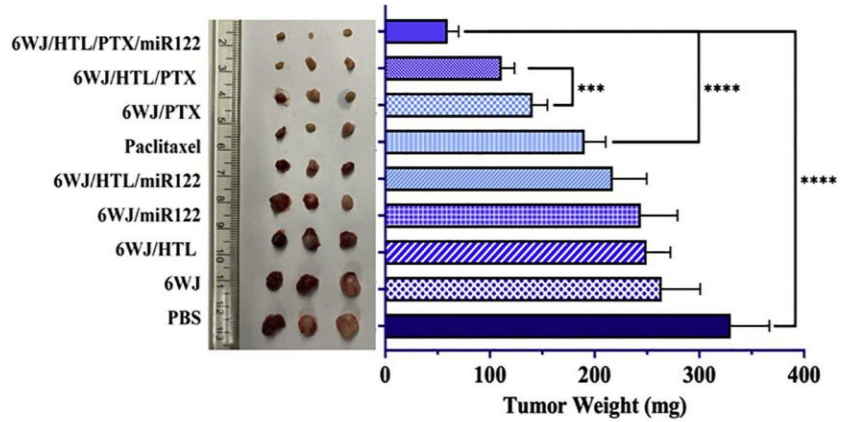
A. Enhanced Drug Solubility



B. Reduced Immunogenicity



C. Enhanced Tumor Suppression



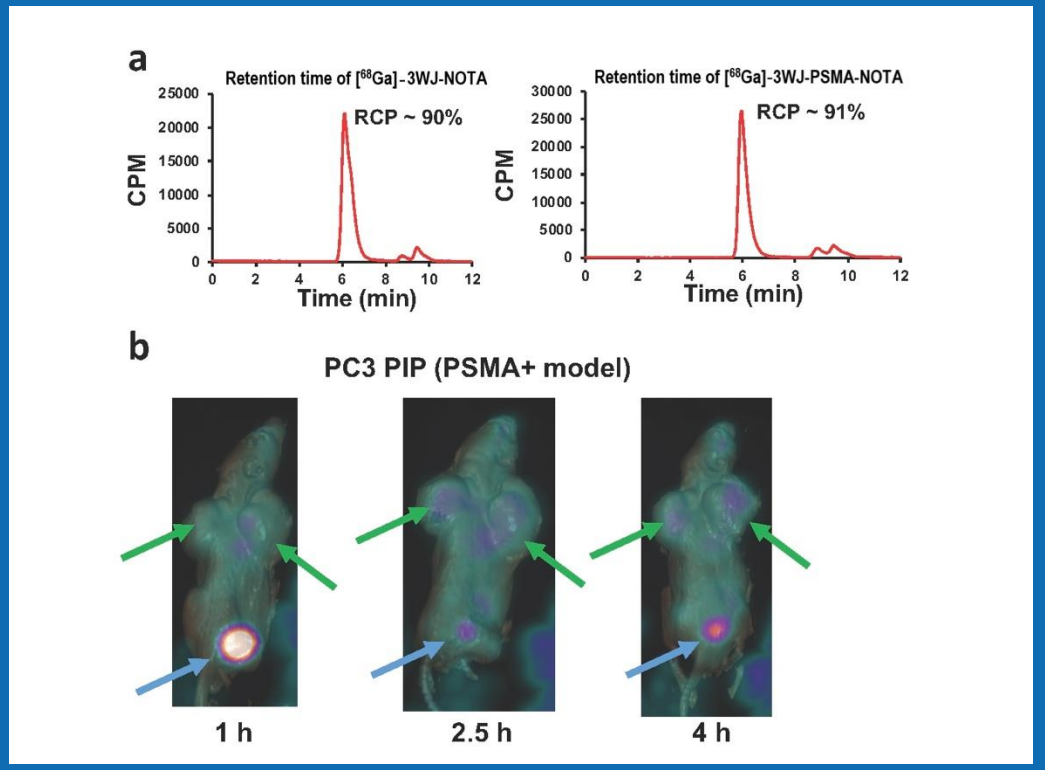
Guo S et.al.& Guo P. Nature Communications, 2020, 11, WO2019156726(A1), Wang H, Elliplli S, Lee W, Li X, Vieweger M, Ho Y, Gu o P. Journal of Controlled Release. 2021

TARGETED RADIATION THERAPY APPLICATIONS

RNA NanoBiotics

Ligands Can be Incorporating to Load Alpha Emitting Radioisotopes into the Targeted RNA Nanoparticles:

- New 4WJ Synthesis protocol allows up to 4 different drugs radioisotopes to be incorporated into particles with click chemistry
- Actinium 225 has become a radioisotope of choice for targeted therapy due to its half-life
- Astatine 211 chemistry well studied and chemical linkers could potentially be incorporated into the nano-structures
- The structure of the RNA Nanoparticles should allow addition of the radioisotope near the point of use



3WJ Nanoparicle with Gallium-68 Radioisotope for Medical Imaging

- **Green Arrow - Tumor**
- **Blue Arrow – Bladder (showing excretion)**

Related Publication: Wang H, **Guo P.** Radiolabeled RNA Nanoparticles for Highly Specific Targeting and Efficient Tumor Accumulation with Favorable In Vivo Bio-disitribution. Molecular Pharmaceutics. 2021 Jul. 18, 8, 2924-2934

Incorporating Additional Cytotoxic Agents Chemistry Established

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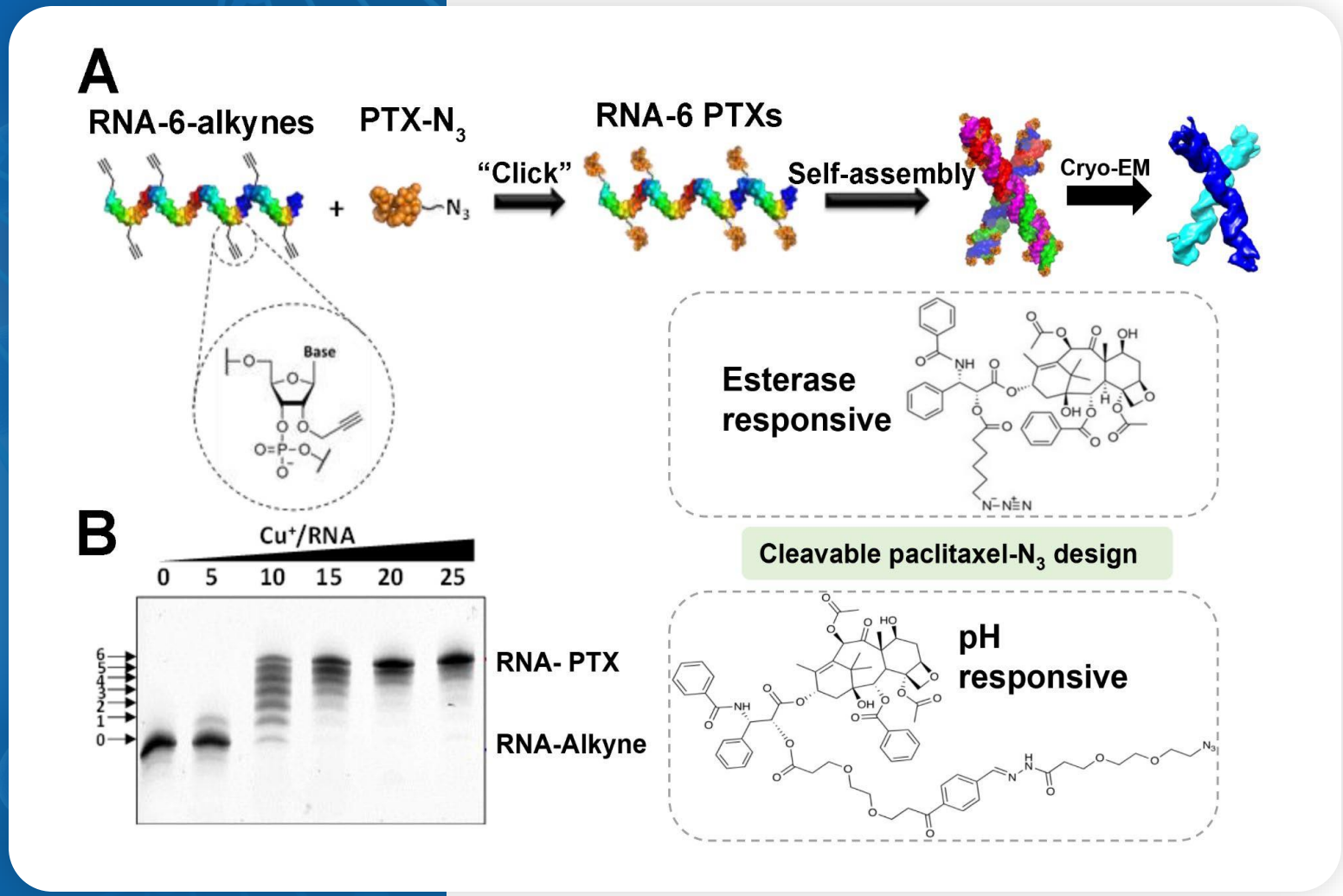
Conjugation of RNA-Paclitaxel (PTX) And Construction of 4WJ-24 PTX Nanoparticles With Esterase Responsive Labile Bond

A. Schematic of RNA-6 PTX chemical conjugation.

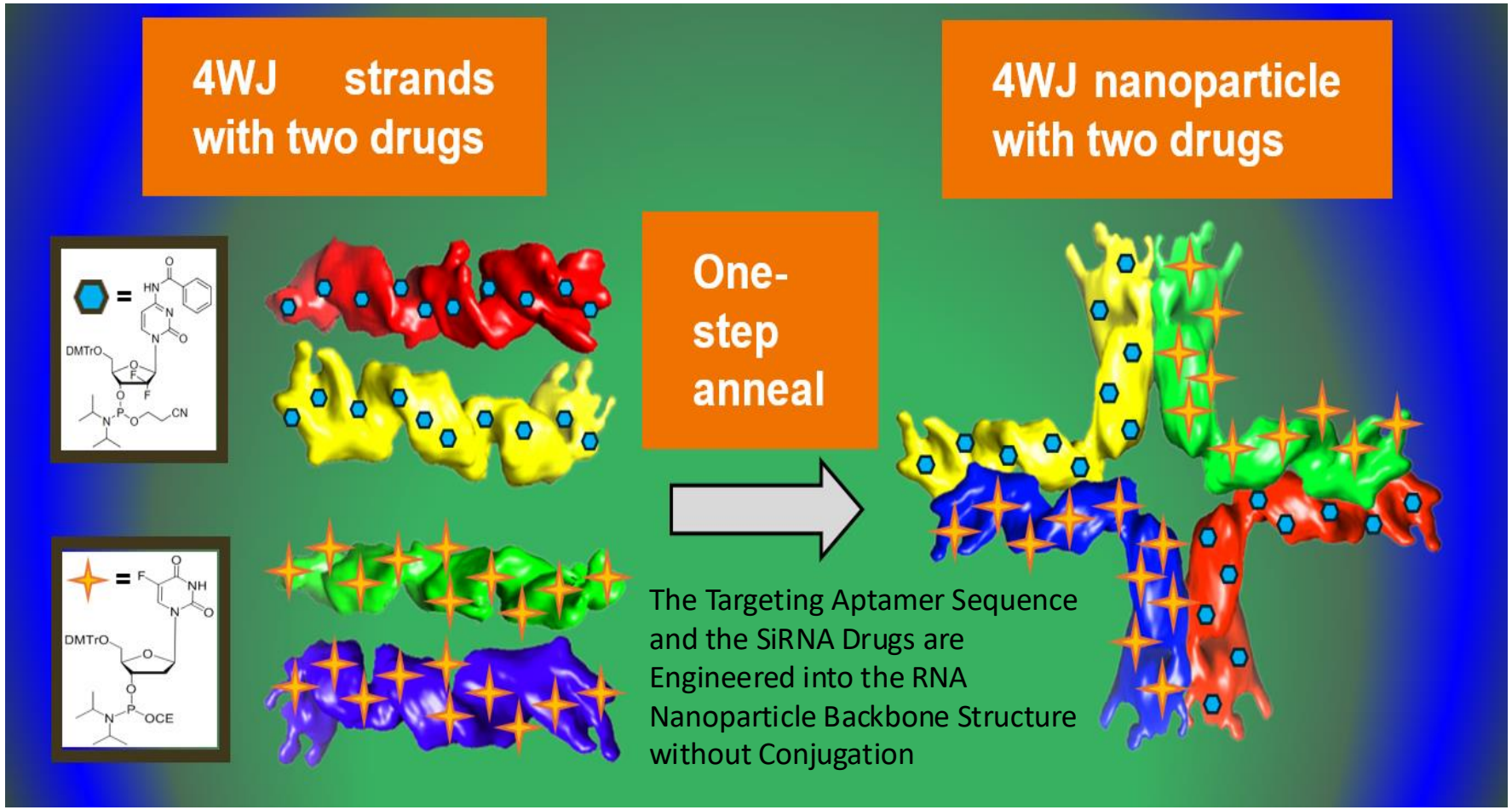
B. Conjugating 4 PTX to one RNA strand evaluated by denaturing PAGE.



Four different Chemotherapeutic can be loaded into Nanoparticles

New 4WJ Synthesis Easily Conjugates in Four Different Chemical Drugs or RadioIsotopes

RNA NanoBiotics



Two papers by Kai Jin et al & P Guo, Mol Therapy, Sep 2024 in press

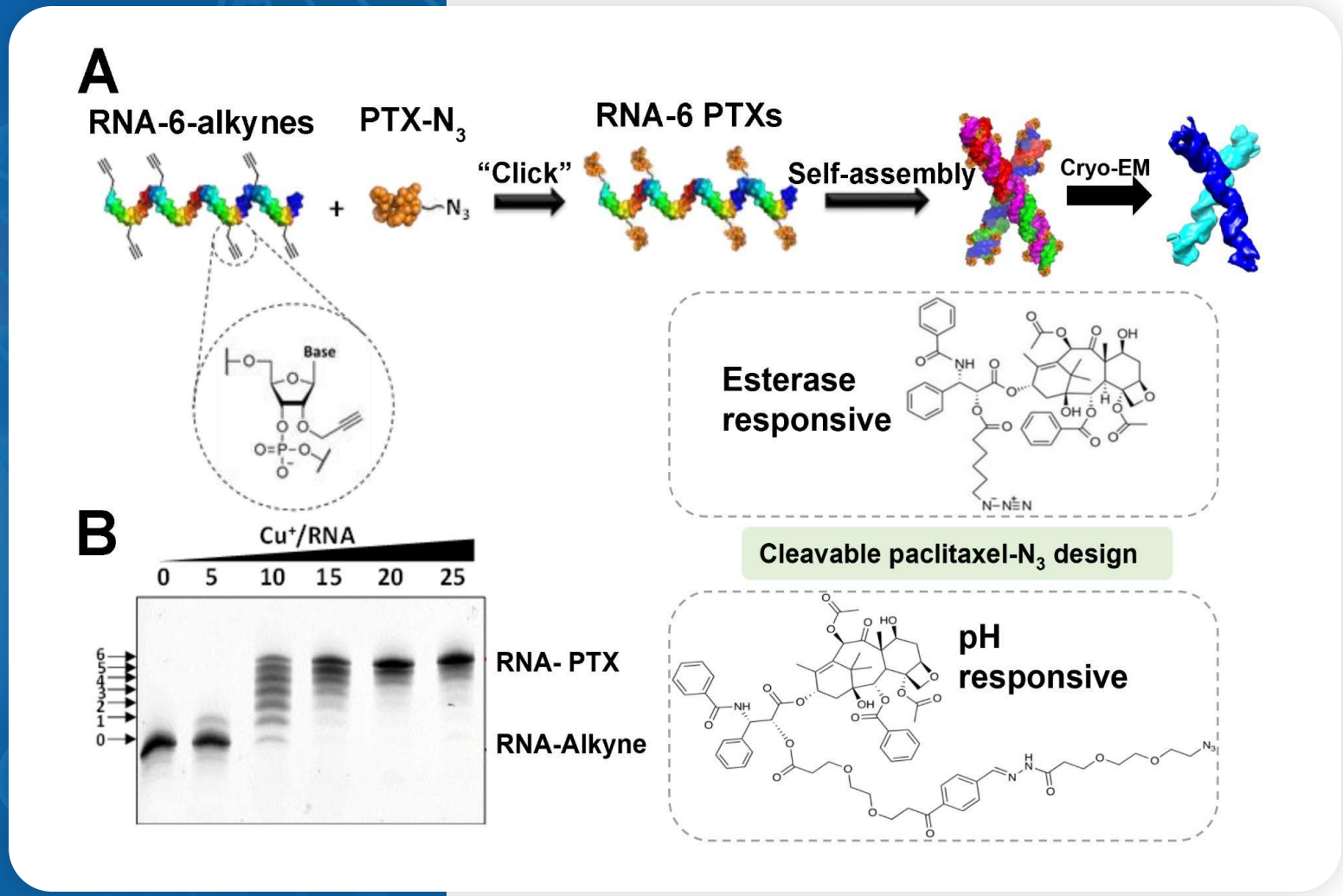
Incorporating Additional Cytotoxic Agents Chemistry Established

RNA NanoBiotics

Conjugation of RNA-Paclitaxel (PTX) And Construction of 4WJ-24 PTX Nanoparticles With Esterase Responsive Labile Bond

A. Schematic of RNA-6 PTX chemical conjugation.

B. Conjugating 4 PTX to one RNA strand evaluated by denaturing PAGE.



Four different Chemotherapeutic can be loaded into Nanoparticles

KEY BENEFIT OF APPROACH IS FAST CLEARANCE IN CIRCULATION & ORGANS, BUT LOW CLEARANCE IN TUMORS

RNA NanoBiotics

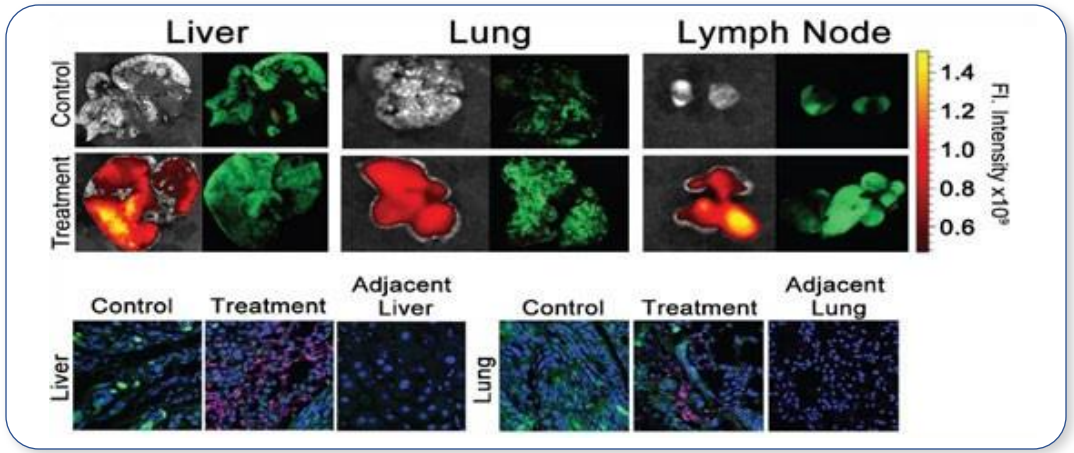
RNA nanoparticles circulate well upon injection

Nanoparticles are cleared within 4 hours in blood

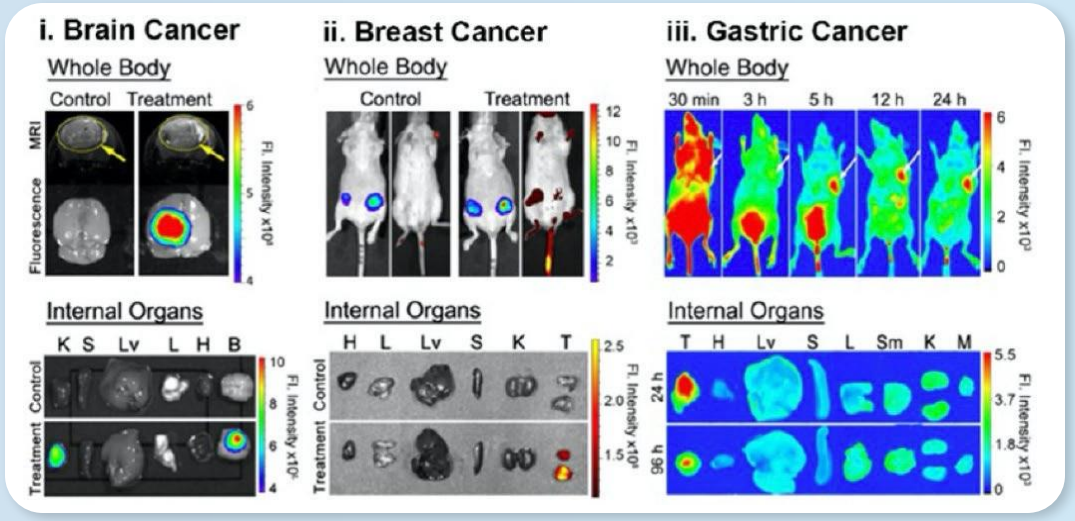
Have high tumor retention for better therapeutics

RNA nanoparticles with ligands tested

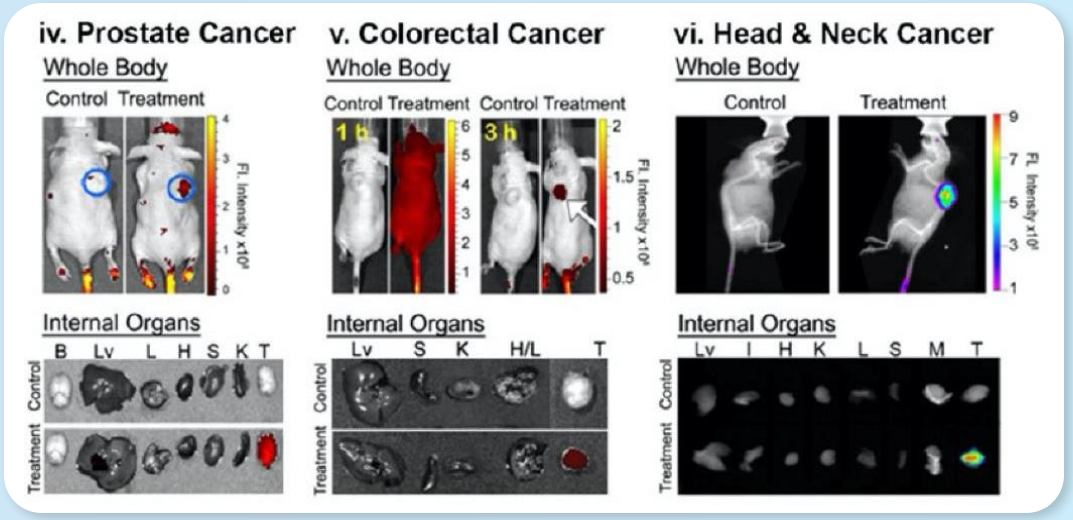
Allows for tumor targeting without accumulation in organs



Colorectal Cancer Metastases to Liver and Lung, Lymph Node



Fast Clearance In Circulation vs Slow Clearance In Tumors

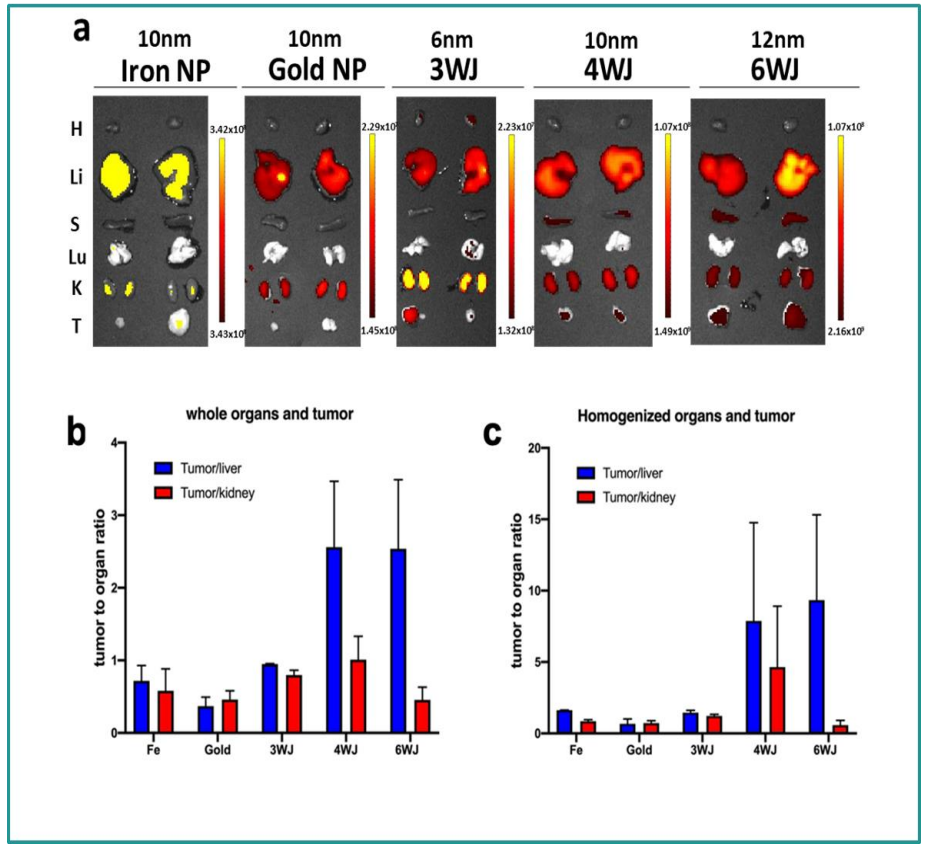


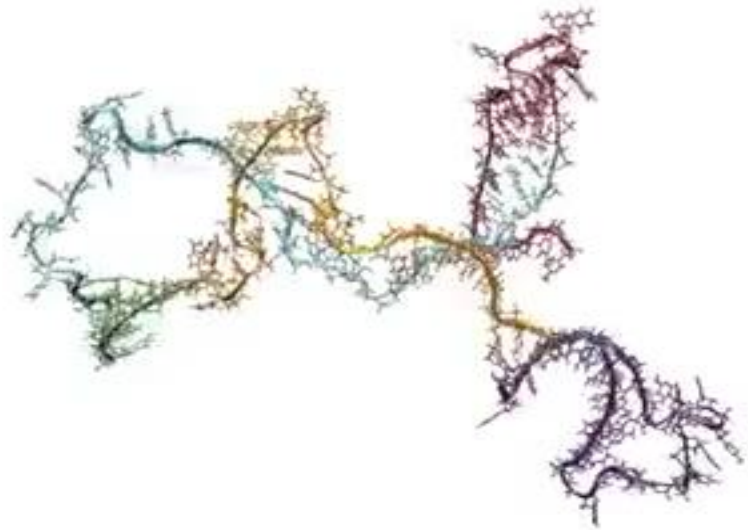
These Benefits Are Critical for Targeted Radiation Therapeutics

Rubbery and Amoeba Properties of RNA Nanoparticles Lead to Fast Renal Excretion

RNA NanoBiotics

The rubbery property of RNA nanoparticles make them more efficiently target to tumor by comparing the retention time in tumor, kidney and liver with iron and gold nanoparticles with the same size





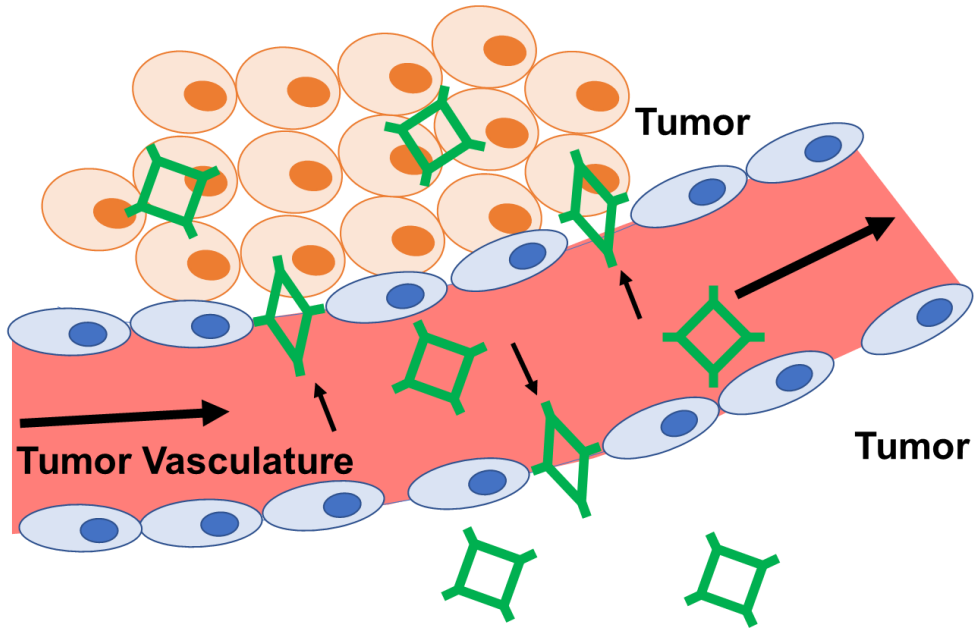
Ghimire C. et al & Guo P. *ACS Nano*. (2020).

Yu A et al.., Molecular Cell, 81: 870. 2021

The Rubbery Properties Benefits

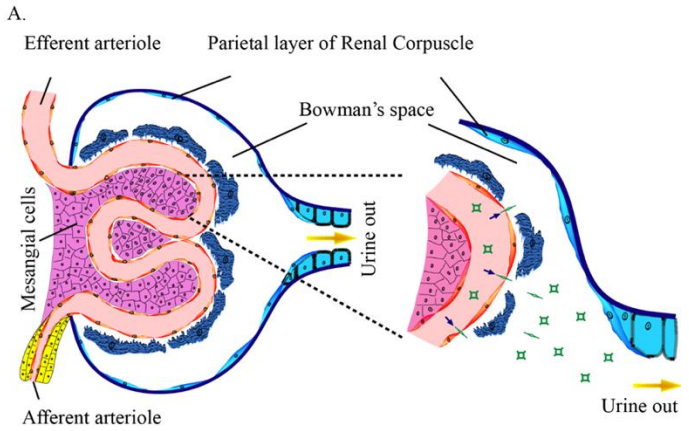
RNA NanoBiotics

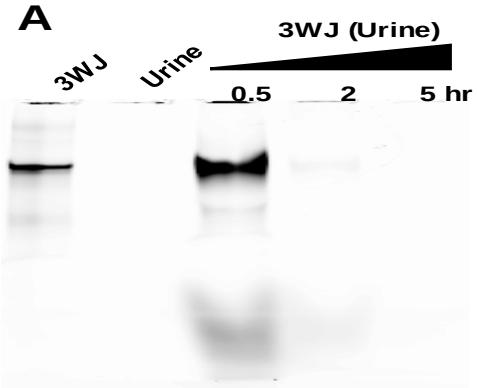
The Rubbery Property of RNA Nanoparticles Enhanced Tumor Accumulation even without Aptamers

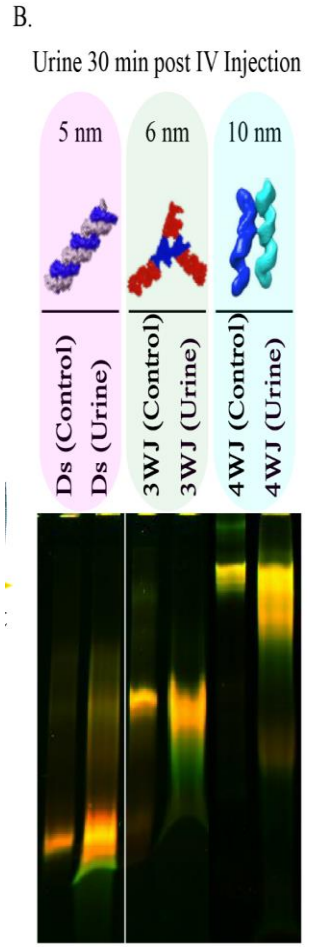


Ghimire C. et al & Guo P. *ACS Nano.* (2020).

Rubbery and amoeba property of RNA leads to fast renal excretion







Binzel D., et al. & **Guo P.** *Chemical Reviews 2021*

Li X., et al. & **Guo P.** *Advanced Drug Delivery Reviews.* 2022

RNA Nanoparticles quickly clear the Kidney's 5 nm Glomerular Filtration Barrier and excreted in the urine

DRUG DEVELOPMENT PIPELINE

RNA NanoBiotics

3
Proprietary Platforms

- RNAi
- RNA/Chemical Drug Complex
- RNA- displaying Exosome

Preclinical Proved To Treat **8** Cancers

- Lung Cancer
- Breast cancer
- Liver cancer
- Prostate Cancer
- Colorectal Cancer
- Ovarian Cancer
- Glioma (Brain Cancer)
- Gastric Cancer

Each Cancer Has
1 to 3

- Different Ligands

Each Cancer Has
2 to 3

- Different Drug Targets

3x8x2x3 = 144
Potential Drug Candidates

We Have **7** Lead Candidates to Choose From
For Our Initial Pipeline Development

CURRENT POTENTIAL DRUG DEVELOPMENT PIPELINE

RNA NanoBiotics

Drug Candidates for IND Submission and Phase I Trial

1	4WJ-FUDR-GEM	Triple Negative Breast Cancer	2026
2	4WJ-miR34	Liver Cancer	2026
3	4WJ-siRNA(RRM2)	Ovarian Cancer	2027
4	4WJ-SN38-EpCAM	KRAS Colon/Lung	2027
5	Folate-4WJ-Exosome/Survivin	Prostate and Breast Cancer	2028
6	Folate-4WJ-Exosome/miR122	Liver Cancer	2028
7	EGFA-4WJ-Paclitaxel	Breast Cancer and Lung Cancer	2029

* Priorities/dates may be re-ranked based on new data and review. Platform related work can continue until priority decision

KEY PUBLICATIONS ON SELECT DRUG CANDIDATES

RNA NanoBiotics

No.	Example Related Publications with Data
1	Yang C, Li Z, Binzel D, **Guo P**, Williams T. **Targeting Oncogenic KRAS in Non-small Cell Lung Cancer** with EGFR Aptamer-conjugated Multifunctional RNA Nanoparticles. Molecular Therapy-Nucleic Acids, 2023 Jul [link][PDF]
2	Liao YC, Cheng TC, Tu SH, Chang J, **Guo P**, Chen L, Ho YS. Tumor targeting and therapeutic assessments of RNA nanoparticles carrying α9-nAchR aptamer and anti- miR-21 on **triple-negative breast cancers**. Molecular Therapy-Nucleic Acids, 2023 Jul [link][PDF]
3	Ellipilli S, Wang H, Binzel D, Shu D, **Guo P**. Ligand-displaying-exosomes using RNA nanotechnology for targeted delivery of multi-specific drugs **for liver cancer** regression. Nanomedicine: Nanotechnology, Biology and Medicine, 2023 Mar, 102667[link][PDF]
4	Wang H, Elliplli S, Lee W, Li X, Vieweger M, Ho Y, **Guo P**. Multivalent rubber-like RNA nanoparticles for targeted co-delivery of paclitaxel and MiRNA to silence the drug efflux transporter and **liver cancer** drug resistance. Journal of Controlled Release. 2021 Feb; 173-184.[link][PDF]
5	Xu Y, Pang L, Wang H, Xu C, Shah H, **Guo P**, Shu D, Qian SY. Specific delivery of delta-5-desaturase siRNA via RNA nanoparticles supplemented with dihomo-y- linolenic acid for **colon cancer** suppression. Redox Biol. 2018 Dec 18;21:101085.[link][PDF]
6	Lee TJ, Yoo JY, Shu D, Li H, Zhang J, Yu JG, Jaime-Ramirez AC, Acunzo M, Romano G, Cui R, Sun HL, Luo Z, Old M, Kaur B, **Guo P**, Croce CM. RNA Nanoparticle-Based Targeted Therapy for **Glioblastoma** through Inhibition of Oncogenic miR-21. Mol Ther. 2017 Jul 5;25(7):1544-1555. [link][PDF]
7	Li X, Jin K, Cheng TC, Liao YC, Lee WJ, Bhullar A, Chen LC, Rychahou P, Phelps M, Ho YS, **Guo P**. RNA four-way junction (4WJ) for spontaneous cancer-targeting, effective tumor-regression, metastasis suppression, fast renal excretion and undetectable toxicity. Biomaterials, 2023 Dec[link][PDF]

Note: This is a subset of related publications and data, Please see full list: https://rnanano.osu.edu/Guo/publications.html