
RNA Origami and Immune Modulation: A Survey

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Abstract

This survey paper delves into the emerging field of RNA origami, highlighting its potential in the precise folding of RNA molecules into complex shapes for diverse applications, particularly in immune modulation. By leveraging principles of nanotechnology, RNA origami facilitates the creation of RNA nanostructures that self-assemble and interact with innate immune receptors, offering innovative strategies for immune regulation. The paper systematically explores the principles of RNA origami design, recent advancements in RNA folding techniques, and the interaction mechanisms between RNA nanostructures and immune receptors. Key applications such as innovative anticoagulants, drug and vaccine delivery systems, and synthetic cells are examined, underscoring the therapeutic potential of RNA origami. Additionally, the survey addresses current challenges, including production and stability issues, design optimization, and integration with biological systems, while proposing future research directions to enhance the scalability and functionality of RNA origami constructs. The study concludes that RNA origami holds transformative potential in nanotechnology and immunology, paving the way for novel therapeutic interventions and expanding the horizons of RNA-based technologies.

1 Introduction

1.1 Structure of the Survey

This survey is systematically structured to elucidate the intricate relationship between RNA origami and immune modulation. It begins with an introduction to RNA origami, underscoring its importance in nanotechnology and immunomodulation. Following this, a comprehensive background section provides essential definitions and outlines key concepts, including RNA origami, self-assembly, and innate immune receptors, along with their interconnections.

The survey then explores the principles of RNA origami and its crucial role in developing complex nanostructures, highlighting advancements in nanotechnology that enable precise RNA folding. This section is further subdivided into discussions on RNA origami design principles, recent innovations in RNA folding techniques, novel constructs, and the impact of environmental factors on RNA origami stability.

Attention shifts to the interactions between RNA nanostructures and innate immune receptors, examining the mechanisms of these interactions and how the structural complexity of RNA-based nanomaterials modulates immune responses. This modulation has implications for biomedical applications, including targeted drug delivery and vaccine development [1, 2, 3, 4]. The survey also investigates potential applications of RNA origami in immune regulation, focusing on innovative anticoagulants, drug and vaccine delivery systems, and the role of RNA origami in synthetic cells and self-assembly processes.

Current challenges in RNA origami and immune modulation are addressed, including issues related to production and stability, design optimization, integration with biological systems, and the technological advancements needed for future research. The paper concludes by synthesizing key

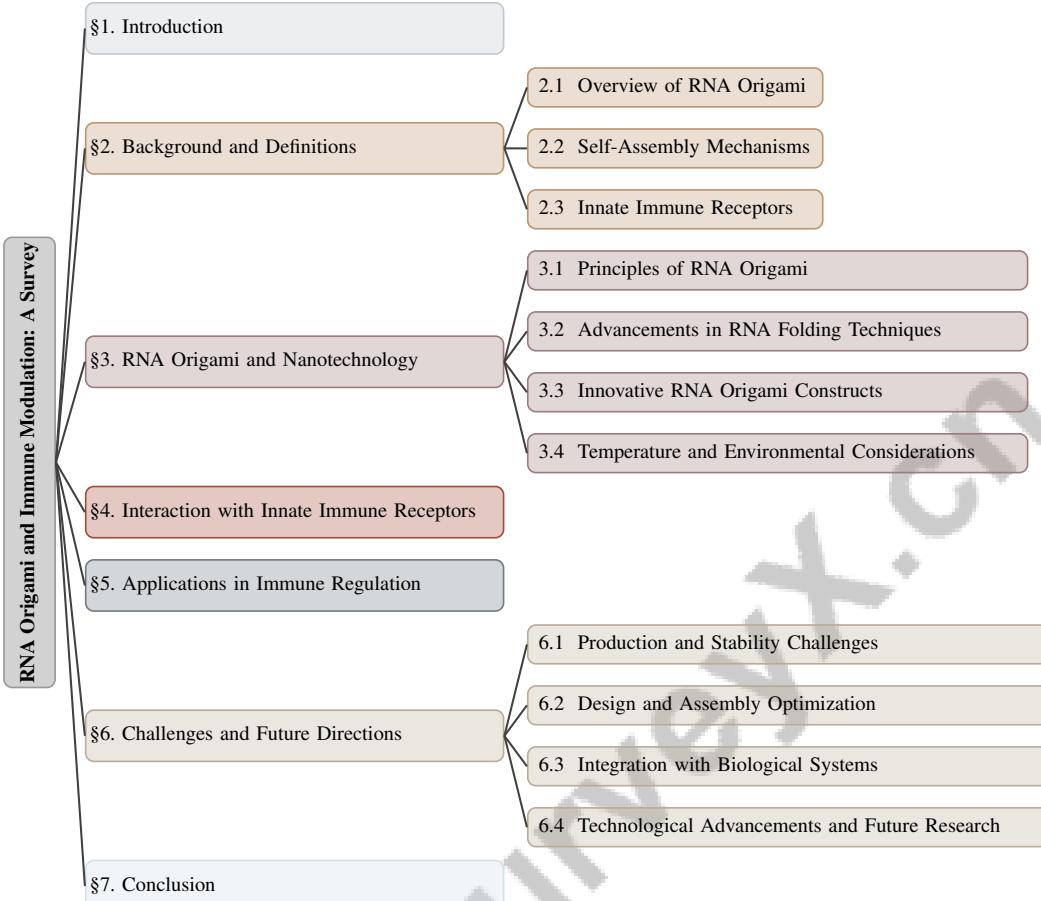


Figure 1: chapter structure

findings and emphasizing the promising role of RNA origami as a versatile platform for enhancing immune modulation strategies, particularly through its use of ribosomal RNA as a stable scaffold for constructing complex nanostructures that improve the delivery and efficacy of therapeutic agents in biomedical applications [2, 3, 4]. The following sections are organized as shown in Figure 1.

2 Background and Definitions

2.1 Overview of RNA Origami

RNA origami is an innovative approach for designing and folding RNA molecules into precise configurations, facilitating applications in biosensing and nanofabrication. Leveraging RNA's ability to form complex secondary and tertiary structures, this technique enables the construction of nanoscale architectures with specific functionalities [5]. Unlike the well-explored DNA origami, RNA origami remains less studied, offering potential for advancements in assembly methods and stability [4].

The modular nature of RNA origami enables it to serve as a template for viral capsid assembly, highlighting its relevance in virology and nanotechnology. It also acts as a molecular hardware alternative to proteins, linking genetic information with structural function [6]. This is particularly promising for hybrid RNA:DNA origami systems, which are being explored for mRNA delivery and artificial ribozyme fabrication [2].

Recent innovations have combined natural rRNA scaffolds with DNA staples to create stable nanostructures, enhancing RNA origami's versatility in constructing complex nanodevices [3]. Additionally, RNA origami has been used to position fluorescent RNA aptamers for Förster Resonance Energy Transfer (FRET) studies, advancing its role in dynamic structural research [7]. Thus, RNA origami is evolving as a crucial tool in nanotechnology, paving the way for future research and applications.

2.2 Self-Assembly Mechanisms

Self-assembly mechanisms are fundamental to RNA origami, utilizing RNA's ability to spontaneously organize into predetermined configurations. This process is driven by RNA's capacity to fold into intricate structures through hydrogen bonding, base stacking, and electrostatic interactions [5]. The stability of these structures is enhanced by complementary interactions between rRNA and DNA staples, exemplified by the rRNA-DNA origami method [3].

Traditional self-assembly approaches face challenges such as kinetic traps, which hinder assembly accuracy and efficiency [8]. Innovations like isothermal folding of RNA origami (IFRO) have been developed to address these issues, involving initial denaturation followed by folding at a constant physiological temperature [9]. Designing interaction matrices for self-assembly remains challenging, with ongoing efforts to encode interactions for structure memorization and retrieval [10].

Advancements in programmable folding of colloidal polymers enable the creation of complex geometries through designed interactions, expanding RNA origami's potential in constructing sophisticated nanostructures [11]. These developments enhance our understanding of RNA origami and open new avenues for applications in nanotechnology and synthetic biology.

2.3 Innate Immune Receptors

Innate immune receptors are crucial components of the immune system, recognizing conserved molecular patterns to defend against pathogens. These receptors, including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs), play key roles in detecting nucleic acids and other pathogen-associated molecular patterns (PAMPs) [4]. RNA origami's relevance lies in its ability to interact with these receptors, potentially modulating immune responses through engineered RNA molecules.

By folding into defined nanostructures, RNA origami offers novel means to influence immune pathways. Recent studies demonstrate RNA nanostructures' potential to engage innate immune receptors, affecting immune signaling [9]. These interactions can be harnessed to activate or suppress immune responses, depending on therapeutic goals.

Integrating RNA origami with hybrid RNA-DNA structures enhances its applicability in biological systems, potentially leading to innovative immune modulation strategies [4]. Theoretical frameworks from polymer physics and statistical mechanics provide insights into RNA folding dynamics, essential for understanding interactions with immune receptors [12].

Research on coat-protein concentration effects in virus-like particle (VLP) assembly offers parallels to RNA origami, as both involve controlled biomolecular assembly influencing immune interactions [1]. By leveraging RNA origami principles and insights into innate immune receptor interactions, novel immunomodulatory therapies can be developed, exploiting natural immune recognition pathways.

3 RNA Origami and Nanotechnology

3.1 Principles of RNA Origami

RNA origami relies on the integration of RNA scaffolds and complementary staple strands to create complex, stable nanostructures. This technique utilizes RNA's ability to form intricate secondary and tertiary structures, crucial for nanoscale self-assembly [9]. Enhancing RNA scaffolds with DNA staples improves assembly efficiency and enables physiological monitoring, expanding RNA origami's applicability in biological contexts [3]. Innovations in RNA origami include RNA Origami Anticoagulants (ROA), engineered for nuclease resistance with thrombin-binding RNA aptamers, demonstrating therapeutic potential, particularly in anticoagulation [13]. Mini DNA-RNA hybrid origami nanobricks, utilizing shorter RNA scaffolds, streamline synthesis and folding processes [4].

Advanced computational design techniques have facilitated the construction of 3D wireframe structures using RNA scaffolds and DNA staples, guided by thermal annealing, showcasing precision in RNA folding [2]. Co-transcriptional folding and assembly of RNA enable rapid production of functional structures, emphasizing RNA origami's efficiency [6]. The Staged RNA Assembly Model (SRAM) allows RNA tiles to dissolve and reform into new structures, offering design flexibility [14]. The apta-FRET system, based on Förster Resonance Energy Transfer (FRET), provides a dynamic

tool for monitoring conformational changes in RNA origami constructs [7]. Innovations in materials science, such as folding mechanisms that enable self-assembly with minimal building block flavors, further enhance RNA origami’s potential [11].

Incorporating non-reciprocal interactions into self-assembly models allows for dynamic transitions between structures, increasing RNA origami’s adaptability [10]. These principles and methodologies highlight RNA origami’s versatility in nanotechnology, facilitating the design and fabrication of structurally complex and functionally diverse nanostructures, paving the way for innovative applications in medicine and beyond.

3.2 Advancements in RNA Folding Techniques

Method Name	Structural Features	Assembly Techniques	Applicable Scenarios
KTE[8]	-	Kinetic-Trap Encoding	Information Processing
PF[11]	Complex Geometries	Programmable Folding	Synthetic Biology
SBAS[15]	Nanostructures Created	Programmable Folding	Biosensing, Synthetic Biology
mDRON[4]	Honeycomb Lattice	Temperature Gradient Anneal	Drug Delivery

Table 1: Overview of recent advancements in RNA folding techniques, highlighting the structural features, assembly techniques, and applicable scenarios of various methods. This table provides a comparative analysis of Kinetic-Trap Encoding, Programmable Folding, and Temperature Gradient Anneal, demonstrating their diverse applications in fields such as synthetic biology, biosensing, and drug delivery.

Recent advancements in RNA folding techniques have significantly improved RNA origami’s precision and efficiency, enabling the creation of complex nanostructures with high fidelity. Kinetic-Trap Encoding leverages polymerization kinetics to encode target structure information, allowing rapid and accurate RNA origami assembly by optimizing folding pathways [8]. Hierarchical interactions in self-assembly have been harnessed to control RNA folding through temperature modulation, facilitating irreversible bond formation and directing assembly into desired geometries [11]. Manipulating environmental conditions such as temperature guides RNA self-assembly into complex, stable configurations, broadening RNA origami’s applications in nanotechnology and synthetic biology.

These advancements enhance RNA origami assembly efficiency by employing ribosomal RNA as a robust scaffold for constructing intricate 2D and 3D nanostructures, while providing insights into RNA folding and stability through comprehensive analyses of secondary and tertiary structures. This understanding is crucial for developing stable RNA-based nanostructures for biomedical applications, including drug delivery and synthetic biology [2, 3, 4, 9]. Integrating these innovative techniques enables the design and fabrication of RNA-based nanostructures with unprecedented accuracy, fostering their application in diverse fields, including drug delivery, biosensing, and novel therapeutic strategies.

As illustrated in Figure 3, which focuses on three main areas of recent advancements in RNA folding techniques—Kinetic-Trap Encoding, Programmable Folding, and RNA Scaffold Techniques—each category highlights specific innovations and applications. Kinetic-Trap Encoding emphasizes high-speed assembly and accurate folding, while Programmable Folding showcases hierarchical interactions and temperature modulation. The RNA Scaffold Techniques utilize robust RNA scaffolds to construct intricate nanostructures. Collectively, these examples underscore RNA origami and nanotechnology’s transformative potential in advancing molecular engineering and biomedical applications [11, 15, 4]. Additionally, Table 1 provides a comprehensive comparison of recent advancements in RNA folding techniques, detailing the structural features, assembly techniques, and applicable scenarios of each method.

3.3 Innovative RNA Origami Constructs

The development of novel RNA origami constructs has expanded the design of nanoscale architectures with diverse applications. These constructs exploit RNA’s inherent properties to form complex, programmable structures tailored for specific functionalities. A significant advancement is RNA origami-based anticoagulants, utilizing thrombin-binding RNA aptamers to inhibit coagulation pathways, demonstrating enhanced nuclease resistance and autonomous folding capabilities, underscoring their therapeutic potential [13]. Table 2 provides a comprehensive summary of innovative

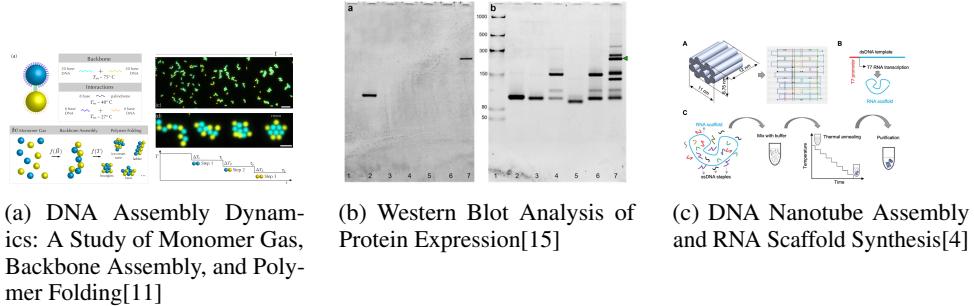


Figure 2: Examples of Advancements in RNA Folding Techniques

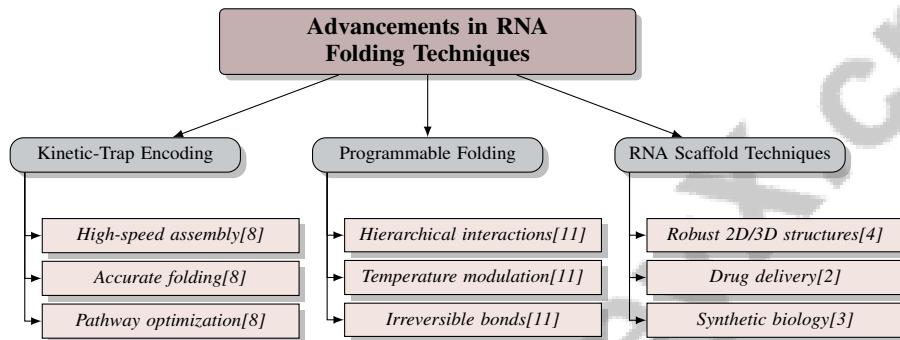


Figure 3: This figure illustrates the recent advancements in RNA folding techniques, focusing on three main areas: Kinetic-Trap Encoding, Programmable Folding, and RNA Scaffold Techniques. Each category highlights specific innovations and applications, such as high-speed assembly and accurate folding in Kinetic-Trap Encoding, hierarchical interactions and temperature modulation in Programmable Folding, and the use of robust RNA scaffolds for constructing intricate nanostructures in RNA Scaffold Techniques.

Method Name	Structural Features	Functional Applications	Integration Techniques
ROA[13]	Self-folding Structures	Therapeutic Applications	Modified Nucleotides
mDRON[4]	Honeycomb Lattice	Drug Delivery	Dna Staple Strands
rRNA-DNA[3]	Complex 3D Nanostructures	Therapeutic Applications, Biosensing	Dna Staples, Fret
apta-FRET[7]	Rna Origami Scaffolds	Biosensor Devices	Fret Systems

Table 2: Overview of RNA origami constructs highlighting their structural features, functional applications, and integration techniques. The table includes various methods such as ROA, mDRON, rRNA-DNA, and apta-FRET, demonstrating their diverse applications in therapeutic, drug delivery, and biosensing domains through innovative integration approaches.

RNA origami constructs, detailing their structural features, functional applications, and integration techniques, which are pivotal in advancing therapeutic, drug delivery, and biosensing technologies.

Beyond therapeutic applications, RNA origami constructs have facilitated hybrid RNA-DNA nanostucture fabrication. By integrating shorter RNA scaffolds with complementary DNA staples, these constructs streamline synthesis and enhance structural stability and functionality, making them suitable for drug delivery and biosensing applications [4]. Incorporating natural rRNA scaffolds into RNA origami designs has diversified these constructs, utilizing rRNA's inherent stability and functional properties to develop robust nanostructures capable of performing complex tasks in biological environments [3]. This approach is critical in creating RNA-based devices for intracellular delivery and molecular sensing, opening new possibilities for targeted therapies and diagnostic tools.

Additionally, integrating Förster Resonance Energy Transfer (FRET) systems into RNA origami constructs allows real-time monitoring of conformational changes, providing valuable insights into these nanostructures' dynamic behavior [7]. This capability is particularly advantageous for studying RNA origami interactions with biological molecules, paving the way for the development of responsive and adaptive nanodevices. Significant progress has also been made in utilizing RNA

origami within synthetic biology, with constructs designed to mimic natural cellular components and processes. By employing programmable folding techniques, researchers have created RNA nanostructures capable of self-assembling into predefined shapes and functions, offering potential applications in synthetic cell construction and artificial ribozyme development [2]. These innovations highlight RNA origami's transformative potential in pushing the boundaries of nanotechnology and biotechnology.

3.4 Temperature and Environmental Considerations

Method Name	Environmental Factors	Folding Dynamics	Structural Stability
PF[11]	Temperature Protocol	Temperature Protocol	Irreversible Bond Formation
rRNA-DNA[3]	Temperature, Magnesium Concentration	Thermal Cycling Protocol	Enhanced Stability
ROA[13]	Ionic Strength	Self-folding Kinetics	Nuclease-resistant Stability

Table 3: Overview of RNA origami methods detailing the impact of environmental factors on folding dynamics and structural stability. The table highlights the role of temperature, ionic strength, and other conditions in guiding self-assembly and enhancing the robustness of RNA nanostructures.

The stability and functionality of RNA origami are profoundly influenced by environmental factors, particularly temperature, which plays a critical role in the folding and assembly of RNA nanostructures. Temperature modulation serves as a key parameter guiding RNA origami self-assembly, affecting folding kinetics and the stability of the resulting structures. The hierarchical nature of interactions in self-assembly processes enables controlled RNA folding through temperature changes, facilitating irreversible bond formation and directing assembly into desired geometries [11]. Table 3 summarizes various RNA origami methods, emphasizing how different environmental factors influence their folding dynamics and structural stability.

Environmental conditions, including ionic strength and pH, significantly impact RNA origami's structural integrity and functionality. Divalent cations can stabilize RNA structures by neutralizing negative charges on the phosphate backbone, enhancing folding fidelity and stability under physiological conditions, crucial for biological applications [3]. RNA origami construct design must consider potential environmental fluctuations that could affect performance *in vivo*. Strategies to enhance RNA nanostructures' robustness against environmental stresses, such as nuclease degradation and thermal denaturation, are essential for successful therapeutic and diagnostic applications [13]. By optimizing environmental conditions and incorporating stabilizing elements, researchers can improve RNA origami's resilience and functionality, broadening its potential applications in nanotechnology and synthetic biology.

4 Interaction with Innate Immune Receptors

4.1 Mechanisms of Interaction

RNA origami structures interact with innate immune receptors through precise molecular recognition and dynamic conformational changes. These structures can mimic pathogen-associated molecular patterns, activating receptors like Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs). This mimicry is facilitated by RNA's versatility as a scaffold, enabling the engineering of diverse 3D shapes that engage the immune system. Recent advancements, such as incorporating ribosomal RNA and hybrid RNA-DNA structures, have improved the stability and functionality of these nanostructures, making them promising candidates for immunotherapy and vaccine development [9, 6, 2, 3, 4]. These interactions are crucial for modulating immune responses by presenting specific motifs recognized by these receptors.

Thrombin-binding RNA aptamers within RNA origami constructs enhance anticoagulation efficacy and allow specific reversal using complementary nucleic acid sequences, demonstrating RNA origami's potential to influence immune pathways through functional motif presentation [13]. Additionally, the conformational dynamics of RNA origami, particularly when encapsulated within structures like the CCMV capsid, can alter RNA motif accessibility, modulating receptor engagement and subsequent immune signaling pathways [5].

The resilience of RNA origami constructs, such as mini DNA-RNA hybrid origami nanobricks, against ribonuclease digestion underscores their robustness in interacting with immune components

without rapid degradation [4]. This stability is further enhanced by using rRNA, which serves as a cost-effective and durable material for molecular origami, maintaining integrity across various environments [3].

Fluorescence-based systems, like the apta-FRET system, allow real-time monitoring of RNA conformational dynamics, providing insights into RNA interactions with immune receptors through specific binding events [7]. These systems enable tracking of conformational changes that facilitate receptor engagement.

RNA origami folding transitions can be conceptualized as phase transitions, offering a theoretical framework for understanding interactions with immune receptors [12]. The assembly of viral capsids, following a nucleation-and-growth pathway, parallels RNA origami assembly dynamics, providing insights into critical thresholds and timing in the interaction process [16].

Experiments increasing coat-protein concentration show a transition from well-formed virus-like particles to RNA-protein condensates, highlighting concentration's role in determining interaction outcomes [1]. Programming the sequence and timing of interactions in colloidal polymer folding enhances RNA origami design for specific immune interactions, enabling precise control over the assembly process [11].

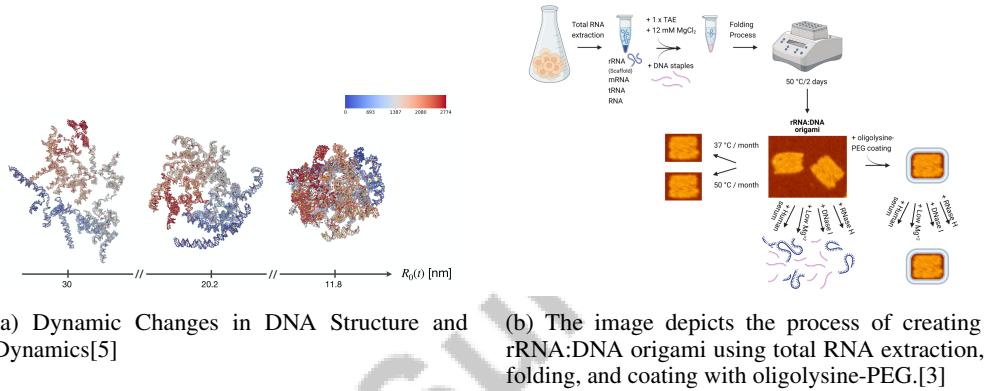


Figure 4: Examples of Mechanisms of Interaction

As shown in Figure 4, understanding the mechanisms of interaction with innate immune receptors requires a focus on dynamic structural changes and molecular-level processes. The first image shows dynamic changes in DNA structure, illustrating transformation stages over time with color-coded ribbons differentiating DNA strands and helices. The second image details the creation of rRNA:DNA origami, highlighting the steps required to construct complex molecular structures. These visualizations provide insights into the molecular dynamics and engineering techniques facilitating interactions with innate immune receptors, forming a basis for further exploration of immune system intricacies [5, 3].

4.2 Structural Complexity and Modulation

The structural complexity of RNA origami significantly influences immune modulation by determining the spatial arrangement and presentation of functional motifs that engage innate immune receptors. The intricate folding patterns and long-range interactions inherent in RNA origami constructs can mimic pathogen-associated molecular patterns, engaging immune receptors such as TLRs and RLRs with high specificity [5]. Studies on RNA2 within capsid structures reveal that these complex formations facilitate stable long-range interactions, crucial for modulating immune responses by altering receptor engagement and signaling pathways.

RNA origami's ability to form stable, complex structures within viral capsids, as demonstrated in molecular dynamics studies, highlights its potential to influence immune modulation through precise molecular recognition and dynamic conformational changes [5]. These interactions are essential for strategically presenting RNA motifs that can either activate or suppress immune responses, depending on therapeutic objectives.

Furthermore, the structural complexity of RNA origami enhances its resilience against enzymatic degradation, allowing sustained interactions with immune receptors and prolonged modulation of immune pathways. This stability is particularly significant for therapeutic applications, as the durability of RNA constructs is critical for effective immune interventions. RNA origami has demonstrated enhanced stability against degradation in various environments, including low magnesium concentrations and the presence of nucleases, supporting its potential use in biomedical settings where robust and long-lasting RNA structures are required for targeted therapeutic effects [13, 3].

By leveraging the intricate structural properties of RNA origami, researchers can engineer sophisticated nanostructures that selectively influence immune responses, paving the way for advancements in immunotherapy and vaccine design. This approach utilizes the unique capabilities of hybrid RNA:DNA origami to create stable, functional scaffolds capable of delivering therapeutic agents, enhancing immune modulation, and facilitating the development of targeted vaccines [2, 3, 4, 6]. The capacity to control the folding and assembly of RNA origami to achieve desired structural complexities provides a powerful tool for manipulating immune pathways and developing targeted immunomodulatory therapies.

In recent years, the innovative use of RNA origami has garnered significant attention in the field of immune regulation. This review explores the various applications of RNA origami, particularly in enhancing therapeutic efficacy and safety. As depicted in Figure 5, the hierarchical structure of these applications can be categorized into three main areas: anticoagulants, drug and vaccine delivery systems, and synthetic cells and self-assembly. Each category not only highlights specific advancements but also outlines potential applications, thereby underscoring the transformative impact of RNA origami in modern therapeutic strategies.

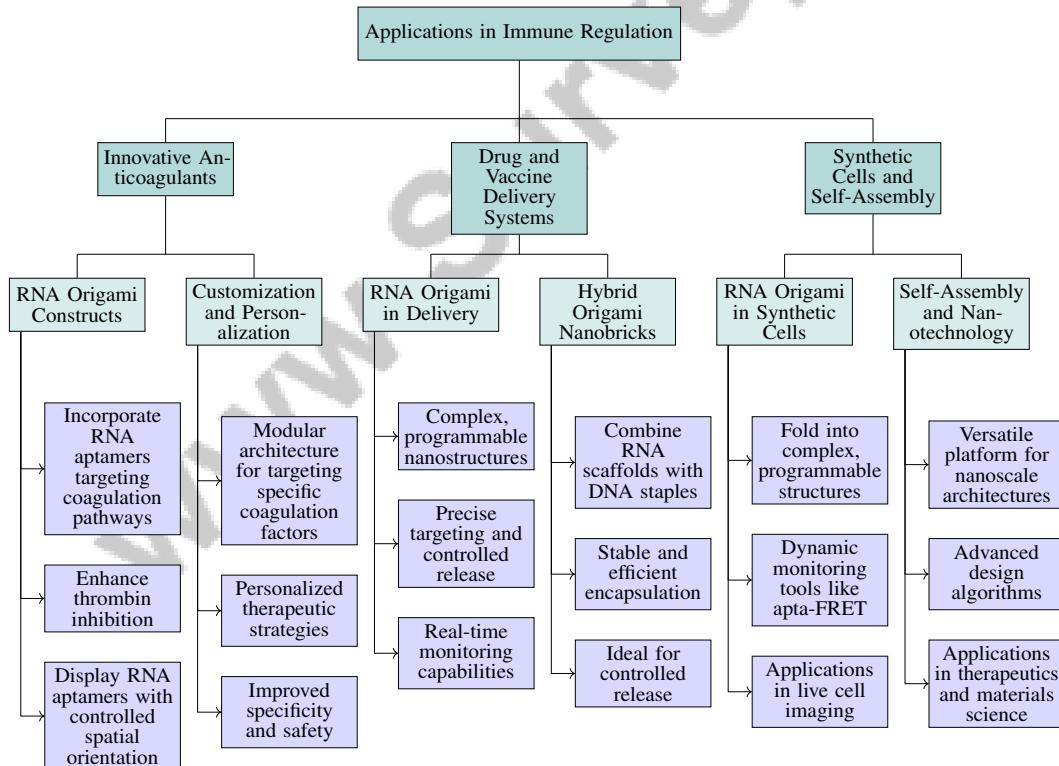


Figure 5: This figure illustrates the hierarchical structure of applications in immune regulation using RNA origami. It categorizes the innovative use of RNA origami into three main areas: anticoagulants, drug and vaccine delivery systems, and synthetic cells and self-assembly. Each area further details specific advancements and potential applications, emphasizing RNA origami's role in enhancing therapeutic efficacy and safety.

5 Applications in Immune Regulation

5.1 Innovative Anticoagulants

RNA origami presents a promising approach for developing advanced anticoagulants by incorporating RNA aptamers that precisely target coagulation pathways. The evolution of self-folding, single-molecule RNA origami constructs has enabled the precise display of thrombin-binding RNA aptamers, which are crucial for maintaining functional integrity in physiological environments due to their enhanced nuclease resistance and autonomous folding capabilities [13]. By integrating multiple thrombin-binding sites within a single RNA structure, these constructs enhance thrombin inhibition—targeting a key enzyme in the coagulation cascade—offering a viable alternative to traditional anticoagulants that often have significant side effects and limited therapeutic windows. The strategic use of self-folding RNA origami to display RNA aptamers with controlled spatial orientation creates a more effective and potentially safer anticoagulant platform, addressing the limitations of conventional therapies [13, 2, 3]. This multivalent approach significantly increases the binding affinity to its target, thereby enhancing therapeutic efficacy through a robust anticoagulation mechanism.

The modular architecture of RNA origami allows for precise customization of anticoagulant constructs to selectively target specific coagulation factors, paving the way for personalized therapeutic strategies that enhance efficacy while minimizing adverse effects associated with conventional anticoagulants. This approach leverages RNA origami's unique capabilities to display RNA aptamers with controlled orientation and spatial arrangement, ultimately improving specificity and safety in anticoagulant therapies [13, 2, 3, 9]. The ability to engineer RNA origami with precise control over folding and functional presentation underscores its potential as a versatile tool in developing next-generation anticoagulants.

Enhancing RNA origami-based anticoagulants enables researchers to explore innovative therapeutic approaches that leverage the distinct characteristics of RNA nanostructures, potentially leading to safer and more effective anticoagulation therapies. These platforms, capable of displaying specific RNA aptamers with precise spatial control, not only improve anticoagulation activity but also address the limitations of conventional anticoagulants, such as side effects and narrow therapeutic windows. Moreover, incorporating nuclease resistance into these structures enhances their stability, making them promising candidates for various clinical settings, including surgeries and thrombotic disease treatments [13, 2, 3, 4].

5.2 Drug and Vaccine Delivery Systems

RNA origami has demonstrated significant potential in enhancing drug and vaccine delivery systems through its ability to form complex, programmable nanostructures. The integration of RNA origami into delivery systems enables precise targeting and controlled release of therapeutic agents, improving treatment efficacy and safety. The split Broccoli aptamer system exemplifies this potential, offering real-time monitoring capabilities crucial for dynamically assessing drug and vaccine delivery [15]. This system serves as a versatile platform for developing biosensors adaptable to various therapeutic contexts, enhancing delivery precision.

The development of mini DNA-RNA hybrid origami nanobricks further underscores RNA origami's potential in drug delivery applications. These constructs, which combine shorter RNA scaffolds with DNA staples, provide a stable and efficient means of encapsulating and delivering therapeutic agents [4]. Their structural stability and functional versatility make them ideal candidates for drug delivery systems, where controlled release and targeted delivery are paramount.

Advancements in RNA origami design have also enabled precise control over viral capsid dimensions, integral to vaccine and gene delivery technologies [17]. By harnessing RNA origami's spatial precision and modularity, researchers can engineer delivery vehicles optimizing the encapsulation and release of vaccines and genetic materials, thereby enhancing their therapeutic potential.

These innovations collectively highlight RNA origami's transformative role in drug and vaccine delivery systems. By leveraging the properties of ribosomal RNA (rRNA), researchers are advancing sophisticated RNA nanostructures that serve as innovative delivery platforms. These platforms enhance therapeutic efficacy through improved targeting of specific cells, controlled release of therapeutic agents, and real-time monitoring of biological responses. Constructing stable 2D and 3D RNA origami utilizing rRNA as a scaffold not only facilitates precise engineering of nanostructures

but also opens new avenues for personalized medicine, enabling tailored therapeutic interventions that adapt to individual patient needs [2, 3].

5.3 Synthetic Cells and Self-Assembly

The application of RNA origami in synthetic cells and self-assembly processes represents a burgeoning area of research with significant potential in synthetic biology and nanotechnology. RNA origami's ability to fold into complex, programmable structures makes it an ideal candidate for constructing synthetic cells, where precise spatial organization and functional integration are paramount. The apta-FRET system exemplifies this potential, providing a dynamic tool for monitoring conformational changes invaluable for studies in synthetic biology and RNA nanotechnology, potentially leading to applications in live cell imaging [7].

In self-assembly, RNA origami offers a versatile platform for constructing nanoscale architectures with high precision. Advanced design algorithms have facilitated the creation of more complex geometries, enabling exploration of different RNA sequences for improved stability and functionality [2]. These advancements enhance the structural integrity of RNA origami constructs and expand their applicability across diverse fields, including therapeutics and materials science.

Utilizing RNA-scaffolded origami in self-assembly processes provides a robust framework for fabricating synthetic cells, where the modularity and programmability of RNA nanostructures can mimic natural cellular components and processes. By optimizing design algorithms and exploring novel RNA sequences, researchers can improve the stability and efficacy of RNA origami constructs, paving the way for innovative applications in synthetic biology and beyond [2].

Incorporating RNA origami into synthetic cells and self-assembly processes presents significant opportunities for advancing nanotechnology and synthetic biology. This innovative approach enables the design and fabrication of functional nanodevices and cellular mimics using RNA as a versatile scaffold, which can be genetically encoded and expressed within lipid vesicles. Recent studies have successfully constructed complex 2D and 3D RNA origami structures that maintain stability under various conditions, paving the way for applications in drug delivery, biosensing, and artificial ribozymes development. By leveraging RNA's unique properties, researchers can explore new avenues for creating robust nanostructures and enhancing synthetic biological systems' functionality [3, 2, 6, 4].

6 Challenges and Future Directions

6.1 Production and Stability Challenges

RNA origami constructs face significant production and stability challenges that limit their broader application. A major issue is scaling up production, as RNA origami's intricate folding patterns and structural variability complicate design and stability compared to DNA-based methods [2]. Advancements in production techniques are needed for large-scale deployment [13]. Stability is another critical issue, as RNA origami structures are prone to degradation in biological environments, necessitating strategies to enhance their stability and preserve functional integrity *in vivo* [4]. Non-specific binding in complex samples can compromise efficacy, requiring mitigation strategies [15].

The encapsulation efficiency and structural integrity of RNA origami depend on specific assembly conditions, emphasizing the need to optimize these parameters for robust construct formation [17]. Despite advanced methods like SRAM enabling complex shape construction with fewer tile types, thermodynamic stability concerns persist, limiting the duration of assembled structures. Impurities in crude RNA extracts can affect folding efficiency and nanostructure quality [3]. *In vitro* assembly may not fully capture *in vivo* complexities, posing challenges for biological system translation [16].

Incomplete folding or assembly can reduce the efficiency and yield of functional structures [9]. Kinetic dead-ends during folding complicate the assembly process, impacting yield [11]. Limitations also arise from reduced capacity compared to equilibrium models and premature shifting due to strong non-reciprocal interactions [10].

Addressing these challenges is crucial for advancing RNA origami in nanotechnology and biotechnology. Strategies to enhance robustness and scalability, such as using ribosomal RNA as a cost-effective

scaffold for stable 2D and 3D nanostructures, can facilitate their use in therapeutic delivery systems, biosensing technologies, and novel synthetic cellular systems [2, 3, 4, 6].

6.2 Design and Assembly Optimization

Optimizing RNA origami design and assembly is vital for enhancing functionality and expanding therapeutic applications. Future research should refine production processes for improved scalability and efficiency, explore additional aptamer combinations for RNA origami anticoagulants, and conduct *in vivo* studies to better understand efficacy and safety profiles [13]. The complexity of biological systems presents challenges, necessitating innovative strategies to integrate RNA origami with biological environments while maintaining structural integrity and functionality [12].

Enhancing Förster Resonance Energy Transfer (FRET) systems within RNA origami is critical for optimization, allowing more accurate monitoring of conformational changes and biological interactions [7]. This could lead to responsive RNA-based nanodevices capable of real-time monitoring and modulation of biological processes.

By implementing targeted optimization strategies, researchers can significantly enhance RNA origami design and assembly, utilizing ribosomal RNA (rRNA) as a cost-effective scaffold. This advancement improves stability and functionality, demonstrated through successful construction of diverse 2D and 3D shapes, facilitating applications in nanotechnology and biotechnology, particularly in biomedical delivery and enzyme nanoreactors [2, 3]. Such developments will advance RNA-based therapeutics and diagnostic tools, propelling RNA nanotechnology forward.

6.3 Integration with Biological Systems

Integrating RNA origami with biological systems requires strategies to enhance biocompatibility and functionality. The split Broccoli aptamer system exemplifies advancements in RNA origami design for biological integration, being non-cytotoxic and cell-permeable, facilitating live cell imaging applications [15]. This underscores the importance of developing RNA constructs that interact seamlessly with cellular environments while maintaining structural integrity.

Future research should prioritize improving the biostability of RNA origami structures to ensure durability *in vivo*, enabling their use in therapeutic and diagnostic applications [4]. Additionally, optimizing RNA origami designs to incorporate active RNA elements, such as ribozymes, could expand their functional repertoire, offering novel mechanisms for cellular modulation and intervention [6].

The design of RNA scaffolds and staple strands is crucial for successful integration with biological systems. Optimizing these components can enhance folding efficiency and stability, facilitating diverse biological applications [9]. Furthermore, refining assembly methods to accommodate a broader range of shapes and improve efficiency is essential for expanding RNA origami versatility in biological contexts [14].

Tackling RNA origami challenges, such as stability and structural integrity, and implementing strategic design optimizations can significantly enhance the integration of ribosomal RNA-based origami with biological systems. This advancement opens new avenues for innovative applications in nanomedicine, including targeted drug delivery and artificial ribozymes development, as well as in synthetic biology, where precise RNA structure engineering can facilitate complex biochemical functions and interactions [2, 3].

6.4 Technological Advancements and Future Research

Future RNA origami research requires significant technological advancements for enhanced precision, scalability, and applicability. Exploring universal self-assembly principles and their integration into biological processes can provide a foundational framework for designing efficient RNA origami constructs [12]. Leveraging these principles can lead to robust and adaptable RNA nanostructures functioning effectively in diverse biological environments.

Integrating error correction mechanisms within RNA origami assembly processes is another critical area. Applying kinetic encoding to information processing tasks in biological and synthetic systems could enhance accuracy and reliability, expanding potential applications [8]. Optimizing assembly

conditions and investigating RNA origami methods' applicability to a broader range of viral capsids and cargo types could enhance these nanostructures' versatility [17].

Understanding RNA spatial distribution within condensates and assembly dynamics at intermediate concentrations warrants further investigation to gain insights into RNA origami formation and function mechanisms [1]. Such understanding can lead to strategies for optimizing RNA origami assembly and stability under various environmental conditions, potentially resulting in new antiviral strategies and therapeutic applications [16].

Optimizing extraction protocols and exploring additional RNA sources are crucial for improving rRNA-DNA origami constructs' stability and scalability under physiological conditions [3]. This will enhance RNA origami's practical applicability in biomedical contexts, where stability and functionality are paramount.

Future research directions also include exploring additional flavors and sequences to enhance foldamer diversity and improve RNA origami folding protocols' robustness [11]. By optimizing the trade-off between structure heterogeneity and interaction sparsity, and exploring time-dependent interactions, researchers can mitigate premature shifts and enhance RNA origami constructs' structural integrity [10].

Recent advancements in RNA origami, particularly through hybrid RNA-DNA structures, 3D wire-frame designs, and ribosomal RNA as scaffolds, present significant opportunities for creating intricate and functional nanostructures. These innovations enhance RNA origami's structural and functional capabilities, paving the way for diverse applications in nanotechnology, synthetic biology, and medicine, including targeted drug delivery, biosensing, and synthetic cellular systems engineering [2, 3, 4, 6].

7 Conclusion

The investigation into RNA origami underscores its transformative potential in the realm of immune modulation through the meticulous crafting of RNA nanostructures. Platforms utilizing RNA origami, particularly those incorporating thrombin-binding aptamers, exhibit enhanced anticoagulant properties, surpassing conventional methodologies and underscoring their therapeutic viability. The strategic design of RNA nanostructures to interact with innate immune receptors presents a pioneering approach to modulating immune responses, paving the way for cutting-edge immunomodulatory therapies.

Furthermore, the role of the electrostatic environment in maintaining RNA structure stability is highlighted as a critical factor in developing RNA origami applications tailored for immune modulation. This understanding is pivotal for creating robust RNA constructs that can withstand physiological conditions, thereby amplifying their therapeutic potential.

Collectively, these insights highlight the innovative potential of RNA origami in both nanotechnology and immunology. Leveraging the distinctive characteristics of RNA nanostructures enables researchers to develop sophisticated strategies for immune regulation, facilitating novel therapeutic interventions and expanding the horizons of RNA-based technological applications.

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