RNA NANOTECHNOLOGY

Jayachandra S. Yaradoddi[1,2,6*], Merja Kontro[2], Sharanabasava V. Ganachari [1*], Sulochana M. B.[3] Dayanand Agsar [4], Rakesh Tapaskar[5] and Ashok S. Shettar[1]

[1] Centre for Material Science, Advanced research in Nanoscience & Nanotechnology, School of Mechanical Engineering, KLE Technological University, Hubballi-580031, Karnataka, India

[2] Department of Environmental Sciences, University of Helsinki, Niemenkatu 73, Lahti 15140, Finland

[3] Department of PG Studies and Research in Biotechnology, Gulbarga University, Kalaburagi-585106, INDIA

[4] Department of PG Studies and Research in Microbiology, Gulbarga University, Kalaburagi-585106, INDIA

[5] Energy Cluster, Centre for research in Renewable & Energy Systems, School of Mechanical Engineering, KLE Technological University, Hubballi-580031, Karnataka, India.

[6] Extremz Biosciences Private Limited (Govt. of Karnataka Funded Start-up), CTIE start up street, KLE Technology University campus, Hubballi-580031, Karnataka, India.

ABSTRACT

DNA, RNA and proteins are seemed to be immensely substantial tools for nano biotechnological applications, this is since their exceptional biochemical properties and role. Particularly RNA is categorized over comparatively high temperature stability, varied organizational pliability and their performance natural circumstances. Above properties made RNA a valued constituent for bio-nanotechnology processes and usefulness, especially RNA Nanotechnology, could synthesize complex molecules using simple molecules through de nova nanostructures having exceptional utility by the strategy, integration and manipulations of most predominant processes which are usually based on different RNA structures and because of their vital biochemical properties. The current chapter emphasis on the basic principles inspires the normal design of RNA nanostructures, pronounces the important methods are used in constructing nanoparticles self-assemblages, further describes the associated challenges and excelled opportunities of RNA nanotechnology in near future.

1. Introduction:

Engineering or manipulation of active systems at nanometer scale in known as Nanotechnology, the principle governing material and device design varies intensely as of those instituted at the macro level. Utmost importantly, all together involves dimishment or trimming of current technologies to up to nanometer scale (Figure 1), where the mode of manipulations micro and macromolecular systems since their beginning of a hierarchy upwards, constructing basic new potential technologies which tends to transform organism's manipulations towards commercialization. That is usually comprises information technology, nano electronics and nanomedicine applied in numerous pharmaceuticals, biotechnological applications and many more to come.

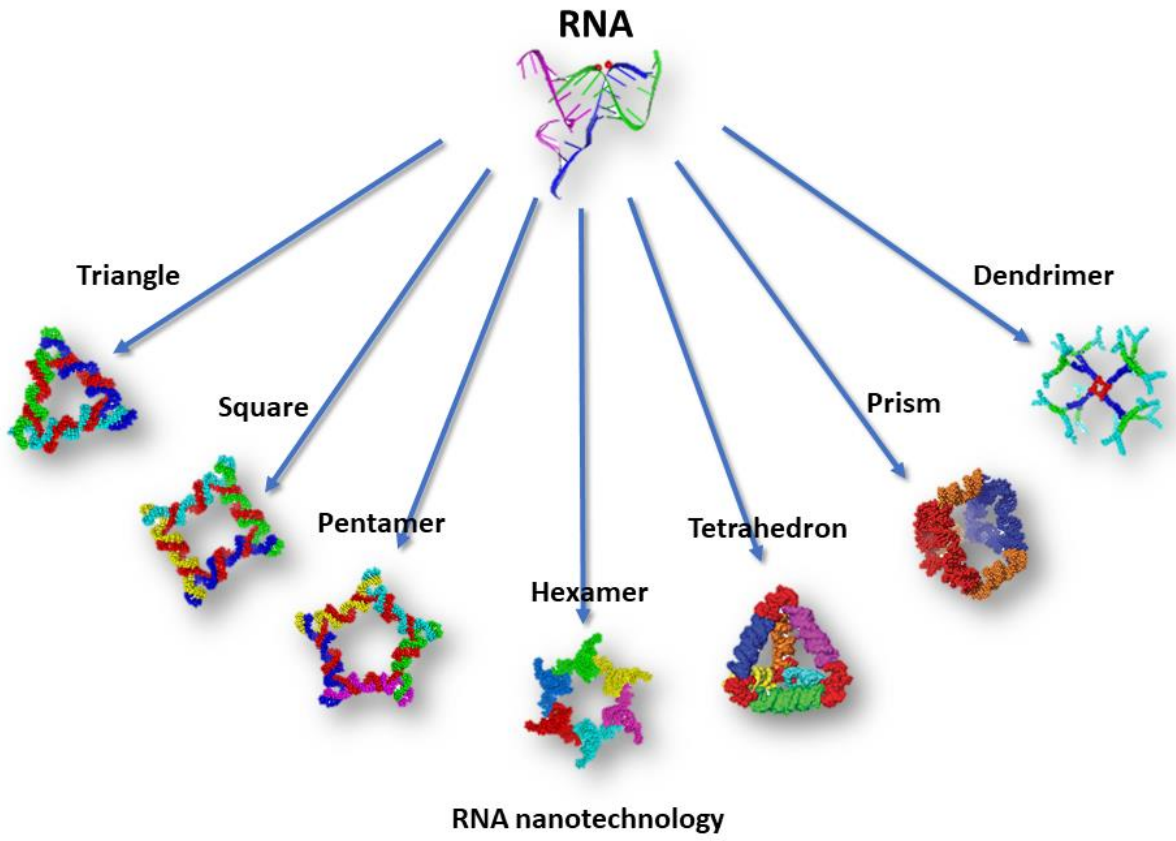


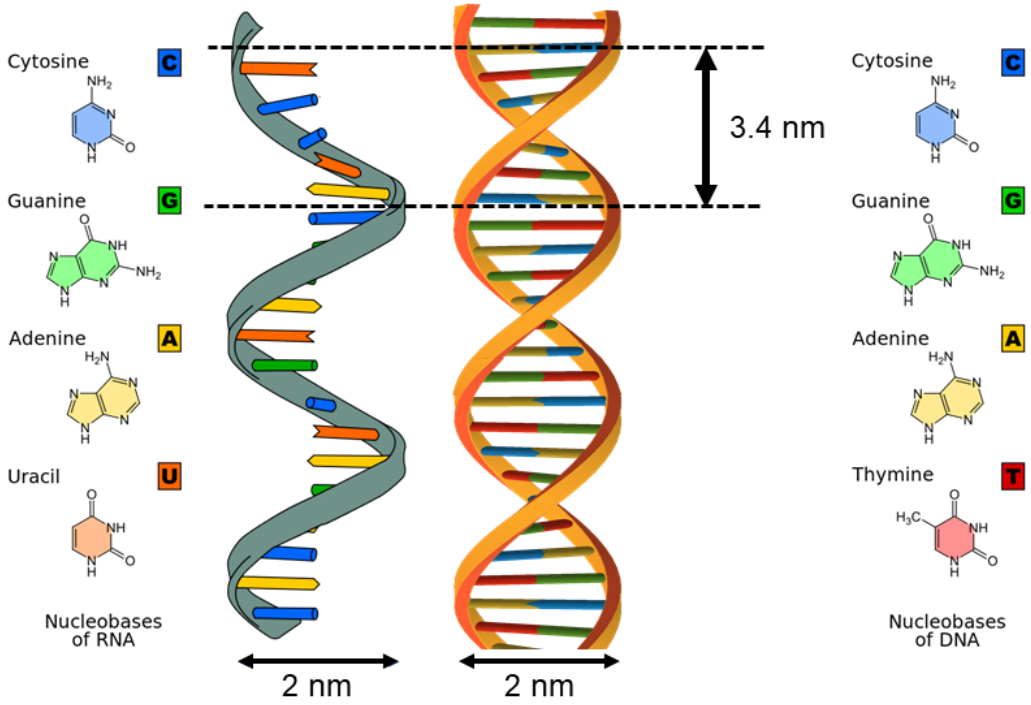
Figure 1. Schematic representation of RNA nanotechnology and its associated applications.

The range of ideas and advantages of nanotechnology interconnect with corresponding broad groups of methods and constituents, because they comprise exceptional properties. One among

such unit is related with the structuring of biochemical processes, systems and biomolecules in innovative forms and functions, which widely called as bio nanotechnology [1].

According to the reports of Nadrian Seeman and coworkers, area of nanotechnology capitalizing the existence of nucleic acids has its genesis. Since 30 years, they commanded the progress of DNA nanotechnology by using DNA structures [2-5]. Concisely, this technology utilizes the properties of DNA especially the complementarity properties for structuring DNA fragments with distinct secondary structures through established Watson and Crick interactions such as A-T and G-C base pairing, comparatively lesser number of basic organizational rules constructed on nucleic acid structural motifs (Figure 2). These basic organizational rules give rise to manipulation and testing of different 3D DNA scaffolds through various interconnection platforms [6-10] and few among them are potentially applied targeted delivery systems through functions as DANA nano capsules [11&12] or DNA nano transporters for other molecular functions [13].

Beside this there is another technology called as DNA-origami, which was introduced by Paul Rothemund [14] and further it has been stretched from its basic constraint for creating various 2D DNA shapes [15] and they are useful models to create 3D objects like pyramidal tetrahedron, nanorobot, functional nanobox or shapes depends upon the structural integrity (transegrity) or helix bundles. In similar fashion Chad and his colleagues have carried out substantial research work using oligonucleotides of DNA in synthesizing nanoparticle probes (NPP) through altering colloidal nanoparticles by relatively small numbers of nucleotides (oligonucleotides) on addition of complementary or corresponding sequence, that permits the self-assemblages of nanoparticles into 2 to 3D (3 dimensional) designs [1].



DNA-RNA structure comparison

Figure 2: Showing comparative model of single stranded RNA and double stranded DNA molecules and their base pairing, and also indicated the uracil nucleotide replaced in place of thymine nucleotide which we could generally see in DNA molecule.

The characterization of RNA as a messenger in especially transcription process was deliberated in 1940s and 1950s [16] its molecular usefulness was described in 1960s [17]. Later stage Tom Ceck and Sidney Altman who confirm the rRNA precursor splicing has transformed the understanding of RNA [1]. Before being catalytic activity supposed to be kept only for proteins, and this innovation led to the chains of additional multitasking functions consigned to RNA. In later year's innovations by Craig Mello and Andrew Fire, concerning to the potentials of RNA on selective regulation of gene expression in animal models by using interference RNA (RNAi) technology, has paved the way for RNA as an exceptionally vital molecules for understandings exclusively their applications in science, molecular medicine and engineering.

Specifically, the capability in understanding in creating suitable functional RNAs directed towards originating the subdivisions such as RNA nanotechnology, breakthrough in RNA nanotechnology occurred after the innovations of Luc Jaeger, Neocles, Eric Westhof and

Peixuan Guo- targeted to overcome the problems associated in synthetic biology and nanomedicine. As per Bramsen *et al* [18], presently there are about 20 different RNA nanotechnology-based drugs under clinical trials. Among them major drugs are now in phase I and phase II trials, unfortunately, yet none of them reached the phase III of the clinical trials.

After achieving initial success, the present technology was overweighed because of huge expectation and deliverables [19], anyhow nowadays the gains by the use of RNA nanotechnology has been progressively improved for smart devices applications. This is reality, because many growing startup companies are working on production of highly innovative RNA nano therapeutic drugs. Which is exclusively strengthening of diverse catalytic activities all together organizes the forms and functions with the specific stoichiometry and orientation in 3D space.

2. The basis of RNA nanotechnology

The strong basis of RNA nanotechnology attracted various fields in relation to concepts of RNA structure and their function that strengthen both along with this, it can immensely increase the design development for defining the sequences required for the accurate folding and assemblages of RNA nano objects. In most of the cases RNA design methods basically works in converse of RNAs standard structure functional identification practices. Generally RNA sequences are presumed and problem at finger is to define the secondary structure of RNA and eventually the RNA three dimensional structures. This is often achieved by the NMR and X-ray crystallography techniques, nevertheless RNA secondary structural information, whether resulted by the laboratory experiments or computational algorithms, is very useful in determination of complex 3 D structure of RNA molecule. Once tested, the 3-dimensional structure later gives stage from which function can be well described and known. In exactly reverse manner RNA nanodesign firstly chooses among the 3-dimensional structure and specific position of scaffold assembly and functional elements and then is the secondary structure and finally primary structure defined. Since from many years' lots of efforts have been put in algorithms development especially for the computational forecasting of secondary and three-dimensional structures of RNA, then works were carried out on de novo with significant contributions using secondary structure.

The main important characteristic features of 3-dimensional RNA structures (Figure 3) have also been deliberated practically, for instance, single molecular imaging or computationally by molecular dyanamics. Given techniques have both provided and also have employed

information gained on structure and function of virsl genomes and non-coding regions of RNAs (example: ribosomes. Therefore, the information acquired from these broad studies on RNA structure and function is now being useful to design nanoparticles using RNA [1].

Nanoparticles ability to form multivalence, degradation tolerant and targeting specific molecules have been established [20] (see Functionalization and Modification). The RNA nanotechnology provides numbers of applications especially for nanomedicine. And it is also prominently accelerated with special attention on designing biocompatible molecules when comparing with other molecules such as liposomal, peptide or artificial nanomaterial conjugates for RNA based functionalities (Figure 4).

Nanoparticles synthesized using RNA can be furnished with traditionally modified with different combinations of therapeutic drugs or biosensors, they demonstrate potential improvement in targeting and penetration of cells or tissues in safe and efficient way [21], they also delivers in mixtures like multi target therapies, concurrent imaging and advanced imaging technologies with proper therapeutics. Apart from this, they promise strict regulation of three-dimensional orientation and stoichiometry of these agents.

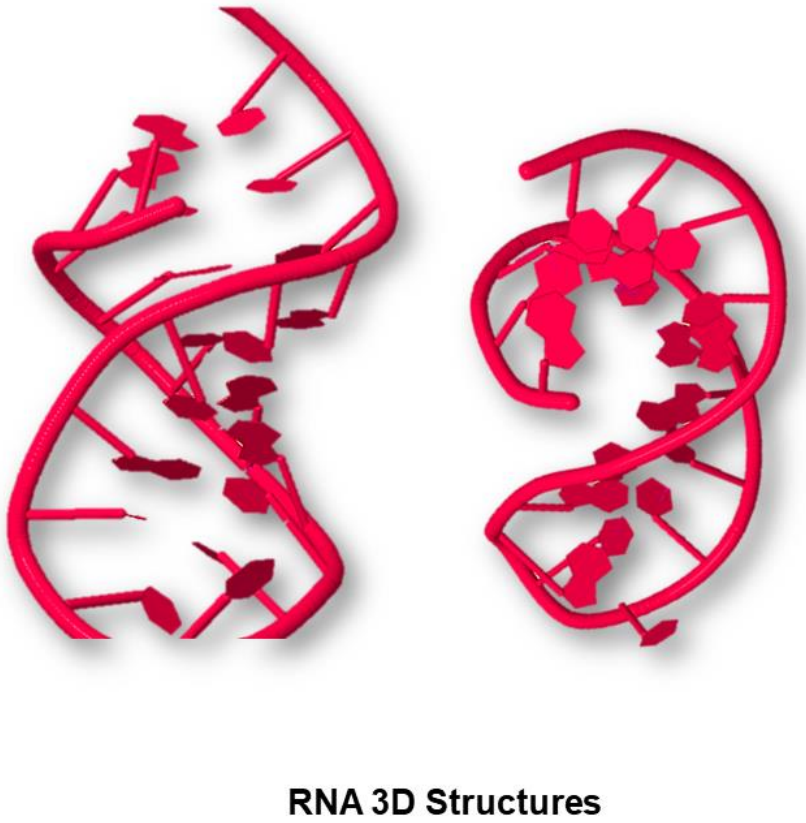


Figure 3: Depicting 3-D structure of RNA molecule.

Lastly, remedies rely on these doctrines based on distinct mechanism by the current drugs and accordingly straightaway it can develop the advantageous potential of current therapies yielding innovative opportunities with a specific focus on therapies towards the treatment of disease caused by the drug resistant microbes they are usually termed as fatal diseases. Easy adaptability of RNA molecule, requires relatively less free energy in RNA strengthening, due to ductility of sequence, selection in structural arrangement, regulator, also by characteristic feature such as self-assemblage kind of RNA is an ultimate component in bionanotechnology applications. Potential familiarization of RNA paradigm patterned, superstructures or designed arrangements. RNA gene sequence can facilitate the development of hexagonal palladium nanoparticles [22].

Encoded self-assemblage nature of RNA ladders can dictate prearrangement in cation-based gold nano particles; intermittently spaced RNA constructions can help to form scaffolds of nanocrystal [23]. Mathematically regular forms like trimers, polygons dimers could be created by RNA molecule [24]. Self-assemblage connection between interlinking coils, self-association by a palindromic order, the constant growth to classified assembly, comfortable association biological empathy makes RNA molecule a very decent constituent to build scaffolds exclusively in group of cells manipulations or engineering [25]. Numerous research laboratories have established aptamers from RNA as a biological sensor.

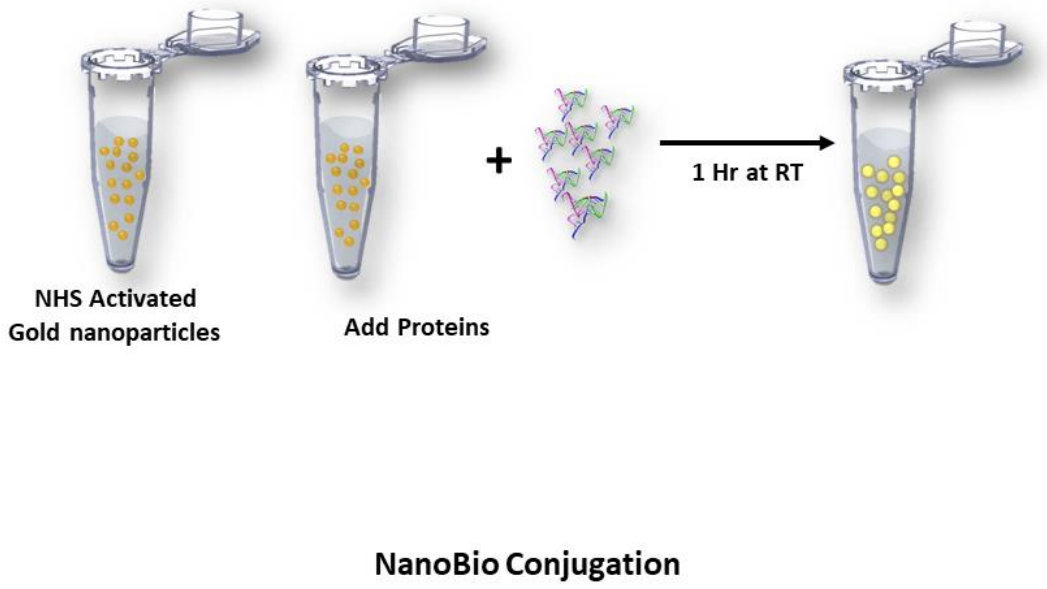


Figure 4: A model combination of natotechnology and biotechnology method in producing industrially important product.

3. Opportunities using RNA nanotechnology

Recent advancement in RNA known for their innovation in nanotherapies or nanomedicine applications that comprise cell detection and binding analysis; well directed transport through receptor molecule facilitated the cellular ingestion, intracellular regulator and computation through gene silencing mechanism, nuclear coat infiltration, and brain plasma barrier transitory. Utmost important medical or beneficial RNA components are discussed below.

3.1 Small interfering RNA (siRNA)

It is generally coiled with nearly 25 nucleotides long and restricts the gene expression by splitting the messenger RNA by a protein-RNA complex named RISC (RNA-induced silencing complex). The small interfering RNA particularly conquers the pronouncing targeted protein whose messenger RNA comprises a gene sequence similar to the sense strand of the interfering RNA. This finding was made in the year 2006 by the scientists Andrew Fire and Craig Mello and they awarded with a Nobel Prize [26].

3.2 Ribozymes

They are typically RNA molecules and catalysis numerous enzymatic and biochemical reactions. Ribozymes are therapeutically very important because they can regulate the gene function by interference and cleaves RNA substrates. Viral genomes RNA and messenger RNA encompasses a gene sequence that corresponds to the catalytic center of ribozyme. Scientists Thomas C. and Sydney Altman were awarded with the Noble Prize in the year 1989. Above this described ribozyme mediated technology has due importance in designing therapeutic drugs development.

3.3 RNA aptamer

These are oligonucleotide molecules and their functions are comparable with the antibodies since they have got potential in identification of particular nucleotides, peptides or organic compounds by establishing binding compartments SELEX [27]; this is one technique to scrutinizing the aptamers unarranged RNA pools developed by Ellington and Szostak [28], and Tuerk and Gold [29]. Through the present technique, numerous RNA aptamer has been designated to target markers applicable towards specific disorders [30].

3.4 Riboswitches

RNA molecular machineries that bind tiny particles that regulates gene expression in reply to organism's requirement. In concerning to the biological regulator mechanism, riboswitches (Figure 5) can identify complex metabolites, and tend to bring early termination of messenger RNA transcription, stops ribosomes from translating messenger RNAs, to cut these messenger RNAs and they activate messenger RNA degradation [31]. Consequently, these RNA switches could be manipulated to produce an innovative age group controller device structured by means of drug-like particles near poise the expression levels of selected genes of living organism. This corresponds the RNA originated gene-regulator device hold potential in upcoming gene therapies by providing nano-scale *cis*-acting modulation [32].

Several RNA constituents that includes small interference RNAs, antisense RNAs, ribozymes, riboswitches, aptamers together with other catalytic or excision RNA shall be simply combined into RNA nanoparticles. The key benefits of RNA nano medicine contain the following: 1) self-assemblage 2) multiple-valency; 3) selected transfer; 4) non-protein 5) nano size; 6) measured mixture of distinct assembly stoichiometry; 7) combination treatment, analysis remedy effects into single particle.

Converse assemblage in RNA gene molecule can tend to multiple-valency. Every component might be discretely functionalized to transport diverse beneficial payloads, reporter genes and pinpointed ligands. Cell-based-specific transfer permits a lesser dose of drug to be administered, consequently that carries reduced lateral effects. The multivalent method comparable to that of combination treatment, where a combination of medications is used to produce a collective result. Multi-valency schemes a supplementary exclusive benefit, in which treatment uncovers the therapeutic possessions may be grouped as nanoparticle exhibited beneath lone administration [33].

Presently, a diversity of additional multivalent nanoparticles has been characterized; though, constructing homologous units and steady duplication in copy numbers among population is interesting. Somewhat ambiguity of construction, stoichiometry can cause random lateral effects or generic noxiousness. Through the RNA nanotechnology, assemblages of similar nanoparticles could be "developed", typically having extremely higher reproducibility rate, distinct assembly, thus easing superiority and protection.

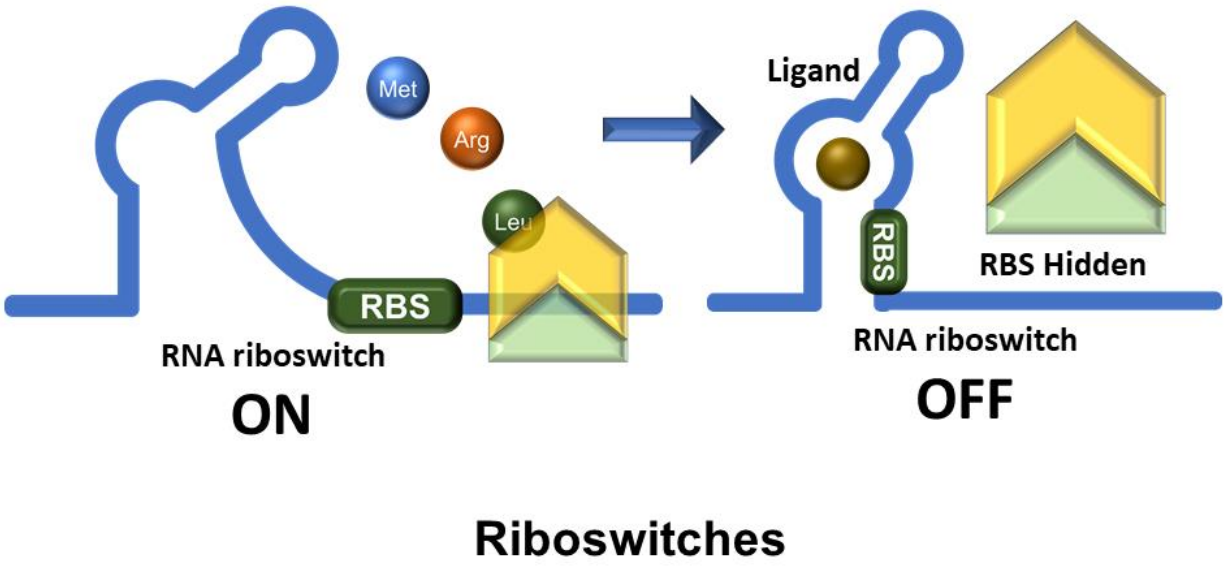


Figure 5: Typical mechanism of action of riboswitches.

Since molecular sizes of the RNA molecules measured in nano-scale remains additional benefit. Especially to active transfer of these nanoparticles to the diseased tissues or organs, numerous studies recommend particles ranging from 10–50 nm are perfect to non-viral vectors as they contain huge adequate to be taken form and sufficient to pass through the cell membrane structures by the cell surface receptor facilitated endocytosis process. Nanoparticle transfer probably best candidature in advancing the pharmacodynamics, bio distribution and pharmacokinetics. Non-protein property can circumvent introduction of foreign particles, permitting repetitive administration in therapy of long-lasting disorders including tumor, diseases caused by viruses, also hereditary disorders. Furthermore, RNA nanoparticles can be categorized by the food and drug administration.

Prospect in RNA nanotechnology in disorder treatment demonstrated in the phi29 pRNA therapeutic organization [33]. Cultivation of the artificial multivalent RNA nanoparticles comprising receptor-binding ligands or aptamers triggered the cell binding and entrance for administered drugs, then programmed cell death that is apoptosis. The distribution effectiveness and therapeutic outcome confirmed later during the animal test trials. The 3-dimensional design, circular arrangement, folding energy, and nucleotide dissimilarity in RNA are useful to produce RNase tolerant RNA nanoparticles through reduced noxiousness and to confirm processing of chimeric RNA complexe inside small interference RNA by Dicer after the transport.

4. Challenges associated with RNA nanotechnology

Formation of RNA nanoparticle comprises coupling of functionalities, module linking, subunit labelling and chemical alteration of nucleic acid fragments. Both chemical and biological methods are applied in producing RNA structure blocks. Though the present technology has raised much heights, still there is an opportunity for the improvement. RNA molecular structure prediction is Solitary of the foremost important contests in RNA nanotechnology is to recognize the exact assembly of the folded RNA nanoparticle. Since the uncommon folding characters, the rule is to elucidate folding of RNA gene molecules are yet to be categorized. By using RNA 2 dimensional prediction system, only up to 70% of structure can be forecasted precisely [34]. However numerous online resources like Mfold, 233 RNA designer, 234 Scaffold, 235 NUPACK, 115 nanofolder, 236 and hyperfold, 182 have been established, forecasting of precise RNA assembly and folding remains tricky. RNA 3 dimensional and 4-dimensional assembly forecast is even more understated. Progressions in bioinformatics tools have further perceptions in RNA structure forecast and computing intermolecular interactions but still more sophisticated tools are most anticipated [35].

4.1 Stability

Natural form of RNA molecule is extremely sensitive to nucleases enzyme especially the RNases. Because of this uncertainty it has become a major limiting issue for its use in nano material structure. With the progression in technology, numerous key measures have been taken into account to increase the stability of RNA gene molecule which comprises; chemical alteration of bases (5-Br-Ura and 5-I-Ura); modifications in phosphate linking and variations like 2′-F, 2′-O-Me, 2′-NH_2 on C2′ carbon [217, 218]. RNA integrity can also be amplified through the synthesis of peptide RNNs, locked nucleic acids with joining and 3′ plugging [36]. Amongst these the 2′-fluorine alteration is most appropriate since its effect on RNA molecular folding and role is negligible [37].

4.1 In vivo half-life and preservation time

In vivo preservation time of a nanoparticle can be its magnitude, making it a critical thing in RNA nanotechnology. Many methodical reports propose 10 nm–100 nm sized particles are optimum for non-viral vector constructed transfer. Particles inside this array are big enough to be reserved in the form and slighter enough to bind the cell receptors and they cross cell membrane [38]. Henceforth ideal extent of RNA nanoparticle is so vital for its biological distribution and advanced preservation duration for in vivo transfer. The half-life of chemically altered RNA nanoparticles was found to be 5–10 h [39]. So, chemicals alterations have

someway reduced, this tricky issue connected to RNA nanoparticle half-life and preservation time but still, there is a space for the improvement.

4.2 Limited carrying capacity

Now, RNA based nanoparticles have restricted carrying extent and is a main problem connected to the RNA nanotechnology. The terminal end of the nanoparticle can be characterized by the molecule, mainly of reduced size. Entire chain classification can intensify the carrying capacity of the nanoparticle nevertheless might leading to misfolding of the nanoparticle due to steric interference eventually proceeded to release of the intended molecule. Computational approaches can be employed to pinpoint the site where the target molecule can be presented without damaging the nanoparticle. The carrying ability of RNA nanoparticles can be augmented by creating dendrimers and RNA dendrimers have been industrialized up to generation 4 [40]. Dendrimers usually can rise the carrying capacity since branched construction and therefore can lead to enlarged transfer of therapeutic RNA molecule. Nevertheless, saturation of therapeutic RNA can lead to gene degradation and dissimilar binding and can cause cytotoxicity within the organisms [41, 42]. So, RNA nanoparticles with minor carrying ability are more advantageous especially in animal models because of their lower toxicity level. Consequently, this can boost the improvement of RNA nanoparticles with high carrying capacity and lower toxicity and it is a great opportunity for researcher in exploring with this specific challenge.

4.3 Scaling up

Because of the high production cost of RNA nanoparticle molecules has limited its applications in pharmaceutical industries. Majority of RNA particles are majorly designed inside the range of chemical combination (Max. of 80 nt). Through the advancement in the technology, the price of oligosynthesis is significantly reduced. Nowadays 10 gm of RNA can be produced per cycle. Though, industrial scale purification is still tricky. Gel electrophoresis and HPLC have limited competences giving low outcome. Recently, ultracentrifugation technique is used for the purification of RNA nanoparticles with significantly high in production [43].

4.4 Endosome escape

RNA based nanoparticles can also be delivered to human body by the way of receptor mediated endocytosis process. Accordingly, chasing the RNA nanoparticle inside the cell is been added foremost important challenge in reference to RNA nanotechnology. Which is just similar to endocytic pathway, then these RNA nanoparticles could develop primary endosomal vesicle.

Once after arrangement, RNA based nanoparticles can be moved to the late endosome and then to lysosomes, where these nanoparticles get entrapped themselves instead reaching the target molecules.

5. Conclusion

In combination of superior design and approaches of RNA based nanoparticles can applied in developing various computational methods and production processes. The current in RNA based nanotechnology have promising applications in synthesis of modular RNA units by establishing broad variety of compact, thermostable constructions or structures. RNA electronics can offer realistically engineered tertiary structures into RNA nanoparticles gathering increases cells or tissue immunity towards the nuclease enzyme exclusively RNases catalysis activities and demonstrated by molecular tiling puzzle.

Comprehensive studies of kissing interactions (these interactions that are made in RNA molecule when two nitrogenous bases between two hairpin loops pair) and later repairs by tertiary folding multi-helix connections have experimentally revealed some of the thermodynamic limitations that dictate RNA folding. Too many contests to comprehending the conceivable of RNA nanotechnology is been continued, nevertheless with additional expansions in computational and design techniques, collectively with an ever-growing library of information of RNA structure constructions and their properties, we can be able pass through the coherent formation of steady complex, multidimensional constructions gifted to perform numerous characters in nanomedicine, pharmacology, synthetic biology, and in other superior fields of bio nanotechnology in overall.

References:

[1] Kirill A. Afonin, Brian Lindsay, Bruce A. Shapiro, (2013). DOI: 10.2478/rnan-2013-0001, RNAN, 1-15

[2] Garibotti, A.V., Liao, S. & Seeman, N.C. (2007). A simple DNA-based translation system. Nano letters 7, 480-483.

[3] Lin, C., Liu, Y. & Yan, H (2009). Designer DNA nanoarchitectures. Biochemistry 48, 1663-1674.

[4] Seeman, N.C. (2010). Nanomaterials based on DNA. Annual review of biochemistry 79, 65-87.

[5] Andersen, F.F. et al (2008).. Assembly and structural analysis of a covalently closed nano-scale DNA cage. Nucleic acids research 36, 1113-1119

[6] Erben, C.M., Goodman, R.P. & Turberfield, A.J. A self-assembled DNA bipyramid (2007). Journal of the American Chemical Society 129, 6992-6993.

[7] Goodman, R.P. et al (2008). Reconfigurable, braced, three-dimensional DNA nanostructures. Nature nanotechnology 3, 93-96.

[8] He, Y. et al (2008). Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra. Nature 452, 198-201.

[9] Shih, W.M., Quispe, J.D. & Joyce, G.F. (2004). A 1.7-kilobase single stranded DNA that folds into a nanoscale octahedron. Nature 427, 618-621.

[10] Aldaye, F.A., Palmer, A.L. & Sleiman, H.F. (2008). Assembling materials with DNA as the guide. Science 321, 1795-1799

[11] Bhatia, D. et al. (2009). Icosahedral DNA nanocapsules by modular assembly. Angewandte Chemie (International ed 48, 4134-4137

[12] Yang, H. et al. (2009). Metal-nucleic acid cages, Nature chemistry 1, 390-396

[13] Lee, H. et al. (2012). Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery. Nature nanotechnology 7, 389-393

[14] Rothemund, P.W. (2006). Folding DNA to create nanoscale shapes and patterns. Nature 440, 297-302

[15] Andersen, E.S. et al. (2008). DNA origami design of dolphin-shaped structures with flexible tails. ACS nano 2, 1213-1218

[16] Geiduschek, E.P. & Haselkorn, R. (1969). Messenger RNA. Annual review of biochemistry 38, 647-676

[17] Lacey, J.C., Jr. & Pruitt, K.M. (1969). Origin of the genetic code. Nature 223, 799-804.

[18] Bramsen, J.B. & Kjems, J. (2012). Development of Therapeutic-Grade Small Interfering RNAs by Chemical Engineering. Frontiers in genetics 3, 154

[19] Krieg, A.M (2011). Is RNAi dead? Mol Ther 19, 1001-1002.

[20] Dibrov, S.M., McLean, J., Parsons, J. & Hermann, T. (2011). Selfassembling RNA square. Proceedings of the National Academy of Sciences of the United States of America 108, 6405-6408

[21] Haque, F. et al. (2012). Ultrastable synergistic tetravalent RNA nanoparticles for targeting to cancers. Nano today 7, 245-257

[22] Gugliotti LA, Feldheim DL, Eaton BE (2004). RNA-mediated metal-metal bond formation in the synthesis of hexagonal palladium nanoparticles. Science.; 304:850–852.

[23] Koyfman AY, et al. (2005). Controlled spacing of cationic gold nanoparticles by nanocrown RNA. J Am Chem Soc.; 127:11886–11887.

[24] Shu D, et al. (2004). Bottom-up assembly of RNA arrays and superstructures as potential parts in nanotechnology. Nano Lett.; 4:1717–1723.

[25] Cayrol B, et al. (2009). A nanostructure made of a bacterial noncoding RNA. J Am Chem Soc.; 131:17270–17276.

[26] Fire A, et al. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature.; 391:806–811.

[27] Ellington A. D. (2009). Back to the future of nucleic acid self-amplification. Nature Chemical Biology.; 5:200–201.

[28] Ellington A.D., Szostak J. W (1990). In vitro selection of RNA molecules that bind specific ligands. Nature.; 346:818–822.

[29] Tuerk C, Gold L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA ploymerase. Science; 249:505–510.

[30] Mi J, et al (2010). In vivo selection of tumor-targeting RNA motifs. Nature Chemical Biology., 6:22–24.

[31] Sudarsan N, et al (2008). Riboswitches in eubacteria sense the second messenger cyclic di-GMP. Science. 321:411–413.

[32] Shahbabian K, Jamalli A, Zig L, Putzer H. (2009). RNase Y, a novel endoribonuclease, initiates riboswitch turnover in Bacillus subtilis. EMBO J.; 28:3523–3533.

[33] Guo S, Tschammer N, Mohammed S, Guo P. (2005). Specific delivery of therapeutic RNAs to cancer cells via the dimerization mechanism of phi29 motor pRNA. Hum Gene Ther.; 16:1097–1109.

[34] Markham NR, Zuker M. (2008). UNAFold. Methods Mol Biol.; 453:3–31.

[35] Yingling YG, Shapiro BA (2017). Computational design of an RNA hexagonal nanoring and an RNA nanotube. Nano Lett.;7: 2328–34. https://doi. org/10.1021/nl070984r.

[36] Patra A, Richert C. (2009). High fidelity base pairing at the 3′-terminus. J Am Chem Soc. 131:12671–81. https://doi.org/10.1021/ja9033654

[37] Liu J, Guo S, Cinier M, Shlyakhtenko LS, Shu Y, Chen C, et al. (2011). Fabrication of stable and RNase-resistant RNA nanoparticles active in gearing the nanomotors

for viral DNA packaging. ACS Nano.; 5:237–46. https:// doi.org/10.1021/nn1024658.

[38] Prabha S, Zhou W-Z, Panyam J, Labhasetwar V. (2002). Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. Int J Pharm.; 244:105–15.

[39] Abdelmawla S, Guo S, Zhang L, Pulukuri SM, Patankar P, Conley P, et al. (2011). Pharmacological characterization of chemically synthesized monomeric phi29 pRNA nanoparticles for systemic delivery. Mol Ther.19: 1312–22.

[40] Sharma A, Haque F, Pi F, Shlyakhtenko LS, Evers BM, Guo P. (2016). Controllable self-assembly of RNA dendrimers. Nanomedicine.,12:835–44.

[41] Wang D, Zhang Z, O'Loughlin E, Lee T, Houel S, O'Carroll D, et al. (2012). Quantitative functions of Argonaute proteins in mammalian development. Genes Dev.; 26:693–704.

[42] Moore CB, Guthrie EH, Huang MT, Taxman DJ. (2010). RNA therapeutics. Methods.; 629:141–58.

[43] Jasinski DL, Schwartz CT, Haque F, Guo P. (2015). Large scale purification of RNA nanoparticles by preparative ultracentrifugation. Methods Mol Biol.; 1297:67–82.

[44] Vrantika Chaudhary, Sumit Jangra and Neelam R. Yadav (2018). Nanotechnology based approaches for detection and delivery of microRNA in healthcare and crop protection. J. Nanobiotechnol. 16:40

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