

REVIEW

RNA and DNA nanoparticles for triggering RNA interference

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Control over the delivery of different functionalities and their synchronized activation in vivo is a challenging undertaking that requires careful design and implementation. The goal of the research highlighted herein was to develop a platform allowing the simultaneous activation of multiple RNA interference pathways and other functionalities inside cells. Our team has developed several RNA, RNA/DNA and DNA/RNA nanoparticles able to successfully complete such tasks. The reported designs can potentially be used to target myriad of different diseases.

Keywords: RNA nanotechnology; RNA/DNA hybrids; RNA interference; siRNAs; FRET

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Along with DNA and proteins, RNA is one of the three major macromolecules that are essential for all known forms of life. However, RNA also shares the functional definition of DNA (informational storage) and proteins (catalytic, structural, transport, and defense). This functional versatility makes RNA a perfect material for building biologically active and meaningful nanostructures. Consequently, RNA nanotechnology has emerged as a significant modality in recent years due to the numerous advantages that it offers pertaining to precise control over the composition and stoichiometry of the delivered RNA-based functionalities as well as other functional moieties^[1-8].

RNA interference (RNAi) is the biological process of specific gene silencing through a natural cellular post-transcriptional regulation process that involves short double stranded-RNAs^[9-11]. The use of RNAi is showing significant potential for various therapeutic applications^[12]. Simultaneous delivery of multiple therapeutic RNAi inducers (siRNAs, miRNAs, shRNAs, etc) to diseased cells is expected to have significant synergistic effects^[13]. The

precise controlled delivery of various RNAi-based therapeutics could be achieved by building programmable RNA scaffolds that can be further functionalized and assembled into RNA nanoparticles of various shapes and compositions^[2,3,14,15].

Recently, we introduced a technique that allows the conditional activation of RNAi in vivo^[16]. The basic idea lies in splitting the functional units into non-functional fragments, followed by their designed conditional re-association and complete restoration of the original function. Using this mechanism, we split the functionality of Dicer substrate RNAs (DS RNAs)^[17] into two RNA-DNA hybrids, which when presented together inside the cell, recognize each other through toehold interactions embedded into the DNA portion of each hybrid, re-associate, and release DS RNAs. Cellular Dicer, an RNaseIII-like enzyme, is further employed to process DS RNAs into short interfering RNAs or siRNAs, which are then utilized by the RNA-induced Silencing Complex, called RISC, to activate RNAi. In order to deliver and conditionally activate split

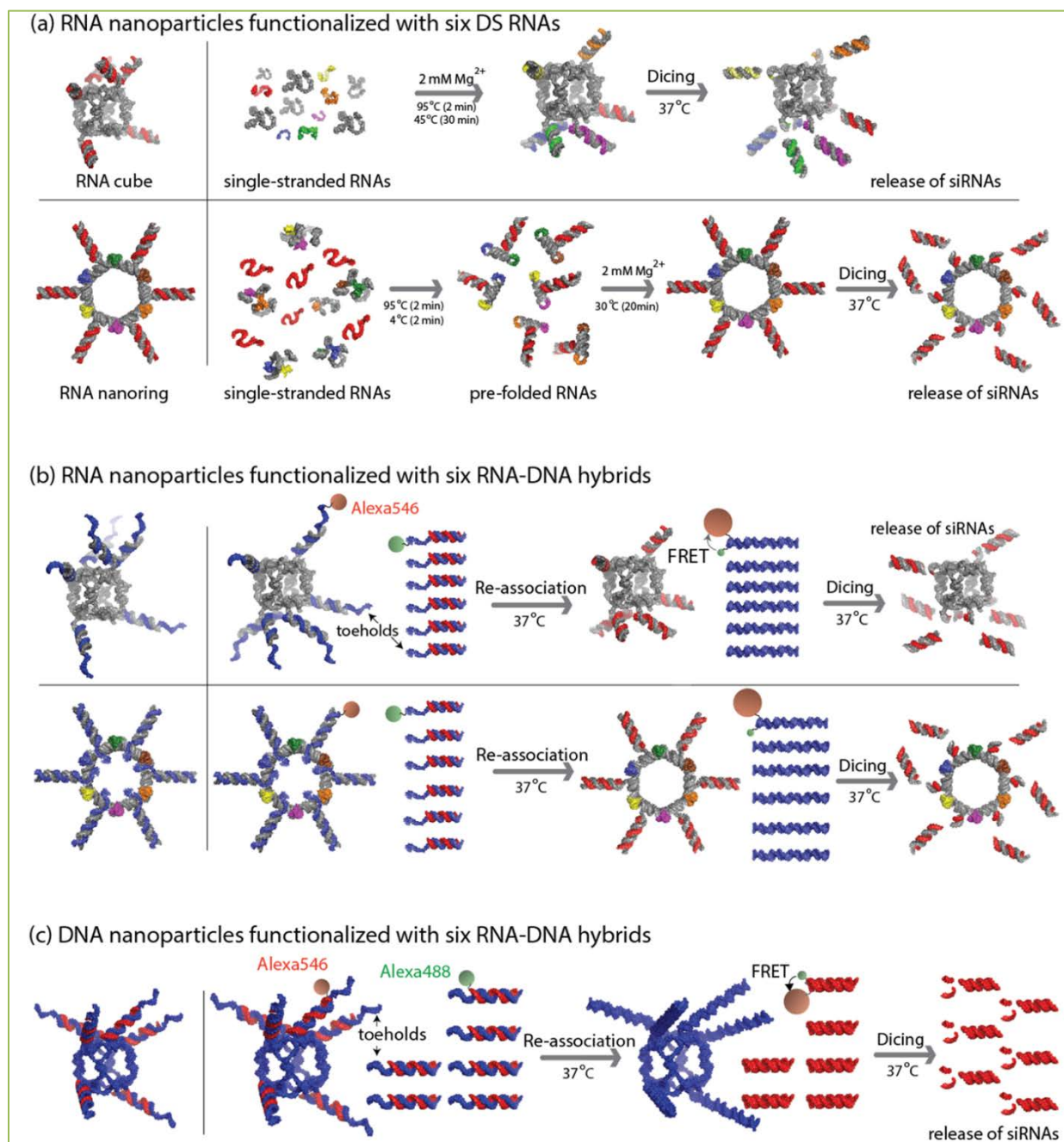


Figure 1. Application of RNA, RNA/DNA and DNA/RNA for RNAi activation. (a) Schematic representation of the assemblies leading to the formation of RNA nanocubes and nanorings functionalized with DS RNAs. (b) Schematic representation of the re-association of the RNA/DNA nanocubes and nanorings initiated by toehold interaction and further release of the siRNAs from the RNA nanorings. (c) Re-association of DNA/RNA nanocubes and hybrids trigger the release of DS RNA further diced into the siRNAs.

functionalities such as DS RNAs, FRET, or RNA aptamers, the inactive hybrids are decorated with complementary ssDNA toeholds that will interact and trigger the re-association process when both of the hybrids get close together within the same cell.

By simply elongating the hybrids, we then demonstrated the ability to simultaneously activate multiple (up to seven)

DS RNAs, aptamers and FRET^[18,19]. However, this approach was limited to the maximum lengths of the single-stranded DNA comprising the hybrids; the results from re-association of long hybrid double DNAs showed some immune-stimulatory effects^[19]. To partially overcome these problems and to diversify the approach, we designed and tested various RNA-DNA and DNA-RNA hybrid nanoparticles consisting of either RNA^[2,20] or DNA^[20] cores

decorated with six RNA-DNA hybrids (Figure 1). Two different previously extensively characterized nanodesigns – nanocubes^[21,22] and nanorings^[23,24] – were used as RNA cores (Figure 1a-b). The resulting functional RNA nanoparticles can be produced either by one-pot assembly^[23] or co-transcriptionally^[25]. However, due to the limitations of the nanoring design strategy^[23], only the nanocubes can be used as the DNA core in the DNA/RNA nanoparticles and only the one-pot assembly protocol is available for their production (Figure 1c). The single-stranded DNA toeholds appending each nanoparticle were designed to initiate the re-association after the addition of the cognate hybrids (Figure 1b-c). The regulated displacement of the DNA partner induced the assembly of the RNA duplexes, which were further processed by the human Dicer enzyme, thus activating RNAi. Various experimental results^[2,20] revealed significant cellular uptake of functionalized nanoparticles through endocytosis. Extensive levels of silencing of the targeted genes were observed and the silencing remained significant throughout the experiment even on the twelfth day post-transfection. Results showed that the silencing only occurred when the nanoparticles and the cognate hybrids were simultaneously delivered into the cells. Overall, the comprehensive cell culture experiments demonstrated FRET and RNAi activation by conditional triggering of the split functionalities in the cells. The use of RNA nanoparticles functionalized with six different siRNAs targeting different parts of the HIV genome^[26] confirmed the successful down-regulation of viral production in HIV infected cells. Another important result presented in the highlighted research^[20] revealed that the DNA-RNA nanoconstructs are potentially better suited for certain therapeutic purposes due to reduced cytokine release.

In conclusion, the novel technique highlighted here could be used to exploit the multiple existing three-dimensional shapes formed by DNAs and RNA-DNA hybrid structures^[27-32].

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