The Kinetics of Particle Release from DNA-Linked Multiparticle Drug Delivery Vehicles

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Statement of Purpose: The versatility of DNA as a reversible materials assembly tool has been demonstrated by several past studies using changes in temperature. We are interested in extending DNA as a tunable and reversible *bio*materials assembly tool and are thus limited to fixed buffer and temperature conditions. Specifically, we are investigating using primary hybridization events between complementary DNA sequences to assemble a model multiparticle drug delivery vehicle in which individual therapeutic agents are packaged together for simultaneous delivery to targeted tissue. In order to release particles from the assembly once delivered to the targeted tissue, we rely on secondary or competitive hybridization events to dissociate duplexes linking complementary particle surfaces[1,2]. Briefly, soluble secondary targets with greater affinity for the immobilized probe sequences effectively displace the original hybridization partners or primary targets to drive disassembly of the DNA-linked particles. Studying the effects of DNA sequence length and concentration on the kinetics of this disassembly process has offered insight into the development of a drug delivery vehicle with tunable release timing.

Methods: EDAC chemistry was used to couple amine-terminated oligonucleotides to carboxylate-modified polystyrene nanoparticles (200 nm) and microspheres (5.04 μm). A range of target lengths (8-16 bases) and concentrations (0.1 - 10.0 μM) were used in these studies. Flow cytometry was used to assess the kinetics and extent of competitive hybridization events between primary targets (intended to drive particle assembly) and secondary targets (intended to drive particle disassembly). First, we incubated probe-functionalized microspheres with soluble, FITC-labeled primary targets and then soluble, unlabeled secondary targets to quantify any changes in the number of primary, labeled duplexes on microspheres due to competitive displacement by secondary targets. Next, we employed DNA-linked colloidal assemblies and soluble, unlabeled secondary targets to quantify the detachment of fluorescent nanoparticles due to competitive hybridization events. These colloidal assemblies or colloidal "micelles" consisted of a nonfluorescent microsphere surrounded by a monolayer of red fluorescent nanoparticles. The uniform size of these colloidal structures allowed for consistent gating of the colloidal micelles before and during nanoparticle detachment. Confocal microscopy was used in conjunction with flow cytometry to visually monitor the disassembly process over time.

Results: Using flow cytometry, we have found that the kinetics of competitive displacement depend strongly on the overall target length, the differences in length of the primary and competitive targets, and the concentration of competitive targets. In general, most, if not all, soluble primary targets were displaced within minutes of exposure to competitive, secondary targets. Disassembly of the colloidal micelles, on the other hand, took several hours to days. As shown in Figure 1, the time required to detach half of the nanoparticles from the colloidal micelles ($t_{1/2}$) is 54 hours at 10 μM secondary target concentration.

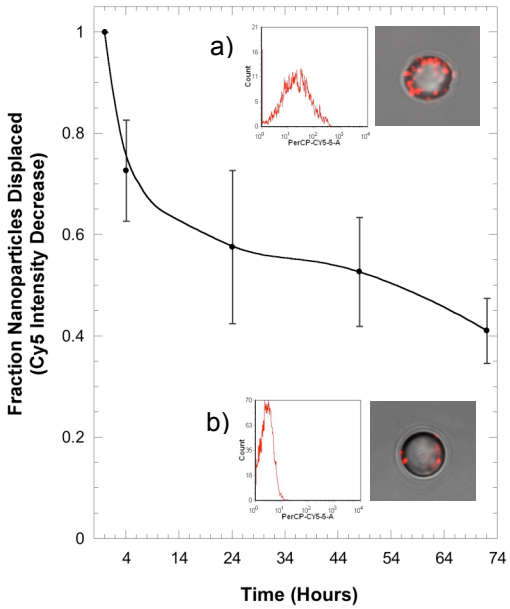

Figure 1. Kinetics of nanoparticle displacement by competitive hybridization. Insets show flow cytometry histograms along with corresponding confocal images of micelles as disassembly occurs at a) 0 hours, and b) 72 hours.

Conclusions: Through careful choice in sequence length and concentration of primary and secondary targets, we propose that DNA provides a unique recognition-based tool to program the assembly and disassembly of biomaterials for applications ranging from drug-delivery to degradable scaffolds. Our focus is now on targeting these DNA-linked assemblies to cells which exhibit upregulation of folate receptors, a common characteristic of many malignant cells, while keeping them protected from enzymatic degradation seen *in vivo*.

References:

1) Tison, C.T. and Milam, V.T. *Langmuir*. **2007**. *23*, 9728-9736.
2) Tison, C.T. and Milam, V.T. *Submitted*.