DNA Analogs for Bio-Inspired Colloidal Assembly and Disassembly
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Introduction
When dealing with sensitive diseases like cancers, it is always a difficult struggle to target tissues with the infection without harming healthy sections. Also, creating a system for these treatments becomes even more unnerving considering that every patient will have a different reaction to the drug or therapeutic treatment in use, making it impossible to create general cures.

One possible solution to this problem is to redesign the drug delivery system used for patients with these illnesses. By coupling the delivery assembly with oligonucleotides complimentary to the diseased tissues, the assembly can be absorbed by these cells and then triggered to disassemble and release its contents into the problem tissue. This way, no healthy tissue is harmed, and with the presence of oligonucleotides these medicines can be personalized to the patient taking them, which will hopefully increase their chances of recovering.

This portion of the research looks into creating layers of red and green fluorescent microspheres (which will one day be the medicine) on a base polystyrene bead to show that coupling and hybridization may be used to create these drug delivery assemblies.

Procedure
Five micron COOH polystyrene beads were suspended and washed with an in house coupling buffer, HCB. After two wash steps of vortexing, sonicating, and centrifuging at 9.9 kg for 3 minutes they were resuspended in the buffer at 1%. Then, through EDAC coupling procedures, the beads were coupled with A20-NH3 DNA and then placed on a rotomixer for 2 hours. After this the beads were washed 3 times in PBS/Tween.

Red and green 200 nm microspheres were prepared similarly. Suspended in HCB they underwent two wash steps (centrifuging at 14 kg for 5 minutes). The same EDAC coupling process was used except that one group (red and green) was coupled with B20(B10)-NH3 and the other with A20-NH3. All samples were at 1% solids in solution. Rotomixing and PBS/Tween wash steps are the same as above.

The bead shown in Figure 1 was made by the following process. Both the five micron beads and the red 200 nm microspheres coupled with B20(B10)-NH3 were brought down to 0.5% solids in solution and sonicated for 30 seconds. Then 5 micro-liters of the 5 micron beads, 25 micro-liters of the 200 nm red microspheres, and 20 micro-liters of PBS/Tween were mixed. This mixture was lightly vortexed and then set on the rotomixer for 48 hours. Ten gentle wash steps followed (NO sonication).

The second layer (green A20) and the third layer (red B20(B10)) were created by keeping the 1:5 ratio and even gentler wash steps.

Results and Discussion
Through the confocal microscope, clear rings of red microspheres could be seen around the base particles. No doublets were seen, and there were few unhybridized microspheres. The second layer was less obvious, but green microspheres were associating with the assemblies and forming a ring-like second layer. The lack of a complete layer of red microspheres coupled with B20(B10) may have led to the second layer settling into the holes, yet still hybridizing with the first red layer. In other views, there were clear relations between one red microsphere and one green microsphere. They were coupled with complementary strands, so this relation is a positive result, giving evidence towards hybridization being the primary form of association.

The third and final layer (example below) was not so clear. Again, it is possible that with the help of incomplete coverage of the base particle these microspheres could have infiltrated the assembly. This could lead to a thickening of the first layer, which is made of the same red coupled microspheres. Another possibility is that these spheres hybridized to the green second layer, but then bent over to form a second bond through the layers and onto the base particle, forming a bridge type structure. This could be a more stable state for the assembly which is why it could have occurred. However, all these explanations are speculations.

Figure 1: A three layered five micron bead with layers of red and green microspheres coupled with short DNA strands. Shift in the layers was caused by movement of the assembly. (Image taken by a confocal microscope)

Conclusion
The hybridization of more than one layer of complimentary microspheres is possible and stable with DNA strands.

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References