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Newsletter # 4: Free/Free selection

We have succeeded in developing a process for free/free selection of aptamers for target molecules. We just finished filing a patent on this, and are not yet ready to publically disclose the basis of our platform. In this newsletter, I will provide a brief overview of the platform and discuss what this means for the whole area of aptamer selection.

Our process works with normal SELEX libraries. We are using 40 nt random regions flanked by primer recognition sequences. First we impose selection for open tertiary structures, then we impose selection for closed structures in the presence of the target. We perform this two step selection in each selection round. This means that we are selecting that subset of aptamers from the initial pool of sequences that not only bind to the target molecule but also change their shape substantially as a result of this binding. We have now selected aptamers for several small molecule targets based on this platform and this approach is working consistently.

I should mention here that another advantage of this approach is that it enables us to characterize binding between the aptamer and the target with a simple syber-green fluorescence test. This is because we are implicitly selecting for a change in the shape of the aptamer upon binding. As a result the double stranded DNA binding dye syber green reports this shape change either through a gain or a decrease in fluorescence. This titrates nicely against target concentration and can be used to generate estimates of k_D

What does our free/free selection system enable?

1.) Development of aptamers for small molecules for which ligand development of any type was not previously possible.

Previously with aptamer selection it was necessary to immobilize small molecules to partition bound aptamer sequences from unbound ones. For a lot of small molecules conjugation of any type results in substantial changes to the structure of the molecule. This is also true for antibody production, as many small molecules do not induce a significant enough antigenic effect to stimulate antibody response unless the small molecule is conjugated to a larger molecule like a protein.

Often these changes are significant enough that aptamers or antibodies raised against the conjugated molecule would not bind to the free form, and the free form is what we are generally interested in. Our new free/free platform overcomes this constraint thus opening up a broader range of target molecules to diagnostic analysis.

Another key advantage of the free/free platform is that all the charge groups on the small molecule are retained and are capable of interacting with the aptamer to form hydrogen bonds, or Van der Waals force interactions. The primary constraint to obtaining better binding affinity between ligands and small molecule targets is the capacity of the small molecule to form hydrogen bonds. By not using any charge group in a target molecule for conjugation, we have the potential to identify aptamers that bind to more sites on the analyte, and thus bind with higher affinity.

2.) Identification of aptamers for complexes in situ.

I am just going to touch on this subject here. Our free/free platform enables us to identify aptamers for complexes between proteins, or between proteins and metabolites, or between metabolites. We can do this in bodily fluids. We do not have to know what the target is before we perform selection. This is really important. We can work with pools of blood serum from different individuals that all exhibit symptoms of the same disease. We counter select with blood serum from healthy patients, and we identify aptamers for what is different between the two groups. We then characterize these differences by using the aptamers in affinity columns and characterizing the identity of what they have bound to.

This positions aptamer selection as a powerful new tool for the discovery of biomarkers for diagnostic or therapeutic development. We are developing business opportunities globally based on this platform. Watch for some exciting announcements on the formation of new subsidiary companies in the very near future.

We are interested in developing joint ventures to apply this platform. Please contact us with your ideas.