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Use of aptamers in diagnostics

Aptamers are not antibodies. The diagnostic research and development field has spent decades developing optimal systems for the use of antibodies in diagnostic devices. This ranges from ELISA platforms to lateral flow to bead based applications. It is a good idea to integrate aptamers as replacements for antibodies in these existing systems because the end-user community has established expertise and invested in the equipment necessary to read ELISAs and lateral flow devices. Aptamers may open the way to new diagnostic approaches, but it does probably make sense to start with what is well established. This newsletter is focused on a discussion of some of the basics that need to be considered when developing aptamer based diagnostics in these platforms. More to the point, this entire newsletter will focus on a discussion of the immobilization of aptamers.

Aptamers tend not to bind to their targets as well as antibodies do. This disadvantage can be overcome to some extent by using the small size of aptamers to increase their concentration per surface area. Aptamers unfortunately cannot just be passively immobilized onto surfaces. Actually they will passively immobilize on surfaces very well, but that is the problem once immobilized they will become passive. They need to be specifically conjugated to surfaces.

1.) Spacers:

A lot of effort and literature has focused on what type of spacer to use between the conjugation moiety and the aptamer. I think that a lot of this is unfortunately aptamer specific. It is better to screen for aptamers that work when they are conjugated to a surface through a C6 spacer. We generally select aptamers with a 40 nt random region. This is usually more than is needed for effective binding. Some of the sequence is superfluous. It has been our experience that when we trim aptamers and test binding in a free/free system we end up with aptamers that do not work as well when immobilized. This is fixed by adding back some of the nucleotides to the 5' end that were there in the longer form.

2.) Conjugation:

This is where most aptamer based diagnostic applications fail. It is difficult to get enough functional aptamer immobilized per unit of surface area. We have tried a number of different approaches and until this last year our best case was still streptavidin coated surfaces and biotinylated aptamers. This did not make a lot of sense, given that the capacity to introduce biotin binding sites through streptavidin on a surface should be much less than the capacity to introduce functional conjugation groups. We have tried many different conjugations to passively immobilized BSA and other proteins, or to ELISA plates that already have functional groups on their surface. We even tried to create functional groups out of plastic surfaces with a KOH treatment. Always, less binding activity than we could achieve with streptavidin coated surfaces. This same observation is true for functional groups on beads.

Finally, we have achieved the improvement that we needed. We coat surfaces with polyethylenimine (PEI) to provide a maximum amount of primary amines per unit area. This also has the function of creating a somewhat three-dimensional surface. We use cyanuric chloride chemistry to attach the aptamers to the PEI. With this approach, we achieve better functionality than with any other approach we have tried.

3.) Blocking:

It is routine to block with BSA, but we have found that this does not work as well with aptamers as it does with antibodies. I suspect that the BSA is rolling right over the aptamers present. It is like using boulders to fill in gaps in your grass lawn. It will fill the gaps, but it reduces access to the yard. We use a variety of small molecules for blocking, including thiolyated short PEG molecules and acetic anhydride. The use of small molecules that will render the remaining surface passive is of critical importance to success. This reduces your zero target values and decreases the standard deviations associated with your target value measurements.

Don't cover a lawn with a forest...

Everything in diagnostics is about signal/noise ratios. What I have described in this newsletter works because it increases signal or it decreases noise. To be successful with aptamers you need to think like an aptamer...