Diabetic Complications Consortium

Application Title: Development and validation of ultrasound molecular imaging agents for diabetic kidney disease by measuring bio-molecular interactions with a novel, sensitive, homogeneous proximity assay (AlphaScreen™)

Principal Investigator: Kennita Johnson

1. Project Accomplishments:

During this grant period, we were able to successfully switch the assay from microbubbles to nanodroplets (ND) to avoid several pitfalls. We were able to develop procedures and validate that the Alpha Assay works well with ND.

Specific Aims:

Aim 1. Develop an in vitro assay to assess and optimize microbubble ligand-receptor interactions.

In the development of this assay, we made the switch from microbubble to nanodroplets (ND). The gas in the microbubbles causes the microbubbles to float to the top of the test plate and diffract the light coming from the bead interactions of the Alpha Assay. This leads to inaccurate and unreliable measurements. Because ND are derived from microbubbles, this is not a departure from the original intent of this award. Using temperature and pressure, microbubbles are condensed to form ND with liquid cores. These liquid-core droplets performed better in the Alpha Assay for allowing the light from the bead interactions to reach the detector unimpeded. ND can also be converted back to microbubbles through temperature and pressure mechanisms as well.

Switching to ND meant that we needed to standardize and validate each step in the process. We started by examining different methods of making ND by comparing the Ouzo method with our standard method. We were able to validate that we could create targeted ND with an A7R-heptapeptide which contains a recognition site to cMyc, which would allow us to attach the donor beads for the Alpha Assay. The Ouzo method created ND with a smaller diameter, which was not beneficial for the current application, because it limited the number of bead sites each ND could hold. We used our standard ND generation method for this work. We also investigated and standardized how to wash ND to remove unincorporated peptide, which could lead to artificially high signal.

The presence of assay signal indicates that both DSPE-P2K-biotin and DSPE-P2K-Myc-A7R were present in the lipid shell, meaning that we were able to create the targeted ND. Figure 1 shows that the binding kinetics for the assay components over 6 hours. Biotin/cMyc-A7R ND signal in the Alpha Assay was still increasing after six hours. Thus, we needed longer incubation durations to reach equilibrium. Figure 2 shows biotinylated cMyc-A7R titrated from 3e8 #/mL to 3e-1 #/mL

using a log dilution. Biotin and cMyc-A7R incorporated at 0.2 mol % on ND surface. The assay was incubated for 24 hours with Alpha Assay readings measured at 2-hour increments. With these longer assay times, ND remained intact for the entire assay period. This further justifies the switch from microbubbles to ND.

2. Publications:

None.

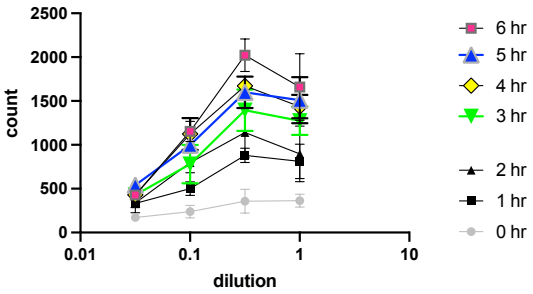

Figure 1. Plotting assay output vs time.

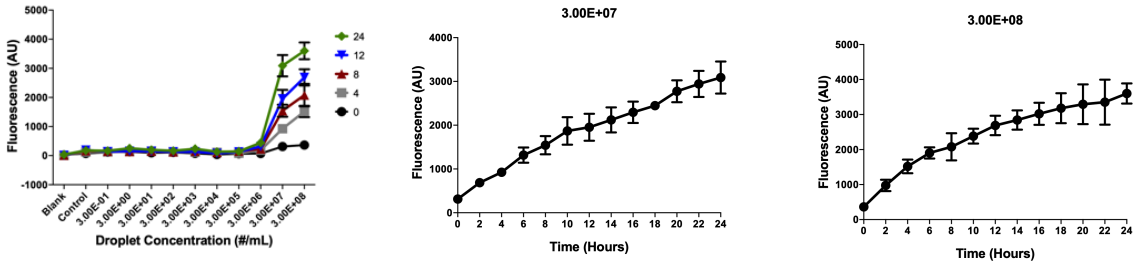

Figure 2. Left: fluorescence vs concentration for hours 0, 4, 8, 12, 24. *Notice that assay signal only begins to increase at higher concentrations.* Center: fluorescence vs time for ND concentration of 3e7 to observe signal increase over 24 hr data collection. Right: fluorescence vs time for ND concentration 3e8 to observe signal increase over 24 hr data collection.