**Exploiting Luminex assays in the Discovery of Nanobodies® at Ablynx/Sanofi**

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1) Background and aim:

Ablynx/Sanofi is dedicated to the development of therapeutic camelid-derived Nanobodies. Many Discovery programs run in parallel, thereby necessitating high-throughput screening capacities to allow a timed characterization of promising lead molecules. With an ever increasing need to screen faster and more efficiently, automated Luminex assays are being developed to propel Nanobody Discovery.

2) Methods:

The focus of the ongoing implementation of the Luminex assay platform has been automation and throughput in 384-well format. We started off to set up automation routines that would facilitate/speed up the daily use: a) taking advantage of the KingFisher Plex instrument from ThermoFisher Scientific, we realized an automated functionalization of up to 96 different bead regions in one unattended run, b) counting of beads has been established using the Luna II cell counter, and c) high recovery 384-well plate washing protocols are in place on the Aquamax4000 device. Further, to leverage the Luminex platform in the Nanobody Discovery process, binding and competition assays have been developed that are currently assessed to screen for immune responses and (off)-target binding. In addition, commercial kits to measure cytokine panels were evaluated.

3) Results:

KingFisher Plex-automated coupling of up to 2 million MagPlex beads/batch using EDC/NHS chemistry yielded bead recoveries between 75 and 95 %. These were reliably determined with the Luna II cell counting device in merely 10 s per bead batch. 384-well microplate washing protocols were optimized for efficient washing steps of MagPlex beads. We found that at least 2000 beads/well are necessary for a robust assay read-out due to an inevitable loss of beads during washing. Immune titer and Nanobody binding assays have been established to assess both target and off-target binding characteristics. For typical screening campaigns of > 2000 Nanobodies per target, the throughput of traditional ELISA assays was found inferior to a 5-plex Luminex assay. The increased dynamic range of Luminex should allow us to identify high affine Nanobodies early in the screening cascade. Lastly, we successfully tested a 3-plex competition assay in which different ligands for 1 receptor were put on MagPlex beads and simultaneously tested for interference with Nanobody/receptor binding.

4) Conclusion:

Luminex assays offer a variety of possibilities to set up Nanobody screening and characterization assays at Ablynx/Sanofi. They allow more information to be queried during the Discovery phase of promising Nanobody leads and thereby saving hands-on time if multiplexing is exploited. The availably of commercial kits allows us to select quicker appropriate read-outs in e.g. cellular assays. The current optimization efforts concentrate on streamlining bead conjugations.