How much material is required for a Biacore SPR experiment?

- 1. Ligand pre-concentration: 3-4 injections of 50-100 μL at 50-100 μg/mL. See Biacore training courses for detailed discussion of ligand pre-concentration.
- 2. Amine coupling: (similar for other immobilization strategies) ~100  $\mu$ L of 50-100  $\mu$ g/mL ligand.
- 3. Specificity analysis and regeneration pilots: 500  $\mu L\,$  1000  $\mu L$  of 500 nM (or higher, depends on  $K_d)$  analyte.
- 4. Surface stability test: 10 injections of 50-100 µL at 500 nM analyte.

5. Typical kinetic analysis: A concentration series of 8 injections for (16 if duplicates) at 50  $\mu$ L /min flow rate for 2 min. per injection: 150  $\mu$ L for each injection, starting at roughly 500 nM - 1000 nM, analyte concentration. Concentration range ideally should run from 0.1 x K<sub>d</sub> – 10-100 x K<sub>d</sub>.