Covalent oriented coupling

1) Couple your capture antibody
2) flow over your his-tagged protein
3) Mix the NHS/EDC reagents yourself (I use the same NHS and EDC aliquots that I also use for activating chip surfaces so you don't have to make those separately, just pull some aliquots from the freezer).
4) Inject the NHS/EDC over the flow-cell(s) of interest.
5) Immidiately inject the ethanolamine (or whatever primary amine quencher you like) after the NHS/EDC mix. I usually put the ethanolamine in the rack and issue the quickinject command while the NHS/EDC activation is happening so that the quench happens as soon as possible after the activation.

That's the basic premise. THe main variable then is how long to inject the NHS/EDC solution. The optimal injection time will balance two parameters. The first is surface stability, and the second is surface activity. Too short an injection and you'll get marginal stabilization, whereas long injections usually give you very stable surfaces. Short injections rarely affect the activity, but longer ones sometimes will. Ideally you know something about the interaction already from measurements on more unstable surfaces (KD, and ideally the off-rate, even if it's collected on a decaying surface, so you have a reference).

Couple antibody to all four flow cells. Sequentially step through the flow cells, one by one, injecting the his-tagged protein and blocking. Do this for three flow cells. In each subsequent flow-cell activated for a shorter period of time. The experiment that worked for TfR was:

FC1: 60 second
FC2: 30 seconds
FC3: 15 seconds
FC4: Don't do anything

Start a sensorgram over all 4 flow cells at 10 ul/min and let it run for one hour.

Process the data and look at the drift over time. There will be two indicators of drift. One will be the loss of material from the flow cells, and the other will be the accumulation of material on the 4th flow cell, captured out of what was lost on the previous cells.

Now, setup an analyte concentration series and run it three times (ideally the same dilutions aliquoted into three tubes, or use the resealable caps so the machine can use the same tubes over and over. Those are a new product out last year). Determine KD and hopefully some kinetics for each

of those runs separately to give yourself an idea of the experimental noise (you could globally fit the three runs together too). Ideally they'll all be the same for the runs and the flow cells. However, what you'll likely see is that the flow cell coupled for the longest time has taken a hit in KD or in off rate. For TfR, the HFE off-rate increased for activations lasting longer than 45 seconds. The increase was, at most, 2-fold, but it was reproducible, so I set the HFE activation around 30 seconds, which also gave stable surfaces.

Even after this procedure sometimes it's good to let the chip 'rest' for an hour or two before beginning the experiment to let it stabilize. It also helps to run startup cycles (blank buffer injections, 5-10 of them, before the experiment even begins) to let everything, including the robotics, to settle down before beginning your samples.