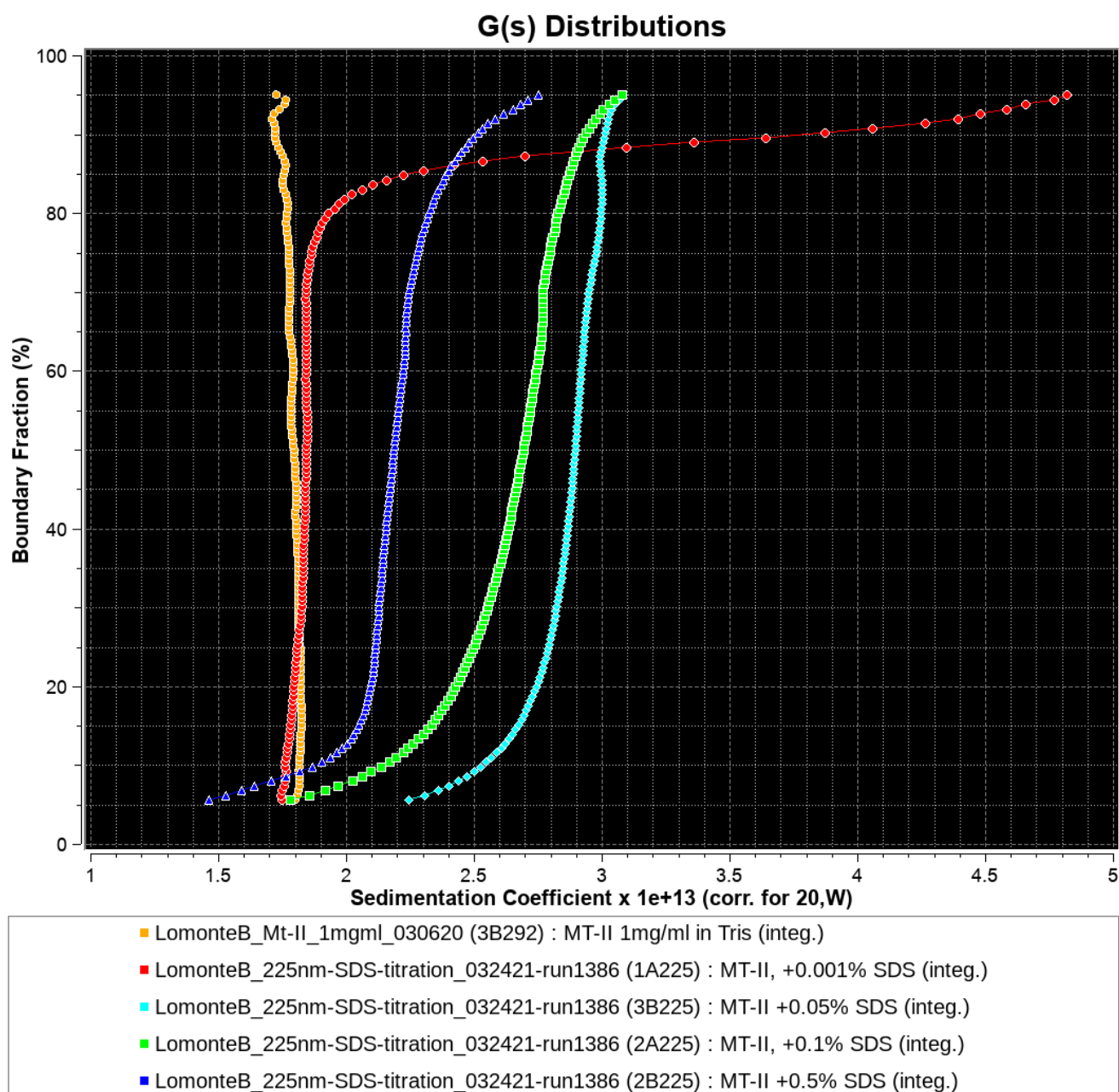


The following AUC experiments were performed with Myotoxin-II:

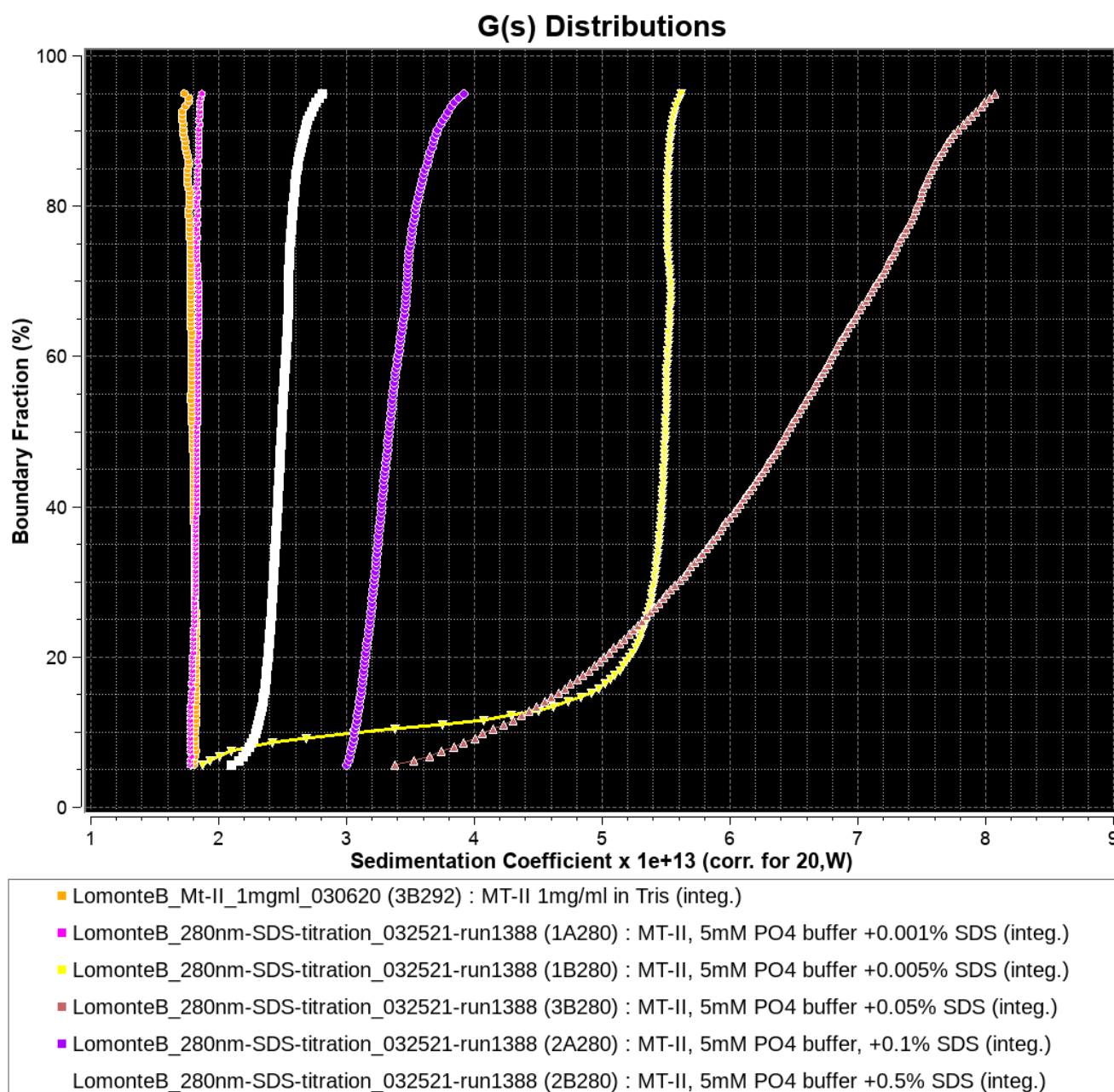
Triple:	[ $\mu$ Molar]	% SDS	Wavelength:
1/A/225	2.3	0.001	225 nm
2/A/225	3.6	0.1	225 nm
2/B/225	3.7	0.5	225 nm
3/B/225	3.4	0.05	225 nm
1/A/280	23.6	0.001	280 nm
1/B/280	23.7	0.005	280 nm
2/A/280	24.7	0.1	280 nm
2/B/280	25.7	0.5	280 nm
3/B/280	21.7	0.05	280 nm

van Holde – Weischet integral s-value distributions are shown below for each concentration set (225 nm = low concentration, 280 nm = intermediate concentration, approx. 8 times higher). For reference, the monomer without SDS is included in each dataset. The striking thing about these experiments is clearly the fact the s-value increases rapidly at the lowest SDS% to a point where most of the material gets actually aggregated, and then as more SDS is added, the thing I would expect to happen for SDS actually occurs, i.e., the breaking up of higher complexes. But, the higher complex does not completely dissolve into monomers, at the highest SDS concentration tried here we seem to get dimers.

Furthermore, what is really important is that the ratio of protein:SDS matters. Any SDS concentration reacts very different when comparing high with low concentration. When intermediate concentration protein is around, the oligomeric state is very different compared to the **same** SDS concentration when only limiting protein is available. I feel AUC provides a highly sensitive signal for the protein:SDS ratio. A possible model may be that a few SDS molecules may link different myotoxin-II together, and these structures are broken up when there is excess SDS present, and some point we also get micelles with embedded myotoxin-2 molecules.

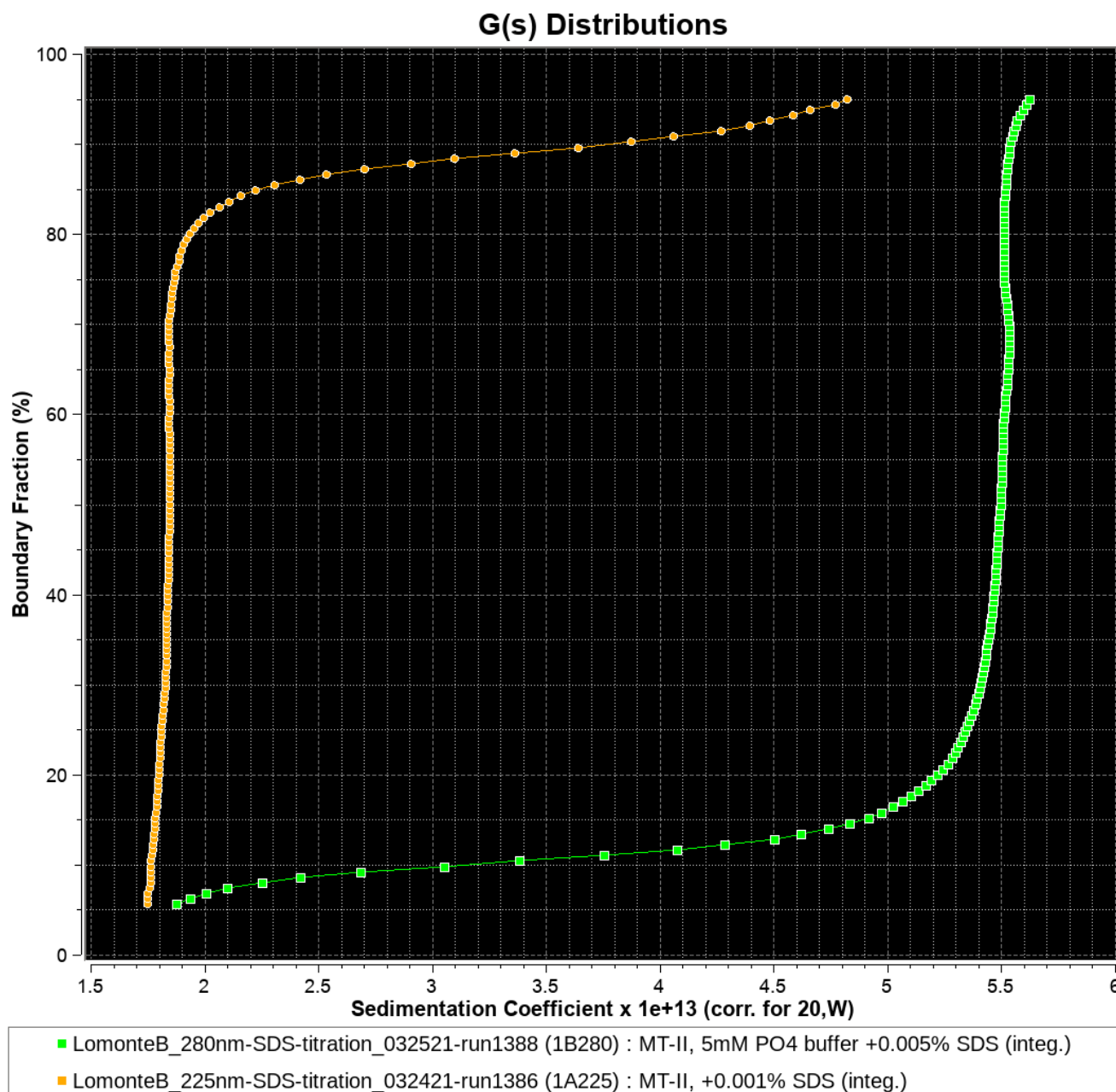


This is an overlay of the 225 nm (low concentration) experiment. I have added the monomeric Myotoxin sample taken in TRIS buffer as a reference (orange). Of note, the 0.005 % SDS sample was aggregated and is not shown. Below I compare the various percentages of SDS as a function of protein concentration, which shows significant differences. As you can see, the s-value initially increases, but then decreases again with higher SDS. This suggests that SDS makes molecules sticky, causing them to aggregate with each other. Once you add enough, these “sticky aggregates” are broken up again, but some oligomerization remains, and may in fact eventually be included in micelles.

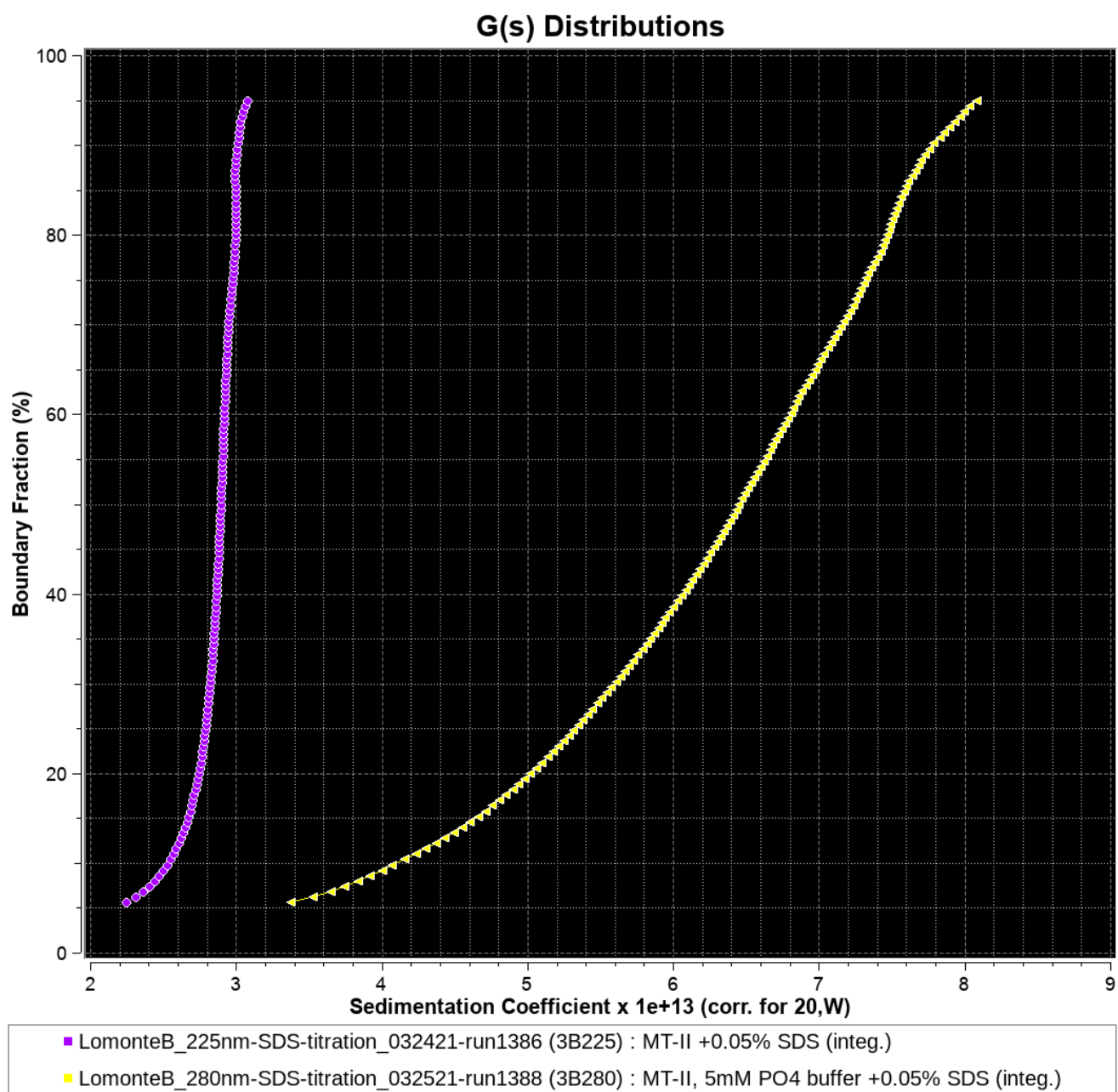


The titration experiment Amy ran provided some outstanding data that is highly intriguing and super interesting. As so often in science, the story is a lot more complicated than just a dimer in SDS. What is clear to me now is that there is a lot more going on than just a dimer as is seen in the SDS PAGE experiment, though that dimer is fully consistent with what we see in the AUC experiment. Both the low and medium high concentration Myotoxin-II data show essentially the same trends. Here is what I see: As you increase the concentration of SDS from very low [SDS] to intermediate, the myotoxin oligomerizes, but not to a dimer, but MUCH higher oligomeric states. Interestingly, there appear to be discrete intermediates detectable, at the right protein/SDS concentration and ratio. For example, at 280 nm where we are measuring around 24  $\mu\text{M}$ , there is 70% of a pure hexamer present when we have 0.005% SDS present. If the SDS is increased 10 fold to 0.05% without changing the protein concentration, myotoxin-II self-associates into much larger structures, without discrete intermediate states. Here is the kicker: If you increase [SDS] further, oligomerization of myotoxin **decreases** again

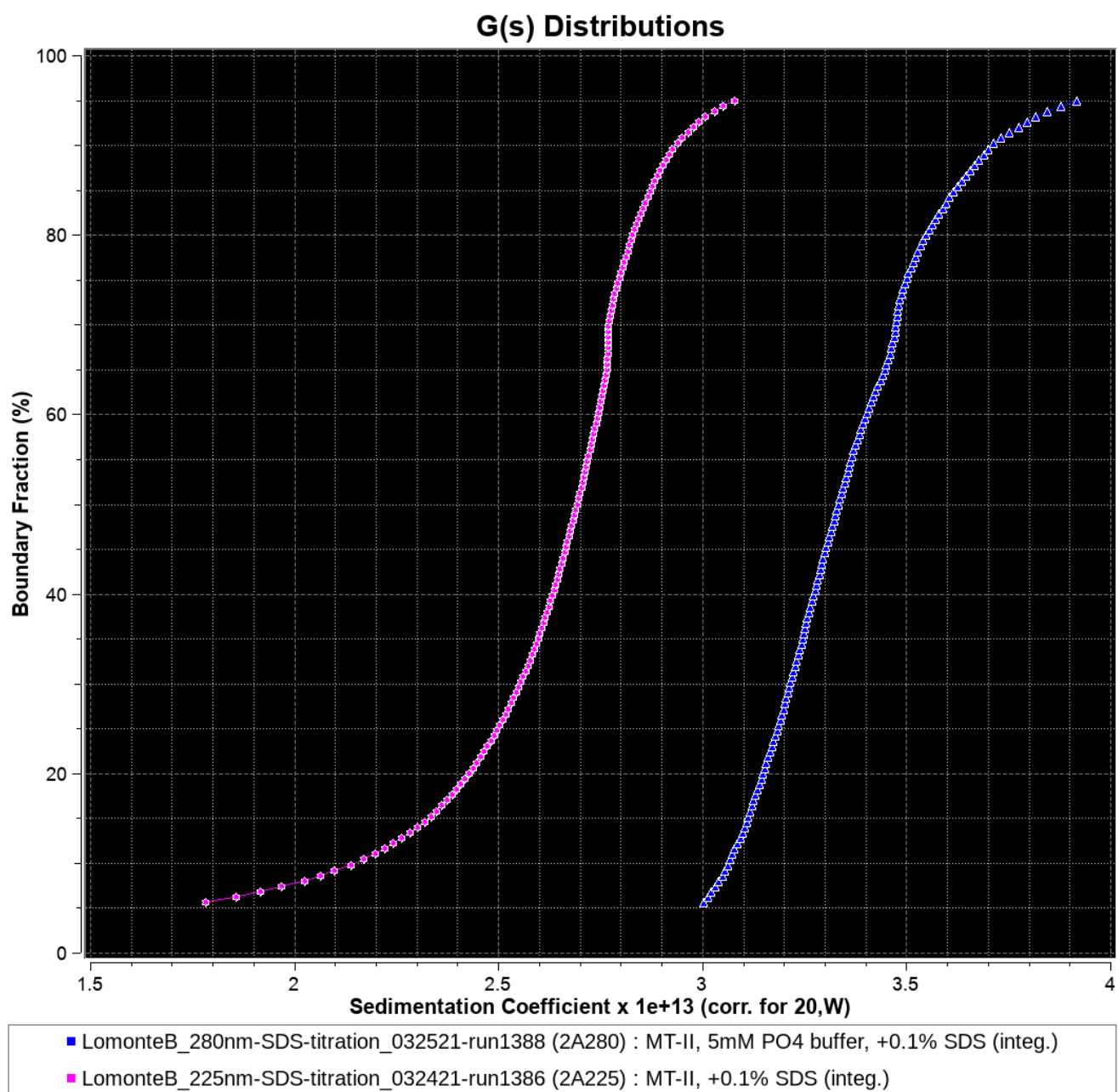
and eventually drops down to the dimer or trimer stage at 0.5% SDS. In that case, SDS appears to be doing what it is normally doing, i.e., degrading inter-molecular interactions.



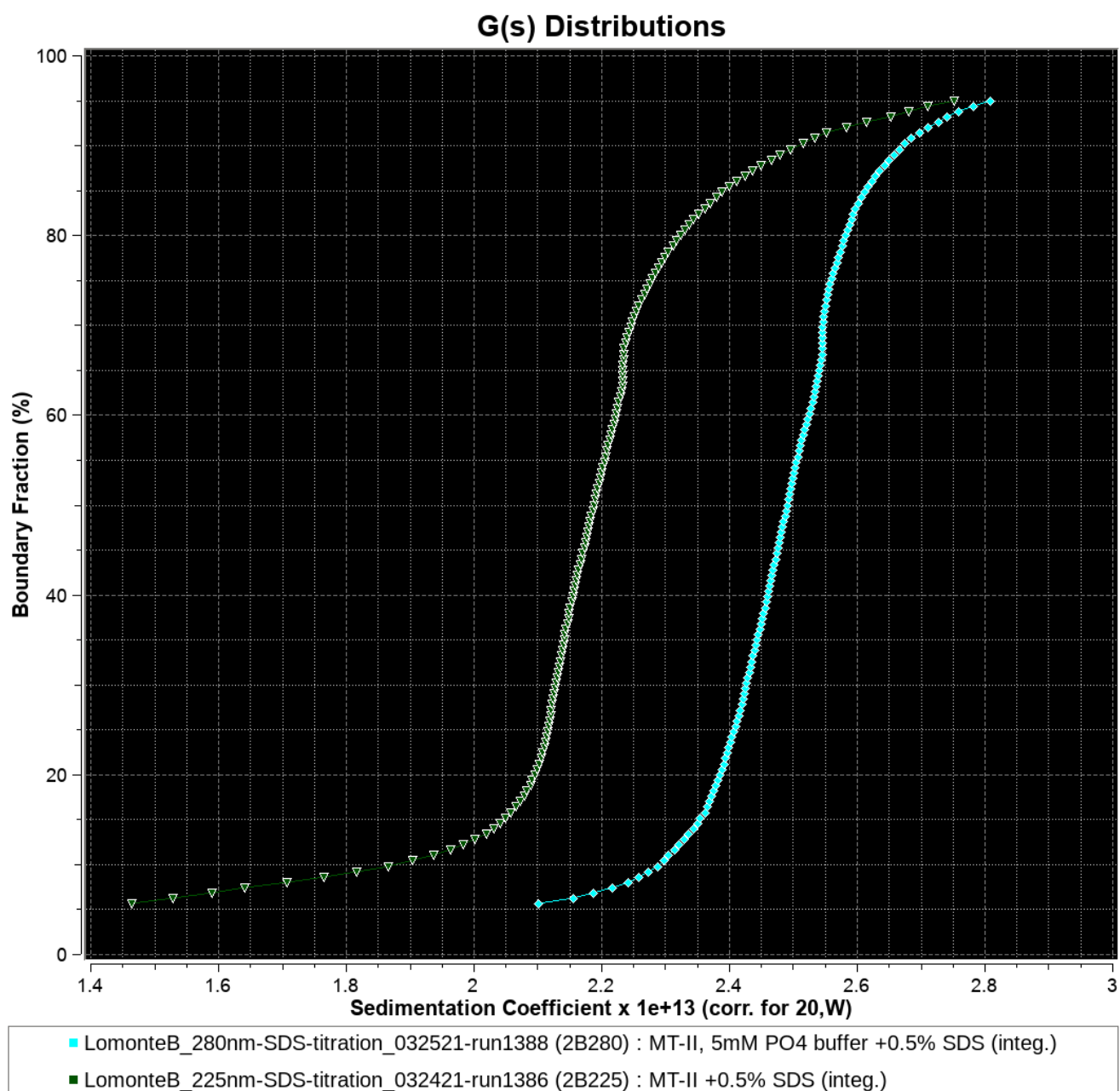
Here is an interesting comparison of the low concentration with 0.001 % SDS and the high concentration sample with 0.005 SDS. What we see is that both samples show an aggregation effect. What is interesting is that the aggregate seems to form a discrete species at 5.5 s and doesn't just keep aggregating to higher oligomeric states. I check the molar mass and it is roughly consistent with a hexamer. Of note is that there is still a small amount of monomer present, but no intermediates. If you recall, the 0.005 % SDS of the low concentration sample aggregated, while the 0.001 % SDS sample of the high concentration showed no visible shift. This suggests that the ratio of SDS concentration to protein concentration is really important.



Here is a comparison of 0.05% for the two concentration. Clearly, the higher protein concentration is closer to the aggregation trigger, while the lower protein concentration is low enough to already disrupt whatever aggregates formed before in lower concentrations of SDS.



For 0.1% SDS the same effect is apparent as for the 0.05% SDS sample, just shifted further to the left due to additional SDS interactions with the aggregates.



For 0.5% SDS we not only are above the critical micelle concentration of SDS, but we also move closer back to the dimer stage, especially for the lower concentration protein sample where the SDS has more of a contribution to the breaking up of aggregates effect.

In summary, for the next NMR experiment, I feel we need to find the SDS concentration at very high protein concentration (20 mg/ml) where we see the most, and the most pure, hexameric structure, since it appears to be homogeneous, as well as no background from SDS, and all the SDS is a) bound to the myotoxin, and b) all SDS molecules are probably in a similar environment, making SDS and protein contacts the biggest source of NMR signals, and c) get maximum protein signal, and minimal SDS background from SDS free in solution.

Amy is now running AUC experiments trying to find this mixture. If you have better ideas please let us know before we waste any sample on experiments that require high concentrations of protein.