Haematologic Technologies
Large Molecule CRO | Research Reagents | CMC

## HCP Watchlist and Knockout Wishlist

Using mass spectrometry to identify and quantify HCPs enables a more rational development of robust purification processes. Obviously, co-purification of proteases can have a significant impact on product quality and stability. We've dealt with many different proteases in our HCP projects over the years. One of the more pernicious proteases we've seen is *Serine Protease HTRA1*. I have come to view HTRA1 as the piranha of the protease world (or *degradomics* if you want to use an *-omics* term). HTRA1 is usually quite easily detected in CCF, but its presence doesn't really matter in highly impure samples – there are plenty of other substrates to occupy its attention. But if HTRA1 is co-purifying after the first affinity column – look out! I've seen multiple cases where HTRA1 can "disappear" the product protein – the product protein just vanishes.

There have been some efforts to generate knockouts for certain HCPs in CHO cells. HTRA1 would be near the top of my wishlist for a knockout. However, I doubt that will be possible. In 2018 Schillinger et al. studied the role of HTRA1 in cell cycle proteomics. They reported that the abundance of 2,872 cellular proteins fluctuated in a HTRA1-dependent fashion, indicating that HTRA1 is not just some superfluous protein. Unless someone can figure out a way around this, it looks like HTRA1 is going to remain on our HCP *watchlist*. Mass spectrometry is one of the best ways to track problematic HCPs like HTRA1. Figuring out how to avoid protease problems early in process development can save a lot of time, effort, stress, heartache…, and money.