PROTEOLYSIS OF NON-IGG MOLECULES IN TRANSIENT HEK293 AND STABLE CHO-K1 BIOPROCESSES

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Since 2006, we use the transient FreeStyle™ 293 System (Thermo Fisher Scientific) as well as an in-house developed stable CHO-K1 platform in order to express recombinant molecules for antibody generation and prototype assay development. Successively, more than 200 different human antigens – secreted and cytosolic – were cloned, expressed, purified via Ni-chelate chromatography and analyzed by SDS-PAGE, UV scan, analytical size exclusion (HPLC/RALS) and dynamic light scattering (DLS).

A major bioprocess issue can be proteolytic degradation, also referred as ‘clipping’, by proteases originating from the host cells, thereby leaving an altered, non- or less functional protein of interest. We experience that approx. 5% of our human non-IgG target molecules are prone to clipping. The use of protein/serum-free chemically defined media in both platforms might also trigger the proteolysis of the protein of interest. So far, this issue was poorly addressed by the scientific community, in part since IgG antibodies – which receive the most attention – tend to be well expressed, robust, and stable molecules. Little is known about the proteases expressed by the host, and about the proteases responsible for clipping. Another challenge is the sheer number of proteases since 100s proteases are known to be present in cell genomes and may be involved in the clipping process.

Outline of the presentation:
1. Examples of HEK- and CHO-derived molecules are presented with their cleavage sites identified by Edman degradation following SDS-PAGE separation and blotting. In general, the proteolysis is not quantitative – rather, a distinct fragmentation ladder is observed by SDS-PAGE analysis. Unfortunately, the cleavage sites do not follow a strict pattern indicating that several different proteases might be in charge, depending on the host cell line as well.
2. Analysis of HEK- and CHO-supernatants by gelatin and caseine zymography reveal the activity of several distinct proteases for each host cell line.
3. The Human Protease Array Kit (R&D Systems) reveals a broad panel of highly expressed cathepsins and metalloproteinases in HEK293 supernatants.
4. Experiments utilizing siRNA as well as co-expression of inhibitor molecules are currently performed in order to track down (and hopefully inhibit) the responsible proteases for each bioprocess.