UNDERSTANDING THE EFFECT OF OXIDATIVE AND ER STRESS ON CHO CELL CULTURE AND PRODUCT QUALITY THROUGH MULTIVARIATE ANALYSIS

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In the past decade, engineering strategies such as media supplementation and process variation have enabled a dramatic increase in antibody production from Chinese hamster ovary (CHO) cells from 1 g/L to over 10 g/L [1]. Out of many methods to improve culture productivity, the application of ER stress and oxidative stress either through chemical or genetic approaches to effect improved yields or product quality has been somewhat equivocal. The reason partially lies on using single stress inducers at a defined concentration to activate ER stress through the unfolded protein response (URP), where the most immediate results have been shown when stressors are used at a high concentrations [2]. However, these high concentrations can be toxic, affecting cell viability; thus the more subtle effects of the stressors at prolonged, but non-toxic concentrations have been underexplored. Further, only recently has the industry raised redox conditions as a potentially measureable, and controllable parameter [3]. Thus, strategies to tune stressors in a predictable manner is highly needed. This project aims to resolve the complex effects of ER and oxidative stress on product quality, yield, and cell viability, as well as to provide an information-rich method to identify process effects on the cell culture.

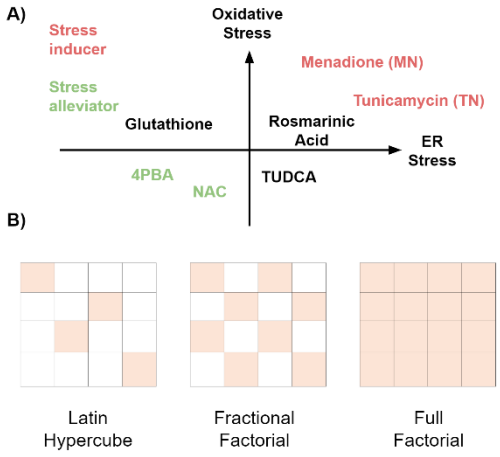

Figure 1 – Experimental design. A) Screening of chemicals that exert either ER or oxidative stress-alleviating or -inducing effect. B) DOE Latin Hypercube design to study the effect of chemicals in a high parameter space with fewer experiments.

In this study, we first conducted a chemical screening to identify four chemicals that would either alleviate or induce ER or oxidative stress during typical CHO antibody production based on measurement of UPR activity and reactive oxygen species (ROS) staining. The chemicals were further screened to determine a concentration that would cause significant stress-inducing or –alleviating effect on CHO cells yet did not demonstrate high toxicity based on viable cell measurements. The selected stressors were utilized at four concentrations within that range for a total of sixteen experiments through a design of experiment (DOE) Latin-Hypercube design (Figure 1) applied to batch culture. Key culture parameters including viability, viable cell density, total cell density, key metabolite concentrations, and antibody titer were measured, as well as product quality attributes such as glycosylation, light chain to heavy chain ratio, and charge variant level. The outcomes were analyzed through correlational, principle component analysis (PCA), and partial least square (PLS) analysis. Overall, we determined that a reductive environment, with low ROS and ER stress, reduced the amount of complex glycoforms in the antibody product. In contrast, an oxidative environment, with higher ROS level and ER stress, showed increased aggregation propensity. Importantly, through a novel multi-attribute assay, we identified N-acetylcysteine (NAC) to be negatively correlated with glycan occupancy. The presence of low levels of tunicamycin (TN), an ER stressor, also led to improved antibody titer and elongated culture duration without compromising preferred glycosylation attributes. An artificial neural network model further enabled visualization of the effect of these chemicals in a large-parameter space, showing at all levels of other chemicals, TN was the prime factor that increased antibody titer. With additional experiments using only TN in a fed-batch culture study, we improved antibody titer by 12.7% using a low level of TN.

In summary, our study serves as an archetype (or case study) for analytical approaches to determining process variations in a large parameter space, and as a step forward for advanced cellular process control. In addition, we identify an unexpected result of NAC on glycosylation, as well as the implementation of TN at non-toxic levels as a beneficial additive to facilitate antibody glycosylation and improve titer.

References: [1] R. Kunert and D. Reinhart. Appl. Microbiol. Biotechnol. 2016.100:3451-3461 [2] K. P. Segar et al. BMC Proc. 2013. 7:67 [3] P. Sinharoy et al. Curr. Opin. Biotechnol. 2021. 71:49-54