DIVISION OF BIOCHEMICAL TECHNOLOGY OF THE AMERICAN CHEMICAL SOCIETY

BIOT

The Best of BIOT Awards: October 04, 2017

Date	Area	Time	Presenter	Institution
Wednesday, October 4th	Upstream Process Development	12:00-12:30 PM	**Rey Martin**	Northwestern University/MedImmune
		Development of a CHO-based cell-free platform for the synthesis of active monoclonal antibodies as a high-throughput		
		12:30-1:00 PM	**Xiaolin Zhang**	University of Delaware
		Identification of genes that rescue deficient DNA double-strand break repair in Chinese hamster ovary cells		

Webinar registration can be made at ACS BIOT WebEx Webinars

"Development of a CHO-based cell-free platform for the synthesis of active monoclonal antibodies as a potential high-throughput screening tool"

Rey Martin, Northwestern University, Evanston, IL/MedImmune, Gaithersburg, MD

Chinese Hamster Ovary (CHO) cells are routinely optimized to stably express monoclonal antibodies (mAbs) at high titers. At the early stages of lead isolation and optimization, hundreds of sequences for the target protein of interest are screened. Typically, cell based transient technology platforms are used for expression screening, but these can be time- and resource-intensive and may also suffer from performance variability. Here, we have developed a cell-free protein synthesis (CFPS) platform utilizing a commercially available CHO extract for the rapid *in vitro* synthesis of active mAbs. Specifically, we optimized reaction conditions to maximize protein yields, established an oxidizing environment to enable disulfide bond formation, and demonstrated the importance of chaperone supplementation and temporal addition of heavy chain and light chain plasmids for intact mAb production. Using our optimized platform, we demonstrate, for the first time to our knowledge, the cell-free protein synthesis of biologically active, intact mAb using CHO cell extracts. We then also explored the utility of our system as a tool for ranking yields of candidate antibodies. Unlike stable or transient transfection-based screening, which requires a minimum of 7 days to obtain mAb product, using our CHO-based CFPS platform, mAb product can be attained within 2 days and it is well-suited for automation. Further development would provide a tool for rapid, high-throughput prediction of expression ranking of mAb producers and has positive implications for the synthesis of difficult-to-express and toxic proteins.

(Read Martin's article; DOI: 10.1021/acssynbio.7b00001).

“Identification of genes that rescue deficient DNA double-strand break repair in Chinese hamster ovary cells”

Xiaolin Zhang, University of Delaware, Newark, DE

Chinese hamster ovary (CHO) cells are widely used to manufacture therapeutic proteins. A high degree of genetic plasticity in CHO cells allows for easy adaptation to various culture conditions and the generation of high-producing clones. However, this inherent genome instability may also result in loss or alteration of the integrated transgenes, leading to production loss or product quality inconsistency, which are key challenges in industrial manufacturing processes.

Efficient DNA double strand break (DSB) repair is critical for cells to maintain genome stability. It is conceivable that observed CHO genome and production instability is related to DSB repair system dysfunction. Here, we compare the DSB repair efficiency between CHO cells and the bEnd.3 mouse endothelial cell line. Moreover, we study various ways to enhance DSB repair efficiency and positively impact protein production stability during 16-week cultures.