



Reduction of charge variants by CHO cell culture process optimization

Zhibing Weng · Jian Jin · ChunHua Shao · Huazhong Li 

Received: 3 November 2019 / Revised: 22 January 2020 / Accepted: 23 January 2020 / Published online: 31 March 2020
© Springer Nature B.V. 2020

Abstract Over the past decade, global interest in the development of therapeutic monoclonal antibodies (mAbs) has risen rapidly. As therapeutic agents, antibodies have shown marked efficacy in combatting a range of cancers and immune diseases with high target specificity and low toxicity (Carla Lucia et al. in *PLoS ONE* 6:e24071, 2011; Donaghy in *MABs* 8:659–671, 2016; Nasiri et al. in *J Cell Physiol* 9:6441–6457, 2018; Teo et al. in *Cancer Immunol Immunother* 61:2295–2309, 2012). Recent advances in cell culture technology, such as high-throughput clone screening, have facilitated antibody production

at concentrations exceeding 10 g/L (Chen et al. in *BMC Immunol* 19:35, 2018; Huang et al. in *Biotechnol Prog* 26:1400–1410, 2010; Lu et al. in *Biotechnol Bioeng* 110:191–205, 2013; Singh et al. in *Biotechnol Bioeng* 113:698–716, 2016). As titers have improved, the industry has begun to focus on the adjustment of target antibody quality profiles to improve efficacy. Cell lines, culture media, and culture conditions impact protein quality (Van Beers and Bardor in *Biotechnol J* 7:1473–1484, 2012). Optimization of critical quality attributes (CQAs), such as charge variants, can be achieved through bioprocess development and is the preferred approach as changes to the cell line or growth media used is considered unfavorable by regulatory bodies (Gawlitze et al. in *Biotechnol Bioeng* 103:1164–1175, 2009; Jordan et al. in *Cytotechnology* 65:31–40, 2013; Pan et al. in *Cytotechnology* 69:39–56, 2016). In this study, the effect of process control and ion supplementation on charge variants of mAbs produced by Chinese hamster ovary (CHO) cells was investigated. Results of this study demonstrated that the concentration of Zn^{2+} , duration of culturing, and temperature affect charge variants of a given mAb. Under the optimum conditions of 3L bioreactors, the most significant was that Zn^{2+} and temperature shift could further improve the quality of antibody. The main peak increased by 12%, and the acid peak decreased by 16%. At the same time, there was no significant loss of titer. This study

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10616-020-00375-x>) contains supplementary material, which is available to authorized users.

Z. Weng · H. Li (✉)
School of Biotechnology, Jiangnan University, Wuxi,
Jiangsu, China
e-mail: hzhli1958@163.com

Z. Weng
e-mail: wengzhib@3s-guojian.com

Z. Weng · C. Shao
Process Science and Production Department, Sunshine
GuoJian Pharmaceutical (Shanghai), Shanghai, China
e-mail: shaoch1984@sina.com

J. Jin
School of Pharmaceutical Sciences, Jiangnan University,
Wuxi, Jiangsu, China
e-mail: jinjian31@126.com

provided supporting evidence for methods to improve charge variants arising during mAb production.

Keywords Charge variants · Chinese hamster ovary cells (CHO) · monoclonal antibodies (mAbs) · Zn²⁺

Introduction

The pharmaceutical industry benefits from robust market demand with global prescription drug sales exceeding 1 trillion USD in 2017. Furthermore, global monoclonal antibody (mAb) market size exceeded \$100 billion USD in 2017, and investment into the development of biologics is expected to continue. Following years of research and development, CHO cells are most commonly used host cell line for antibody production due to their ability to execute appropriate post-translational modifications of mAbs necessary for therapeutic utility (Chu and Robinson 2001; Lim et al. 2010). Monoclonal antibodies are complex heterogeneous glycoproteins with multiple post-translational modifications; therefore a number of different variants exists for a particular mAb (Brorson and Jia 2014; Higel et al. 2016; Houde et al. 2010). Each variant of a mAb has unique biochemical and biophysical properties associated with their specific post-translational modifications (Higel et al. 2016; Mueller 2017; Zheng et al. 2014). The majority of mAbs developed and tested by Chinese biopharma company pipelines are biosimilar, therefore mAbs should have molecular properties similar to those of the original targets (Li et al. 2015; Silva et al. 2014). However, controlling post-translational modifications both in and outside of cells is difficult as the underlying mechanisms are complex and often poorly understood (Begona and Leyre 2012; Walsh 2010). Common issues in the development of biosimilar mAb include aggregates, fragments, and differentially glycosylated mAbs (Berkowitz et al. 2012; Read et al. 2011; Wei et al. 2006). Charge variants can be identified by use of cation exchange chromatography (IEC), whereby the acidic mAb peak elutes before the major peak and the basic mAb peak elutes later (Khawli et al. 2015; Lingg et al. 2013). For example, the IEC elution profile of the mAb assessed in this study is shown in Fig. 1, where the early- and late-eluting peaks are indicative of the acidic and basic

charge variants, respectively. These charge variants have the potential to alter molecular properties of mAbs, and therefore their safety and efficacy profiles, thus presenting significant regulatory concern (Beck et al. 2013; Chirino and Mire-Sluis 2004; Dada et al. 2015; Hintersteiner et al. 2016; Rea et al. 2011; Shukla and Thömmes 2010). To date, the relationship between charge variant production and presence of impurities during mAb production has been elucidated (Khawli et al. 2015; Kishishita et al. 2015; Prabhu and Gadgil 2019). For biosimilar mAb production, the frequency of charge variants must be adjusted to correspond to the original drugs (Begona and Leyre; Vázquez-Rey and Lang 2011). Traditionally, IEC has been used in the purification of mAbs. Unfortunately, this approach greatly reduces product yield. Optimization of cell culture processes in the production of mAbs can alter charge variant frequencies (Kishishita et al. 2015; Seo et al. 2016). Therefore, in the present study we investigated methods to optimize the production of charge variants and to improve mAb production quality.

Materials and methods

Cell lines

The cell line used in this paper was developed by Sunshine Guojian (A Member of 3SBio). According to the published amino acid information of Trastuzumab, the recombinant anti-HER2 humanized mAb DNA sequence was synthesized and inserted into the pUC57 intermediate vector. The PCR method was utilized to amplify the antibody heavy chain and light chain sequence from the pUC57 plasmid and a signal peptide was added to the N-terminus of the corresponding sequence. Meanwhile, AvrII/BstZ17I restriction site was added to the ends of heavy chain Open Reading Frames (ORFs), EcoRV/PacI restriction site was added to the ends of light chain ORFs. Freedom pCHO1.0 plasmid (Gibco, ThermoFisher, USA) was selected as the expression vector. The heavy chain ORFs and the light chain ORFs were cloned into the multiple cloning site AvrII/BstZ17I and EcoRV/PacI of pCHO1.0 respectively. The recombinant plasmid was named pCHO-VHL. See Supplementary information Figure 1 for detail plasmid map information. After the antibody gene

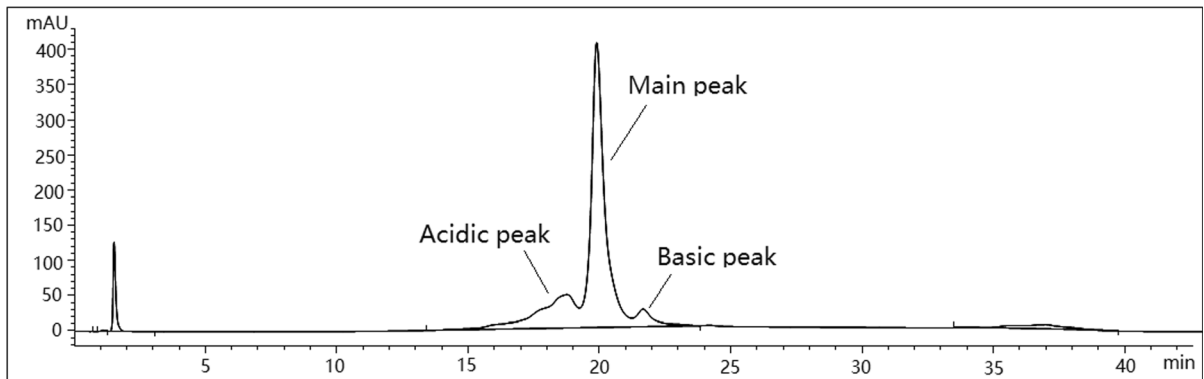


Fig. 1 Cation exchange chromatography elution profile. The acidic mAb peaks elute before the main peak and the basic mAb peaks elute after the main peak. As shown in this figure, the peaks of approximately 18 min are the acid charge peaks, the

sequence accuracy was confirmed, the plasmid was transfected into host cell CHO-DG44 cell (Thermo Fisher, USA). The transfected cells were screened by pressure screening, clonal screening, single cell sub-cloning screening and engineered cell strain producing trastuzumab was obtained. These cells were finally stored in liquid nitrogen tanks.

Cell culture media

The basic medium used during the cultivation was 300F (Sunshine GuoJian, CHINA), and the nutrient medium 300S (Sunshine GuoJian, CHINA) was added with 0.008 mol/L glutamine(Gln) during the cultivation, and the solution used was 30% Glucose solution, 10% AF(antifoam) and 2 mol/L Na_2CO_3 solution. 300F and 300S produced by Sunshine Guojian was chemically defined media. Dissolved the medium powder in water for injection (WFI) and kept agitating for 3 h and weighed the medium to a final volume. The media were filtered through sterilizing grade filter in biosafety cabinet(Esco, Germany). The sterile media should be stored at 4–8 °C avoiding light. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, MnCl_2 and CuSO_4 metal salts were purchased from Sigma.

Cell culture method

One vial of working cell bank (WCB) was thawed in a water bath at 37 °C, Shook the vial until thawed completely and inoculated into a 125 mL shake flask. The CHO cells in shaking flask were cultured in CO_2 shaking incubators (INFORS Multitron, Switzerland)

largest peak being the main one followed by the basic charge peaks. The horizontal axis represents the retention time of HPLC and the vertical axis represents the absorbance unit (mAU)

incubated at 36.5 °C, 5% CO_2 , 145 rpm. During scale-up operations, cells were cultivated in fed-bath mode in 3L bioreactors (Eppendorf DASGIP, Germany) at an inoculation density of 0.9×10^6 cells/mL and set the other parameters as following: 35–37 °C, pH 6.8–7.1, 40% dissolved oxygen, 200–400 rpm. All experiments were maintained under the same conditions except when concentration of Zn^{2+} , Cu^{2+} , Mn^{2+} ions or temperature were altered (Ex: 36.5 °C to 35 °C on Day 0 /Day 2/Day 3/Day 5). Nutrient solutions were added daily between Day 3 and 13. Culture media were sampled for analysis daily between Day 11 and 15.

Online measurements and offline analysis

CHO cells density was counted by use of an automated cell counter (Countstar, ALIT, China), Glucose and lactate levels were measured by use of a biochemical analyzer (Roche, Switzerland), pH and Dissolved oxygen (DO) were measured by use of sensors (Mettler Toledo, Switzerland), Charge variants and titer were analyzed by use of high performance liquid chromatography (Agilent 1260, USA).

Results

Influence of culture temperature shift

Temperature impacts cell growth and can affect metabolism by altering the effective rate of energy

transfer within cells (Sengupta et al. 2011; Si et al. 2014). During the later stages of antibody production, lower temperatures can improve cell survival (Yoon et al. 2003). To study the effects of temperature variation on mAb production, a series of experiments were conducted. Culture parameters were as follows: flasks were incubated at 5% CO₂ with the shaking speed of 145 rpm, an initial cell density of 0.9×10^6 cells/mL and a final pH of 6.8–7.1. As shown in Fig. 2 and Supplementary Table 1, in order to evaluate the influence of temperature shift, the three shake flasks were shifted from 36.5 to 35 °C on day 2, day 3 or day 5 respectively. The other two flasks were cultured at 36.5 °C and 35 °C respectively. All batches were harvested on Day 14.

Previous studies have demonstrated that lower temperatures yield a higher proportion of cells in the G1 phase, and thereby improve antibody titer (Fogolín et al. 2004; Kaufmann et al. 1999; Oguchi et al. 2006; Schatz et al. 2003). In contrast, the temperature is reduced, the decrease in protein titers always occurs when the temperature does not significantly affect the viability of the cells. Some other studies have similar results to the manuscript (Bloemkolk et al. 1992;

Fogolín et al. 2004; Reuveny et al. 1986; Sureshkumar and Mutharasan 1991), lower temperatures reduce titers and impact the glycoprotein charge heterogeneity (Fig. 2). It is likely that a shift in temperature extended the transit time of antibodies through the endoplasmic reticulum and Golgi apparatus, thereby promoting glycosylation (Bollati-Fogolín et al. 2005; Gomez et al. 2012; Si et al. 2014), similar to previous reports where it was demonstrated that lower temperatures reduce erythropoietin (EPO) sialic acid content (Ahn et al. 2008; Kaneko et al. 2010). Post-translational modifications such as sialylation can alter the charge distribution of mAbs, and might explain results of this study (Clark et al. 2004). As demonstrated in Fig. 2, a shift in cell culture temperature on Day 3 resulted in an acceptable mAb titer and fewer charge variants, thus indicating the utility of these culture conditions.

Influence of culture duration

Following inoculation, cell culture growth proceeded through a number of phases; latent phase (Day 0–Day 1), logarithmic growth phase (Day 2–Day 6), and

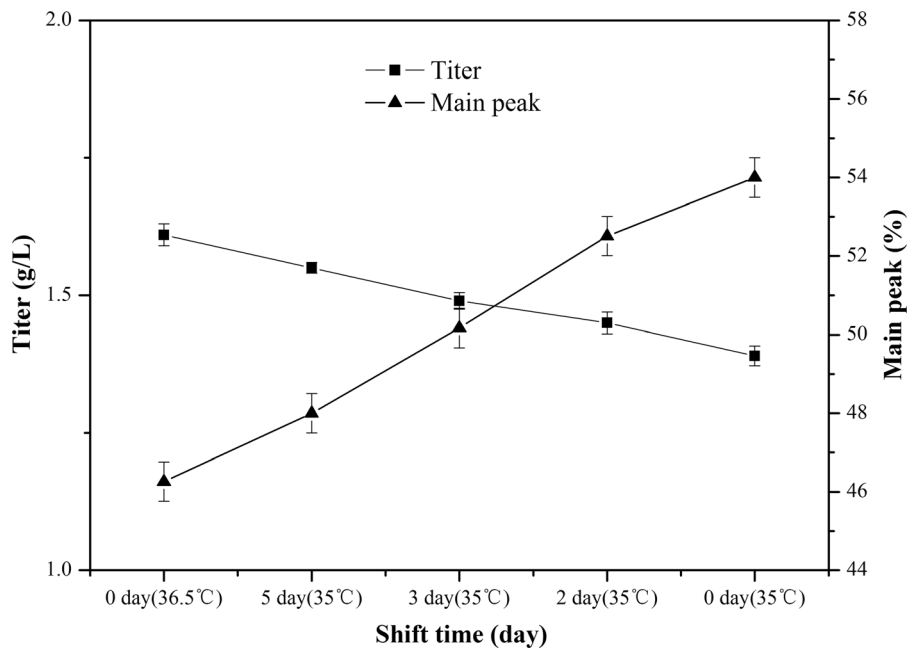


Fig. 2 The different effect of temperature shift on titer and charge variants. The three shake flasks were shifted from 36.5 to 35 °C on day 2, day 3 or day 5 respectively. The other two flasks were cultured at 36.5 °C and 35 °C respectively. All batches

were harvested on Day 14. Titer and charge variants of Day 14 as shown in this figure were the averages of triplicates of three experiments

stagnant phase (Day 7–Day 15). In this study, the effect of culture duration on the growth of CHO cells, mAb titer, and charge variant frequency were investigated. Parameters of cell cultivation were as described above and cell culture media was sampled daily on day 11–15.

Results demonstrate that antibody titer and size of acidic charge variant peak increased significantly with incubation time (Fig. 3). In contrast, main peak size decreased significantly and the peak associated with the acidic species between Day 11 and 15 reached a maxima of approximately 20%. The peak size of the basic charge variant remained constant over time. According to the previous research find, increase in sialic acid content or deamidation of asparagine residues was likely associated with an increase in the presence of acidic charge variants (Du et al. 2012; Leblanc et al. 2017). Thus, incubation time is a critical parameter which can be adjusted according to desired outcomes in order to maintain appropriate mAb product quality. Results of this experiment demonstrate an optimal harvest times on day 14.

Influence of metal ion concentration

The impact of ion concentrations on cell growth, mAb titer, and charge variant was investigated. Ions tested included Zn^{2+} , Cu^{2+} and Mn^{2+} in various concentrations. Culture conditions were as described in the previous two studies and temperature were shifted

from 36.5 to 35 °C on day 3, all cell batches were harvested on Day 14.

Mn^{2+} and Zn^{2+} function to catalyze enzymatic reactions, whereas, Cu^{2+} often functions to inhibit the activity of enzymes (Luo et al. 2012; Radhakrishnan et al. 2018). By varying concentration of the selected ions, effects on enzymatic reactions involved in mAb production by CHO cells was investigated. As presented in Fig. 4, It was demonstrated that higher concentrations of Zn^{2+} (40 μ M) reduced the size of acidic charge variant peaks and increased the size of main peaks relative to lower concentration of Zn^{2+} (4 μ M) (Fig. 4d). At the same time, cultures with Zn^{2+} expressed higher protein than cultures with Mn^{2+} and Cu^{2+} . Compared with no additive culture and 4 μ M, found that Zn^{2+} concentration increased the protein expression significantly at 40 μ M almost 12.24% and 5.45% (Fig. 4c). Mn^{2+} and Cu^{2+} had little effect on the acidic charge variant peak, whereas Cu^{2+} somewhat reduced the size of the main peak and increased the size of basic charge variant peaks (Fig. 4d). A high Cu^{2+} concentration (400 μ M) negatively impacted cell density, viability, and mAb titer (Fig. 4a–c).

CPB is an exocrine protease, each of which contains an ion of zinc, which hydrolyzes the C-terminal residues of peptide chains, such as lysine, glycine or arginine. Previous work has shown that CPB activity is enhanced by maintaining a proper Zn^{2+} concentration, eventually leading to charge

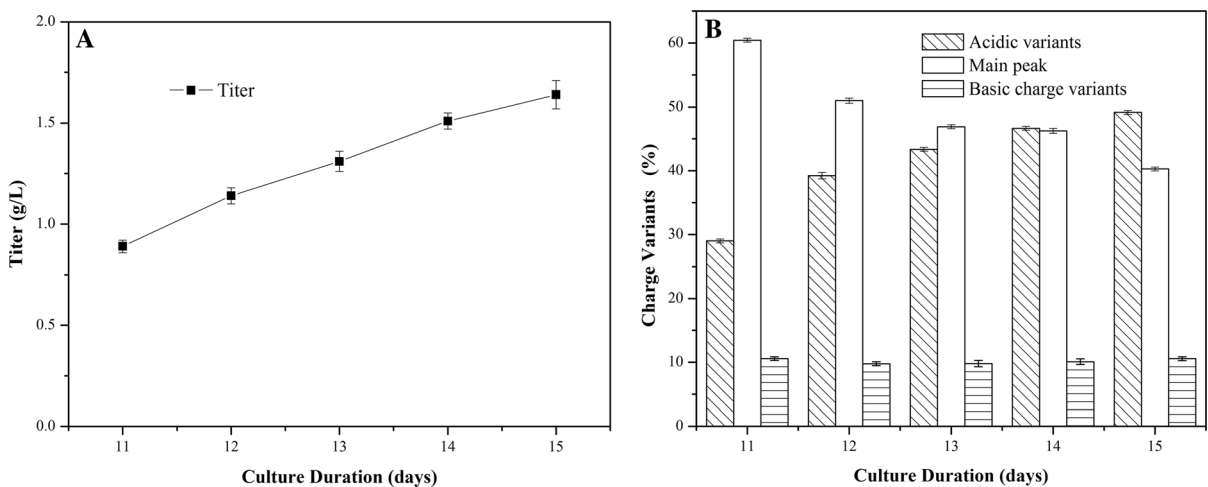


Fig. 3 The effect of culture duration on charge variants and titer. The mAb titer increased with the extension of culture time. Acidic variants increased and main peak decreased with the

extension of culture time. **a** mAb titer. **b** Distribution of charge variants. All data points were the averages of triplicates of three experiments

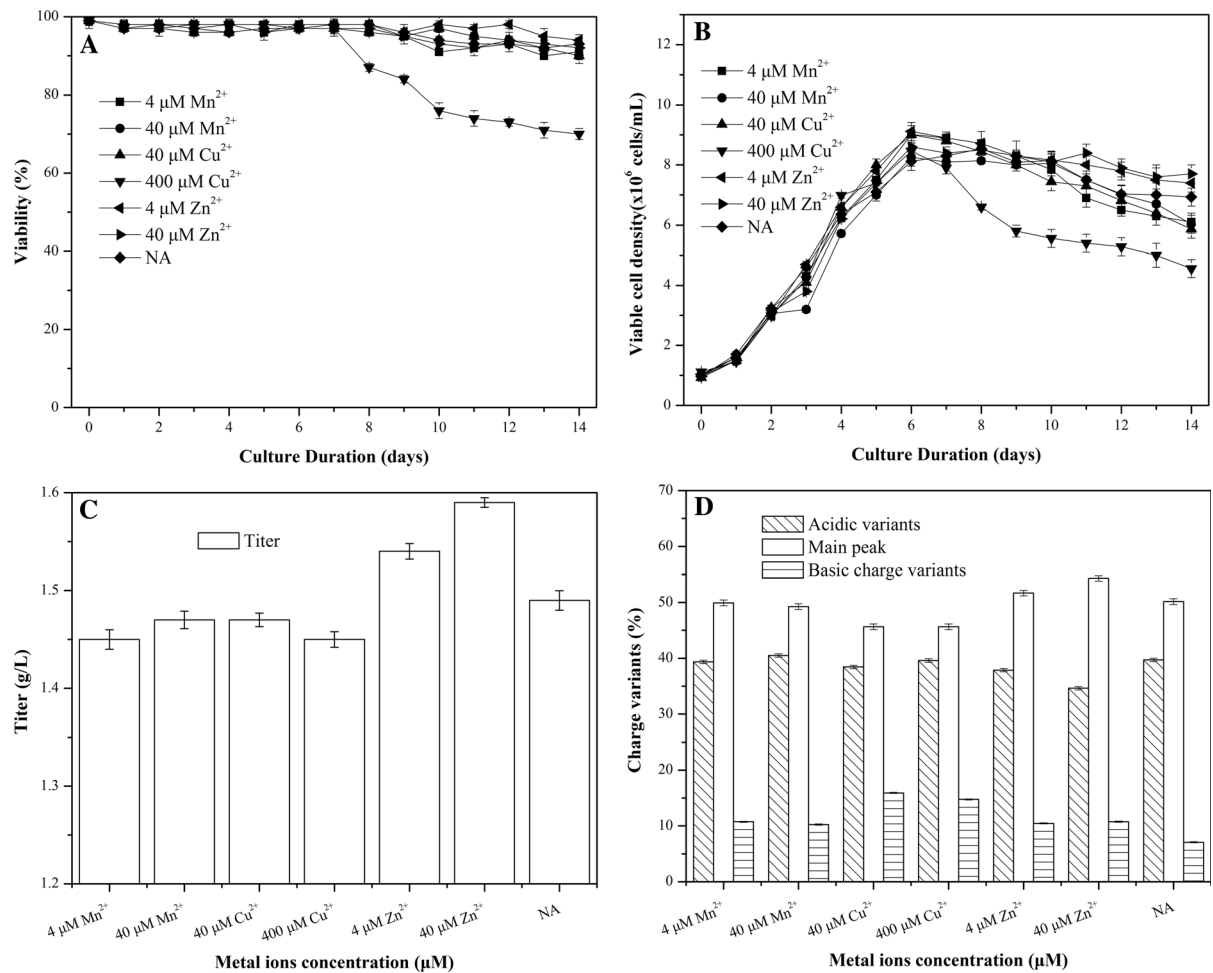


Fig. 4 The effect of metal ions on cell growth, titer and charge variants. High Cu^{2+} concentration (400 μM) negatively impacted cell density, viability, and mAb titers. In contrast, high concentrations of Zn^{2+} (40 μM) promote expression and

reduce acidic peaks. All batches were harvested on Day 14. **a** Cell growth. **b** Viable cell density (VCD). **c** mAb titer. **d** Distribution of charge variants. All data points were the averages of triplicates of three experiments

Optimization of cell culture processes

To investigate the effect of varying Zn^{2+} concentrations on cell growth, mAb titer, and charge variants frequencies, cells were grown by use of media containing 30 μM to 150 μM of Zn^{2+} . Cells were initially seeded at a density of 1.0×10^6 cells/mL, and growth conditions were as described in the preceding section.

As presented in Fig. 5, a certain range of Zn^{2+} concentrations promote expression and reduce acidic peaks. In contrast, high Zn^{2+} concentration (150 μM)

negatively impacted cell density and viability (Fig. 5a, b). The size of the main mAb peak increased as Zn^{2+} concentration increased from 30 to 110 μM . However, when zinc concentration was equal to 150 μM , main peak size decreased (Fig. 5d). The experiment showed that when the expression of protein reached the highest peak, Zn^{2+} concentrations were 70 μM (Fig. 5c), and when the concentration reached 110 μM , the main mAb peak peaked (Fig. 5d). Therefore, we focus on 100 μM , with a range of plus or minus 30 μM , the concentrations of Zn^{2+} between 70 and 130 μM were selected as optimal.

Based on results of our experiments, Zn^{2+} concentration and cell culture temperature were deemed

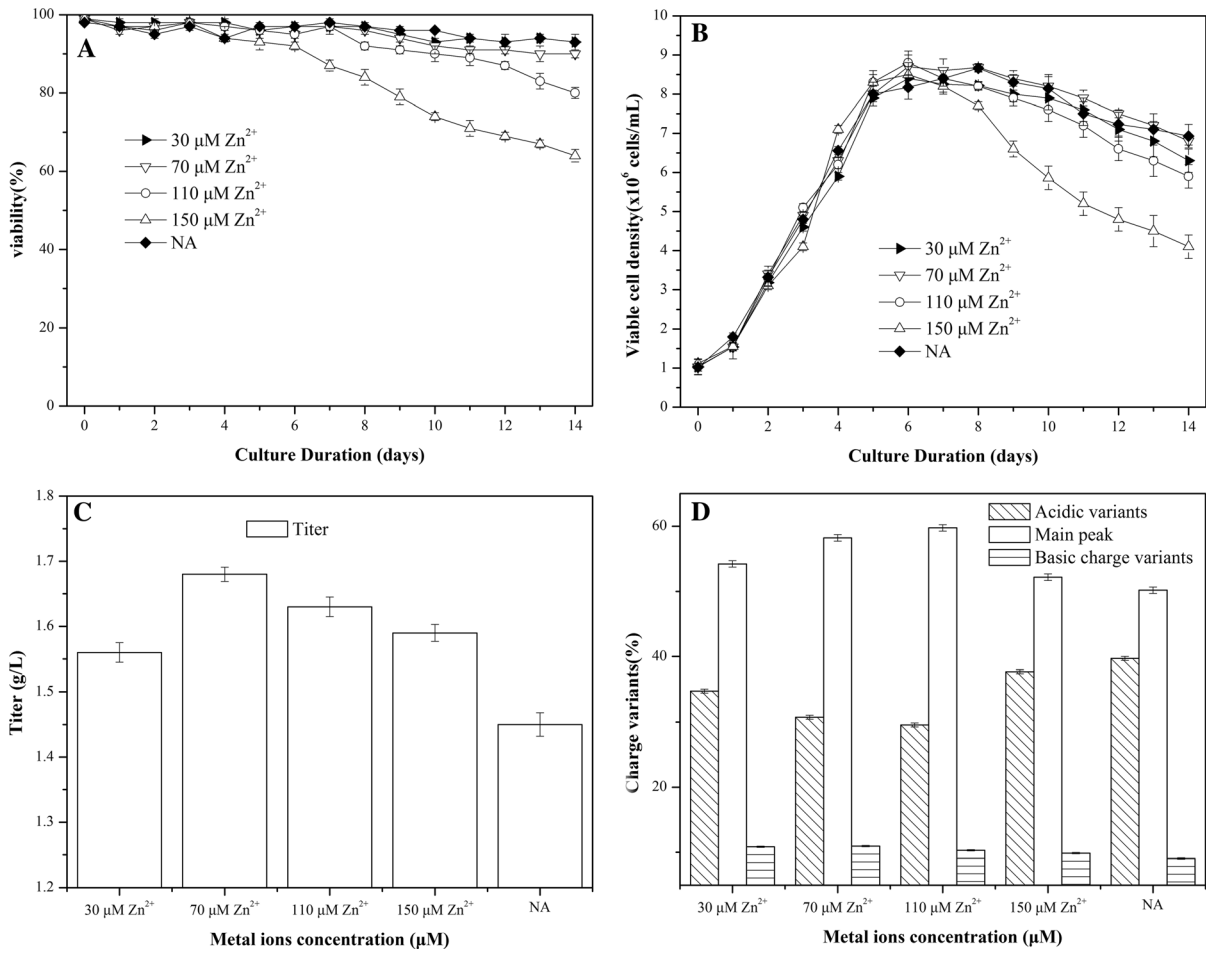


Fig. 5 The effect of Zn²⁺ on cell growth, mAb titer, charge variants. A certain range of Zn²⁺ concentrations promote expression and reduce acidic peaks. In contrast, high Zn²⁺ concentration (150 μM) negatively impacted cell density and

viability. All batches were harvested on Day 14. **a** Viability. **b** Viable cell density (VCD). **c** mAb titer. **d** Distribution of charge variants. All data points were the averages of triplicates of three experiments

critical process parameters impacting the quality of produced mAb. Therefore, experiments were designed to assess the effect of temperature and Zn²⁺ concentration on cell growth, mAb titer, and charge variant frequencies. Culture conditions were as described in the preceding two sections, with varying Zn²⁺ concentration and culture temperature as outlined in Table 1.

As mentioned previously, the frequency of basic variants processed by CPB remained stable across culture conditions. Therefore, focus was given to the main peak (++++), acidic charge variant peak (+++), and titer (++) as the key outcomes of this study, whereby the “+” symbol represents relative weight. Following response surface modeling (Fig. 6),

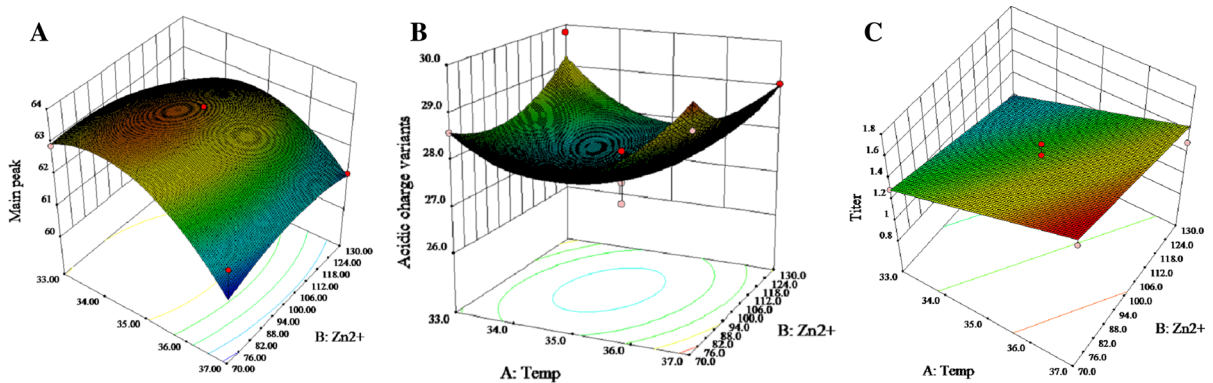
the DoE software indicated that the best control point to minimize the frequency of charge variants was to culture cells at a temperature of 35.13 °C on day 3 with media Zn²⁺ concentration equal to 79.82 μM.

Process validation (3L)

To validate finding of this study at a larger scale, the above mentioned temperature (temperature shift from 36.5 to 35 °C on day 3), harvest time (day 14), and metal ion concentration results (80 μM Zn²⁺) were used in the culturing of cells in large 3L bioreactors in duplicate (reactor 1 and reactor 2). A third reactor was used as a negative control with temperature maintained at 36.5 °C and a Zn²⁺ concentration equal to 80

Table 1 DoE design space coordinates employed for response surface modeling of the control of mAb charge variants

Run	Temperature (°C)	Zn ²⁺ (μM)	Acidic variants (%)	Main peaks (%)	Basic variants (%)	Titer (g/L)
1	37	70	29.19	61.77	9.03	1.7
2	37	100	29.01	60.92	10.07	1.6
3	37	130	29.04	61.16	9.80	1.3
4	33	70	28.59	62.90	8.51	1.3
5	33	100	27.97	63.39	8.64	1.1
6	33	130	29.81	60.95	9.23	0.9
7	35	70	28.14	62.68	9.17	1.5
8	35	100	26.66	63.67	9.67	1.4
9	35	100	27.84	63.29	8.87	1.4
10	35	100	27.14	63.16	9.69	1.5
11	35	130	27.54	63.15	9.31	1.3
Control	37	0	35.45	54.15	10.39	1.3

**Fig. 6** DoE response surface predict MAb main peak, acidic variants and titer as a function of temperature and Zn²⁺. **a** RSM of main peak. **b** Contour line of main peak. **c** RSM of acidic variants

μM. As presented in Fig. 7, it was found that a shift in temperature changed glycoprotein charge heterogeneity and increased main peak size relative to the negative control reactor (Fig. 7a–c). Addition of 80 μM Zn²⁺ further reduced the frequency of charge variants and increased main peak by 12% and a concomitant decrease in the acidic peak of 16% (Fig. 7d).

Discussion

Many factors including cell line, media, and culture processes significantly influence antibody quality (Rouiller et al. 2014). Under current regulatory

guidelines or in consideration of other limitations, cell lines or media cannot be changed, therefore optimization of other CQAs such as culture duration, temperature, and metal ion concentrations can be used to improve enzyme activity and production of mAb. Enzymes are vital to mAb maturation and in vivo occurs in the endoplasmic reticulum or Golgi apparatus which are the major site of glycosylation in mammalian cells.

Post-translational modifications arising in vitro or in vivo can affect antibody quality. Following secretion from cells, mAbs are subject to additional modifications mediated by enzymes released from host cells. During later stages of cell culture, cells

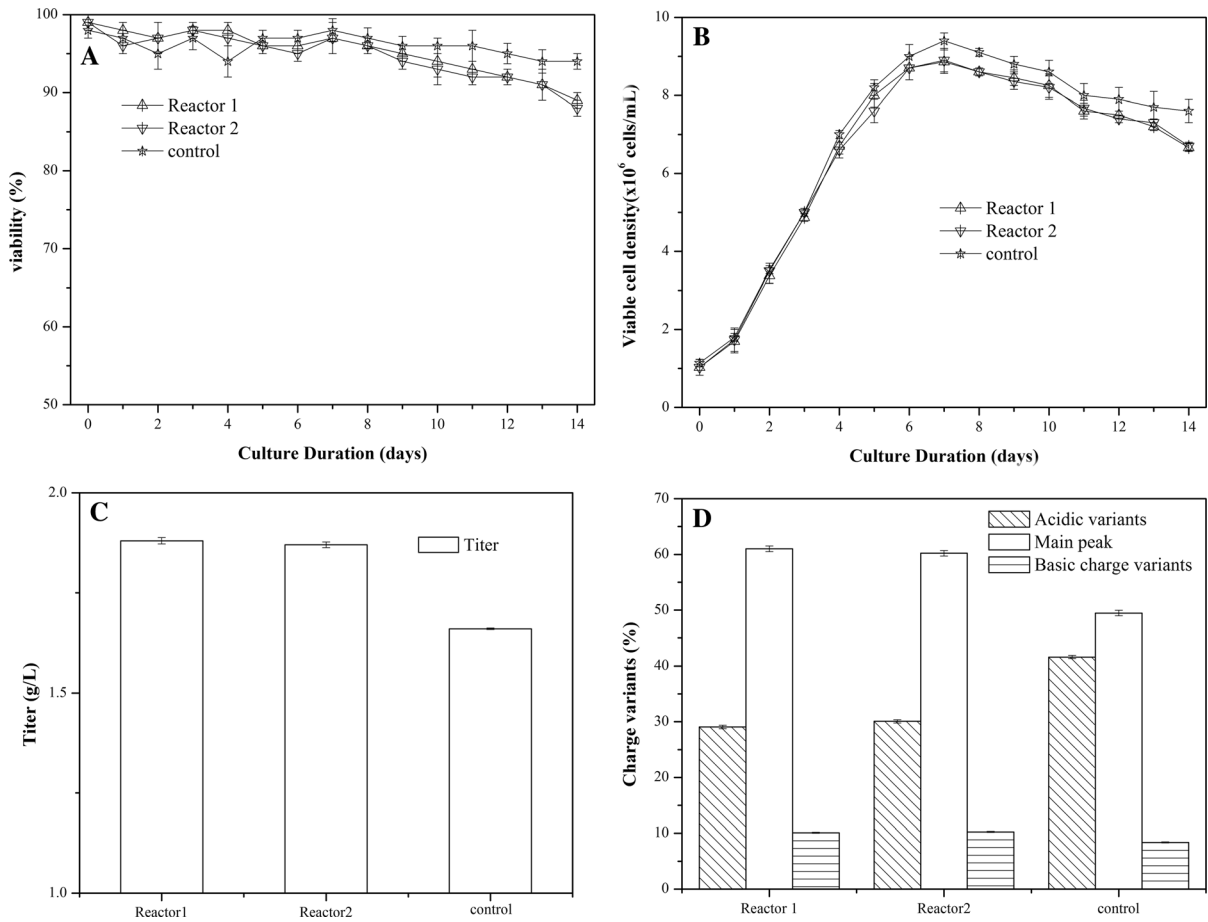


Fig. 7 The effect of Zn^{2+} on cell growth, mAb titer, charge variants. The parameters of two parallel reactors as following: temperature shift from $36.5\text{ }^{\circ}\text{C}$ to $35\text{ }^{\circ}\text{C}$ on day 3 and $80\text{ }\mu\text{M}$ Zn^{2+} , the third reactor was used as a control with temperature

maintained at $36.5\text{ }^{\circ}\text{C}$ and $80\text{ }\mu\text{M}$ Zn^{2+} . All batches were harvested on Day 14. **a** Viable cell density (VCD). **b** Viability. **c** mAb titer. **d** Distribution of charge variants. All data points were the averages of triplicates of three experiments

begin to die leading to a significant release of active enzymes into cell culture media. High enzyme concentrations and longer culture durations can thus significantly alter the frequency of particular variants of proteins of interest. Therefore, process parameters can be optimized to elicit desired quality standards for a given mAb. Previous studies have demonstrated that sialic acid content, deamination of asparagine residues, presence of non-classical disulfide linkages, and high mannose content are post-translational modifications leading to the development of acidic charge variants (Du et al. 2012; Leblanc et al. 2017). In contrast, the presence of C-terminal lysine, tryptophan oxidation, methionine and glycine amidation can all contribute to the presence of basic charge variant species (Khawli et al. 2015).

As demonstrated harvest time, culture temperature, and Zn^{2+} concentration impact charge variant frequency of mAbs produced in CHO cells and impact the frequency of acidic charge variant species. In this study, it was demonstrated that optimization of critical process parameters (CPPS) and critical material attributes (CMAs), can be achieved by optimization of CQA for robust manufacturing. It is vital that future work expand upon these results in order to define broadly acceptable operational ranges in which mAb production can be robustly optimized in order to facilitate biosimilar production workflows.

Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest in this work.

Ethics approval Gibco Freedom CHO-DG44 cell and freedom pCHO1.0 mammalian cell expression vector in this paper were purchased commercially from ThermoFisher scientific and institutional approval was not needed.

References

- Ahn WS, Jeon JJ, Jeong YR, Lee SJ, Yoon SK (2008) Effect of culture temperature on erythropoietin production and glycosylation in a perfusion culture of recombinant CHO cells. *Biotechnol Bioeng* 101:1234–1244
- Beck A et al (2013) Analytical characterization of biosimilar antibodies and Fc-fusion proteins. *TrAC Trends Anal Chem* 48:81–95
- Begona C, Leyre Z (2012) Therapeutic monoclonal antibodies: strategies and challenges for biosimilars development. *Curr Med Chem* 19:4445–4450
- Berkowitz SA, Engen JR, Mazzeo JR, Jones GB (2012) Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars. *Nat Rev Drug Discov* 11:527–540
- Bloemkolk JW, Gray MR, Merchant F, Mosmann TR (1992) Effect of temperature on hybridoma cell cycle and MAb production. *Biotechnol Bioeng* 40:427–431
- Bollati-Fogolín M, Forno G, Nimtz M, Conradt HS, Etcheverrigaray M, Kratje R (2005) Temperature reduction in cultures of hGM-CSF-expressing CHO cells: effect on productivity and product quality. *Biotechnol Prog* 21:17–21
- Brorson K, Jia AY (2014) Therapeutic monoclonal antibodies and consistent ends: terminal heterogeneity, detection, and impact on quality. *Curr Opin Biotechnol* 30:140–146
- Carla Lucia E et al (2011) A neutralizing RNA aptamer against EGFR causes selective apoptotic cell death. *PLoS ONE* 6:e24071
- Chen Q et al (2018) A novel approach for rapid high-throughput selection of recombinant functional rat monoclonal antibodies. *BMC Immunol* 19:35
- Chirino AJ, Mire-Sluis A (2004) Characterizing biological products and assessing comparability following manufacturing changes. *Nat Biotechnol* 22:1383–1391
- Chu L, Robinson DK (2001) Industrial choices for protein production by large-scale cell culture. *Curr Opin Biotechnol* 12:180–187
- Clark KJR, Chaplin FWR, Harcum SW (2004) Temperature Effects on Product-Quality-Related Enzymes in Batch CHO Cell Cultures Producing Recombinant tPA. *Biotechnol Prog* 20:1888–1892
- Dada OO, Jaya N, Valliere-Douglass J, Salas-Solano O (2015) Characterization of acidic and basic variants of IgG1 therapeutic monoclonal antibodies based on non-denaturing IEF fractionation. *Electrophoresis* 36:2695–2702
- Donaghy H (2016) Effects of antibody, drug and linker on the preclinical and clinical toxicities of antibody-drug conjugates. *MAbs* 8:659–671
- Du Y, Walsh A, Ehrick R, Xu W, May K, Liu H (2012) Chromatographic analysis of the acidic and basic species of recombinant monoclonal antibodies. *MAbs* 4:578–585
- Fogolín MB, Wagner R, Etcheverrigaray M, Kratje R (2004) Impact of temperature reduction and expression of yeast pyruvate carboxylase on hGM-CSF-producing CHO cells. *J Biotechnol* 109:179–191
- Gawlitzeck M, Estacio M, Fürch T, Kiss R (2009) Identification of cell culture conditions to control N-glycosylation site-occupancy of recombinant glycoproteins expressed in CHO cells. *Biotechnol Bioeng* 103:1164–1175
- Gomez N et al (2012) Culture temperature modulates aggregation of recombinant antibody in cho cells. *Biotechnol Bioeng* 109:125–136
- Higel F, Seidl A, Sorgel F, Friess W (2016) N-Glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins. *Eur J Pharm Biopharm* 100:94–100
- Hintersteiner B et al (2016) Charge heterogeneity: Basic antibody charge variants with increased binding to Fc receptors. *mAbs* 8:1548–1560
- Houde D, Peng Y, Berkowitz SA, Engen JR (2010) Post-translational modifications differentially affect IgG1 conformation and receptor binding. *Mol Cell Proteomics* 9:1716–1728
- Huang YM, Hu W, Rustandi E, Chang K, Yusuf-Makagiansar H, Ryll T (2010) Maximizing productivity of CHO cell-based fed-batch culture using chemically defined media conditions and typical manufacturing equipment. *Biotechnol Prog* 26:1400–1410
- Jordan M, Voisard D, Berthoud A, Tercier L, Kleuser B, Baer G, Broly H (2013) Cell culture medium improvement by rigorous shuffling of components using media blending. *Cytotechnology* 65:31–40
- Kaneko Y, Sato R, Aoyagi H (2010) Changes in the quality of antibodies produced by Chinese hamster ovary cells during the death phase of cell culture. *J Biosci Bioeng* 109:281–287
- Kaufmann H, Mazur X, Fussenegger M, Bailey JE (1999) Influence of low temperature on productivity, proteome and protein phosphorylation of CHO cells. *Biotechnol Bioeng* 63:573–582
- Khawli LA et al. (2015) Charge variants in IgG1: isolation, characterization, in vitro binding properties and pharmacokinetics in rats. *Mabs* 2:613–624
- Kishishita S, Nishikawa T, Shinoda Y, Nagashima H, Okamoto H, Takuma S, Aoyagi H (2015) Effect of temperature shift on levels of acidic charge variants in IgG monoclonal antibodies in Chinese hamster ovary cell culture. *J Biosci Bioeng* 119:700–705
- Leblanc Y, Ramon C, Bihoreau N, Chevreux G (2017) Charge variants characterization of a monoclonal antibody by ion exchange chromatography coupled on-line to native mass spectrometry: case study after a long-term storage at + 5 °C. *J Chromatogr B* 1048:130–139
- Li W, Kerwin JL, Schiel J, Formolo T, Davis D, Mahan A, Benchaar SA (2015) Structural Elucidation of post-translational modifications in monoclonal antibodies. In: State-of-the-art and emerging technologies for therapeutic monoclonal antibody characterization vol 2. *Biopharmaceutical characterization: the NISTmAb case study*, vol

1201. ACS symposium series, vol 1201. American Chemical Society, Washington, pp 119–183. <https://doi.org/10.1021/bk-2015-1201.ch003>
- Lim Y, Wong NS, Lee YY, Ku SC, Wong DC, Yap MG (2010) Engineering mammalian cells in bioprocessing - current achievements and future perspectives. *Biotechnol Appl Chem* 55:175–189
- Lingg N, Tan E, Hintersteiner B, Bardor M, Jungbauer A (2013) Highly linear pH gradients for analyzing monoclonal antibody charge heterogeneity in the alkaline range. *J Chromatogr A* 1319:65–71
- Lu F, Toh PC, Burnett I, Li F, Li J (2013) Automated dynamic fed-batch process and media optimization for high productivity cell culture process development. *Biotechnol Bioeng* 110:191–205
- Luo J, Zhang J, Ren D, Tsai W-L, Li F, Amanullah A, Hudson T (2012) Probing of C-terminal lysine variation in a recombinant monoclonal antibody production using Chinese hamster ovary cells with chemically defined media. *Biotechnol Bioeng* 109:2306–2315
- Mueller MM (2017) Post-translational modifications of protein backbones: Unique functions, mechanisms and challenges. *Biochemistry* 57:177–185
- Nasiri H, Valedkarimi Z, Aghebati-Maleki L, Majidi J (2018) Antibody-drug conjugates: promising and efficient tools for targeted cancer therapy. *J Cell Physiol* 9:6441–6457
- Oguchi S, Saito H, Tsukahara M, Tsumura H (2006) pH Condition in temperature shift cultivation enhances cell longevity and specific hMab productivity in CHO culture. *Cytotechnology* 52:199–207
- Pan X, Streefland M, Dalm C, Wijffels RH, Martens DE (2016) Selection of chemically defined media for CHO cell fed-batch culture processes. *Cytotechnology* 69:39–56
- Prabhu A, Gadgil M (2019) Nickel and cobalt affect galactosylation of recombinant IgG expressed in CHO cells. *Biometals* 32:11–19
- Radhakrishnan D, Robinson AS, Ogunnaike BA (2018) Controlling the glycosylation profile in mAbs using time-dependent media supplementation. *Antibodies* 7:1
- Rea JC, Moreno GT, Lou Y, Farman D (2011) Validation of a pH gradient-based ion-exchange chromatography method for high-resolution monoclonal antibody charge variant separations. *J Pharm Biomed Anal* 54:317–323
- Read EK, Park JT, Brorson KA (2011) Industry and regulatory experience of the glycosylation of monoclonal antibodies. *Biotechnol Appl Biochem* 58:213–219
- Reuveny S, Velez D, Macmillan JD, Miller L (1986) Factors affecting cell growth and monoclonal antibody production in stirred reactors. *J Immunol Methods* 86:53–59
- Rouiller Y, Périlleux A, Vesin M-N, Stettler M, Jordan M, Broly H (2014) Modulation of mAb quality attributes using microliter scale fed-batch cultures. *Biotechnol Prog* 30:571–583
- Schatz SM, Kerschbaumer RJ, Gerstenbauer G, Kral M, Dorner F, Scheiflinger F (2003) Higher expression of fab antibody fragments in a CHO cell line at reduced temperature. *Biotechnol Bioeng* 84:433–438
- Sengupta N, Rose ST, Morgan JA (2011) Metabolic flux analysis of CHO cell metabolism in the late non-growth phase. *Biotechnol Bioeng* 108:82–92
- Seo JS et al (2016) Characteristics of human cell line, F2N78, for the production of recombinant antibody in fed-batch and perfusion cultures. *J Biosci Bioeng* 121:317–324
- Shukla AA, Thömmes J (2010) Recent advances in large-scale production of monoclonal antibodies and related proteins. *Trends Biotechnol* 28:253–261
- Si NS, Sellick C, Lee K, Mason A, Kontoravdi C (2014) How does mild hypothermia affect monoclonal antibody glycosylation? *Biotechnol Bioeng* 112:1165–1176
- Silva Ad et al (2014) Target-directed development and pre-clinical characterization of the proposed biosimilar rituximab GP2013. *Leuk Lymphoma* 55:1609–1617
- Singh N, Arunkumar A, Chollangi S, Tan ZG, Borys M, Li ZJ (2016) Clarification technologies for monoclonal antibody manufacturing processes: Current state and future perspectives. *Biotechnol Bioeng* 113:698–716
- Sureshkumar GK, Mutharasan R (1991) The influence of temperature on a mouse–mouse hybridoma growth and monoclonal antibody production. *Biotechnol Bioeng* 37:292–295
- Teo PZ, Utz PJ, Mollick JA (2012) Using the allergic immune system to target cancer: activity of IgE antibodies specific for human CD20 and MUC1. *Cancer Immunol Immunother* 61:2295–2309
- Van Beers MMC, Bardor M (2012) Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins. *Biotechnol J* 7:1473–1484
- Vázquez-Rey M, Lang DA (2011) Aggregates in monoclonal antibody manufacturing processes. *Biotechnol Bioeng* 108:1494–1508
- Walsh G (2010) Post-translational modification of protein biopharmaceuticals. *Drug Discov Today* 15:773–780
- Wei W, Singh S, Zeng DL, King K, Nema S (2006) Antibody structure, instability, and formulation. *J Pharm Sci* 96:1–26
- Yoon SK, Song JY, Lee GM (2003) Effect of low culture temperature on specific productivity, transcription level, and heterogeneity of erythropoietin in Chinese hamster ovary cells. *Biotechnol Bioeng* 82:289–298
- Yoshimoto N, Itoh T, Inaba Y, Ishii H, Yamamoto K (2013) Structural basis for inhibition of carboxypeptidase B by selenium-containing inhibitor: selenium coordinates to zinc in enzyme. *J Med Chem* 56:7527–7535
- Zheng K, Yarmarkovich M, Bantog C, Bayer R, Patapoff TW (2014) Influence of glycosylation pattern on the molecular properties of monoclonal antibodies. *MAbs* 6:649–658

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.