Supplementary Information for

Efficient Antibody Assembly in *E. coli* Periplasm by Disulfide Bond Folding Factor Co-expression and Culture Optimization

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MATERIALS AND METHODS

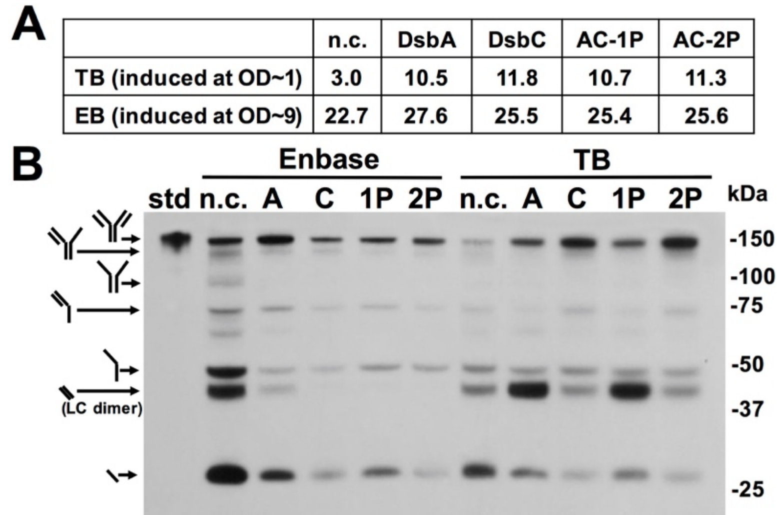
IgG Cloning and Expression. To construct an IgG periplasmic expression vector pMAZ-Her, VH and VL genes encoding anti-HER2 mAb trastuzumab (Herceptin) were chemically synthesized and cloned into *Nhe*I/*Hind*III and *Nco*I/*Not*I sites on pMAZ360 [16]. pMAZ-Her was co-transformed into Jude-I harboring each folding factor plasmid pBAD-DsbA/-DsbC/AC-1P/AC-2P. Transformed cells were cultivated in TB or EnBase media at 25 °C. After induction with 0.2% arabinose and 0.1 mM IPTG, periplasmic fractions were prepared for Western blotting analysis (non-reducing) using goat anti-human IgG-HRP (Jackson ImmunoResearch).

RESULTS

Production of Aglycosylated IgG. To further demonstrate that DsbA/C can promote proper disulfide formation and protein folding, we next attempted to produce aglycosylated IgG in *E. coli*

periplasm. The VH and VL fragments of mAb trastuzumab were cloned into a periplasmic IgG expression vector carrying P_{Lac} and pelB leaders, and co-transformed into Jude-I cells with each plasmid pBAD-DsbA/-DsbC/AC-1P/AC-2P. Transformed cells were cultured in TB or EnBase media at 25 ^{o}C with induction. After 24 hr, OD_{600} of TB culture without folding factor was 3.0, while cell densities OD_{600} reached 10.5-11.8 with DsbA/C co-expression (**Supplementary Fig 1A**), suggesting these folding factors significantly improved the cell growth, likely by reducing the toxicity effects of IgG expression on host cells. Similarly, DsbA/C enhanced cell growth in EnBase media from OD_{600} 22.7 (no folding factor) to 25.4-27.6. Western blotting analysis of periplasmic preparations using goat anti-human IgG-HRP indicated that without DsbA/C co-expression, in addition to the band associated with full IgG, as many as six partially assembled antibody fragments were detected, especially for unassembled LC and HC, and LC dimer (**Supplementary Fig 1B**). With DsbA/C co-expression in EnBase media, these fragmented species were much less present, and amounts of fully assembled IgG were significantly increased for the DsbA construct, suggesting improvement of the quality for IgG production. In TB media, all the DsbA/C constructs resulted in more than 5-fold enhancement of the IgG production, and the unassembled fragments were not increased for DsbC and AC-2P constructs. Using construct AC-2P in EnBase, expressed IgG were further purified for quantification. Results indicated that 2 mg purified aglycosylated IgG was obtained per liter of culture.

SUPPLEMENTARY FIGURE 1



	n.c.	DsbA	DsbC	AC-1P	AC-2P
TB (induced at OD~1)	3.0	10.5	11.8	10.7	11.3
EB (induced at OD~9)	22.7	27.6	25.5	25.4	25.6

Supplementary Figure 1. Assembly of aglycosylated full IgG with DsbA/C co-expression. (A) OD_{600} after 24 hour induction at 25 ^{o}C. **(B)** Western blotting results developed with anti-human IgG-HRP. 2 mg IgG was purified per litter of EnBase media when construct DsbAC-2P was co-expressed.