Generation of Recombinant CHO TurboCell™ Lines for the Production of PASylated Human DNase I

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Introduction

Recombinant human deoxyribonuclease I (rhDNase I) has been used for reducing sputum viscosity in the treatment of cystic fibrosis for more than 20 years. However, the short half-life of rhDNase I requires a high dosing frequency, which is associated with elevated risk of lung infections. Therefore, we have set out to generate a recombinant CHO cell line producing a rhDNase I with prolonged half-life by applying PASylation® technology.

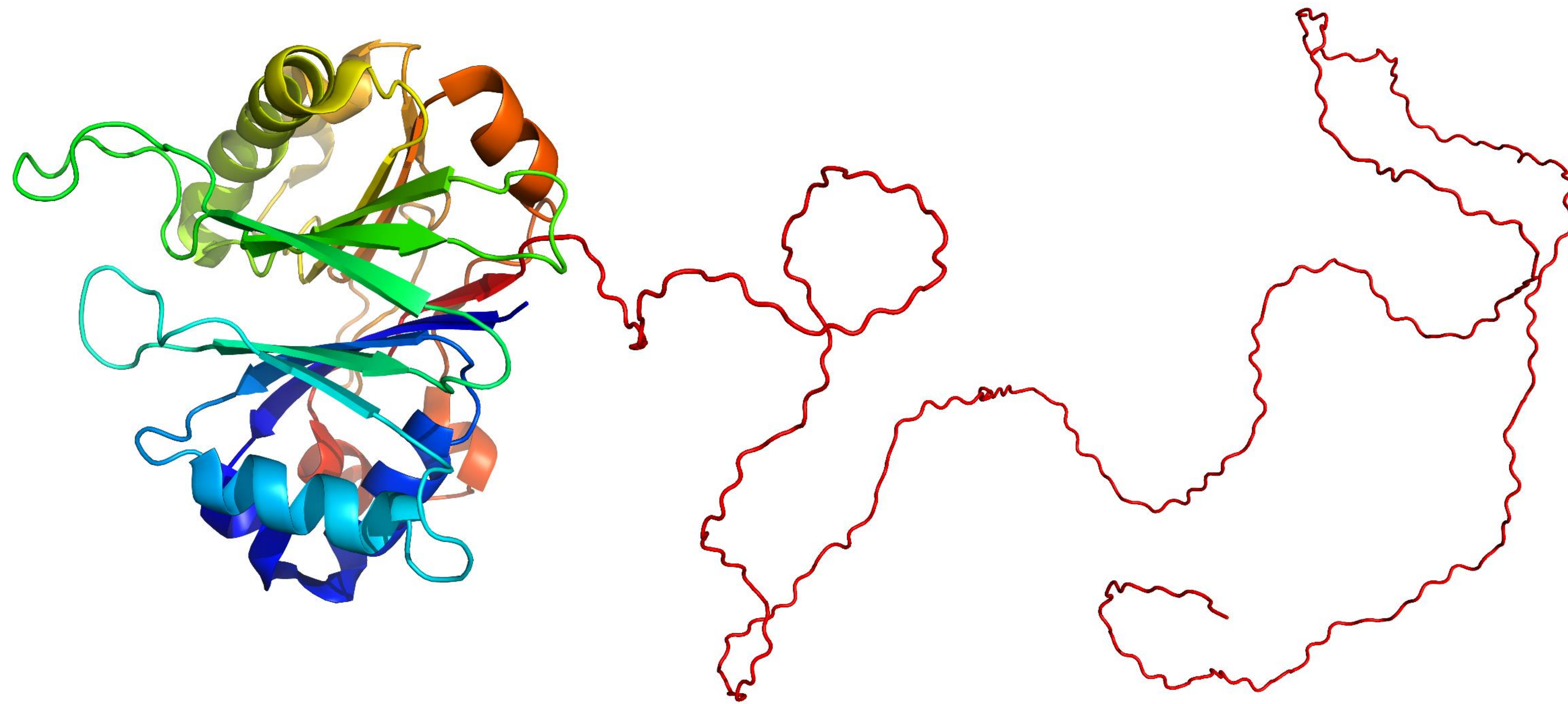

Figure 1: Computer model of N-terminally PASylated (red) recombinant human DNase I (rainbow). (PyMOL 2.2.0)

Aim

Generation of a CHO cell line expressing a PASylated rhDNase I (Figure 1) by site-directed gene integration. Based on the genetically encoded highly soluble disordered polypeptide chain made of Pro, Ala and/or Ser (PAS), which is fused with the enzyme, an increased half-life by enlarging the hydrodynamic volume and shielding of the therapeutic protein is expected[2,3].

Cell Line Generation

The process for manufacturing a PASylated rhDNase I comprises both cell line generation and upstream/ downstream development (Figure 2) starting with:

- **Cloning of PAS- and P/A-DNase I fusion protein constructs**
- **CHO transfection into Rentschler TurboCell™ for stable site-directed GOI integration**

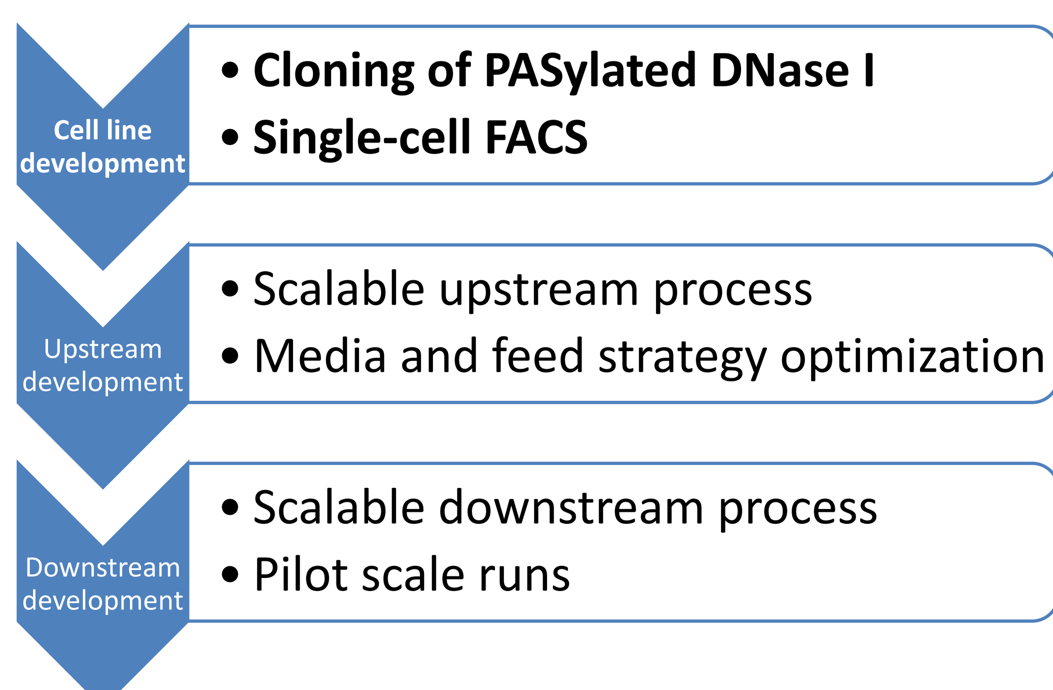

Figure 2: Experimental approach and development plan.

DNase Activity Assay

DNase I expression levels were quantified by a modified Quant-iT™ PicoGreen™ (PG) activity assay[1].
DNA degradation was shown to be linear between 10 and 100 pg of bovine pancreas (bp) DNase I (Figure 3).

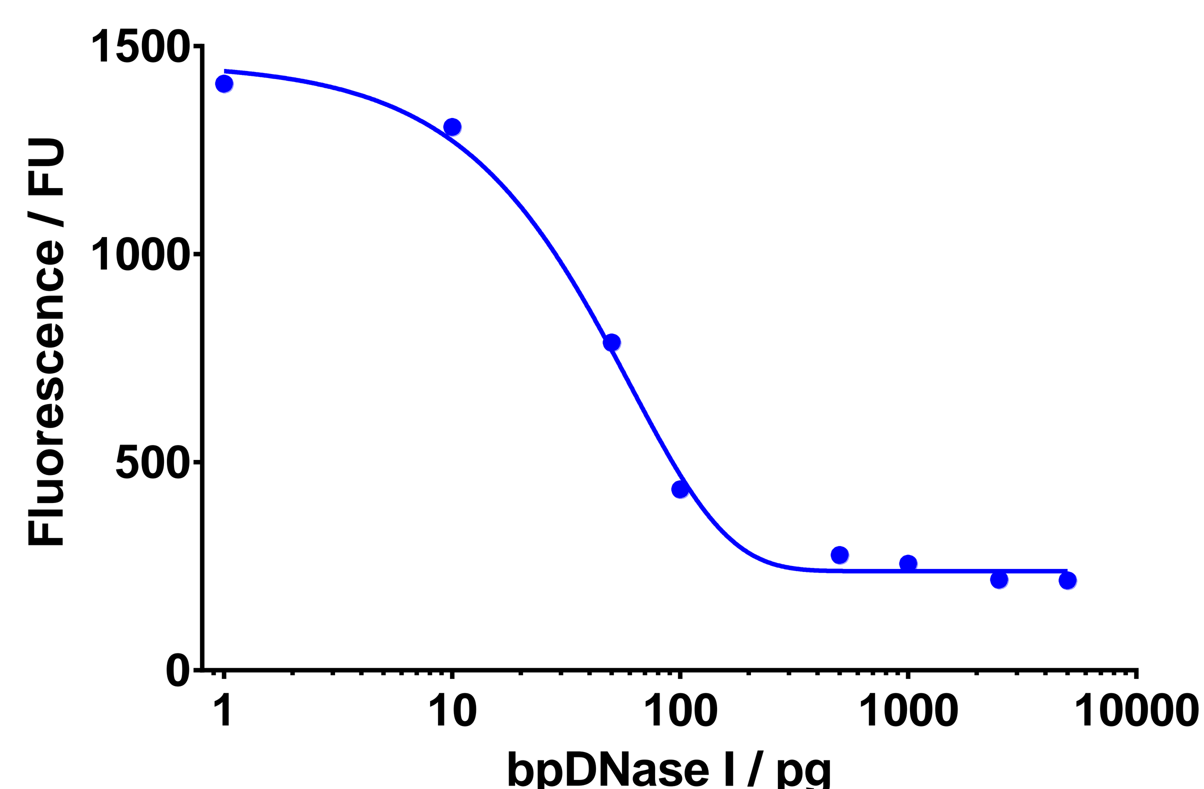

Figure 3: Reduction in fluorimetric signal at 520 nm by bpDNase I-catalyzed DNA hydrolysis (excitation at 480 nm). Single exponential decay fit (GraphPad Prism 6.07). The fluorescent PG intercalates into double-stranded DNA.

Results

Transient transfection of the constructs P/A(600)-DNase I, P/A(400)-DNase I-P/A(400), PAS(600)-DNase I and wtDNase I (4 µg and 3x10^6 cells per transfection) resulted in TurboCell™ CHO pools expressing PASylated and wtDNase I, respectively. Successfull expression was verified by the DNase activity assay using the respective cell culture supernatant from 4-day cultures (Figure 4). At ten-fold serial dilution of each supernatant, a DNase I mediated decrease in flourimetric signal was observed for all constructs. The secreted PASylated proteins with an apparent molecular size above 250 kDa were analyzed by denaturing SDS-PAGE (Figure 5).

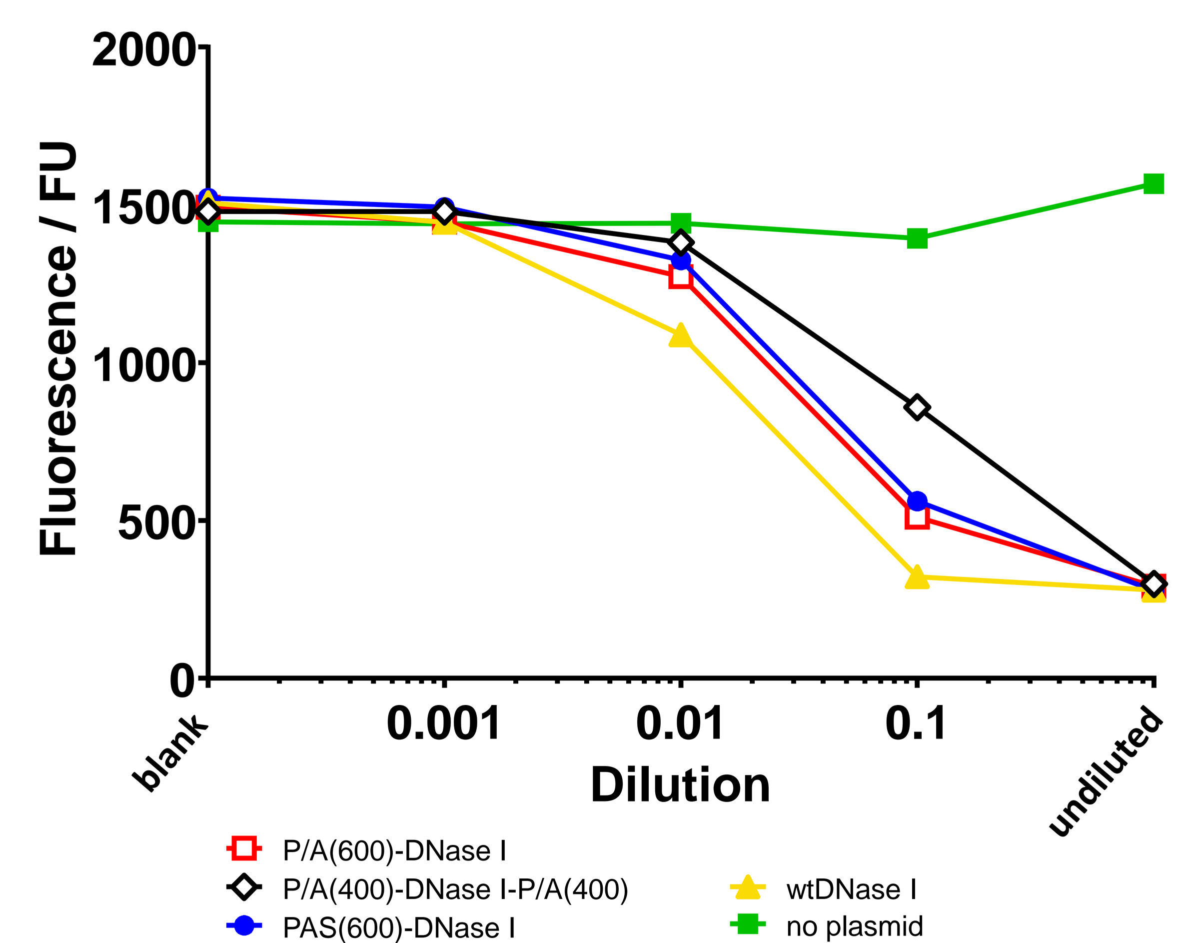

Figure 4: DNase I activity assay using supernatants from cultivation day 4 for different PASylated DNase I constructs and recombinant wtDNase I after transient transfection of CHO cells. (GraphPad Prism 6.07)

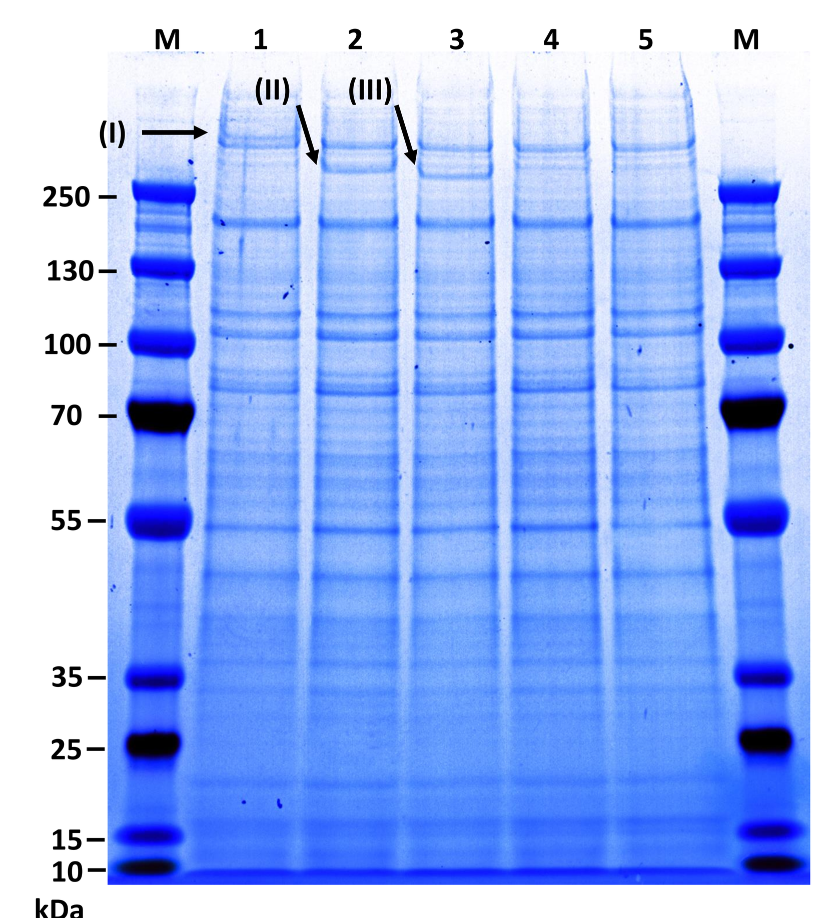

Figure 5: 4-12% Bis-TRIS SDS-PAGE (reducing), stained with Coomassie blue, of day 4 CHO cell culture supernatants (16.5 µl); M: PageRuler Ladder, Lane 1: P/A(400)-DNase I-P/A(400) (I), Lane 2: P/A(600)-DNase I (II), Lane 3: PAS(600)-DNase I (III), Lane 4: wtDNase I (not visible) Lane 5: Control w/o plasmid.

References

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