Research Square

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Numaswitch – an efficient high-titer expression platform to produce peptides and small proteins

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Abstract

The production of peptides as active pharmaceutical ingredients (APIs) by recombinant technologies is of emerging interest. A reliable production platform, however, is still missing due the inherent characteristics of peptides such as proteolytic sensitivity, aggregation and cytotoxicity. We have developed a new technology named Numaswitch solving present limitations. Numaswitch was successfully employed for the production of diverse peptides and small proteins varying in length, physicochemical and functional characteristics, including Teriparatide, Linaclotide, human β-amyloid and Serum amyloid A3. Additionally, the potential of Numaswitch for a cost-efficient commercial production is demonstrated yielding > 2 g Teriparatide per liter fermentation broth in a quality meeting API standard.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

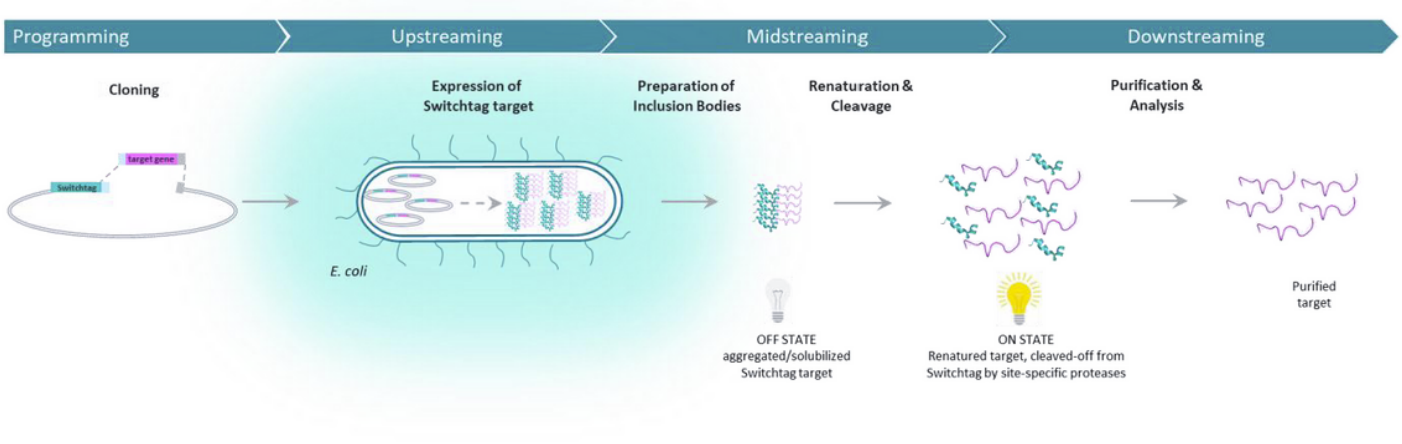

Figure 1

Schematic view of the Numaswitch approach. Being integrated into an expression cassette and expressed in E. coli, Switchtag targets form inclusion bodies (IBs) inside the cytoplasm (IB-tag). The IBs are extracted and solubilized. Switchtag targets are subsequently quantitatively renatured in the presence of Ca2+ (renaturation tag). After renaturation targets are separated from the Switchtag by site-specific proteases releasing traceless products without any non-desired amino acids remaining attached.

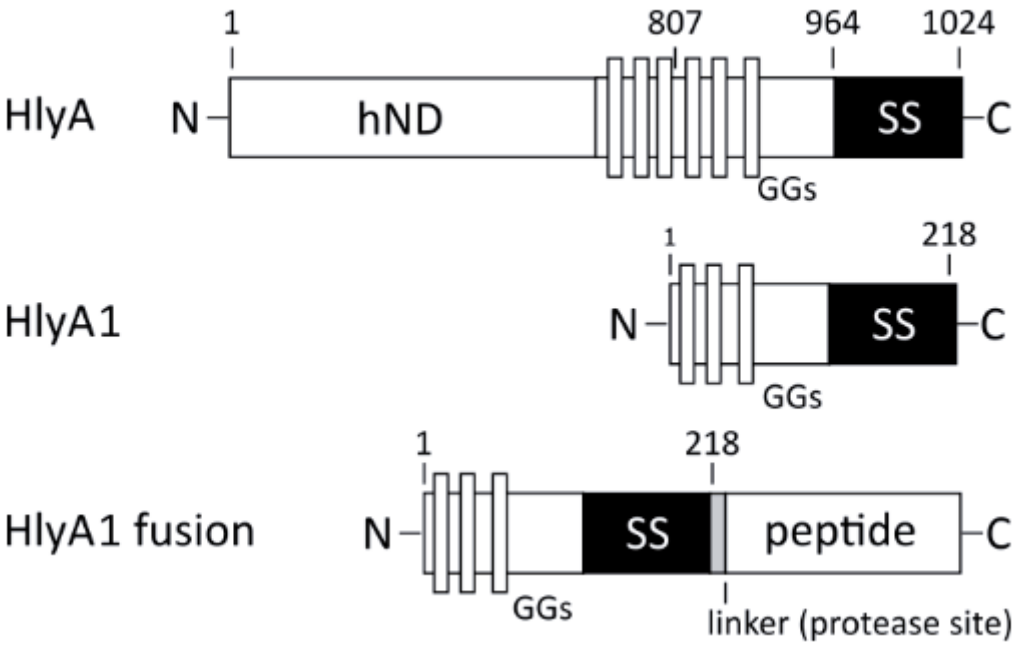

Figure 2

Schematic view of HlyA, HlyA1 and a HlyA1 fusion. HlyA consist of a hydrophobic N-terminal domain (hND), an RTX domain (RTX) characterized by the presence of so-called GG repeats (GGs) and a C terminal secretion signal (SS, ~60 aa). In HlyA1 and HlyA1 fusions the C-terminal 218 aa of HlyA are present. Between HlyA1 and fused targets a Tobacco Edge Virus (TEV) cleavage site is inserted. Numbers indicate the position in the corresponding amino acid sequence.

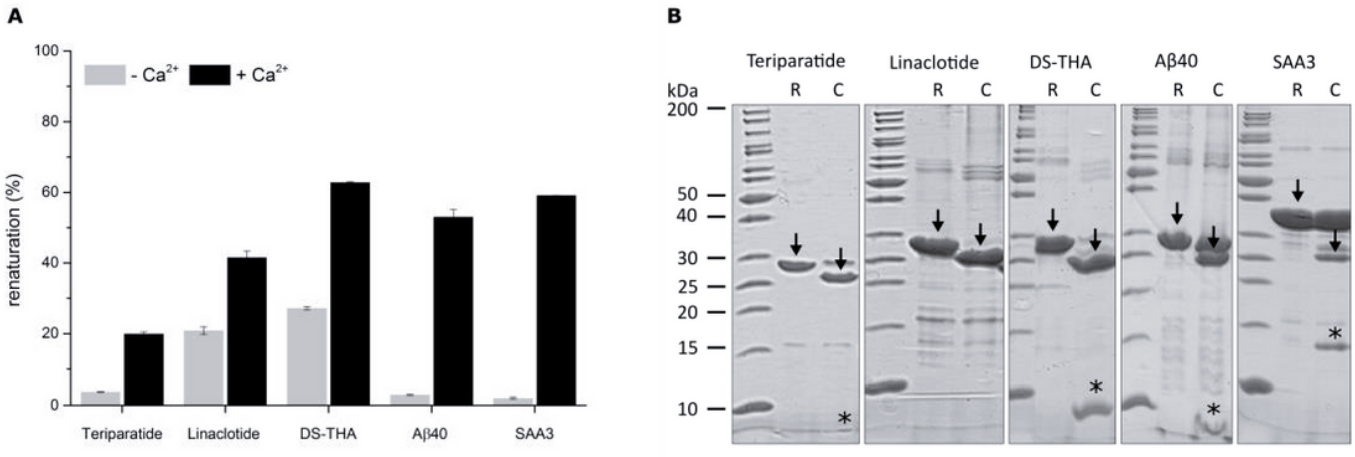

Figure 3

Renaturation efficiencies and TEV protease cleavage of HlyA1 fusions. A Renaturation efficiencies (%) of HlyA1 fusions in the presence or absence of 10 mM Ca2+ ions. 100% renaturation efficiency corresponds to the adjusted protein concentration for the renaturation reaction. Error bars indicate the SD (n = 3). B SDS-PAGE analysis (Coomassie-stained) of renatured HlyA1 fusions prior to and after TEV protease cleavage. Arrows indicate the HlyA1 fusions after renaturation (R) and HlyA1 after TEV protease cleavage (C). Signals were found for each target but Linaclotide. Due to visualization limits of Coomassie staining, the location is highlighted (*). Release of targets was further assessed by RP-HPLC analysis (Figure S3).

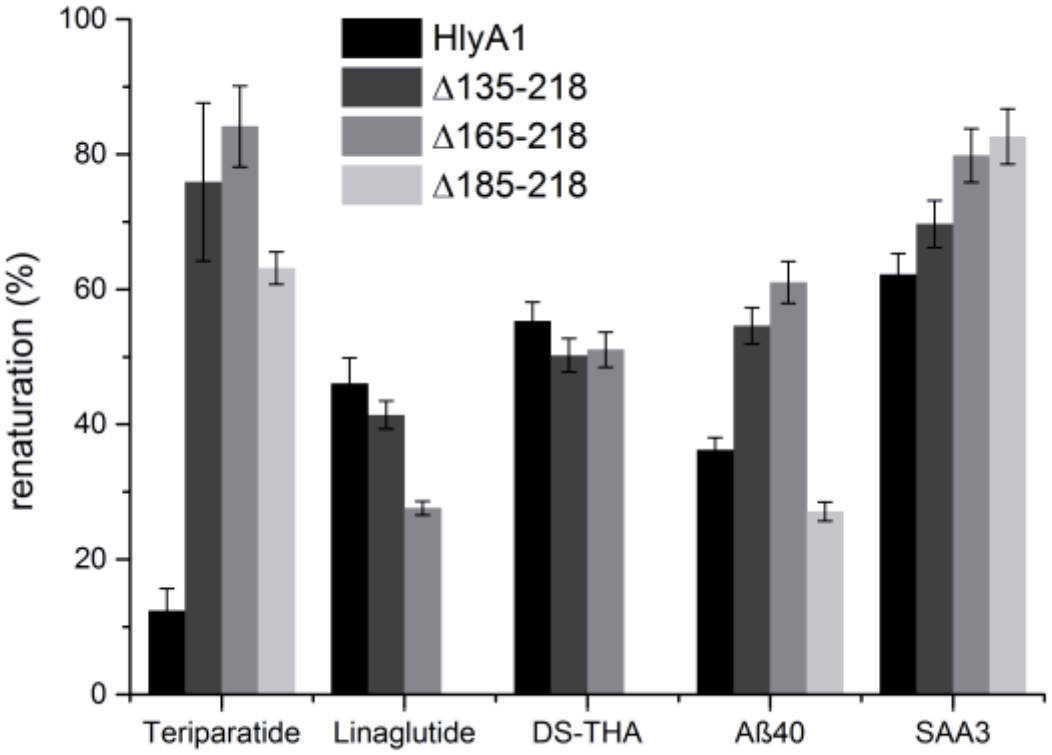

Figure 4

Renaturation efficiencies of HlyA1 fusions and truncated HlyA1 fusion variants. Shown are the renaturation efficiencies (%) in Tris-based buffer in the presence of 10 mM Ca2+ ions. Error bars indicate the SD, measurements were performed in triplicates.

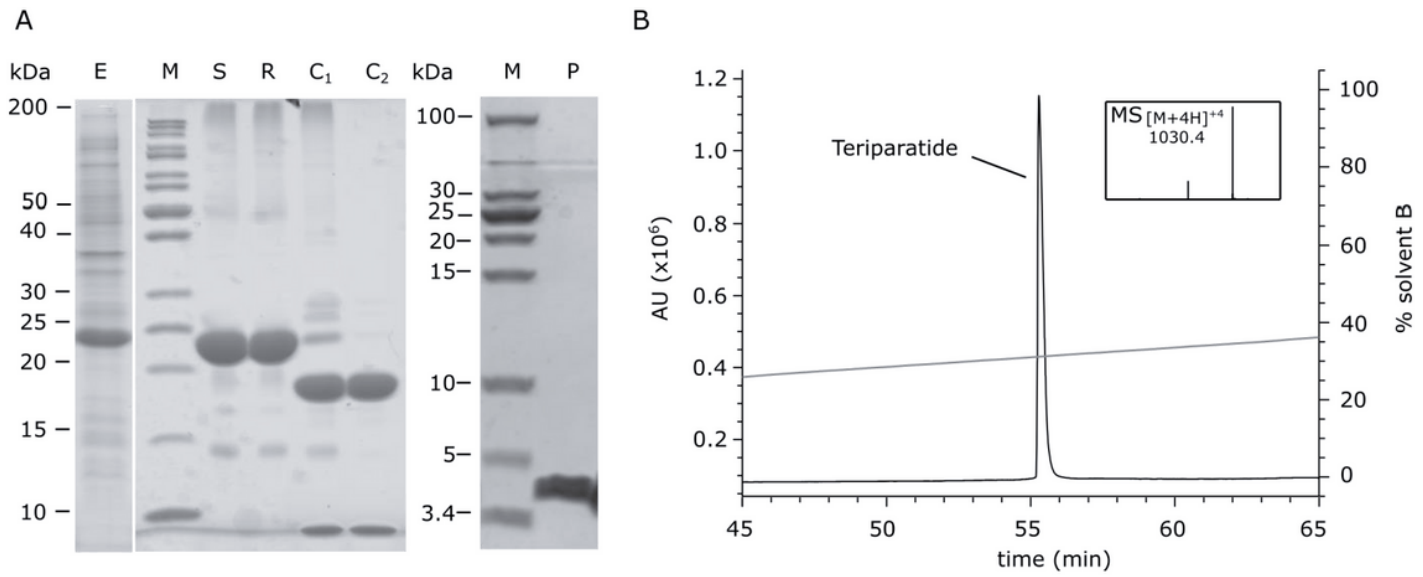

Figure 5

Numaswitch applied to produce Teriparatide A SDS-PAGE analysis of different process steps; E. coli cells from high cell density fermentation (E), solubilized Switchtag Teriparatide IBs (S), renaturation reaction (R), TEV protease cleavage (C1: crude, C2, cleared) and produced Teriparatide after CEX, RP FLASH purification and TFA/acetate exchange (P). M: Molecular weight marker with shown masses. B HPLC/MS analysis of purified Teriparatide revealed > 99.6% purity of lyophilized Teriparatide.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AMBexpressNumaswitchSIsubmitted20210212.pdf