430c Refolding Kinetics of a Recombinant Fusion Protein without Chaotropic Agents

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Refolding of biopharmaceutical proteins at an industrial scale is often hampered by the required extreme dilution of the refolding protein, the excessive use of auxiliary materials such as chaotropic agents and a disappointingly low yield of native functional protein. Therefore the pharmaceutical industry strives to the development of cleaner and more cost-efficient processes. Detailed molecular insight in the refolding process and the competing reactions such as misfolding and in particular aggregate formation have been the subject of theoretical studies. Documentation of real industrial systems –including the impact on process design and economy- is modest, and is the topic of the current work. This work reports the oxidative batch dilution refolding of a 12.7 kDa fusion protein which is produced by bacterial fermentation. The solubilization of its inclusion bodies could be achieved at alkali pH and without chaotrophic agents. Additionally, the refolding yield obtained was high using industrially relevant protein concentration which is approximately 100 to 400 times larger than the optimum reported for this refolding technique. In this paper, the refolding kinetics was assessed, and the underlying refolding and parallel aggregation kinetics were studied. The overall result was a substantial improvement of refolding yield of the final (non-fusion) protein, compared to reported yields for that product.

The process integrated implementation of the refolding method required a parallel development of purification technology, in this case the development of an anion exchange method allowed baseline separation of the native protein from the resulting aggregates, leading to a relatively compact process. Future work is targeted at the identification of mechanisms that lead to the reduction or complete prevention of aggregates and misfolded monomers