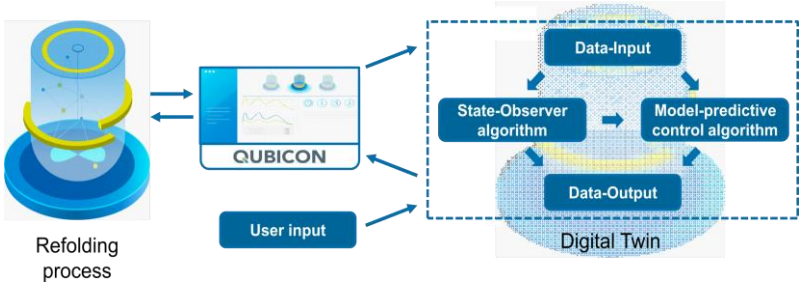
SUCCESS STORY

**CHASE**
**Chemical Systems Engineering**

Programme: COMET – Competence Centers for Excellent Technologies

Programme line: COMET-Centre (K1)

Type of project: Model based process design, 10/19 – 09/23, multi-firm




Schematic overview of an integrated platform approach for model-based monitoring and control of a protein refolding process.
(© Bilfinger; agreed modification by CHASE/Pauk)

# CONTROLLING OF REFOLDING KINETICS BY COMBINING PAT AND MODELING

## PROCESS UNDERSTANDING ALONG QUALITY BY DESIGN PRINCIPLES AND PROCESS MONITORING SERVES AS A BASIS FOR A CONTROLLED AND REPRODUCIBLE REFOLDING ENVIRONMENT

Overexpression of recombinant proteins in Escherichia coli results in misfolded and non-active protein aggregates in the cytoplasm, so-called inclusion bodies (IB). In recent years, a change in the mindset regarding IBs could be observed: IB are no longer considered as an unwanted waste product, but as a valid alternative to produce product with high yield, purity and stability in short process times. However, solubilization of IBs and subsequent refolding is necessary to obtain correctly folded and active product.

This protein refolding process is a crucial downstream unit operation - commonly done in batch or fed-batch dilution mode. Drawbacks of the state-of-the-art include: the large volume of buffers and capacities of re-folding tanks, issues with uniform mixing, challenging analytics at low protein concentrations, reaction kinetics in non-usable aggregates and generally low re-folding yields.

There is no generic platform procedure available and a lack of robust control strategies. The introduction of Quality by Design is the method-of-choice to provide a controlled and reproducible refolding environment. However, reliable online monitoring techniques to describe the refolding kinetics in real-time are scarce.

Gathering process understanding along Quality by Design principles and process monitoring could serve

Federal Ministry
Republic of Austria
Climate Action, Environment, Energy, Mobility, Innovation and Technology

Federal Ministry
Republic of Austria
Digital and Economic Affairs

Version 01/2020

as a basis for a controlled and reproducible refolding environment.

We propose the implementation of a combined approach by applying on-line PAT analytics and modelling in the controlled environment of a fully automated refolding vessel, equipped with the advanced process control software Qubicon® (provided by Bilfinger).

This integrated approach follows the strategy that certain aspects of this complex reaction process can be monitored directly, while the rest can be estimated via modelling and can subsequently be used for realtime process control. For monitoring we are currently using a combination of HPLC and photometric methods to assess product quantity and quality attributes. During refolding temperature, pH, dissolved oxygen content and agitation are controlled.

In addition, our Digital Twin solution provides us with an estimation of current refolding kinetics which will be used to optimize refolding yields via model predictive control (MPC).

**Project coordination (Story)**
Christoph Herwig, Univ.-Prof. DI Dr.
Key Researcher
TU Wien
T +43 676 4737217
christoph.herwig@tuwien.ac.at

**Competence Center CHASE GmbH**
Altenbergerstrasse 69
4040 Linz
T +43 664 9658923
Patrick.pammer@chasecenter.at
www.chasecenter.at

**Project partner**

- Bilfinger, Austria
- TU Wien, Austria

This success story was provided by the centre management and by the mentioned project partners for the purpose of being published on the FFG website. CHASE is a COMET Centre within the COMET – Competence Centers for Excellent Technologies Programme and funded by BMK, BMDW and the provinces Upper Austria and Vienna. The COMET Programme is managed by FFG. Further information on COMET: www.ffg.at/comet