An integrated approach for monitoring and control of refolding kinetics by combining PAT and modelling

Himmelfahrtstagung 2021 – Novel downstream technologies and integrated bioprocesses

Chika Linda Igwe[1,2], *Jan Niklas Pauk*[1,2], *Christoph Herwig*[2]

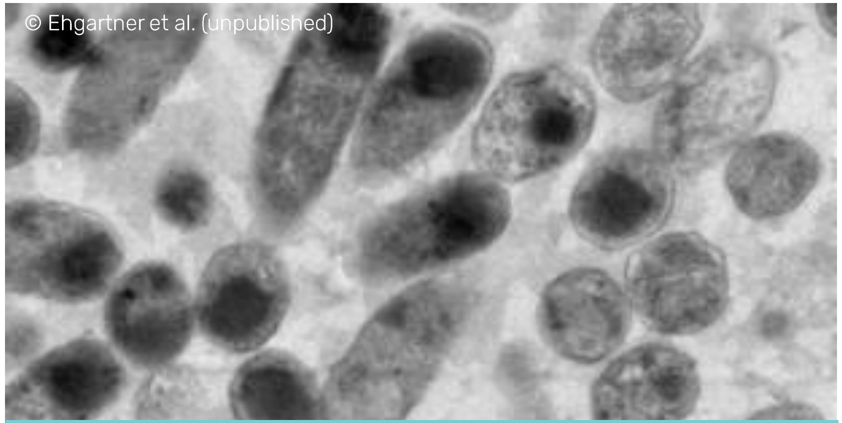
[1] *Competence Center CHASE GmbH, Altenbergerstraße 69, 4040 Linz, Austria*

[2] *TU Vienna, Gumpendorferstraße 1a, 1060 Vienna, Austria*

The Challenge

E. coli is commonly used for the production of biopharmaceuticals.

+ Cheap substrates
+ Fast growth
+ High product titers
+ Ease in genetic manipulation



- Extensive DSP
- Inclusion body formation

Inclusion Bodies

Benefits & Industrial Relevance

Product	Therapeutic Indication	Manufacturer
Betaseron (interferon beta 1b)	Multiple Sclerosis	Bayer
Humalog (lispro)	Diabetes	Eli Lilly
Humulin (rh insulin)	Diabetes	Eli Lilly
Lantus (long-acting insulin glargine)	Diabetes	Aventis
Neulasta (pegfilgrastim)	Neutropenia	Amgen
Neupogen (filgrastim)	Neutropenia	Roche
Pegasys (peginterferon 2a)	Hepatitis	Genentech
Pegintron (peginterferon 2b)	Hepatitis	Merck & Co.

- Ease of isolation
- High product yield and purity
- Mechanic stability
- Expression of toxic proteins

Inclusion Bodies

Downstream Processing

IB Isolation	Solubilization	Processing Solubilizate	Protein Refolding	Processing Refolded Protein	Protein Purification
– Denaturants – Detergents	– Denaturants – Detergents – Reducing agents	– Concentration – Buffer exchange – Clarification	– Dilution – Dialysis – Diafiltration – On-column refolding – High-pressure refolding	– Concentration – Buffer exchange – Clarification	– Chromatography – Filtration

Inclusion Bodies

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Improving low recovery yields by optimizing the refolding process mode

Current Strategies and Challenges

Black Box

Limited process knowledge available

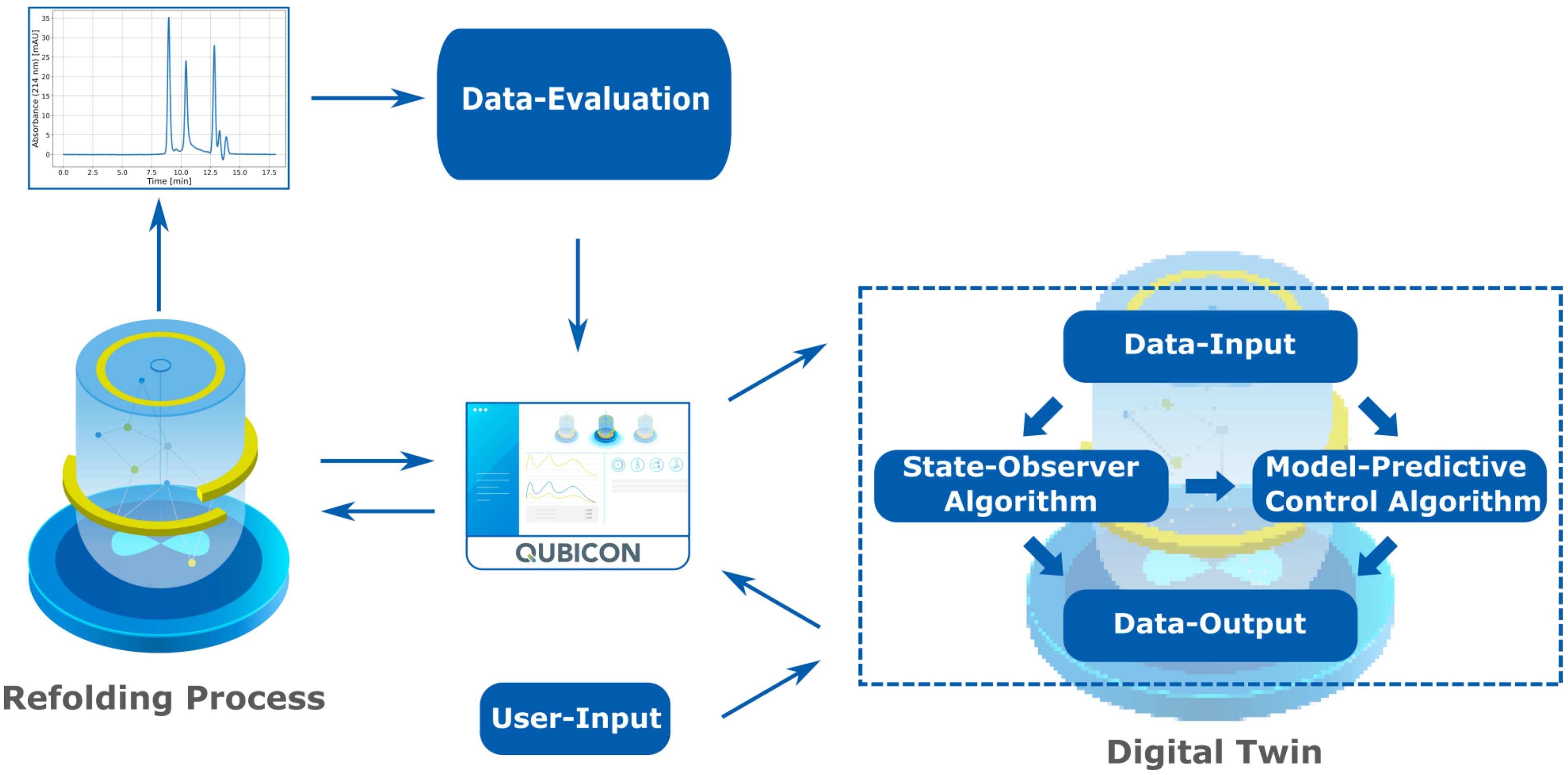
- – Empirical
- – Product specific
- – Uneconomical
- – High variability
- – Low yields

- \+ Knowledge-based
- \+ Transferable
- \+ Robust control
- \+ RT optimization
- \+ High yields

Grey Box

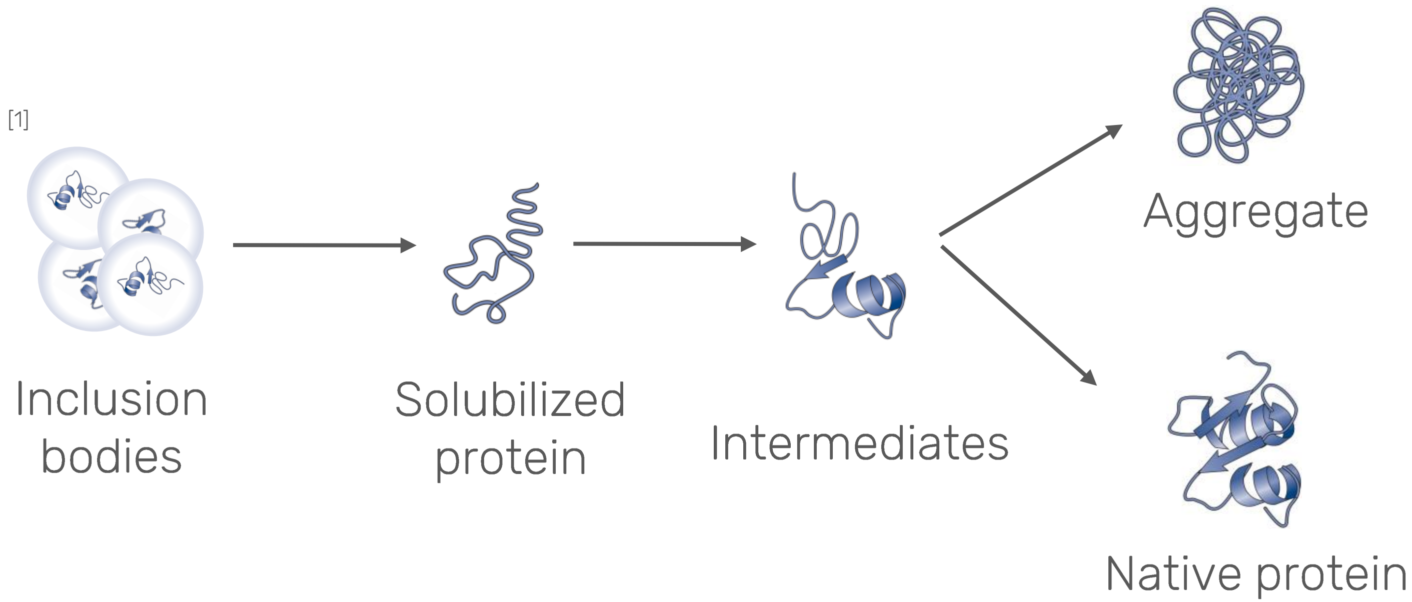
Transferable process knowledge available

Platform Refolding Process



Enhanced Control of Refolding

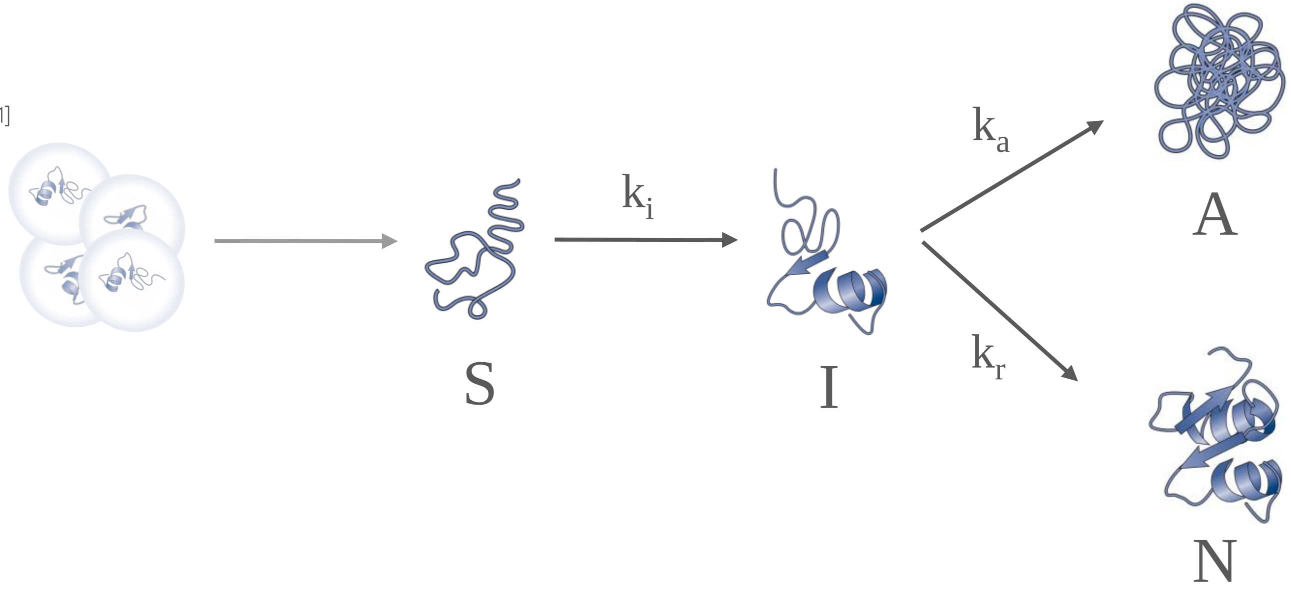
Protein Refolding Models

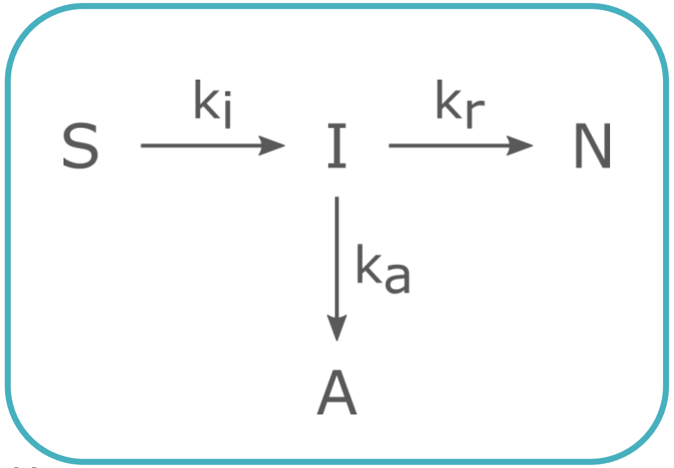


[1] Tyedmers, et al.,(2010). *Nat Rev Mol Cell Biol* **11**, 777-788.

Protein Refolding Models

[1]





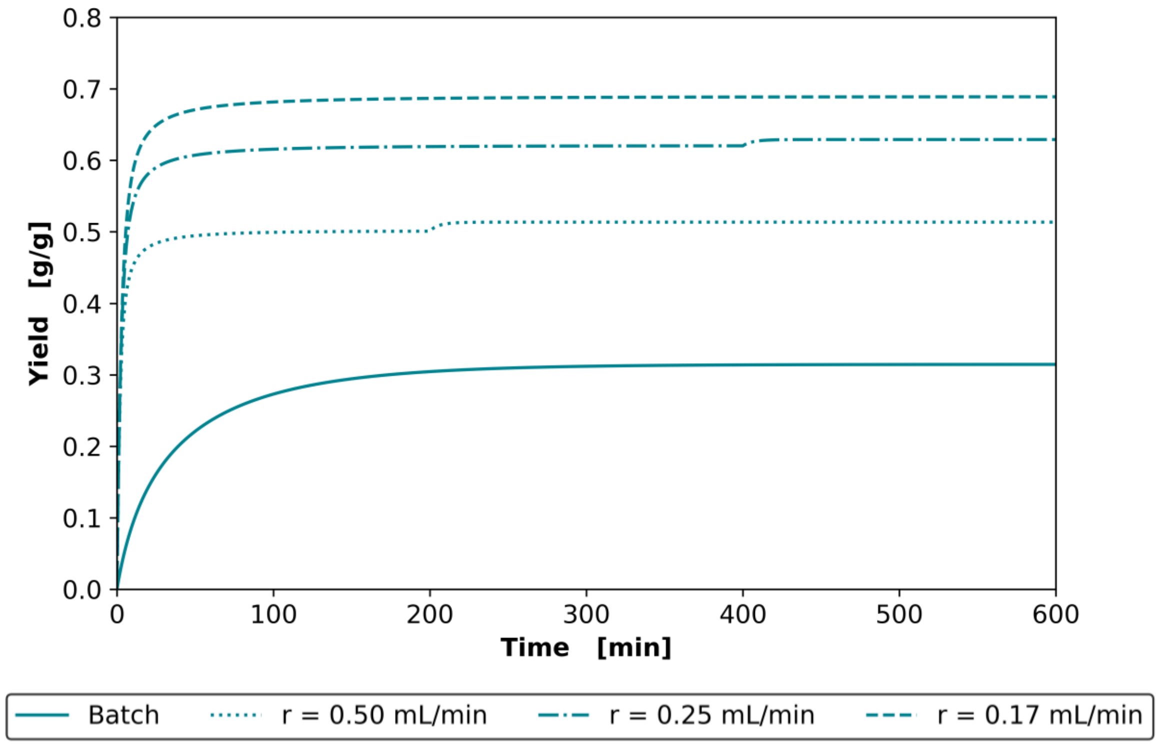
[2]

Adaption of model to complex proteins to develop a generic tool library

[1] Tyedmers et al. (2010). *Nat Rev Mol Cell Biol* **11**, 777-788.
[2] Hevehan DL, De Bernardez Clark E (1997).Biotechnol Bioeng 221-30.

Protein Refolding Process

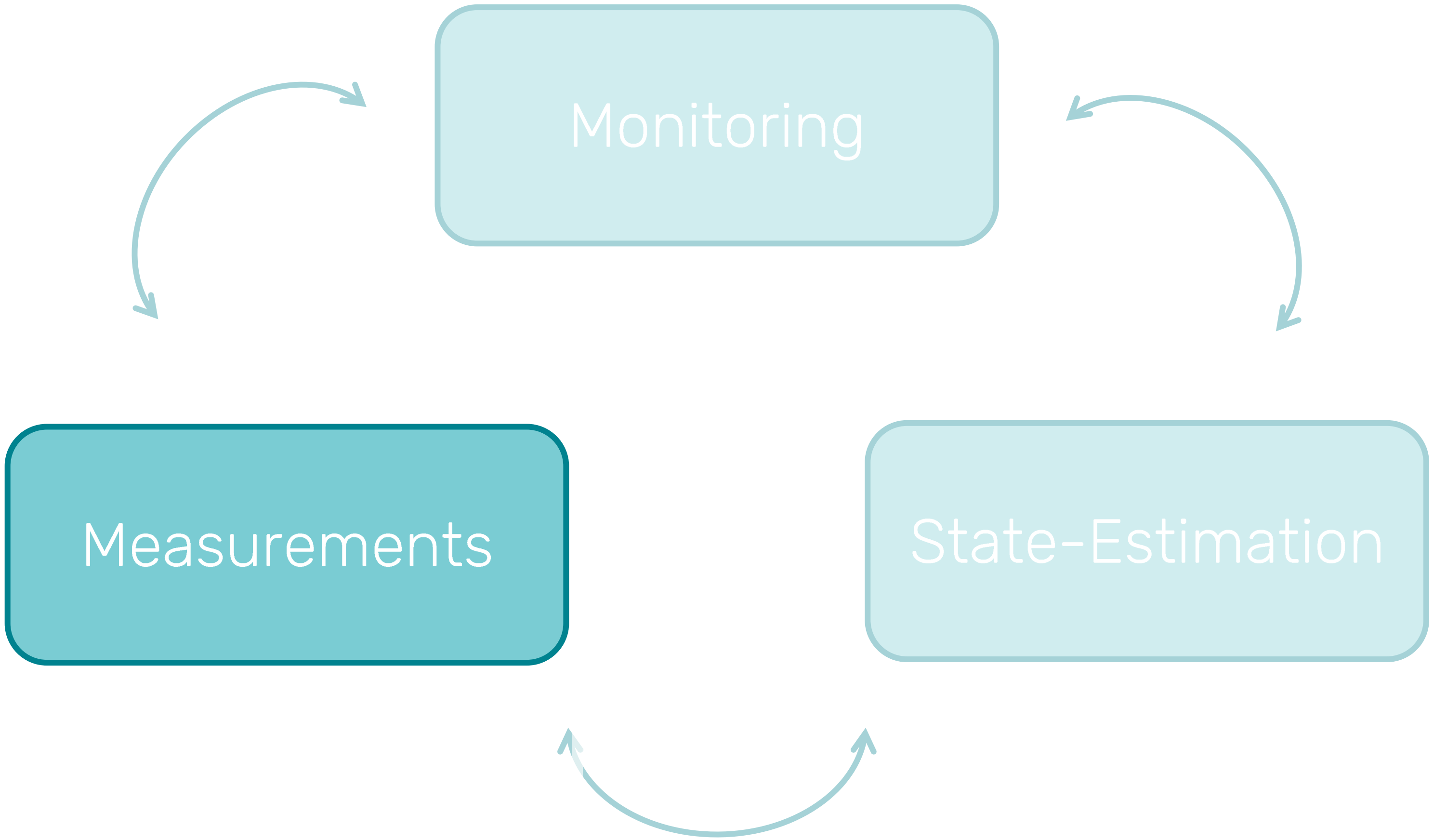
- Fed-batch refolding process with consequent batch period vs. batch process
- Final protein concentration: 1 g/L
- Feed rates (r): 0.17, 0.25, 0.5 mL/min
- Yield depends on solubilized protein concentration in vessel



Fed-batch or continuous is the process mode of choice for efficent IB refolding

Monitoring of Refolding Processes

Monitoring of Refolding Processes



Measurement of Protein States

Tools & Methods

Method	Type	Information	Limit of Detection
DOT and Redox signal	Inline	Soft-Sensor approach S-S bond formation	Independent of protein concentration
Raman Spectroscopy	Inline Atline	Secondary structure and dynamics S-S bond formation	1 mg/ml
ATR-FTIR	Inline	Secondary structure and dynamics	0.01 mg/ml
Size Exclusion Chromatography	Offline Atline	Differentiation between states	0.01 mg/ml
Reversed-Phase Chromatography	Offline Atline	Differentiation between states	0.01 mg/ml
Enzymatic Assay	Offline Atline	Activity, native structure	Depending on enzyme and assay

[1]

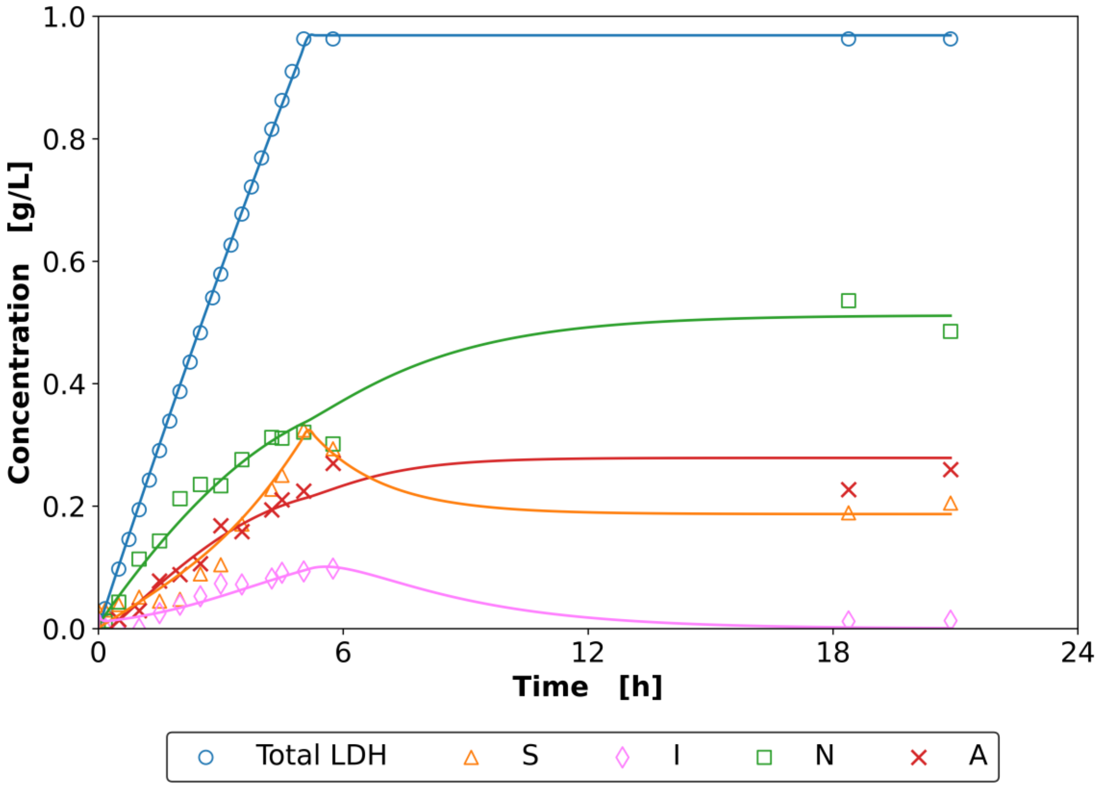
[1] Pauk et al.(2021). Appl Microbiol Biotechnol 105, 2243-226.

Experimental Setup

Measurement of Protein States

Application – Lactate Dehydrogenase (LDH)

- Simple model protein
- Fed-batch with subsequent batch phase
- Constant feed rate
- Result:
  - Rapid refolding to active form
  - Accumulation of solubilized protein lowers yield



Final protein concentration: 0.96 g/L, feed-rate: 0.33 mL/min, final denaturant concentration: 0.33 M, refolding in phosphate buffer (pH 6, 1 mM EDTA, 20 µM NADH), pH control (NaOH, HCl) , solubilization with 4 M Guanidin Hydrochloride

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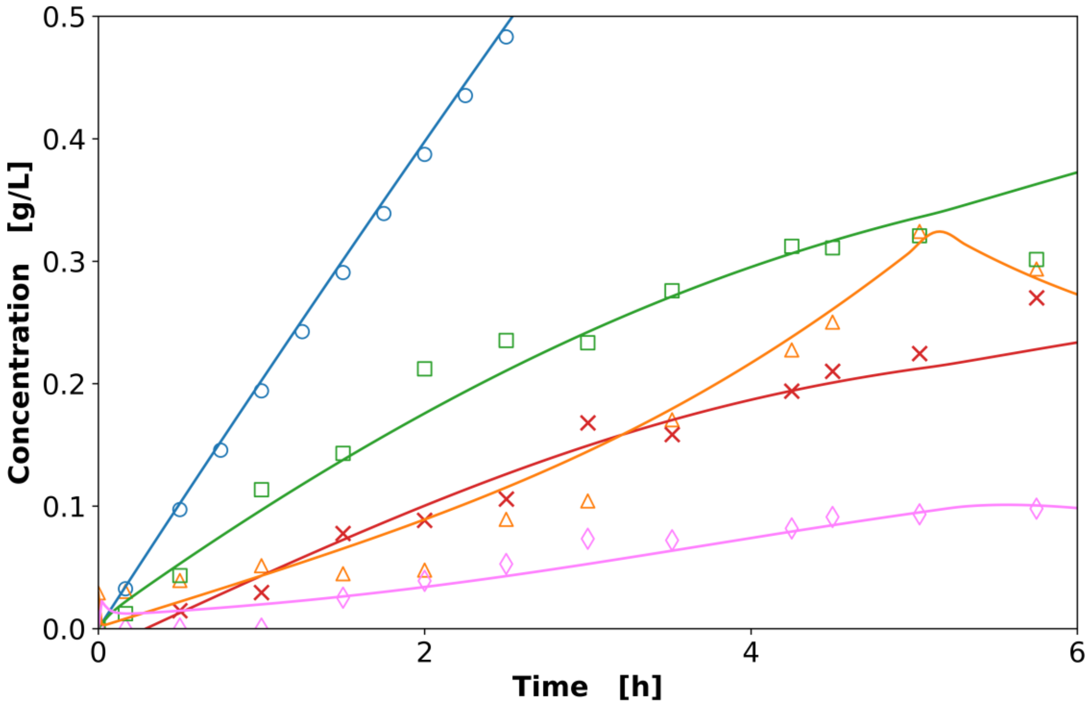


Optimization of recovery yield requires real-time adaption of feed rate

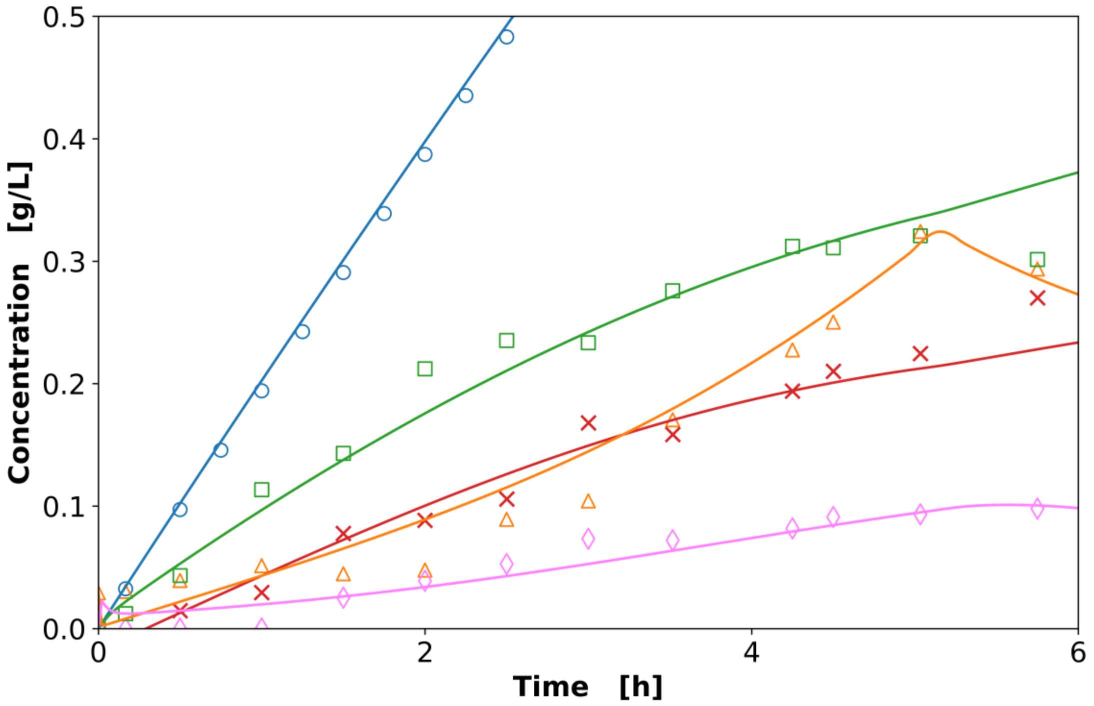
Measurement of Protein States

Limitations

Delay between sampling and response

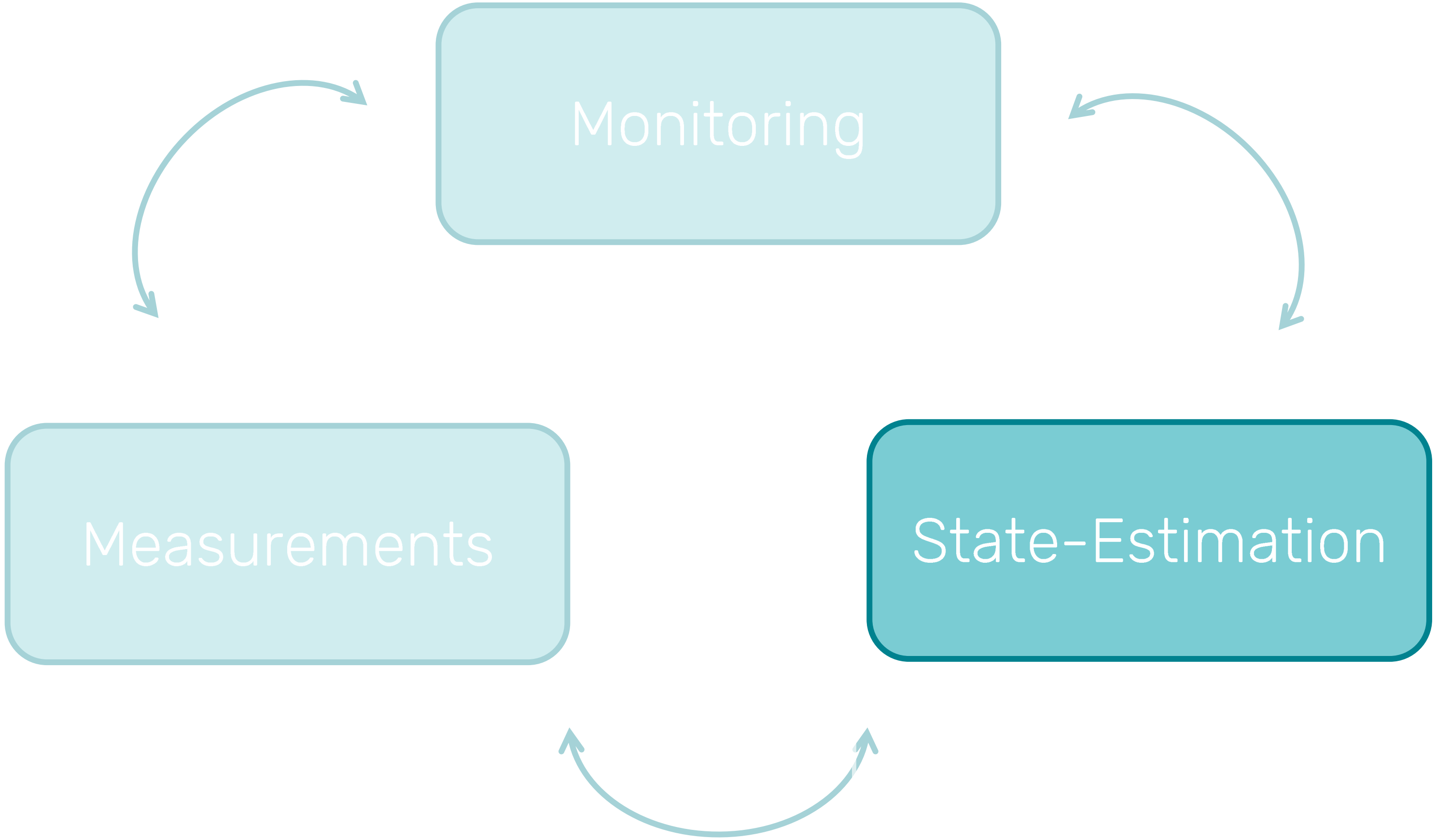
Time shift between different measurements

Not all states measurable and at every measurement cycle



Estimation of remaining protein states needed for effective monitoring

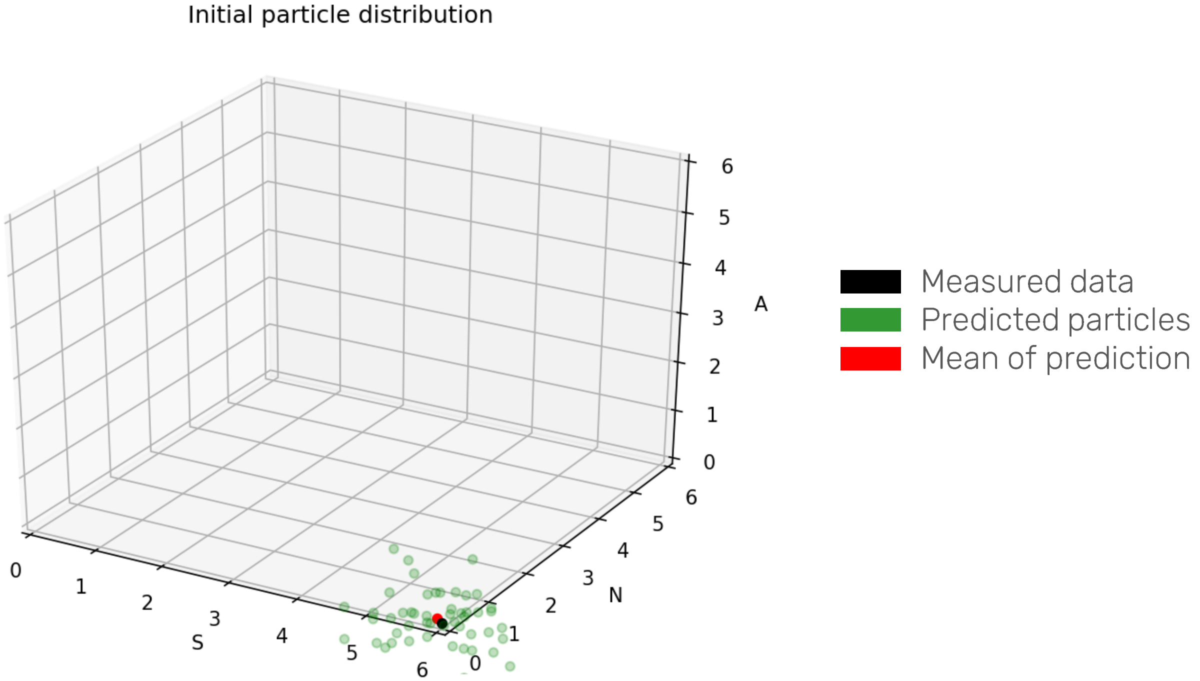
Monitoring of Refolding Processes



State-Estimation

Application – Particle Filter

- Filter intialization
- Prediction
- Update
- Resample
- Compute estimate



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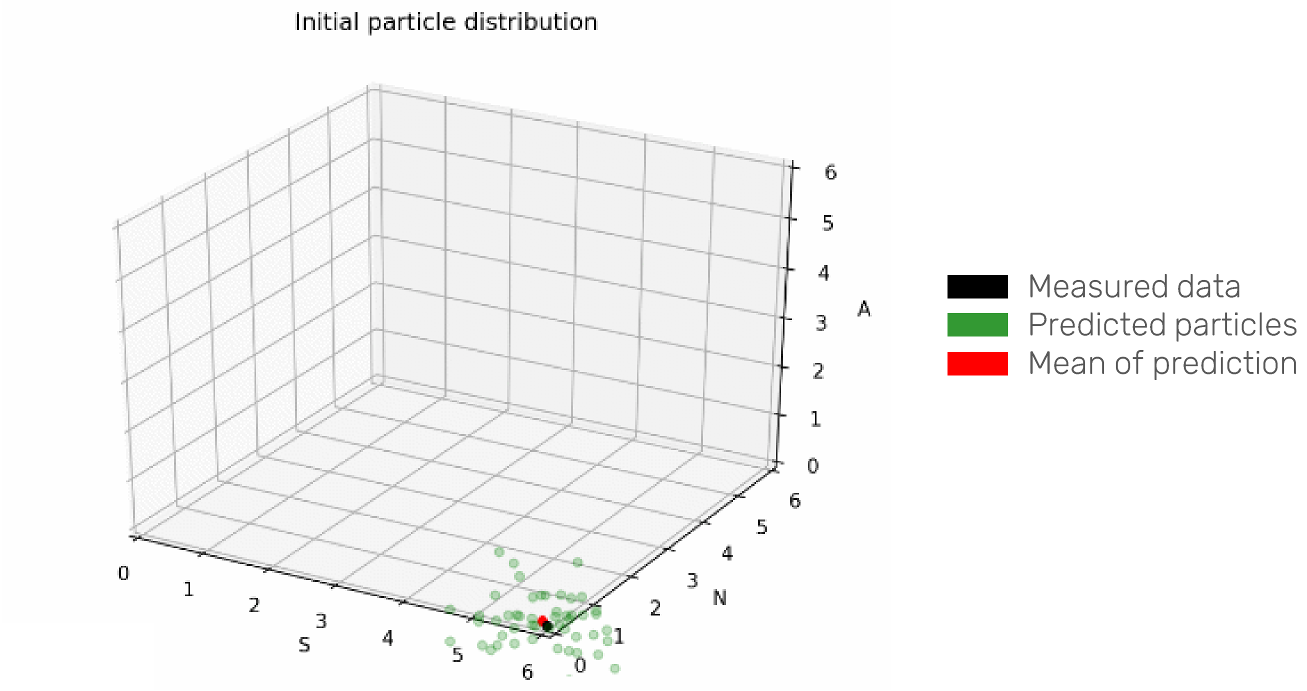
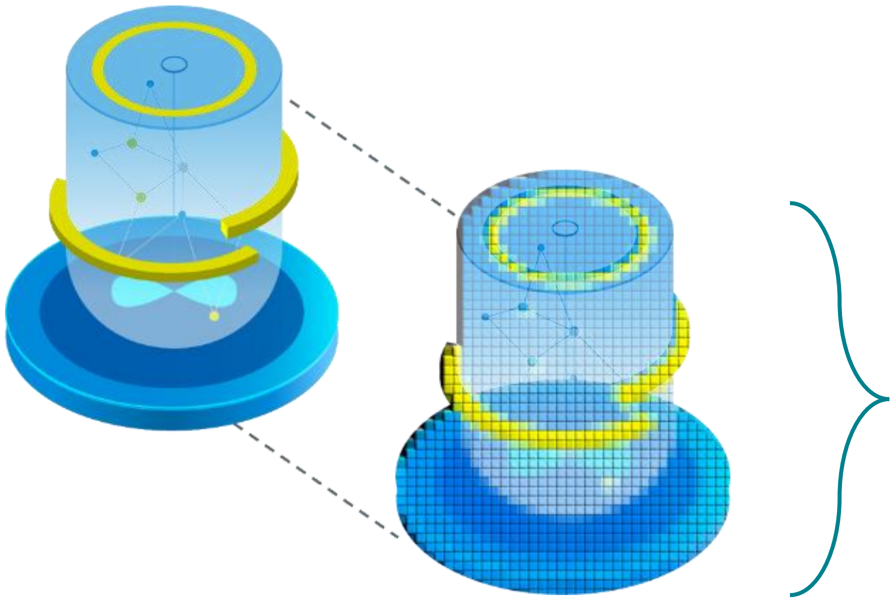


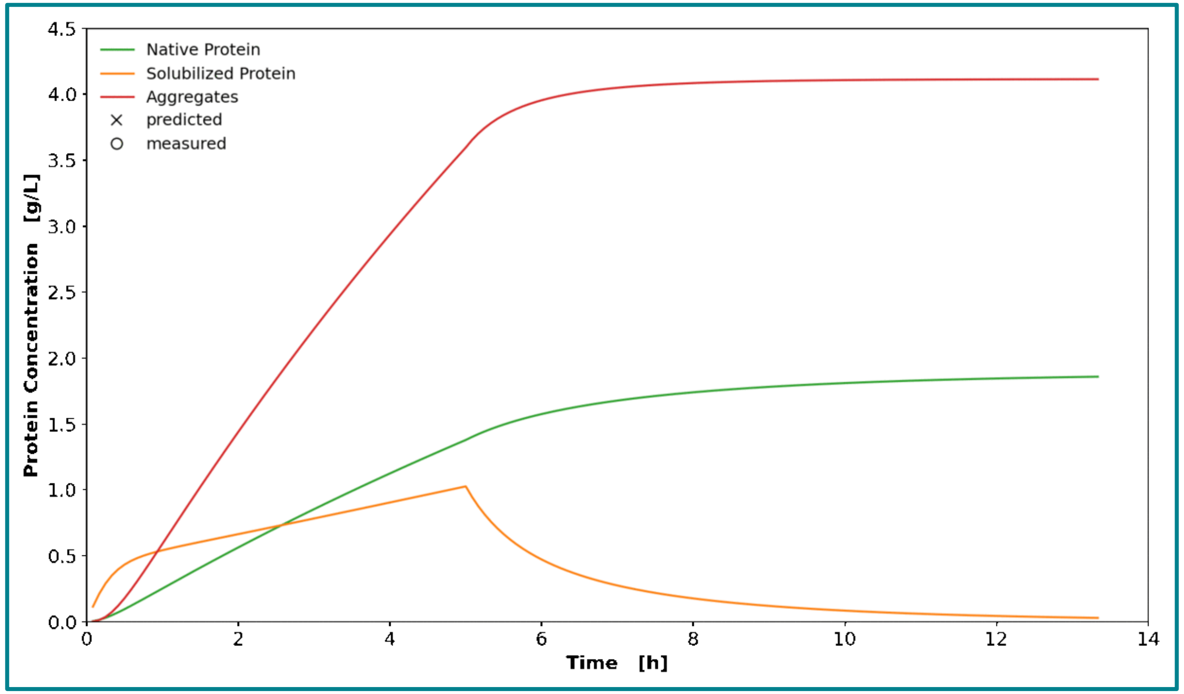
State-estimation is the basis for model-based-control

State-Estimation

Application



Basis for model-based-control



Digital Twin: control of the system by a process model

Novelty & Benefits

Platform technology for monitoring and control of protein refolding using fed-batch dilution

Online monitoring of refolding kinetics

Optimal control of KPIs

Adaptability to new products

CHASE

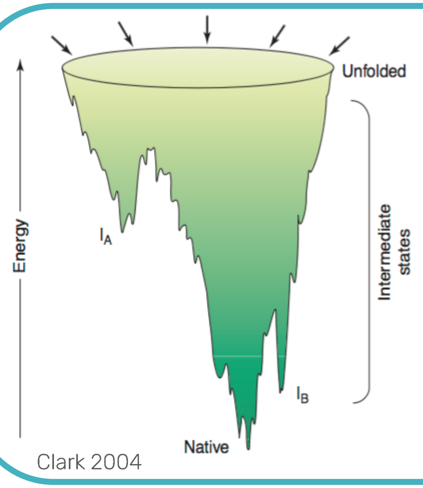
Chika Linda Igwe

Competence Center CHASE GmbH
Altenbergerstraße 69, 4040 Linz, Austria
chika.igwe@chasecenter.at

BILFINGER

TU WIEN

Protein Refolding Models



$$S \xrightarrow{k_i} I \xrightarrow{k_r} N, \quad I \xrightarrow{k_a} A$$

$$\frac{dc_{SL}}{dt} = -\left(k_r \cdot c_{SL} + k_a \cdot c_{SL}^2\right) + \frac{F_R \cdot c_{SR}}{V_L} - \frac{F_R \cdot c_{SL}}{V_L}$$

$$\frac{dc_{NL}}{dt} = k_r \cdot c_{SL} - \frac{F_R \cdot c_{NL}}{V_L}$$

$$\frac{dc_{AL}}{dt} = k_a \cdot c_{SL}^2 - \frac{F_R \cdot c_{AL}}{V_L}$$

$$\frac{dc_{PL}}{dt} = \frac{F_R \cdot c_{SR}}{V_L} - \frac{F_R \cdot c_{PL}}{V_L}$$

$$\frac{dc_{DL}}{dt} = \frac{F_R \cdot c_{DR}}{V_L} - \frac{F_R \cdot c_{DL}}{V_L}$$

$$\frac{dV_L}{dt} = F_R$$

$$k_r = a_r\left(1 + c_{DL}\right)^{b_r}$$
$$k_a = a_a\left(1 + c_{DL}\right)^{b_a}$$

Reaction rates depend on denaturant concentration

Measurement of Protein States

Method	Type of analysis	Information	Necessary protein amount
CD	Offline Online	Far UV-CD: secondary structure Near UV-CD: tertiary structure	Far UV-CD: 0.25 mg/ml Near UV-CD: 2.5 mg/ml
DLS	Offline At line Online	Tertiary and quaternary structure Quantification	0.05 mg/ml
DOT and Redox Sensor	Online	Soft-Sensor approach Monitor refolding of proteins with S-S bonds	Independent of protein concentration
Fluorescence Spectroscopy	Offline Online	Tertiary and quaternary structure	0.015 mg/ml depending on protein size
ATR-FTIR	Offline At line	Secondary structure and dynamics	0.01 mg/ml
Raman Spectroscopy	Online	Secondary structure and dynamics	1 mg/ml
QCL-IR	Offline	Secondary structure	0.25 mg/ml
RP-HPLC	Offline Online	Differences in hydrophobicity Oxidized, reduced protein species	0.01 mg/ml
SEC	Offline Online	Aggregates and native protein	0.01 mg/ml

Control of Refolding Processes

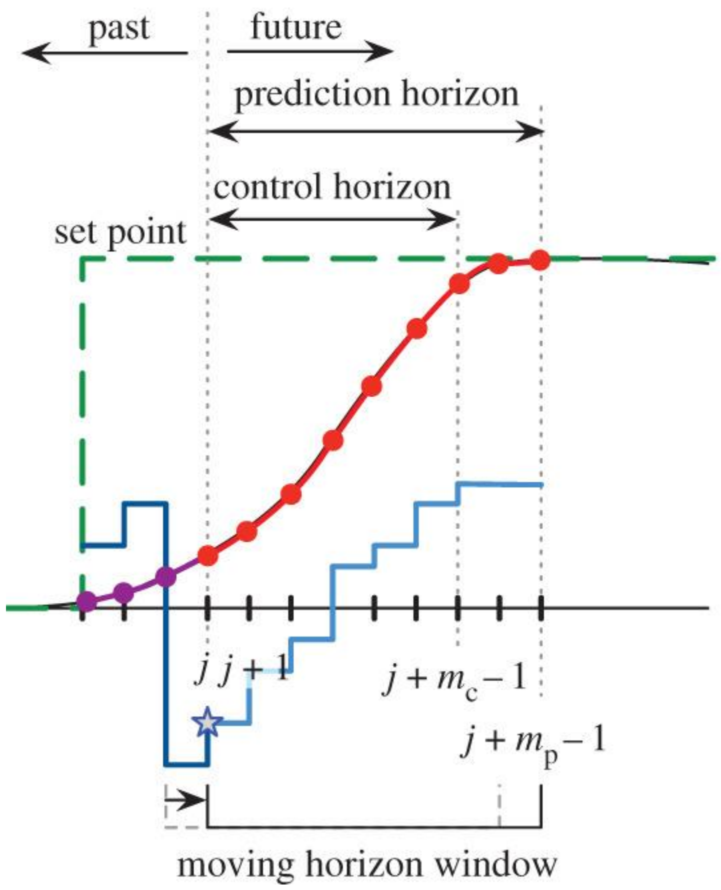
Requirements	Benefits of model-based control
Adaptable to process changes	Adaptable to process changes
Non-linear process control	Utilization of state predictions
Fast action against process disturbance	Addition of constraints

Process Control using Model Predictive Control

Online Optimization process

Application of first control step

Repetition of these steps



Kaiser et al. 2018

Platform Refolding Process

Benefits of Model-Based Control

- Adaptable to process variations
- Utilization of state predictions
- Addition of constraints
- Fast action against process disturbance