Antibody production and modification using a eukaryotic cell-free system based on CHO cell lysates

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The concept of antibody-drug conjugates (ADCs) is theoretically simple, but it is difficult to combine their components into an optimized and functional therapeutic agent. By using traditional conjugation chemistries, the resulting products are heterogeneous regarding the number of attached drugs as well as the location of drug linkage. This results in different ADC subpopulations, each having its own specific characteristics, making the characterization of the ADC formulation as a whole very difficult.

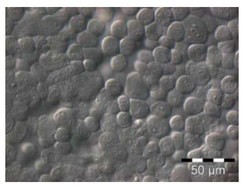
Against this background, we develop biochemical tools for the site-specific modification of antibodies based on cell-free protein synthesis (CFPS) and amber suppression. CFPS is a versatile, flexible and fast approach for the synthesis and modification of proteins. Using this technology, antibodies can be synthesized in a very fast and flexible manner in an open reaction - which allows for direct manipulation of reaction conditions to optimize protein folding and incorporation of non-canonical amino acids.

Here we demonstrate the synthesis of different antibody formats (IgG, scFv-Fc, scFv) in a microsome-containing cell-free system based on translationally active Chinese hamster ovary (CHO) cell lysates. Besides cell-free synthesis of non-labeled antibodies, we show residue-specific labeling as well as site-specific labeling of antibodies by amber suppression.

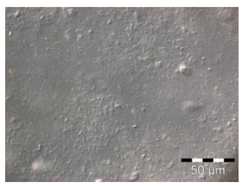
1. Lysate production pipeline

Fermentation of CHO cells up to 30 L scale

Cell harvest



Preparation of highly productive cell lysate



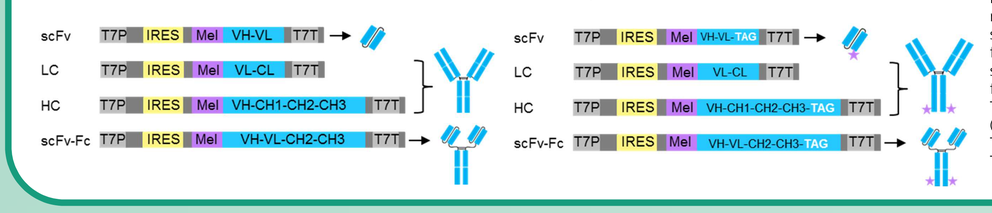
Eukaryotic lysates:

- Storable at – 80°C
- Stable for years
- Reproducible performance

Template design

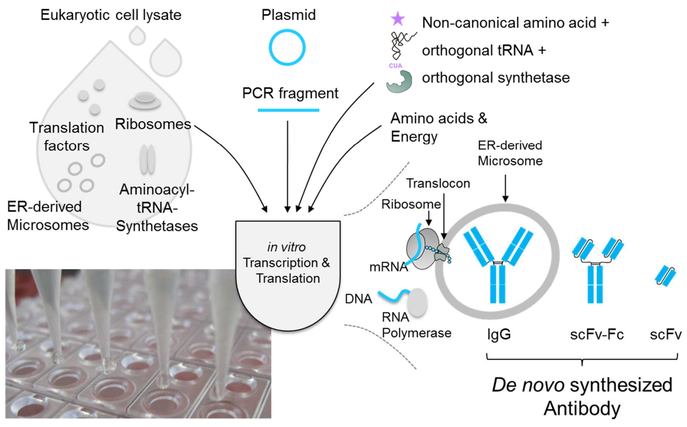
Templates without amber stop codon

Templates with amber stop codon (TAG) (for incorporation of ncaa)



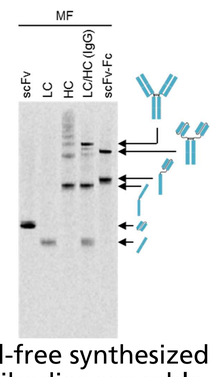
Abbreviations:
HC: Antibody heavy chain
IRES: Internal ribosome entry site
LC: Antibody light chain
Mel: Melittin signal sequence
ncaa: non-canonical amino acid
scFv: Single-chain variable fragment
scFv-Fc: single-chain variable fragment Fc fusion
TAG: Amber Stop Codon (flexible position)
T7P: T7 promoter
T7T: T7 terminator

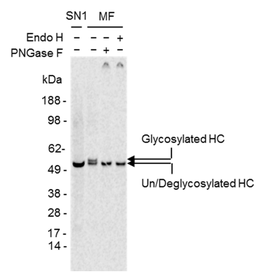
2. Cell-free antibody synthesis

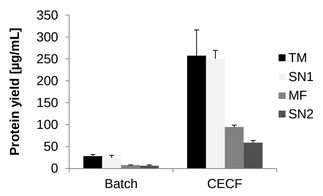


- Rapid *de novo* synthesis of proteins (batch: 3 h)
- Synthesis based on PCR products
- HTS-compatible
- Open system
- Easy optimization of reaction conditions
- Scalable reactions
- Protein yields:
  - 10 – 50 µg/ml (batch mode)
  - 100 – 1000 µg/ml (Continuous exchange cell-free, CECF, mode)

Results (Stech et al., 2017)

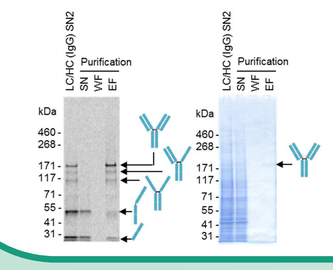


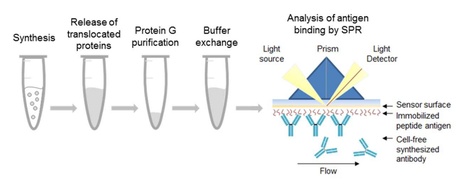


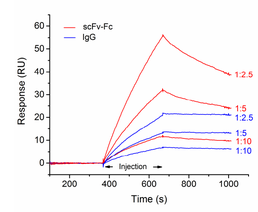


Abbreviations:
CECF: Continuous-exchange cell-free system
HC: Antibody heavy chain
LC: Antibody light chain
MF: Microsomal fraction
scFv-Fc: single-chain variable fragment Fc fusion
SN1: Supernatant fraction 1
SN2: Supernatant fraction 2
TM: Translation mixture

- Cell-free synthesized antibodies assemble to heterotetramers
- Cell-free synthesized antibodies are glycosylated (ER-level glycosylation)
- Antibody yields can be increased by switching from batch to CECF reaction mode





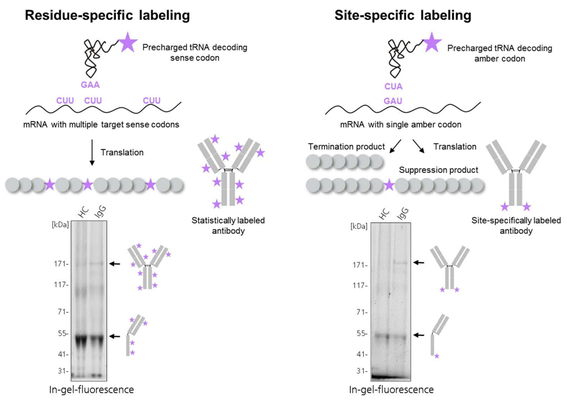


- Cell-free synthesized and Protein G purified antibodies are functionally active

3. Antibody labeling by amber suppression

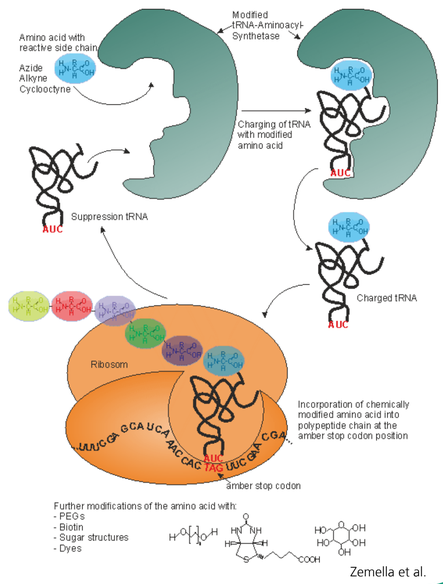
Two labeling strategies:

1) Pre-charged tRNAs



- Cell-free synthesized antibodies can be labeled with fluorescent dyes (here: Bodipy-TMR-lysine)

2) Orthogonal system



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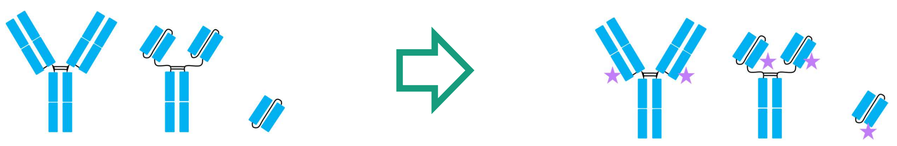
Ongoing research

Cell-free synthesis → Chemoselective modification



1) Cell-free synthesis of antibodies and co-translational incorporation of p-azido-L-phenylalanine (AzF)
2) Subsequent chemoselective coupling of Dylight-650-phosphine (Staudinger ligation)

1) Cell-free synthesis of antibodies and co-translational incorporation of propargyloxyphenylalanine (pPa)
2) Subsequent chemoselective coupling of Sulfo-Cy5 (Cu(I)-catalyzed azide-alkyne click chemistry reaction)

- Cell-free synthesized antibodies can be site-specifically labeled with azide/alkyne groups, allowing subsequent chemoselective reactions (Unpublished data intentionally not shown)

For more detailed information please refer to:
Stech et al.: *Cell-free synthesis of functional antibodies using a coupled in vitro transcription translation system based on CHO cell lysates.* Sci Rep. (2017) 7:12030.
Thoring et al.: *A high-yield production technology for synthesis of "difficult-to-express" proteins based on a novel continuous exchange CHO cell-free system.* Sci Rep. (2017) 7(1).
Stech et al.: *Cell-free eukaryotic systems for the production, engineering and modification of scFv antibody fragments.* Engineering in Life Sciences 14 (2014), 387-398.
Stech, M. and Kubick, S.: *Cell-free synthesis meets antibody production: A review.* Antibodies 4 (2015), 12-33.
Zemella et al.: *Cell-Free protein synthesis: Pros and cons of prokaryotic and eukaryotic systems.* ChemBioChem (2015) 16, 2420-2431.

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