# AI-Protein Engineering aiProtein Engineering aiProtein®

## **RevoAb™** Special Edition

RevoAb™ Delivers *ai*Protein® s Core Technology Straight to You In early access until March 2026

aiProtein®: RevolKa's innovative Machine Learning-driven protein engineering platform

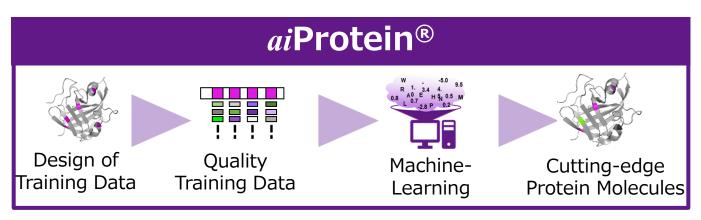


This service optimizes antibody properties, including protein yield (host expression levels), thermal/structural stability, solubility and more at little cost of antigen-binding potencies (affinity). The optimization is powered by RevolKa's advanced artificial-intelligence (AI)-driven protein engineering platform, *ai*Protein®. This innovative AI technology designs high-performance antibodies with a high probability of success. This service also offers multiproperty optimization and supports a wide range of antibody modalities, including monoclonal IgG, scFv, and VHH can be optimized. Antibody humanization, as well as affinity recovery after humanization is also available.

#### ♠aiProtein®: RevolKa's innovative AI-driven protein engineering platform

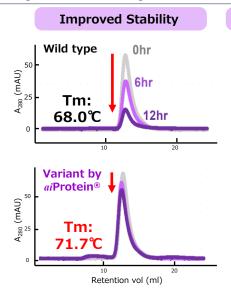
- 1. Design of training data set customized to a target protein
- 2. Quality training data by RevolKa's wet lab capability
- 3. Low-n, fine-tuned machine-learning technology

#### = Cutting-edge protein molecules for industrial use

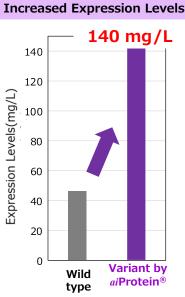


#### ◆Case studies of aiProtein ® Full-Package Service

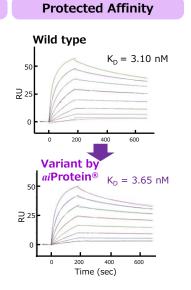
### Case Study(1): Improved Expression and Stability of Nivolumab



Thermal stability of nivolumab and an *ai*Protein®-optimized variant were tested at 60 °C for 0, 6, and 12 hours. Size-exclusion chromatography (SEC) analysis demonstrated extremely high stability of the *ai*Protein® variant.

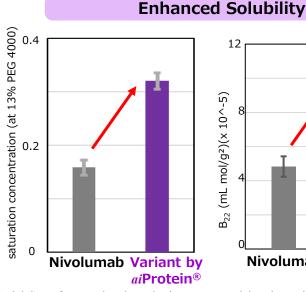


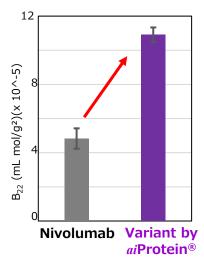
The nivolumab variant achieved a yield of 140 mg/mL (3-fold higher than nivolumab) in a transient Expi293F mammalian cell secretion system.



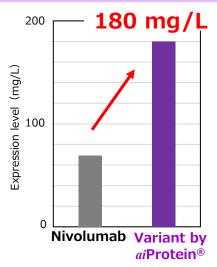
Nivolumab and the variant showed a comparable antigen binding and dissociation constant values in a surface plasmon resonance analysis.

#### Case Study(3): Improved Solubility and Yield of Nivolumab





#### **Increased Expression Levels**



The Nivolumab variant antibody achieved a yield of 180 mg/L (3-fold higher than nivolumab) in a transient Expi293F mammalian cell secretion system.

Solubility of monoclonal antibody was tested by the polyethylene glycol (PEG) method. A Nivolumab variant generated by aiProtein® showed higher saturation concentration under a 13% PEG condition compared to Nivolumab (left). Consistent with this result, second virial coefficient(B<sub>22</sub>), representing protein colloidal stability, was also higher for the variant than that of wild-type (right), suggesting that the variant can be potentially formulated at a high concentration.

The binding affinity of the variant was comparable to that of wild-type.

## Case Study(3): Improved Expression and Stability of Humanized VHH

Variant by aiProtein®

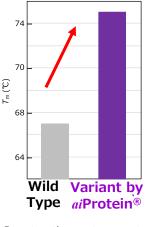
**Increased Expression levels** 

300

200

Wild Type

#### **Improved Stability**

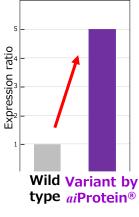


## A variable heavy domain of heavy chain (VHH) that showed severe aggregation after humanization was optimized by by *ai*Protein® to improve yields in *E.coli* BL21(DE3) due to aggregation issues. The variant VHH exhibited a significant increase in yields (left) and monodispersity in size-exclusion chromatography analysis.

Denaturation temperature (Tm),representing structural stability of the protein, was measured using a thermal shift assay. The variant VHH generated by aiProtein® exhibited a significant increase in thermal stability of 6 °C.

## Case Study(4): Improved Expression and Stability of Diabody

#### **Increased Expression Levels Enhanced Stability**



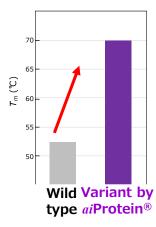
A difficult-to-express diabody was optimized by *ai*Protein® to improve yields in *E. coli* BL21(DE3).

Quantitative SDS-PAGE analysis showed a 5-fold increase in yield

the

generated variant.

aiProtein®-



Denaturation temperature (Tm), representing structural stability of the protein, was measured using a differential scanning calorimetry assay. The variant VHH generated by *ai*Protein® exhibited a significant increase in thermal stability of more than 15 °C.

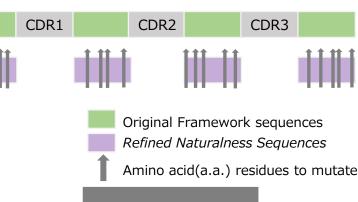
#### **♦** Core Technologies of *ai*Protein<sup>®</sup>

#### Core Technology -1

Framework Engineering **Driven by Refined Naturalness Design** 

#### Core Technology -2

Low-n Machine Learning with as few as 100 Training Data



Machine learning

Generation of promising antibody sequences with optimized mutations

#### Bringing Core Technology -1 to You



In early access until March 2026



#### <u>All you need</u>

Submit target sequence



**Delivery** ≤ 2 weeks\*2



#### What you can get

Up to 3 designs\*1 for

- Enhancing developability
- Protected affinity



## <u>Pricing</u> ≤\$700



#### Design concept

Engineering frameworks driven by RevolKa's refined naturalness design



Remain with you



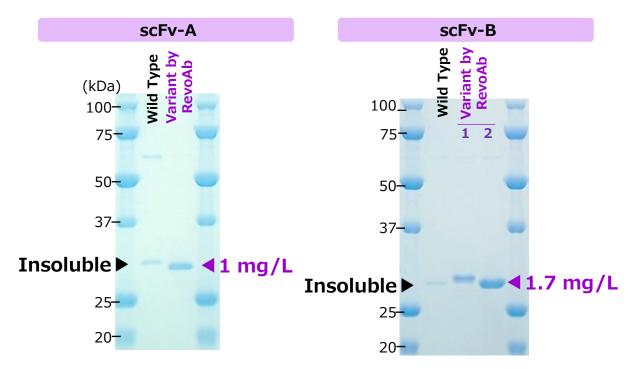
#### **Confidentiality**

R-free design

#### ◆ RevoAb<sup>TM</sup>: Case Study

#### **Improved Expression of Difficult-to-Express scFv**

Two examples of scFv (left: scFv-A, right: scFv-B)



Two difficult-to-express single-chain variable fragments (scFvs): scFv-A (left) and scFv-B (right), were optimized by *ai*Protein® to improve yields in *E. coli* BL21(DE3). The scFv-A and scFv-B variants generated by *ai*Protein® (scFv-A: 1 variant, scFv-B: 2 variants) exhibited a significant increase in yields. Binding affinity to the antigen of the two scFv-B variants was comparable to that of the wild-type. Affinity of the scFv-A variant was not tested.

## ◆ RevoAb<sup>TM</sup> + aiProtein<sup>®</sup>: A Complete Antibody Optimization Pipeline

RevoAb<sup>TM</sup> identifies the candidate mutations, then aiProtein<sup>®</sup>'s AI finds the perfect combinations for next-level antibody performance!

✓ Next-Gen, Multi-Properties Optimization

10% OFF for projects with the same antibody as RevoAb™!

#### Don't miss your chance!

Request Form: RevoAb: Request Form

Contact us: support-revotune@revolka.co.jp

FAQ: https://revoab.revolka.com/



Request Form

#### **♦ Flow of** *ai*Protein <sup>®</sup> Full-Package Service

Stability Optimization Expression Level Optimization

Affinity Recovery Customized Service

01

Initial consultation for experimental plan and estimates

02

Design of antibody variants for AI training and collection of training data

03

Building an AI-model specifically designed for the customer's antibody with the training data

Generation of optimized variant sequences to test

04

Selection of candidate variants to report (approximately 5 variants)



**Option** 

Validation of the candidate variants

#### Delivery of the report including deliverables

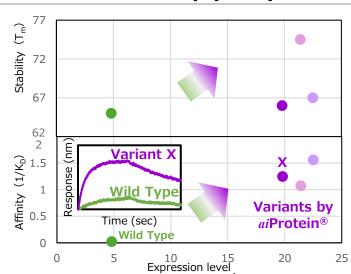
#### Service and Deliverables

- A preliminary technical consultation will be placed before starting a project to share customer's antibody information and properties of interest.
- The lead time from the submission of the customer's antibody protein sequence to the delivery of optimized antibody sequences is 8 to 11 months depending on requirements in experiments, such as protein expression system.
- The deliverables include approximately 5 protein sequences of optimized variants and experimental data regarding improved properties. All of the variants will be experimentally validated by RevolKa.

#### *ai*Protein®:

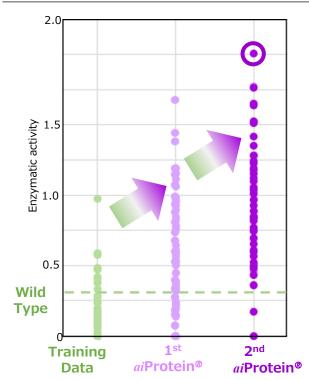
## ML-guided-Versatile, Multi-Properties Engineering \*Please note that the following examples are outside RevoAb™'s scope. For inquiries, kindly contact RevolKa. biz-contact@revolka.co.jp

#### Case Study(5): Improved Affinity, **Expression and Stability of Anti-COVID-19 Antibody (VHH)**



Promising variants with variations in affinity, stability, and yield

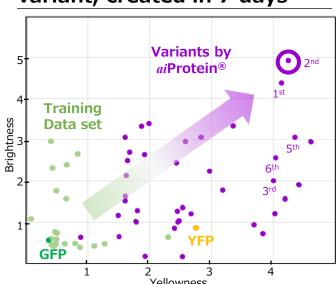
#### Case Study(7): Improved **Expression and Stability of SortaseA**



6× Higher Activity with Improved Expression

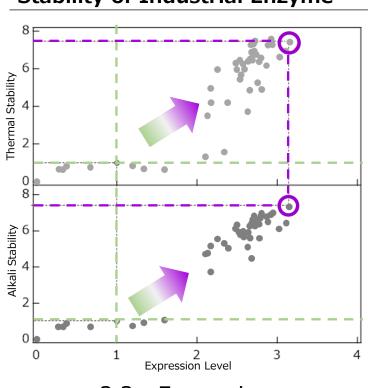
Saito, Y. et al. (2021) ACS Catal. 11, no. 23, 14615-14624. DOI:10.1021/acscatal.1c03753

#### Case Study(6): At a 30-year variant, created in 7 days



Dramatic improvement through prediction of all variants Y. et al. (2018) ACS Synth. Biol. 7, 2014-2022. DOI:10.1021/acssynbio.8b00155.

#### Case Study(8): Improved Expression, Thermal and Alkali Stability of Industrial Enzyme

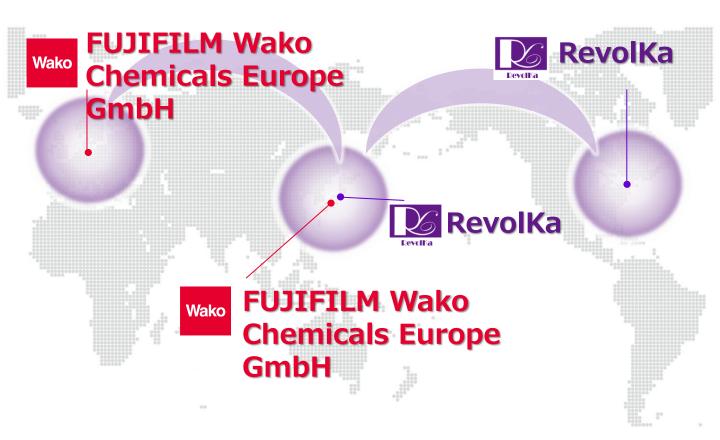


3.3× Expression, 7.5× Thermostability, 7.5× Alkali Stability



#### aiProtein®: RevolKa's innovative Machine Learning-driven protein engineering platform

#### ▶ Contact





US: https://labchem-wako.fujifilm.com/us/category/95358.html EU: https://labchem-wako.fuiifilm.com/europe/category/95358.html





Europe



https://www.revolka.com/index.html

Contact : biz-contact@revolka.co.jp



RevolKa