Higher-Order Structure, Stability, and Similarity Assessment of VHH Antibodies Using CD Spectroscopy

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[3]RePHAGEN Co., Ltd., Uruma, Okinawa 904-2234, Japan, [4]ELTE NAP Neuroimmunology Research Group, Department of Biochemistry, Institute of Biology, ELTE Eötvös Loránd University, Budapest H-1117, Hungary
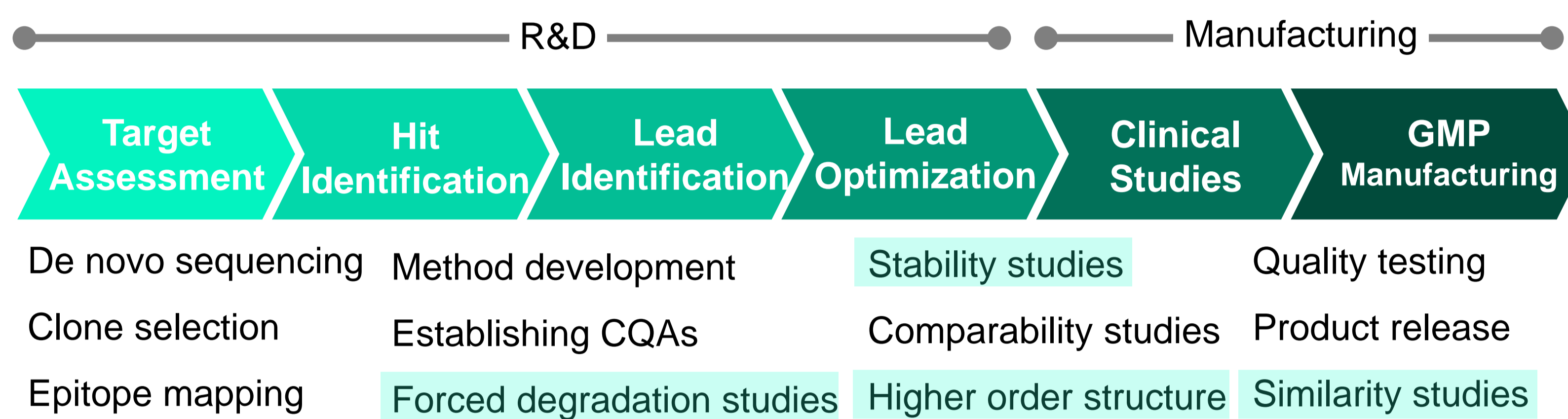
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SUMMARY

- VHH* antibodies are attracting attention as next-generation modalities
- Higher-order structure (HOS) is a critical quality attribute (CQA) responsible for VHH antibody functions
- CD spectroscopy is a suitable technique for protein HOS assessment

*Variable domain of heavy chain antibodies

R&D and Manufacturing Scheme for Therapeutic Antibodies

R&D — Manufacturing

| Target Assessment	Hit Identification	Lead Identification	Lead Optimization	Clinical Studies	GMP Manufacturing
De novo sequencing	Method development	Stability studies		Quality testing	
Clone selection	Establishing CQAs	Comparability studies		Product release	
Epitope mapping	Forced degradation studies	Higher order structure	Similarity studies		

INSTRUMENT

- Protein secondary and tertiary structure
- Wide protein concentration range
- Simple measurement/analysis procedures

J-1000 series
CD spectrophotometer
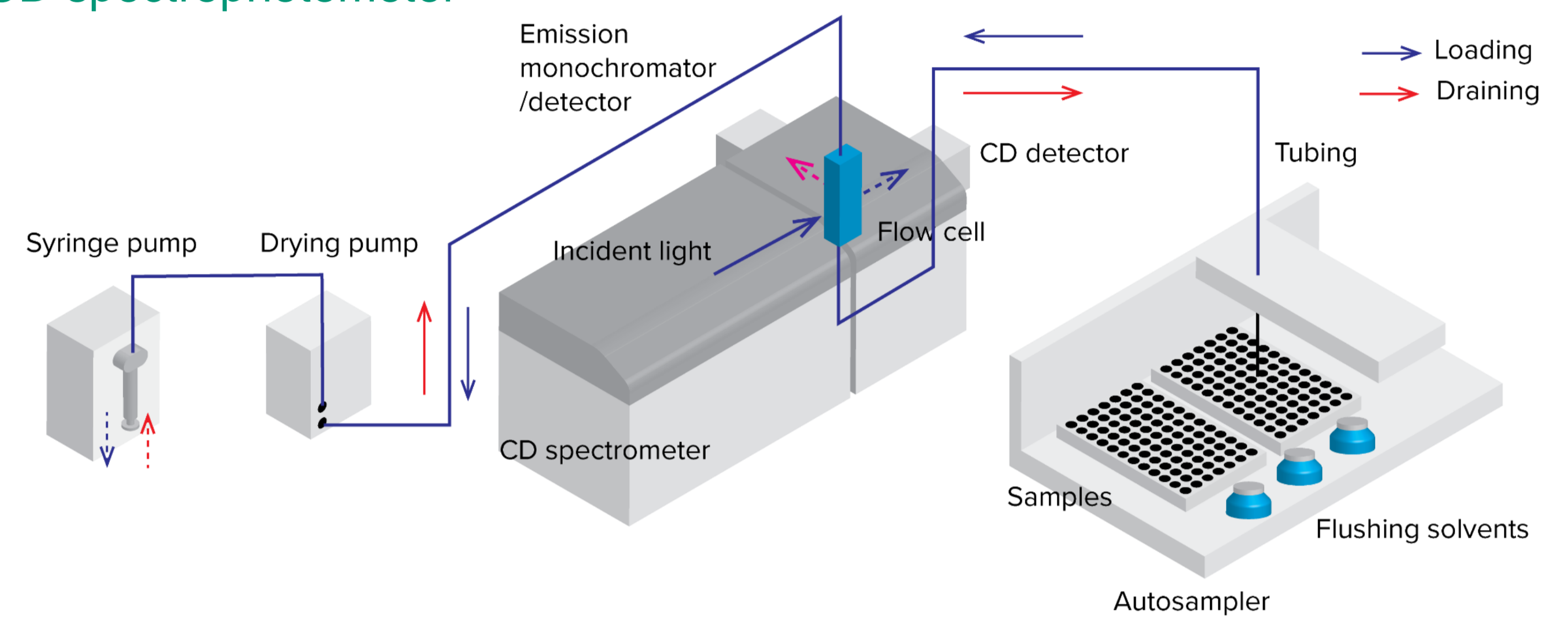
Schematic diagram of automated screening system

1. SECONDARY STRUCTURE STUDY
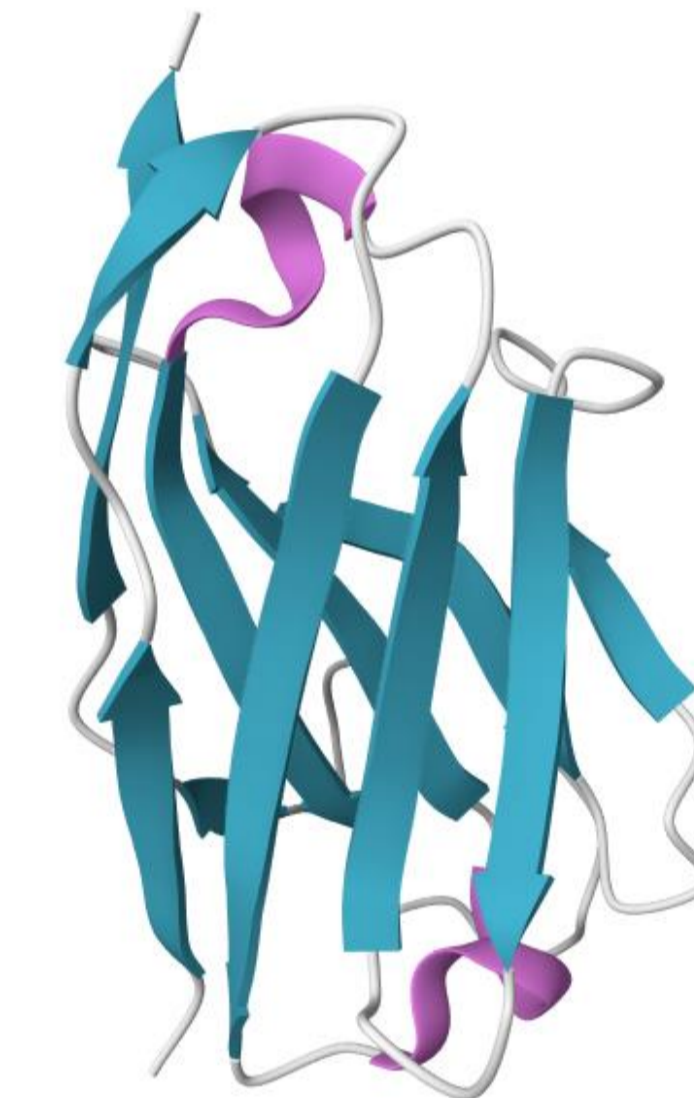
Secondary Structure Fractions
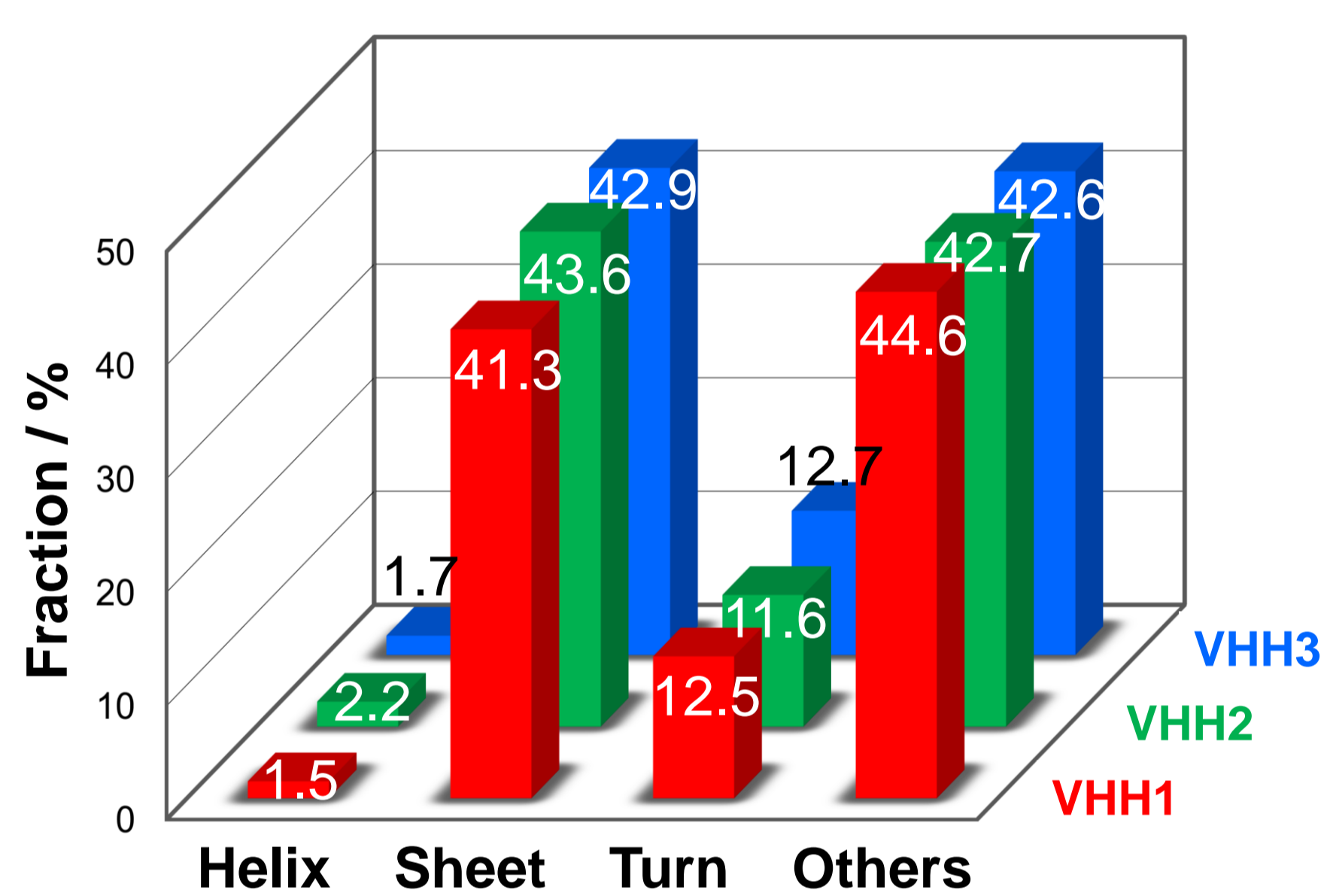
Secondary structure compositions of anti-SARS-CoV-2 VHH antibodies (VHH1, VHH2, and VHH3, prepared with different sequence) were estimated from their CD spectra using BeStSel, a high-performance algorithm developed by Dr. József Kardos and Dr. András Micsonai (https://bestsel.elte.hu)[2-5]. The VHH antibodies were provided by RePHAGEN Co., Ltd (https://rephagen.com/en/).

STRUCTURE OF VHH ANTIBODIES

Heavy-chain antibodies

Crystal structure of VHH

Image from the RCSB PDB (rcsb.org) of PDB ID 5U65[1]

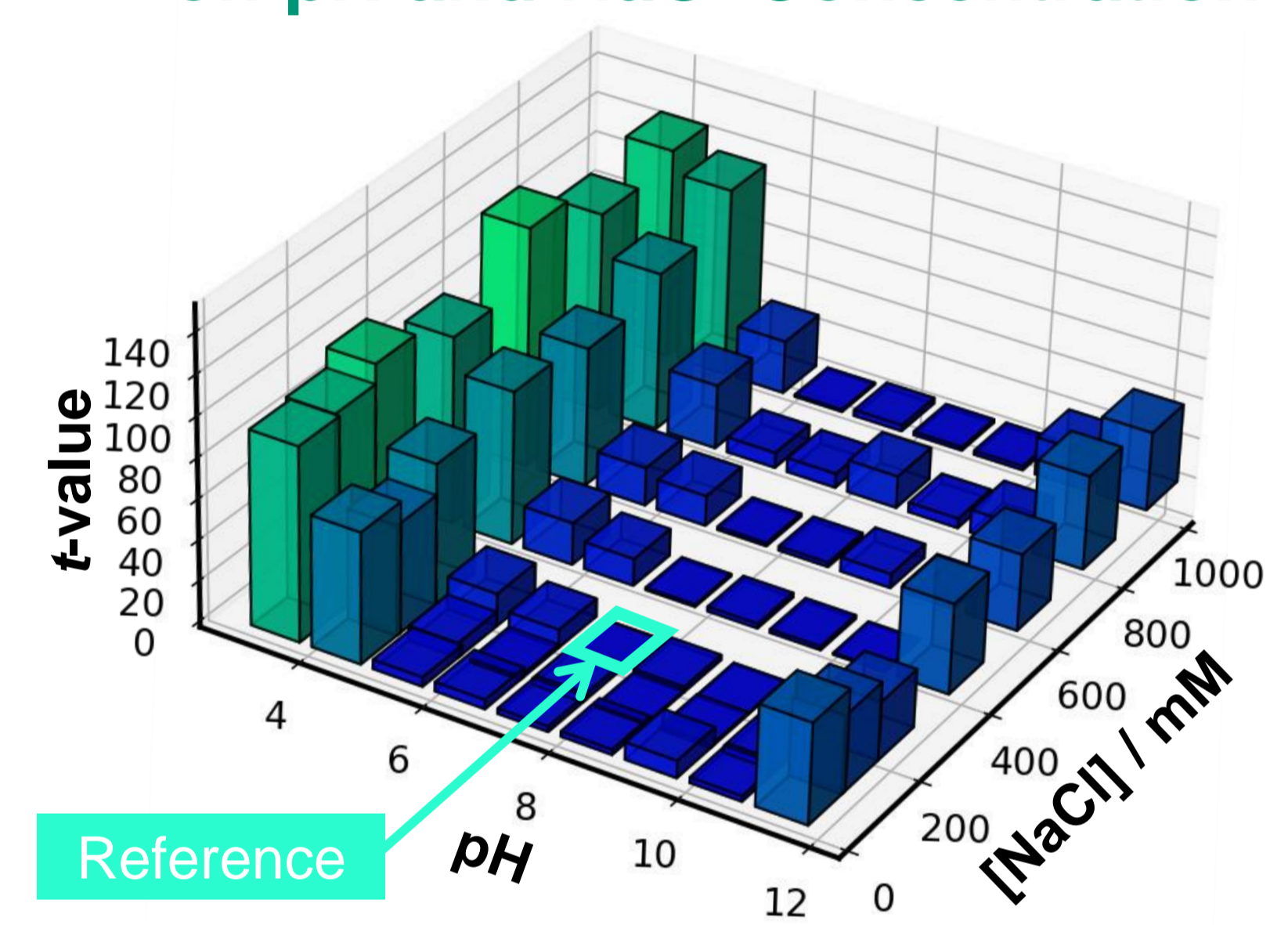## 3. STABILITY STUDY

pH AND SALT CONCENTRATION

Dependance of Spectral Change on pH and NaCl Concentration

Spectral change in anti-HSA VHH antibody (VHH4) was statistically evaluated using the JASCO qHOS program. A VHH4 at pH 7 and NaCl concentration of 200 mM was set as a reference. The CD spectral change between the reference and VHH4s under different combinations of pH and NaCl concentrations was quantified by t-values. The larger t-value is, the more spectral difference they show.

2. SIMILARITY STUDY

CD Spectra and Similarity Test (TOST) Results

Secondary Structure (Far-UV Region)


Tertiary Structure (Near-UV Region)
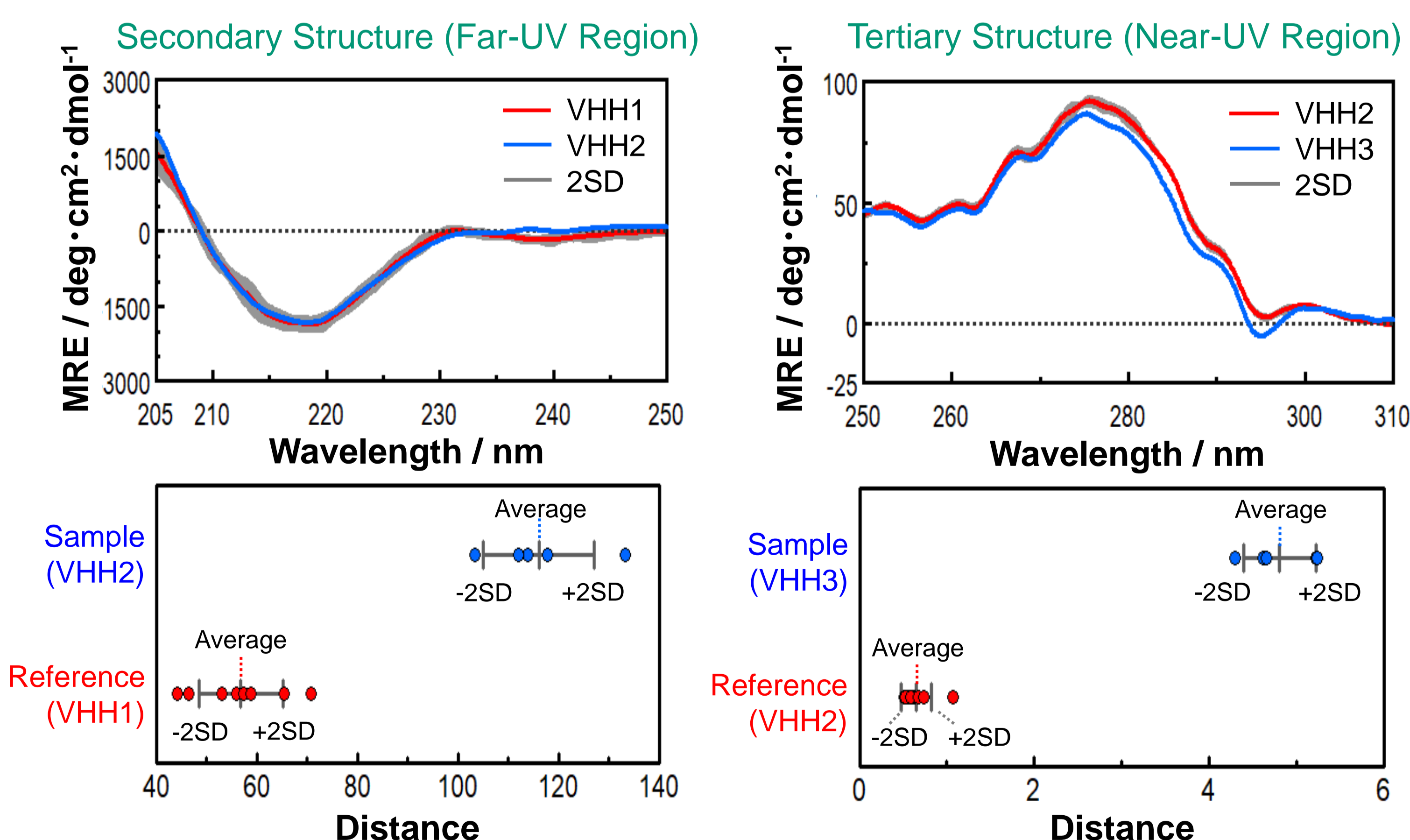
Similarities between anti-SARS-Cov-2 VHH antibodies with different sequences were statistically evaluated from their CD spectral shapes using JASCO qHOS program[7]. TOST (two one-sided t-test) was used to determine whether there was equivalence between two different VHH antibodies.

HEAT

Denaturation Curve

$T_m = 65$ °C

Changes in Secondary Structure Fractions
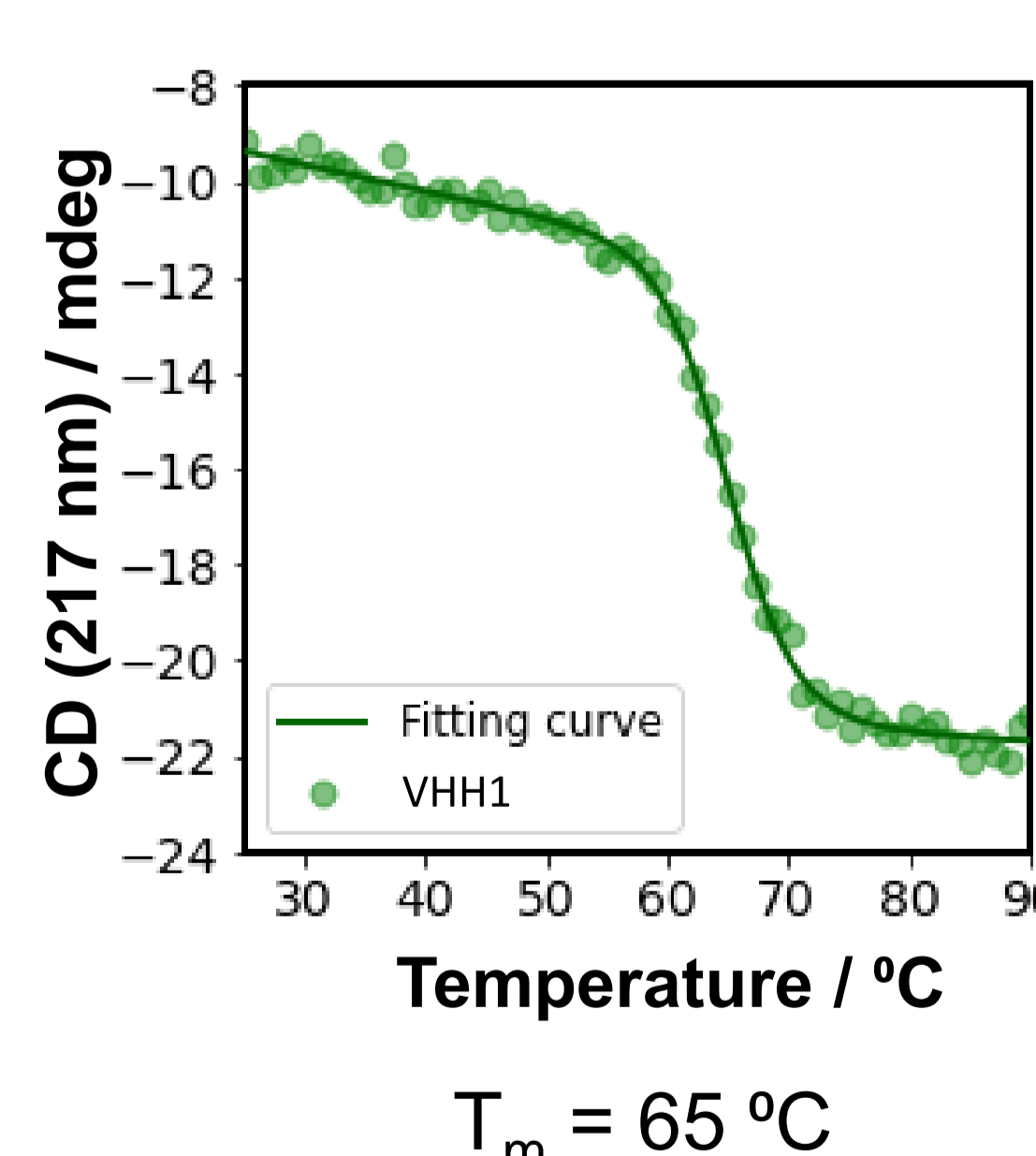
- Melting temperature (T_m) of VHH1 was calculated from the CD denaturation curve to check the thermal stability. The CD signal at 217 nm is originated from β-sheets.
- Refolding ability of VHH1 was assessed by monitoring changes in secondary structure fractions while increasing and decreasing the temperature.
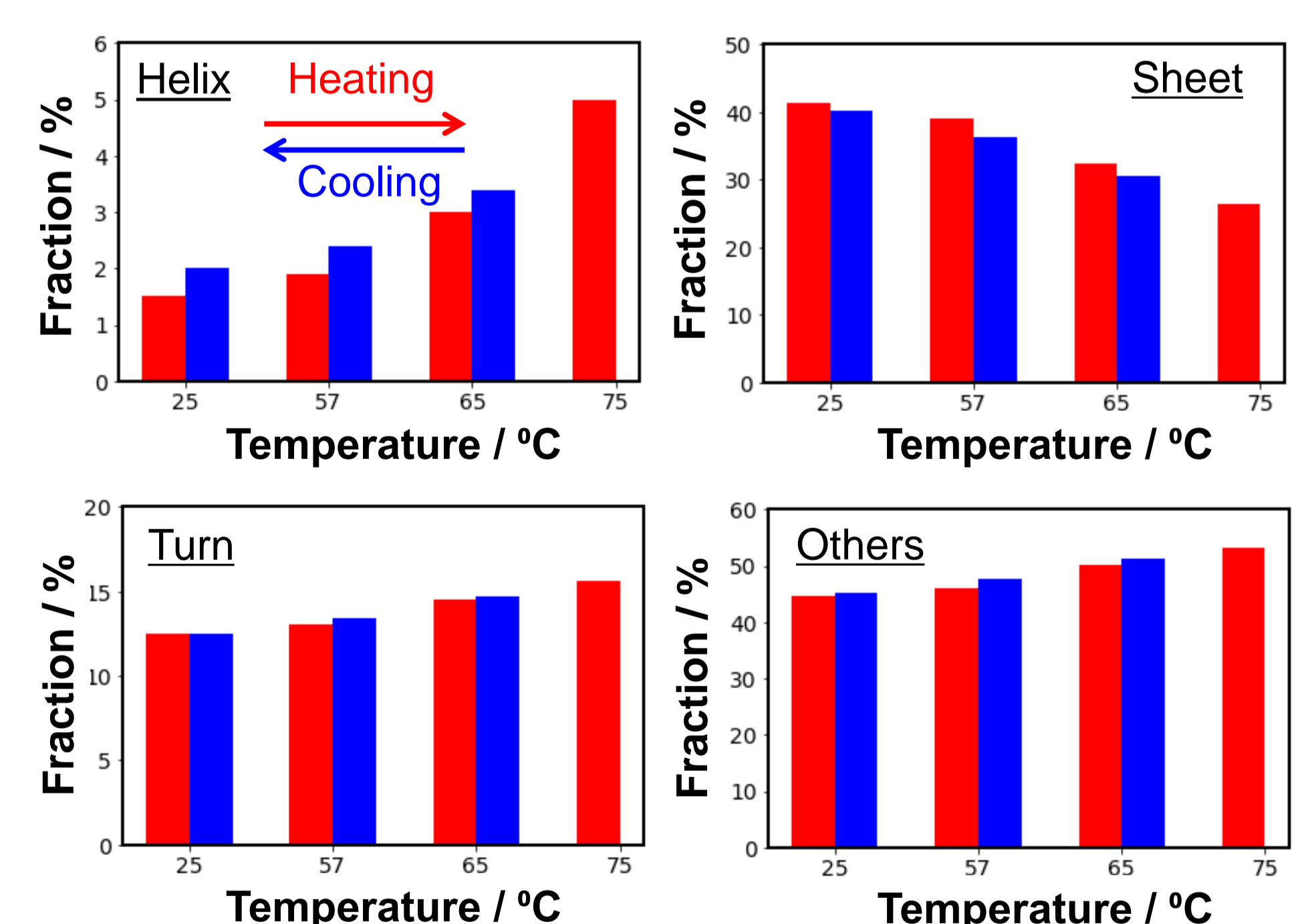
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7. Oyama T, et al. Performance Comparison of Spectral Distance Calculation Methods. Appl Spectrosc. 2022, 76(12), 1482-1493. DOI: 10.1177/00037028221121687

BeStSel

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