

# In Silico Antibody Characterisation

Sample Report

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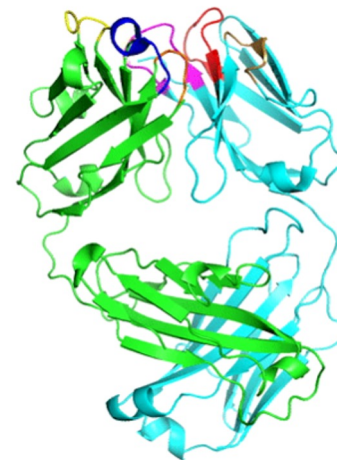
# Sequence and structure of mAb123

Fv Heavy chain:

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQA  
PGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNKNTLFLQ  
MNSLRAEDTAVYYCATNDDYWGQGTLVTVSS

Fv Light chain:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQ  
APRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVY  
YCQQSSNWPRTFGQGTKVEIK



Legend:

HCDR1

HCDR2

HCDR3

LCDR1

LCDR2

LCDR3

3D structure of mAb123 in cartoon. CDRs in sequence and structure are colour co-ordinated.

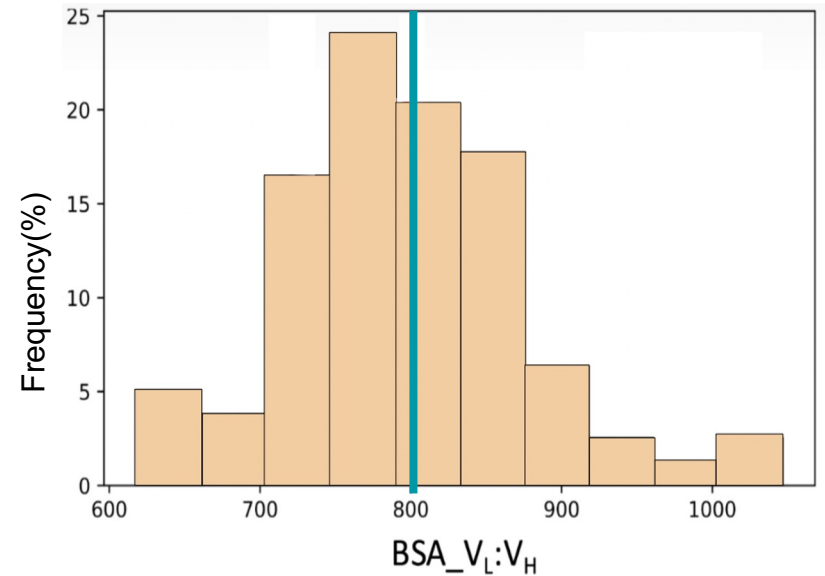
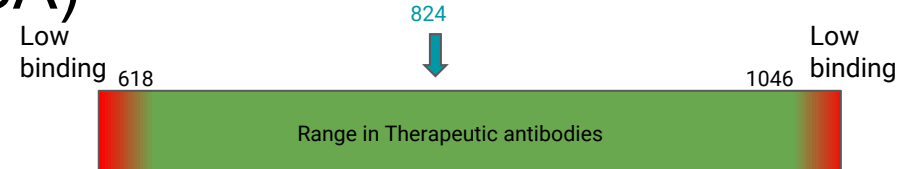
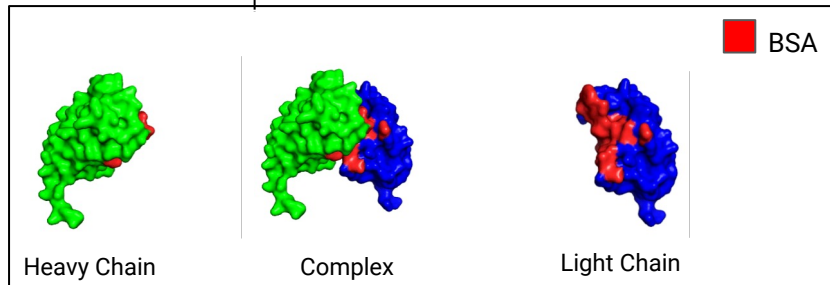
# VL-VH Buried Surface Area (BSA)

mAb123: 824 Sq.A

**Definition** Interface area between the VL and VH chains.

**Significance** Contributes to the stability of Fv region and indicates compatibility between VL-VH.

**Implications** Good. Buried Surface Area is in preferred range<sup>4</sup>



Buried Surface Area distribution of therapeutic antibodies.

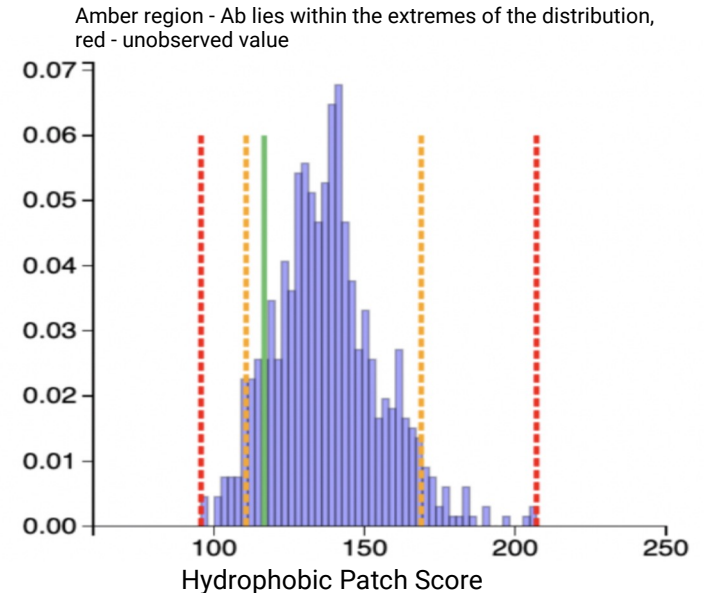
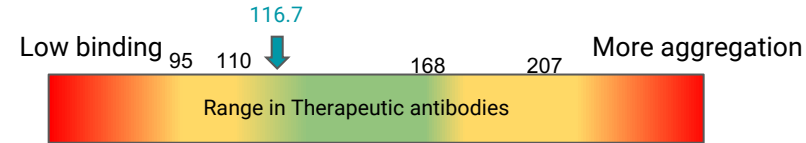
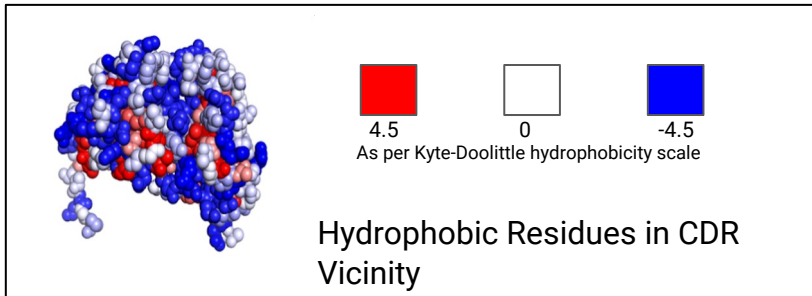
# Hydrophobic Patch

mAb123: 116.6931

**Definition** Assess the likelihood of aggregation of hydrophobic residues in antibodies

**Significance** Distributions of charged and nonpolar residues can influence solution behavior.

**Implication** Good. Hydrophobic Patch score is in preferred range<sup>3</sup>



Hydrophobic Patches of therapeutic antibodies. Distribution in blue, red and amber lines represents invalid and valid ranges

# Patches of Positive Charges

mAb123: 1.2186

## Definition

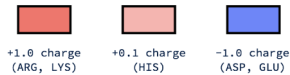
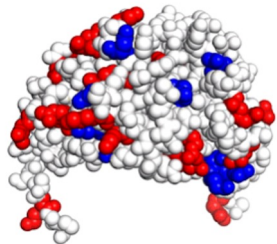
Regions within the CDRs that have a high concentration of positively charged residues.

## Significance

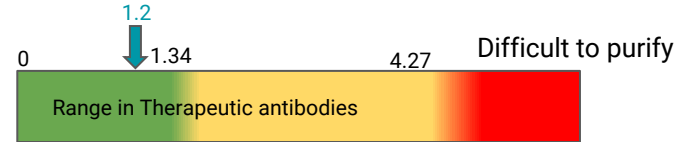
Signifies high rates of clearance and poor expression levels.

## Implications

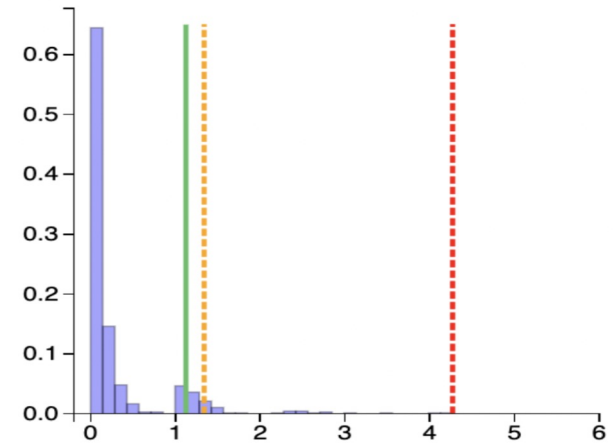
Good. Positive Patch Charge score is in preferred range<sup>3</sup>



Positive Charge Patch Residues



Amber region - Ab lies within the extremes of the distribution, red - unobserved value



Positive Patch Charge Score

Positive Patches of therapeutic antibodies

Distribution in blue, red and amber lines represents invalid and valid ranges of positive charge patches.

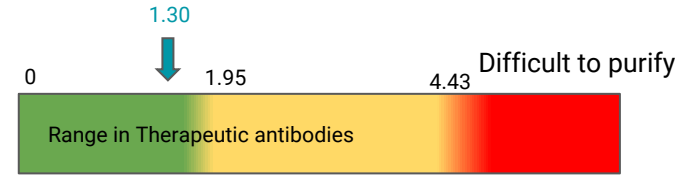
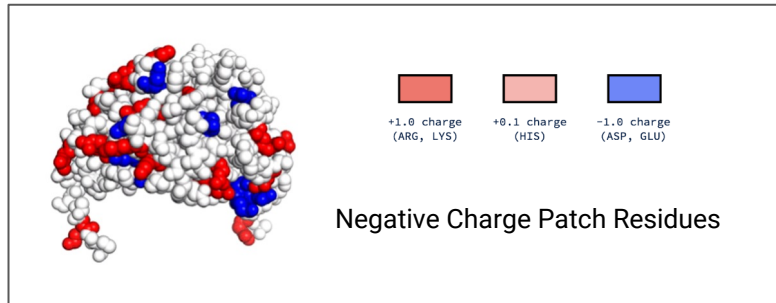
# Patches of Negative Charges

mAb123: 1.306

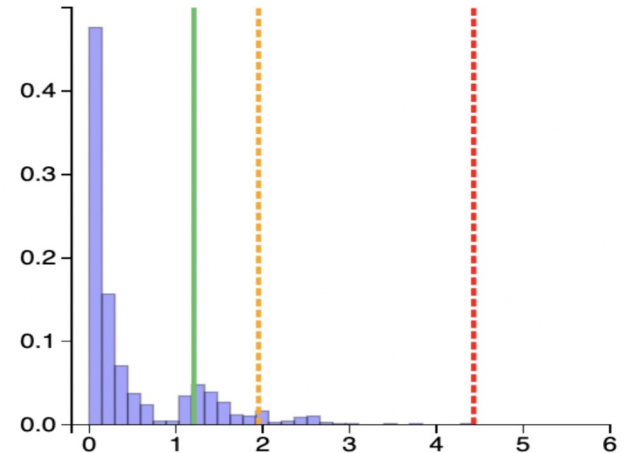
**Definition** Regions within the CDRs that have a high concentration of negatively charged residues

**Significance** Signifies high rates of clearance and poor expression levels.

**Implications** Good. Negative Patch Charge score is in preferred range<sup>3</sup>



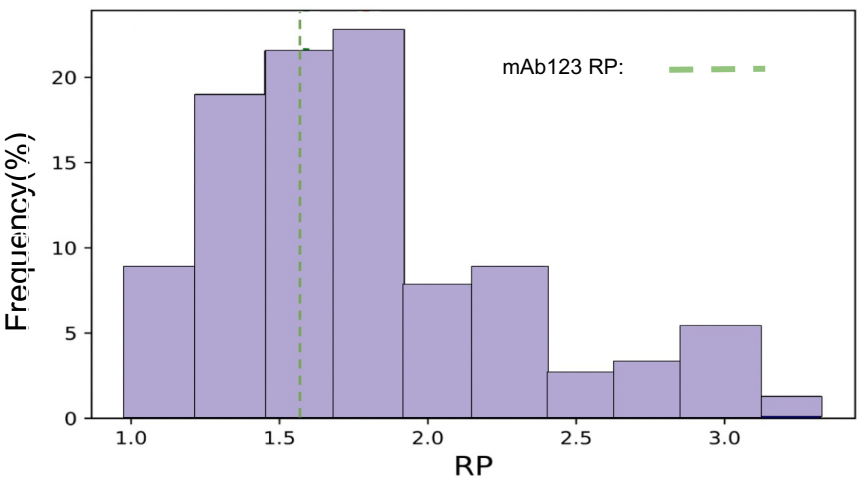
Amber region - Ab lies within the extremes of the distribution, red - unobserved value



Negative Patch Charge Score  
Negative Patches of Therapeutic Antibodies  
Distribution in blue, red and amber lines represents invalid and valid ranges of negative charge patches.

# Ratio of Charged to Hydrophobic Patches

mAb123: 1.7



Ratio of charged patch to hydrophobic patch distribution of therapeutic antibodies.

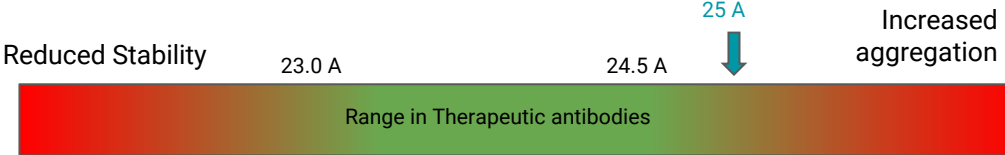
- Definition** Surface areas covered by charged and hydrophobic patches
- Significance** Large surface patches - undesirable aggregation & high viscosity
- Implications** Good. Ratio of Charged to Hydrophobic Patches is in preferred range<sup>4</sup>



# Hydrodynamic radius

mAb123: 25 A

- Definition** Refers to antibody size in a solution, considering weight and shape.
- Significance** It is connected to Ag diffusivity in a solution.
- Implications** Value of mAb123 is outside the reported range. Identification and modification of antibody regions contributing to the large hydrodynamic radius is suggested.



Distribution of hydrodynamic radius in therapeutic antibodies - Work in Progress

# Isoelectric point (pI)

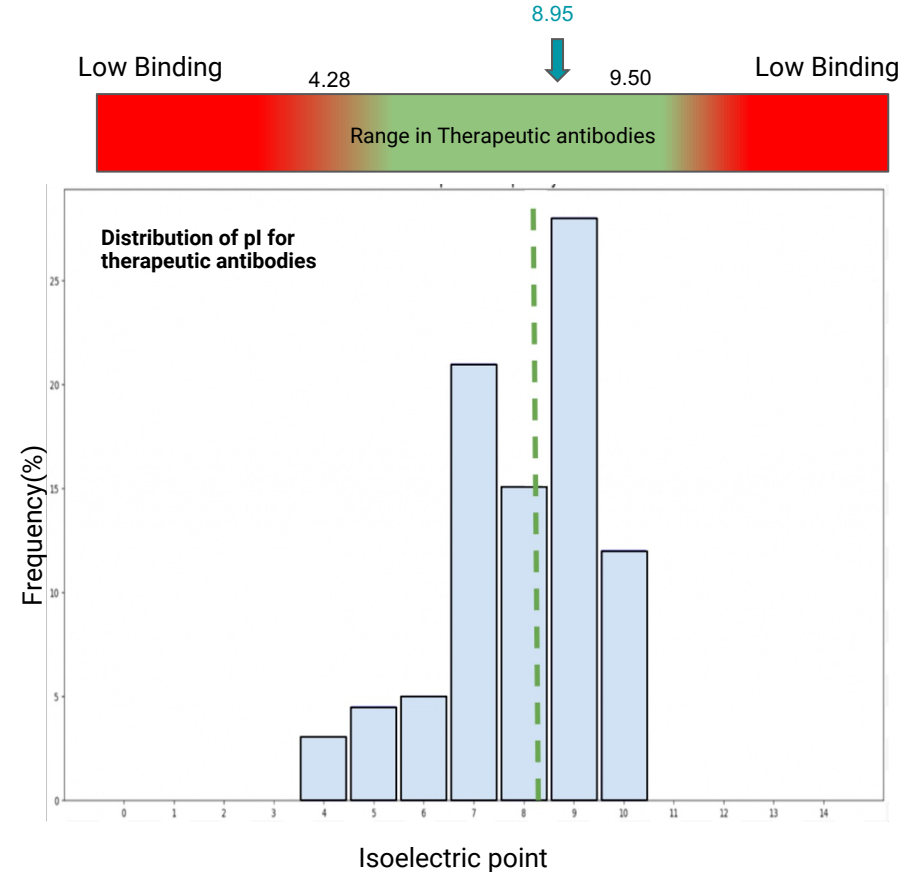
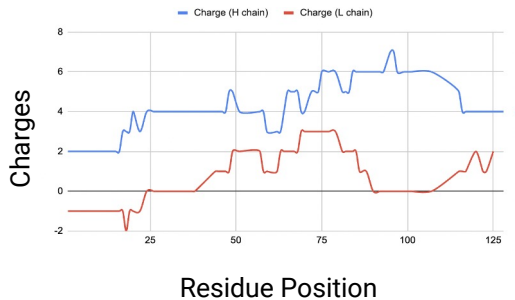
mAb123: 8.95

**Definition** pH at which a protein carries no net electrical charge.

**Significance** pI of an antibody influences its solution properties in vitro and PK/PD in vivo

**Implication** Good. pI is in preferred range<sup>1</sup>

Position Vs Charge of residues at pH 7.4



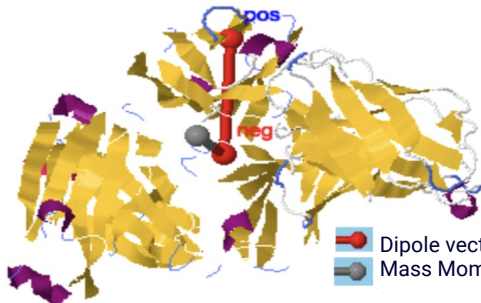
# Dipole Moment

mAb123: 507

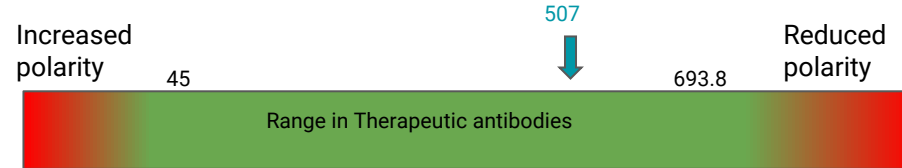
**Definition** | Separation between positively and negatively charged residues.

**Significance** | Distributions of charged and nonpolar residues can influence solution behavior

**Implications** | Good. Dipole moment is in preferred range



Dipole vector : [43.24, 73.58, -62.02]  
Mass Moments vector: [1558.47, 793.99, 703.97]



Distribution of dipole moment in therapeutic antibodies - Work in Progress

# Humanness Evaluation

mAb123: 93%

- Definition** Assess the extent to which the antibody conforms to human characteristics.
- Significance** A more human-like antibody is less likely to cause an immune response in patients
- Implication** Good. Humanness Evaluation is in preferred range<sup>2</sup>

Ab	Identity
mAb123(whole)	93%
Heavy chain	86%
Light chain	100%

93%



High immunogenicity > 80%

User threshold ( fraction of the human population should contain a given peptide for it to be human) - 80%

Distribution of humanness in therapeutic antibodies - Work in Progress

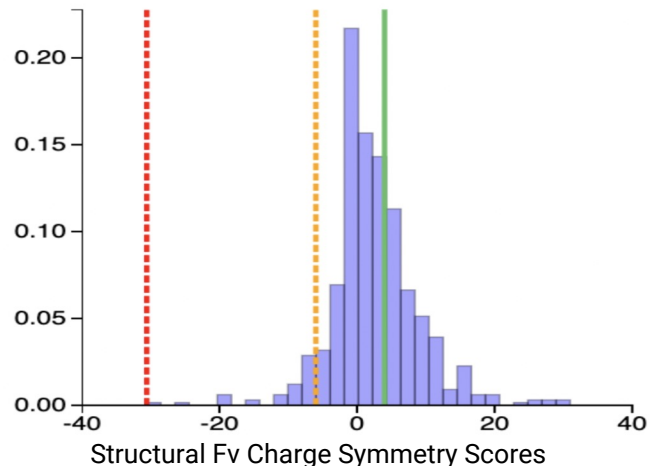
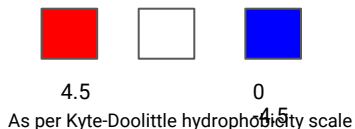
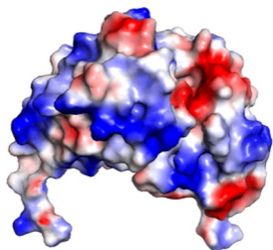
# Structural Fv Charge Symmetry Parameter (SFvCSP)

mAb123: 4.0

**Definition** Metric to assess the electrostatic charge symmetry within the variable domains (Fv)

**Significance** A negative score suggests an imbalance in charges while a positive score indicates a more balanced charge distribution.

**Implications** Good. Structural Fv Charge Symmetry score is in preferred range<sup>3</sup>



Structural Charge Symmetry of Antibodies.  
Distribution in blue, red and amber lines represents invalid and valid ranges of SFvCSP scores

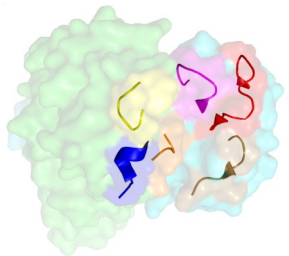
# Complementarity-determining Region (CDR) Length

mAb123: 40

**Definition** | The total length of CDRs in an antibody

**Significance** | CDR length can affect the specificity and affinity of the antibody

**Implication** | Good. CDR length is in preferred range<sup>3</sup>



Legend:

HCDR1

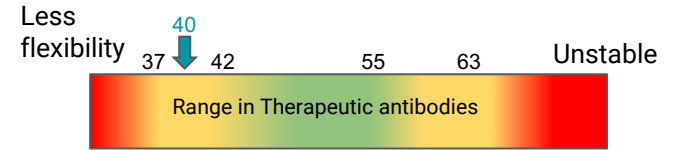
HCDR2

HCDR3

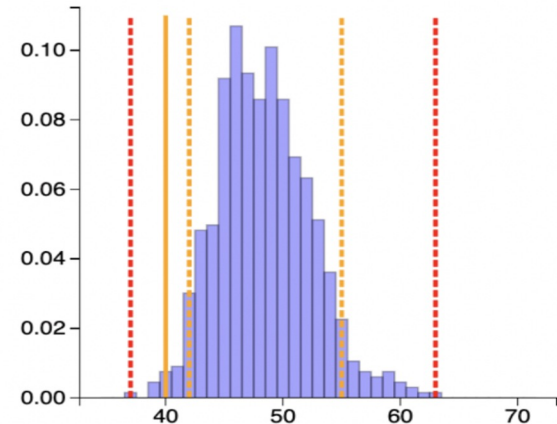
LCDR1

LCDR2

LCDR3



Amber region - Ab lies within the extremes of the distribution, red - unobserved value

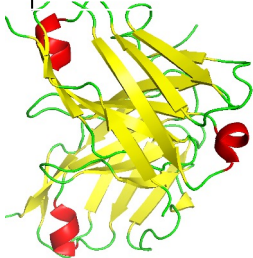


CDR Length distribution of therapeutic antibodies. Distribution in blue, red and amber lines represent invalid and valid ranges of cdr lengths

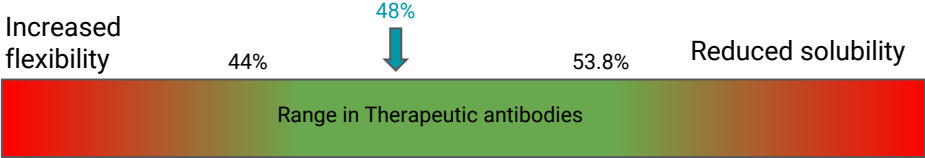
# Protein Strand Ratio

mAb123: 48%

- Definition** Amino acids in strand conformation divided by the total antibody amino acids
- Significance** Strands boost antibody stability and target binding
- Implications** Good. Protein strand ratio is in preferred range<sup>5</sup>



mAb123 secondary structure. Helix in red, strand in yellow, loops in green



Distribution of protein strand ratio in therapeutic antibodies - Work in Progress

# References

1. Bailly, Marc, et al. "Predicting antibody developability profiles through early stage discovery screening." *MAbs*. Vol. 12. No. 1. Taylor & Francis, 2020.
2. Gao, Sean H., et al. "Monoclonal antibody humanness score and its applications." *BMC biotechnology* 13.1 (2013): 1-12.
3. Raybould, Matthew IJ, et al. "Five computational developability guidelines for therapeutic antibody profiling." *Proceedings of the National Academy of Sciences* 116.10 (2019): 4025-4030.
4. Ahmed, Lucky, et al. "Intrinsic physicochemical profile of marketed antibody-based biotherapeutics." *Proceedings of the National Academy of Sciences* 118.37 (2021): e2020577118.
5. Licari, Giuseppe, et al. "Embedding Dynamics in Intrinsic Physicochemical Profiles of Market-Stage Antibody-Based Biotherapeutics." *Molecular Pharmaceutics* 20.2 (2022): 1096-1111.