

## Supplementary Table

**Table S1.** K<sub>D</sub> values of HF family against Her2

Constructs	K <sub>D</sub> (M) ± SD <sup>1</sup>
H0	7.11E-09 ± 1.04E-08
HF1	5.81E-09 ± 6.85E-09
HF2	1.40E-09 ± 8.46E-10
HF3	3.88E-09 ± 4.83E-09
HF4	2.13E-09 ± 1.61E-09

Data are mean±SD unless indicated

<sup>1</sup>K<sub>D</sub> calculated by BLI at 25°C. Mean of 5 independent experiments.

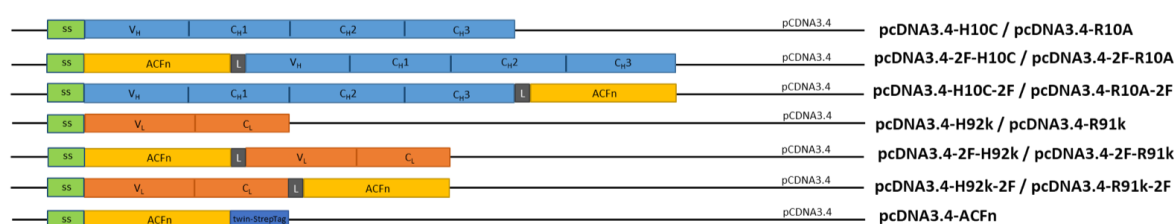


Figure S1: Scheme of plasmids construction needed for both Ab and BsAb production

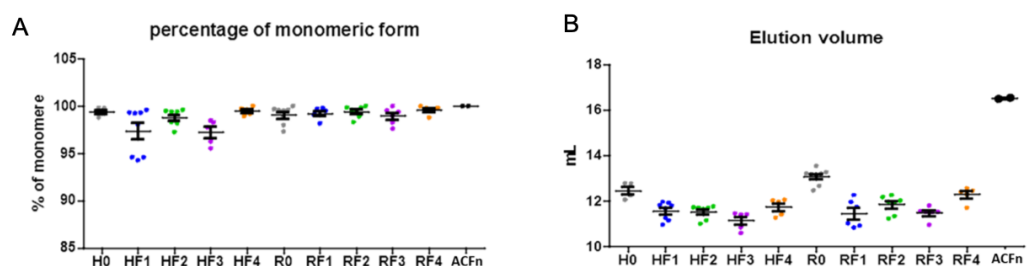


Figure S2: (A) Percentage of monomeric form and (B) elution volume for each protein after analysis of size-exclusion chromatography for each production

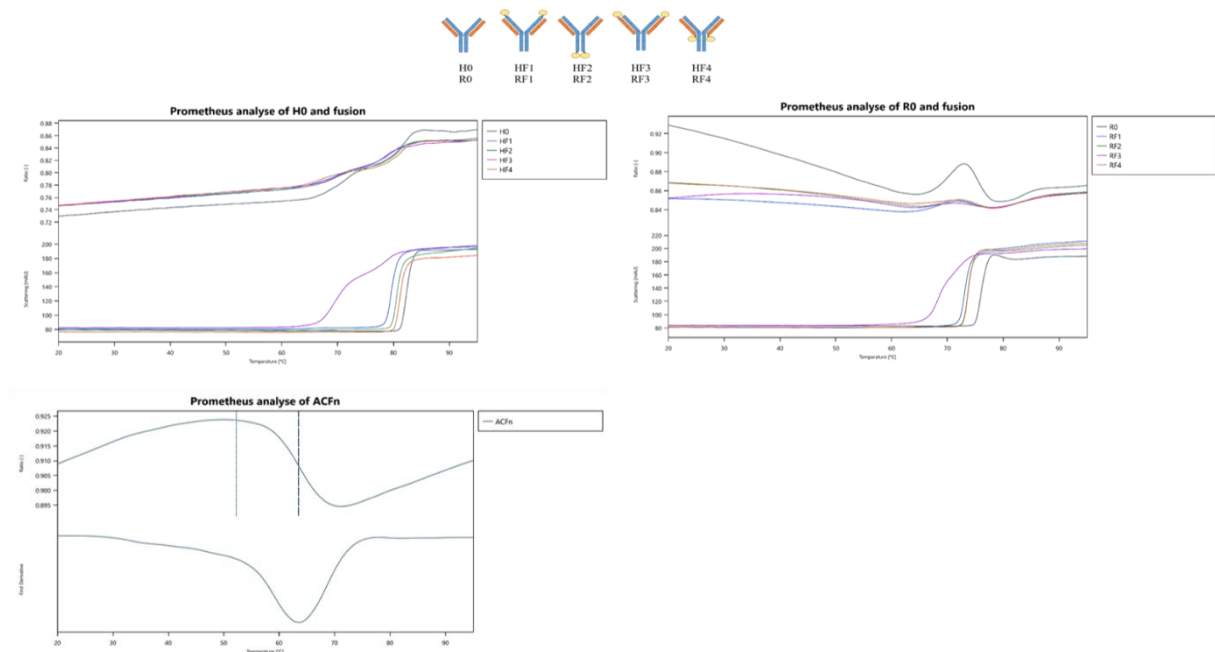


Figure S3: Profile of curves obtained by Prometheus for nanoDSF analyse

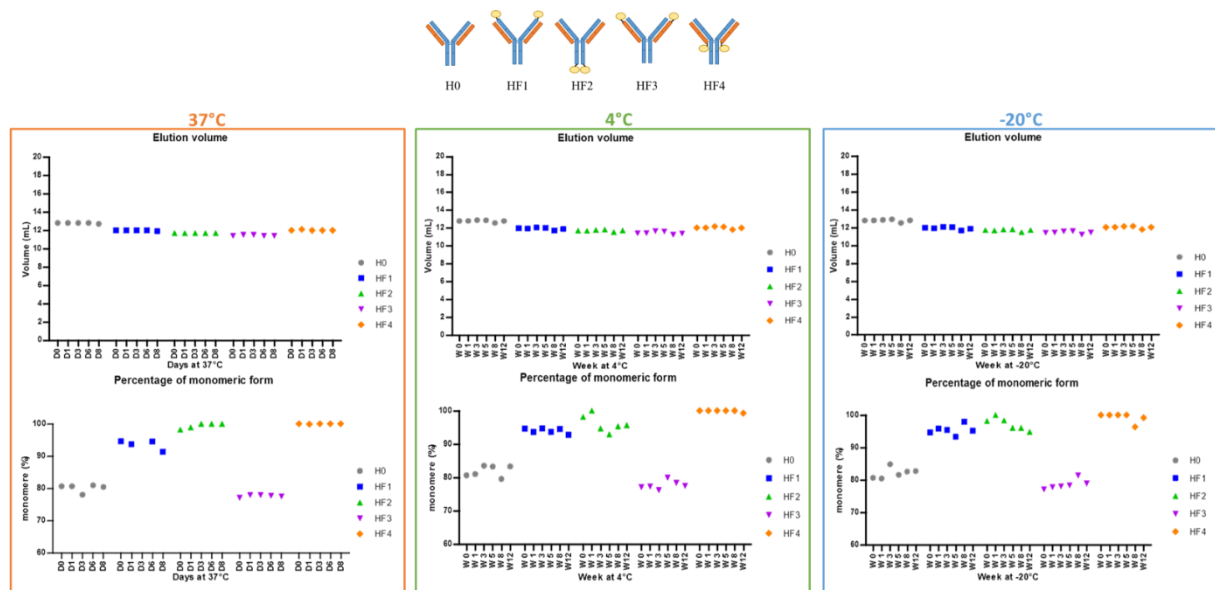
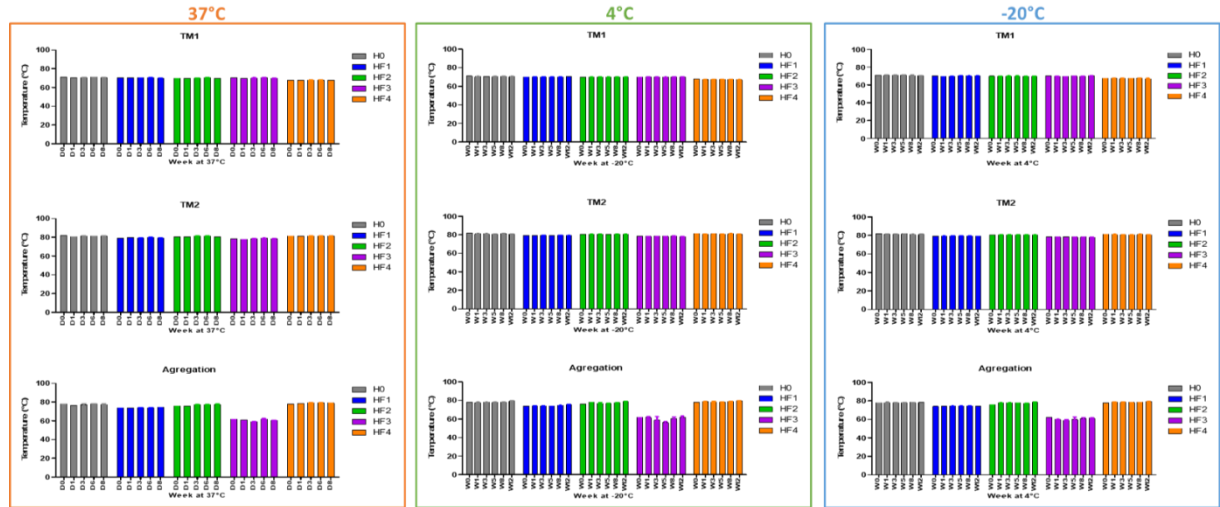
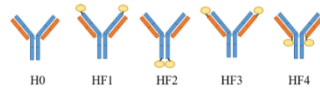
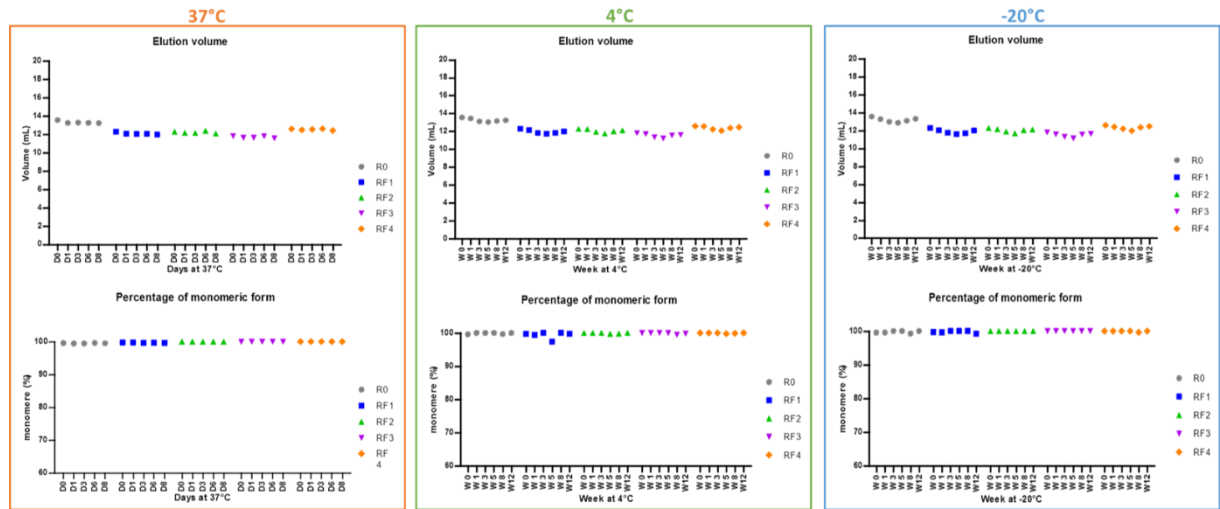
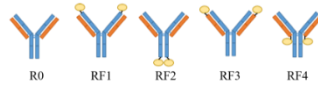


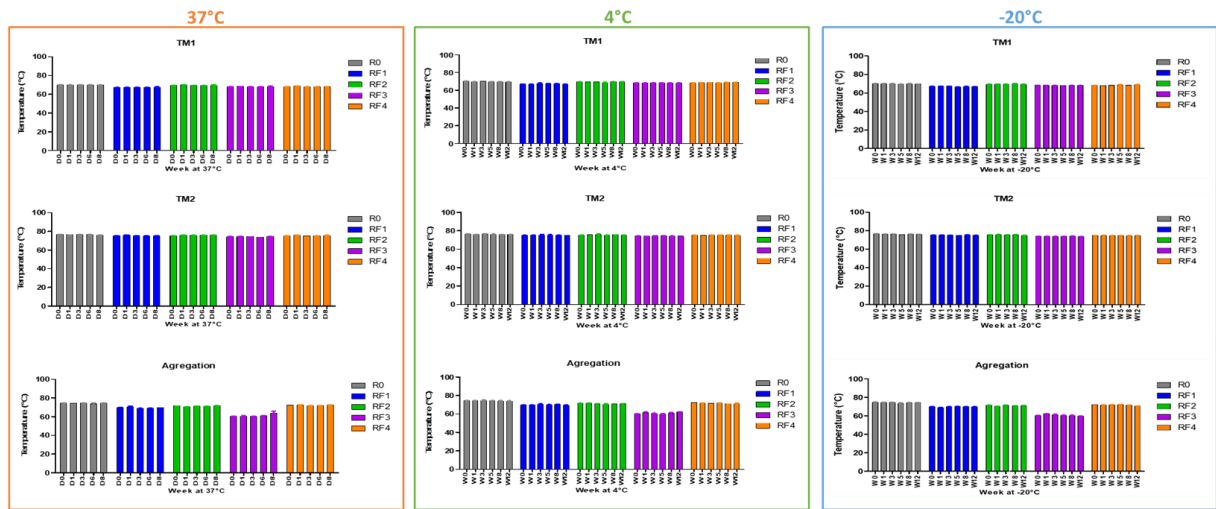
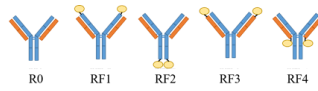
Figure S4: Kinetics of protein stability during storage for height days at 37°C or twelve weeks at +4°C or -20°C. Analyze by size-exclusion chromatography on a calibrated Superdex 200 10/300 GL column of elution volume and percentage of monomeric form of each antibody for Trastuzumab and related BsAb (HF1-HF4).



**Figure S5:** Kinetics of protein stability during storage for height days at 37°C or twelve weeks at +4°C or -20°C. Thermal and aggregation analysis by Prometheus for Trastuzumab and BsAb (HF1-HF4).

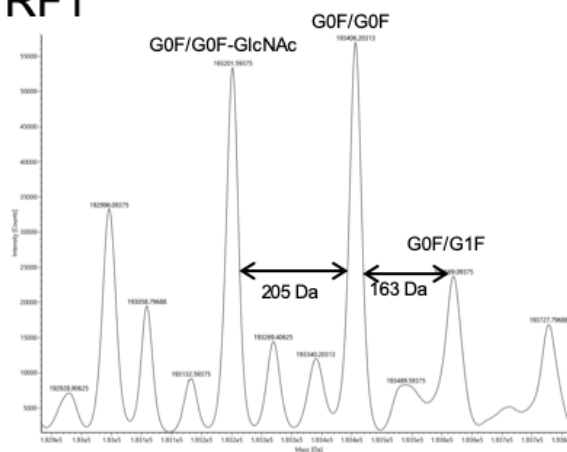


**Figure S6:** Kinetics of calibrated protein stability during storage for height days at 37°C or twelve weeks at +4°C or -20°C. Analyze by size-exclusion chromatography of a calibrated Superdex 200 10/300 GL column of elution volume and percentage of monomeric form of each antibody for Rituximab and related BsAb (RF1-RF4).

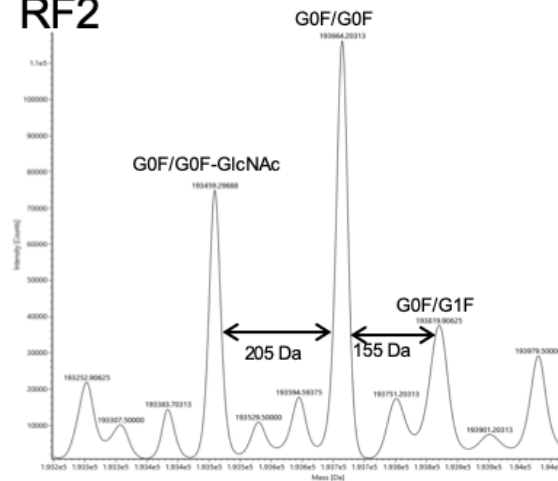


**Figure S7:** Kinetics of protein stability during storage for height days at 37°C or twelve weeks at +4°C or -20°C . Thermal and aggregation analysis by Prometheus for Rituximab and related BsAb (RF1-RF4)

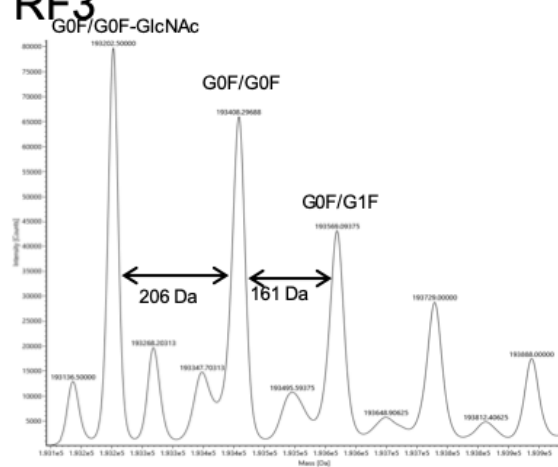
RF1



RF2



RF3



RF4

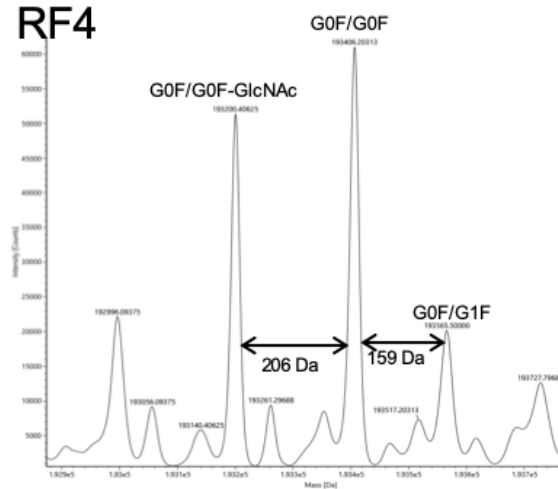


Figure S8: Mass spectra of HF BsAb

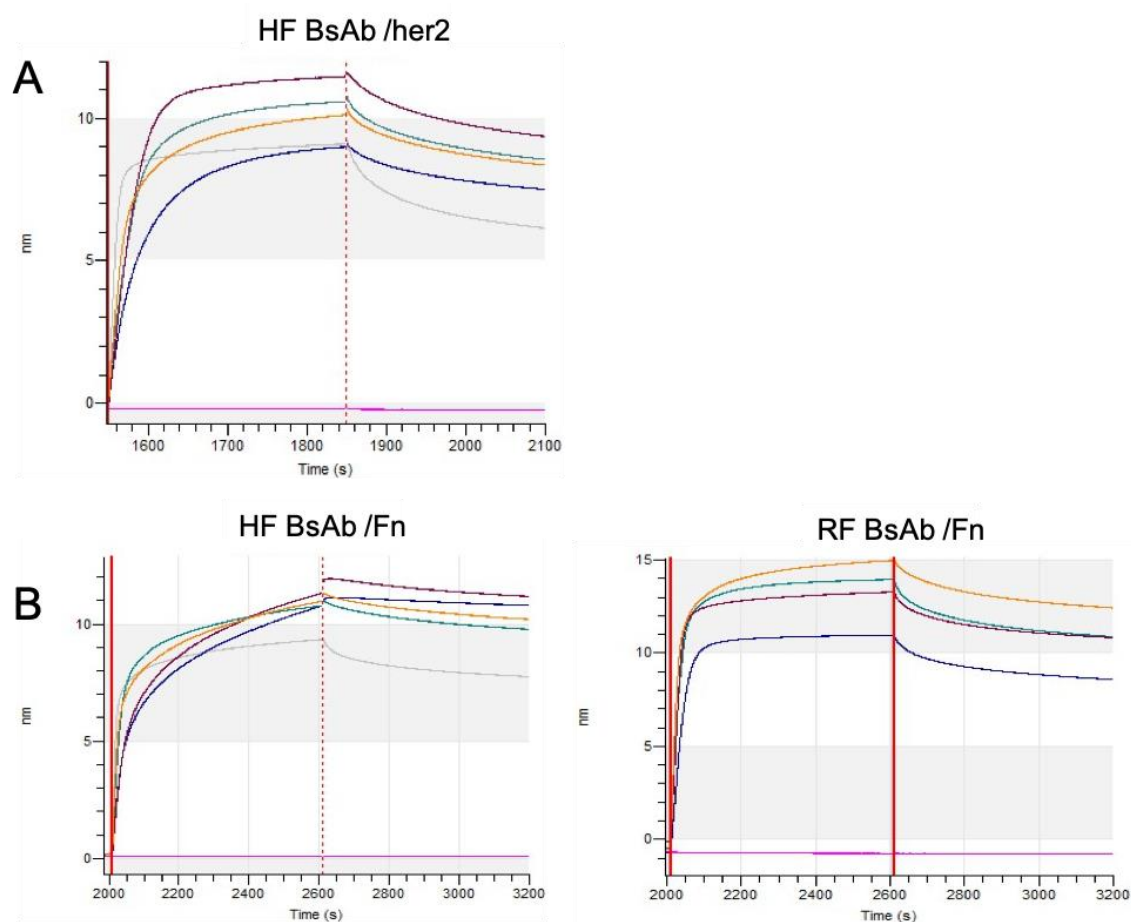


Figure S9: Sensorgrams of (A) H0-H4 at 200 nM on Her2, (B) H0-H4 and R0-RF4 at 200 nM or Fn obtained by BLI. H0/R0 in gray, HF1/RF1 in blue, HF2/RF2 in green, HF3/RF3 in red, HF4/RF4 in orange, buffer in pink.