Supplementary information

for

Modification of the kinetic stability of immunoglobulin G by solvent additives

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Figure S1

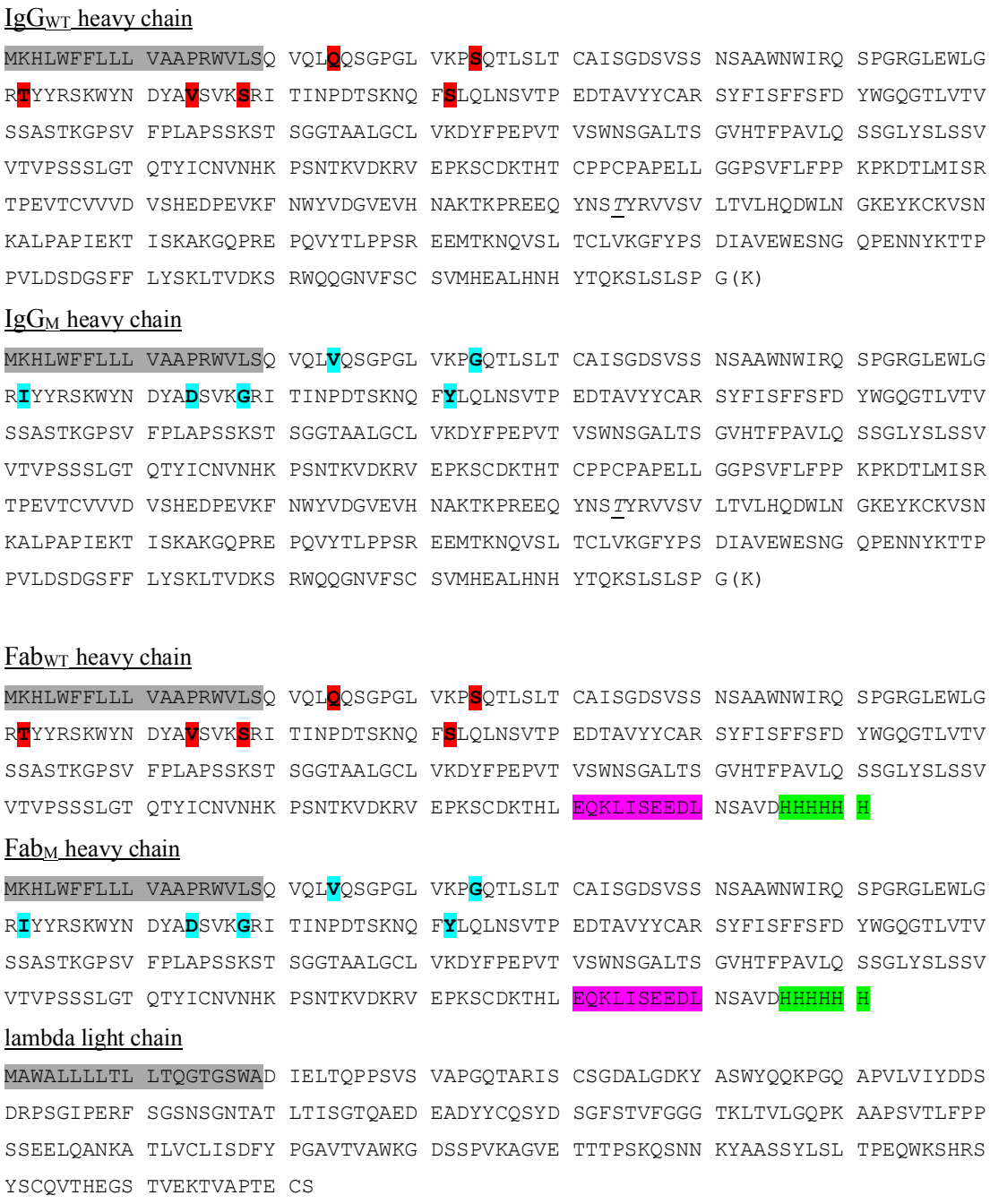

Figure 1. Sequences of the IgG and Fab fragments used in this study. Mutated residues in the respective heavy chains are highlighted in either red for the IgG$_{WT}$ and in turquois for the IgG$_{M}$ constructs. The cleaved signal sequences in both the heavy and light chains are marked in grey and the myc- and his-tag present only in the heavy chains of the Fab constructs in pink or green, respectively. Considering the cleavage of IgG heavy chain C-terminal lysine (therefore these residues are stated in brackets) the resulting pI values for the IgG$_{WT}$ and the IgG$_{M}$ are 6.89 or 6.65, respectively, while those for the Fab$_{WT}$ and the Fab$_{M}$ are 6.28 or 6.14, respectively

Figure S2

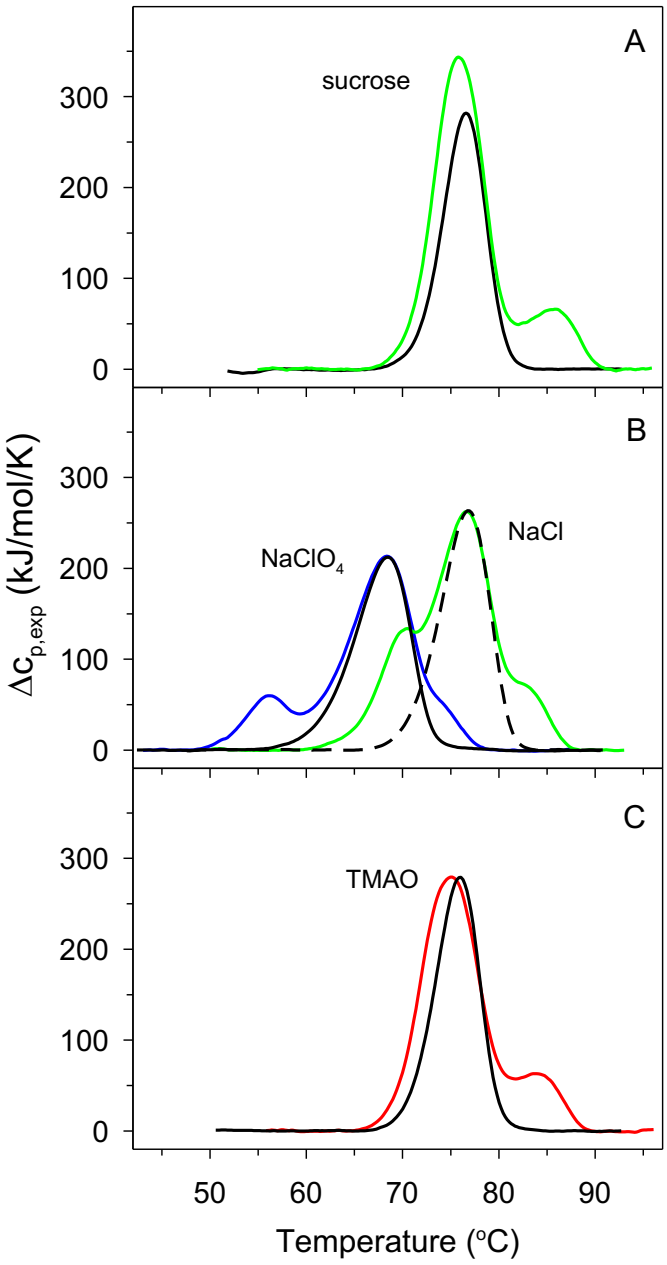

Figure S2. Comparison of DSC scans of IgG_{WT} in the presence of representative additives with the corresponding scans of Fab_{WT} fragments under identical conditions. The DSC scans of IgG are shown in color, corresponding DSC scans of Fab fragment are shown in black lines (DSC scan of Fab_{WT} in the presence of 1 M NaCl is shown in dashed black line). All DSC measurements were performed at a protein concentration 0.5 mg/ml in corresponding buffers at a scan rate of 1 K/min.

Figure S3

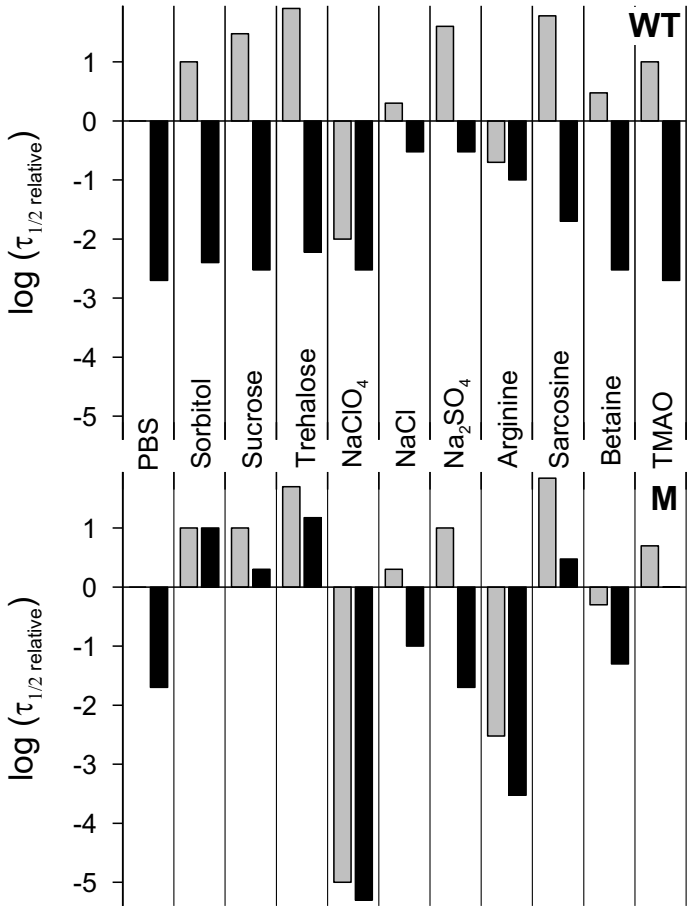

Figure S3. Comparison of relative lifetimes of individual Fab fragments (grey bars) and the Fab fragments in the context of the full-length IgG (black bars) in the presence of studied additives. The respective lifetimes are expressed as the logarithm (log_{10}) of relative lifetimes of the Fab fragments relative to the individual Fab_{WT} (upper part) or Fab_M (lower part) in the presence of PBS at 37°C. Thus, the respective Fab in PBS is set to zero in this plot.

Figure S4

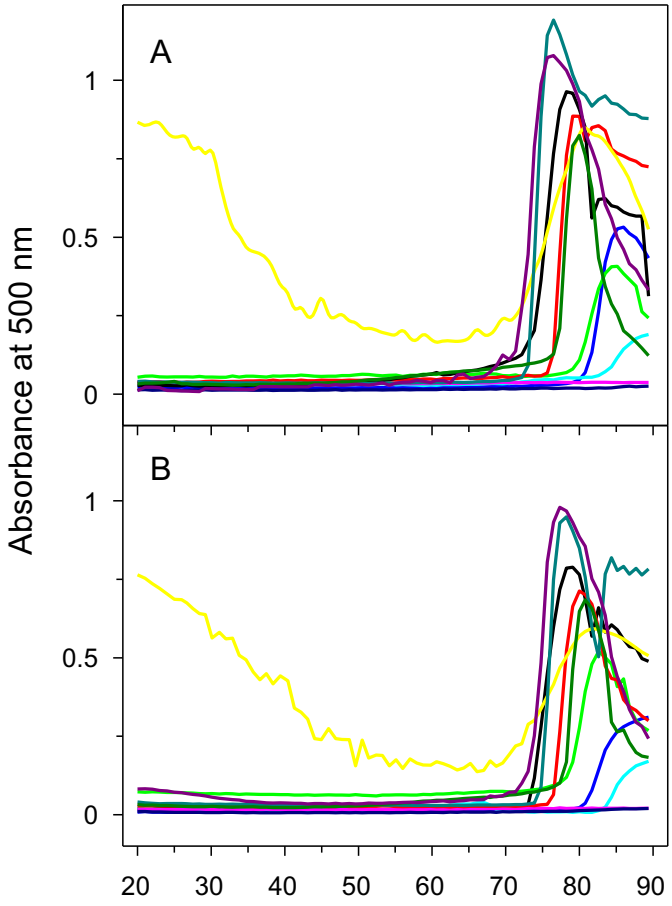

Figure S4. Temperature dependence of absorbance at 500 nm for IgG_{WT} (A) and IgG_{M} (B) in the presence of studied additives: PBS (black), sorbitol (blue), sucrose (cyan), trehalose (green), $NaClO_4$ (magenta), NaCl (red), Na_2SO_4 (yellow), arginine (dark blue), sarcosine (dark cyan), betaine (dark green), and TMAO (dark magenta).

Figure S5

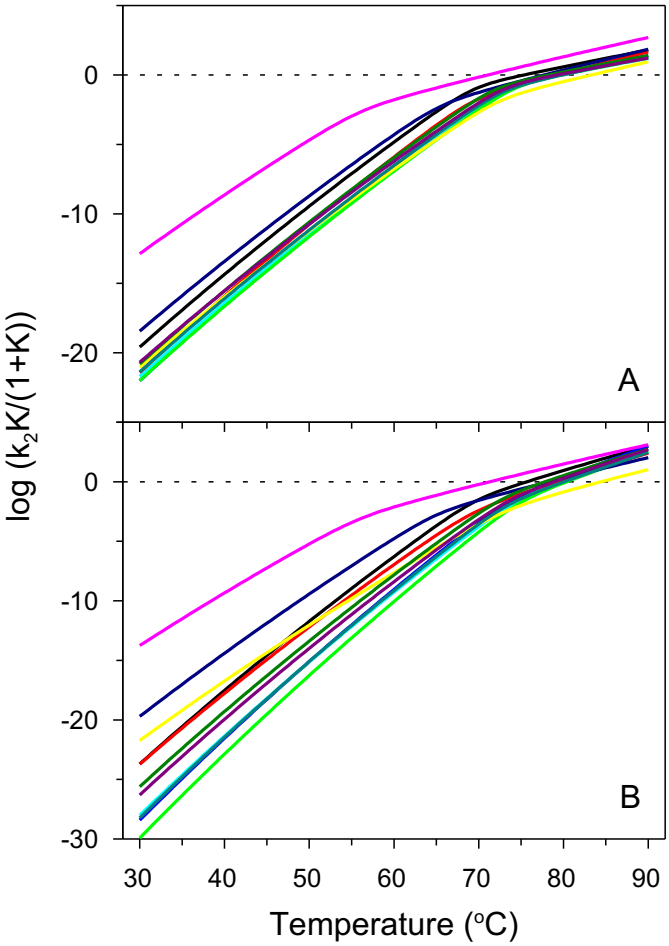

Figure S5. Temperature dependence of $\log\left(k_2 \frac{K}{1+K}\right)$ of IgG_{WT} (A) and IgG_M (B) in the presence of studied additives: PBS (black), sorbitol (blue), sucrose (cyan), trehalose (green), $NaClO_4$ (magenta), NaCl (red), Na_2SO_4 (yellow), arginine (dark blue), aarcosine (dark cyan), betaine (dark green), and TMAO (dark magenta).

Figure S6

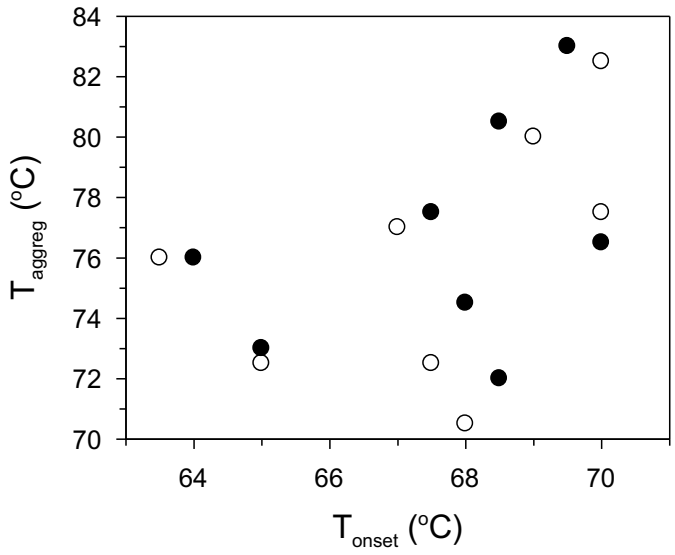

Figure S6. Correlation of onset temperatures, T_{onset}, and aggregation temperatures, T_{aggreg}, for IgG_{WT} (white circles) and IgG_{M} (black circles). The temperatures are listed in Table S3.

Table S1. Fitting parameters for thermal transitions of Fab_{WT} fragment and IgG_{WT}

		T_1 (°C)	ΔH_1 (kJ/mol)	T_2^* (°C)	E_{a2} (kJ/mol)	$\tau_{1/2,\ 37°C}$ (x factor)	ΔH_2 (kJ/mol)	T_3^* (°C)	E_{a3} (kJ/mol)	ΔH_3 (kJ/mol)	R^2
PBS	Fab_{WT}	-	-	75.1 ±0.1	443 ±4	1	1273 ±10	-	-	-	0.9893
	IgG_{WT}	69.3 ±0.1	656 ±4	75.3 ±0.1	295 ±2	~0.002	1539 ±6	84.4 ±0.1	390 ±4	471 +3	0.9997
Sorbitol	Fab_{WT}	-	-	77.5 ±0.1	476 ±4	~10	1908 ±12	-	-	-	0.9911
	IgG_{WT}	73.0 ±0.1	703 ±15	78.4 ±0.1	293 ±10	~0.004	1738 ±23	86.7 ±0.2	388 ±15	484 ±13	0.9976
Sucrose	Fab_{WT}	-	-	78.6 ±0.1	482 ±4	~30	1670 ±11	-	-	-	0.9904
	IgG_{WT}	74.3 ±0.1	681 ±5	79.5 ±0.1	279 ±3	~0.003	1544 ±7	88.6 ±0.1	380 ±4	523 ±4	0.9998
Trehalose	Fab_{WT}	-	-	79.5 ±0.1	495 ±4	~80	2094 ±14	-	-	-	0.9920
	IgG_{WT}	74.2 ±0.1	680 ±5	79.7 ±0.1	292 ±3	~0.006	1786 ±7	88.4 ±0.1	383 ±4	547 ±4	0.9998
$NaClO_4$	Fab_{WT}	-	-	71.5 ±0.1	353 ±1	~0.01	1623 ±4	-	-	-	0.9984
	IgG_{WT}	56.2 ±0.1	428 ±3	71.1 ±0.1	341 ±2	~0.003	1502 ±29	76.5 ±0.2	308 ±23	415 ±28	0.9977
NaCl	Fab_{WT}	-	-	79.3 ±0.1	422 ±2	~2	1784 ±7	-	-	-	0.9961
	IgG_{WT}	69.4 ±0.1	623 ±2	79.2 ±0.1	377 ±1	~0.3	1664 ±8	85.5 ±0.1	365 ±7	523 ±8	0.9996
Na_2SO_4	Fab_{WT}	-	-	85.8 ±0.1	427 ±3	~40	1826 ±8	-	-	-	0.9961
	IgG_{WT}	72.4 ±0.1	570 ±3	83.5 ±0.1	349 ±3	~0.3	1660 ±50	87.1 ±0.1	372 ±26	536 ±50	0.9993
Arginine	Fab_{WT}	-	-	78.1 ±0.1	375 ±1	~0.2	1923 ±2	-	-	-	0.9999
	IgG_{WT}	65.5 ±0.1	545 ±2	77.7 ±0.1	367 ±2	~0.1	1551 ±12	84.5 ±0.2	339 ±13	410 ±12	0.9984
Sarcosine	Fab_{WT}	-	-	79.1 ±0.1	494 ±4	~60	1953 ±12	-	-	-	0.9927
	IgG_{WT}	72.8 ±0.1	640 ±4	79.8 ±0.1	317 ±2	~0.02	1747 ±8	87.1 ±0.1	375 ±5	578 ±6	0.9997
Betaine	Fab_{WT}	-	-	77.4 ±0.1	446 ±3	~3	1777 ±10	-	-	-	0.9933
	IgG_{WT}	71.9 ±0.1	668 ±4	78.2 ±0.1	289 ±3	~0.003	1598 ±7	86.8 ±0.1	367 ±4	585 ±4	0.9997
TMAO	Fab_{WT}	-	-	78.0 ±0.1	471 ±3	~10	1691 ±10	-	-	-	0.9928
	IgG_{WT}	73.0 ±0.1	656 ±3	79.2 ±0.1	276 ±3	~0.002	1355 ±6	87.2 ±0.1	357 ±4	505 ±4	0.9998

Fitting parameters for thermal transitions of Fab_{WT} fragment and IgG_{WT} were obtained from fits of experimental data using Eq. 4 and 6, respectively. All measurements were performed at a scan rate of 1 K/min. The shelf-life $\tau_{1/2}$ is related to the calculated shelf-life of the Fab_{WT} at 37°C, which is set to 1. The changes in T_2^*, E_{a2}, and $\tau_{1/2}$ in the presence of the additives are color-coded in three different categories (indicated by three different strengths of either green (increase in parameters, i.e., longer half-life or higher stability) or red (decrease in parameters)), based on the intensity of the changes: for T_2^*, category 1

represents an in/decrease between 1° and 3°C, category 2 is between 3° and 5°C, while category 3 indicates changes by more than 5°C. For E_{a2}, category 1 represents an in/decrease up to 20 kJ/mol, category 2 is between 20 and 50 kJ/mol, while category 3 indicates changes by more than 50 kJ/mol. For $\tau_{1/2}$, category 1 represents an in/decrease of the shelf life between 2- and 10-fold, category 2 is between 10- and 30-fold, while category 3 indicates changes by more than 30-fold.

Table S2. Fitting parameters for thermal transitions of Fab_M fragment and IgG_M

		T_1 (°C)	ΔH_1 (kJ/mol)	T_2^* (°C)	E_{a2} (kJ/mol)	$\tau_{1/2,\ 37°C}$ (x factor)	ΔH_2 (kJ/mol)	T_3^* (°C)	E_{a3} (kJ/mol)	ΔH_3 (kJ/mol)	R^2
PBS	Fab_M	-	-	77.7 ±0.1	614 ±5	1	1439 ±10	-	-	-	0.9906
	IgG_M	69.6 ±0.1	612 ±4	75.6 ±0.1	510 ±4	~0.02	1754 ±8	84.6 ±0.1	338 ±5	588 ±4	0.9994
Sorbitol	Fab_M	-	-	80.6 ±0.1	628 ±4	~10	1487 ±7	-	-	-	0.9961
	IgG_M	73.3 ±0.1	597 ±5	78.7 ±0.1	652 ±4	~10	1692 ±10	87.1 ±0.1	347 ±10	523 ±8	0.9988
Sucrose	Fab_M	-	-	81.1 ±0.1	621 ±4	~10	1404 ±8	-	-	-	0.9945
	IgG_M	74.6 ±0.1	601 ±5	79.7 ±0.1	607 ±4	~2	1596 ±9	88.6 ±0.1	347 ±8	522 ±7	0.9990
Trehalose	Fab_M	-	-	82.4 ±0.1	635 ±4	~50	1593 ±9	-	-	-	0.9943
	IgG_M	74.9 ±0.1	642 ±4	80.3 ±0.1	639 ±3	~15	1823 ±8	89.1 ±0.1	331 ±7	591 ±7	0.9993
$NaClO_4$	Fab_M	-	-	72.5 ±0.1	407 ±3	~$1x10^{-5}$	1279 ±7	-	-	-	0.9931
	IgG_M	56.1 ±0.1	403 ±4	71.3 ±0.2	398 ±4	~$5x10^{-6}$	1224 ±116	75.5 ±0.2	303 ±39	747 ±114	0.9953
NaCl	Fab_M	-	-	80.5 ±0.1	598 ±3	~2	1356 ±6	-	-	-	0.9965
	IgG_M	69.3 ±0.1	531 ±4	79.2 ±0.1	549 ±4	~0.1	1475 ±30	85.7 ±0.3	280 ±22	634 ±29	0.9969
Na_2SO_4	Fab_M	-	-	86.6 ±0.1	564 ±2	~10	1381 ±5	-	-	-	0.9977
	IgG_M	73.4 ±0.1	450 ±5	84.4 ±0.2	458 ±9	~0.02	1124 ±215	87.9 ±0.3	283 ±86	591 ±207	0.9962
Arginine	Fab_M	-	-	79.0 ±0.1	473 ±3	~0.003	1430 ±7	-	-	-	0.9953
	IgG_M	65.1 ±0.1	540 ±3	78.3 ±0.1	425 ±3	~0.0003	1589 ±45	84.2 ±0.3	279 ±25	622 ±44	0.9973
Sarcosine	Fab_M	-	-	81.9 ±0.1	649 ±3	~70	1455 ±6	-	-	-	0.9967
	IgG_M	73.2 ±0.1	623 ±5	80.0 ±0.1	608 ±4	~3	1825 ±13	87.1 ±0.2	357 ±15	557 ±13	0.9981
Betaine	Fab_M	-	-	79.1 ±0.1	583 ±4	~0.5	1378 ±7	-	-	-	0.9957
	IgG_M	72.9 ±0.1	599 ±9	77.7 ±0.1	547 ±9	~0.05	1471 ±14	86.6 ±0.2	354 ±11	530 ±10	0.9979
TMAO	Fab_M	-	-	80.0 ±0.1	625 ±5	~5	1249 ±7	-	-	-	0.9931
	IgG_M	73.5 ±0.1	563 ±7	78.8 ±0.1	592 ±5	~1	1380 ±13	86.9 ±0.2	345 ±14	444 ±10	0.9981

Fitting parameters for thermal transitions of Fab_M fragment and IgG_M were obtained from fits of experimental data using Eq. 4 and 6, respectively. All measurements were performed at a scan rate of 1 K/min. The shelf-life $\tau_{1/2}$ is related to the calculated shelf-life of the Fab_M at 37°C. The changes in T_2^*, E_{a2}, and $\tau_{1/2}$ in the presence of the additives are color-coded in three different categories (indicated by three different strengths of either green (increase in parameters, i.e., longer half-life or higher stability) or red (decrease in parameters)), based on the intensity of the changes: for T_2^*, category 1 represents an in/decrease

between 1° and 3°C, category 2 is between 3° and 5°C, while category 3 indicates changes by more than 5°C. For E_{a2}, category 1 represents an in/decrease up to 20 kJ/mol, category 2 is between 20 and 50 kJ/mol, while category 3 indicates changes by more than 50 kJ/mol. For $\tau_{1/2}$, category 1 represents an in/decrease of the shelf life between 2- and 10-fold, category 2 is between 10- and 30-fold, while category 3 indicates changes by more than 30-fold.

Table S3. Parameters characterizing the thermal transitions of IgG_{WT} and IgG_M

IgG_{WT}	T_{onset}* (°C)	T_{agg}* (°C)	$\log(\tau_{rel,IgG}^2/\tau_{rel,Fab})$**	$\log[k_2K/(1+K)]$
PBS	65.0	72.5	-5.40	-15.89
Sorbitol	69.0	80.0	-5.80	-18.14
Sucrose	70.0	82.5	-6.52	-17.97
Trehalose	70.0	77.5	-6.35	-18.22
$NaClO_4$	50.5	-	-3.04	-9.88
NaCl	63.5	76.0	-1.35	-17.53
Na_2SO_4	66.5	-	-2.65	-17.46
Arginine	60.5	-	-1.30	-14.89
Sarcosine	67.5	72.5	-5.18	-17.65
Betaine	67.0	77.0	-5.52	-17.08
TMAO	68.0	70.5	-6.40	-17.05
IgG_M	T_{onset} (°C)	T_{agg} (°C)	$\log(\tau_{rel,IgG}^2/\tau_{rel,Fab})$	$\log[k_2K/(1+K)]$
PBS	65.0	73.0	-3.40	-19.33
Sorbitol	68.5	80.5	1.00	-23.56
Sucrose	69.5	83.0	-0.40	-23.31
Trehalose	70.0	76.5	0.65	-24.94
$NaClO_4$	50.5	-	-5.60	-10.63
NaCl	64.0	76.0	-2.30	-19.51
Na_2SO_4	68.0	-	-4.40	-18.20
Arginine	60.0	-	-4.52	-15.96
Sarcosine	68.0	74.5	-0.89	-23.44
Betaine	67.5	77.5	-2.30	-21.14
TMAO	68.5	72.0	-0.70	-21.81

* The experimental error in determination of T_{onset} and T_{agg} is estimated to be ±0.5 °C.

**The parameter $\tau_{rel,IgG} \cdot \frac{\tau_{rel,IgG}}{\tau_{rel,Fab}}$ is expressed in logarithmic form.