Supplementary information

for

Modification of the kinetic stability of immunoglobulin G
by solvent additives

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Figure S1

IgG<sub>WT</sub> heavy chain
MKHLWFLLL VAAPRWVLSDQ VQLQSGPGL VKFQTLSLTLT CAISSGVSS NSAANNIRQ SPGRGELWG
RTYRYSKWN DYADSVKQRI TINPDTSQKQ FLQLNSVTP EDTAVYYCAR SYFISFFSD YWGGQLVTIV
SSASTKGPST FPFLAPSSKST SGGAAGLCLV KDLYFPSEPVT VSWSGALTTS GVTQFAPAVLQ SGLYLSLSS
VTVPSSSLGTL QYICNVPNHK PSNTKVDRKVR EPKSCDKHTT CPPCPAPELL GGPSVFLLFP KPKDTLIMSR
TPEVTGADV VSHEDPEVKF NWYVQGVEVH NAKTPEREQQ YNASTYRVVSV LTVLHQDLNLN GKEYKCKVSN
KALPAPIETIK ISAKAQQPER EQYVTLPFSR EEMTNKQVSL TCLVKGFFPS DIAVEMESNG QPENNYKTP
PVLDSDGFF LYSKLTVDKS RWWQGQVFSC SVMHEALNH YTQQKLSSLP G(K)

IgG<sub>M</sub> heavy chain
MKHLWFLLLL VAAPRWVLSDQ VQLQSGPGL VKFQTLSLTLT CAISSGVSS NSAANNIRQ SPGRGELWG
RTYRYSKWN DYADSVKQRI TINPDTSQKQ FLQLNSVTP EDTAVYYCAR SYFISFFSD YWGGQLVTIV
SSASTKGPST FPFLAPSSKST SGGAAGLCLV KDLYFPSEPVT VSWSGALTTS GVTQFAPAVLQ SGLYLSLSS
VTVPSSSLGTL QYICNVPNHK PSNTKVDRKVR EPKSCDKHTT CPPCPAPELL GGPSVFLLFP KPKDTLIMSR
TPEVTGADV VSHEDPEVKF NWYVQGVEVH NAKTPEREQQ YNASTYRVVSV LTVLHQDLNLN GKEYKCKVSN
KALPAPIETIK ISAKAQQPER EQYVTLPFSR EEMTNKQVSL TCLVKGFFPS DIAVEMESNG QPENNYKTP
PVLDSDGFF LYSKLTVDKS RWWQGQVFSC SVMHEALNH YTQQKLSSLP G(K)

Fab<sub>WT</sub> heavy chain
MKHLWFLLL VAAPRWVLSDQ VQLQSGPGL VKFQTLSLTLT CAISSGVSS NSAANNIRQ SPGRGELWG
RTYRYSKWN DYADSVKQRI TINPDTSQKQ FLQLNSVTP EDTAVYYCAR SYFISFFSD YWGGQLVTIV
SSASTKGPST FPFLAPSSKST SGGAAGLCLV KDLYFPSEPVT VSWSGALTTS GVTQFAPAVLQ SGLYLSLSS
VTVPSSSLGTL QYICNVPNHK PSNTKVDRKVR EPKSCDKHTT EQKLISEEDEL NSAVFILSHNM I

Fab<sub>M</sub> heavy chain
MKHLWFLLLL VAAPRWVLSDQ VQLQSGPGL VKFQTLSLTLT CAISSGVSS NSAANNIRQ SPGRGELWG
RTYRYSKWN DYADSVKQRI TINPDTSQKQ FLQLNSVTP EDTAVYYCAR SYFISFFSD YWGGQLVTIV
SSASTKGPST FPFLAPSSKST SGGAAGLCLV KDLYFPSEPVT VSWSGALTTS GVTQFAPAVLQ SGLYLSLSS
VTVPSSSLGTL QYICNVPNHK PSNTKVDRKVR EPKSCDKHTT EQKLISEEDEL NSAVFILSHNM I

lambda light chain
MAWALLLLLL LTTGQGNSWD IELQPQPSVS VAPGQTARIS CSGDLDKDY ASNYQKPKGQ APVLIYDSD
DRPSIGPERF GSNSGNTAT LTGQTAED EADYYCQYSYD SGFSTVGGG TLTVLFQQPK AAPSVTLFPP
SSEELQANKA TLVCLISDFY PGAVTVWKG DSSPVKAGVE TTTPKQSNN KYAASSLYL TPEQWKSHRS
YSCOVTHEGS TVKETVAPTE CS

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<sub>WT</sub> and in turquoise for the IgG<sub>M</sub> 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<sub>WT</sub> and the IgG<sub>M</sub> are 6.89 or 6.65, respectively, while those for the Fab<sub>WT</sub> and the Fab<sub>M</sub> 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. 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 FabWT (upper part) or FabM (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.** Temperature dependence of absorbance at 500 nm for IgG<sub>WT</sub> (A) and IgG<sub>M</sub> (B) in the presence of studied additives: PBS (black), sorbitol (blue), sucrose (cyan), trehalose (green), NaClO<sub>4</sub> (magenta), NaCl (red), Na<sub>2</sub>SO<sub>4</sub> (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 IgGWT (A) and IgGM (B) in the presence of studied additives: PBS (black), sorbitol (blue), sucrose (cyan), trehalose (green), NaClO₄ (magenta), NaCl (red), Na₂SO₄ (yellow), arginine (dark blue), aarcosine (dark cyan), betaine (dark green), and TMAO (dark magenta).
Figure S6.

Figure S6. Correlation of onset temperatures, $T_{\text{onset}}$, and aggregation temperatures, $T_{\text{agg}}$, for IgG$_{\text{WT}}$ (white circles) and IgG$_{\text{M}}$ (black circles). The temperatures are listed in Table S3.
**Table S1.** Fitting parameters for thermal transitions of FabWT fragment and IgGWT

|         | T1 (°C) | ΔH1 (kJ/mol) | T2 (°C) | ΔH2 (kJ/mol) | T1/2, 37°C (x factor) | ΔH3 (kJ/mol) | T3 (°C) | E3 (kJ/mol) | ΔH3 (kJ/mol) | R²  |
|---------|---------|--------------|---------|--------------|---------------------|--------------|---------|-------------|--------------|-----|
| PBS     |         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 75.1    | 443          | 1                   | 1273         | -       | -           | -            | 0.9893 |
| IgGWT   | 69.3    | ±0.1         | 565     | ±4           | 75.3                | 295          | ±2      | ~0.002      | 1539         | ±0.1  |
|         |         |              |         |              |                     |              |         |             |              | 0.9977 |
| Sorbitol|         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 77.5    | 476          | ~10                 | 1908         | -       | -           | -            | 0.9911 |
| IgGWT   | 73.0    | ±0.1         | 703     | ±15          | 78.4                | 293          | ±10     | ~0.004      | 1738         | ±23   |
|         |         |              |         |              |                     |              |         |             |              | 0.9976 |
| Sucrose |         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 78.6    | 482          | ~30                 | 1670         | -       | -           | -            | 0.9904 |
| IgGWT   | 74.3    | ±0.1         | 681     | ±5           | 79.5                | 279          | ±3      | ~0.003      | 1544         | ±7   |
|         |         |              |         |              |                     |              |         |             |              | 0.9998 |
| Trehalose|         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 79.5    | 495          | ~80                 | 2094         | -       | -           | -            | 0.9920 |
| IgGWT   | 74.2    | ±0.1         | 680     | ±5           | 79.7                | 292          | ±3      | ~0.006      | 1786         | ±7   |
|         |         |              |         |              |                     |              |         |             |              | 0.9998 |
| NaClO4  |         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 71.5    | 353          | ~0.01               | 1623         | -       | -           | -            | 0.9984 |
| IgGWT   | 56.2    | ±0.1         | 428     | ±3           | 71.1                | 341          | ±2      | ~0.003      | 1502         | ±29   |
|         |         |              |         |              |                     |              |         |             |              | 0.9977 |
| NaCl    |         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 79.3    | 422          | ~2                  | 1784         | -       | -           | -            | 0.9961 |
| IgGWT   | 69.4    | ±0.1         | 623     | ±2           | 79.2                | 377          | ±1      | ~0.3        | 1664         | ±8   |
|         |         |              |         |              |                     |              |         |             |              | 0.9996 |
| Na2SO4  |         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 85.8    | 427          | ~40                 | 1826         | -       | -           | -            | 0.9961 |
| IgGWT   | 72.4    | ±0.1         | 570     | ±3           | 83.5                | 349          | ±3      | ~0.3        | 1660         | ±50   |
|         |         |              |         |              |                     |              |         |             |              | 0.9993 |
| Arginine|         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 78.1    | 375          | ~0.2                | 1923         | -       | -           | -            | 0.9999 |
| IgGWT   | 65.5    | ±0.1         | 545     | ±2           | 77.7                | 367          | ±2      | ~0.1        | 1551         | ±12   |
|         |         |              |         |              |                     |              |         |             |              | 0.9984 |
| Sarcosine|        |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 79.1    | 494          | ~60                 | 1953         | -       | -           | -            | 0.9927 |
| IgGWT   | 72.8    | ±0.1         | 640     | ±4           | 79.8                | 317          | ±2      | ~0.02       | 1747         | ±8   |
|         |         |              |         |              |                     |              |         |             |              | 0.9997 |
| Betaine |         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 77.4    | 446          | ~3                  | 1777         | -       | -           | -            | 0.9933 |
| IgGWT   | 71.9    | ±0.1         | 668     | ±4           | 78.2                | 289          | ±3      | ~0.003      | 1598         | ±7   |
|         |         |              |         |              |                     |              |         |             |              | 0.9997 |
| TMAO    |         |              |         |              |                     |              |         |             |              |     |
| FabWT   | -       | -            | 78.0    | 471          | ~10                 | 1691         | -       | -           | -            | 0.9928 |
| IgGWT   | 73.0    | ±0.1         | 656     | ±3           | 79.2                | 276          | ±3      | ~0.002      | 1355         | ±6   |

Fitting parameters for thermal transitions of FabWT fragment and IgGWT 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 T1/2 is related to the calculated shelf-life of the FabWT at 37°C, which is set to 1. The changes in T2, E2, and T1/2 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 T2, 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 $E_a$, 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 FabM fragment and IgG_M

|                | T1 (°C) | ΔH1 (kJ/mol) | T2 (°C) | ΔH2 (kJ/mol/x factor) | T12,37°C (°C) | ΔH3 (kJ/mol) | R²   |
|----------------|---------|--------------|---------|------------------------|---------------|--------------|------|
| PBS FabM       | -       | -            | 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±5 | 338±4 | 0.9994 |
| Sorbitol FabM  | -       | -            | 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±5 | 347±10 | 0.9988 |
| Sucrose FabM   | -       | -            | 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±5 | 347±8 | 0.9990 |
| Trehalose FabM | -       | -            | 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±7 | 331±7 | 0.9993 |
| NaClO4 FabM    | -       | -            | 72.5±0.1| 407±3                 | ~1x10⁵        | 1279±7       | -    | -    | 0.9931 |
| IgG_M          | 56.1±0.1| 403±4        | 71.3±0.2| 398±4                 | ~5x10⁶        | 1224±116     | 75.5±39 | 303±114 | 0.9953 |
| NaCl FabM      | -       | -            | 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±22 | 280±22 | 0.9969 |
| Na₂SO₄ FabM    | -       | -            | 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±86 | 283±207 | 0.9962 |
| Arginine FabM  | -       | -            | 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±27 | 279±25 | 0.9973 |
| Sarcosine FabM | -       | -            | 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±15 | 357±13 | 0.9981 |
| Betaine FabM   | -       | -            | 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±11 | 354±11 | 0.9979 |
| TMAO FabM      | -       | -            | 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±14 | 345±14 | 0.9981 |

Fitting parameters for thermal transitions of FabM 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 τ_{1/2} is related to the calculated shelf-life of the FabM at 37°C. The changes in T2', Eα2, and τ_{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 T2', 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<sub>WT</sub> and IgG<sub>M</sub>

| IgG<sub>WT</sub> | T<sub>onset</sub> (<°C>) | T<sub>agg</sub> (<°C>) | log(τ<sub>rel,IgG</sub> / τ<sub>rel,Fab</sub>) ** | log[k<sub>2K/(1+K)</sub>] |
|-----------------|------------------|------------------|-------------------|-------------------|
| 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<sub>4</sub> | 50.5             | -                | -3.04             | -9.88             |
| NaCl            | 63.5             | 76.0             | -1.35             | -17.53            |
| Na<sub>2</sub>SO<sub>4</sub> | 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<sub>M</sub> | T<sub>onset</sub> (<°C>) | T<sub>agg</sub> (<°C>) | log(τ<sub>rel,IgG</sub> / τ<sub>rel,Fab</sub>) ** | log[k<sub>2K/(1+K)</sub>] |
|-----------------|------------------|------------------|-------------------|-------------------|
| 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<sub>4</sub> | 50.5             | -                | -5.60             | -10.63            |
| NaCl            | 64.0             | 76.0             | -2.30             | -19.51            |
| Na<sub>2</sub>SO<sub>4</sub> | 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<sub>onset</sub> and T<sub>agg</sub> is estimated to be ±0.5 °C.
** The parameter τ<sub>rel,IgG</sub> <sup>τ<sub>rel,IgG</sub> / τ<sub>rel,Fab</sub></sup> <sup>τ<sub>rel,Fab</sub></sup> is expressed in logarithmic form.