Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Fluorescence dye-based detection of mAb aggregates in CHO culture supernatants

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Fig. S1 Different extrinsic fluorescence dyes used for analysis of mAb aggregates in acetate buffer. MAb aggregates were induced with 500 mmol L$^{-1}$ NaCl using a mAb concentration of 1 mg mL$^{-1}$. Fluorescence intensities of NaCl-stressed samples were normalized to the corresponding unstressed control for each dye. ANS (50 µmol L$^{-1}$), Bis-ANS (2 µmol L$^{-1}$), thioflavin T (50 µmol L$^{-1}$) and Congo Red (20 µmol L$^{-1}$) were used for analysis of mAb aggregates. Analysis was performed on a SpectraMax M5e microplate reader (Molecular Devices) with excitation at 410 nm, emission at 490 nm, and a cut-off filter of 475 nm. Experiments were performed in replicates (n = 2 ± SD). *P<0.05, **P<0.01, ***P<0.001 for a two-tailed t-test. P-values were calculated by comparison to unstressed mAb.
Fig. S2 ThT concentration dependency of mAb aggregate detection. Different thioflavin T concentrations were used to detect 0.25 mg mL\(^{-1}\) mAb induced by pH 5/65°C in SFM4CHO cell culture medium (a) or 1 mg mL\(^{-1}\) mAb induced by 0.5 mol L\(^{-1}\) NaCl in 20 mmol L\(^{-1}\) acetate buffer (b). Experiments were performed in replicates (n = 3 ± SD)