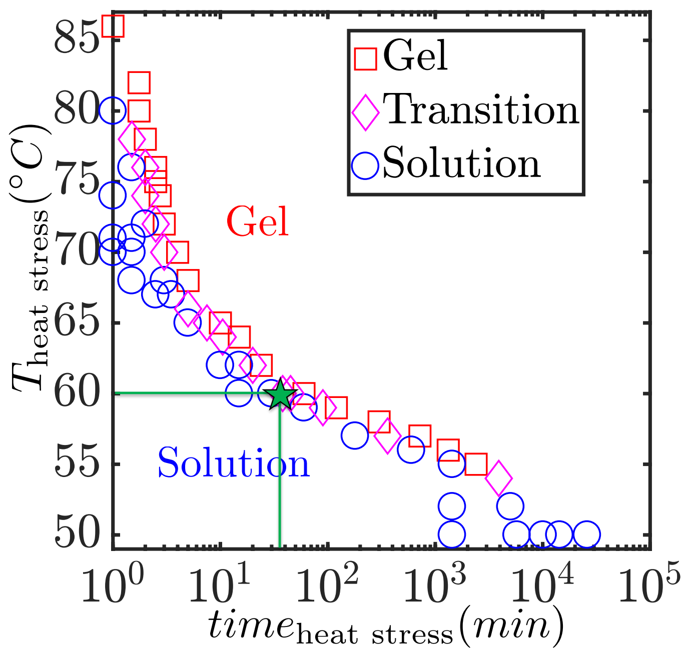
Clogging of microfluidic constrictions by monoclonal antibody aggregates: role of aggregate shape and deformability - Supplementary Information

Charles Duchêne,[1] Vasco Filipe,[2] Sylvain Huille,[2] and Anke Lindner[1,*]

[1] *PMMH - ESPCI, UMR 7636 CNRS, PSL Research University, Sorbonne University, University Paris Diderot, 7-9 quai Saint-Bernard, 75005 Paris, France*

[2] *Sanofi Biopharmaceutics Development, Impasse des Ateliers, 94400 Vitry-sur-Seine, France.*

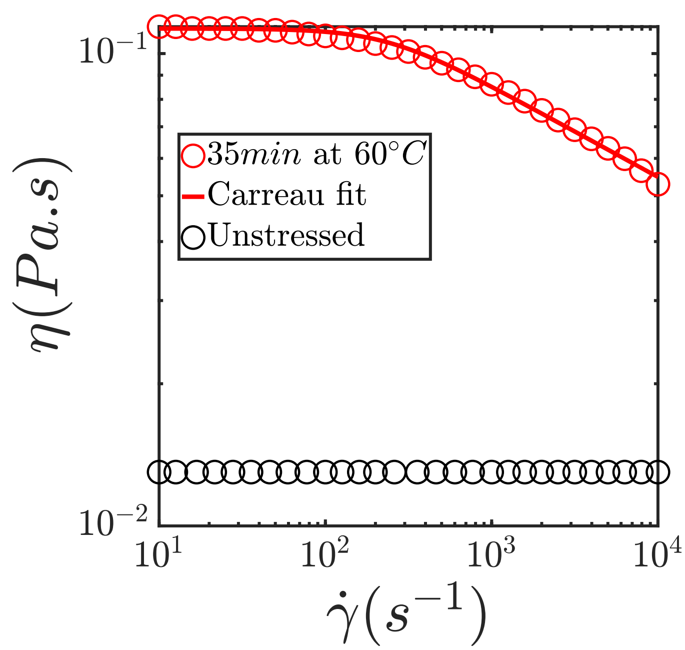
I. HEAT STRESS CONDITIONS FOR AGGREGATE FORMATION



Supplementary Figure 1. Temperature of heat stress $T_{\text{heat stress}}$ as a function of time. Blue circles represent solutions with no visible differences with the unheated solution, magenta diamonds correspond to the appearance of a large number of aggregates visible by eye and a transition towards a gel like structure, represented by red circles. The optimal condition (35min at 60°C) is highlighted with a green star.

A specific temperature and duration has been chosen ($T = 60$°C for 35min) for the heat stress applied to the mAb solution. These conditions were determined after many tests at different temperatures (between 50°C and 80°C) and for different durations (between 1min and 2.16×10^4min = 15 days). During the heating process (Supplementary Fig. 1) we observed the solution visually and also preformed rheological tests. For short heating or heating at low temperature, no qualitative changes can be observed in the solution (blue circles). However at a given moment very large aggregates, visible by eye, appear (magenta diamonds) and a transition towards a gel like structure (red circles) is observed. Heating at 60°C for 35min has been chosen, as the heating time is not too long and the applied temperature remains below the melting temperature $T_m = 68$°C for our mAb. As shown on Fig. 1 (a) in the Main Document, a sufficient number of large aggregates is created under these conditions.

* anke.lindner@espci.fr



Supplementary Figure 2. Viscosity η as a function of shear-rate $\dot{\gamma}$: unstressed solution ($\circ$) solution stressed for 35min at 60°C ($\circ$) with $T = 25$°C. Carreau fit (–) for the stressed solution: $\eta = \frac{\eta_0}{(1+(\tau\dot{\gamma})^2)^m}$ with $\eta_0 = 0.113$Pa.s, $\tau = -4.2 \times 10^{-3}$s and $m = 0.096$.

II. RHEOLOGICAL PROPERTIES

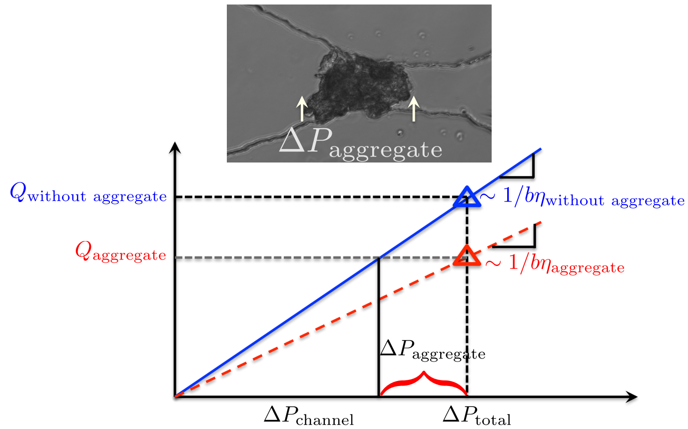
The rheological properties of the mAb solutions have been measured before and after heat stress using a cone plate geometry (CP 60-1) on a rheometer Physica MCR 501, from Anton Paar. During heating the protein conformation is changed and the hydrophobic heart opened, leading to attractive reactions between proteins and aggregate formation [1]. This structure change also influences the viscosity of the protein solution as seen on Supplementary Fig. 2 displaying the viscosity η as a function of the shear-rate $\dot{\gamma}$ for a solution before and after heat stress (35min at 60°C). During heating the viscosity at small shear rates is increased by nearly an order of magnitude. The heated solutions show shear-thinning for shear rates above 100 s^{-1} and the viscosity can be adjusted with the Carreau model: $\eta = \frac{\eta_0}{(1+(\tau\dot{\gamma})^2)^m}$ with $\eta_0 = 0.113$Pa.s, $\tau = -4.2\times10^{-3}$s and $m = 0.096$. Note that typical the shear-rates in the microchannel are larger than 10^3 s^{-1} and viscosities occurring in the microfluidic experiments are thus always below 0.1Pa.s.

III. PRESSURE DIFFERENCE AROUND A CLOG

The pressure difference around an aggregate $\Delta P_{\text{aggregate}}$ clogging a constriction is calculated from the total pressure difference ΔP_{total} and the pressure drop occurring along the microchannel $\Delta P_{channel}$. The total pressure difference ΔP_{total} is imposed by means of the pressure controller and the flow-rate Q is measured at the outlet of the microchannel in the presence Q_{clog} or absence of a clog Q_0. The device has a hydrodynamic resistance $R_h = \Delta P_{\text{total}}/Q_0$ [2], where $R_h = \eta C_h$. For a non-zero flow rate Q_{clog}, a pressure drop will thus occur along the microchannel also in the presence of an aggregate. This pressure drop along the channel can be quantified as

$$\begin{aligned}\Delta P_{\text{channel}} &= \eta(Q_{clog})\, C_h\, Q_{clog} \\ &= \Delta P_{total} \frac{Q_{clog}}{Q_0} \frac{\eta(Q_{clog})}{\eta(Q_0)}\end{aligned} \tag{1}$$

Note that the correction for the viscosity with and without aggregate becomes necessary due to the shear thinning character leading to a change in viscosity for different flow rates.



Supplementary Figure 3. Schematic explanation of the relevant pressure in a clogging situation. The pressure difference applied to the aggregate $\Delta P_{\text{aggregate}}$ can be calculated as the difference between the total applied pressure and the pressure drop occurring along the micro-channel (see section III). The photography above the graph represents the pressure drop $\Delta P_{\text{aggregate}}$.

The pressure at the aggregate is then obtained as $\Delta P_{aggregate} = \Delta P_{\text{total}} - \Delta_{channel}$.

[1] W. Wang, S. Nema and D. Teagarden, *International Journal of Pharmaceutics*, 2010, **390**, 89–99.
[2] P. Tabeling, *Introduction à la microfluidique*, Belin, 2003.