**Supplementary Information for “Rapid mixing of sub-microliter drops by magnetic micro-stirring”**

**Estimate of temperature increase of drop due to viscous dissipation during mixing**

The rotating external magnetic field provides energy and does work to rotate the stir bar inside the drop, against the drag force. First the drag force $F_d$ is estimated, and from it the viscous energy dissipated. The drag equation:

$$F_d = \frac{1}{2} \rho v^2 C_D A$$

$\rho$ is the density of the fluid (1000 kg/m$^3$ for water or an aqueous protein solution), $v$ the velocity of the moving bar, $C_D$ the drag coefficient and $A$ the reference area (projected area in the direction perpendicular to the velocity). For a ‘worst case’ (highest energy dissipated) we assume the bar is oriented with its largest surface area perpendicular to the flow. This results in the highest drag coefficient (about 2 for a long, flat plate, see ref. Hoerner). The spinning speed is 1000 rpm and the bar is estimated to move around a circular trajectory inside the drop of radius 400 µm, giving its velocity $v = 2\pi(1000/60s) \times 400\mu m = 0.042$ m/s.

This results in a drag force

$$F_d = \frac{1}{2} \times 1000 \text{kg/m}^3 \times (0.042 \text{m/s})^2 \times 2 \times (400 \times 200 \times 10^{-12} \text{ m}^2) = 141\text{nN}$$

The total work $W$ done during 1 second of mixing (1000/60= 16.7 revolutions happen during that time) is then:

$$W = 2\pi \times 400 \mu m \times 1.4\mu N \times 16.7 = 5.9\text{nJ}$$

If we assume all this work is converted into heat, the resulting temperature increase $\Delta T$ in a hemispherical drop of 500nl is

$$\Delta T = \frac{5.9\text{nJ}}{500 \times 10^{-9} \text{ kg} \times 4184 \frac{\text{J}}{\text{kgK}}} = 3\ \text{µK}$$

It is then reasonable to state that, using a ‘safety factor’ of 10 (accounting for the imperfections of this simplified model), an upper limit for the estimated temperature increase is about 30 µK.

**References:**
**Calculation of protein bound to surface of stir bar**

Each stir bar is a rectangular box with a surface area of $1.78 \times 10^{-7} \text{ m}^2$ (thickness = $1.5 \times 10^{-5} \text{ m}$, width = $2 \times 10^{-4} \text{ m}$, length = $4 \times 10^{-4} \text{ m}$). Assuming the proteins are roughly spherical, the following maximum binding of a protein monolayer to the stir bar is obtained:

**Trypsin**

Diameter = $4.5 \times 10^{-9} \text{ m}$ (PDB: 3MFJ), circular area = $\pi (2.25 \times 10^{-9} \text{ m})^2 = 1.59 \times 10^{-17} \text{ m}^2$. Assuming the densest packing of equal diameter circles in a plane (Weisstein), the moles of trypsin bound would be

$$\frac{1}{6} \pi \sqrt{3} \times \frac{\left(1.78 \times 10^{-7} \text{ m}^2\right)}{1.59 \times 10^{-17} \text{ m}^2} = 1.69 \times 10^{-14} \text{ mol}$$

For a 500 nl reaction, the concentration bound is

$$\frac{1.69 \times 10^{-14} \text{ mol}}{5 \times 10^{-7} \text{ l}} = 3.37 \times 10^{-8} \text{ M}$$

**Human serum albumin**

Diameter = $6.5 \times 10^{-9} \text{ m}$ (PDB: 1BM0), circular area = $\pi (3.25 \times 10^{-9} \text{ m})^2 = 3.32 \times 10^{-17} \text{ m}^2$. Assuming the densest packing of equal diameter circles, the moles of HSA bound would be

$$\frac{1}{6} \pi \sqrt{3} \times \frac{\left(1.78 \times 10^{-7} \text{ m}^2\right)}{3.32 \times 10^{-17} \text{ m}^2} = 8.08 \times 10^{-15} \text{ mol}$$

For a 500 nl reaction, the concentration bound is

$$\frac{8.08 \times 10^{-15} \text{ mol}}{5 \times 10^{-7} \text{ l}} = 1.62 \times 10^{-8} \text{ M}$$

Reference: