Electronic Supplementary Material (ESI) for Analytical Methods.

A handheld orbital mixer for processing viscous samples in low resource settings

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Supplemental Material

S1. Nomenclature

t	Time (s)
$C_{HFITC,b}$	Concentration of His-FITC in bulk solution (mol/m^3)
$C_{HFITC,s}$	Concentration of unbound His-FITC on the bead surface (mol/m^3)
$C_{HFITC \cdot NiNTA}$	Concentration of His-FITC bound to NiNTA (mol/m^3)
C_{NiNTA}	Concentration of free NiNTA binding sites (mol/m^3)
$C_{HFITC,s}^{QSS}$	Quasi-steady state concentration of unbound His-FITC on the bead surface (mol/m^3)
V	Total sample volume (m^3)
k_m	Mass transfer coefficient (m/s)
a_i	Total bead surface area (m^2)
k_{on}	Kinetic on-rate (m^3/mol*s)
k_{off}	Kinetic off-rate (1/s)

S2. Mass Transfer Model Derivation

The system of interest has four biochemical species: poly-His FITC (HFITC) in the bulk fluid, unbound HFITC on the surface of the magnetic beads, nickel(II) nitrilotriacetic acid (NiNTA), and HFITC bound to NiNTA. Performing a species mole balance on these four species results in three coupled differential equations, and one algebraic expression (eqs. (S1)-(S4)).

$$V\frac{dC_{HFITC,b}}{dt} = -k_m a_i\left(C_{HFITC,b} - C_{HFITC,s}\right) \tag{S1}$$

$$V\frac{dC_{HFITC,s}}{dt} = k_m a_i\left(C_{HFITC,b} - C_{HFITC,s}\right) - V\left(k_{on}C_{HFITC,s}C_{NiNTA} - k_{off}C_{HFITC\cdot NiNTA}\right) \tag{S2}$$

$$V\frac{dC_{HFITC\cdot NiNTA}}{dt} = V\left(k_{on}C_{HFITC,s}C_{NiNTA} - k_{off}C_{HFITC\cdot NiNTA}\right) \tag{S3}$$

$$C_{NiNTA}(t) = C_{NiNTA}(0) - C_{HFITC\cdot NiNTA}(t) \tag{S4}$$

To simplify the model, we make the following assumptions:

1. The beads and the HFITC are homogenously dispersed in solution
2. Unbound HFITC on the surface of the beads is at quasi-steady state
3. The amount of NiNTA on the beads is in a large enough excess that the concentration of NiNTA remains a constant

While Assumption (1) is dependent on the quality of mixing, it is a reasonable assumption after inspection of the high-speed video shown in Supporting Material. Assumption (2) will depend on the mass transfer rate relative to the kinetic binding and dissociation rates. Under most circumstances there will be an initial transient period, but the unbound surface concentration will reach a steady-state value. With a known volume of beads added and the approximate binding capacity supplied by the manufacturer (up to 70 mg His-tagged protein/mL bead stock), the NiNTA concentration is in large enough excess for Assumption (3) to be valid.

After making these asssumptions, equations (S1)-(S4) can be simplified to:

$$\frac{dC_{HFITC,b}}{dt} = -\frac{k_m a_i}{V}\left(C_{HFITC,b} - C_{HFITC,s}^{QSS}\right) \tag{S5}$$

$$\frac{dC_{HFITC \cdot NiNTA}}{dt} = \left(k_{on} C_{HFITC,s}^{QSS} C_{NiNTA}^{0} - k_{off} C_{HFITC \cdot NiNTA}\right) \tag{S6}$$

$$C_{HFITC,s}^{QSS} = \frac{\frac{k_m a_i C_{HFITC,b}}{V} + k_{off} C_{HFITC \cdot NiNTA}}{\frac{k_m a_i}{V} + k_{on} C_{NiNTA}^{0}} . \tag{S7}$$

Equations (S5)-(S7) are now linear, and can be analytically solved:

$$C_{HFITC,b}(t) = \frac{V k_{off}}{k_m a_i \sigma_1} + \frac{V k_{on} C_{NiNTA}^{0} e^{\frac{-k_m a_i \sigma_1 t}{V\left(\frac{V k_{on} C_{NiNTA}^{0}}{k_m a_i} + 1\right)}}}{k_m a_i \sigma_1} \tag{S8}$$

$$\sigma_1 = \frac{V k_{off}}{k_m a_i} + \frac{V k_{on} C_{NiNTA}^{0}}{k_m a_i} \tag{S9}$$

$$C_{HFITC \cdot NiNTA}(t) = \frac{V k_{on} C_{NiNTA}^{0} C_{HFITC}^{0}}{k_m a_i \sigma_2} - \frac{V k_{on} C_{NiNTA}^{0} C_{HFITC}^{0} e^{\frac{-k_m a_i \sigma_2 t}{V\left(\frac{V k_{on} C_{NiNTA}^{0} C_{HFITC}^{0}}{k_m a_i} + 1\right)}}}{k_m a_i \sigma_2} \tag{S10}$$

$$\sigma_2 = \frac{V k_{off}}{k_m a_i} + \frac{V k_{on} C_{NiNTA}^{0} C_{HFITC}^{0}}{k_m a_i} . \tag{S11}$$

S3. Mass Transfer Model Parameters

A table of parameters used to fit k_m to equation (S8) is shown in Table S1.

Table 1. Model parameters.

Parameter	**Value (units)**
C_{NiNTA}^{0}	10.0017 (mol/m^3)
a_i	0.0036 (m^2)
C_{HFITC}^{0}	0.166 (mol/m^3)
V	1.05E-7 (m$^{3)}$
k_{on}	1e4 (m^3/mol*s)

k_{off} 1.4e-4 (1/s)

An approximate $k_{on}$and k_{off} were estimated from the K_D value of 1.4e-8 M, for a hexa-histidine tag binding to a NiNTA sensor (from Knecht et al[1]). Surface area and ligand concentration were estimated from manufacturer specifications.

S4. Mass Transfer Model Results

The data shown in Fig. 4 is presented again with the fit from the mass transfer model solution given by eq. (S8) (Figure S1). The model agrees qualitatively for each viscosity. While the model consistently undershoots the steady-state (infinite time) solution, this can be attributed primarily to the estimation of unknown parameters: ligand concentration, interfacial area, and on-/off-rates. Inspection of eq. (S8) shows an additive constant term that, in the limit of $t \to \infty$, would underestimate the experimental data. More accurate approximations of these parameters would be expected to result in a better fit.

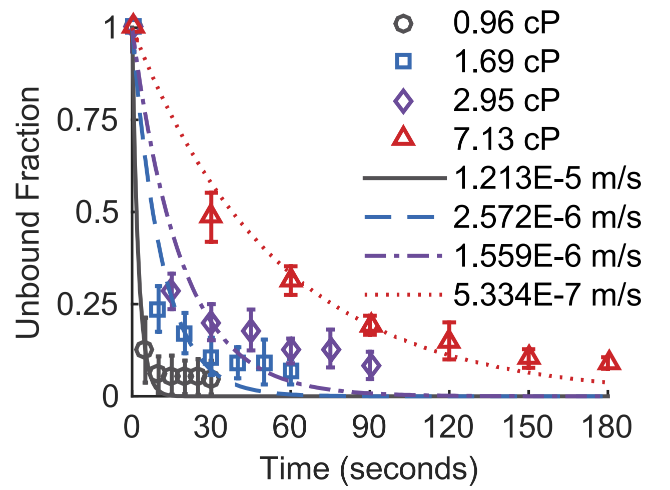

Figure S1. The experimental mixing data (mean +/- standard deviation, n=3) with the model fit from eq. (S8).

S5. References

1. Knecht, S., D. Ricklin, A. N. Eberle and B. Ernst. Oligohis-tags: Mechanisms of binding to ni2+-nta surfaces. *J. Mol. Recognit.* 22:270-279, 2009.