Label-free microscale thermophoresis for the study of lactoferrin-drug interaction

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Abstract

In this study, a microscale thermophoretic (MST) method was developed to investigate lactoferrin-drug interaction using label-free system which depends on the intrinsic fluorescence of one interacting partner. The interaction between lactoferrin and the iron chelator deferiprone was first investigated to evaluate the excretion of deferiprone into mother milk during lactation. Furthermore, the interaction between lactoferrin and amphotericin B was investigated for possible explanation of the observed synergistic effect as antifungal effect. The experiments were performed on Monolith NT.115 LabelFree® (NanoTemper Technologies, Munich, Germany). Different concentrations of the intended drugs in the range of 0.007- 250 μM were titrated against 120 nM fixed concentration of lactoferrin which was dissolved in 0.1 M tris buffer at pH 7.4. The measurements were performed using standard capillaries at 20% excitation power and medium MST power. The results indicated a significant interaction between lactoferrin and deferiprone however, no significant interaction between lactoferrin and amphotericin B was observed. The data were analyzed using NT Analysis software which was provided by NanoTemper Technologies. The estimated binding constant for lactoferrin-deferiprone interaction was (8.9 x 10 -6 ± 1.6 SD) which is reported for the first time. This significant binding between lactoferrin and deferiprone may potentiates the drug secretion into mother milk. The technique shows fast and simple approach to study protein-drug interaction without complicated labelling procedures.

Experimental

A. Instrument

The developed method was carried out using Monolith NT.115 LabelFree® microscale thermophoresis (NonoTemper technologies, Munich, Germany). Instrument principle and picture is shown in Figure 1.

B. Method

❖ All samples were prepared in 0.1 M Tris buffer and the pH was adjusted at 7.4.

❖ In each experimental set three different concentrations of each partner were prepared for MST scanning thereafter, fixed concentration of LF were selected according to the fluorescence signals in scanning test and serial concentrations of drug were prepared for measurements. All sample preparations summarized in Table 1.

❖ MST setting was summarized in Table 2.

Data analysis:

The used MST is supported with analysis software to fit the data curve with two different binding parameters K_D (binding constant) or EC50 from Hill equation.

Herein, K_D from low of mass action fit with the obtained data as follow:

$$A + T \leftrightarrow AT$$

$$F(C_T) = F_u + (F_b - F_u) \times \frac{C_{AT}}{C_A}$$

$$\text{Fraction bound} = \frac{1}{2CA} \times (C_T + C_A + K_d - \sqrt{(C_T + C_A + K_D)^2 - 4(C_T C_A)}$$

F_u Fluorescence in unbound state, F_b Fluorescence in bound state, K_D Dissociation constant, C_{AT} Concentration of complex, C_A Concentration of Molecule A (fluorescent) and C_T Concentration of titrated molecule

Results

❖ LF shows binding affinity to CP20 (Figure 2).

❖ LF-AmB thermophoretic curve shows no discrimination between bound and unbound molecules (Figure 3)

❖ Dissociation constant (KD) was estimated as summarized in Table 3.

Table 3. Calculated K_D constant with statistical parameters.

Fit Results (K_D)	
Fit Model:	K_D
Bound	857.596
Unbound	869.026
K_D	$8.9 \times 10^{-6} \pm 1.6 SD$
Target Conc.	0.000000121
K_D Confidence:	$\pm 4.1 \times 10^{-6}$

Conclusion

The observed interaction between LF and CP20 predicts drug excretion into mother milk as drug bounded fraction. No interaction was observed between LF and AmB under the used MST conditions. Thus, the synergistic effect of LF-AmB is not probably due to direct interaction between them. MST labelFree system provides valuable information for binding behavior and exhibit flexibility to study the molecular interactions without demand to extra labeling procedures.

Overview

Lactoferrin (LF) is known as a multifunctional protein which possess antimicrobial, antitumor, anti-inflammatory, protease inhibition and iron chelation effects [1]. LF is present in all biological fluids thus, it considered good protein target for interaction studies of drug pharmacokinetics, drug efficacy and drug design [2,3]. Deferiprone (CP20) -iron chelators- is highlighted in several reports for treatment of many disease states such as infectious diseases, cancers, neurodegenerative diseases [4,5]. In contrast, LF provides synergism with Amphotericin B (AmB) against several fungal species. However, the synergism is attributed to protein-drug interaction which elaborate specific properties for antifungal activity[6,7]. For this purpose, two different approaches were carried out to investigate LF-drug interaction. First approach is study the interaction between LF and CP20 to predict the drug excretion into mother milk as protein bounded fraction and second approach to investigate the interaction between LF and AmB using label-free microscale thermophoresis (MST). The technique is used to measure the molecular interactions through temperature gradient in microscale size with no restriction to buffering system or immobilization procedures [8,9].
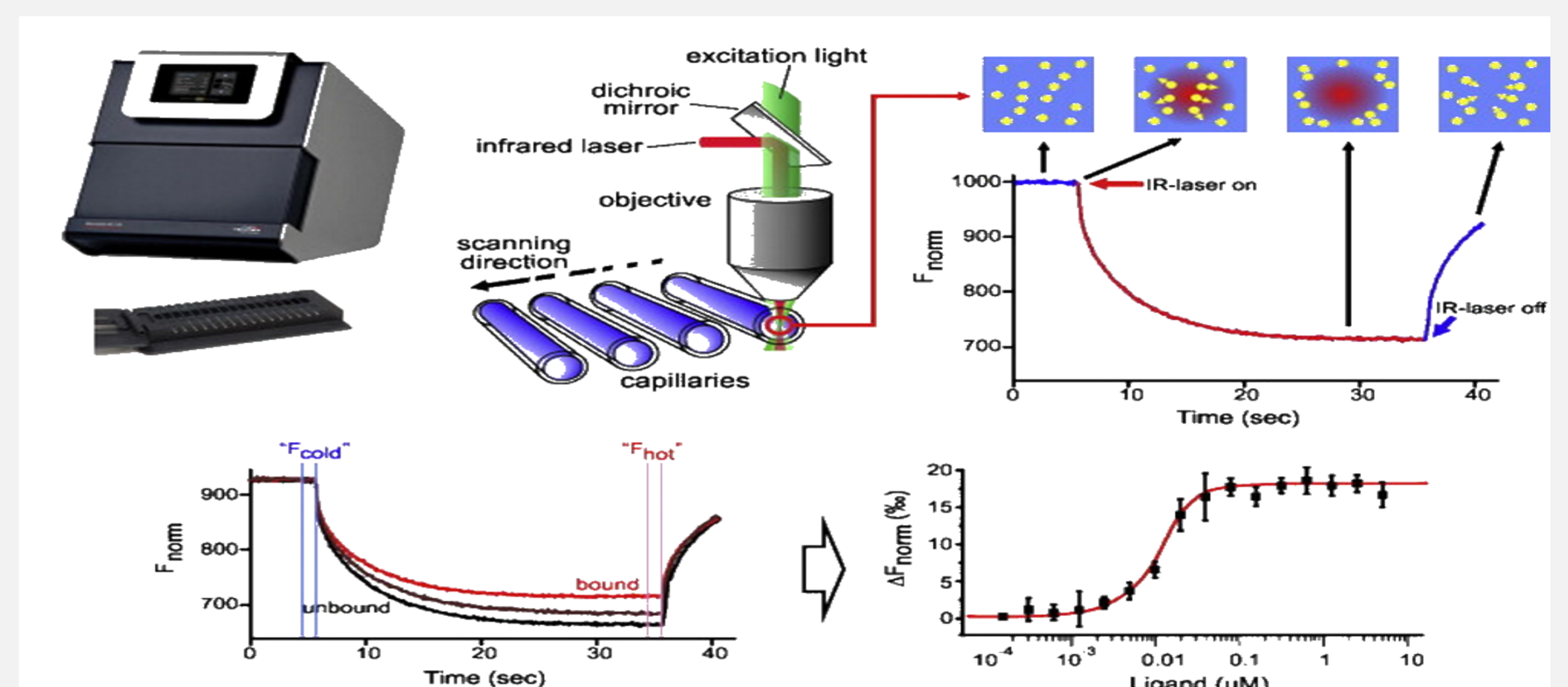

Figure 1. Principle of MST

Table 1. Sample preparation for different experimental sets.

	MST scanning	MST measurements
LF (target)	60, 120, 240 nM	Fixed at: 120 nM
CP20 (ligand)	125, 250,500 nM	Range of: $0.007 - 250$ μM
AmB (ligand)	125, 250, 500 nM	Range of: $0.007 - 250$ μM
Tris Buffer (solvent)	0.1 M	-

Table 2. MST setting.

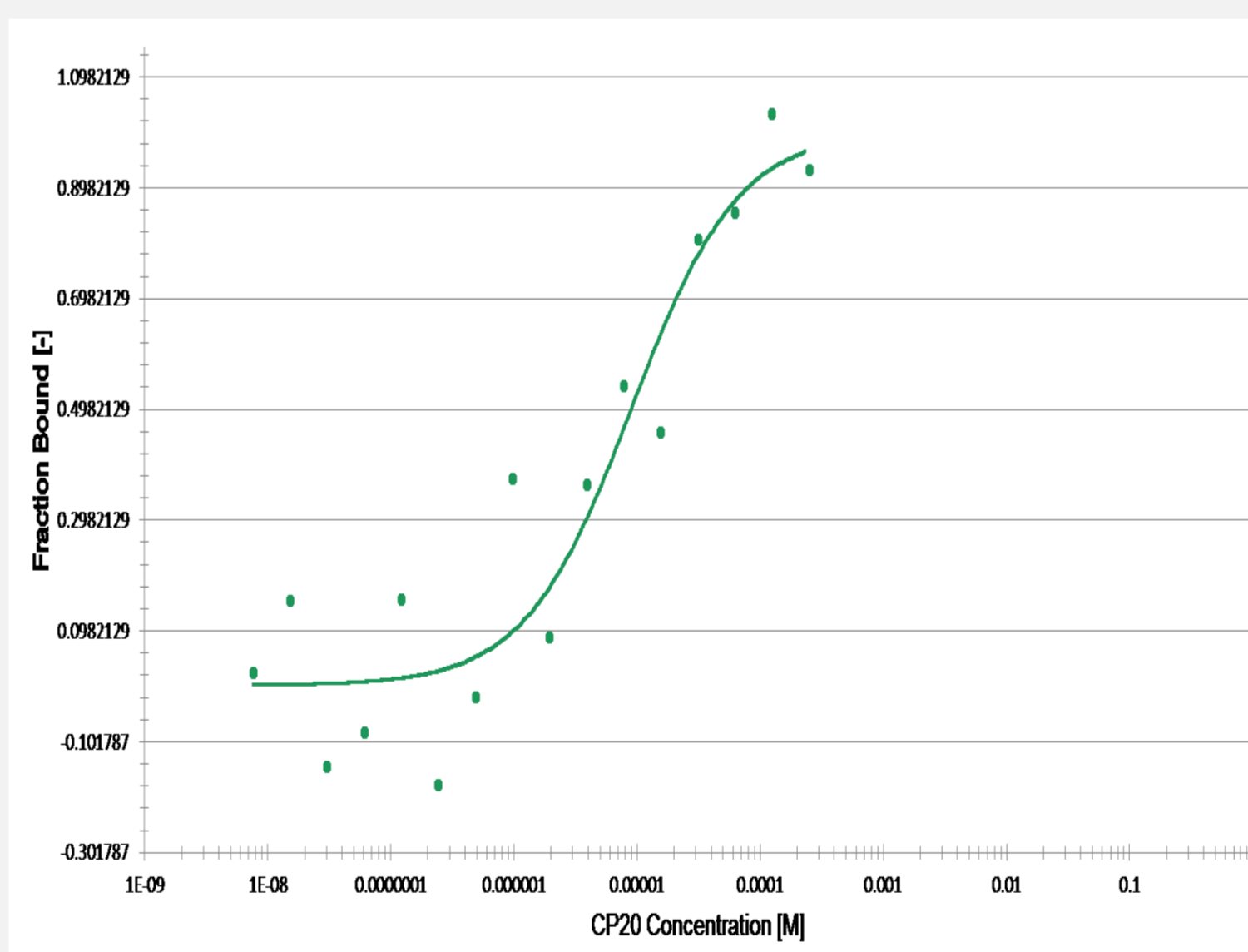| MST parameter	Set
MST-Power:	Medium
Excitation-Power:	20%
Excitation type:	Label-Free
Thermostat Setpoint:	(disabled)

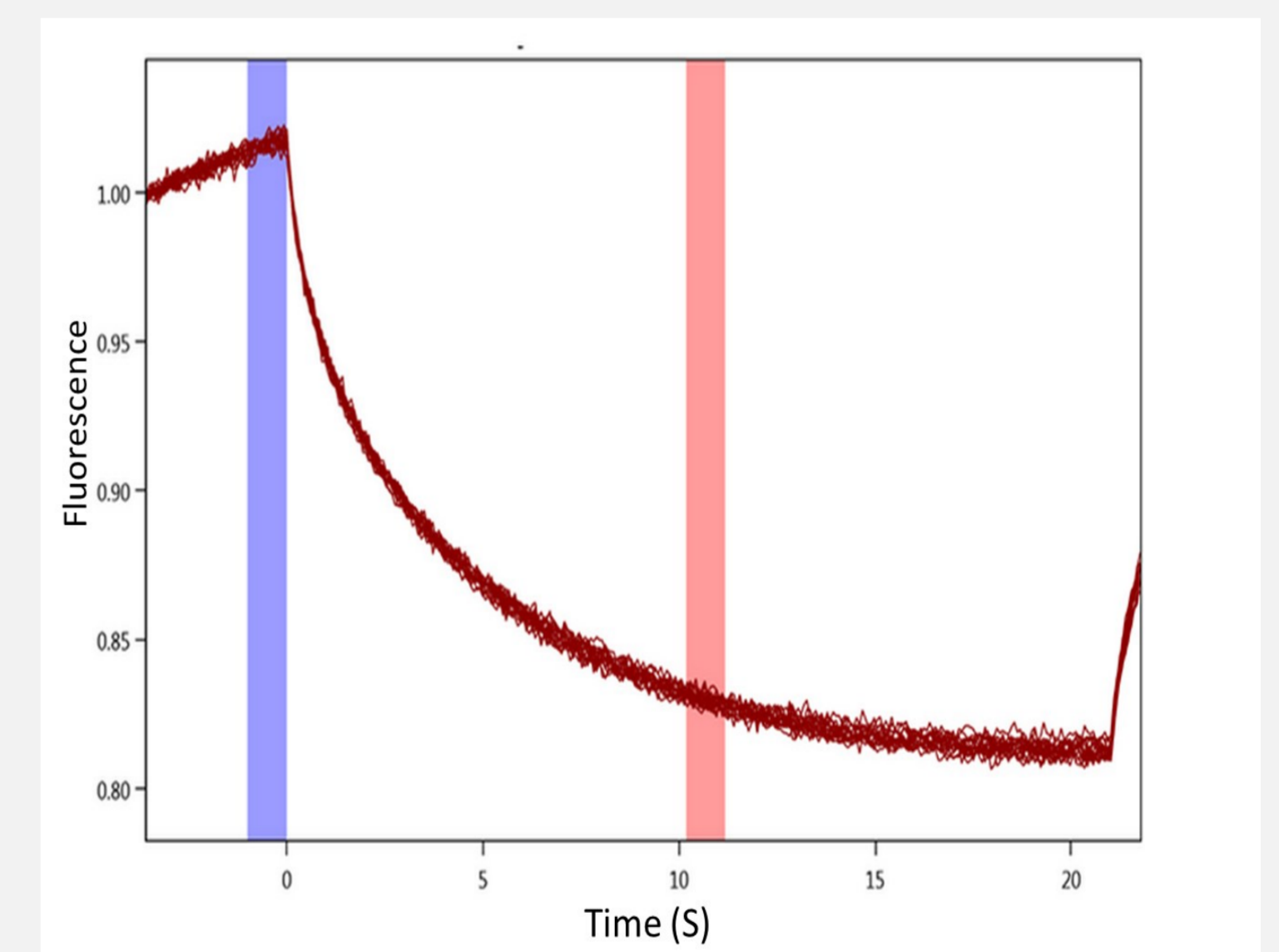
Figure 2. Binding curve for LF-CP20.

Figure 3. Thermophoretic curve for LF-AmB.

References

1. Farnaud, S. & Evans, R. W. Lactoferrin - A multifunctional protein with antimicrobial properties. *Mol. Immunol.* **40,** 395–405 (2003).
2. Guo, M., Lu, X., Wang, Y. & Brodelius, P. E. Comparison of the interaction between lactoferrin and isomeric drugs. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **173,** 593–607 (2017).
3. Talebi, R., Ahmadi, A., Afraz, F. & Abdoli, R. Parkinson's disease and lactoferrin: Analysis of dependent protein networks. *Gene Reports* **4,** 177–183 (2016).
4. Mounsey, R. B. & Teismann, P. Chelators in the treatment of iron accumulation in parkinson's disease. *Int. J. Cell Biol.* **2012,** (2012).
5. Kontoghiorghes, G. J. Iron mobilization from transferrin and non-transferrin-bound-iron by deferiprone. Implications in the treatment of thalassemia, anemia of chronic disease, cancer and other conditions. *Hemoglobin* **30,** 183–200 (2006).
6. Zarember, K. A., Cruz, A. R., Huang, C. Y. & Gallin, J. I. Antifungal activities of natural and synthetic iron chelators alone and in combination with azole and polyene antibiotics against Aspergillus fumigatus. *Antimicrob. Agents Chemother.* **53,** 2654–2656 (2009).
7. Lai, Y. W. *et al.* Synergy and antagonism between iron chelators and antifungal drugs in Cryptococcus. *Int. J. Antimicrob. Agents* **48,** 388–394 (2016).
8. Entzian, C. & Schubert, T. Studying small molecule-aptamer interactions using MicroScale Thermophoresis (MST). *Methods* **97,** 27–34 (2016).
9. Jerabek-Willemsen, M., Wienken, C. J., Braun, D., Baaske, P. & Duhr, S. Molecular interaction studies using microscale thermophoresis. *Assay Drug Dev. Technol.* 9, 342–353 (2011).