

## TECHNICAL NOTE

# Binding Analytics of an Aptamer to Small Molecule Determined by Microscale Thermophoresis

### INTRODUCTION

Aptamer development has historically been limited to one-target at a time. **Base Pair Biotechnologies** has addressed this severe limitation by successfully multiplexing the conventional aptamer selection process. Our platform technology is a patent pending approach to aptamer discovery that allows us to offer de novo aptamer discovery services at unprecedented speed and throughput. Our expertise in aptamer and related assay development allows us to support our customers in a wide range of novel applications.

Our discovery services include validation of aptamer binding by characterizing the aptamer:target dissociation constant (Kd) before delivery of aptamer materials to the customer for further testing. Analytical methods such as Surface Plasmon Resonance (SPR) and Biolayer Interferometry (BLI) are typically used for larger targets. Recently, however, we have explored and validated Microscale Thermophoresis (MST) developed by **NanoTemper Technologies GmbH** (München, Germany). In the case of aptamers to small molecules, MST has no lower molecular weight limit and can therefore “see” into a range that SPR and BLI cannot. NanoTemper Technologies GmbH develops, produces and markets innovative, high quality instruments for bio-medical research based on NanoTemper's unique and proprietary MST technology.

Microscale Thermophoresis (MST) is based on a physical principle used for the first time in bioanalytics. It detects changes in the hydration shell, charge or size of molecules allowing measurement of a wide range of biomolecular interactions under close-to-native conditions. MST can be performed label-free, immobilization-free, in any buffer or complex biofluid. NanoTemper's unique technology is ideal for basic research applications requiring flexibility in the experimental scale, as well as for pharmaceutical research applications including small molecule profiling.



**Figure 1:** NanoTemper Technologies GmbH Monolith NT series of instruments are based on detection of fluorescent dye (Monolith NT.115 Series) or molecule fluorescence (Monolith NT.Label Free) that exhibit in range of 280nm (excitation) and 360nm (emittance). Consumable products include Protein Labelling Kits, MST Control Kits, and Binding Assay Development Kits.



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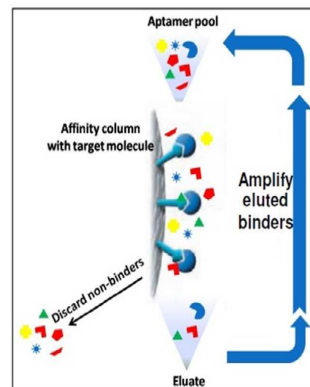
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## BACKGROUND

### Aptamer selection

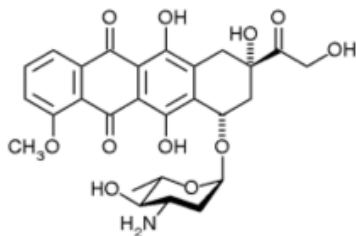
Aptamers are single-stranded DNA or RNA oligonucleotides selected to have unique three dimensional folding structure for binding to a variety of targets such as proteins, peptides, and even small molecules with affinity and specificity rivaling that of antibodies. They are typically selected in vitro by a process commonly referred to as “SELEX” [1,2] as depicted in **Figure 2**. Using a proprietary variant of this process, Base Pair Biotechnologies is developing aptamers to multiple targets simultaneously.

**Figure 2:** Overview of SELEX for Production of DNA Aptamers. A randomized library, flanked by two constant regions for PCR priming is constructed. The library is allowed to bind with the target and partitioned from the non-binding population. Following repeated rounds of selection and enrichment, high affinity DNA ligands are cloned and sequenced.



### Aptamer target for this study

Epirubicin (**Figure 3**) is an anthracycline cytotoxic agent used as a component of adjuvant therapy in patients with evidence of axillary node tumor involvement following resection of primary breast cancer [3]. In the conventional SELEX process, such small molecules are typically immobilized to some sort of solid matrix for convenient partitioning of binding and non-binding nucleic acids. While epirubicin and the closely related doxorubicin have convenient primary amine “handles” for covalent immobilization, in this selection we investigated a “quick and dirty” approach in which the hydrophobic molecule was simply passively adsorbed on a highly hydrophobic surface. Following several rounds of such aptamer selection, aptamer clones were evaluated by MST as described below.



**Figure 3:** Structure of Epirubicin Hydrochloride





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## BACKGROUND CONTINUED

### Microscale Thermophoresis (MST)

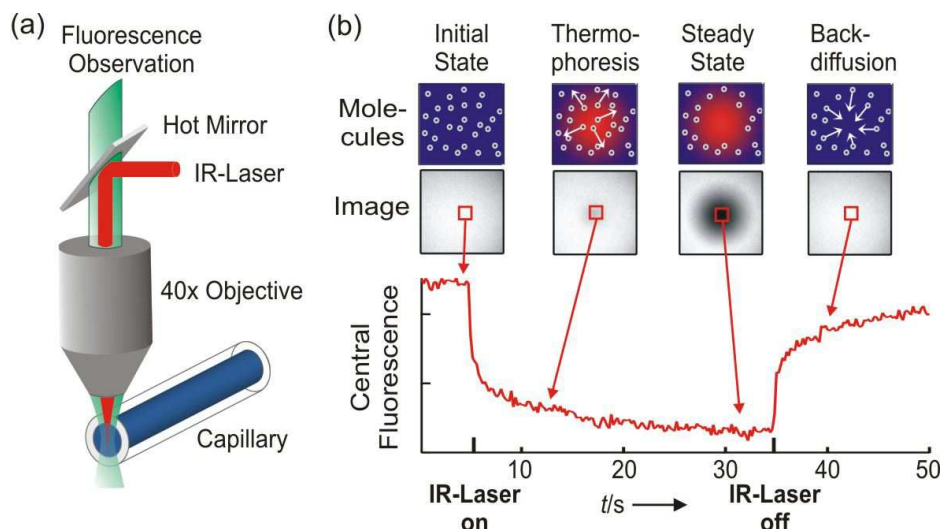
MST allows fast, immobilization-free and label-free quantification of biomolecule interactions with high sensitivity and accuracy as well as low sample consumption. As the name “Microscale Thermophoresis” implies, the method is based on thermophoresis - the directed movement of particles in a microscopic temperature gradient.

A temperature difference  $\Delta T$  in space leads to a depletion of the solvated biomolecules in the region of elevated temperature (Figure 3, below), quantified by the Soret coefficient  $S_T$ :  $c_{\text{hot}}/c_{\text{cold}} = \exp(-S_T\Delta T)$ . This thermophoretic depletion depends on the interface between molecule and solvent. Under constant buffer conditions, thermophoresis probes the size, charge and solvation entropy of the molecules. The thermophoresis of a protein typically differs significantly from the thermophoresis of a protein-ligand complex due to binding induced changes in the protein’s size, charge and solvation entropy. Even if a binding event does not significantly change the size or charge of a protein, MST can still detect the binding due to binding-induced changes in the solvation entropy of the molecules involved.

In practice, for MST analysis an infrared-laser is used to generate precise microscopic temperature gradients within thin glass capillaries that are filled with a sample in a buffer of choice. The fluorescence of molecules is used to monitor the motion of molecules along these temperature gradients. The fluorescence can be either intrinsic (e.g. tryptophane) or of an attached dye or fluorescent protein (e.g. GFP). For the data below, various concentrations of epirubicin were presented to cy5-labeled aptamer and the binding constant or  $K_d$  was determined.

**Figure 4: Thermophoresis assay.**

**(a)** The solution inside the capillary is locally heated with a focused IR-laser, which is coupled into an epifluorescence setup using a hot mirror. **(b)** The fluorescence inside the capillary is recorded and the normalized fluorescence in the heated spot is plotted against time. The IR-laser is switched on at  $t=5$  s and the fluorescence decreases as the temperature increases and the labeled aptamers move away from the heated spot due to thermophoresis. Once the IR-laser is switched off the molecules diffuse back.



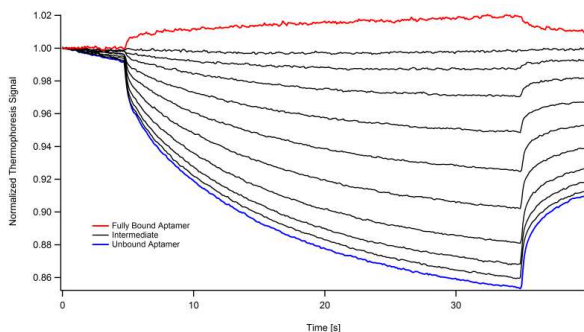


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## RESULTS

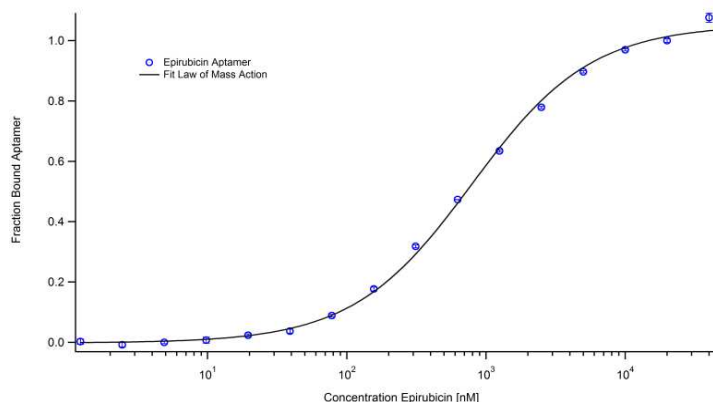
Raw data from the aptamer:epirubicin interaction are shown in **Figure 5** below. Of note are the significant fold-signal changes with increasing epirubicin concentration and the excellent signal to noise of the experiment.



**Figure 5:** Thermophoresis time traces. The thermophoresis time traces of an unbound aptamer (blue) differ significantly from the time traces of an aptamer bound to epirubicin (black and red).

Raw, time-dependent data as shown in **Figure 5** are transformed to a binding curve (**Figure 6**) by normalizing the thermophoresis values at a given time (here  $t=30s$ ) to the fraction of bound molecules and plotting this against the varying epirubicin concentration results. Standard analysis of this curve results in an apparent  $K_d = 711 \pm 30$  nM with a signal-to-noise ratio of 65.

**Figure 6:** Aptamer-epirubicin binding. The fraction of bound fluorescently labeled aptamer is plotted versus the titrated epirubicin concentration. The values are the mean values from three independent measurements, the error bars are mostly smaller than the symbols. The signal-to-noise ratio is 65. The nonlinear fit (black line) with the law of mass action derives a  $K_d$  of  $711 \text{ nM} \pm 30 \text{ nM}$ .



## CONCLUSION

While the affinity of the epirubicin aptamer was not particularly high, we generated this particular aptamer using an experimental procedure. More importantly, we were pleased at the utility of MST technology not only for measuring the binding response to small molecules but also for measurement of moderate affinity interactions in free solution. Based on the results of this first interaction with the method, we expect to analyze several additional small molecule aptamers selected at Base Pair using MST in the near future.

### References:

1. Tuerk C, Gold L: Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 1990,249:505-10.
2. Ellington AD, Szostak JW: In vitro selection of RNA molecules that bind specific ligands. *Nature* 1990, 346:818-22.
3. [http://www.pfizerpro.com/page\\_not\\_found?rid=/wyeth\\_html/home/minisites/oncology/ellence/pi/description.html](http://www.pfizerpro.com/page_not_found?rid=/wyeth_html/home/minisites/oncology/ellence/pi/description.html)

