

Evaluation of a new optical microscale thermophoresis technology for fragment based drug discovery

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Introduction

Fragment-based drug discovery (FBDD) is an emerging technology in drug discovery that has been successfully applied to challenging targets. Fragment screening is generally driven by biochemical, or biophysical methods such as X-ray, nuclear magnetic resonance (NMR) or surface plasmon resonance (SPR). The biophysical methods play a pivotal role for the successful prosecution of FBDD projects. Recently a new powerful technology has become available, which detects changes in the hydration shell of molecules. Microscale thermophoresis (MST) is ideal for measuring enzyme activity and biomolecule interactions under close-to-native conditions without surface immobilization of target proteins. Virtually any biochemical process relating to the interaction, size, stability or conformation of biomolecules and biochemical complexes can be measured with high sensitivity in buffer, cell lysate or serum. Here we present a case study, where MST was used to study the interaction of p38 α kinase (MAPK14) with fragments identified in a SPR screening campaign.

Principles of microscale thermophoresis

MST is a powerful new technology, which measures changes in the hydration shell of molecules. 150 years ago Charles Soret and Carl Ludwig discovered that molecules move in temperature gradients, a physical effect called thermophoresis. The solvation entropy and the hydration shell of molecules were identified as the driving forces for thermophoresis. With the availability of IR-lasers, precise microscale temperature gradients can be produced within capillaries to study the specific movement of biomolecules along these gradients. Any modifications in the primary, secondary, tertiary or quaternary structure of biomolecules cause changes in their hydration shell and result in a relative change of movement along the temperature gradient. Thus, MST can be used to determine binding affinities, binding kinetics and activity kinetics of biomolecules with high accuracy and sensitivity.

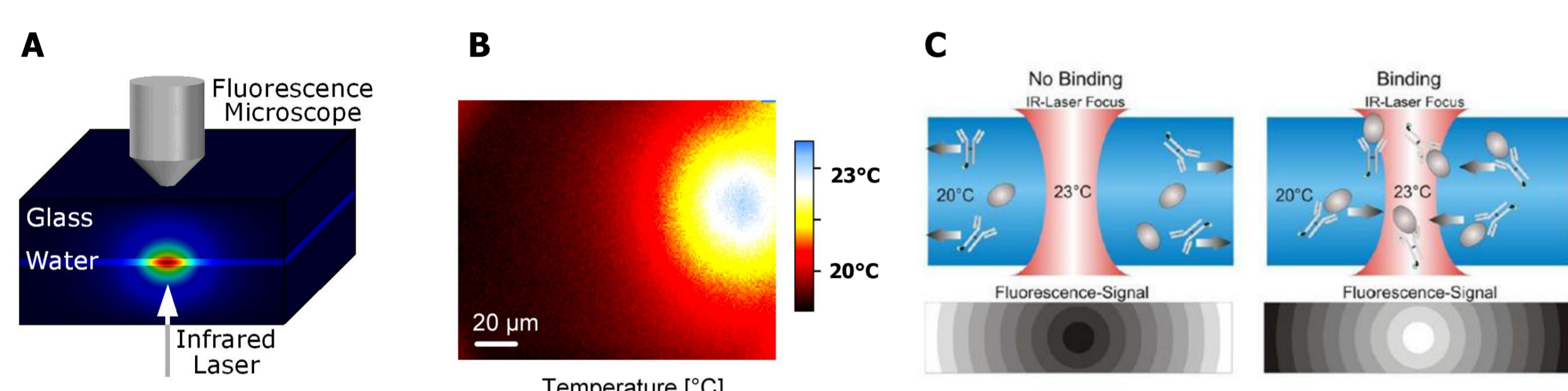


Figure 1. (A) Schematic overview of the generation of precise microscale temperature gradients by IR lasers. (B) Illustration of a microscale temperature gradient induced by IR laser. (C) Schematic overview of the movement of unbound and bound biomolecules in microscale temperature gradients.

Case study p38 α : assay validation

A case study with p38 α was carried out to evaluate the value of the MST technology for FBDD. The MST assay was developed at NanoTemper and validated with the well characterized p38 α inhibitors BIRB 796 and SB202190. Usually one of the binding partners is labeled with a fluorescent dye (e.g. p38 α in this case). The concentration of the labeled binding partner is kept constant (e.g. 50 nM in this case) and increasing amounts of unlabeled binding partner are added. A schematic overview of a typical MST experiment is represented in Figure 2. Results of two representative experiments are shown in Figure 3. The determined K_d values of 5 nM for BIRB 796 and 100 nM for SB202190 were in the range of published values.

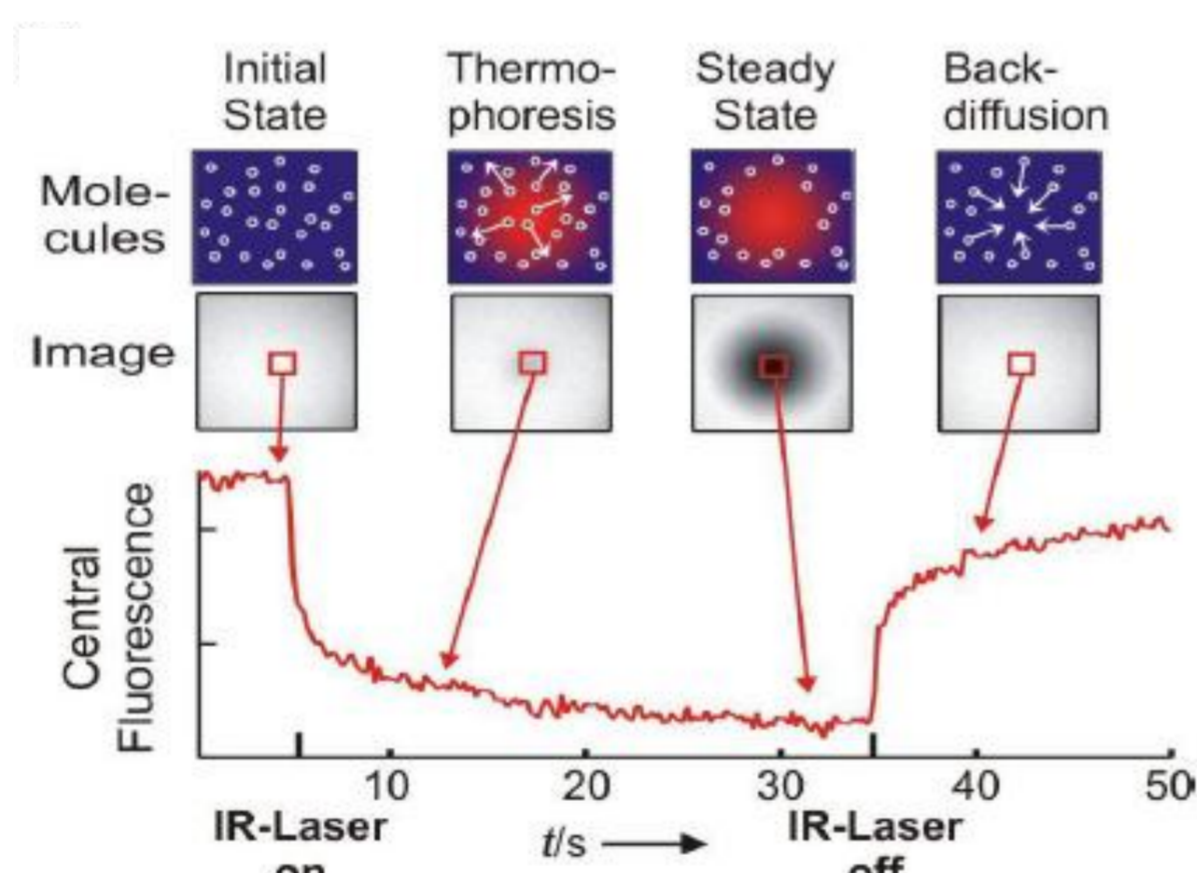


Figure 2. Illustration of a typical MST experiment. Samples are heated with an IR-laser within a capillary and central fluorescence is recorded with a CCD camera. While the IR-laser is switched on at 5 s, the fluorescent molecules start to migrate along the temperature gradient and a decrease in central fluorescence is observed. When the laser is switched off at 35 s, the temperature gradient disappears and the molecules diffuse back.

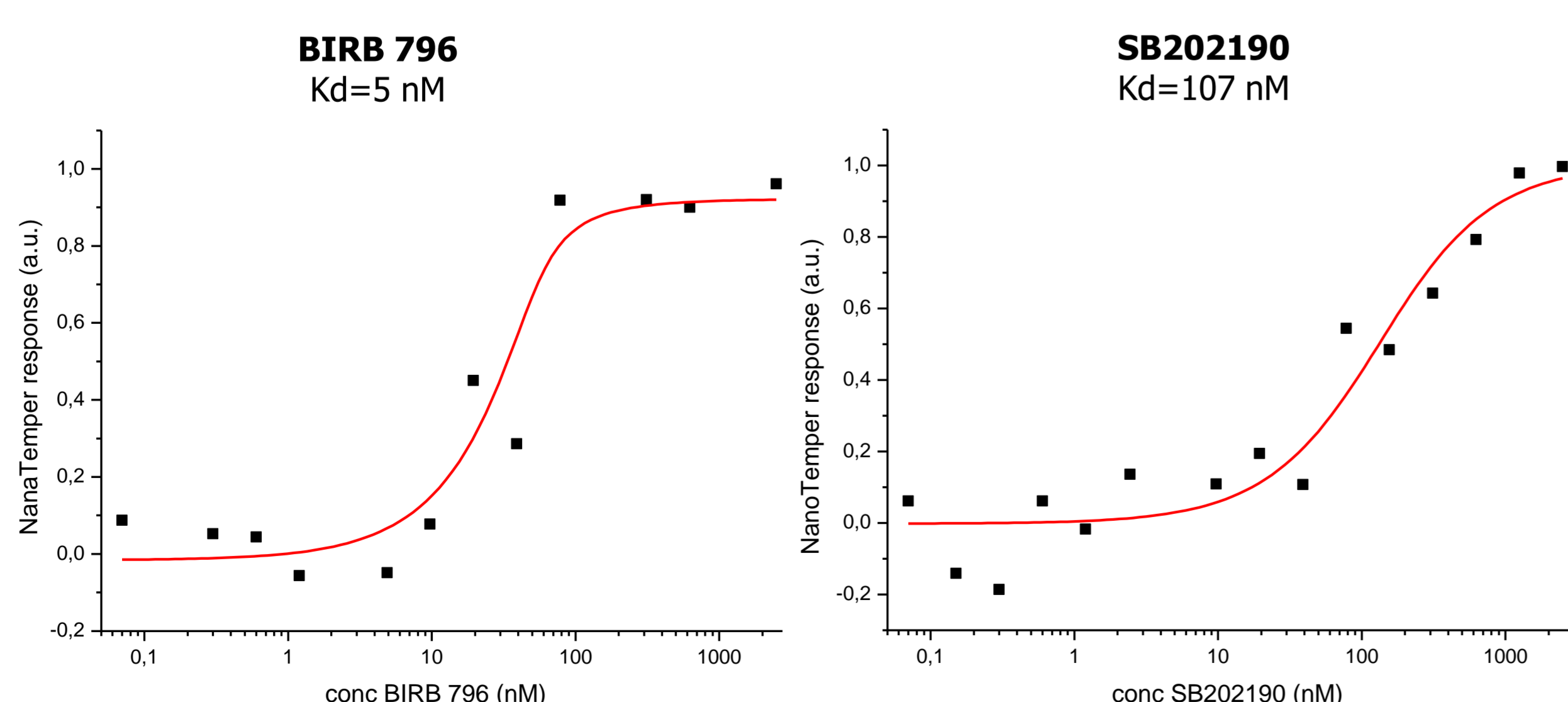


Figure 3. Determination of K_d values for the two p38 α inhibitors BIRB 796 and SB202190 in the MST assay. Serial dilutions of test compounds were incubated with labeled p38 α for 0, 3 or 9 h at 30°C before thermophoretic analysis. Illustrated dose response curves represent the results of the 9 h incubation experiments, which were not significantly different from the 3 h results.

Case study p38 α : results

Three fragments which were originally identified in a SPR based fragment screen on a BiacoreTM T100 instrument and one inactive control fragment were selected for analysis by MST. As illustrated in Figure 4, binding to p38 α was detected for Fragment A, Fragment C and Fragment D. While the binding curves for Fragment A and Fragment D had positive amplitudes, the curve for Fragment C had a negative amplitude. Due to solubility limits, the fragments could not be tested at concentrations above 1 mM and thus saturation binding could not be determined. Nevertheless, for Fragment A and Fragment C K_d values of 893 μ M and 1152 μ M were estimated based on fitted sigmoidal binding curves. No significant interaction with p38 α was observed for Fragment B.

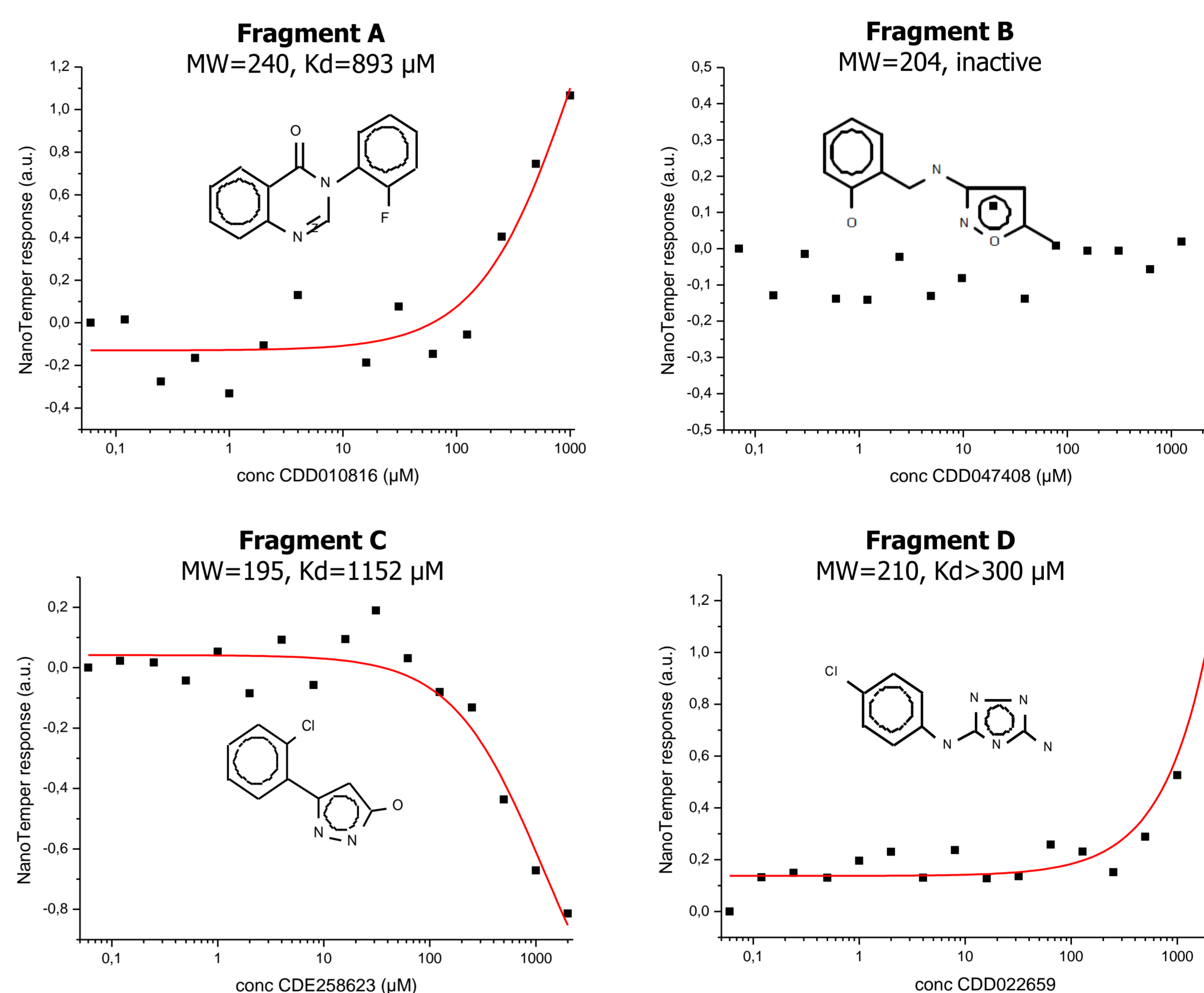


Figure 4. Determination of K_d values for 4 fragments in the MST assay. Serial dilutions of test fragments were incubated with p38 α kinase for 4 h at 30°C before thermophoretic analysis.

Case study p38 α : discussion

We have evaluated NanoTemper's proprietary optical MST technology for FBDD. A case study was carried out using the well characterized p38 α and a selection of three verified hit fragments, which were originally identified in an SPR-based fragment screen, and a negative control fragment. The selected fragments were tested by MST, in a functional assay, and the three active binders were analyzed by co-crystallization with p38 α for structure and mode of binding determination. The activities of the fragments determined by different biophysical and biochemical assay technologies correlated well and are summarized in Table 1. The relevance of the inverse amplitude of the binding curve observed for Fragment C in the MST assay is unknown. It is hypothesized that the direction of the amplitude could provide new information regarding the mode of interaction, which could so far not be obtained by the use of alternative technologies.

Assay	Fragment A	Fragment B	Fragment C	Fragment D
MST K _d	893 μ M	no binding	1152 μ M	>300 μ M
SPR K _d	780 μ M	no binding	1590 μ M	1380 μ M
X-ray	ATP binding site	n.d.	ATP binding site	distal site
Caliper IC50	1040 μ M	n.d.	4090 μ M	4200 μ M

Table 1. Summary of fragment activities on p38 α determined by different assay technologies.

Final conclusions

- p38 α binding fragments originally identified by SPR were confirmed by MST, in a functional Caliper-based assay and by co-crystallization.
- The fragment with the lowest K_d as determined by SPR and MST was the most potent inhibitor in the functional Caliper-based assay.
- An inactive control fragment did not interact with p38 α in the MST assay.

From this case study it is concluded that NanoTemper provides a new powerful biophysical technology, which is worthwhile pursuing for fragment based drug discovery. Additional kinetic interaction studies for the determination of on- and off-rates of the fragments are currently ongoing.