Case Study

Measuring the unmeasurable:
How to capture out-of-range target engagement kinetics for an advanceable clinical candidate

How do you validate the binding affinity of a promising lead candidate with a half-life exceeding the traditionally measurable range?

Discover how our biophysics team adapted an SPR chaser assay to enable label-free characterization of compounds with nontypical dissociation rates, generating IND-enabling data to support advancement of an oncology candidate into the clinic.

In this study, we characterized the interaction of an oncology clinical candidate compound (**MRTX1719**) with two forms of **P**rotein A**r**ginine **M**ethyl **T**ransferase **5** (**PRMT5**:MEP50) bound to either the cofactor SAM or the metabolite MTA (hereafter PRMT5•SAM and PRMT5•MTA). The goal was to determine the affinity and kinetic properties of the compound for each form of PRMT5 and compare this with the known specificity for killing of MTAP-deleted cells over a wild type congenetic cell line. PRMT5 is a class II PRMT that catalyzes the sequential and symmetric transfer of methyl groups to arginine residues of substrate proteins thereby epigenetically regulating genes involved in oncogenesis. The compound was provided without information on target selectivity or K_D values, and our team was instructed to determine the affinity for each form of the target using SPR.

Background

Surface Plasmon Resonance (SPR) is a label-free biophysics method, which is typically used in small molecule hit discovery to characterize binding to target proteins. Using a flow injection system, the binding of a small molecule to an immobilized protein target is monitored in real time. The resulting sensorgrams, acquired at various compound concentrations, allow for the determination of both the affinity and kinetics (k_{on} and k_{off}) of the observed interaction (Figure 1). However, even with the most modern and sensitive instruments, the ranges of kinetics that can be practically and robustly measured are 10^{3}-10^{6} M^{-1} s^{-1} for k_{on} and 10-10^{-5} s^{-1} for k_{off}, which correspond to an affinity range of 100 pM to 10 µM. This affinity range covers the majority of clinical candidates, and is therefore a leading technique for this purpose; however, some mechanisms of action require a much higher affinity entering territory, which is not typically accessible via SPR.

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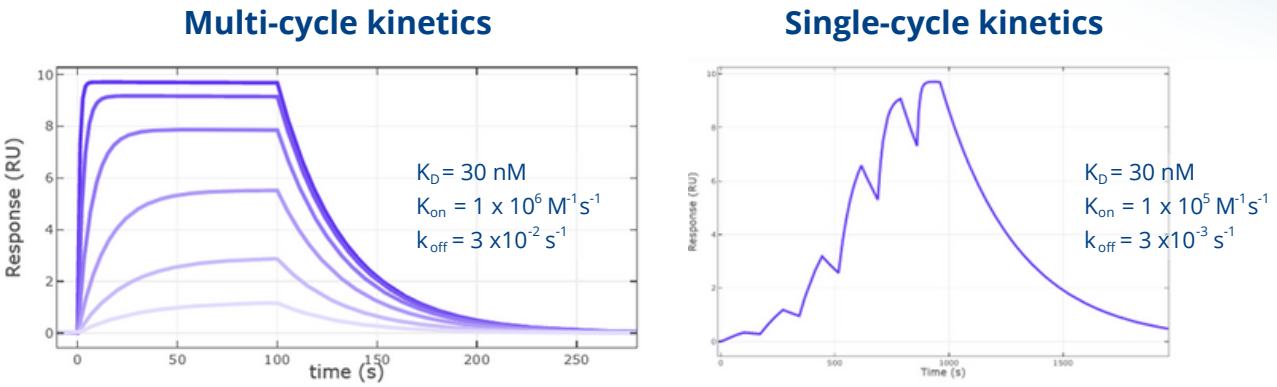


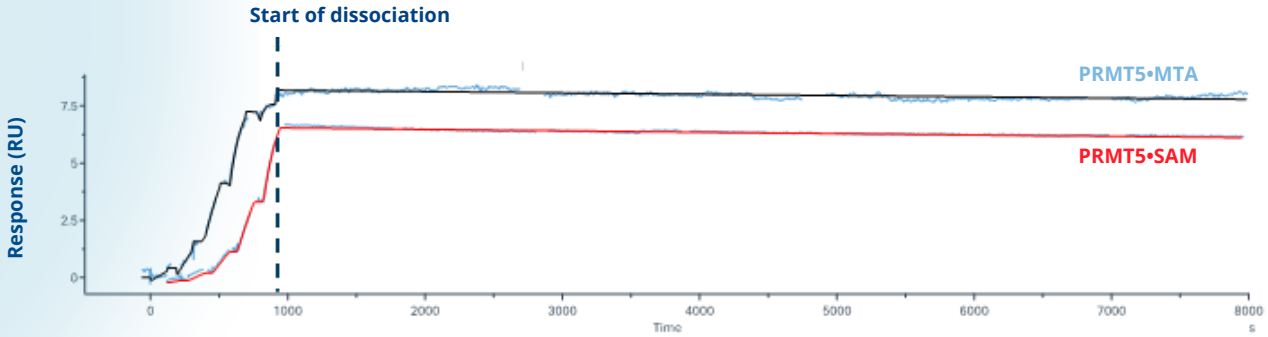
Figure 1. *An example of typical SPR sensorgrams that can be generated from modern SPR instruments. The binding can be observed in multi-cycle kinetics mode (left) where an analyte (small molecule) was injected at increasing concentrations ranging from 4 nM to 1 µM and full dissociation was monitored for 150 seconds after each injection. The binding can also be observed in single-cycle kinetics mode (right) where an analyte was injected from 4 nM to 1 µM without allowing complete dissociation and the dissociation was measured for 1,000 seconds only after the injection of the highest analyte concentration.*

The challenge

When characterizing the affinity of MRTX1719 for PRMT5•SAM and PRMT5•MTA, we discovered that the k_{off} of the dissociating complex was too slow ($k_{off} < 10^{-5}$ s^{-1}) to be determined by simply monitoring the dissociation using SPR. In single-cycle kinetics experiments with a long dissociation period (2 hours), no significant amount of MRTX1719 dissociated (Figure 2). As a result, it was not possible to measure sufficient dissociation to determine k_{off}. Furthermore, when the dissociation observation period was extended beyond 2 hours, the dissociation data was negatively affected by interference from drift in the SPR signal (an intrinsic limitation of the system which can best be seen in the blue trace of Figure 2) and this further prevented us from direct k_{off} determination.

Compounds with such slow dissociation rates are uncommon. SPR is the only established method* to directly measure small molecule dissociation from a target protein, therefore characteristics which fall outside the traditionally measurable scope can present unique research challenges.

*Accurate at the time of publication.

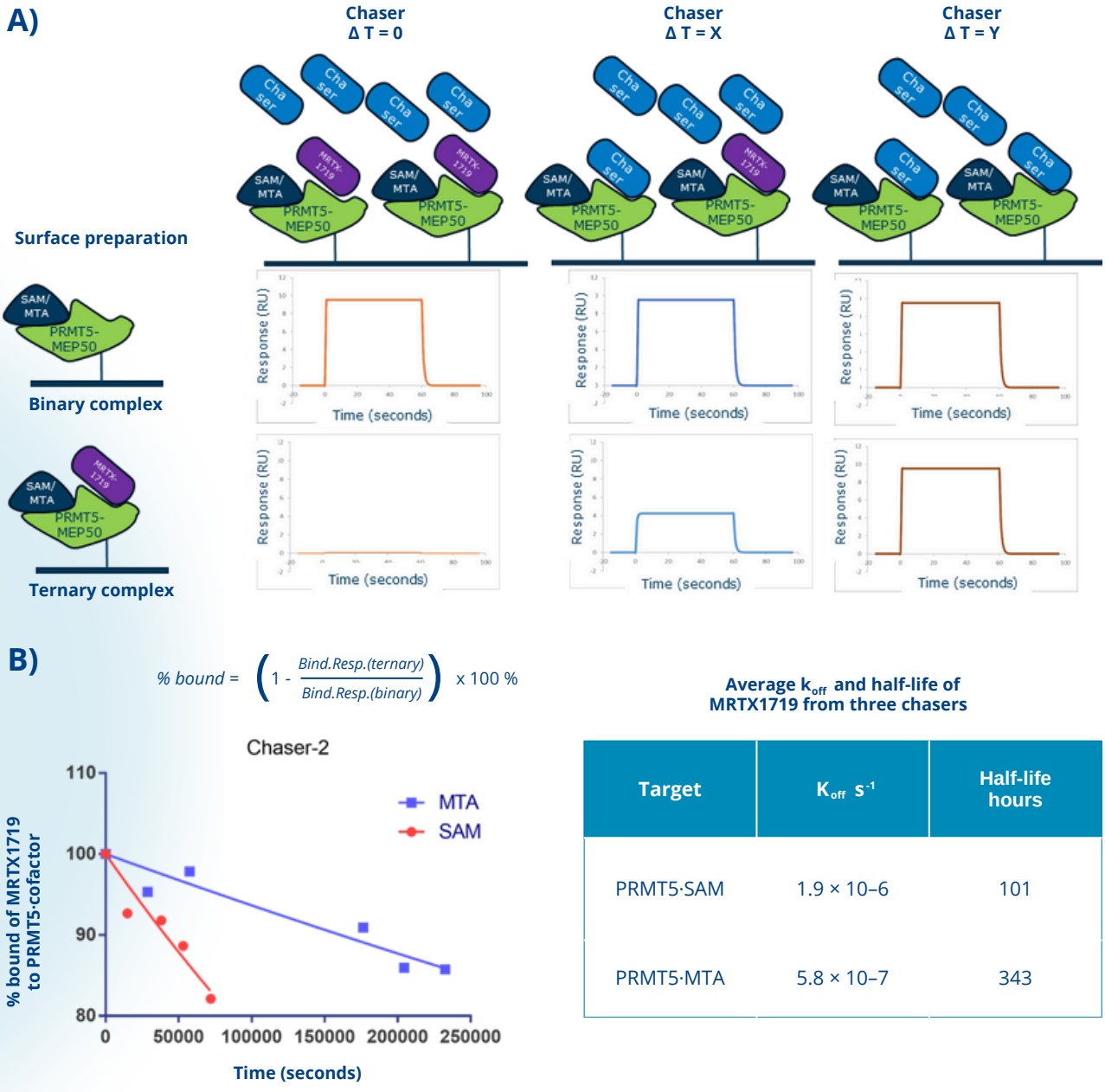


Single cycle kinetic (SCK) injection from 0.37 to 30 nM

Figure 2. *The dissociation of MRTX1719 from the PRMT5•MTA/SAM complex was very slow, with a k_{off} below 10^{-5} sec^{-1} (half-life >19 hours), and therefore no significant difference was observed in the dissociation of MRTX1719 from PRMT5•MTA (blue trace) compared to PRMT5•SAM (red trace).*

Working the problem

As we were unable to directly observe and measure the dissociation event, we instead determined the dissociation rate constant of MRTX1719 indirectly by designing an adapted SPR chaser assay. This method, originally pioneered in 2016 (Ref. 1 and 2), enables the measurement of small molecule dissociation from an immobilized protein over a half-life greater than 24 hours. For the present study, the ternary complex of MRTX1719•PRMT5•SAM (or •MTA) was first pre-formed on the sensorchip surface of an SPR instrument and allowed to dissociate over a period of up to ~56 hours. We selected three different chaser compounds with amenable kinetic characteristics, all of which compete with MRTX1719 for binding to PRMT5. The binding of the chaser molecules to PRMT5•cofactor was assessed at different points during the dissociation of MRTX1719 (Figure 3A). As the chasers and MRTX1719 competed for the same binding site on PRMT5•cofactor, the amount of bound chaser reflected the fraction of free binding sites made available by dissociation of MRTX1719. This allowed the determination of the remaining MRTX1719 bound to the protein (% bound) at the time of chaser injection (Figure 3B). The K_D and half-life were determined by fitting the MRTX1719 occupancy over time to a single exponential decay function: $Y = Y_0 \cdot \exp(-k_{off} \cdot X)$ (Figure 3B).



Average k_{off} and half-life of MRTX1719 from three chasers

Target	K_{off} s^{-1}	Half-life hours
PRMT5·SAM	1.9 × 10–6	101
PRMT5·MTA	5.8 × 10–7	343

Figure 3. *Graphical concept of* ***A)*** *SPR chaser assay: 1) Block target with the compound of interest (MRTX1719), 2) use a chaser (competitive binder) to assess whether the compound has dissociated, and 3) monitor the chaser binding level over time, which increases as the compound of interest dissociates.* ***B)*** *% PRMT5 bound to MRTX1719 was determined from the chaser binding data and it was then fitted to the exponential decay model to determine the dissociation rate constant of MRTX11719 for PRMT5 bound to either SAM or MTA.*

By using a chaser assay, we successfully determined the k_{off} and K_D values of MRTX1719 for the two forms of PRMT5 (Table 1 in Figure 3B), which was not possible with a direct binding assay. MRTX1719 exhibited a long dissociation half-life of 14 days when bound to PRMT5•MTA and a shorter half-life of 4.6 days when bound to PRMT5•SAM. These represent some of the longest complex half-lives reported by an SPR experiment at the time of publication.

Enabling the development of compounds with longer half-lives

The affinity of MRTX1719 was K_D = 9.4 pM for PRMT5•SAM and K_D = 0.14 pM for PRMT5•MTA. The 67-fold higher selectivity for PRMT5•MTA over PRMT5•SAM observed in SPR experiments correlated well with 74x greater effect on cell viability in the HCT116 MTAP-del vs HCT116 MTAP-WT assay (Ref. 3). This finding was reported as a part of a fragment-based drug discovery approach to MRTX1719, where one of the PRMT5•MTA-selective fragment hits was successfully developed into a clinical study candidate for MTAP-del cancer, with support of structure-based drug design (SBDD).

ZoBio, which was acquired by Oncodesign Services in early 2024, supports research like this through the provision of services such as screening fragment libraries, characterizing hits and their analogues, and providing high resolution 3D structures of compound complexes. You can learn more about our contribution to this structure-based development of MRTX1719 and another lead series in a related paper published in the *RSC Medicinal Chemistry Journal* (Ref. 4).

Ref. 1. E. Chan-Penebre et al., *Nat. Chem. Biol.* 11, 432–437 (**2015**).
Ref. 2. J. G. Quinn, K. E. Pitts, M. Steffek, M. M. Mulvihill, *J Med Chem.* 61, 5154–5161 (**2018**).
Ref. 3. C. R. Smith et al., *J. Med. Chem.* 65, 1749–1766 (**2022**).
Ref. 4. C. R. Smith et al., *RSC Medicinal Chem.* (**2022**), doi:10.1039/d2md00163b.

More information

Oncodesign Services is a leading CRO specializing in drug discovery and preclinical services. In 2024, it acquired ZoBio, a boutique CRO with gene-to-lead expertise in small molecules. The Oncodesign-ZoBio group contributes to the development of innovative therapies from target to preclinical candidates through stand-alone and integrated capabilities in medicinal chemistry, computer-assisted drug design, protein production, biophysics, structural biology, DMPK, in vitro / in vivo pharmacology, and in vivo pharmaco-imaging.

The group supports a global portfolio of clients from laboratories based in Dijon (France), Leiden (The Netherlands), Montreal (Canada) and Paris (France).

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