Nsp12 – Materials and Methods

Nsp12 was labeled in assay buffer following the labeling protocol as specified in NanoTemper Technologies RED-NHS 2nd Generation labeling kit. The assay buffer for all experiments was 20 mM Hepes pH 7.5, 150 mM NaCl, 1 mM MgCl2, 2.5 mM DTT, 0.005% TWEEN® 20, and a final volume of 20 μL per datapoint was used for both the 8-pt-screen and the affinity screen (12 points) in Dianthus 384-well microwell plates.

The compound library and Suramin were dissolved in 100% DMSO at a concentration of 20 mM. For the 8-pt-screen, we diluted the compounds from 20 mM stock solutions to 4 mM and Suramin to 0.8 mM in 100% DMSO and transferred the first dilution into a conventional microwell plate. The second and all following 3-fold dilution steps were prepared in DMSO in the microwell plate, using at each step 5 µl of the preceding dilution and 10 µl of DMSO. Then, 19.5 µl of labeled nsp12 at a final concentration of 25 nM was transferred into the Dianthus 384-well microplate, and 0.5 µl of compound dilution series was added and mixed thoroughly. Final concentrations were 2.5% DMSO and 100 µM – 45.7 nM compound, 3-fold dilution series. Final Suramin concentrations were 20 µM – 9.14 nM, 3-fold dilution series. For the 12-pt affinity screen, concentrations were adapted to obtain final compound concentrations of 250 µM – 1.41 nM and Suramin concentrations of 100 µM – 0.56 nM, 3-fold dilution series for both.

After the Dianthus 384-well microplates were loaded with the compounds + nsp12 mix, they were equilibrated for 30 min at RT and centrifuged for 30 sec at 400 x *g* before loading into the Dianthus NT.23PicoDuo. The system was set to 25°C as set temperature. The samples were first measured for 1 sec without heating and for 5 sec with the IR-laser turned on. The two optical systems in Dianthus were used in parallel. Measured fluorescence values collected are displayed as relative fluorescence, where the fluorescence obtained at ambient temperature is normalized to one, and as normalized fluorescence (Fnorm) which describes the ratio between fluorescence values (F1) after and the fluorescence values (F0) prior to IR laser activation and is typically given in ‰. The dissociation constant or KD, is obtained by fitting a dose-response curve to a plot of Fnorm vs. ligand concentration.

References for Dianthus:

* APPLICATION NOTE “Fast molecular interaction screening of epigenetic gene regulator G9a with fragments from a large chemical space”, ©2019 NanoTemper Technologies, Inc. South San Francisco, CA, USA. All Rights Reserved.