

Appendix 1. The binding constants of NNI binding to NS5B570-Con1 measured by Surface Plasmon Resonance ^a

Compound	k_{on} ($M^{-1} s^{-1}$)	k_{off} (s^{-1})	$t_{1/2}$ (min)	K_d [μM]
NNI-1	1.7×10^6	0.023	0.5	0.01
HCV-796	2.9×10^4	7.9×10^{-4}	15	0.02
NNI-3	2.9×10^6	0.026	0.4	0.01
NNI-4	7.7×10^3	0.010	1.2	0.2

^a The Biacore S51 biosensor (BIACORE Life Sciences, Uppsala, Sweden) was used to determine the compound binding parameters as described (20).

1) Change the footnote of Appendix 1 to below:

^a Data obtained with a Biacore S51 biosensor (GE Healthcare) as previously described (20).

2) Addition of Appendix 2:

Appendix 2. Method of SPR binding experiments

SPR (surface plasmon resonance) assays were performed similarly to (20) but with the following modifications. Both NS5B570-Con1 and NS5B750-BK proteins were coupled to a sensor chip using standard amine coupling (Biacore manual, GE Healthcare). Briefly, aliquots (5 μ l of 5 mg/ml) of either NS5B570-Con1 or NS5B750-BK were rapidly diluted with 150 μ l of 50 mM MES pH 6.0, 50 mM NaCl, and 10 mM MgCl₂ and immediately injected for five minutes over an activated CM5 chip surface at 10 μ l/min and blocked immediately with a 1:1 mix of running buffer (50 mM Hepes pH 8.0, 150 mM NaCl, 10 mM MgCl₂, 5 mM DTT) and 1 M ethanolamine. This resulted in surfaces coupled with 10,000-15,000 RUs of NS5B with > 90% the expected compound binding capacity. Compound preparation, data collection, data reduction, and fitting were carried out as described in (20).