Supplemental material:

Kinetic analysis.

The sensorgrams were fitted by the Single Cycle Kinetics (SCK) or by the Single Cycle Kinetics On a Decaying Surface methods (SCKODS) as described previously *(Karlsson et al., 2006; Palau & Di Primo, 2012)* assuming a simple Langmuir 1:1 model according to Equations (1) and (2), for the association and dissociation phases:

$$\frac{dRU}{dt} = k_a[C]RU_{\max} - (k_a[C] + k_d)RU \qquad (1)$$

$$\frac{dRU}{dt} = -k_d RU_{t0}.e^{-k_d(t-t0)} \qquad (2)$$

where RU is the signal response; RU_{max}, the maximum response level; RU_{t0}, the response at the beginning of the dissociation phase, [C] the molar concentration of the injected sample, k_a and k_d, the association and dissociation rate constants, respectively. The dissociation equilibrium constant, K_D, was calculated as k_d/k_a.

Table S1: Equilibrium and rate constants for $SL2_{kistem}$-5BSL3.2 complexes.

Complexes	k_a (10^4 M^{-1} s^{-1})	k_d (10^{-3} s^{-1})	K_D (nM)
$SL2_{kistem}$-5BSL3.2[a]	4.59 ± 0.08	4.52 ± 0.09	98.5 ± 1.4
$SL2_{kistem}$-5BSL3.2[b]	5.35 ± 0.16	5.45 ± 0.02	102 ± 3

[a]The kinetic parameters were determined from direct curve fitting of the sensorgrams reported in Fig. 2B. [b]The kinetic parameters were determined by direct curve fitting of the sensorgrams reported in Fig. 5. The values are the average and standard deviations of at least two independent experiments performed with duplicate injections.

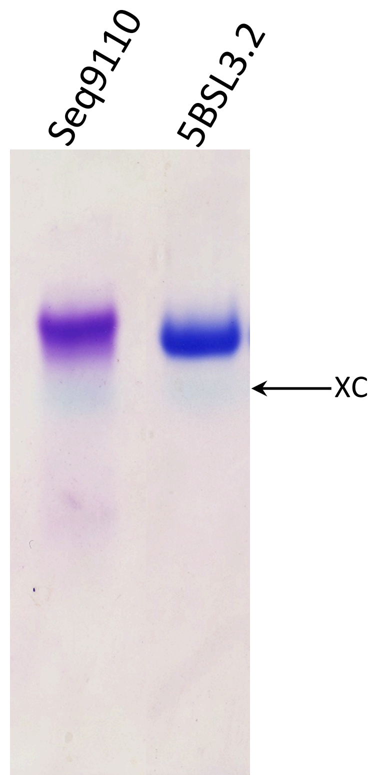

Figure S1: Native acrylamide gels of stem-loops Seq9110 and 5BSL3.2 (see Fig. 1B for the sequences and the secondary structures). 4 μg of Seq9110 and 5BSL3.2 were prepared in 50 mM Tris-acetate buffer, pH 7.3 at 20°C, containing 3 mM magnesium acetate (migration buffer) and loaded on 15% (W/V) 75:1 acrylamide/bis(acrylamide) native gels as described in Materials and Methods. XC refers to as xylene cyanol dye. The gels were stained by "Stains-all".

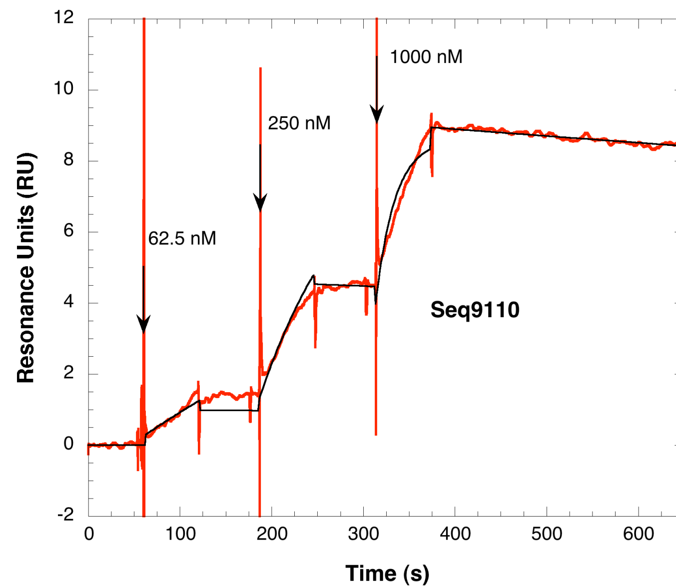

Figure S2: Kinetic analysis of Seq9110 binding to biotinylated 5BSL3.2. Seq9110 prepared in the running buffer (10 mM sodium phosphate buffer, pH 7.2 at 20°C, containing 50 mM sodium chloride, 3 mM magnesium chloride and 0.05% Tween-20) was injected sequentially in the order of increasing concentrations, 62.5 nM (first arrow from the left), 250 nM (second arrow) and 1000 nM (third arrow). The red curves represent the experimental data and the black line the fit to a Langmuir 1:1 model. Regeneration was achieved with a 2 min pulse of a mixture of 40% formamide, 3.6 M urea and 30 mM EDTA prepared in milliQ water.

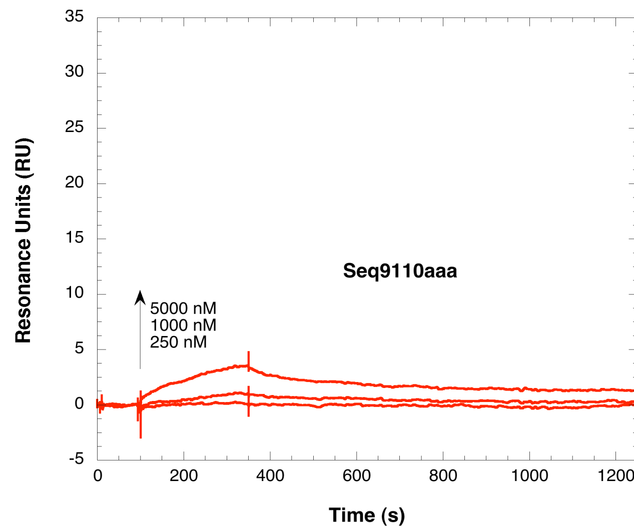

Figure S3: Kinetic analysis of Seq9110aaa binding to biotinylated 5BSL3.2. The experiments were carried out by the classical method, which consists in injecting increasing concentrations of the sample with a regeneration step after each injection. The samples were prepared in the running buffer (10 mM sodium phosphate buffer, pH 7.2 at 20°C, containing 50 mM sodium chloride, 3 mM magnesium chloride and 0.05% Tween-20) and injected at 50 µl/min.

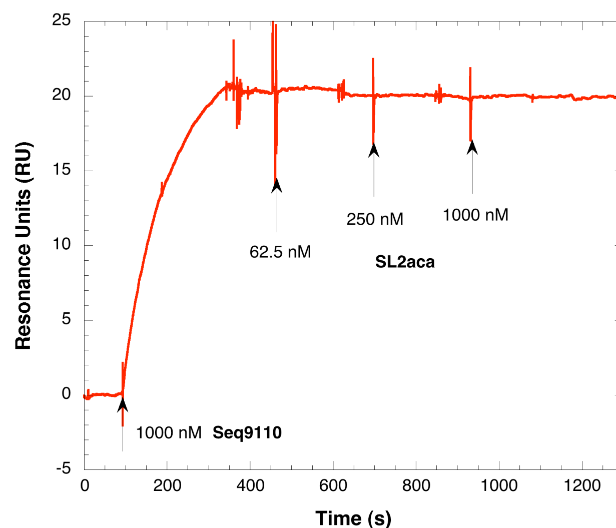

Figure S4: Kinetic analysis of SL2aca binding to 5BSL3.2 while Seq9110 dissociates from biotinylated 5BSL3.2. Seq9110 was first injected (first arrow from the left) over biotinylated 5BSL3.2. SL2aca was injected sequentially in the order of increasing concentrations as indicated. The samples were prepared in the running buffer (10 mM sodium phosphate buffer, pH 7.2 at 20°C, containing 50 mM sodium chloride, 3 mM magnesium chloride and 0.05% Tween-20) and injected at 50 µl/min.

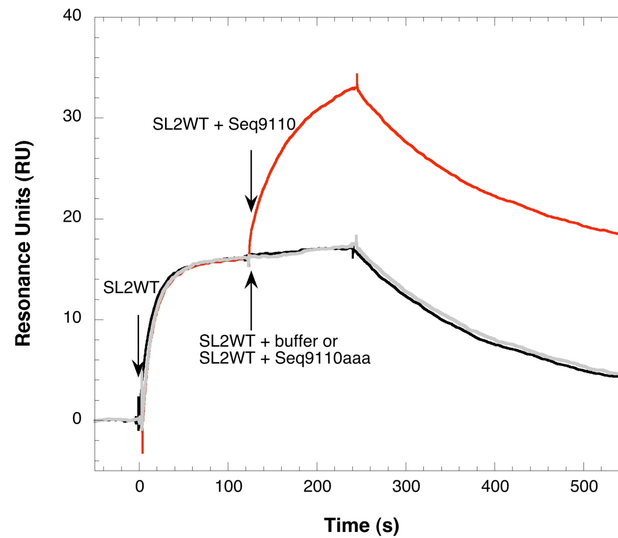

Figure S5: Kinetic analysis of the $SL2_{WT}$-Seq9110-5BSL3.2 ternary complex. In this experiment $SL2_{WT}$ was first injected at 1 µM (first arrow) to saturate the binding site on the apical loop of biotinylated 5BSL3.2. Seq9110 (5 µM) was then simultaneously injected with $SL2_{WT}$ (second arrow, red curve). A mutated sequence of Seq9110, Seq9110aaa (see Fig.1B, the three Gs were replaced by three Cs), designed to prevent binding with the internal loop of 5BSL3.2 (grey curve) behaves as buffer (black curve). The samples were prepared in the running buffer (10 mM sodium phosphate buffer, pH 7.2 at 20°C, containing 50 mM sodium chloride, 3 mM magnesium chloride and 0.05% Tween-20) and injected at 50 µl/min.

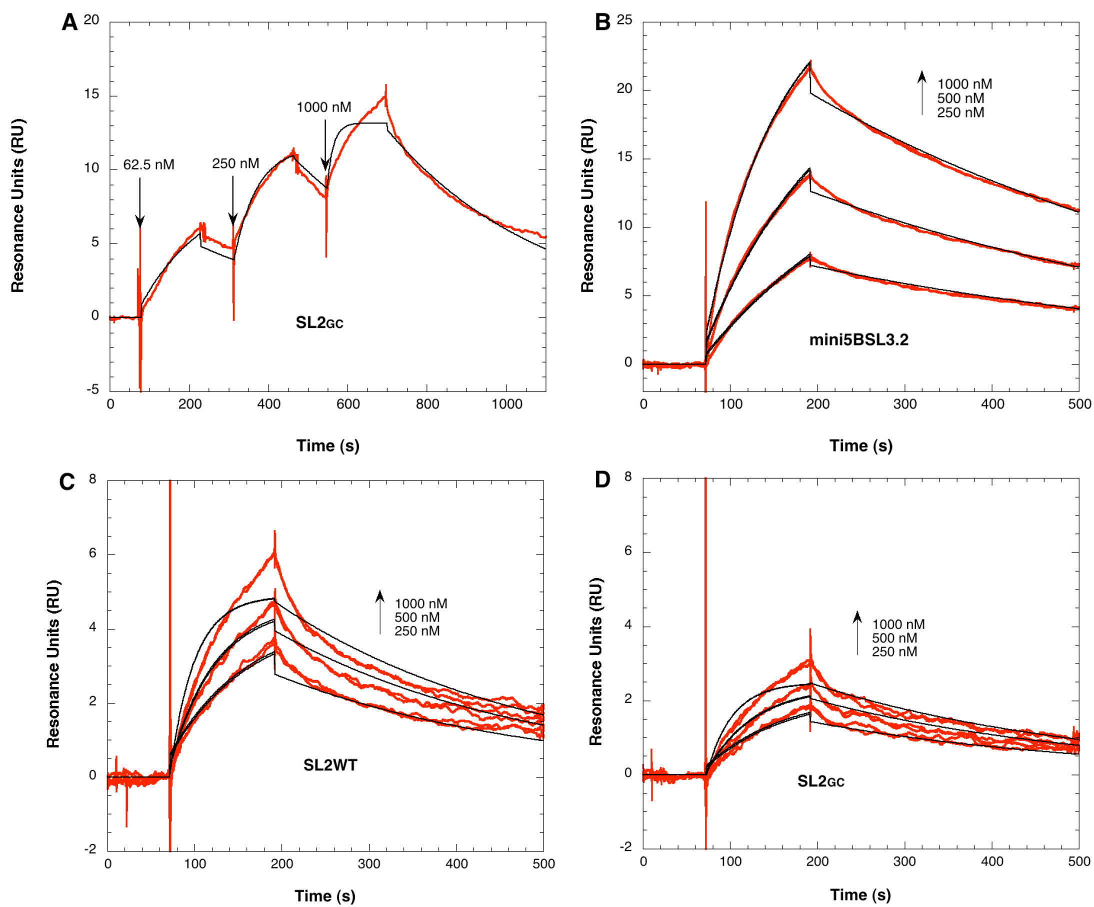

Figure S6: Kinetic analysis of complexes formed between 5BSL3.2 or $SL2_{WT}$ immobilized on the sensor chip surface and SL2 hairpins or mini5BSL3.2, a truncated version of 5BSL3.2 (Fig. 1B). The samples were prepared in the running buffer (10 mM sodium phosphate buffer, pH 7.2 at 20°C, containing 50 mM sodium chloride, 3 mM magnesium chloride and 0.05% Tween-20) and injected at increasing concentrations as indicated by the arrows. The red curves represent the experimental data (duplicate injections) and the black line the fit to a Langmuir 1:1 model. Regeneration was achieved with a 2 min pulse of a mixture of 40% formamide, 3.6 M urea and 30 mM EDTA prepared in milliQ water. A) Injection of $SL2_{GC}$ over 5BSL3.2. B) Injection of mini5BSL3.2 over $SL2_{WT}$. C) Injection of $SL2_{WT}$ over $SL2_{WT}$. D) Injection of $SL2_{GC}$ over $SL2_{WT}$.

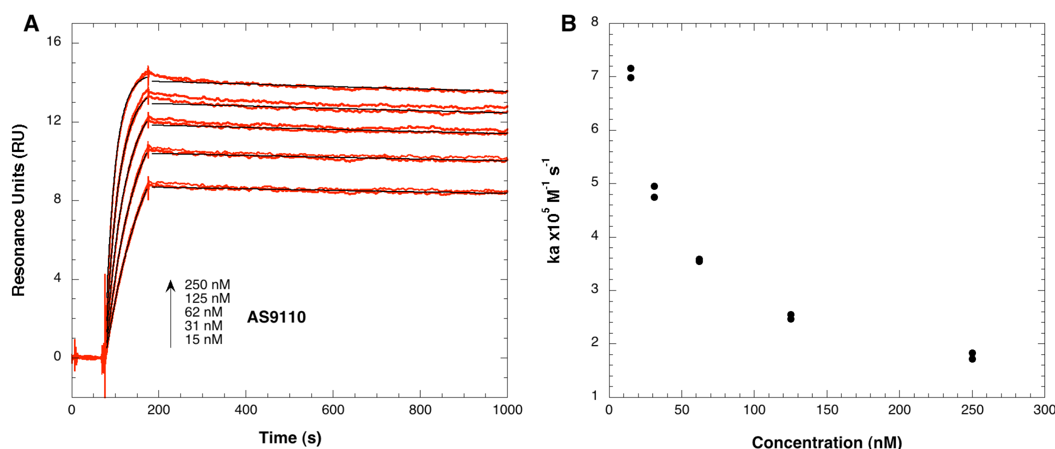

Figure S7: Kinetic analysis of the 5BSL3.2-AS_{9110} complex. The samples were prepared as described in the Materials and Methods. They were injected at increasing concentrations (in duplicate) as indicated by the arrows (Fig. S7A). The red curves represent the experimental data. As mentioned in the manuscript the data could not be fitted by global analysis that imposes one association rate constant, k_a, and one dissociation rate constant, k_b, for all sensorgrams. We tried to analyze separately the association and dissociation phases. The dissociation phases could be actually fitted by global analysis as shown in Fig. S7A (black lines). The dissociation rate constant is equal to $4.14\ 10^{-5} \pm 0.03\ 10^{-5}\ s^{-1}$. If this value is used to fit separately each association phase, curve fitting works but when the resulting association rate constants, k_a, are plotted as a function of the concentration of the sample injected (Fig. S7B), k_a is clearly not constant as it should be for a 1:1 model of interaction. We have to admit that we are not able to find an explanation for this behavior. We checked for trivial reasons such as errors in the dilutions, influence of the flow rate or the level of immobilization of the target, unsuccessfully. This would need further investigations.