Supporting Information

Rapid Crosslinking of an RNA Internal Loop by the Anticancer Drug Cisplatin

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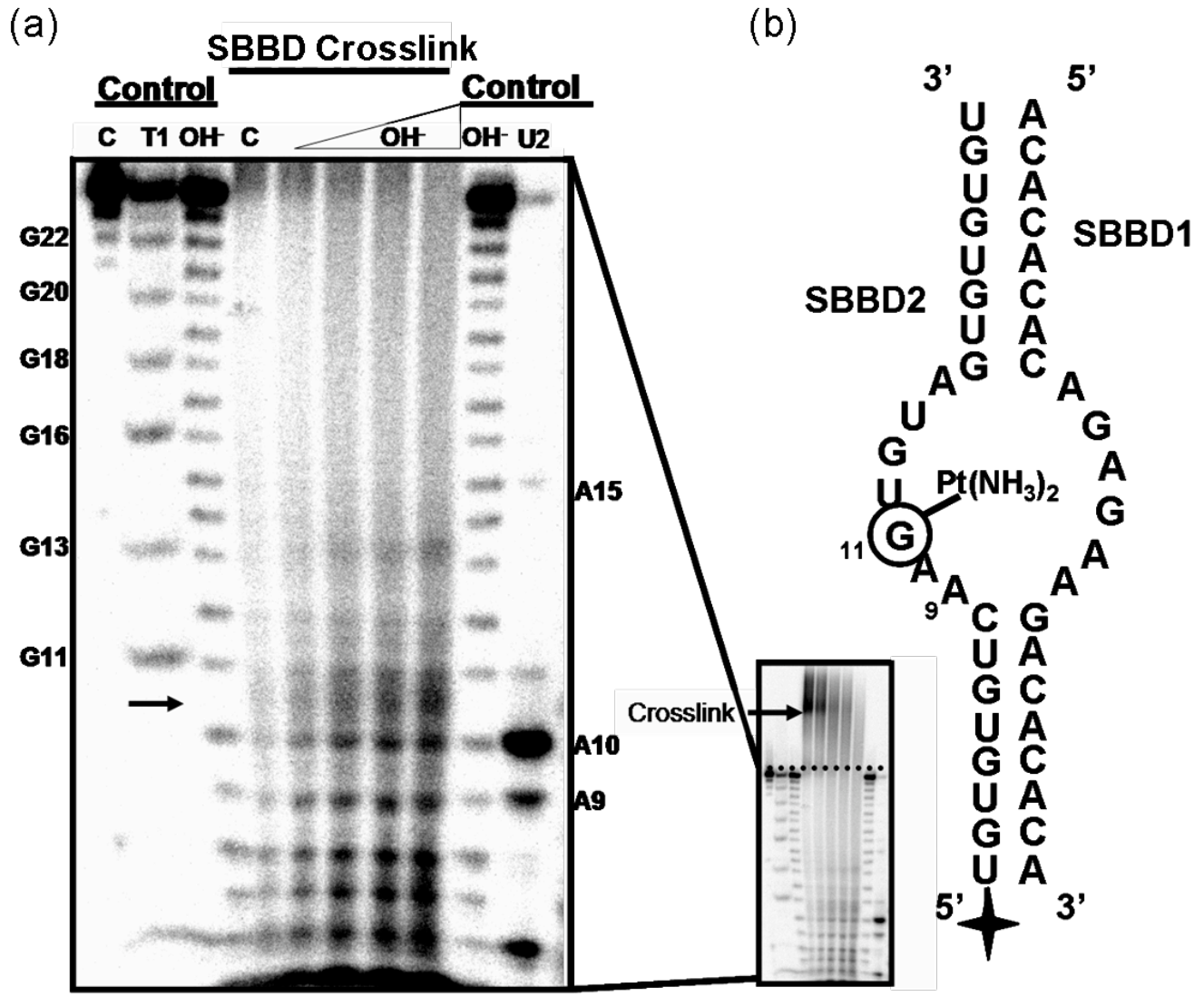


Figure S1. (a) 20% denaturing PAGE analysis of the products of partial alkali hydrolysis of the SBBD crosslink with 5'-end-labeled SBBD2. The crosslinking site G11 on SBBD2 corresponds to G9 in BBD. Lanes: Control- **C:** 5' end-labeled SBBD2. **T1:** G specific sequence ladder generated by partial nuclease digestion of SBBD2 RNA by T1 RNase. **U2:** A specific sequence ladder generated by partial nuclease digestion of SBBD2 RNA by U2 RNAse. **OH^-:** reference alkali hydrolysis ladder for SBBD2. SBBD crosslink Samples- **C:** dPAGE-isolated SBBD2 crosslink **OH^- Lanes:** dPAGE-isolated SBBD crosslink treated under alkali hydrolysis for increasing amounts of time. (b) The predicted secondary structure of hybridized SBBD1 and SBBD2 RNAs with the location of the major $[Pt(NH_3)_2]^{2+}$ on the SBBD2 strand highlighted.

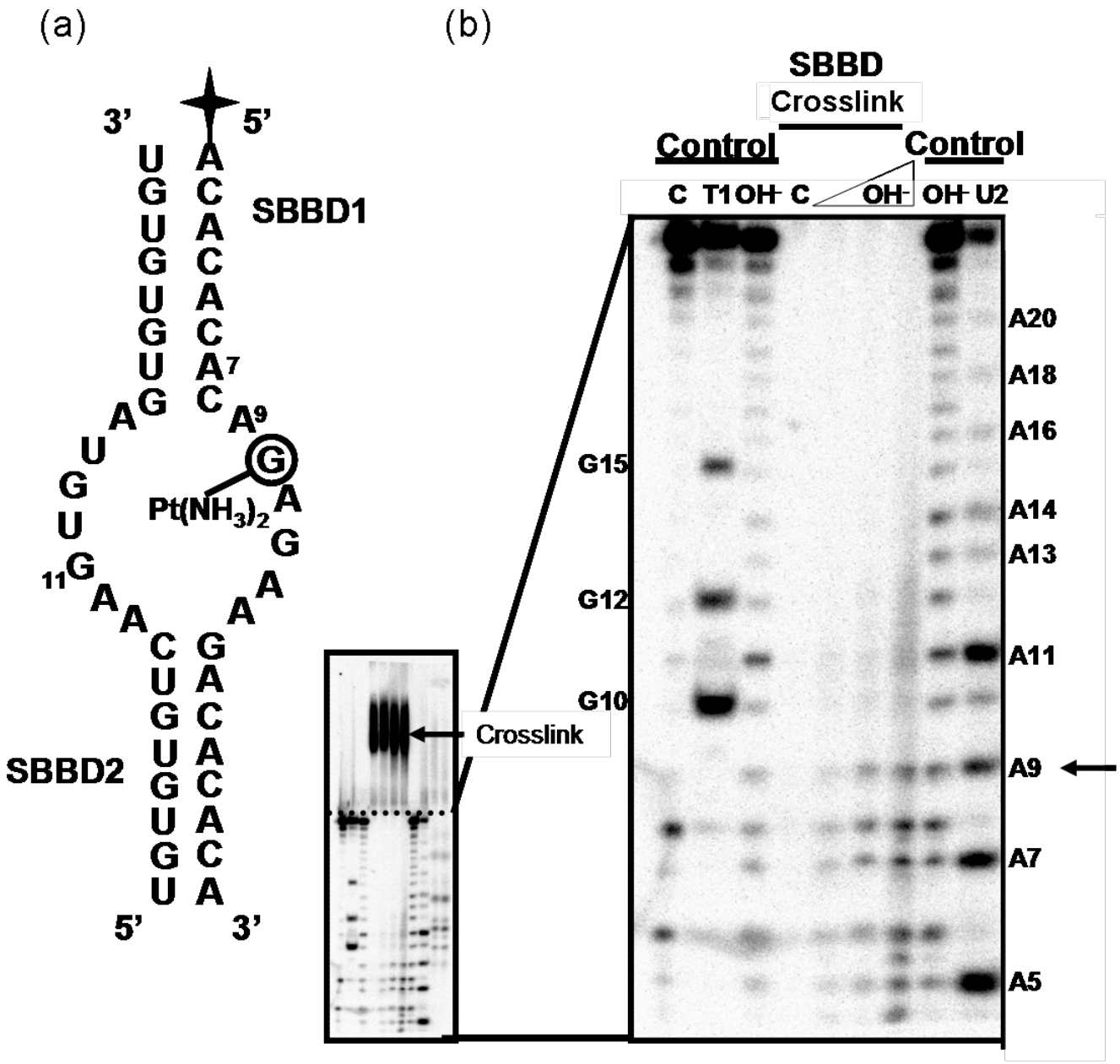


Figure S2. (a) The predicted secondary structure of hybridized SBBD1 and SBBD2 RNAs with the location of the major platinum induced crosslink on the SBBD1 strand highlighted. The crosslinking site G10 on SBBD1 corresponds to BBD site G31. (b) 20% dPAGE analysis of the products of partial alkali hydrolysis of the SBBD crosslink with 5'-end-labeled SBBD1. Lanes: Control- **C:** 5' end-labeled SBBD1. **T1:** G specific sequence ladder generated by partial nuclease digestion of SBBD1 RNA by T1 RNase. **U2:** A specific sequence ladder generated by partial nuclease digestion of SBBD1 RNA by U2 RNAse. **OH^-:** Reference alkali hydrolysis ladder for SBBD1. SBBD Crosslink Samples- **C:** dPAGE-

isolated SBBD crosslink. **OH^- Lanes:** dPAGE-isolated SBBD crosslink treated under alkali hydrolysis for increasing amounts of time.

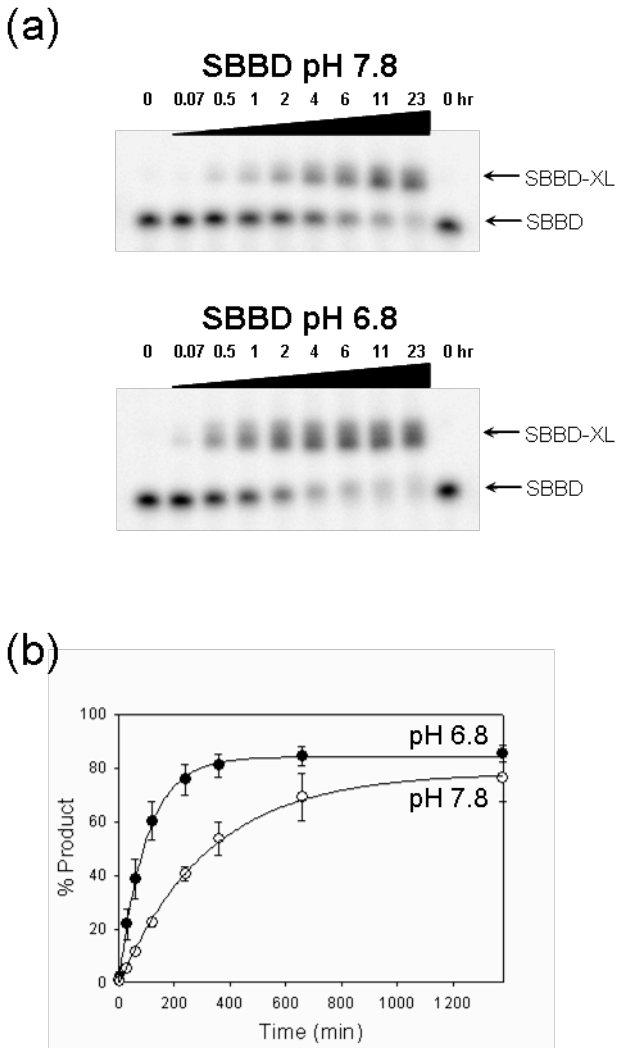
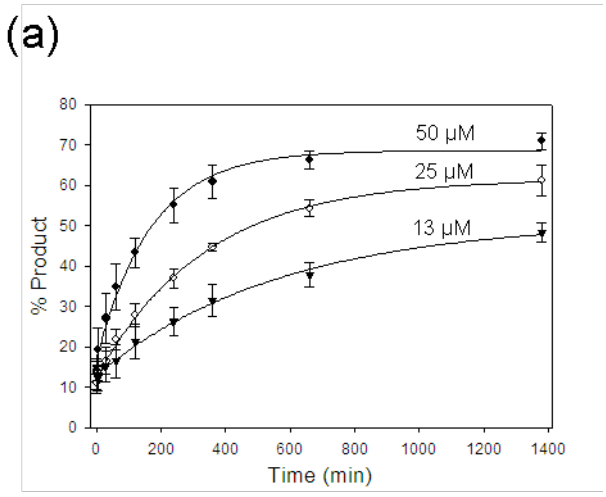


Figure S3. (a) dPAGE radiograms depicting products of cisplatin binding to SBBD in pH 6.8 and 7.8 over time. (b) Comparison of the reaction rates of SBBD in pH 6.8 (filled circles) and 7.8 (open circles). Conditions: (a) Reactions were performed with 0.1 μM SBBD duplex with either 50 μM aquated cisplatin in 5 mM TEA (pH 7.8) or 25 μM aquated cisplatin in 5 mM MOPS (pH 6.8). Both reactions were in 100 mM $NaNO_3$, 1 mM $Mg(NO_3)_2$, and at 37 °C. Reactions analyzed by 20% dPAGE.



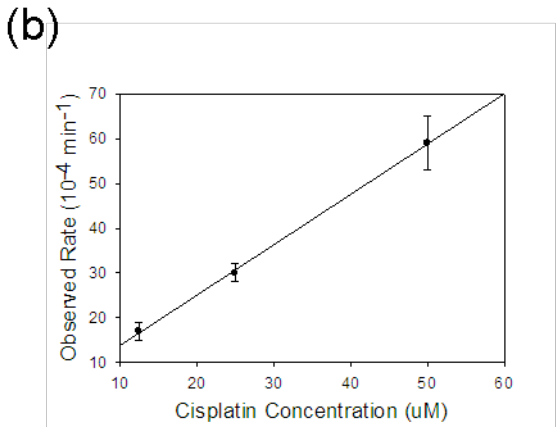


Figure S4. (a) BBD kinetics with 50 µM (filled circles), 25 µM (open circles), and 12.5 µM (triangles) aquated cisplatin. (b) Observed rate constant (k_{obs}) versus cisplatin concentration. Conditions: Reactions were performed in 100 mM $NaNO_3$, 1 mM $Mg(NO_3)_2$, and 5 mM TEA (pH 7.8) at 37 °C.

Table S1. Platination Rates of Oligonucleotide Constructs[a]

Construct	μM Pt	pH	k_{obs} (10^{-5} s^{-1})	k_{rxn2} (M^{-1} s^{-1})[b]
BBD	50	7.8	9.8(1.0)	2.0(2)
BBD	25	7.8	5.0(3)	2.0(1)
BBD	12.5	7.8	2.8(3)	2.3(2)
BBD	25	6.8	21.3(1.8)	8.5(7)
RNA HP	50	7.8	8.3(2)	1.7(3)
DNA HP	50	7.8	1.7(2)	0.33(3)
SBBD	50	7.8	5.2(3)	1.1(1)
SBBD	25	6.8	17.0(5)	6.8(2)

a All experiments used 0.1 μM oligonucleotide (or 0.1 μM SBBD duplex) and were performed at 37 °C with 100 mM $NaNO_3$ and 1 mM $Mg(NO_3)_2$. Trials at pH 7.8, and 6.8 were done in 5 mM TEA and MOPS respectively.

b Because all kinetics were performed under pseudo-first order conditions, second order rate constants were obtained by dividing k_{obs} by the concentration of aquated cisplatin used.