# SUPPORTING INFORMATION

## Catalytic MetalloDrugs Targeting HCV IRES RNA

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## Experimental Methods

### *Materials.*

RNA was purchased from Dharmacon, part of Thermo Fisher Scientific (Lafayette, CO). Peptides were purchased from Genemed Synthesis Inc (South San Francisco, CA) with C-terminal amidation. The sequence for the IRES SL IIb RNA used was 5'-Fluorescein-GGCAGAAAGCGUCUAGCCAUGGCGUUAGUAUGCC-3', for the IRES SL IV RNA was 5'-Fluorescein-GGACCGUGCACCAUGAGCACGAAUCC-3', and for HIV Rev Response Element (RRE) RNA was 5'-Fluorescein-UUGGUCUGGGCGCAGCGCAAGCUGACGGUACAGGCC-3'. All RNA was annealed by heating to 95° C and then cooled slowly to room temperature before use.

### *RNA Binding Experiments.*

RNA binding experiments were performed in the presence of 84 nM GGHYrFK-amide (**1**) in 20 mM HEPES (pH 7.4), 100 mM NaCl. For GGHYrFK-amide, serial additions of unlabeled IRES RNA were added and tyrosine emission was monitored ($\lambda_{ex}$ = 280 nm, $\lambda_{em}$ = 313 nm). Binding data for GGHYrFKGGGYGRKKRRQRRR-amide (**1**-Tat, **2**) and GGHYrFKGGGKDEL-amide (**1**-KDEL, **3**) were obtained by adding serial additions of peptide to 5'-fluorescein labeled IRES SLIIb and monitoring at 518 nm (Ex = 485 nm). Data was then fit to a one-site binding model.

***Reaction Kinetics via Fluorescence.***

HCV IRES RNA cleavage was monitored *in vitro* by fluorescence using 5' fluorescein end-labeled RNA with excitation and emission wavelengths of 485 nm and 518 nm, respectively. Reactions were carried out in reaction volumes of 100 µl in the presence of 1 mM ascorbate and 1 mM $H_2O_2$ in 20 mM HEPES buffer (pH = 7.4), 100 mM NaCl with 100 nM of the catalyst and analyzed according to the change in fluorescence observed as the reaction occurred. Both a time-dependence and a concentration-dependence of RNA substrate were observed. The initial velocities were fit to a line using Origin 7.0 software and $k_{obs}$ obtained. These values were then used to obtain the Michaelis-Menten parameters.

***HCV Cellular Replicon Assay.***

A stable cell line ET (luc-ubi-neo/ET) was employed in the assay; specifically, a Huh7 human hepatoma cell line that contains three cell culture-adaptive mutations.[34] The composition of the HCV RNA replicon with a stable luciferase (Luc) reporter and replicon is shown diagrammatically in Fig. S2, wherein the Luc reporter is used as an indirect measure of HCV replication. The activity of the Luc reporter is directly proportional to HCV RNA levels and positive control antiviral compounds behave comparably using either Luc or RNA endpoints. The HCV RNA replicon antiviral evaluation assay examined the effects of compounds at six half-log concentrations each. Human interferon alpha-2b was included in each run as a positive control compound. Sub-confluent cultures of the ET line were plated out into 96-well plates that were dedicated for the analysis of cell numbers (cytotoxicity) or antiviral activity, and various concentrations of metallodrugs and controls were added to the appropriate wells the following day. Cells were processed 72 h later when the cells were still sub-confluent. Six half-log serial dilutions of the compound were performed, and values derived for $IC_{50}$ (the concentration that inhibited virus replication by 50%), $TC_{50}$ (the concentration that lowered cell viability by 50%) and TI (the selectivity index: $TC_{50}/IC_{50}$). HCV RNA replicon levels were assessed as the replicon-derived Luc activity. The toxic concentration of drug that reduced cell numbers (cytotoxicity) was assessed by the CytoTox-1 cell proliferation colorimetric assay (Promega).

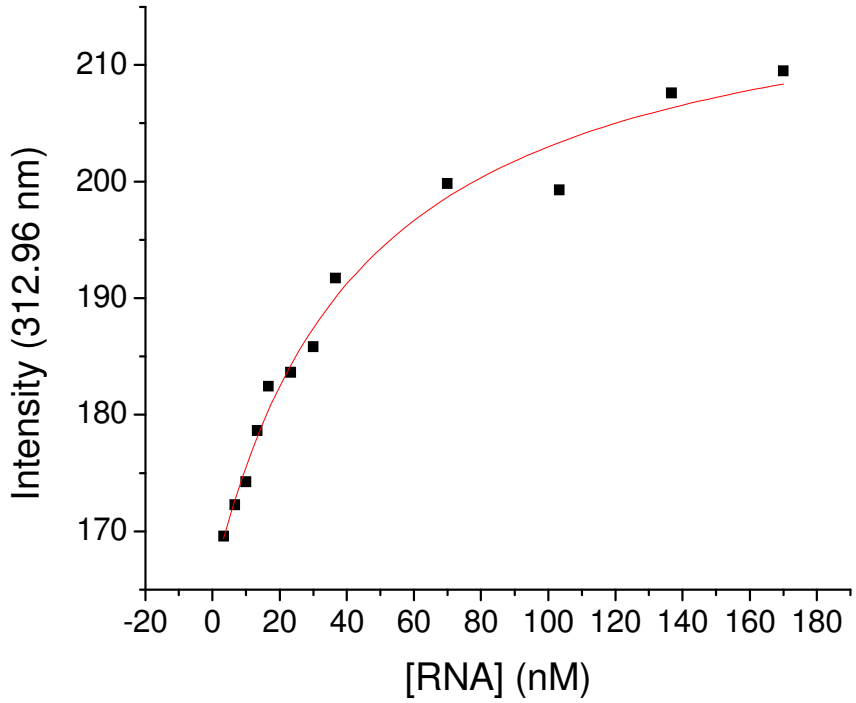


**Fig. S1**. Binding curve for GGHYrFK-amide (**1**). [**1**] = 84 nM, [HEPES] = 20 mM, [NaCl] = 100 mM, pH = 7.4. The tyrosine on **1** was excited at 280 nm and monitored at 312.96 nm.

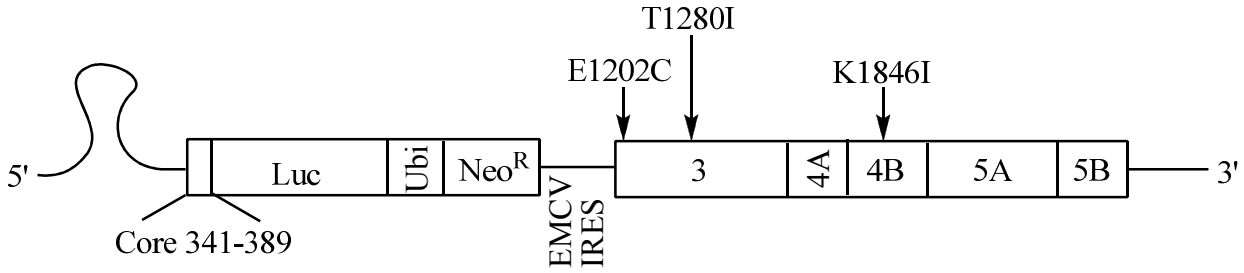


**Fig. S2**. Composition of HCV RNA replicon used in cellular assays[1] by Southern Research Institute. The HCV RNA replicon ET contains the 5' end of HCV (with the HCV Internal Ribosome Entry Site (IRES) and the first few amino acids of the HCV core protein) which drives the production of a firefly luciferase (Luc), ubiquitin (Ubi), and neomycin phosphotransferase ($Neo^R$) fusion protein. Ubiquitin cleavage releases the Luc and $Neo^R$ proteins. The EMCV IRES element controls the translation of the HCV structural proteins NS3-NS5. The NS3 protein cleaves the HCV polyprotein to release the mature NS3, NS4A, NS4B, NS5A and NS5B proteins that are required for HCV replication. At the 3' end of the replicon is the authentic 3' NTR of HCV. The sites of the three cell culture adaptive mutations are shown as arrows.

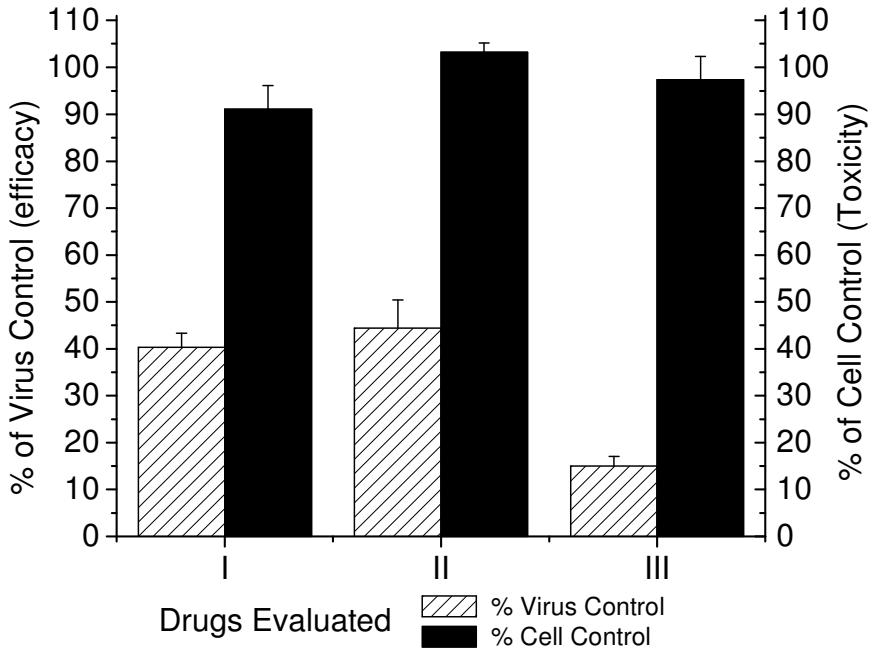


**Fig. S3**. Combination treatment of **1**-Cu with rIFNα-2b showing additive-synergistic effects (I, 0.2 μM **1**-Cu; II, 0.2 IU/mL rIFNα-2b; III, 0.2 μM **1**-Cu + 0.2 IU/mL rIFNα-2b).

**Table S1.** Effect of uptake sequences on binding and reactivities of metallodrugs targeting IRES SL IIb.

| Compound | $IC_{50}$ (μM) | $K_D$ (nM)[a] | Slope (RFU*$min^{-1}$* $\mu M^{-1}$) |
|---|---|---|---|
| **1**-Cu | 0.58 | 44 | 0.111 |
| **2**-Cu | 1.09 | 174 | 0.09 |
| **3**-Cu | 0.67 | 656 | 0.143 |

[a] $K_D$ values listed are for the free peptides.

## Reference

1. Pietschmann, T., V. Lohmann, A. Kaul, N. Krieger, G. Rinck, G. Rutter, D. Strand, and R. Bartenschlager.. *J. Virol*. 2002 **76**, 4008-4021.