Supporting Information

Biological Stability and Activity of siRNA in Ionic Liquids**

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1 Experimental Procedures

Materials: CD45 siRNA, phosphate buffered saline (PBS) tablets, acrylamide/bisacrylamide 40% (19:1) and ammonium persulfate were purchased from Sigma Aldrich. eGFP siRNA, 10 x Tris-Borate-EDTA, ultrapure N,N,N′,N′-tetramethylethylenediamine (TEMED), Dulbecco Modified Eagle medium (DMEM), Fetal Calf Serum (FCS), Optimem, RNAiMAX, alamarBlue reagent and Hanks Balanced Salt Solution (HBSS) were purchased from Life Technologies. The siRNA ladder, microRNA marker and 4x loading dye were purchased from New England Biolabs care of Genesearch. The miRNA-21-mer was from Trilink Biotechnologies and a kind gift from Dr Lillian Lee, RMIT University, Melbourne. Choline dihydrogen phosphate (CDP) and the buffered ionic liquid (IL) were respectively prepared as per the literature procedure[1-3]. Further dilutions of the CDP- IL were carried out with water to achieve final compositions of 50% (w/w) IL and 20%(w/w) IL. Hela eGFP cells were purchased from CellBiolabs.

Cell Culturing: The activity of eGFP siRNA after storing it in 20% (w/) IL and 50% (w/w) IL was examined. Typically, HeLa GFP cells were plated in a 48 well plate with 30 000 cells/well in DMEM with 10% FCS. The cells were incubated at 37^{o}C with 5% CO_2 and grown for 24 hours. HeLa eGFP cells were then transfected with eGFP siRNA (15 pmole siRNA per well) according to the manufacturer's instructions (for Lipofectamine) and incubated for 48 hours.

To measure cytotoxicity, an identical plate was prepared and following the 48 h incubation, alamarBlue was added according to the manufacturer's instructions. HBSS was used instead of media and the plate was read 24 h after alamarBlue addition.

Gel Electrophoresis: All gel making and running apparatus was purchased from Bio-Rad. Native polyacrylamide gel electrophoresis (PAGE) and 7M urea polyacrylamide gel electrophoresis, was used to qualitatively determine the stability of siRNA. The gels were prepared using short plates and spacer plates assembled in a gel cassette assembly system before securing it in a casting stand and immediately after pouring the gel a 10 well comb was inserted. The gels were left to polymerize for at least an hour before usage and if necessary stored at 4^{o}C for up to one week maximum.

The gels were used to run the siRNA samples in a Mini-PROTEAN Tetra cell using a PowerPac. Urea PAGE, the gels were run for 15 minutes prior to loading to heat the gel. All gels were run at 70V for 75 minutes for as the loading dye approached the end of the gel.

Following the run, the gels were stained with 1x SYBR Safe prepared in 1 X TBE for 20 minutes. Once stained, gels were visualised using a Molecular Imager Gel Doc XR System with Quantity One software. **UV-Vis Spectroscopy:** The melting temperature curves for eGFP siRNA were measured using a Cary UV-VIS, controlled by a thermostat and using a thermal package. eGFP siRNA was prepare at 1 μM in its respective buffer and heated to 95^{o}C for three minutes before slowly cooling to room temperature and conducting the measurements. The absorbance was measured using a wavelength of 275 nm, with a slit average of 2 nm, at an average time of 0.2 s and ramping at 1^{o}C/min. The thermal package was then used to derive the 1st derivative, and conduct the two state process for van't Hoff's model. The raw data measured was smoothed using a 25 pt algorithm using OriginPro 8.

Flow Cytometry: eGFP gene knockdown was determined by flow cytometry. Following a 48 hour transfection with eGFP siRNA, HeLa eGFP cells were trypsinized, washed three times in PBS before resuspending in PBS with 2% FCS. The samples were kept on ice and analysed by flow cytometry on a BD FACS Calibur. Data was analysed using Flow Jo V10. Just prior to analysis by flow cytometry, propidium iodide was added to stain dead cells.

Circular Dichroism Spectroscopy: The siRNA samples were prepared at stock concentrations and degraded at 37^{o}C for 1 hour before diluting the solution to 1.5 μM to measure on the circular dichrosim (CD) spectrometer. The samples were run from 200 nm to 350 nm and an accumulation of three measurements, per sample was obtained. The resulting data was smoothed by a 25 pt algorithm using OriginPro 8.

2 siRNA sequence

CD45 siRNA duplex

5'dTdTCUGGCUGAAUUUCAGAGCA3'
3'GACCGACUUAAAGUCUCGUdTdT5'

miRNA-21-mer

5'UAGCUUAUCAGACUGAAGUUGA3'

3 Additional Results

3.1 siRNA degradation mechanism

Figure S1 below illustrates both CD45 siRNA and eGFP siRNA profiles in 10% native PAGE and 12% urea PAGE (7 M). In order to help identify the double stranded and single stranded siRNA as well as the effect of CDP-IL on the siRNA under denaturing conditions. Evidently the CDP-ILs is also able to restrict the destabilization of double stranded siRNA as less single stranded siRNA can be seen upon increasing amount of IL in Figure S1 (b) and (d).

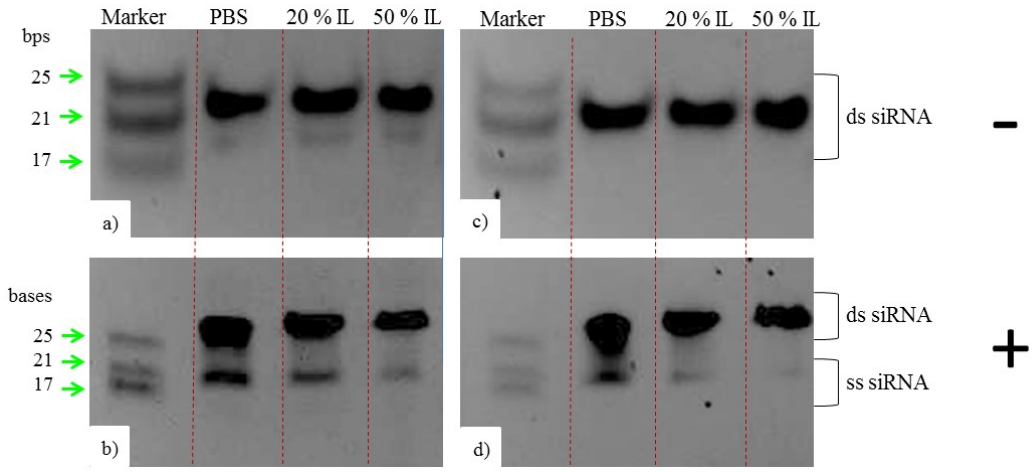

Figure S1: Degradation mechanism of CD45 siRNA and eGFP siRNA profiled. CD45 siRNA (a) Native PAGE (b) Urea PAGE and eGFP siRNA (c) Native PAGE and (d) Urea PAGE. The positive denotes denaturing heat treatment at 95^{o}C for 5 minutes and the negative denotes no heat treatment.

3.2 siRNA degradation kinetics

Figure S2 below shows CD45 siRNA in 12% urea PAGE (7 M). After the four hours there is primarily single stranded CD45 siRNA remaining.

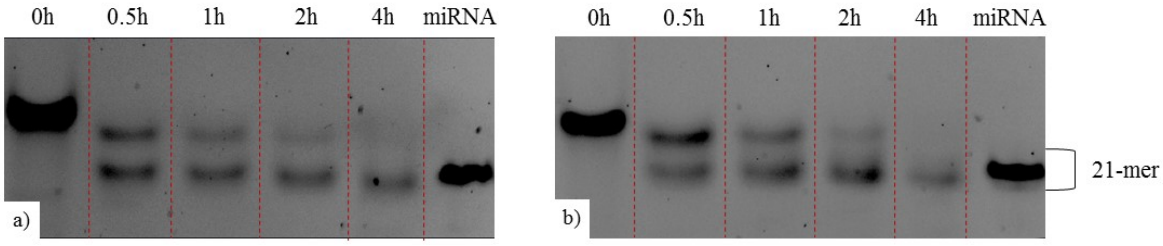

Figure S2: Degradation kinetics of CD45 siRNA on native PAGE gels. (a) 20% IL (b) 50% IL. 0h denotes no treatment with RNase A. 21mer single-stranded miRNA sizer marker was loaded into the last lanes of both gels.

3.3 Time Lapse Profile of RNase A concentration on CD45 siRNA in PBS, 20% IL and 50% IL

Figure S2 below is a continuation of Figure 2 in the main text over a period of almost 4 months. The concentration of RNase A is 0.25mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml.

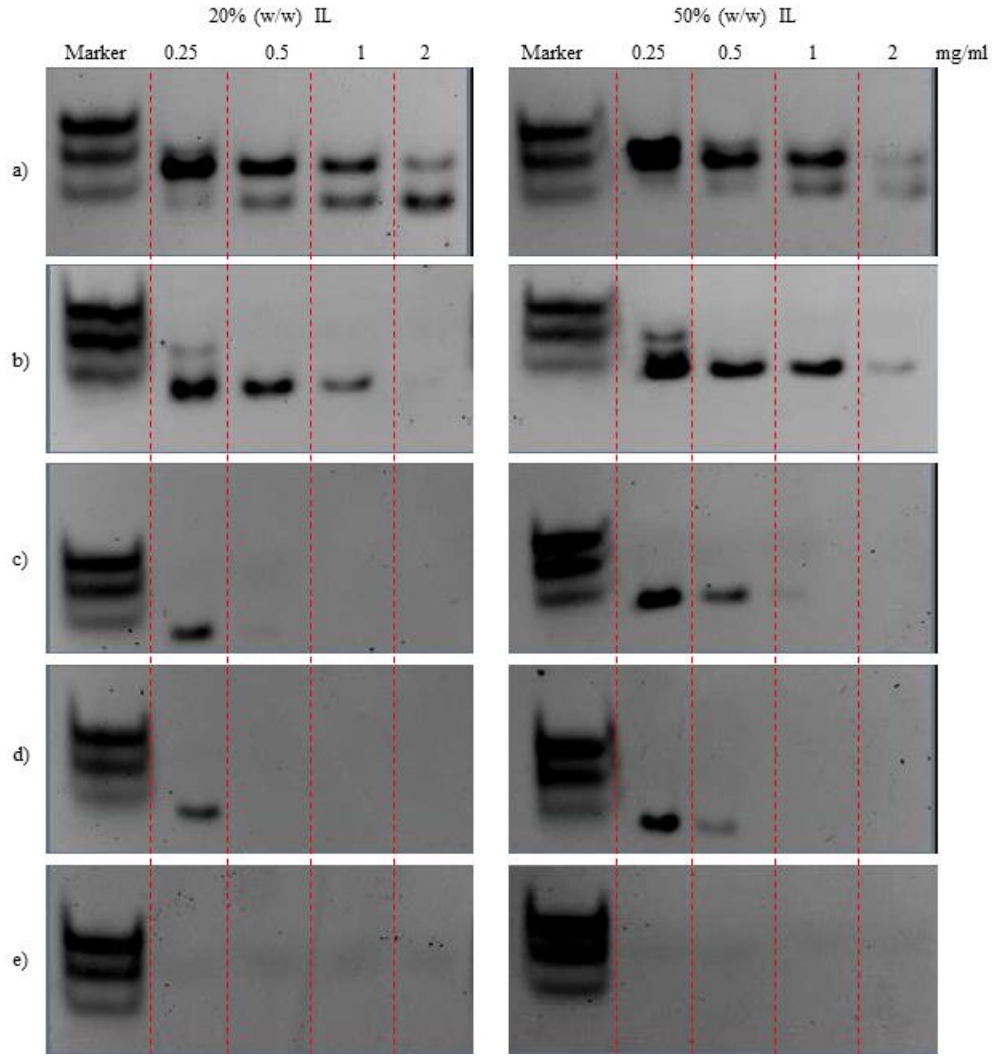

Figure S3: Degradation profile of CD45 siRNA in PBS, 20% (w/w) IL and 50% (w/w) IL. Native gel electrophoresis images of stock samples treated with RNase A on Day 0 for 1 hour at 37^{o}C and thereafter an aliquot was taken for the following days from the same stock at: a) Day 0; b) Day 13; c) Day 55; d) Day 97; e) 111 of incubation.

3.4 Time Lapse Degradation Profile of eGFP siRNA in PBS, 20% IL and 50% IL

Native PAGE gels were run periodically over a long period of time to investigate the stability of eGFP siRNA. The siRNA was treated with 1 mg/ml of RNase A for 1 hour at 37°C on Day 3 in PBS, 20% (w/w) IL and 50% (w/w) IL.

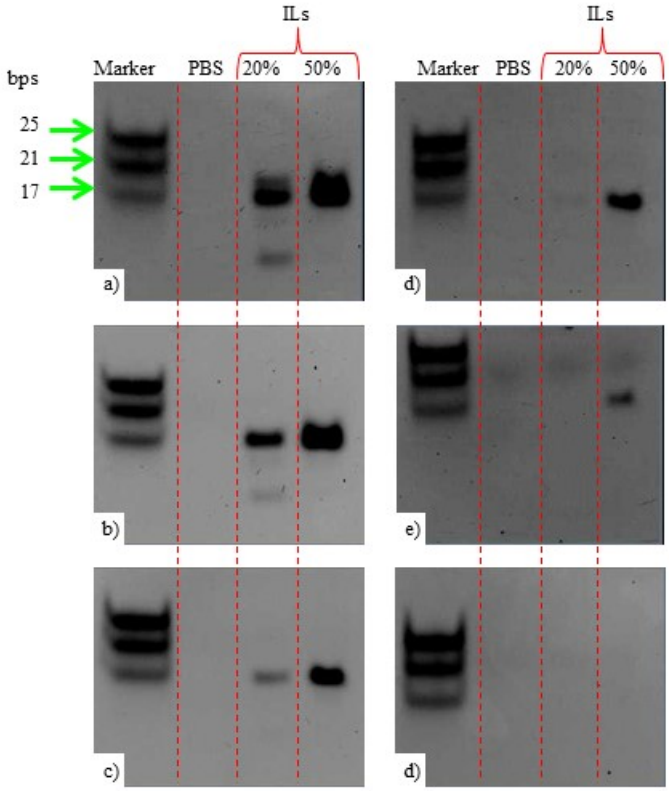

Figure S4: Degradation profile of eGFP siRNA in PBS, 20% (w/w) IL and 50% (w/w) IL. Native PAGE images of stock samples treated with RNase on Day 0 for 1 hour at 37°C and thereafter an aliquot was taken for the following days from the same stock at: a) Day 0; b) Day 3; c) Day 9; d) Day 28; e) Day 63; and f) Day 98 of incubation.

3.5 Melting Temperature Curves

Melting temperature curves were run on a Cary UV-Vis with a 6 cell Peltier. The peak of the siRNA in each buffer was first determined before the melting temperature was run at a slit of 2 nm and average time of 0.2 s. Each sample was run in triplicates before conducting thermodynamic analysis.

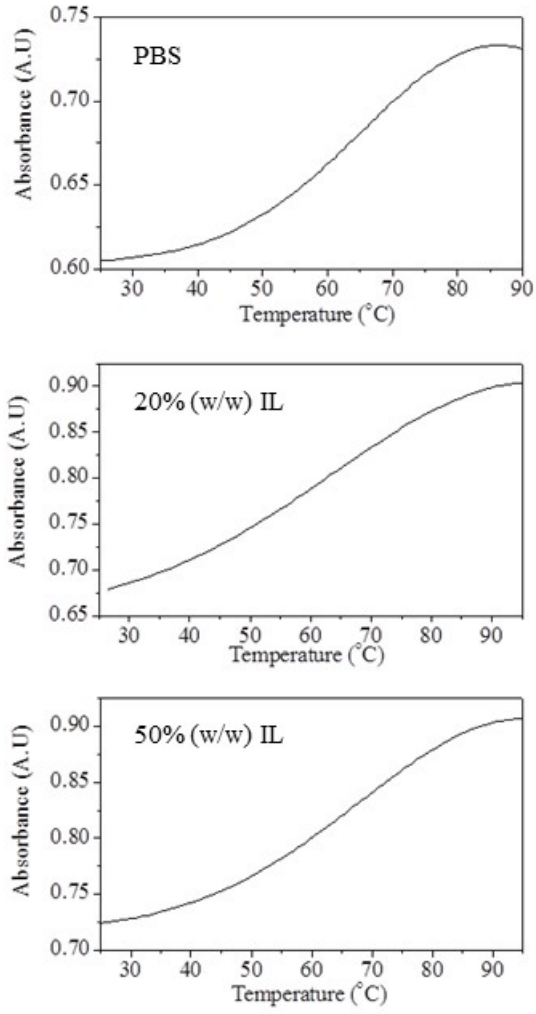

Figure S5: Melting Temperature profile of eGFP siRNA in PBS, 20% (w/w) IL and 50% (w/w) IL.

4 References

1. K. Fujita, D. R. MacFarlane and M. Forsyth, *Chemical communications*, 2005, 4804-4806.

2. R. Vijayaraghavan, B. Thompson, D. MacFarlane, R. Kumar, M. Surianarayanan, S. Aishwarya and P. Sehgal, *Chem. Commun.*, 2009, **46**, 294-296.

3. D. R. MacFarlane, R. Vijayaraghavan, H. N. Ha, A. Izgorodin, K. D. Weaver and G. D. Elliott, *Chemical Communications*, 2010, **46**, 7703-7705.