Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Inhibited Fragmentation of mAbs in Buffered Ionic Liquids**

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1 Experimental Sections

Materials: EGFR mAb was kindly provided by Monash Antibody Technology Facility, with special thanks to Professor Edouard Nice and Dr Caroline Laverty. Phosphate buffered saline (PBS) tablets, and SDS-PAGE kit (Fluka) were purchased from Sigma Aldrich. Proteinase K, Dulbecco Modified Eagle medium (DMEM), Fetal Calf Serum (FCS) were purchased from Life Technologies. The SDS-PAGE broad range marker and loading dye were purchased from BioRad. Rabbit anti-mouse IgG-PE was purchase from Santa Cruz Biotechnology. bCDHP is prepared, purified and characterised as described previously [1] by partial neutralisation of locally prepared CDHP with choline hydroxide solution (20 wt% in water). The target proton activity (pH = 7.2) of the mixture is initially determined and subsequently confirmed by determination of the pH titration curve of the CDHP diluted in water at 0.1 M concentration. Final water- bCDHP mixtures are made by dilution with deionisied water. HeLa cells were purchased from CellBiolabs.

SDS-PAGE: All gel making and running apparatus was purchased from Bio-Rad. Sodium Dodecyl Sulfate (SDS) polyacrylamide gel electrophoresis (PAGE), was used to qualitatively determine the stability of mAbs. 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 SDS-PAGE gels were prepared to be 10% separation which was left to polymerize for 20-30 minutes before pouring the 8% stacking 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 mAbs samples in a Mini-PROTEAN Tetra cell using a PowerPac. All gels were run at 150V for 55 minutes or as the loading dye approached the end of the gel. Following the run, the gels were stained with coomassie brilliant blue for 2 hours

and de-stain using de-staining solution and regularly checked in order to prevent complete de-staining. Once de-stained well, gels were scanned using a Canon Scanner.

Cell Culturing & Flow Cytometry: The activity of EGFR mAb before and after proteinase K digestion was tested in PBS, 20% (w/w) bCDHP and 50% (w/w) bCDHP and examined. Typically, HeLa cells were plated directly into FACS tubes with 250 000 cells/tube and washed three time before incubating in PBS with 1% BSA on ice for 20 minutes. Then they were incubated with EGFR mAb for 1 hour on ice. Following the one hour, they were again washed three times and incubated with the secondary antibody on ice for another hour. Subsequently HeLa cells were washed again three times before re-dispersing in PBS with 1% BSA. The samples were kept on ice and analysed by flow cytometry on a CyAn Flow Cytometer. Data was analysed using Flow Jo V10.

Circular Dichroism Spectroscopy: The EGFR mAb samples were prepared at stock concentrations (500 μg/mL) and degraded at 37^{o}C with proteinase K for various time intervals (10 mins, 1 hr, 12 hr, 24 hr, 48 hr and 7 days) before diluting the solution to 50 μg/μL to measure on the circular dichrosim (CD) spectrometer. The samples were run from 200 nm to 250 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 References

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