

Finally the absorbance at 550 nm of the plates was read with the Tecan plate reader. Absorbance values were blanked against DMSO and the absorbance of cells exposed to medium only (i.e. no polymer or polyplex added) were taken as 100 % cell viability (i.e. the control).

2.3.4 Flow Cytometry: General Procedure for the Analysis of Cells

To exclude non-viable cells and debris from the analysis by flow cytometry, the viable cell population was determined. Viable cells have different scatter characteristics to non-viable cells due to their morphology; the scatter plot was therefore used to select the viable cell population. Flow cytometry was used in Chapter 5 to assess cell association and relative binding affinity of ligands for uPAR. Following incubation with fluorophores all subsequent steps were conducted at 4 °C. After treatment with fluorophores and rinsing with PBS adherent cells were scraped in PBS and centrifuged (2000 RCF, 2 min, 4 °C), the supernatant removed and the cell pellet re-suspended in 300 µl of PBS (4 °C). U937 cells were centrifuged after treatment with fluorophores and rinsed by re-suspension in PBS/BSA (0.1 %, 4 °C) twice. The method was derived from that described by Ormerod et al. (2000). In the side/forward scatter plot viable cells were selected as a region and this region used to collect events, 10000 events were collected for each sample. Forward and side scatter settings for the different cell lines are shown in Table 2.3, as is the gain for FL-1 (530 nm).

2.3.5 Evaluation of Protein Content Using the Bicinchoninic Acid (BCA) Assay

The BCA assay is widely used to quantify protein (Smith et al., 1985). The method reported previously was used and scaled accordingly. The protein content of cell homogenates was determined to standardise the amount loaded in western blots (Chapter 5), and to establish the specific activity of luciferase in transfection experiments (Chapters 4 and 6). Briefly, in both cases BCA was added to a CuSO₄ pentahydrate solution (4 % w/v) at a ratio of 1:50 and 200 µl of this reagent was added to 20 µl of cell lysate (Sections 2.3.7 and 2.3.8.1) and incubated at 37 °C for 25 min. The absorbance of the copper-I-BCA complex was measured at 550 nm using the Tecan plate reader. Calibration standards of bovine serum albumin (BSA) in homogenisation buffer were treated identically to samples and used to quantify the amount of protein in the fractions (Fig. 2.3).