Determination of Gd^{III} content in cells.

At the end of the uptake experiment, the medium was removed and cells washed three times with EBSS buffer. Cells were then collected in 200μL EBSS (Earl's balanced salt solution) and were sonicated for 10s for a complete lysis, added with the same volume of HCl 37% and left at 120˚C overnight. Upon this treatment all Gd^{III} was solubilized as free aquo-ion. By measuring the water proton relaxation rate of these solutions, it is possible to determine its concentration.[1] Relaxation rate measurements were performed at 20 MHz and 25˚C on a Spinmaster spectrometer (Stelar, Mede, Italy), by using a conventional Inversion Recovery pulse sequence. The obtained R_{1obs} data are related to the concentration of the paramagnetic species according to the formula:

$$R_{1obs} = R_{1W} + [Gd^{III}] \cdot r_{1p}{}^{Gd(III)}$$

where R_{1W} is the relaxation rate of pure water (0.38 s^{-1}) and $r_{1p}{}^{Gd(III)}$ the millimolar relaxivity of the Gd^{III} aquo-ion (13.5 $mM^{-1}s^{-1}$ in 6M HCl, 25 °C). The moles of Gd^{III} obtained in this way were normalized against the weight (in milligrams) of cellular proteins. The protein concentration of each sample was determined from cell lysates by the Bradford method[2] using bovine serum albumin as the standard.

References

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2) M.M. Bradford *Anal. Biochem.* 1976, **72**:142-146.