# **Supplementary Information for**

## Polymeric PARACEST MRI contrast agents as potential reporters for gene therapy

Yunkou Wu,<sup>1</sup> Christiane E. Carney,<sup>2</sup> Michael Denton<sup>3</sup>, Elaine Hart<sup>3</sup>, Piyu Zhao,<sup>1</sup> Daniel N. Streblow,<sup>3</sup>

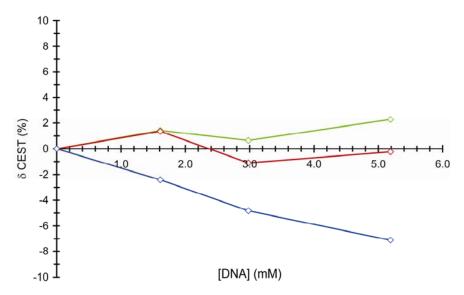
A. Dean Sherry<sup>1,4</sup> and Mark Woods,<sup>2,5</sup>

- 1) Department of Chemistry, University of Texas at Dallas, P.O. Box 830668, Richardson, Texas, 75083.
- 2) Department of Chemistry, Portland State University, P.O. Box 751, Portland, OR 97207 Tel: + 1 503 725 8238, Fax: + 1 503 725 9525
- 3) Vaccine and Gene Therapy Institute, Oregon Health and Sciences University, 505 NW 185th Avenue. Beaverton, Oregon 97006.
- 4) Advanced Imaging Research Center, University of Texas Southwestern Medical Center, 5325 Harry Hines Blvd, Dallas, Texas 75235.
- 5) Advanced Imaging Research Center, Oregon Health and Sciences University, 3181 S.W. Sam Jackson Park Road. L452, Portland, Oregon 97239.

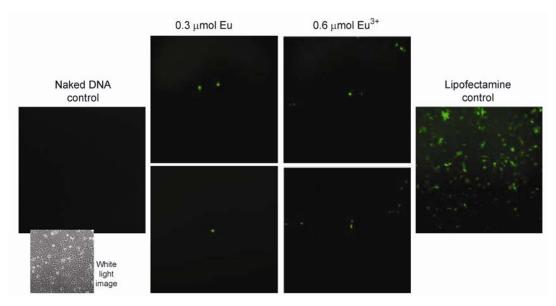
Tel: + 1 503 418 5530, Fax: + 1 503 418 1543

E-mail: mark.woods@pdx.edu or woodsmar@ohsu.edu

### Supplementary Information for Wu et. al.

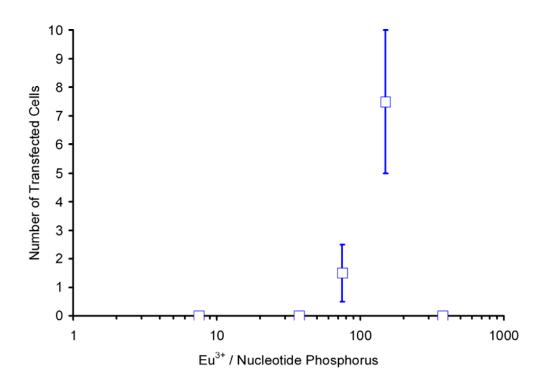


**Fig. S1.** The observed change in CEST ( $\delta$  CEST) as a function of added DNA concentration, expressed as a percentage of the CEST observed when [DNA] = 0 mM. Data is shown for a 5 mM solution of Eu1 (blue, B<sub>0</sub> = 500 MHz, B<sub>1</sub> = 600 Hz, irr. time = 2s 298 K, pH 7.0, PBS); Eu2 (red, B<sub>0</sub> = 600 MHz, B<sub>1</sub> = 600 Hz, irr. time = 3s 298 K, pH 7.5, TBS) and Eu3 (green, B<sub>0</sub> = 500 MHz, B<sub>1</sub> = 600 Hz, irr. time = 2s 298 K, pH 7.0, PBS).



**Fig. S2.** Images demonstrating the transfection of HEK293 cells with GFP using Eu1 at 0.3  $\mu$ mol Eu<sup>3+</sup> of (left column) and 0.6  $\mu$ mol Eu<sup>3+</sup> (right column) with 1.4  $\mu$ g of the plasmid DNA under a fluorescence microscope at 10× magnification. The image on the left shows the result of a control experiment transfecting with naked DNA (a white light image is provided to demonstrate the presence of the cells); the image on the right demonstrates the result of a control experiment using lipofectamine.

### Supplementary Information for Wu et. al.



**Fig S3.** An optimization curve showing that the transfection efficiency of the GFP plasmid improves with increasing amount of Eu1 employed as transfection agent, to a point. After an Eu<sup>3+</sup>/nucleotide phosphorus ratio of 150 is exceeded transfection appears to cease altogether.

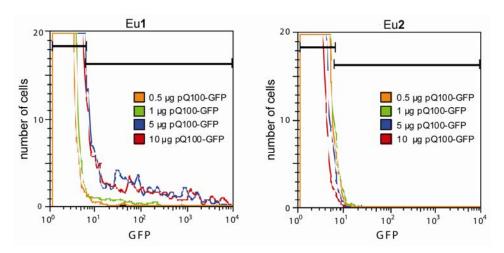
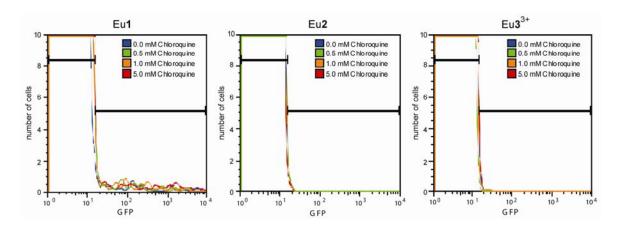
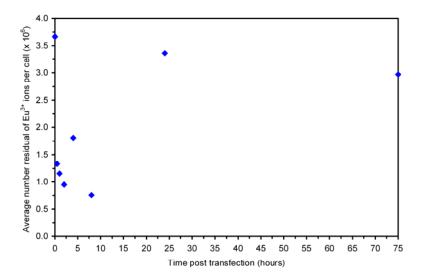


Fig. S3. The extent GFP gene expression in HEK293 cells determined by flow cytometry using Eu1 (left) and Eu2 (right).  $0.6 \mu mol$  of Eu<sup>3+</sup> was used with  $0.5 \mu g$  (orange),  $1.0 \mu g$  (green),  $5.0 \mu g$  (blue) and  $10.0 \mu g$  (red) of plasmid DNA.

### Supplementary Information for Wu et. al.



**Fig. S5.** The extent GFP gene expression in HEK293 cells determined by flow cytometry using Eu1 (left), Eu2 (centre) and Eu3<sup>3+</sup> (right) in the presence of varying amounts of chloroquine. These data show that there is no change in ether the transfection rate or level when chloroquine is employed indicating that the gene delivery system does not get trapped in the entry mechanism.



**Fig. S6.** The amount of Eu<sup>3+</sup> found in cells by ICP-MS after 4 hours of exposure to Eu1 and DNA followed by incubation in fresh media (t = 0 is the end of the 4 hours of transfection). Points at 4, 24 and 75 hours post-transfection seem to be subject to error consistent with the low quantities of Eu<sup>3+</sup> taken into cells, nonetheless Eu1 does appear to leave cells fairly quickly after transfection except for a small quantity which seems relatively long-lived in cells.