# Chapter 7

## A Brief Study on the Distribution of Polyamide-Dye Conjugates Between Cells

Imaging of live cells incubated with polyamide-dye conjugates shows that many conjugates can enter the nuclei of living cells unaided by transfection reagents (1, 2). Kinetic experiments of DNA binding polyamides show that dissociation rates for hairpin polyamides bound at match-sites are slow (3). Insight into how polyamides distribute between cells might have implications for eventual studies in animal models.

Polyamides **1** and **2,** which target the core sequence 5'-WGGWCW-3', were synthesized as previously described (1). Polyamide **1** is a conjugate of fluorescein isothiocyanate (FITC) and 2 of tetramethylrhodamine (TMR) (Figure 7.1). These polyamides were selected based on previous data that showed nuclear localization of these polyamides in cell culture. MCF7 cells were grown in 24 well plates for 24 hours under standard culturing conditions as previously described. Then, polyamide **1** or **2** was added to a concentration of 2 μM and allowed to incubate with the cells for at least 24 hours. Cells were then washed with fresh media and trypsinized. Trypsinized cells that were pre-incubated with **1** or **2** were combined or kept separate and plated onto glass-bottom culture dishes and allowed to grow for an additional 16-24 hours. Confocal microscopic imaging of the FITC- and TMR- conjugates **1** and **2** was as previously described (1).

As expected, **1** and **2** each localized in the nucleus of MCF7 cells (Figure 7.2). When cultures of cells pre-incubated with **1** and **2** were combined, cells initially incubated with **1** retained **1** while those initially incubated with **2** retained **2**, suggesting minimal distribution between cells over the time of the experiment. Experiments using Hoechst have shown similar results. One implication is that for a heterogeneous group of cells exposed to polyamide, the cells that divide relatively infrequently might be expected

to retain polyamides for a longer time as compared to cells that divide more frequently and 'dilute' out the polyamide to daughter cells. Examples of cells that divide infrequently are stem cells of tumors and normal tissue (4, 5). Possible applications might include the selective labeling of cells for the purposes of tracking their migration *in vivo*. For these applications, selection of orthogonal fluorophores would allow the labeling of multiple groups of cells.

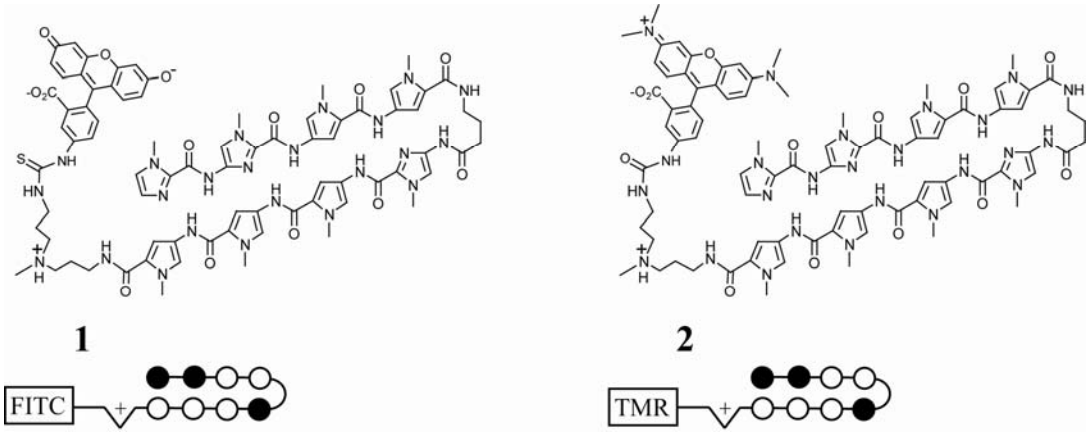


**Figure 7.1** Structures of polyamides **1** and **2**. **1** is a FITC-conjugate and **2** is a TMR-conjugate.

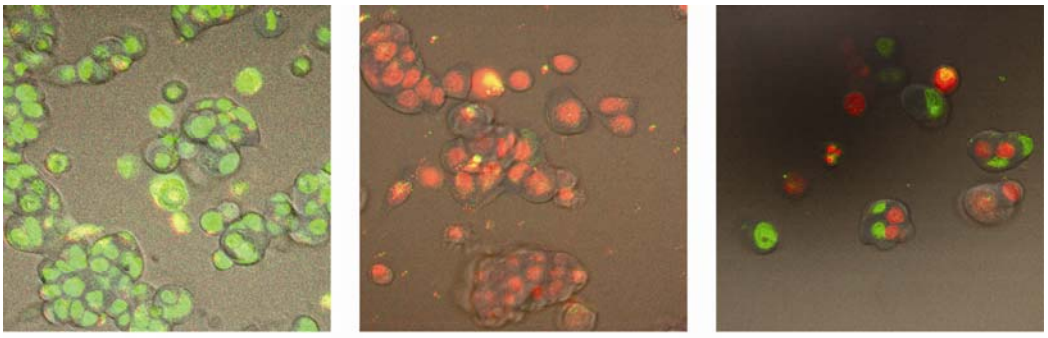


**Figure 7.2** Cellular localization of **1** and **2** in MCF7 cells. FITC-conjugate **1** is represented as green and TMR-conjugate **2** is represented as red.

**References**

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2. Edelson, B. S., Best, T. P., Olenyuk, B., Nickols, N. G., Doss, R. M., Foister, S., Heckel, A. & Dervan, P. B. (2004) *Nucleic Acids Res.* **32,** 2802-2818.

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