OLIGONUCLEOTIDE OPTICAL SWITCHES FOR INTRACELLULAR SENSING

S. Tombelli[1], A. Giannetti[1], C. Trono[1], B. Adinolfi[2], P. Nieri[2], G. Sotgiu[3], G. Varchi[3], F. Baldini[1]

[1]Istituto di Fisica Applicata Nello Carrara, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, - 50019 Sesto Fiorentino (FI), Italy

[2] Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno Pisano, 6 - 56126 Pisa, Italy

[3] Istituto per la Sintesi Organica e la Fotoreattività, Consiglio Nazionale delle Ricerche, Via P. Gobetti 101 - 40129 Bologna, Italy

The understanding, following and monitoring of intracellular signal transduction pathways or the evaluation of the effectiveness of administrated drugs are only two examples where intracellular optical nanosensors can play a fundamental role. Oligonucleotide optical switches are among the most promising optical nanosensors proposed in the recent years. They are suitable molecules capable to turn on or to modify their light emission upon the molecular interaction with well-defined molecular targets [1]. Among this type of probes, molecular beacons (MBs) have been used in a variety of applications, including intracellular sensing

We describe here the design, implementation and characterization of structured polymethylmethacrylate (PMMA) NPs for intracellular mRNA monitoring. In particular, cationic core–shell NPs were used, made up of a core of PMMA, surrounded by a shell bearing cationic groups [2]. The PMMA NPs have been synthesized to include fluoresceine molecules in order to follow the nanoparticles trafficking inside the cell. The PMMA nanoparticles have been characterized as potential intracellular nanocarriers of a MB, for tumor mRNA sensing. In particular, survivin targeting MBs have been used with Atto647N and Blackberry 650 as fluorophore/quencher pair. Two different survivin targeting MBs were tested differing in sequence and length. The MBs were initially characterized in vitro for analytical characterization. Further studies were conducted in order to verify the functionality of the MB once attached to the NPs. Bear and MB-coated PMMA NPs were then tested with adenocarcinomic human alveolar basal epithelial cells (A549) in terms of cell vitality and internalization.

Acknowledgments: Work supported by the Tuscany Region with the project NANOCELL (by PAR FAS Linea 1.1.a.3)

[1] A. Giannetti, S. Tombelli, F. Baldini, Analytical Bioanalytical Chemistry (2013) in press (DOI 10.1007/s00216-013-7086-8).

[2] P. Rimessi, et al. Molecular Therapy, 17 (2009) 820–827.