Electronic Supporting Information

Time-Resolved Lanthanide Luminescence for Lab-on-a-Chip Detection of Biomarkers on Cancerous Tissues

Bo Song, Venkataragavalu Sivagnanam, Caroline D. B. Vandevyver, Ilkka Hemmilä, Hans-Anton Lehr, Martin A. M. Gijs and Jean-Claude G. Bünzli

Reagents. Eu-W8044 and Tb-W14016 luminescent complexes (see Figure S1) were from PerkinElmer Human Health, Turku, Finland. The 5D10 mAb was purchased from the University of Hasselt, Biomedical Research Institute, Diepenbeek, Belgium. Avidin (A9275), fibronectin (FN) (F1141), poly-l-lysine (P4832) and anti-mouse IgG produced in goat (M8645) were purchased from Sigma. FITC-conjugated streptavidin (19538-050) and 4',6-diamidino-2-phenylindole (DAPI, D1306) were obtained from Invitrogen. Biotin conjugated anti-mycoplasma (11296744) was purchased from Roche. Biotin-conjugated Substance P was obtained from the Department of Biochemistry, University of Lausanne, Switzerland. The monoclonal antibody 5D10 was biotinylated using the Immunoprobe™ Biotinylation Kit (BK101, Sigma) according to the manufactures instructions. Polyclonal rabbit anti-human c-erbB-2(Her2) oncoprotein (A 0485) and biotinylated anti-IgG (K0679) were purchased from DAKO. Anti-human ER mouse monoclonal antibody (RTU-ER-6F11) was obtained from Novocastra Lab Ltd.

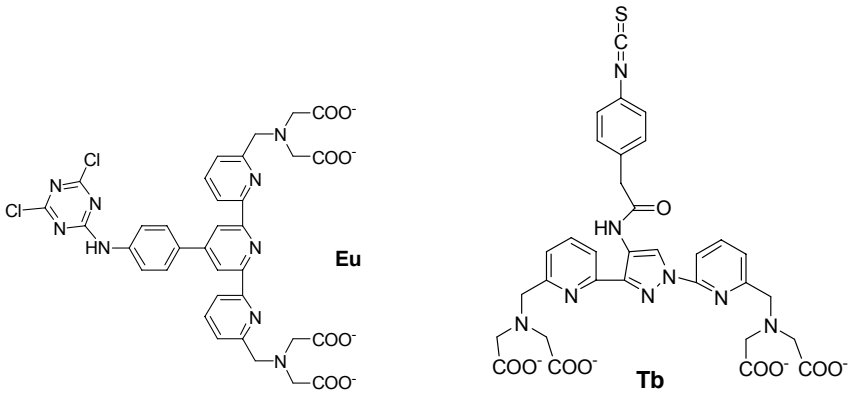

Figure S1. Formulae of the Eu-W8044 (left) and Tb-14016 (right) chelates

Microfluidic channel fabrication. The microfluidic channel master structure was realized following a reported method[5] by generating 200 µm wide microfluidic channel patterns in a 100 µm thick SU8 photoresist layer with conventional photolithography. A 10:1 mixture of PDMS pre-polymer and the curing agent were cast over the master and then cured at 70 °C for 4 h. The cured PDMS replica was peeled off from the mold and access holes with 0.5 mm diameter for the inlet and outlet tube connections were made by piercing the replica with a blunt needle. The PDMS replica and a glass slide were subjected to air plasma (500 W, 4 mbar) for 20 s and then immediately brought into contact, resulting in permanent binding. For the breast cancer tissue assays, a 4 µm thick microtome section of tissue was deposited on a glass substrate after which the PDMS replicated structure was mechanically clamped to seal the channel.

Cell lines and culture conditions. We used the following cell lines: human cervical adenocarcinoma cell line HeLa (ATCC CCL-2) and human breast adenocarcinoma cell line MCF-7 (ATCC HTB-22). Cells were cultivated in 75 cm^2 culture flasks using RPMI 1640 (Sigma, R8758, UK) supplemented with 5 % fetal calf serum (FCS), 2 mM L-glutamine, 1 mM sodium pyruvate, 1 % non-essential amino-acids, 1 % 4-(2-hydroxyethyl)- monosodium salt (HEPES) (all from Gibco Cell Culture, Invitrogen, Basel, Switzerland). Cultures were maintained at 37 °C under 5 % CO_2 and 95 % air atmosphere. The growth medium was changed every other day until the luminescent experiments. Cell density and viability, defined as the ratio of the number of viable cells over the total number of cells, of the cultures were determined using trypan blue staining and a Neubauer improved hemacytometer (Blau Brand, Wertheim, Germany).

Lanthanide complex-labeling of proteins. Lanthanide-labeled avidin, 5D10 mAb and anti-mouse IgG Ab were prepared according to the protocol by Mukkala et al.:[1] *e.g.,* 1 mg of anti-mouse IgG Ab and 20-fold excess of Ln complex (124 nmol) were mixed in 500 µl of 0.1 M sodium carbonate solution (pH 9.3), and incubated overnight at 4 ^{0}C. The Ln-labeled anti-mouse IgG Ab was purified by Sephadex G25 column (354678, GE healthcare) and eluted with PBS. The complex:protein ratio was determined by a DELFIA assay (DELFIA Inducer and DELFIA Enhancer, PerkinElmer Human Health, Wallac Oy, Turku, Finland).

Luminescent and fluorescent microscopy parameters. For the immunocytochemical assay, the following settings were used for single detection of Eu-W8044: excitation: 340 nm (band pass (BP) 70 nm); emission: long pass filter (LP) 420 nm; excitation pulse length: 10 µs; delay time: 100 µs; gate time: 600 µs; exposure time: 30 s. For multiplex detection: Eu- W8044: excitation: 340 nm (BP 70 nm); emission: LP 585 nm; for Tb- W14016: excitation: 340 nm (BP 70 nm); emission: 545 nm (BP 35 nm). The fluorescence of FITC was recorded using classical fluorescent microscopy (excitation: 480 nm (BP 30 nm) filter, emission: 530 nm (BP 30 nm) filter, exposure time: 10 s). The time-resolved measurement conditions for the immunohistochemical assay were as described above, except for the exposure time, which was 60 s. The fluorescence of DAPI was collected with conventional fluorescence microscopy (excitation: 365 nm (BP 80 nm) filter, emission: 450 nm (BP 65 nm) filter, exposure time: 5 s).

Immunocytochemical luminescent detection. Figure S2 represents a statistical comparison between the time-resolved luminescent Eu-W8044-based fluorescent and FITC-based detection of the 5D10 Ag on cancer cells. For each immunocytochemical experiment illustrated in Figure 2c, three luminescence/fluorescence images were taken - on different locations of the microchannel - and the luminescence/fluorescence intensity of 5 cells/image was calculated using the program AxioVision Rel. 4.5 (Zeiss). The average recorded luminescence/fluorescence intensity is shown in Figure S2. The signal-to-noise ratio, defined as the intensity for the 5D10 Ag detection on MCF-7 cells (+) divided by the intensity for the 5D10 Ag detection on HeLa cells (-), showed to be 1.6 times higher for time-resolved luminescent detection (Eu-W8044), compared to the classical fluorescent detection (FITC).

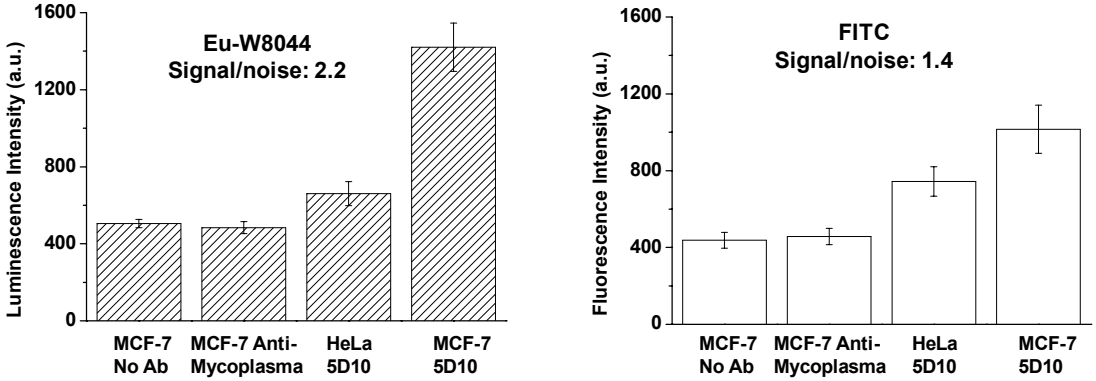

Figure S2. Comparison between the average time-resolved luminescent Eu-W8044-based and classical fluorescent FITC-based detection of the 5D10 Ag on positive (+) MCF-7 cells and on HeLa cells for negative control (-).

Reference

[1] V. M. Mukkala, M. Helenius, I. Hemmilä, J. Kankare, H. Takalo, *Helv.Chim.Acta* **1993**, *76,* 1361-1378.