Bioconjugated Lanthanide Luminescent Helicates as Multilabels for Lab-on-a-Chip Detection of Cancer Biomarkers

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Electronic Supporting Information

6 pages

1. Methods and reagents

The Eu-W8044 luminescent complex (see Figure S1) was obtained from PerkinElmer Human Health, Wallac Oy, Turku, Finland and used without further purification

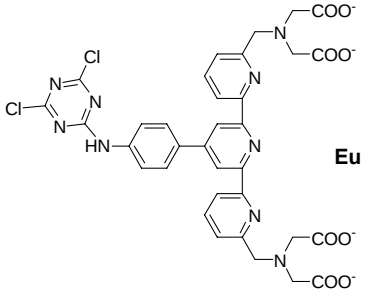

Figure S1. Formula of the Eu-W8044 chelate.

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2. Bioconjugation of the helicate and test of the resulting detection probe

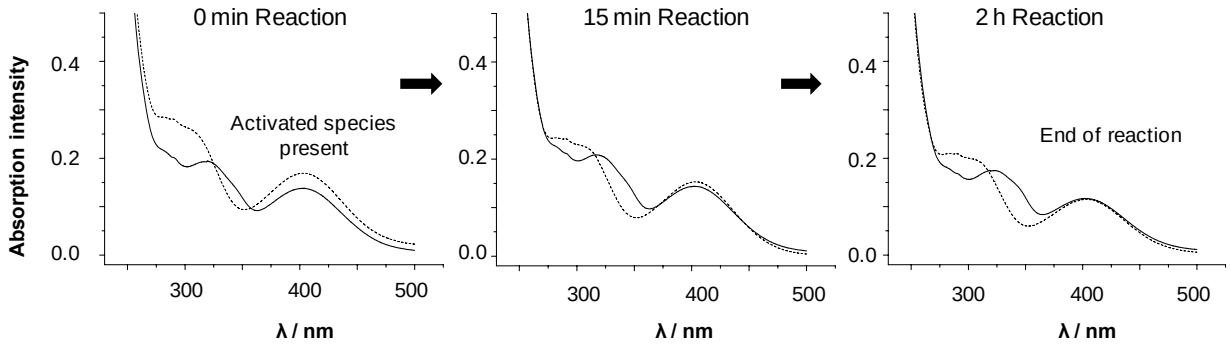

Figure S2. Absorption spectra before (—) and after (·····) addition of 20 µL of NaOH 5 M at different time intervals for monitoring the coupling step. Procedure: 10 µL of the reacting mixture were diluted to 300 µL by 0.01 M phosphate buffer (pH 7) at the given time intervals; NaOH induces hydrolysis of the activated ester and thus generation of the HNSA dianion. The absorbance at 406 nm stabilized after 2 h indicating the completion of the coupling reaction.

Table S1. Optimization of the bioconjugation reaction; reagent equivalents are with respect to avidin; t_{act}: activation step duration; $t_{cpl.}$: coupling step duration; L/P = luminophore/protein ratio; solvent PBS 0.1 M. Final adopted conditions: entry **8**.

Nr	$[Eu_2(L^{C2COOH})_3]$ /eq.	EDCH, NSA / eq.	t_{act}	t_{cpl}	pH_{cpl}	L/P
1	100	1000	15 min	2 h	8.2	1.8
2	8	80	15 min	2 h	8.2	1.7
3	6	60	15 min	2 h	8.2	1.1
4	10	500	15 min	2 h	8.2	1.4
5	1	10	12 h	48 h	7.4	0.12
6	4	40	15 min	2 h	7.4	0.78
7	10	100	15 min	2 h	9.1	2.2
8	**10**	**100**	**15 min**	**2 h**	**8.2**	**3.2**

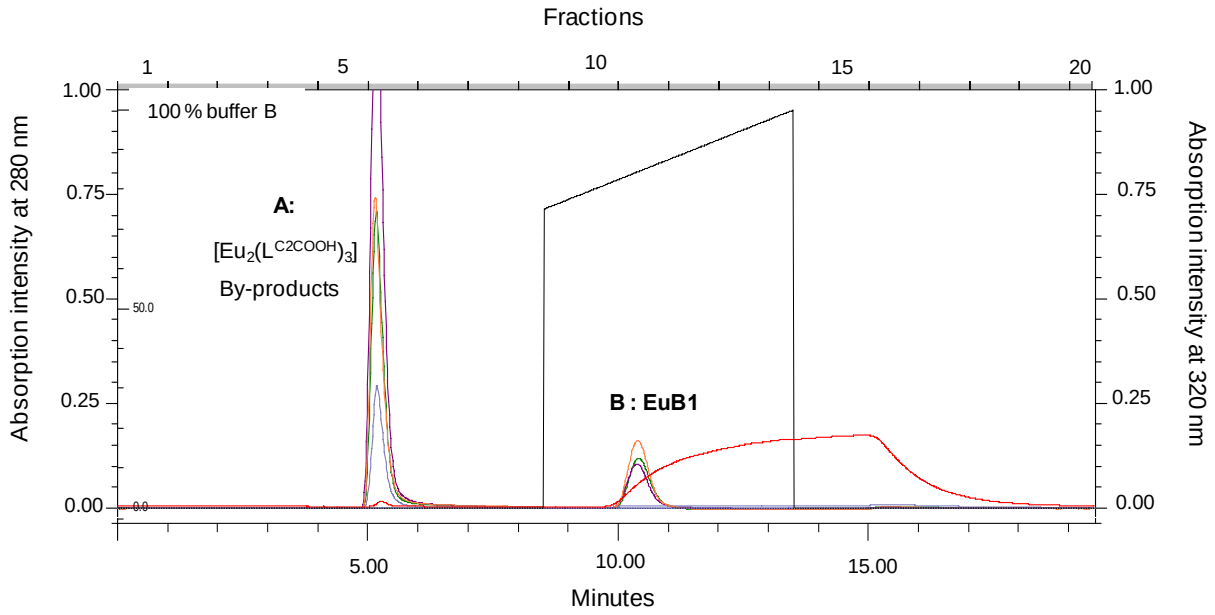

Figure S3. Chromatogram obtained during purification of **EuB1** using a cation exchange column and a gradient of solvents: 0-1 M NaCl in PBS buffer (pH 7.4) at a flow rate of 1 mL/min. The black line (—) indicates the % of buffer B (0.01 M PBS, 1 M NaCl, pH 7.4), (—) conductivity, (—) absorption at 406 nm, (—) absorption at 320 nm, (—) absorption at 280 nm, and (—) absorption at 244 nm.

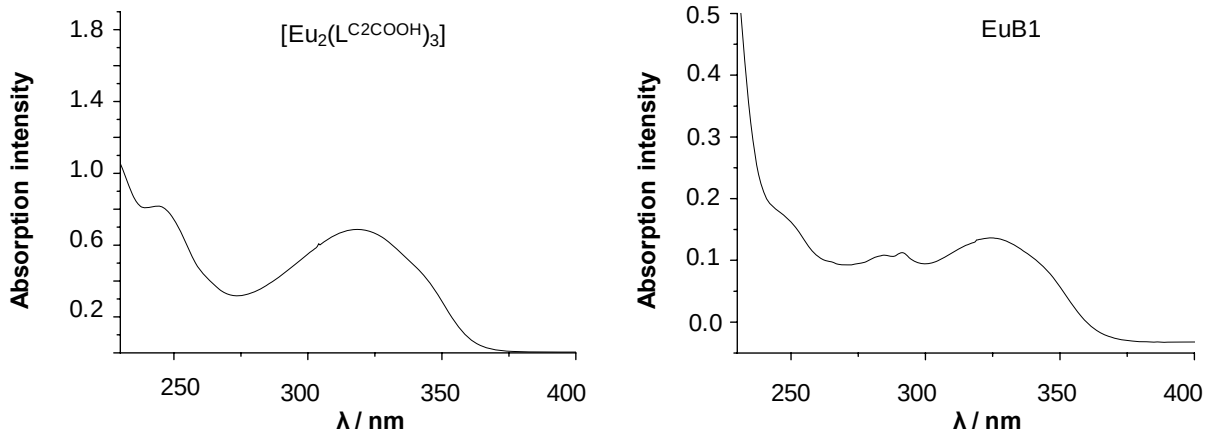

Figure S4. UV/Vis spectra of $[Eu_2(L^{C2(CO2H)})_3]$ (left) and the Eu-labelled avidin complex **EuB1** (right) in 0.01 M PBS aqueous solution at pH 7.4.

Table S2. Crystal-field sublevels of $[Eu_2(L^{C2(CO2H)})_3]$ and the bioconjugated analogue **EuB1** measured at room temperature in 0.01 M PBS aqueous solution pH 7.4.

Transitions	$[Eu(L^{C2(CO2H)})_3]$ (ν / λ) [$cm^{/1}$ / nm]	$[Eu(L^{C2(CO2H)})_3]^{3-}$ Crystal-field sub-levels / cm^{-1}	**EuB1** (ν / λ) [cm^{-1} / nm]	**EuB1** Crystal-field sub-levels / cm^{-1}
$^5D_0 \rightarrow {}^7F_0$	17277 / 578.8	0	17268 / 579.1	0
$^5D_0 \rightarrow {}^7F_1$	17004 / 588.1	273, 453	16995 / 588.4	273, 444
	16824 / 594.4		16824 / 594.4	
$^5D_0 \rightarrow {}^7F_2$	16258 / 615.1	1019, 1122	16258 / 615.1	1010, 1121
	16155 / 619.0		16147 / 619.3	
$^5D_0 \rightarrow {}^7F_3$	15430 / 648.1	1847, 1947	15430 / 648.1	1838, 1938
	15330 / 652.3		15330 / 652.3	
$^5D_0 \rightarrow {}^7F_4$	14548 / 687.4	2729, 2805,	14548 / 687.4	2720, 2796,
	14472 / 691.0	2899, 3034	14472 / 691.0	2896, 3025
	14378 / 695.5		14372 / 695.8	
	14243 / 702.1		14243 / 702.1	

Table S3. Relative corrected intensities of $^5D_0 \rightarrow {}^7F_J$ transitions of $[Eu_2(L^{C2(COOH)})_3]$ and **EuB1** in 0.01M PBS aqueous solution pH 7.4 at room temperature.

Complex	$^7F_1$	$^7F_2$	$^7F_3$	$^7F_4$
$[Eu(L^{C2(CO2H)})_3]$	1.00	1.01	0.11	1.80
EuB1	1.00	0.95	0.10	1.77

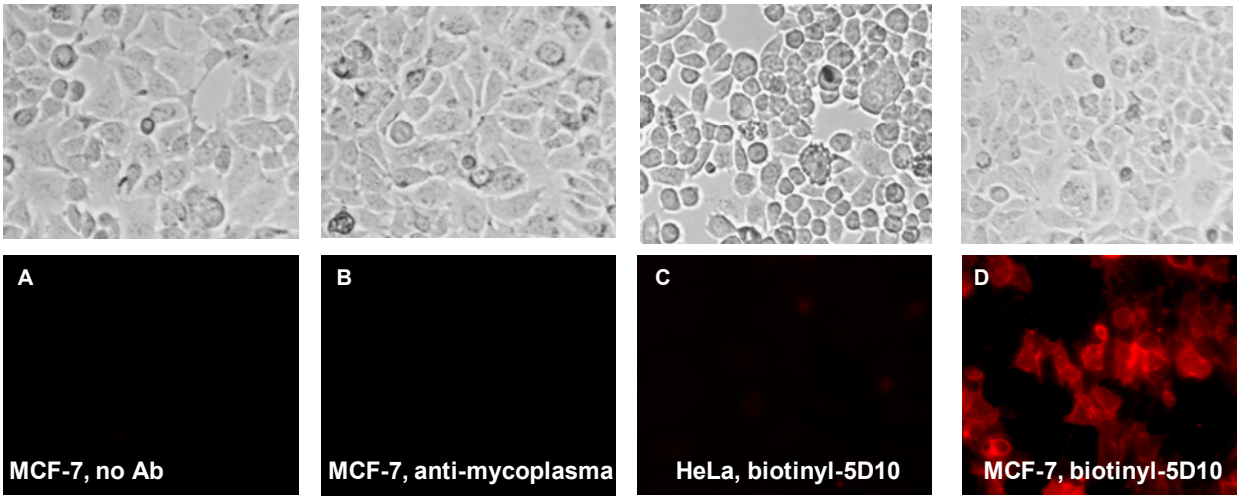

Figure S5. Microscopy images of the immunoluminescence assay of **EuB1** in MCF-7 and HeLa cell lines. Negative controls: (A) no Ab, MCF-7 cells; (B) anti- mycoplasma mAb and MCF-7 cells; (C) biotinylated 5D10 and HeLa cells. Positive control: (D) biotinylated 5D10 and MCF-7. Top row: bright field images. Bottom row: TR images: excitation, BP filter 340 nm (70 nm); emission, LP filter LP (420 nm); delay time, 100 μs; exposure time, 60 s.

Experiments in microchannels

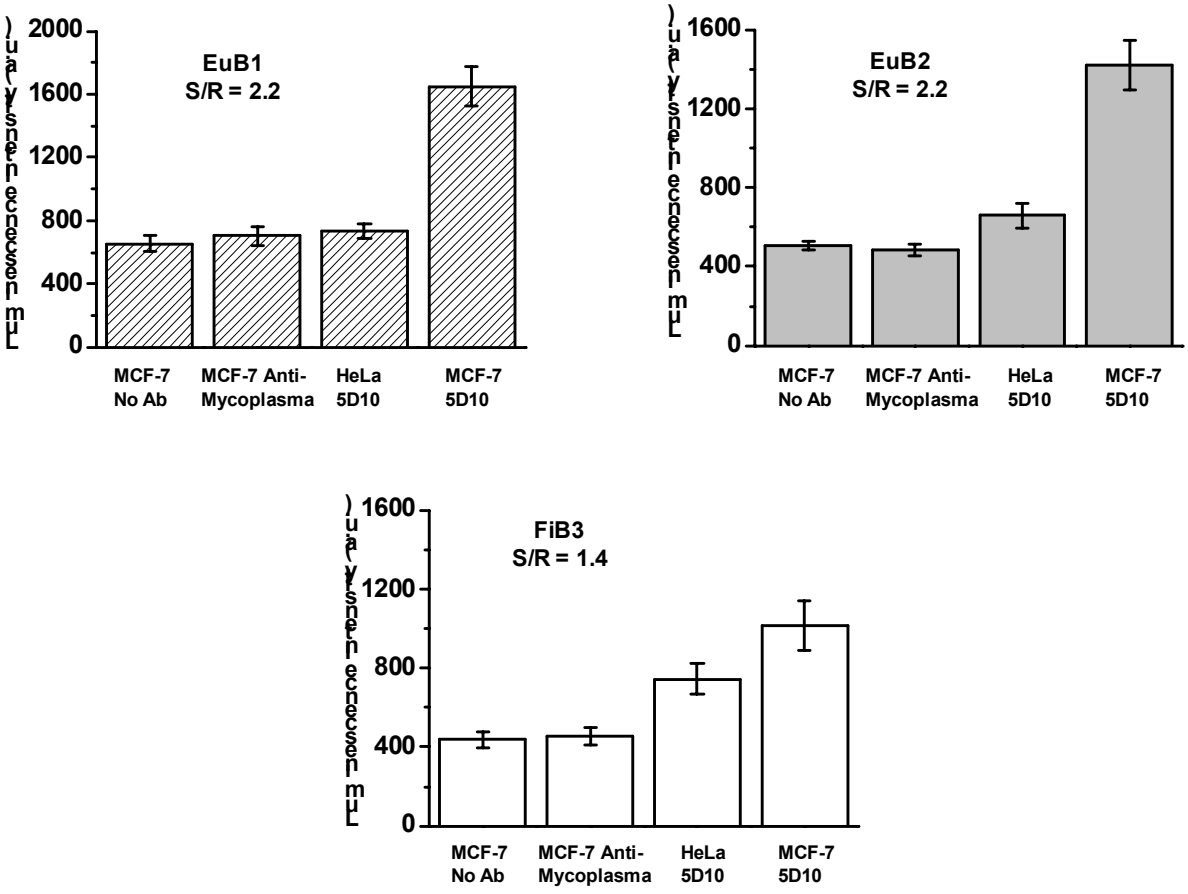

Figure S6. Comparison between the time-resolved lanthanide-based luminescence and organic dye-based fluorescence detection of the antigen recognized by 5D10 on MCF-7 (+) and HeLa (-) cells.