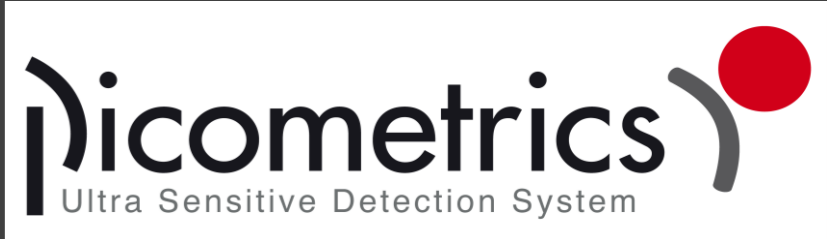


CE-SDS-L(ed)IF: Comparative studies of three fluorescent derivatization methods for the analysis of therapeutic monoclonal antibodies



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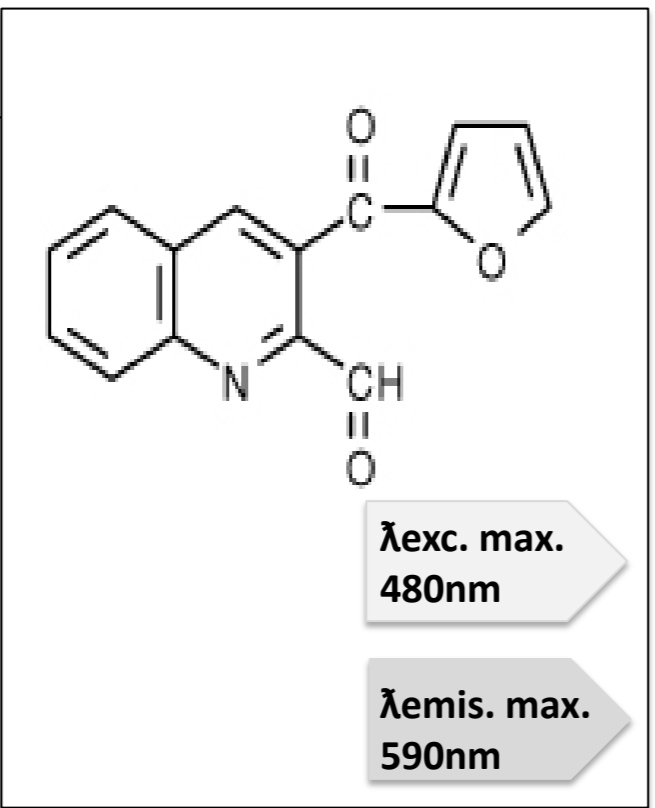


Introduction : CE-SDS is an important separation technique in biopharmaceutical manufacturing and CE-SDS-LIF has become a popular method for characterization and quantification of MAb’s. Dyes such as 5-TAMRA.SE or FQ are widely used for labeling MAb’s for fluorescence detection and many different CE methods have been described in the literature. In this work, the Hunt & Nashabeh’s procedure [i] was chosen using 5-TAMRA.SE, 3-(2-furoyl)-quinoline-2 carboxaldehyde (FQ) and Naphthalenedialdehyde (NDA) as derivatizing agents. The derivatives were detected at the wavelengths using appropriate Light-Emitting-Diodes for excitation and the sensitivity obtained from each derivative was compared. For this work, a Picometrics detector with a Light-Emitting-Diode (LED) was integrated into a Capillary Electrophoresis (CE) system from Agilent Technologies. The high divergent light beam of the LED was focused on the capillary using an original patented optical arrangement. CE-L(ed)IF provides an extremely powerful and flexible tool for the analysis of therapeutic monoclonal antibodies in pharmaceutical quality control analysis.

Instrumentation and method :
System : CE Instrument: Agilent 7100 CE; Detector: Picometrics Zetalif. Capillary : 33 cm total length ; 19 cm effective length.
Method : Buffer of commercial Beckman SDS kit; Injection : - 10kV, 10s, Separation : -16kV

FQ derivatization method

Fluorescence derivatization method of Mab’s with FQ and CE/LIF analysis with two light sources : 488nm solid state laser and 480nm/30nm LED source are presented.



Preparation of FQ-Labeled rMAbs: rMAb (2mg/mL) was mixed in 35 μL of 0.1 M citrate-phosphate (pH 6.5), 2% SDS, 10 mM NEM, 1 mM KCN, and 25 nmol FQ; Labeling reactions were incubated for 5 min at 75°C.

Figure 1 shows that the S/N ratio obtained on the main peak with the laser is 3900 and 3700 with the LED.

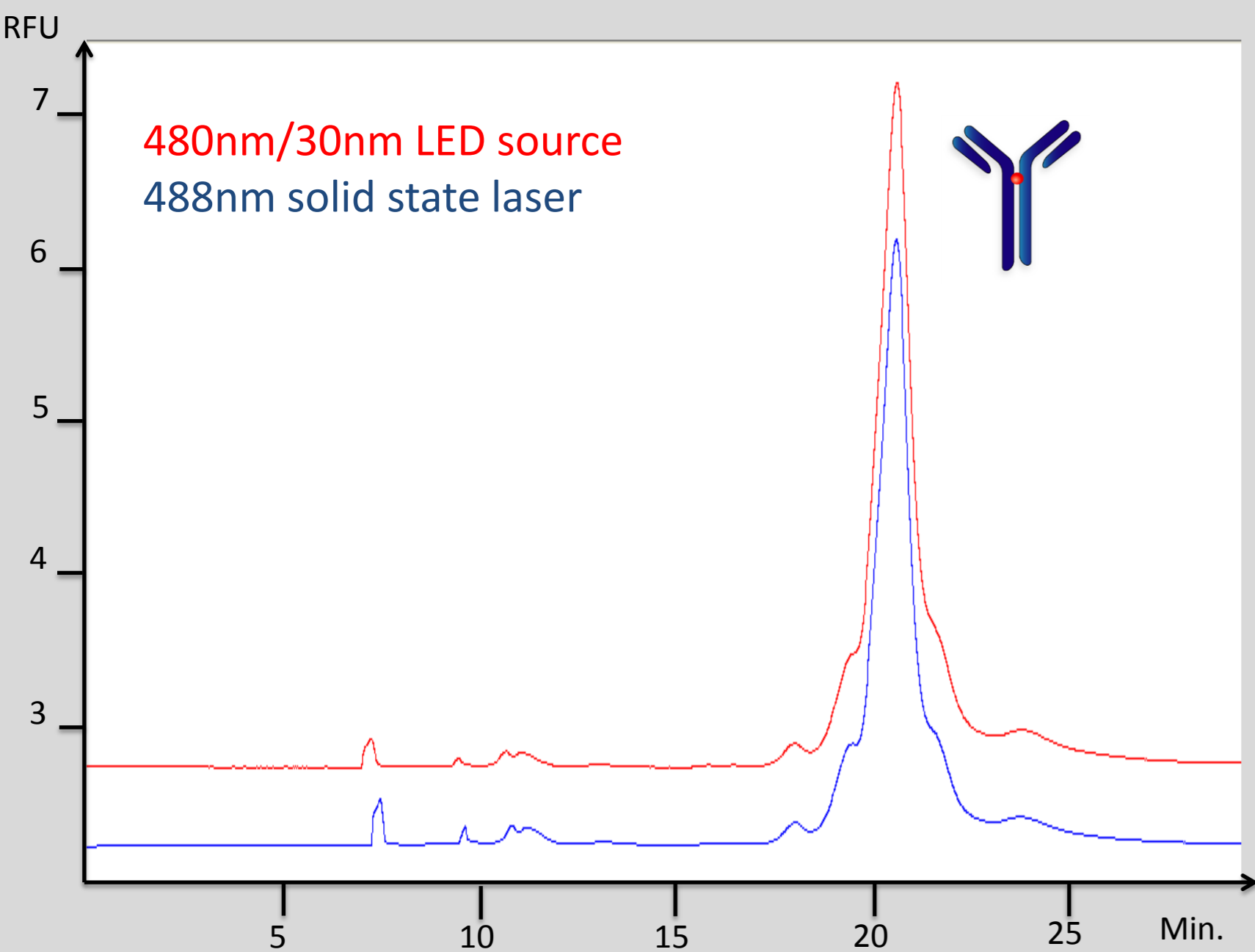
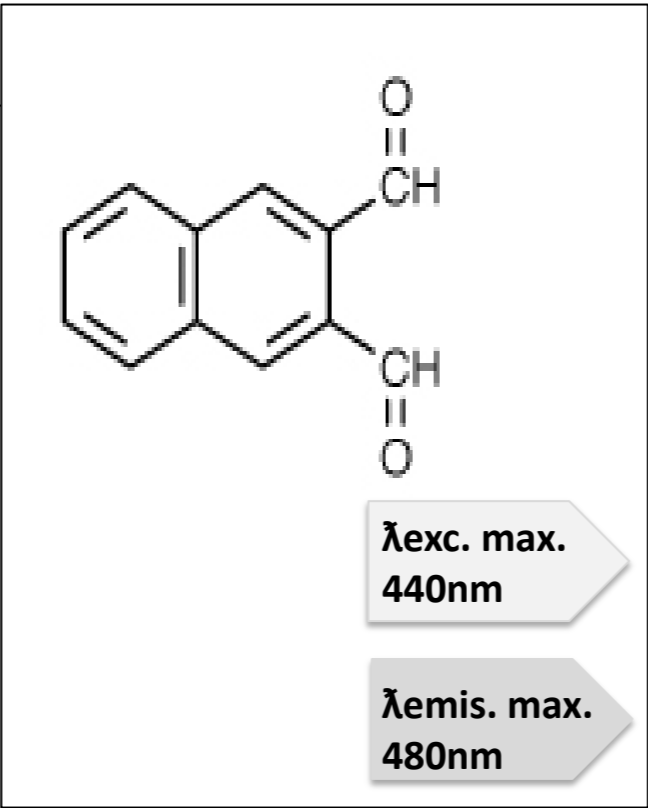


Figure 1 : Analysis of human Ig labeled with FQ using a 488nm laser (blue) and a 480nm LED (red)

Sensitivity is similar with the 480nm LED and the 488nm laser.

NDA derivatization method

Fluorescence derivatization method of Mab’s with NDA and CE/LIF analysis with two light sources : 410nm solid state laser and 450nm/30nm LED source are presented.



Preparation of NDA-Labeled rMAbs: rMAb (2mg/mL) was mixed in 35 μL of 0.1 M citrate-phosphate (pH 6.5), 2% SDS, 10 mM NEM, 1 mM KCN, and 25 nmol NDA; Labeling reactions were incubated for 5 min at 75°C.
In figure 2, the S/N ratio obtained on the main peak is 2700 with the 410nm laser and 6700 with the 450nm LED.

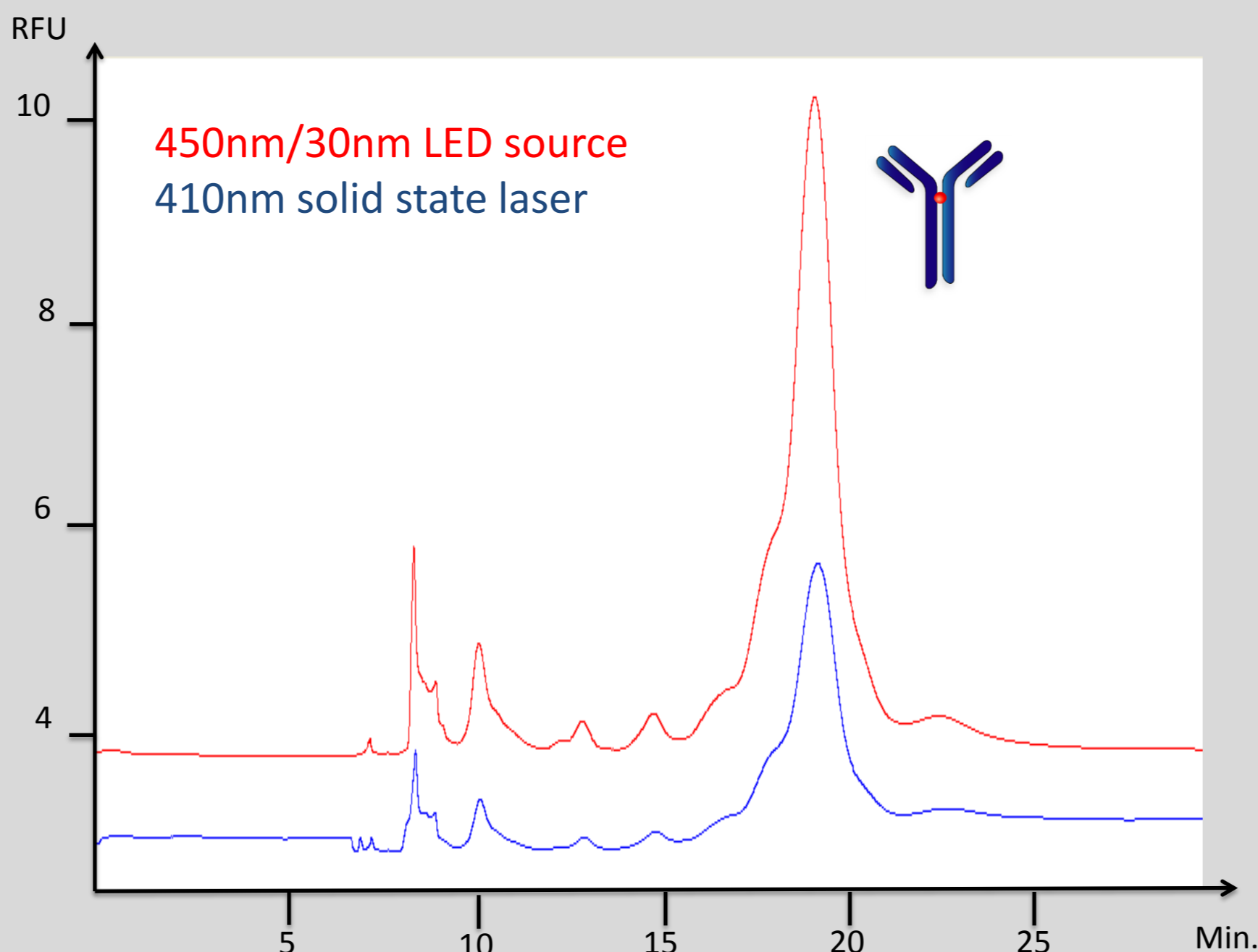
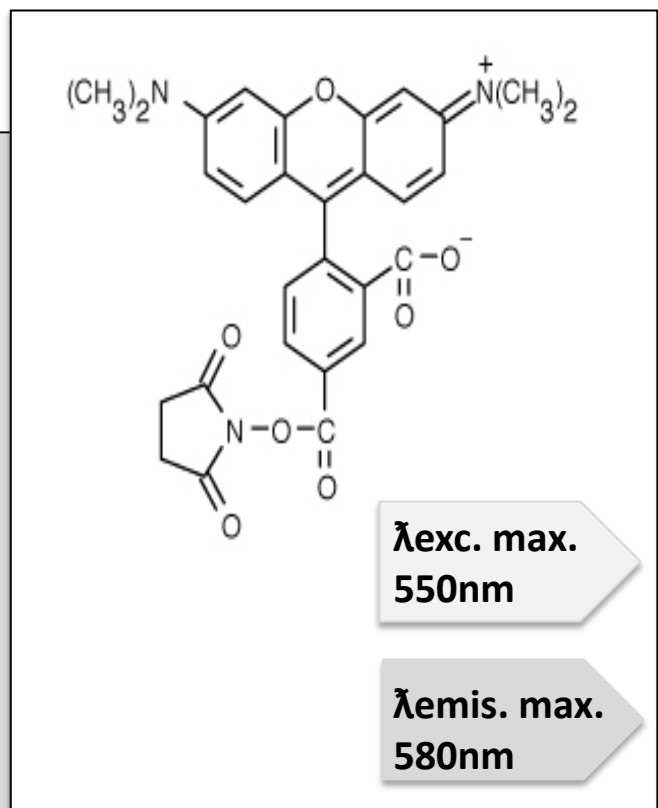


Figure 2 : Analysis of human Ig labeled with NDA using a 410nm laser (blue) and a 450nm LED (red)

The use of a 450nm wavelength, which corresponds to the maximum λexc of the NDA, shows an increase in sensitivity by a factor 2.5 compared to the use of 410nm.

5-TAMRA.SE derivatization method

Fluorescence derivatization method of Mab’s with 5-TAMRA.SE and CE/LIF analysis with three light sources : 488nm solid state laser, 480nm/30nm LED source and 530nm/30nm LED source are presented.



Preparation of 5-TAMRA.SE-Labeled rMAbs: rMAb samples (2mg/mL) were buffer exchanged into 0.1 M sodium bicarbonate, pH 8.3, using a NAP-5 column. 10μL of 5-TAMRA.SE (1.4 mg/mL) dissolved in DMSO was then added to 190 μL of rMAb solution and the resultant mixture incubated for 2 h at 30°C. After incubation, 190 μL of the antibody-dye conjugate was loaded onto a second NAP-5 column and collected in 700 μL of 0.1 M sodium bicarbonate, pH 8.3. Nonreduced SDS-rMAb conjugates were prepared by mixing 100 μL of the rMAb-dye conjugate and 100μL of the CE-SDS sample buffer.

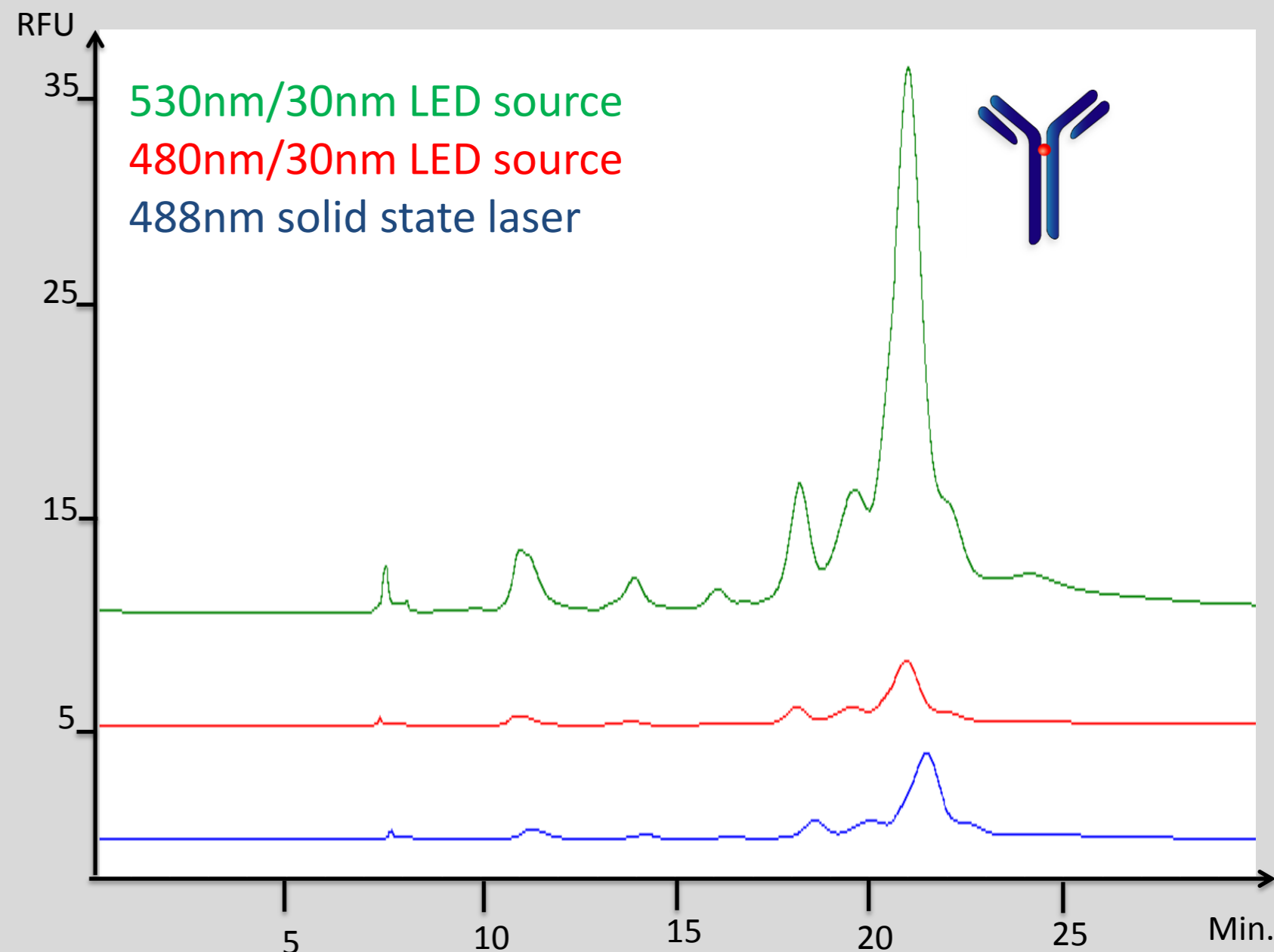


Figure 3 : Analysis of human Ig labeled with 5-TAMRA.SE using a 488nm laser (blue), a 480nm LED (red) and a 530nm LED (green)

S/N ratio obtained on the main peak is 4100 with the 488nm laser, 1400 with the 480nm LED and 25800 with the 530nm LED.
The 530nm LED increases the sensitivity by a factor 6 compared to the 488nm laser.

Conclusion : This poster presents three methods of protein derivatization with 5-TAMRA.SE, FQ and NDA. FQ and NDA are two fluorogenic dyes whose derivatization method is similar and no purification after labeling is required whereas the method with 5-TAMRA.SE is longer due to the excess of dye that has to be removed. The performance of different light sources (laser and LED) were performed with the analysis of a human Ig. For 5-TAMRA.SE, the best sensitivity is obtained with a 530nm LED. With FQ, sensitivities obtained with the 480nm LED and 488nm laser are very similar. With NDA, the 450nm LED magnifies the ratio S/N by a factor 2.5 compared to the laser 410nm. The LED light sources allow more wavelengths ranges to better match the maximum λexc’s dyes.

References :
[i] Hunt G, Nashabeh W. Anal. Chem. 1999, 71, 2390-2397
[ii] Michels DA, Brady LJ, Guo A, Balland A. Anal. Chem. 2007, 79, 5963-5971
[iii] Salas-Solano, O.; Tomlinson, B.; Du, S.; Parker, M.; Strahan, A.; Ma, S. Anal.Chem. 2006, 78, 6583-6594.

	FQ	NDA	5-TAMRA.SE
Derivatization method	<ul style="list-style-type: none">- Fluorogenic reagent, no purification after labeling;- Rapid method of labeling (5min at 75°C)	<ul style="list-style-type: none">- Fluorogenic reagent, no purification after labeling;- Rapid method of labeling (5min at 75°C)	<ul style="list-style-type: none">- The excess dye should be removed with NAP-5 column- Long period of time required for labeling and purification (2 hours at 30°C + purification)
Ratio S/N on the main peak	3700 with 480nm LED 3900 with 488nm laser	2700 with 410nm laser 6700 with 450nm LED	1400 with 480nm LED 4100 with 488nm laser 25800 with 530nm LED