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Abstract

This study reports on the development of a novel instrument for capillary electrophoresis (CE) coupled with laser induced fluorescence (LIF) detection that is inspired by the Lego-toy concept. The Lego CE-LIF design is an evolution of purpose-made CE instrumentation, allowing the users to construct their own analytical device with a high degree of standardization (*i.e.* a "standard" setup) without requirement of mechanical and electronic workshop facilities. To allow instrument reproduction outside the original fabrication laboratory, which is not trivial for in-house-built CE systems, the new design is based on unprecedent 'plugging' hyphenation of various off-the-shelf parts available for microfluidics, optics and electrophoresis. To render the operation with Lego CE-LIF optimal, we developed a new background electrolyte (BGE), using for the first time extremely high concentrations of zwitterionic and large weakly charged species for much improvement of detection sensitivity. The Lego CE-LIF was demonstrated for separation and detection of oligosaccharides labelled with 8-aminopyrene-1,3,6-trisulfonic acid (APTS). The new gel-free BGE for oligosaccharide analysis also allowed simplification of the conventional CE-LIF protocol used with commercial instruments while keeping satisfactory separation performances. Furthermore, the new BGE is fully compatible with a non-thermostatted Lego CE instrument thanks to low current and therefore low heat generation under application of a high voltage.

1. Introduction

After almost 40 years of development and instrument commercialization, capillary electrophoresis (CE) is now among established analytical techniques and becomes the method of choice for several classes of analytes, notably DNA, glycans, therapeutic proteins, chiral molecules, and inorganic ions [1]. The majority of the works on CE have been carried out using bench-top commercial instruments. While such systems offer robustness with a high degree of automation and standardization, their high prices render them often not accessible to researchers in academia, especially to laboratories with limited budget and modest infrastructure. In this context, in-house built CE instrumentation has appeared as an affordable alternative to satisfy the urgent need for inexpensive and simple analytical devices for versatile applications. Indeed, such purpose-made instruments are not only much less expensive than commercial systems but can also be constructed as portable versions [2-4] or with flexible configurations adapted to different needs [5]. Over 10 years, our groups have demonstrated the use of purpose-made CE devices for various analytical screening applications, notably quality control of antibiotics [6, 7], environmental monitoring [8-10], food control [11, 12], forensics [13] and clinical analyses [14]. With the aim to open further the access to CE instrumentation, Kuban *et al*. have recently presented a review detailing all steps required to construct an open-source CE system [15]. This is indeed part of the action plan of the European network promoting portable, affordable and simple analytical platforms [16]. Nevertheless, a mechanical and electronic workshop, even modestly equipped, is often required for construction of purpose-made and open-source CE systems. Exception can be found only for the simplest CE setup with syphoning injection in which an operator only needs a ready-to-use high voltage (HV) module, one capillary and different small vials to carry out electrophoretic separations. Nevertheless, this mode of injection which is the least reproducible, together with manual capillary flushing with a plastic syringe is not fully

appreciated by users due to a high risk of contamination during operation and from the air [6]. Construction of more elaborated versions avoiding the syphoning injection normally require some electronic and mechanical skills that are not always available in analytical laboratories with routine operations. This hinders the wider adoption of in-house built CE instrumentation.

With the goal to drastically improve the CE popularity as a simple and affordable approach to the population, we present herein a Lego CE concept to facilitate technology/ methodology transfer between different laboratories and eliminate the workshop facility requirement. This design is inspired by the Lego toy concept, in which the users with no mechanical and electronic competences can easily assemble a CE system from different commercially available ready-to-use electrophoretic and microfluidic modules. This new CE instrument was coupled with a laser induced fluorescence (LIF) detector that was also constructed with the Lego design, using off-the-shelf optical, electronic and microfluidic parts. The performance of Lego CE-LIF was evaluated in terms of injection reproducibility and detection sensitivity. A demonstration of the Lego CE-LIF system was made with separation and detection of oligosaccharides labelled with 8-aminopyrene-1,3,6-trisulfonic acid (APTS). Such analyses are commonly carried out on commercial bench-top CE instruments using conventional buffers containing inorganic ions that may not be optimal for CE-LIF. To significantly improve the CE-LIF detection performance, a new background electrolyte (BGE) was therefore developed using for the first time zwitterionic and large weakly charged species at very high concentrations to allow excellent stacking of fluorescently labelled oligosaccharides. The new BGE for CE-LIF is inspired from low-conductivity buffers for CE coupled with capacitively coupled contactless conductivity detection (C^4D) [17, 18] and electrolytes buffered with an isoelectric ampholyte for CE with indirect photometric detection [19]. Comparison was made with a conventional BGE used for this purpose to highlight the

advantageous features of our new buffer for CE-LIF. This optimization was inspired by our recent work on reinvestigation of CE-LIF conditions for proteins and peptides analyses [20]. Finally, different modes of pressure-assisted electrophoresis was demonstrated with the Lego-CE-LIF instrument, using the new BGE for CE-LIF, for the optimized separations of labelled oligosaccharides.

2. Experimental

2.1. Chemicals and reagents

All chemicals for preparation of buffers were of analytical or reagent grade and purchased from Sigma-Aldrich (Lyon, France). Glucose oligosaccharides (dextran ladder) and fluorescent reagents (APTS and fluorescein isothiocyanate FITC) were bought from Sciex (Villebon sur Yvette, France). β-Alanine, 2-(N-morpholino)ethanesulfonic acid (MES), acetic acid, lithium hydroxide monohydrate, tris(hydroxymethyl)aminomethane (Tris) and 2-(Cyclohexylamino)ethanesulfonic acid (CHES) were used for preparation of background electrolyte (BGE) solutions.

2.2. Apparatus and Material

Method development for establishment of new BGEs for CE-LIF was performed with a Beckman Coulter PA800+ system (Sciex Separation, Brea, CA) coupled with a LIF detector ($\lambda_{excitation}$: 488 nm, $\lambda_{emission}$: 520 nm). Instrument control was carried out using Karat 8.0 software (Sciex Separation). A standalone LED induced fluorescence (LEDIF) detector was purchased from Adelis (Zetalif, Picometrics, Toulouse, France). Data acquisition (for Zetalif or Lego LIF detector) was done with a Mini-corder ER181 data acquisition system (eDAQ Europe, Warszawa, Poland) connected to the USB-port of a personal computer. Polyimide coated fused silica capillaries of 50 µm id and 375 µm od (TSP050375, Polymicro, CM

Scientific, Silsden, UK) or UV transparent coated fused silica capillaries of 50 µm id and 375 µm od (TSH050375, CM Scientific, Silsden, UK) were used for all CE experiments. Deionized water was purified using a Direct-Q3 UV purification system (Millipore, Milford, MA, USA). pH values of buffer solutions and samples were acquired with a SevenCompact pH meter (Mettler Toledo, Schwerzenbach, Switzerland). Selection of BGE compositions and buffer ionic strength (IS) calculations were based on simulations with the computer program PhoeBus (Analis, Suarlée, Belgium).

For Lego CE instrumentation, all fluid connections were made with 0.02 in. inner diameter (id) and 1/16 in. outer diameter (od) Teflon tubing from Upchurch Scientific (Oak Harbor, WA, USA). The electrophoresis module was based on a dual polarity high voltage power supply with ±30 kV maximum output (HVPS for CZE, Villa Labeco, Slovakia). The high voltage safety cage is a regular Perspex box purchased from Amazon. The microfluidic manifold is composed of different modules purchased from Fluigent (Paris, France), including an ElectroWell (or FluiWell 4C) setup and a pressure controller (Flow EZ). Capillary flushing and sample injection were done with a device to generate either vacuum (MZ 2NT, Vacuubrand, Wertheim, Germany) or compressed air (FLPG Plus, Fluigent).

For modular LIF setup, the current amplifier (DC-100kHz, AMP120), the fiber patch cables (1000µm, M35L01 and 600 µm, M53L01), an optical breadboard (MBH4545/M), spacers (BA2S7/M), the in-line fiber optic filter mount (FOFMS/M-UV) and cover (FOFM-CV), the 488nm notch filter (NF488-15) and the FITC emission filter (MF530-43) were purchased from ThorLabs (Maisons-Laffitte, France). The photosensor module with PMT tube (H10721-210), the fiber adapter (E5776-51) was purchased from Hamamatsu Photonics (Massy, France). A microfluidic manifold Assy 5 port (P-154, Upchurch) was used for optical cell

147 setup. The 488 nm laser module (488L-14A, Integrated Optics) was purchased from Acal Bfi
148 (Evry, France).
149
150 ***2.3. Methods***
151 *Preparation and storage of fluorescently labelled oligosaccharides*
152 The preparation of fluorescently labelled glucose oligosaccharides was performed according
153 to the protocol of Reider *et al.* [21]. Briefly, 2 mg of dextran ladder was added in a 200 µL
154 PCR tube, followed by addition of 4 µL of 40 mM APTS in 20% acetic acid (AcOH), 4 µL of
155 20% AcOH, 2 µL of 1M sodium cyanoborohydride (NaBH3CN) in tetrahydrofuran (THF).
156 The mixture was incubated at 70°C for 30 mins with open vial cap. After the reaction the
157 samples were diluted in 100 µL deionized water, aliquoted and stored at -20°C. Further
158 dilution of this stock solution was carried out before CE-LIF analysis.
159
160 *CE-LIF of oligosaccharides*
161 Analyses of APTS-labeled oligosaccharides were carried out with a BGE composed of either
162 858 mM β-Alanine and 822 mM MES (IS = 50 mM, pH 5.04) (BGE 1); 364 mM Beta-
163 Alanine and 538 mM MES (IS =25 mM, pH=4.75) (BGE 2); or 25 mM LiOH and 47 mM
164 acetic acid (IS = 25 mM, pH=4.75) (BGE 3). The optimized BGE (BGE 1) was adopted for
165 the rest of the study. CE separations with the Lego-CE-LIF system were implemented using
166 fused-silica capillaries with ID of 50 µm, the total length of 45 cm and effective length of 23
167 cm under a separation voltage of -25 kV. The fused silica capillaries were preconditioned with
168 1 M NaOH for 5 min, water for 5 min, 1M HCl for 5 min, water for 5 min, and the BGE 1 for
169 15 min prior to use. Between runs the capillary was rinsed with the BGE for 5 min.
170
171 **3. Results and Discussion**

3.1. System design and performance

3.1.1. Lego CE

Our Lego concept is essentially an evolution of in-house-made compact CE whose first version was introduced by Hauser *et al.* in 1998 [22] and open-source CE introduced by Kuban *et al.* in 2019 [15]. The Lego CE setup is a balance between costly bench-top commercial CE instruments and low-cost in-house-built devices that are hardly reproduced from one laboratory to another. The logic behind the Lego CE concept is demonstrated in Fig. S1 in the electronic supplementary information (ESI). For construction of in-house-built and open-source CE instruments, people normally have to rely on technical drawings that are either provided by the host laboratories (for the in-house-built ones) or available on-line and free-of-charge (for the open-source ones) to reproduce the electronical, mechanical and fluidic modules. To understand and follow these technical drawings, specific knowledge and skills are normally required, which are unfortunately not always available in the majority of analytical laboratories. These challenges could on the other hand overcome with our Lego CE design with a high degree of standardization, and without recourse to any technical drawings for construction of plug-and-play modules. The Lego CE design we developed here is based on unprecedented hyphenation (specifically for CE instrumentation) of various off-the-shelf parts available for microfluidics and electrophoresis. More concretely, we used a high-accuracy miniature pressure controller setup, a high voltage generator for CE, a device for gas compression or vacuum generation and a fluidic interface dedicated to microfluidic operation to build the CE system. A simplified schematic drawing of the Lego CE system is shown in Fig. 1. Compressed air generated from an air compressor or a gas tank is driven to a stand-alone pressure controller (*i.e*. Flow EZ 1000 mbar, Fluigent) to provide precise pressure for hydrodynamic injection (generally from 30 - 100 mbar) and capillary flushing (1000 mbar). Alternatively, similar operations could be carried out from the opposite side of the capillary,

using a vacuum generator and a pressure controller (*i.e*. Flow EZ -800 mbar, Fluigent) providing negative pressures for injection (-30 mbar to -100 mbar) and capillary flushing (-800 mbar). If both negative and positive pressures are desired, a Flow EZ push-pull module can be used for pressure manipulation in the range of -800 mbar to +1000 mbar. This offers setup flexibility to users. Note that any pressure in the range from 0 to 1000 mbar (or from 0 to - 800 mbar) can be set and monitored for injection or capillary flushing purposes either with physical knob and a digital screen integrated on the pressure controller or with a computer-linked control program (see Fig. S2). In addition, pressure assistance during electrophoresis, which is not trivial in in-house-made CE instruments, can also applied to accelerate the analysis time or improve the separation resolution, as pressure can be precisely controlled and monitored during application of high voltages (see section below). In in-house made CE instruments [6, 9, 13, 23-27], a desired pressure value can sometimes not be precisely set and monitored. Thus, optimization of hydrodynamic injection in these cases is generally done with injection time variation rather than pressure adjustment. Both optimization modes (time and pressure) are now available in our Lego CE version. Solutions to be injected in the capillary (i.e. sample, BGE or other generating solutions) can be easily changed by plugging the corresponding vial to the fluiwell or electrowell interface (Fig. S2A and B in ESI for their setup). In our case with the electrowell (Fig. S2C), a platinum electrode is already integrated in this interface so a ground connection can be made easily without any further module modification. If the fluiwell interface is used instead on the GND side, a steel tubing commonly used for HPLC can be employed for ground connection. In this case the capillary end is centered and extruded from the GND steel electrode so that they are both in contact with the working solution (Fig. 1). For high voltage generation, a commercial module containing a ± 30kV Spellman unit with an integrated digital display was employed, allowing control and monitoring of the voltage and current during electrophoresis. The high voltage

side was isolated using a Perspex box sold for cosmetic or arrangement purposes that can be purchased online. Alternatively, any cage made from electrically isolating materials (e.g. poly(methyl methacrylate), mica, polyvinyl chloride (PVC)) could be used [28]. The total cost for construction of this Lego CE system from these off-the-shelf components is estimated to be 5000 Euros.

3.1.2. Lego LIF

The detection module is one of the most critical parts of the whole CE instrument. Among all detection types commonly employed for CE, fluorescence detection, or laser-induced fluorescence (LIF) detection in particular, is often used to improve significantly the detection sensitivity, especially for determination of biomolecules such as proteins and peptides. The popularity of fluorescence detection in CE however is often hindered (at least partially) by very high purchase costs of commercial LIF or LED-induced-fluorescence (LEDIF) detectors. Efforts to produce purpose-made fluorescence detectors adapted to modest budgets and infrastructure were already communicated [29-31], but normally require electronic and mechanical skills and workshop, with 3D-printing facilities in some cases [29, 31]. For teaching purpose, Thompson *et al.* introduced a low-cost CE-LEDIF device for testing some fluorophore standards [32]. As part of the Lego-CE instrument, we developed a new Lego LIF detector. The Lego-LIF design exploits off-the-shelf components commonly used in microfluidics and optics in order to minimize (or eventually eliminate) the need for workshop and skills that are not always available in laboratories with routine analyses. The schematic design of Lego LIF detector is demonstrated in Fig. 2 whereas a photo of the system can be seen in Fig. S3 in ESI. A miniature LIF module from Integrated Optics, powered with a USB cable from a personal computer, was used for the first time in CE-LIF to provide the excitation wavelength of 488 nm which is most commonly used for fluorescence detection of

biomolecules. The incident excitation light was set perpendicular to the optical window of the separation capillary using a black microfluidic interface. This interface plays the role of an optical cell, allowing excellent light alignment. The emission light was collected from an outlet of the interface situated perpendicular to both incident light and the capillary (Fig. 2). The emission light was then passed through an optical band-pass filter (or FITC 530 nm emission filter). A notch filter for 488 nm can be optionally added to block any residual excitation light. The filtered light was then diverted to a photomultiplier tube (photo sensor) to convert incident photons into electric current signals. These were subsequently converted into voltage signals and amplified using a trans-impedance amplifier, prior to analog-to-digital conversion and data acquisition into a computer. All these optical, microfluidic and electronic components are ready-to-use modules and can be plugged together using the adaptors provided by the suppliers. Users can choose different laser / LED types for the light source from various suppliers, depending on the budget available and the target applications. In our particular case where cost-effectiveness and miniaturization are the two most important criteria, a miniature USB-powered high-performing laser module was chosen. The overall cost for such Lego LIF detector was estimated to be 5000 euros, which is much cheaper (less than 25 %) than the purchase cost of a commercial fluorescence detector for CE.

3.1.3. Performance evaluation

To evaluate the injection function of the new Lego CE, a series of tests were implemented with injection of a standard FITC solution at different pressures and injection times conventionally used in commercial CE systems. The reproducibility data for peak areas obtained at different injection pressures and times are shown in table 1. Good injection reproducibility was achieved at any injection pressure and time, except for the case of 30 mbar over 5 sec. The poorest reproducibility in this condition could be probably due to the too

short time for pressure manipulation at relatively low pressure range. The reproducibility for migration time was excellent (RSD % < 0.5 %) under a delivery pressure of 400 mbar, proving again the added value of this system, exhibiting precise pressurization. For evaluation of detection signals, the performance of Lego LIF detector was compared to that of a commercial LEDIF detector, using the same separation capillary and CE conditions. Electropherograms for analysis of FITC at 110 nM that were obtained with both detectors are shown in Fig. 3, whereas comparison data are presented in table 2. Very good linearity (R^2 better than 0.997) was acquired, whether the calibrations were made with peak areas or peak heights, proving a very good response of the Lego LIF to the variation of FITC concentrations. The detection sensitivity was approximately 10 times better for the commercial LIF (see table 2). This can be explained by the fact that no focusing lens or special optical setups were employed for the Lego LIF as otherwise required for a costly commercial fluorescence detector. In addition, the photosensor module with PMT tube used for Lego LIF is a miniaturized and inexpensive version, which might perform less well than the one used for the commercial counterpart. In the former one, no electronic filtering was included in the photomultiplier tube or trans-impedance amplifier module, whereas this feature was already integrated in the latter one. This explains the more noisy background for the raw signal of Lego LIF (see Fig. 3A), which was not the case for the commercial detector (no signal difference between Fig. 3C and 3D). The lack of electronic filtering in the Lego LIF was therefore compensated by digital filtering function offered by the data acquisition module, allowing significant reduction of background noise and improvement of detection sensitivity (Fig. 3B). The LOD values presented in table 2 were achieved for the filtered signals.

3.2. Separation and detection of fluorescently labelled oligosaccharides with Lego CE-LIF

Glucose-oligosaccharides are often used as the ladder reference for analyzing N-glycans released from glycoproteins, serving for quality control of therapeutic glycoproteins and diagnostic purposes [33, 34]. For oligosaccharides and glycans labeling, APTS is the most frequently used fluorescent agent whereas BGEs containing inorganic ions are often used for CE-LIF separation of labelled oligosaccharides and glycans [35, 36]. The electroosmotic flow (EOF) is normally suppressed so that the negatively charged APTS-tagged oligosaccharides (and glycans) can migrate against the EOF to arrive at the LIF detector for detection. All BGE compositions reported so far for CE-LIF analysis in general, and CE-LIF for such purpose in particular contain inorganic ions (*e.g*. phosphate, borate etc.) and / or use inorganic acid and base (typically NaOH and HCl) for pH adjustment. These BGEs with low UV absorbing feature, while being well adapted to UV detection, may not be optimal for LIF detection. We have recently demonstrated that a much-improved performance for CE-LIF detection of proteins and peptides could be achieved with our new BGEs for CE-LIF thanks to a better stacking effect and lower current generation [20]. With a similar rationality, we optimized the BGE composition for CE-LIF of labelled oligosaccharides this time. The principle behind this strategy is illustrated in Fig. 4. By using a very dense zone of zwitterionic and large weakly charged ions in the BGE to block the sample zone, the target analytes will be well stacked in the sample-BGE boundary. While this stacking phenomenon can be observed using conventional BGE containing inorganic ions, this effect is expected to be pushed up to the maximum with our new BGE strategy. The use of extremely high BGE concentrations, while not readily possible with inorganic ions due to high current generation, is now feasible thanks to the very low electrophoretic movement of the large and / or zwitterionic molecules constituting the BGE. Via simulation with the Phoebus program, we compared the properties

of different new BGEs for CE-LIF at pH 4.75 that have never been used before for such purpose (table 3). The lithium acetate buffer at the same pH, frequently employed by different groups for CE-LIF of oligosaccharides and glycans [35, 36], was used as a reference for these comparisons. Among these new BGEs, beta-alanine / MES exhibits the best buffer capacity and was expected to provide the best stacking effect due to the highest components' concentrations (364 mM beta alanine et 538 mM MES). Another BGE composed of Naphtyl-1-amine and MES, which is thought to offer equivalent performance to that of beta-alanine/MES, is not considered due to the presence of a carcinogenic agent. Separation performance for CE-LIF of APTS-labelled oligosaccharides was thus compared between LiOH/Acetic acid and beta-alanine/MES BGEs, using first a commercial instrument (Fig. 5A and 5B). At IS of 25 mM, the signals of oligosaccharides obtained with beta-alanine / MES were two times higher than those obtained with LiOH / acetic acid buffer. With equivalent background noises observed, this confirms a much better LIF sensitivity with the new beta-alanine / MES BGE. The electroosmotic flow mobility was found a bit higher for beta-alanine/MES buffers, which explains the longer migration times of oligosaccharide peaks. To further improve the stacking effect, the IS of beta-alanine/MES was doubled and the electropherogram for these conditions is shown in Fig. 5C. Conveniently, with beta-alanine/MES BGE, an increase in IS from 25 to 50 mM only leads to a tolerable increase in the generated current (from 13 to 25 µA under 30 kV). The beta-alanine/MES BGE (IS 50 mM) led to a much higher peak sensitivity (almost 3 times) than the conventional LiOH/Acetic acid buffer for the first 5 peaks. For slower-migrating ones (due to the presence of a higher EOF magnitude with our new BGE), the peaks were more broadened, leading to a less performance in detection sensitivity. Compared to previously communicated CE-LIF conditions for this purpose [35, 36], our new BGE offered higher signals. Noted also that low

current generation (leading to low Joule heating) was achieved and no bubble formation was observed when working with our non-thermostatted system.

The Lego CE-LIF was then used with this buffer for separations of APTS-labelled oligosaccharides. The CE-LIF electropherogram obtained is shown in Fig. 6A. Excellent peak shapes and separation resolutions were achieved for glucose units GU1 till GU6. To compensate for the peak retardation when using beta-alanine/MES BGE, pressure assistance could be applied during electrophoresis, which is not a complication when using the Flow EZ pressure controller. As can be seen in Fig. 6B, the peaks arrived faster to the detector and more glucose units could be visualized under the pressure assistance at 30 mbar. The pressure-assisted electrophoresis can even be finely tuned by using a pressure gradient. By applying a pressure of 30 mbar at 0s and then 20 mbar at 5 min, the fast arrival of the first four peaks could be maintained, whereas separation resolution for the slower ones, which could correspond to the sizes of large N-glycans of glycoproteins, was improved (see Fig. 6C). Note that the unit displayed for LIF signals in Fig. 5 was RFU as the electropherograms were obtained with a LIF detector from Sciex, whereas that in Figs. 3 and 6 was in a mV scale as the signals were converted with an external data acquisition module. With this demonstration, we expect to open a door for various applications exploiting both hydrodynamic and electrokinetic principles with Lego CE-LIF. We also provide here a tool that could be tuned to get it adapted for any kind of prospective glycan analysis. Indeed by playing on voltages and pressures we would achieve the best separation performances whatever the kind of glycans to be analyzed (i.e. N- or O-glycans, small or longer ones or even a mixture of these types).

4. Conclusions

We successfully developed a new Lego CE-LIF instrument that can be constructed from off-the-shelf modules. Recourse to mechanical and electronic workshops can therefore be avoided. A high degree of standardization with an affordable construction cost can be achieved with this Lego CE-LIF design. The Lego design would allow the users to setup their own analytical devices at a cost at least 70 % cheaper than the purchase price of a commercial system while keeping a high degree of standardization (*i.e*. a 'standard' setup) and facilitation of technology transfer that are not offered by in-house-made versions. This design was demonstrated for separation of fluorescently labelled oligosaccharides that serve as a reference for glycoprotein-derived glycan analysis. We also successfully developed a new BGE based on large weakly charged and zwitterionic molecules at very high concentrations for such analyses. This new BGE matches well to the Lego CE-LIF operation in terms of low current generation (to avoid Joule heating in a non-thermostatted system), and high stacking effect for improved LIF detection sensitivity. Various applications of Lego CE-LIF are envisaged in different domains in order to increase the popularity of such design as an interesting alternative to in-house-built hardly standardizable CE instrumentation.

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The authors declare no conflict of interest.

Table 1. Salient performance data for the test on injection reproducibility realized with the Lego CE system. Analyte: FITC 1 µM; delivery pressure: 400 mbar; silica capillary with l_{eff} of 35 cm and L_{tot} of 60 cm

Injection pressure	**Injection time**	**Peak area (mV·s) (mean value)**	**RSD % (n = 4) Peak area**
30 mbar	5 s	0.66	10.8
	10 s	1.06	2.37
	20 s	2.03	3.00
50 mbar	05 s	0.97	1.26
	10 s	1.68	4.08
	20 s	3.10	1.51
100 mbar	5 s	1.70	5.88
	10 s	3.33	1.85
	20 s	6.13	1.49

Table 2. Data on comparison on LOD and linearity between 2 LIF detectors.

Analyte: FITC 1 µM. CE conditions : BGE composed of Tris / CHES (IS 20 mM, pH 8.4), silica capillary with l_{eff} of 25 cm and L_{tot} of 45 cm; high voltage of 25 kV with normal polarity; hydrodynamic injection at 50 mbar over 10s.

Detector	**Calibration range (nM)**	**Linearity (R^2) with peak area**	**Linearity (R^2) with peak height**	**LOD (nM)**
Lego LIF	30-1000	0.999	0.997	14
Commercial LIF (ZetaLIF)	3-1000	0.999	0.999	1.2

457 **Table 3**. Inorganic-species-free BGE compositions at IS of 25 mM and pH of 4.75, simulated

458 with Phoebus program

459

BGE compositions	I (µA) at 30kV 50µm 65cm	buffer capacity (mmol/l.pH)	Expected quality	Remark
LiOH 25mM + Acetic acid 47 mM	16	28	Reference	
Acetic acid 47mM + His 26mM	14	30		similar to LiOH / Acetic acid
Pyridine 32mM + MES 538mM	16	71	+	carcinogenic
TRIS 25mM + MES 538mM	11	59	+	
Beta-alanine 364 mM + ANISIC Acid 36mM	13	70	+	
Beta-alanine 364 mM + Sorbic Acid 48mM	14	79	+	
Beta-alanine 358 mM + Phenylphosphonic acid 24mM	7	53	+	
Beta-alanine 364 mM + Furoic Acid 26mM	13	54	+	
Beta-alanine 364 mM + methanesulfonic acid 25mM	17	53	+	
Naphtyl-1-Amine 170mM + MES 538mM	9	105	++	carcinogenic
Beta-alanine 364 mM + MES 538mM	13	109	+++	

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Figure captions:

Fig. 1. Schematic drawing of Lego CE design. GND: Ground electrode.

Fig. 2. Schematic drawing of Lego LIF design

Fig. 3. Electropherograms for CE-LIF separation of FITC 1µM using A) Lego LIF detector without digital filter; B) Lego LIF detector with digital filter; C) commercial LEDIF detector without digital filter; and D) commercial LEDIF detector with digital filter. CE conditions : BGE composed of Tris / CHES (IS of 20 mM, pH 8.4); silica capillary with L_{eff} of 25 cm and L_{tot} of 45 cm; high voltage of 25 kV with normal polarity; hydrodynamic injection at 50 mbar over 10s.

Fig. 4. Principle of our new BGE optimization strategy for CE-LIF of labelled oligosaccharides

Fig. 5. Electropherograms for CE-LIF separations of oligosaccharide ladders carried out with a commercial PA800+ system, using A) conventional LiOH/Acetic acid BGE (IS 25 mM, pH 4.75); B) beta-alanine / MES BGE (IS 25 mM, pH 4.75); and C) beta-alanine / MES BGE (IS 50 mM, pH 5.04). CE conditions: HV -25 kV; capillary of 50 µm ID with total length of 30 cm and effective length of 20 cm; hydrodynamic injection at 50 mbar over 10s.

Fig. 6. Electropherograms for CE-LIF separations of oligosaccharide ladders using the Lego CE-LIF instrument. CE conditions: BGE composed of beta-alanine/MES with IS of 50 mM and pH 5.04; HV -25 kV; fused silica capillary with L_{tot} of 45 cm and

L_{eff} of 23 cm; hydrodynamic injection at 50 mbar over 10s. A) Without pressure assistance; B) With pressure assistance at 30 mbar from t = 0s; C) With pressure gradient: 30 mbar at t = 0s, then 20 mbar at t = 5 min

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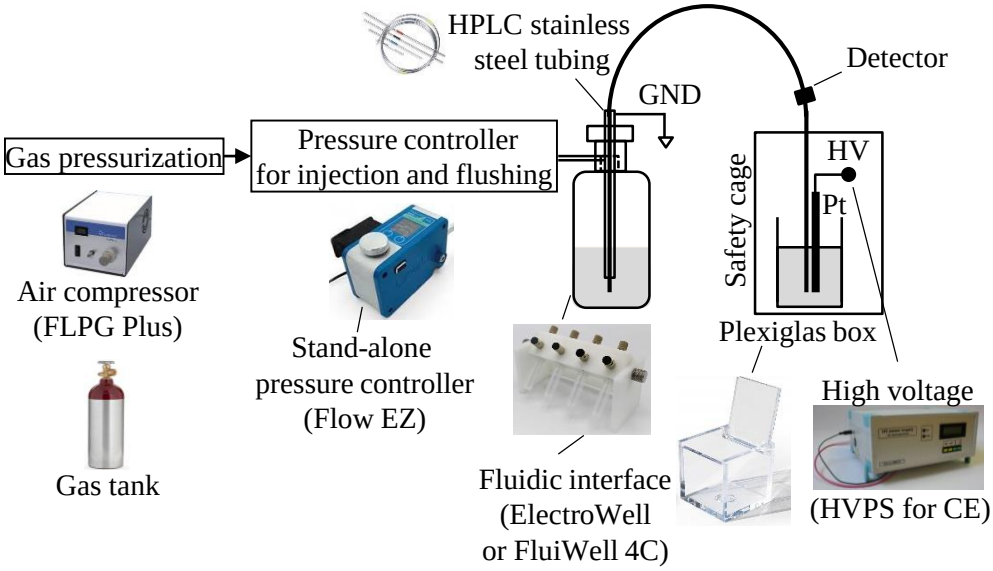
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Gas tank
Fluidic interface
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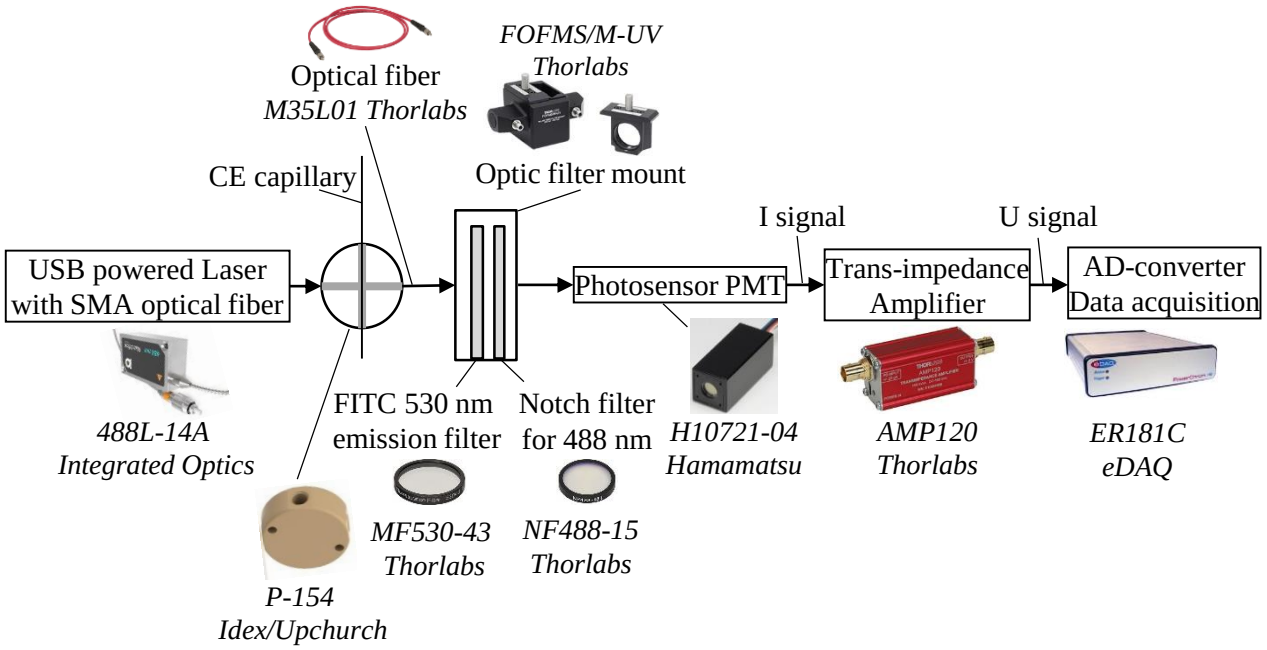
Figure 1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with SMA optical fiber
Photosensor PMT
Trans-impedance
Amplifier
AD-converter
Data acquisition
488L-14A
Integrated Optics
FITC 530 nm
emission filter
Notch filter
for 488 nm
H10721-04
Hamamatsu
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Figure 2

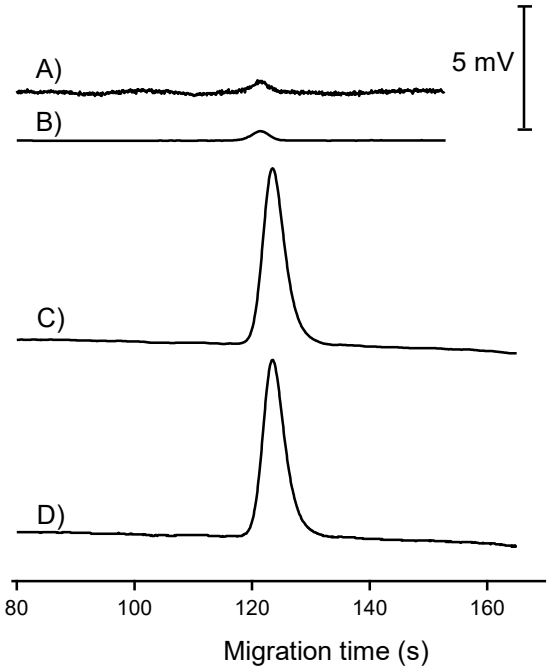


Figure 3

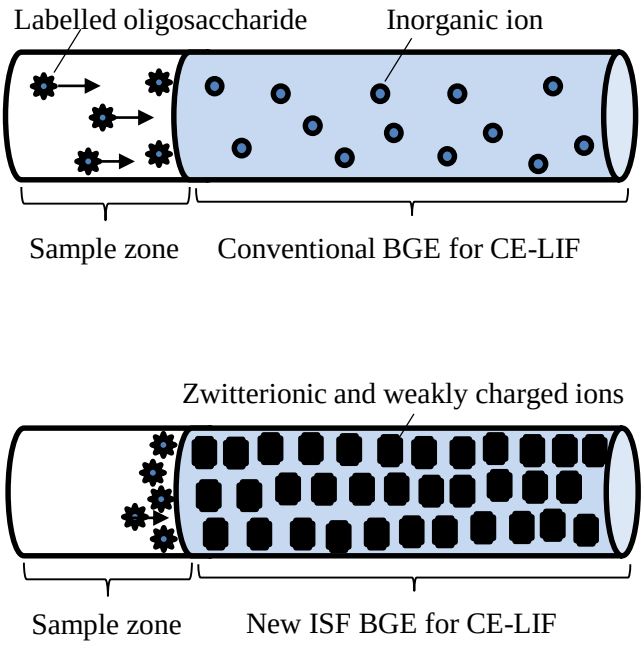
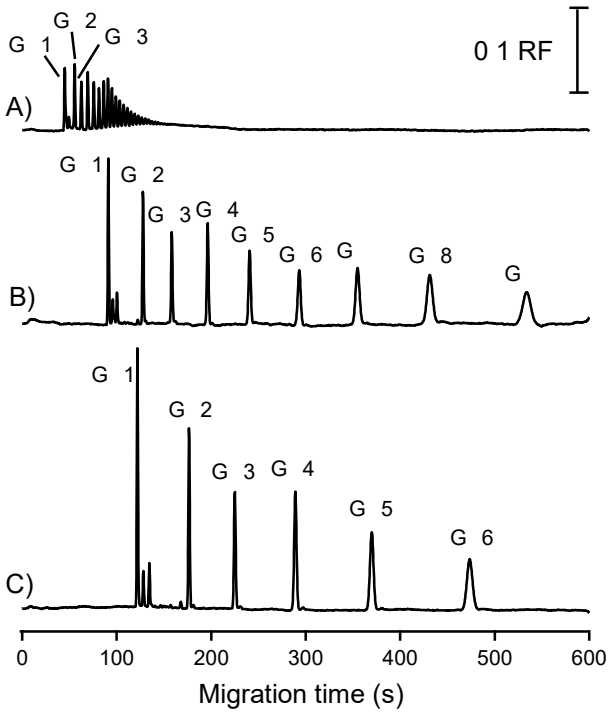

Labelled oligosaccharide
Inorganic ion
Sample zone
Conventional BGE for CE-LIF
Zwitterionic and weakly charged ions
Sample zone
New ISF BGE for CE-LIF

Figure 4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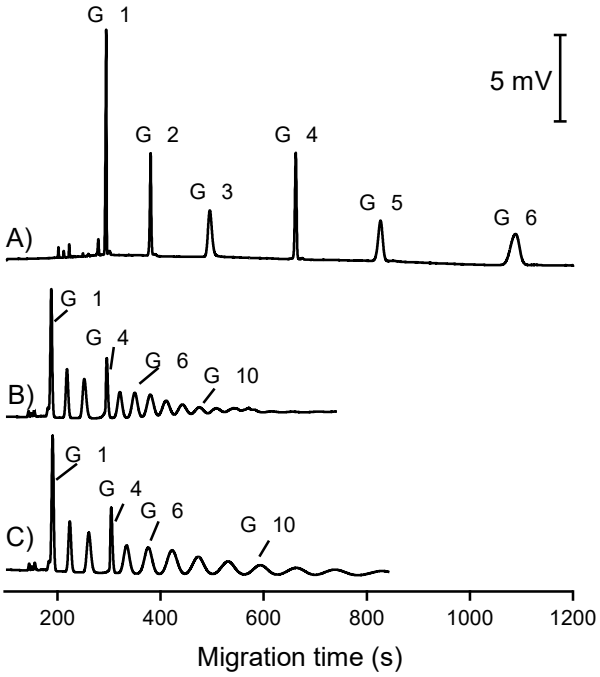
Migration time (s)

Figure 5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B)
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G 4
G 6
G 10
C)
200
400
600
800
1000
1200
Migration time (s)

Figure 6

Lego-toy-inspired instrumentation for capillary electrophoresis with laser induced fluorescence detection.

By *Théo Liénard-Mayor, Jasmine S. Furter, Myriam Taverna, Hung Viet Pham, Peter C. Hauser* and Thanh Duc Mai **