



HAL
open science

Combining ionic diode, resistive pulse and membrane for detection and separation of anti-CD44 antibody

Imad Abrao-Nemeir, Oumaima Zaki, Nathan Meyer, Mathilde Lepoitevin,
Joan Torrent, Jean-Marc Janot, Sebastien Balme

► To cite this version:

Imad Abrao-Nemeir, Oumaima Zaki, Nathan Meyer, Mathilde Lepoitevin, Joan Torrent, et al.. Combining ionic diode, resistive pulse and membrane for detection and separation of anti-CD44 antibody. Journal of Membrane Science, 2022, 649, pp.120391. 10.1016/j.memsci.2022.120391 . hal-03929840

HAL Id: hal-03929840

<https://hal.umontpellier.fr/hal-03929840>

Submitted on 9 Jan 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Combining ionic diode, resistive pulse and membrane for 2 detection and separation of anti-CD44 antibody.

3
4 Imad Abrao-Neimer^a, Oumaima Zaki^a, Nathan Meyer^{a,b}, Mathilde Lepoitevin^c, Joan Torrent^b, Jean-Marc
5 Janot^a, Sebastien Balme^{*a}
6

7 ^a Institut Européen des Membranes, UMR5635 University of Montpellier ENCSM CNRS, Place Eugène
8 Bataillon, 34095 Montpellier cedex 5, France.

9 ^b INM, University of Montpellier , INSERM, Montpellier, France

10 ^c Institut des Matériaux Poreux de Paris (IMAP), UMR 8004 CNRS, Ecole Normale Supérieure de Paris,
11 Ecole Supérieure de Physique et de Chimie Industrielles de Paris, PSL Université, 75005 Paris, France
12

13 **Abstract**

14 The need for antibody protein is growing for both diagnosis and therapy. In this work, we combine
15 two strategies based on single nanopore sensing and multipore membrane to separate, detect
16 and identify antibodies. This proof of concept was done for the anti-CD44. In a first step, a single
17 nanopore with a bullet shape were designed using track-etching of polymer film and
18 functionalized, with a PEG spacer and CD44 antigen. The detection of anti-CD44 was evidenced
19 by an inversion of the current rectification. After demonstrating the ability to specifically detect
20 the antiCD44 in a single nanopore, a multipore membrane with cylindrical nanopore was designed
21 following the same protocol and used to elute a solution of anti-CD44. In a second step, the elution
22 product was analysed using a single SiN nanopore showing that the anti-CD44 and CD44 antigen
23 are extracted from the membrane. The analysis of the amplitude of the current blockade shows
24 that the complex is dissociated.
25

26 **1. Introduction**

27 The increasing need for the identification of proteins as biomarkers as well as their synthesis as
28 therapeutic agents requires the development of innovative solutions for their analysis, production
29 and identification [1,2]. The protein extraction from complex biological matrices is usually done
30 using numerous methods including dialysis, centrifugation, electrophoresis, chromatography, 2D

31 gel or immunoprecipitation [3–5]. More recently, microfluidic techniques met a great interest
32 because of the possibility of multiplexing channels and low volume required (10^{-9} to 10^{-18} L) [6,7].
33 This interest is emphasized by proteomic advances. They require that the separation is connected
34 to a detector with a high sensitivity, providing information about the presence of the target
35 protein [8]. Membrane chromatography for protein separation has emerged several decades ago
36 for analysis [9,10] and more recently for production [11–14]. However, they stayed as a concept
37 for protein analysis due to numerous bottlenecks such as low surface volume ratio, limiting the
38 quantity of protein bonding [15,16]. This makes impossible to detect the eluted analyte directly
39 from the membrane to common detection devices.

40 On the other hand, the sensing technology based on single polymers and solid-state nanopore
41 has demonstrated numerous achievements in the area of protein detection, discrimination, and
42 identification of assembly [17]. There are basically two ways to detect protein using such
43 nanopore. The first one is based on ionic diode properties [18,19]. Such properties are usually
44 obtained in asymmetrical (conical or bullet-like shape) nanopore made in polymer film using
45 track-etching technique [20–23]. Because of the asymmetrical shape and the surface charge, the
46 current-voltage (I-V) response is not linear [24–26]. Thus, when the target binds to the probe
47 grafted inside the nanopore, it induces a modification of inner surface wall properties that is
48 immediately followed by a modification of the ionic transport generating a change in the ionic
49 diode signal [27,28]. This type of nanopore was previously used to detect several proteins such as
50 lysozyme [29–34], antibodies[35], and biopolymers [36]. Despite the possibility to specifically
51 detect a target, the ionic diode is not suitable for a fine characterization of proteins or provide
52 information on their possible structural modification [17]. To do so, a second way can be used
53 involving resistive pulse sensing (RPS) [37,38]. The RPS consists of applying a constant voltage
54 through a single nanopore and record the ionic current. The passage to an analyte induces a

55 current perturbation characterized by an amplitude and duration. The resistive pulse has already
56 been used for protein or antibody analysis [39–43]. The current perturbations provide a
57 fingerprint of the protein or protein assembly [44–52]. The RPS sensing can be done at protein
58 concentrations in the nM range and does not require labelling. This technique is also gaining
59 interest since the development of dielectric breakdown manufacturing that allows single
60 nanopore fabrication, making it more accessible than electrons or ions beam that require
61 expensive facilities [53,54].

62 Combining these different single nanopore and classical membrane approaches could be an
63 interesting way to detect, separate and characterize a protein with minimum steps and a go/no-
64 go information during the separation step. It is interesting to note that the investigations
65 combining single and multipore membranes mainly deal with osmotic energy production. This
66 combined approach provides a fundamental understanding of ionic transport at the single
67 nanopore scale and evaluation of the efficiency on multipore membranes [55,56]. Conceptually
68 for proteins, the separation and detection steps will involve the use of a track-etched membrane
69 that is scalable from a single nanopore membrane and high density of nanopore membrane (10^5
70 to 10^{11} pores/cm²). Placed in paralleled, the single nanopore membrane "sensor" validate
71 whether the analyte is present in the solution while multipore the membrane separates the
72 analyte from the matrix. These two singles and multipore membranes can be produced and
73 functionalized with a probe following exactly the same conditions [23,57], and thus the sensor
74 have the same physico-chemical properties as separation membrane. In the second step, the
75 target protein separated by the multipore membrane will be analysed using a single SiN nanopore
76 by resistive pulse sensing.

77 Here, we demonstrate a new approach combining different single and multi-pore membrane
78 technologies for separation, detection and analysis of antibodies. We selected the CD44 antigen

79 (AgCD44) and its associated antibodies (anti-CD44) due to their involvement in cancer [58] or as
80 a monoclonal antibody-based therapeutic agent [59]. For this purpose, single pores are produced
81 by track etching method and functionalized with AgCD44 as probe. Then, we demonstrate that
82 the AgCD44 grafting does not alter its specificity to detect anti-CD44 before producing multipore
83 membranes under the same conditions to selectively capture anti-CD44. In a second step, the
84 complex anti-CD44/AgCD44 is eluted from the multipore membrane and analysed by a single SiN
85 nanopore using the resistive pulse method. This second step will aim to demonstrate if the elution
86 process maintains the complex AgCD44/anti-CD44.

87 **2. Materials and Method**

88 **2.1. Materials**

89 The 13 μm thick PET films with biaxial orientations were purchased from Goodfellow (ES301130).
90 EDC ([≥99.0%](#), 03449), HCl (30721) NaOH ([≥98%](#), 30620), MES ([≥99.5%](#), M8250), NaCl ([≥99.5%](#) ,
91 S7653), Ethylenediamine ([≥99%](#), E26266), NiCl₂ ([98%](#), 339350), ethylenediaminetetraacetic acid
92 (EDTA) ([98.5-101.5%](#), E5513), CD44 Antigen (APREST83079), BSA (A9418), lysozyme (05281),
93 Avidin (A9275), Anti-avidin (B9655), Anti-BSA (SAB4301142), L-DOPA ([≥98%](#), D9629) and PBS tab
94 buffer (P4417) were purchased from Sigma-Aldrich. NHS-PEG-NTA (228PG2-NSNT-2K) were
95 purchased from Nanocs. KCl ([≥ 99.5%](#), POCL-00A) was purchased from Labkem. Dowfax 2A1 (lot
96 #TO20884587-02) was purchased from EZkem. CD44 antibody (ANT-242) was purchased from
97 PROSPEC.

98 **2.2. Membrane design**

99 **2.2.1. Single and multipore track-etched membrane**

100 The 13 μm thick PET films were irradiated by heavy swift ion Kr (9 MeV) at GANIL (Caen, France).
101 The single pores were obtained from a single track while the multipore membranes were obtained
102 with a fluency of 10^8 ion/ cm^2 [55]. To obtain a bullet-shaped nanopore (single and multipore
103 membranes), only one side of the film was exposed to UV at 312 nm for 26 hours avoiding
104 surfactant adsorption[60,61]. The chemical etching was done using a solution containing 6 M
105 NaOH and 0.05% Dowfax, heated at 60°C for 6 min. Then, the pore was rinsed with ultrapure
106 water into successive baths of 10 min, 15 min, 30 min, one hour and then overnight.. To obtain
107 cylindrical nanopore (multipore membrane only), the irradiated polymer films were activated by
108 12 h per side exposition to UV at 312 nm[62]. Then the chemical etching was done under NaOH
109 4M solution at 40°C for 4 min to obtain nanopore with a diameter around 100 nm.

110 **2.2.2. Track-etched nanopore and membrane functionalization**

111 Single and multipore PET membrane were functionalized using the same procedure. The
112 membrane (single or multipore) was immersed overnight in a solution of ethylenediamine (1:100)
113 in MES buffer 0.1 M containing 0.05 M of EDC (pH was adjusted to 4.5 ± 0.5 with HCl) [63]. Then,
114 a small amount of NHS-PEG-NTA was added to 1 mL of PBS for 12 hours. The membranes (single
115 and multipore) were then rinsed by Milli-Q water and incubated in a BSA solution at 1 mg/mL for
116 3 hours. Next, NiCl_2 solution 100 mM was added for 1 hour. Finally, CD44 antigen His-tag was
117 added to the tip side of the nanopore and incubated for 3 hours. This was followed by incubation
118 of the membrane with three different anti-CD44 (3 $\mu\text{g}/\text{mL}$) for 1 hour each. The washing solution
119 was prepared with 500 mM imidazole and 100 mM EDTA, the pH was adjusted to 8.

120 **2.3. Ionic diode measurement**

121 The current-voltage (I-V) measurements were performed with an eONE XV amplifier. The current
122 was measured by Ag/AgCl electrodes. One electrode is connected to the working electrode the

123 amplifier and the second one is connected to the ground. The working electrode and the ground
124 were placed on the tip and the base side of nanopore respectively (Figure 1e). The I-V curves were
125 recorded using PBS solution 1X after each step of the functionalization and antibody detection.

126 **2.4. Filtration**

127 For the filtration, 1.3 cm diameter multipore membranes (pore density 10^8 pore/cm²) were
128 functionalized and then placed into a filtration system made of Teflon ($r= 0.6$ cm) into which the
129 sample is injected via a syringe. The filtration process was done using Harvard apparatus PHD
130 2000 perfusion, and consists of three steps: first, the passage of 250 μ L of the anti-CD44 (3.37
131 μ g/mL) at a flow rate of 167 μ L/h. Next, the membrane was washed with PBS before to remove
132 any molecules not bound to the immobilized receptors. Finally, 250 μ L of the washing solution
133 was passed through at a flow rate of 167 μ L/hr to remove the complex AgCD44/Anti-CD44.

134

135 **2.5. Resistive pulse sensing**

136 **2.5.1. SiN drilling and coating**

137 The single nanopore was drilled inside stressless SiN, 12 nm thick (Norcada) by dielectric
138 breakdown using Northern Nanopore instruments. Briefly, the SiN film was washed in piranha
139 ($H_2SO_4:H_2O_2$, 3:1) solution at 90°C for 1 hour. Then, it was rinsed with Milli-Q water and dried
140 thoroughly using an air gun, before being placed in the microfluidic cell (Northern Nanopore). The
141 system was then flushed with 600 μ L of propan-2-ol before being filled with deionized and
142 degassed water. After that, the cell was filled with a solution of KCl (1 M)/ HEPES (8.3 mM), the
143 pH is then adjusted to 8 by KOH (1 M). Then a potential ramp from 0 to 5 V followed by a slower
144 ramp from 5 to 14 V was applied across the microchip until a pore opening is detected. Once the

145 nanopore is formed, the solution was changed to NaCl 1.5 M, HEPES 8.3 mM at pH 8 (adjusted
146 using NaOH 1 M) for the conditioning step. The latter consists to apply a voltage box from -3 V to
147 3 V for several cycles until the nanopore reached the desired diameter, here about 9.5 nm. After
148 nanopore opening and characterization by measurement of the conductance, the nanopore was
149 incubated with saturated L-DOPA ($c = 10\mu\text{g}/\text{ml}$) solution in degassed deionized water for 2 hours.
150 Then the current was measured for 1 min using -300 mV and +300 mV in order to assess the noise
151 level of the nanopore.

152 **2.5.2. Protein detection and analysis**

153 Protein detection through SiN nanopore (diameter about 9.5 nm) was performed by resistive
154 pulse using electrolyte solution NaCl 2 M, PBS 1X, pH 7.4. The solution of AgCD44, anti-CD44 and
155 complex AgCD44/anti-CD44 use for calibration was prepared at 20 nM. The protein solution was
156 placed on the half-cell connected to the working electrode, the ground was placed on the
157 opposite side (figure 1) then applying a voltage of -300 mV for 5 min for each protein solution.
158 The ionic current was recorded at sampling rate 200 kHz with a Bessel filter at 10 kHz. The data
159 acquisition was performed with a HEKA EPC800 amplifier coupled with a LIH 8+8 acquisition card
160 using patchmaster software (HEKA electronics, Germany). The current drop induced by the
161 proteins were detected using a custom-made LabVIEW software “Peak Nano Tools”. The current
162 traces were filtered at 5 kHz with a Butterworth filter. The threshold for event detection was
163 determined as 6 times the standard deviation after correction of baseline fluctuations by a
164 Savitzky–Golay filter. The current drop call events were characterized by a dwell time, and an
165 amplitude of the blockade of the current as $\Delta I/I_0$.

166 **Fluorescence measurement**

167 The fluorescence correlation spectroscopy was performed using lab-made confocal
168 spectrometer previously described [42]. Anti-CD44 was labelled with the alexa-fluor 640 under
169 buffer NaCl 500 mM, Tris 5 mM, pH 7.5 at a concentration around 1 nM.

170 **3. Results and discussion**

171 **3.1. Track etched design and functionalization**

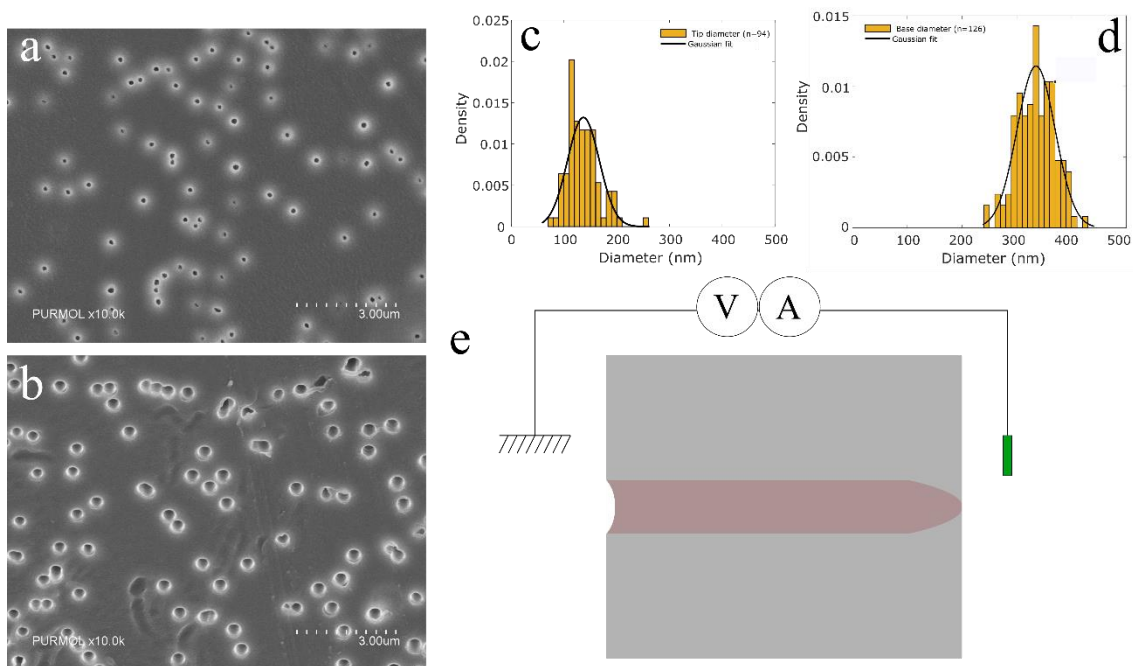
172 Single and multipore nanopore membranes were obtained by the track-etched technique on PET
173 films (13 μm) To obtain the sensors, we designed a single asymmetric nanopore for their ionic
174 diode properties. To this end, bullet-like shape nanopore was achieved by the addition of Dowfax
175 2:1 surfactant in the etching solution thanks to the different etching rate due to the exposure or
176 not of the PET surface to UV light [64] (Figure 1e). The diameters d_{tip} and D_{base} were determined
177 by scanning electron microscopy (SEM) on multipore membranes with high pore density obtained
178 under the same conditions (Figure 1a-b). For an etching time of 6 minutes at a temperature of 60
179 $^{\circ}\text{C}$, the histogram of measured d_{tip} and D_{base} are centred at 130 +/- 20 nm and 320 +/- 50 nm
180 respectively (Figure 1c-d). Because it exists variability in nanopore size, each single nanopore was
181 characterized by ionic conductance measurements. After the chemical etching, the I-V curves
182 recorded in PBS 1X are non-linear due to the negative charge of the carboxylate moieties. The
183 origin of this current rectification is the non-homogeneous distribution of ions along the nanopore
184 as previously reported[65,66] . It depends on the electrolyte concentration and the nanopore
185 shape and surface charge. Thereby, a weak modification of the nanopore surface state due to a
186 functionalization step or the binding of an analyte induce a modification of I-V curve shape that
187 can be characterized by a change of the rectification factor (eq 1)

$$188 R_f = \log \left| \frac{I_{(+1.5V)}}{I_{(-1.5V)}} \right| \quad (1)$$

189 Under our conditions, a positive value of R_f reflects a global negative charge of the nanopore
190 surface. On the contrary, an inversion of R_f toward negative values is induced by a global positive
191 surface charge of the nanopore. The functionalization of the single nanopore requires 4 steps
192 (Figure 2a) before binding the AgCD44. Due to the variability of the nanopore, the rectification
193 factors are given by the average value and the error bars correspond to the standard errors
194 obtained on 8 independent single nanopores. For each I-V curve, at least 10 measurements were
195 performed and averaged. The first functionalization step consists of adding ethylenediamine to
196 the carboxylate moieties. The success of this step is evidenced by a decrease or an inversion of
197 the R_f value due to a partial conversion of the COO^- of the PET by positive charges of the NH_3^+
198 moieties. In the second step, the NHS-PEG-NTA is grafted in the presence of EDC. We observe that
199 the current rectification increases due to the replacing of NH_3^+ moieties by negative charge of
200 carboxylate moieties of PET-NTA. This confirms that the functionalization was successful. The next
201 steps consist of the addition of the AgCD44 with a histidine tag after addition of Ni^{2+} . It is obvious
202 that the addition of PEG as a spacer will not avoid the non-specific adsorption of AgCD44. Indeed,
203 the reported density for carboxylic groups inside a PET nanopore is about 0.2 COOH/nm^2 [67]. In
204 addition, the successive functionalization steps do not have a 100 % yield. Considering that, there
205 are spaces between the PEG-NTA chains where the AgCD44 can be adsorbed. This could lead to a
206 modification of the nanopore surface and thus play a role in the R_f value.

207 There are several strategies to reduce non-specific adsorption [68]. The most effective is high
208 density PEG grafting. This option is not suitable as it is limited to the number of grafting points in
209 the nanopore, in our case, that would be the carboxylate moieties' density. Another solution is to
210 adsorb BSA in the free spaces. We used this second strategy. After adsorption of BSA on the
211 nanopore using a solution at 1 mg/mL in PBS, we notice a decrease in the R_f . This confirms the
212 presence of free space between the PEG-NTA. We determined an optimal incubation time of 3

213 hours as after the measured rectification factor stays constant. Once BSA was adsorbed, Ni^{2+} was
 214 added for 3h. The chelation of Ni^{2+} on the carboxylate moieties and tertiary amine of NTA
 215 functions induces an inversion of the rectification factor due to an addition of positive charge in
 216 the nanopore. We notice that the values of the current and the thus the R_f are not strictly identical
 217 for all the single nanopores. This is due to the variability in the size of the nanopore and the
 218 functionalization rates. However, the trends are identical regardless the nanopore.

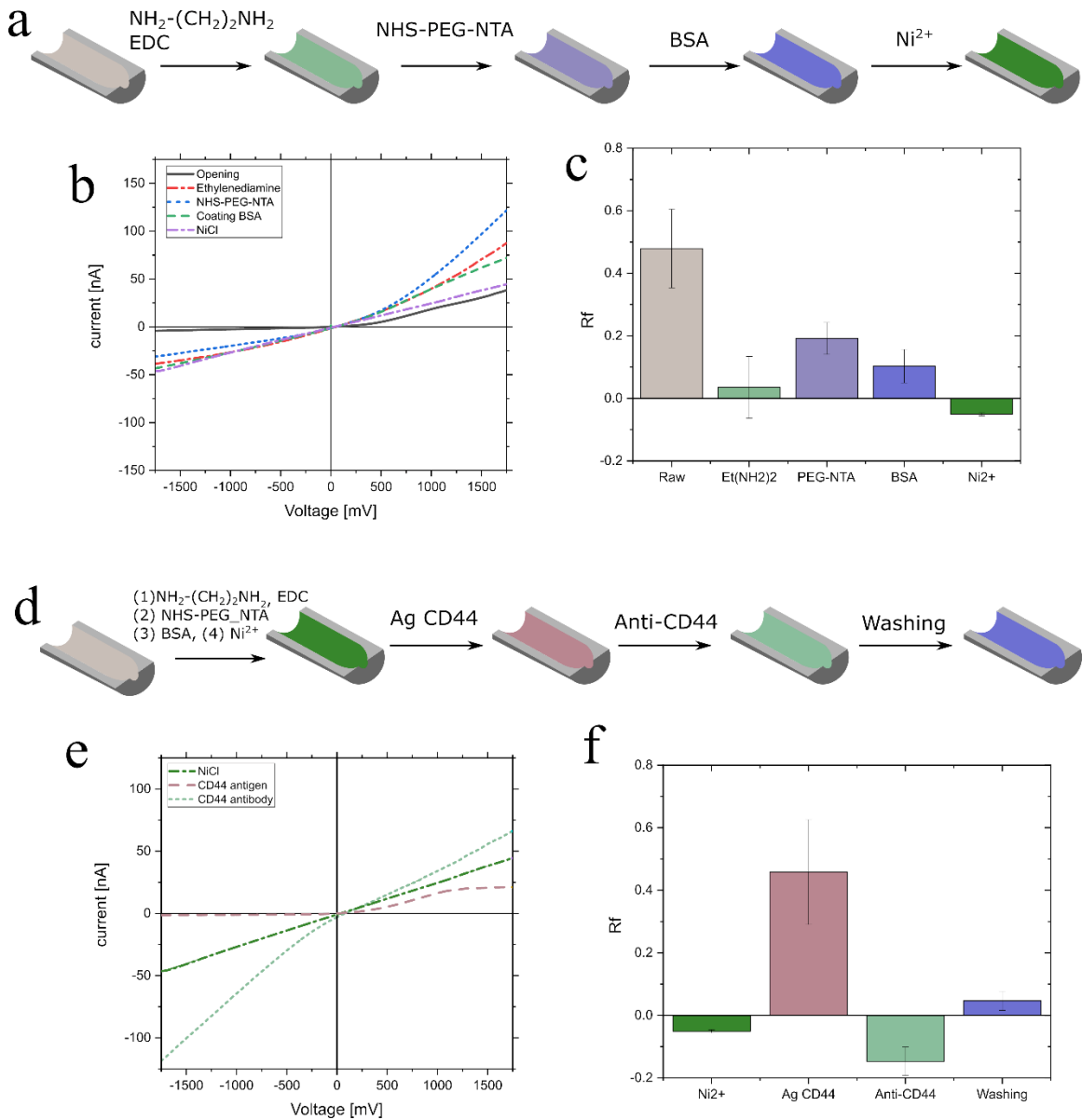


219
 220 *Figure 1 : SEM of bullet shape multipore membrane open under the same condition that single*
 221 *nanopore. (a) is the tip side and (b) the base and the associate histogram of diameter (c) and (d)*
 222 *respectively. (e) Scheme of single bullet shape nanopore for the measurement of the current of*
 223 *the ionic diode under an applied potential.*

224 **3.2. Anti-CD44 detection**

225 Our aim is to detect the anti-CD44. For this, the probe selected is the AgCD44 with a histidine-tag
 226 because the two amines of imidazole moieties specifically chelate the Ni^{2+} loaded on the PEG-NTA
 227 grafted inside the nanopore. The nanopore incubates for 3 hours in the AgCD44-his-tag solution

228 and then the I-V curves are recorded. We observe an inversion of the R_f which is in good
229 agreement with a negative global charge of the AgCD44 at pH 7.2 (IEP 4.9 calculated from IPC).
230 We therefore attempted to detect the anti-CD44 in PBS solution at a concentration of 3 $\mu\text{g/ml}$.
231 After incubation for 90 min, the R_f reverses toward negative values. We note at this point that the
232 successive inversion of the R_f recorded after the Ni^{2+} addition, AgCD44 and antiCD44 occurred on
233 all single nanopores ($n=8$). This allows, despite the variability of the R_f values inherent to the
234 distribution of single nanopore size (as shown for multipore membrane on figure 1), to control of
235 the last steps of the functionalization and the detection of the antibody. We rinsed the nanopore
236 with an imidazole solution that contains EDTA In order to release the AgCD44/anti-CD44 complex.
237 After washing, the rectification reverses to reach a range close to the step before the addition of
238 Ni^{2+} suggesting that the complex is filtrated. However, this will be confirmed later by experiments
239 using multipore membranes and further analysis of the filtrate solution.



240

241 *Figure 2 : (a) Illustration of the functionalization steps of the single and multipore nanopore*

242 *membranes (b) I-V curve obtained after each functionalization steps using PBS 1X (c) histogram*

243 *of mean R_f value obtained from 8 individual singles nanopores ($d_{tip} = 130 \pm 20$ nm and $D_{base} =$*

244 *320 ± 50 nm). (d) Illustration of the detection and elution steps of the single and multipore*

245 *nanopore membranes (e) I-V curve obtained after each detection and washing steps (f)*

246 *histogram of mean R_f value obtained from 8 individual singles nanopore. The error bars are the*

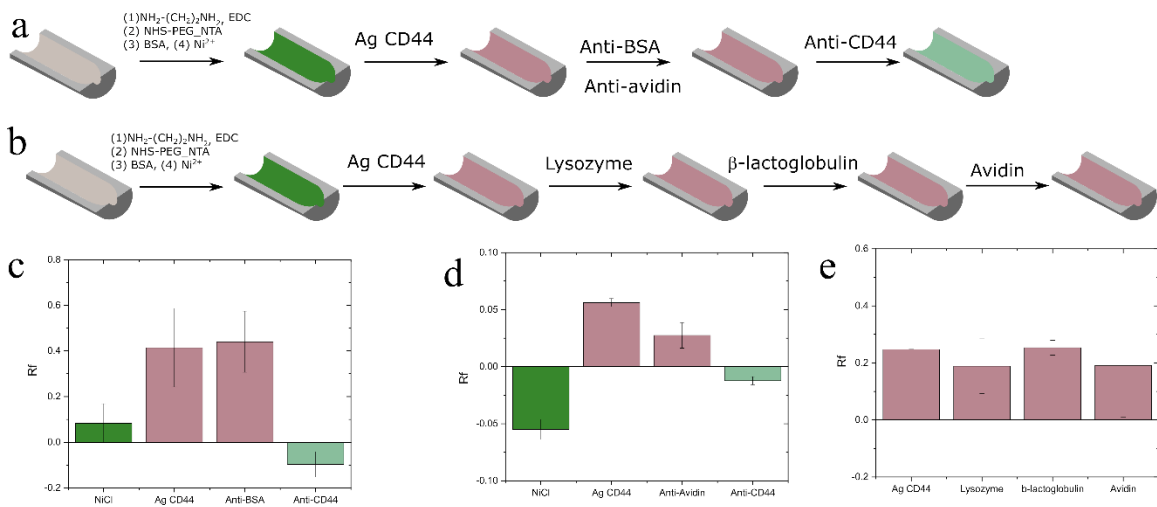
247 *means error obtain from the 8 individual singles nanopore.*

248

249 At this stage, we have to evaluate the selectivity of the single nanopore against antibodies and
250 other proteins. After 3h incubation with anti-BSA (n=6 independent nanopore), we did not
251 observe any inversion of the rectification factor (figure 3). This means that there is no BSA binding
252 site accessible in the nanopore from the coating. Similar results were obtained using anti-avidin
253 (n=3 independent nanopore). Regardless the antibody, a small variation of R_f may occur due to
254 non-specific interactions or adsorption. Nevertheless, this does not compromise the efficiency of
255 the nanopore sensor. Indeed, after incubation with anti-BSA or anti-avidin, the nanopore remains
256 functional to detect anti-CD44 as shown by the inversion of the current rectification (figure 3).

257 We have also evaluated whether the addition of different proteins induces a disruption of the R_f
258 due to non-specific adsorption. For this purpose, a series of proteins was incubated in the AgCD44-
259 functionalized nanopore. After washing with PBS, the I-V response remained the same, as the
260 rectification factor (Figure 3e). This means that the selected proteins do not modify the surface
261 properties of the nanopore and therefore their eventual adsorption can be neglected. After
262 incubating these proteins, the nanopore sensor remains functional. However, it should be noted
263 that without an optimal BSA coating, the R_f is modified after protein incubation.

264



265

266

Figure 3 : (a) Illustration of selectivity tests performed for single nanopore membranes against

267

(a) antibodies and (b) proteins (c) histogram of mean R_f value obtained from n individual single

268

nanopores after incubation with (c) anti-BSA ($n=6$), (d) anti-avidin ($n=3$) and (e) various proteins

269

($n=3$). The error bars are the means error obtain from the n individual single nanopore. The

270

nanopore size are $d_{tip} = 130 \pm 20$ nm and $D_{base} = 320 \pm 50$ nm.

271

3.3. Separation by multipore membrane and sensing of eluted anti-CD44 at the single

272

molecule scale

273

We have previously demonstrated on a single nanopore membrane that functionalization with

274

AgCD44 was efficient in detecting anti-CD44. The washing with imidazole solution suggested that

275

the complex AgCD44/anti-CD44 should be removed from the pore. To verify this and thereby

276

validate our approach to antibody separation, we designed multipore membranes containing

277

cylindrical pores of 100 nm of ± 20 nm diameter following strictly the same steps as the single

278

nanopore. After grafting the AgCD44, anti-CD44 solutions were filtered before washing with

279

imidazole\EDTA solution. The eluted solution obtained with unlabelled anti-CD44 was analysed

280

by single SiN nanopore of 9.7 nm diameter and 12 nm thickness obtained by dielectric breakdown.

281

In the first step, solutions of AgCD44, anti-CD44 and the complex anti-CD44/AgCD44 were

282 analysed by the nanopore. The current traces show for each sample current blockades that can
 283 be assigned to the analyte. The amplitude of the current blockades for AgCD44 and anti-CD44 are
 284 centred at 0.03 and 0.04 respectively. This may seem counter-intuitive given the difference in
 285 volume of the two proteins (i.e.: 61 nm³ and 356 nm³ respectively). We notice, however, that the
 286 size of anti-CD44 is larger than the nanopore and therefore only bumping events can be recorded.
 287 This is confirmed by a shorter dwell time recorded for the anti-CD44 than the AgCD44 (Figure 4).
 288 Conversely, the AgCD44 antigen can translocate into the nanopore. Based on the calculation
 289 proposed by Yusko *et al.* [69], it is possible to obtain a theoretical value for the amplitude of the
 290 current blockage by assuming a spheroidal oblate geometry of the AgCD44.

$$\frac{\Delta I}{I_0} = \frac{\Lambda \gamma}{\pi r_p^2 (l_p + 1.6r_p)} S\left(\frac{r_p}{2R_h}\right) \quad (2)$$

291 where Λ is the protein volume, γ is a scaling factor, $S\left(\frac{r_p}{2R_h}\right)$ correction factor that calculates the
 292 effective hydrodynamic radius R_h of the protein inside the nanopore (equation 4).

$$S\left(\frac{r_p}{2R_h}\right) = \frac{1}{1 - 0.8\left(\frac{R_h}{r_p}\right)^3} \quad (3)$$

293 In the equation 2, γ depends on the shape of the protein, for a spherical one it is equal to 1.5.
 294 For a spheroid, the value of γ depends on the orientation (parallel \parallel or perpendicular \perp) of the
 295 protein relative to the electrical field. Form factors relative to the orientation γ_{\parallel} and γ_{\perp} are given
 296 by equation 5 [69] .

$$\gamma_{\parallel} = \frac{1}{1 - n_{\parallel}} \text{ and } \gamma_{\perp} = \frac{1}{1 - n_{\perp}} \quad (4)$$

297 Where n_{\parallel} and n_{\perp} are dependent on the ratio m of two hydrodynamic radii. The antibodies have
 298 an oblate shape $m=a/b$. The form is given by the equation 5 and 7

$$n_{\parallel} = \frac{1}{1 - m^2} \left[1 - \frac{m}{\sqrt{1 - m^2}} \cos^{-1}(m) \right] \quad (5)$$

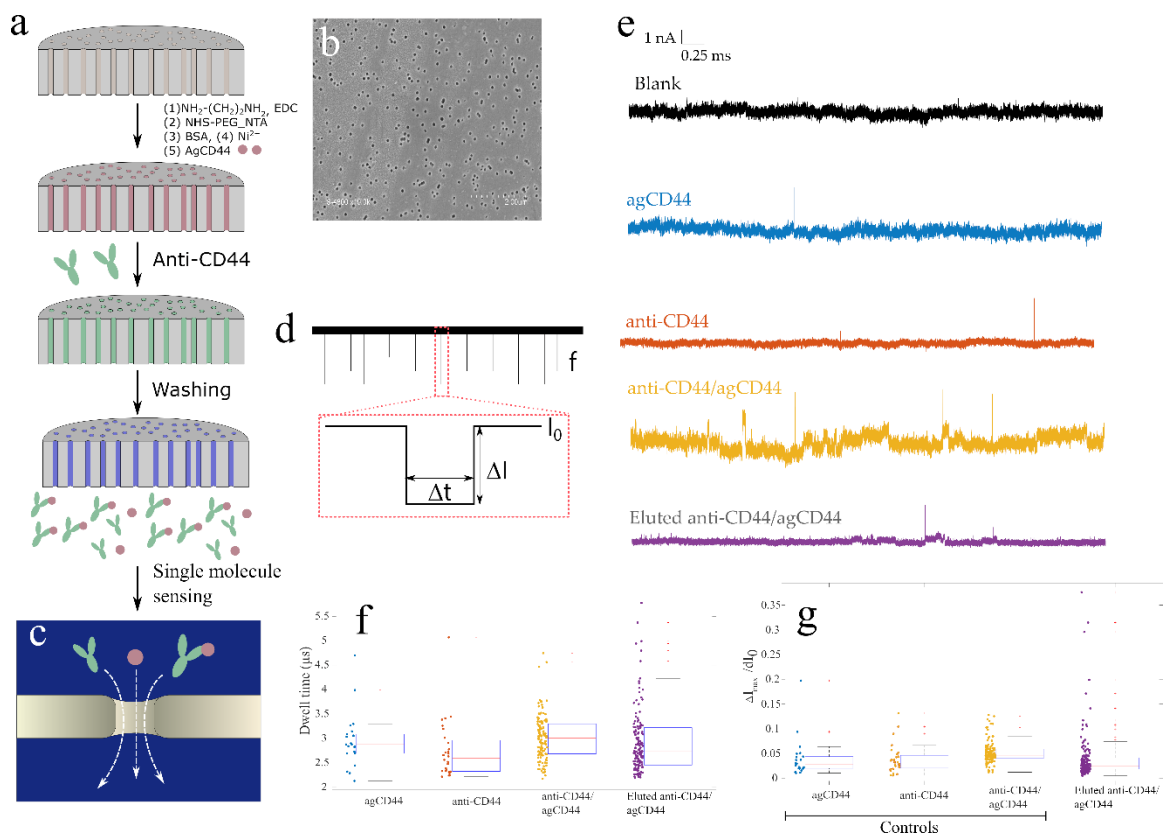
$$n_{\perp} = \frac{(1 - n_{\parallel})}{2} \quad (6)$$

299

300 Taking as radius $a = 2$ nm and $b = 2.7$ nm (from the PBD structure), the expected $\Delta I/I_0$ (according to
 301 a parallel and perpendicular orientations 0.05 and 0.09 respectively) are larger than measured
 302 one (centred to 0.03). The experimental values lower than expected could be due to a change in
 303 the structure of the protein under the effect of the electric field in the pore [70]. We also observe
 304 that the dwell time is ms scale is larger than the theoretical one as previously reported for the
 305 experiment conducted at sampling rate of 200 kHz [42,71]. This was assigned to the ability to
 306 detect only the protein that interacts with the nanopore [42]. The $\Delta I/I_0$ obtained for the
 307 AgCD44/anti-CD44 complex are centred on 0.06 (Figure 4). This shift toward larger values can be
 308 attributed to a larger volume of the complex than that of the anti-CD44, thus inducing a larger
 309 blocking amplitude during the bump. Interestingly, the dwell time increase regarding the
 310 antiCD44 that can be assigned to the larger volume of the complex and thus an increase of the
 311 diffusion coefficient.

312 The eluted solution from the multipore membrane was then analysed by the same single SiN
 313 nanopore. To do so, we measured current blockade during 25 min with a frequency rate of 0.11
 314 events/s. Even if this capture rate is low, it confirms that proteins are eluted from the membrane.
 315 The analysis of the $\Delta I/I_0$ reveals that the distributions are centred on 0.03. This corresponds well
 316 with the expected values for non-complexed anti-CD44 and AgCD44. In addition, the distribution
 317 of dwell time is spread suggesting that translocation and bumping are recorded. These results
 318 could be explained by the dissociation of AgCD44/anti-CD44 complex during the washing process.

319 In order to confirm this, filtration experiments with anti-CD44 labelled with alexa fluor 647 were
 320 performed. In order to observe a fluorescence signal in the elution product, we used a confocal
 321 system to perform fluorescence correlation spectroscopy measurements. First, a fluorescence
 322 signal was observed for the filtration product confirming the presence of anti-CD44 in the elution
 323 product. Self-correlation function analysis did not reveal a significant increase in the diffusion
 324 coefficient of antiCD44 due to its complexation with AgCD44. This is consistent with the nanopore
 325 analysis which suggests that the washing with imidazole\EDTA allow the elution of the anti-CD44
 326 and AgCD44 but separates the two entities.



327
 328 *Figure 4 : a. Scheme of the filtration process through a multipore membrane. b. scanning*
 329 *electron microscope image of the multipore membrane, c. Scheme of the detection process by a*
 330 *solid state nanopore, d. Scheme of the resistive pulse showing an event having a current*
 331 *blockade $\Delta I/I_0$ and dwell time Δt , e. Traces of each of the measured proteins (AgCD44 in blue,*

332 *anti-CD44 in orange, the complex in yellow) and the eluted solution in violet shown here by the*
333 *membrane trace, f. Boxplot for the logarithm of the dwell time of all the events obtained from*
334 *each protein and the filtered membrane solutions, e. boxplots for the current blockade of all the*
335 *protein and filtered solutions.*

336 **4. Conclusion**

337 In summary, we designed single nanopores ionic diode sensors to detect anti-CD44 by a simple I-
338 V curve measurement. The use of a bullet-like shape allows a good reproducibility, despite
339 variability inherent to the individual measurement. The multipore membrane obtained under
340 strictly the same conditions allowed the capture of the antibodies and their analysis by two
341 methods (nanopore and FCS). The elution product contains the uncomplexed antibody. Thus, we
342 show that it is possible to design a membrane based on single and multipore track-etched
343 technique for the separation of antibodies with an associated sensor allowing the parallel
344 detection of the antibody capture from the bulk solution. Next, using SiN nanopore sensors,
345 further analysis on the elution product allows to obtain information on the assembly of the two
346 entities antibody and antigens. We expect that the combination of a multipore membrane with
347 the ionic diode sensor in a microfluidic system could provide a novel versatile nanopore-based
348 device for an unprecedented fast preparation of analytical samples or the production of
349 antibodies. In addition, the parallel detection of traces of a target antibody could allow a pre-
350 control of the sample before a deeper and costly analysis.

351

352 **Acknowledgments**

353 This work was founded by Agence Nationale de la Recherche (ANR-19-CE42-0006, NanoOligo) and
354 l'institut Carnot Chimie Balard Cirimat (ref16CARN000801). Single tracks have been produced in

355 GANIL (Caen, France) in the framework of an EMIR project. The authors thank E. Balanzat at
356 CIMAP Caen for support to produce tracked polymer film.

357

358 **References**

- 359 [1] J.K. Aronson, R.E. Ferner, Biomarkers-A General Review, *Current Protocols in*
360 *Pharmacology* 76 (2017) 9.23.1-9.23.17.
- 361 [2] O. Yang, M. Qadan, M. Ierapetritou, Economic Analysis of Batch and Continuous
362 Biopharmaceutical Antibody Production: A Review, *J Pharm Innov* 14 (2019) 1–19.
- 363 [3] S. Liu, Z. Li, B. Yu, S. Wang, Y. Shen, H. Cong, Recent advances on protein
364 separation and purification methods, *Advances in Colloid and Interface Science* 284
365 (2020) 102254.
- 366 [4] Z. Zhu, J.J. Lu, S. Liu, Protein separation by capillary gel electrophoresis: a review,
367 *Analytica Chimica Acta* 709 (2012) 21–31.
- 368 [5] C.K. Larive, S.M. Lunte, M. Zhong, M.D. Perkins, G.S. Wilson, G. Gokulrangan, T.
369 Williams, F. Afroz, C. Schöneich, T.S. Derrick, C.R. Middaugh, S. Bogdanowich-
370 Knipp, Separation and analysis of peptides and proteins, *Anal. Chem.* 71 (1999)
371 389R-423R.
- 372 [6] K.K.R. Tetala, M.A. Vijayalakshmi, A review on recent developments for
373 biomolecule separation at analytical scale using microfluidic devices, *Analytica*
374 *Chimica Acta* 906 (2016) 7–21.
- 375 [7] M. Sonker, V. Sahore, A.T. Woolley, Recent advances in microfluidic sample
376 preparation and separation techniques for molecular biomarker analysis: A critical
377 review, *Analytica Chimica Acta* 986 (2017) 1–11.
- 378 [8] H.J. Issaq, The role of separation science in proteomics research, *Electrophoresis* 22
379 (2001) 3629–3638.
- 380 [9] R. Ghosh, Protein separation using membrane chromatography: opportunities and
381 challenges, *Journal of Chromatography A* 952 (2002) 13–27.
- 382 [10] S. AHMED, A. LUTES, T. BARBARI, Specific capture of target proteins by
383 oriented antibodies bound to tyrosinase-immobilized Protein A on a polyallylamine
384 affinity membrane surface, *Journal of Membrane Science* 282 (2006) 311–321.
- 385 [11] D. YU, M. MCLEAN, J. HALL, R. GHOSH, Purification of monoclonal antibody
386 from tobacco extract using membrane-based bioseparation techniques, *Journal of*
387 *Membrane Science* 323 (2008) 159–166.
- 388 [12] L. Wang, R. Ghosh, Fractionation of monoclonal antibody aggregates using
389 membrane chromatography, *Journal of Membrane Science* 318 (2008) 311–316.
- 390 [13] J. Wang, E.W. Jenkins, J.R. Robinson, A. Wilson, S.M. Husson, A new multimodal
391 membrane adsorber for monoclonal antibody purifications, *Journal of Membrane*
392 *Science* 492 (2015) 137–146.
- 393 [14] A.L. Zydney, New developments in membranes for bioprocessing – A review,
394 *Journal of Membrane Science* 620 (2021) 118804.
- 395 [15] E. Klein, Affinity membranes: a 10-year review, *Journal of Membrane Science* 179
396 (2000) 1–27.

- 397 [16] G. Brisson, M. Britten, Y. Pouliot, Electrically-enhanced crossflow microfiltration
398 for separation of lactoferrin from whey protein mixtures, *Journal of Membrane*
399 *Science* 297 (2007) 206–216.
- 400 [17] N. Meyer, I. Abrao-Nemeir, J.-M. Janot, J. Torrent, M. Lepoitevin, S. Balme, Solid-
401 state and polymer nanopores for protein sensing: A review, *Advances in Colloid and*
402 *Interface Science* 298 (2021) 102561.
- 403 [18] P.Y. Apel, I.V. Blonskaya, O.L. Orelovitch, S.N. Dmitriev, Diode-like ion-track
404 asymmetric nanopores: Some alternative methods of fabrication, *Nuclear*
405 *Instruments and Methods in Physics Research Section B: Beam Interactions with*
406 *Materials and Atoms* 267 (2009) 1023–1027.
- 407 [19] P.Y. Apel, I.V. Blonskaya, O.L. Orelovitch, P. Ramirez, B.A. Sartowska, Effect of
408 nanopore geometry on ion current rectification, *Nanotechnology* 22 (2011) 175302.
- 409 [20] K.-J. Kim, P.V. Stevens, A.G. Fane, Porosity dependence of pore entry shape in
410 track-etched membranes by image analysis, *Journal of Membrane Science* 93 (1994)
411 79–90.
- 412 [21] V. Chavan, C. Agarwal, A.K. Pandey, J.P. Nair, P. Surendran, P.C. Kalsi, A.
413 Goswami, Controlled development of pores in polyethylene terephthalate sheet by
414 room temperature chemical etching method, *Journal of Membrane Science* 471
415 (2014) 185–191.
- 416 [22] S. Dutt, P. Apel, N. Lizunov, C. Notthoff, Q. Wen, C. Trautmann, P. Mota-Santiago,
417 N. Kirby, P. Kluth, Shape of nanopores in track-etched polycarbonate membranes,
418 *Journal of Membrane Science* 638 (2021) 119681.
- 419 [23] T. Ma, J.-M. Janot, S. Balme, Track-Etched Nanopore/Membrane: From
420 Fundamental to Applications, *Small Methods* 4 (2020) 2000366.
- 421 [24] I.V. Blonskaya, N.E. Lizunov, K. Olejniczak, O.L. Orelovich, Y. Yamauchi, M.E.
422 Toimil-Molares, C. Trautmann, P.Y. Apel, Elucidating the roles of diffusion and
423 osmotic flow in controlling the geometry of nanochannels in asymmetric track-
424 etched membranes, *Journal of Membrane Science* 618 (2021) 118657.
- 425 [25] Z.S. Siwy, Ion-Current Rectification in Nanopores and Nanotubes with Broken
426 Symmetry, *Adv. Funct. Mater.* 16 (2006) 735–746.
- 427 [26] Z. Siwy, E. Heins, C.C. Harrell, P. Kohli, C.R. Martin, Conical-Nanotube Ion-
428 Current Rectifiers: The Role of Surface Charge, *J. Am. Chem. Soc.* 126 (2004)
429 10850–10851.
- 430 [27] M. Ali, B. Yameen, J. Cervera, P. Ramírez, R. Neumann, W. Ensinger, W. Knoll, O.
431 Azzaroni, Layer-by-layer assembly of polyelectrolytes into ionic current rectifying
432 solid-state nanopores: insights from theory and experiment, *J. Am. Chem. Soc.* 132
433 (2010) 8338–8348.
- 434 [28] G. Laucirica, Y.T. Terrones, V. Cayón, M.L. Cortez, M.E. Toimil-Molares, C.
435 Trautmann, W. Marmisollé, O. Azzaroni, Biomimetic Solid-State Nanochannels for
436 Chemical and Biological Sensing Applications, *TrAC Trends in Analytical*
437 *Chemistry* (2021) 116425.
- 438 [29] M. Ali, S. Nasir, W. Ensinger, Bioconjugation-induced ionic current rectification in
439 aptamer-modified single cylindrical nanopores, *Chemical communications*
440 (Cambridge, England) 51 (2015) 3454–3457.

- 441 [30] M. Ali, P. Ramirez, M.N. Tahir, S. Mafe, Z. Siwy, R. Neumann, W. Tremel, W.
442 Ensinger, Biomolecular conjugation inside synthetic polymer nanopores via
443 glycoprotein-lectin interactions, *Nanoscale* 3 (2011) 1894–1903.
- 444 [31] M. Ali, M.N. Tahir, Z. Siwy, R. Neumann, W. Tremel, W. Ensinger, Hydrogen
445 peroxide sensing with horseradish peroxidase-modified polymer single conical
446 nanochannels, *Analytical chemistry* 83 (2011) 1673–1680.
- 447 [32] M.N. Tahir, M. Ali, R. Andre, W.E.G. Müller, H.-C. Schröder, W. Tremel, W.
448 Ensinger, Silicatein conjugation inside nanoconfined geometries through
449 immobilized NTA-Ni(II) chelates, *Chem. Commun.* 49 (2013) 2210–2212.
- 450 [33] M. Lepoitevin, M. Bechelany, E. Balanzat, J.-M. Janot, S. Balme, Non-Fluorescence
451 label protein sensing with track-etched nanopore decorated by avidin/biotin system,
452 *Electrochimica Acta* 211 (2016) 611–618.
- 453 [34] M. Lepoitevin, B. Jamilloux, M. Bechelany, E. Balanzat, J.-M. Janot, S. Balme, Fast
454 and reversible functionalization of a single nanopore based on layer-by-layer
455 polyelectrolyte self-assembly for tuning current rectification and designing sensors,
456 *RSC advances* 6 (2016) 32228–32233.
- 457 [35] I. Duznovic, A. Gräwe, W. Weber, L.K. Müller, M. Ali, W. Ensinger, A. Tietze, V.
458 Stein, Ultrasensitive and Selective Protein Recognition with Nanobody-
459 Functionalized Synthetic Nanopores, *Small* (Weinheim an der Bergstrasse,
460 Germany) 17 (2021) e2101066.
- 461 [36] T. Ma, E. Balanzat, J.-M. Janot, S. Balme, Single conical track-etched nanopore for
462 a free-label detection of OSCS contaminants in heparin, *Biosensors & bioelectronics*
463 137 (2019) 207–212.
- 464 [37] J. Li, D. Stein, C. McMullan, D. Branton, M.J. Aziz, J.A. Golovchenko, Ion-beam
465 sculpting at nanometre length scales, *Nature* 412 (2001) 166–169.
- 466 [38] L. Xue, H. Yamazaki, R. Ren, M. Wanunu, A.P. Ivanov, J.B. Edel, Solid-state
467 nanopore sensors, *Nat Rev Mater* 5 (2020) 931–951.
- 468 [39] R. Hu, J.V. Rodrigues, P. Waduge, H. Yamazaki, B. Cressiot, Y. Chishti, L.
469 Makowski, D. Yu, E. Shakhnovich, Q. Zhao, M. Wanunu, Differential Enzyme
470 Flexibility Probed Using Solid-State Nanopores, *ACS nano* 12 (2018) 4494–4502.
- 471 [40] J. Larkin, R.Y. Henley, M. Muthukumar, J.K. Rosenstein, M. Wanunu, High-
472 bandwidth protein analysis using solid-state nanopores, *Biophysical Journal* 106
473 (2014) 696–704.
- 474 [41] P. Waduge, R. Hu, P. Bandarkar, H. Yamazaki, B. Cressiot, Q. Zhao, P.C. Whitford,
475 M. Wanunu, Nanopore-Based Measurements of Protein Size, Fluctuations, and
476 Conformational Changes, *ACS nano* 11 (2017) 5706–5716.
- 477 [42] S. Balme, P.E. Coulon, M. Lepoitevin, B. Charlot, N. Yandrapalli, C. Favard, D.
478 Muriaux, M. Bechelany, J.-M. Janot, Influence of Adsorption on Proteins and
479 Amyloid Detection by Silicon Nitride Nanopore, *Langmuir* 32 (2016) 8916–8925.
- 480 [43] L. Reynaud, A. Bouchet-Spinelli, J.-M. Janot, A. Buhot, S. Balme, C. Raillon,
481 Discrimination of α -Thrombin and γ -Thrombin Using Aptamer-Functionalized
482 Nanopore Sensing, *Anal. Chem.* 93 (2021) 7889–7897.
- 483 [44] E.C. Yusko, B.R. Bruhn, O.M. Eggenberger, J. Houghtaling, R.C. Rollings, N.C.
484 Walsh, S. Nandivada, M. Pindrus, A.R. Hall, D. Sept, J. Li, D.S. Kalonia, M. Mayer,
485 Real-time shape approximation and fingerprinting of single proteins using a
486 nanopore, *Nature Nanotech* 12 (2017) 360–367.

- 487 [45] E.C. Yusko, P. Prangio, D. Sept, R.C. Rollings, J. Li, M. Mayer, Single-Particle
488 Characterization of A β Oligomers in Solution, *ACS nano* 6 (2012) 5909–5919.
- 489 [46] H. Chae, D.-K. Kwak, M.-K. Lee, S.-W. Chi, K.-B. Kim, Solid-state nanopore
490 analysis on conformation change of p53TAD-MDM2 fusion protein induced by
491 protein-protein interaction, *Nanoscale* 10 (2018) 17227–17235.
- 492 [47] J. Li, D. Fologea, R. Rollings, B. Ledden, Characterization of Protein Unfolding
493 with Solid-state Nanopores, *PPL* 21 (2014) 256–265.
- 494 [48] M. Firnkes, D. Pedone, J. Knezevic, M. Döblinger, U. Rant, Electrically facilitated
495 translocations of proteins through silicon nitride nanopores: conjoint and competitive
496 action of diffusion, electrophoresis, and electroosmosis, *Nano letters* 10 (2010)
497 2162–2167.
- 498 [49] D. Fologea, B. Ledden, D.S. McNabb, J. Li, Electrical characterization of protein
499 molecules by a solid-state nanopore, *Applied physics letters* 91 (2007) 539011–
500 539013.
- 501 [50] J. Houghtaling, C. Ying, O.M. Eggenberger, A. Fennouri, S. Nandivada, M.
502 Acharjee, J. Li, A.R. Hall, M. Mayer, Estimation of Shape, Volume, and Dipole
503 Moment of Individual Proteins Freely Transiting a Synthetic Nanopore, *ACS nano*
504 13 (2019) 5231–5242.
- 505 [51] L. Reynaud, A. Bouchet-Spinelli, C. Raillon, A. Buhot, Sensing with Nanopores and
506 Aptamers: A Way Forward, *Sensors (Basel, Switzerland)* 20 (2020).
- 507 [52] Y.-L. Ying, R.-J. Yu, Y.-X. Hu, R. Gao, Y.-T. Long, Single antibody-antigen
508 interactions monitored via transient ionic current recording using nanopore sensors,
509 *Chem. Commun.* 53 (2017) 8620–8623.
- 510 [53] M. Waugh, K. Briggs, D. Gunn, M. Gibeault, S. King, Q. Ingram, A.M. Jimenez, S.
511 Berryman, D. Lomovtsev, L. Andrzejewski, V. Tabard-Cossa, Solid-state nanopore
512 fabrication by automated controlled breakdown, *Nat Protoc* 15 (2020) 122–143.
- 513 [54] H. Kwok, K. Briggs, V. Tabard-Cossa, Nanopore fabrication by controlled dielectric
514 breakdown, *PLoS one* 9 (2014) e92880.
- 515 [55] S. Balme, T. Ma, E. Balanzat, J.-M. Janot, Large osmotic energy harvesting from
516 functionalized conical nanopore suitable for membrane applications, *Journal of*
517 *Membrane Science* 544 (2017) 18–24.
- 518 [56] T. Ma, E. Balanzat, J.-M. Janot, S. Balme, Nanopore Functionalized by Highly
519 Charged Hydrogels for Osmotic Energy Harvesting, *ACS Appl. Mater. Interfaces* 11
520 (2019) 12578–12585.
- 521 [57] M. Lepoitevin, T. Ma, M. Bechelany, J.-M. Janot, S. Balme, Functionalization of
522 single solid state nanopores to mimic biological ion channels: A review, *Advances in*
523 *Colloid and Interface Science* 250 (2017) 195–213.
- 524 [58] H. Xu, M. Niu, X. Yuan, K. Wu, A. Liu, CD44 as a tumor biomarker and therapeutic
525 target, *Exp Hematol Oncol* 9 (2020) 36.
- 526 [59] H.A. Runnels, G.L. Weber, J. Min, E.M. Kudlacz, J.F. Zobel, C.B. Donovan, M.A.
527 Thiede, J. Zhang, R.B. Alpert, M.A. Salafia, A.J. Milici, D. Burdette, R.R. Bell, J.S.
528 Beebe, X. Xu, PF-03475952: a potent and neutralizing fully human anti-CD44
529 antibody for therapeutic applications in inflammatory diseases, *Adv Therapy* 27
530 (2010) 168–180.

531 [60] P.Y. Apel, I.V. Blonskaya, S.N. Dmitriev, O.L. Orelovitch, A. Presz, B.A.
532 Sartowska, Fabrication of nanopores in polymer foils with surfactant-controlled
533 longitudinal profiles, *Nanotechnology* 18 (2007) 305302.

534 [61] P.Y. Apel, I.V. Blonskaya, N.E. Lizunov, K. Olejniczak, O.L. Orelovitch, M.E.
535 Toimil-Molares, C. Trautmann, Osmotic Effects in Track-Etched Nanopores, *Small*
536 (Weinheim an der Bergstrasse, Germany) 14 (2018) e1703327.

537 [62] S. SIRIJARUKUL, E. BALANZAT, E. VASINA, P. DEJARDIN, Flat sheet
538 membrane with controlled variation of pore density and pore size in a direction
539 parallel to the surface☆, *Journal of Membrane Science* 296 (2007) 185–194.

540 [63] K. Kececi, L.T. Sexton, F. Buyukserin, C.R. Martin, Resistive-pulse detection of
541 short dsDNAs using a chemically functionalized conical nanopore sensor,
542 *Nanomedicine (London, England)* 3 (2008) 787–796.

543 [64] P.Y. Apel, I.V. Blonskaya, S.N. Dmitriev, O.L. Orelovich, B.A. Sartowska, Ion track
544 symmetric and asymmetric nanopores in polyethylene terephthalate foils for
545 versatile applications, *Nuclear Instruments and Methods in Physics Research Section*
546 *B: Beam Interactions with Materials and Atoms* 365 (2015) 409–413.

547 [65] Z.J. Grzywna, Z. Siwy, C.L. Bashford, Nonlinear theory for ionic transport through
548 track-etched nuclear membranes, *Journal of Membrane Science* 121 (1996) 261–
549 269.

550 [66] D.Y. Butylskii, N.D. Pismenskaya, P.Y. Apel, K.G. Sabbatovskiy, V.V. Nikonenko,
551 Highly selective separation of singly charged cations by countercurrent
552 electromigration with a track-etched membrane, *Journal of Membrane Science* 635
553 (2021) 119449.

554 [67] T. Ma, N. Arroyo, J. Marc Janot, F. Picaud, S. Balme, Conformation of Polyethylene
555 Glycol inside Confined Space: Simulation and Experimental Approaches,
556 *Nanomaterials (Basel, Switzerland)* 11 (2021).

557 [68] D. Coglitore, J.-M. Janot, S. Balme, Protein at liquid solid interfaces: Toward a new
558 paradigm to change the approach to design hybrid protein/solid-state materials,
559 *Advances in Colloid and Interface Science* 270 (2019) 278–292.

560 [69] E.C. Yusko, J.M. Johnson, S. Majd, P. Prangkio, R.C. Rollings, J. Li, J. Yang, M.
561 Mayer, Controlling protein translocation through nanopores with bio-inspired fluid
562 walls, *Nature Nanotech* 6 (2011) 253–260.

563 [70] A. Carlsen, V. Tabard-Cossa, Mapping shifts in nanopore signal to changes in
564 protein and protein-DNA conformation, *Proteomics* (2021) e2100068.

565 [71] C. Plesa, S.W. Kowalczyk, R. Zinsmeister, A.Y. Grosberg, Y. Rabin, C. Dekker,
566 Fast translocation of proteins through solid state nanopores, *Nano letters* 13 (2013)
567 658–663.
568