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Author(s)	Frauenlob, Martin; Guo, Honglei; Kurokawa, Takayuki; Gong, Jian Ping
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1 Supporting information

2 to

3 Origin of surface charge of double network hydrogels prepared by  
4 sequential polymerization

5 Martin Frauenlob<sup>1,2</sup>, Honglei Guo<sup>3</sup>, Takayuki Kurokawa<sup>3,\*</sup> and Jian Ping Gong<sup>3,4\*</sup>

6 <sup>1</sup>Graduate School of Life Science, Hokkaido University, N21W11, Kita-ku, Sapporo, Hokkaido 001-  
7 0021, Japan

8 <sup>2</sup>Group of Biomaterials and Microfluidics Core Facility, Institut Pasteur, 25-28 Rue du Docteur Roux,  
9 75015 Paris, France

10 <sup>3</sup>Faculty of Advanced Life Science, Hokkaido University, N21W11, Kita-ku, Sapporo 001-10 0021,  
11 Japan

12 <sup>4</sup>Institute for Chemical Reaction Design and Discovery (WPI-ICReDD), Hokkaido University, 8  
13 N21W10, Kita-ku, Sapporo 001-0021, Japan

14  
15 \*Corresponding author (E-mail: [kurokawa@sci.hokudai.ac.jp](mailto:kurokawa@sci.hokudai.ac.jp); [gong@sci.hokudai.ac.jp](mailto:gong@sci.hokudai.ac.jp))

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## Supporting Information

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### 19 1.1 Experimental section

#### 20 1.1.1 Materials

21 The monomers 2-acrylamido-2-methylpropanesulfonic acid (AMPS, 1<sup>st</sup> network) was obtained by  
22 Toagosei Co., Ltd., Japan. The acrylamide (AAM, 2<sup>nd</sup> network), the crosslinker N,N'-  
23 methylenebis(acrylamide) (MBAA), and initiator 2-oxoglutaric acid were obtained from Wako Pure  
24 Chemical Industries, Ltd., Japan. All reagents were used as received, and all solutions were prepared  
25 in double-distilled water.

#### 26 1.1.2 Single network, double network, and co-polymer hydrogel synthesis

27 All hydrogels were synthesized based on the formulations in **Table 1** and **Table S1** via free-radical  
28 polymerization by 365 nm-UV light for 8 hours in an argon atmosphere. The double network (DN)  
29 hydrogels were prepared as previously described using glass slides as a molding material, and no  
30 compression in the polymerization steps was applied to create a 2<sup>nd</sup> network surface layer<sup>1</sup>.

31 Briefly, the 1<sup>st</sup> network was synthesized from an aqueous solution 1 M AMPS containing 3 mol%  
32 crosslinker MBAA and 1 mol% 2-oxoglutaric acid as initiator, poured between two glass plates spaced  
33 by 1 mm. The poly 2-acrylamido-2-methylpropanesulfonic acid (PAMPS) hydrogel rectangles of  
34 different sizes were cut out to reach volume ratios  $V_r$  of 0,02 to 0,001 ( $V_r = V_{PAMPS}/V_{AAM}$ ) of PAMPS  
35 hydrogel ( $V_{PAMPS}$ ) in AAM 2<sup>nd</sup> network precursor solution ( $V_{AAM}$ ). For the sample PAMPS-w, an as-  
36 prepared PAMPS hydrogel with 2 cm<sup>3</sup> (geometry:  $l \times b \times h$ , 4×5×0,1 cm; 2 mL) washed in 100 mL water  
37 ( $V_r$  0,02) for 15 days. Here, the 100 mL washing water was exchanged daily, and ionic conductivity was  
38 measured. PAMPS and PAMPS-w hydrogels were equilibrated for 3 days in 50mL of 2<sup>nd</sup> network  
39 precursor solution containing 2,0 M AAM monomer, 0,1 mol% MBAA crosslinker, and 0,1 mol% 2-  
40 oxoglutaric acid. The PAMPS hydrogels soaked in AAM solution were sandwiched between two glass  
41 plates and polymerized to DN hydrogels (PAMPS/PAAM). After equilibration and removal of the PAMPS

42 hydrogel from the 2<sup>nd</sup> network precursor solution, this waste solution was used to synthesize  
 43 polyacrylamide (PAAm) single network (SN) hydrogels (PAAm-ps).

44 Further, a control group out of poly(AAm-co-AMPS) hydrogel was synthesized to demonstrate how the  
 45 PAMPS concentration affects the electric potential. Here, the PAMPS concentration was regulated by  
 46 the concentration of AMPS in the mixture in the range of 20 – 0,02 mM. The mixture solution contains  
 47 2,0 M AAm, 0,1 mol% MBAA crosslinker, and 0,1 mol% 2-oxoglutaric acid. Since this control group tries  
 48 to mimic the residual AMPS content in the 2<sup>nd</sup> network precursor solution, it is also referred to as  
 49  $C_{AMPS,R}$  for the reason of simplification. To synthesize the poly(AAm-co-AMPS) hydrogels, the aqueous  
 50 AAm and AMPS precursor solutions were mixed by the ratios in **Table S1**. The mixtures were  
 51 polymerized between two glass plates spaced by 1 mm. Before experimental use, all hydrogels were  
 52 equilibrated in water and in  $1 \times 10^{-4}$  M NaClaq for MET measurement.

53 **Table S1: Poly(AAm-co-AMPS) hydrogel formulation with varied  $C_{AMPS,R}$  concentration.**

Total volume (mL)	Volume of PAAm precursor sol.* (mL)	Volume of 1 M AMPS precursor sol. ( $\mu$ L)	$C_{AMPS,R}$ (mM)	$C_{AMPS,R}/C_{AAm}$ (M/M)
50	49	1000	20	1/98
50	49,5	500	10	1/198
50	49,9	100	2	1/998
50	50	10	0,2	1/10000
50	50	1	0,02	1/100000

54 \* 2,0 M AAm, 0,1 mol% crosslinker, and 0,1 mol% initiator.

### 55 1.1.3 Determining unreacted $C_{AMPS,R}$ of the hydrogel via the ionic conductivity of bath 56 solution

57 The unreacted AMPS monomer concentration ( $C_{AMPS,R}$ ) in the hydrogel bathing solution was monitored  
 58 on a conductivity meter (FE30 KIT, Mettler Toledo). The evaluation included the correlation between  
 59 ionic conductivity and the concentration of the dissolved AMPS monomers via a dilution series ranging  
 60 from  $10^{-1}$  to  $10^{-6}$  M, using water or 2<sup>nd</sup> network precursor solution containing AAm monomer  
 61 (formulation in **Table 1**) as diluting agent (**Figure 1A**). Hereby, we assume that other stable and highly  
 62 charged molecules in the hydrogel precursor solution, such as crosslinker and initiator, are totally  
 63 consumed during the 8 hours of polymerization because they are used at a much smaller concentration

64 than the monomer. In the AAm solution, the lower detection limit of  $C_{AMPS}$  lies at a concentration of  
65 0,1 mM, as the conductivity approaches a constant value of 340  $\mu\text{S}/\text{cm}$ , which is characteristic for the  
66 pure 2<sup>nd</sup> network precursor solution of 2 M AAm containing 0,1 mol% crosslinker and 0,1 mol%  
67 photoinitiator (**Table S2**). The calibration curves for AMPS in water and AMPS in 2<sup>nd</sup> network precursor  
68 solution were fitted by Origin (OriginLab Corp.) software. With these calibration curves, we calculated  
69  $C_{AMPS,R}$  in the washing water of the PAMPS-w hydrogel immersed in 100 mL water and the equilibration  
70 of PAMPS and PAMPS-w hydrogel in 2<sup>nd</sup> network precursor solution of AAm at multiple time points.  
71 Hydrogels were stored at 25 °C in the dark on a horizontal shaker plate at 60 rpm. It needs to be noted  
72 that the calculated  $C_{AMPS,R}$  was corrected by the daily decrease in conductivity of the control AAm bath  
73 solution containing no PAMPS hydrogel since the UV/VIS reactive 2-oxoglutaric acid is consumed  
74 slightly over time.

#### 75 1.1.4 Determine surface charge density $C_G$ via the microelectrode technique (MET)

76 To investigate the effect of  $C_{AMPS,R}$  on the hydrogel surface charge density  $C_G$  the electric potential  
77 difference  $\Delta\varphi$  between the inside and outside of the hydrogel was measured via MET<sup>2</sup>. Before the  
78 sampling, hydrogels were washed in water until the washing water reached a conductivity of less than  
79 2,0  $\mu\text{S}/\text{cm}$ . Then the hydrogel was immersed in the bath aqueous solution of NaCl  $1 \times 10^{-4}$  M ( $C_S$ ) (Debye  
80 length is 30,4 nm) until it reached equilibrium at a conductivity of  $\sim 15$   $\mu\text{S}/\text{cm}$  at 25 °C. The hydrogels  
81 were probed with a fiber-filament glass capillary microelectrode filled with 3 M KCl of 180 nm outer  
82 diameter and 128 nm inner diameter (P-1000 capillary puller - Sutter Instrument Co., U.S.). The  
83 microelectrode was inserted at a speed range of 0,5 – 2  $\mu\text{m}/\text{s}$ . The surface charge density  $C_G$  at the  
84 hydrogel surface was calculated via **Eq. 1** by using the averaged electric potential differences  
85 ( $\Delta\varphi$  between blue arrows in **Figure 3**,  $n = 3$ ) between the bathing solution (**Figure 3**, white area) and  
86 the hydrogel surface region (**Figure 3**, light grey area). The activity coefficients are considered as 1 ( $\gamma_s$ ,  
87  $\gamma_G = 1$ ) since the mobile ion concentration in salt solution and the surface layer of DN hydrogels are  
88 very low.

89 1.1.5 Determination of the volumetric swelling ratio of hydrogels with varied  $C_{AMPS,R}$  in the  
90 precursor solution

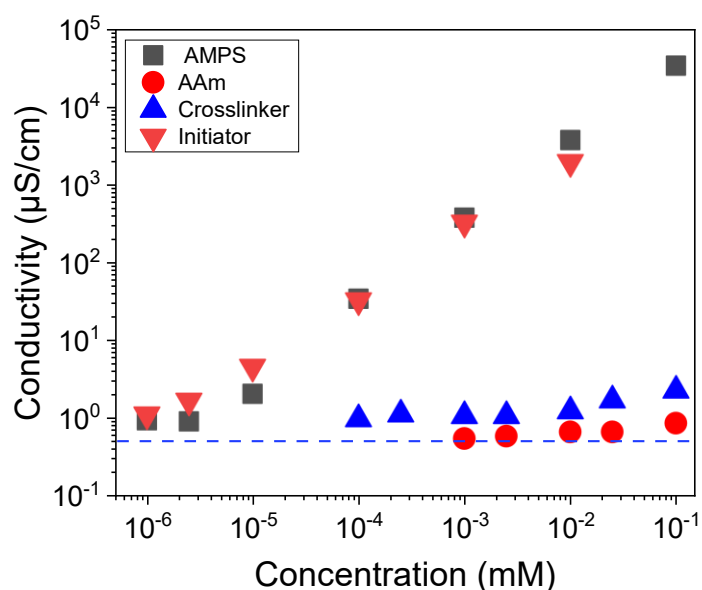
91 The volumetric swelling ratio of hydrogels  $Q = (t_1/t_0)^3$ , was determined from the thickness of hydrogels  
92 in the as-prepared state after polymerization of PAAm ( $t_0$ ) and in the equilibrated state in  $1 \times 10^{-4}$  M  
93 NaCl solution ( $t_1$ ) for PAAm-ps and Poly(AAm-co-AMPS) hydrogels. For PAMPS/PAAm DN hydrogels,  
94 the surface layer's swelling ratio should be considered. The bulk DN hydrogel hardly swells in the  $1 \times 10^{-4}$   
95 M NaCl solution, as the volumetric swelling ratio  $Q = (t_1/t_0)^3$  of the as-prepared PAMPS/PAAm  
96 hydrogel ( $t_0$ ) (which is the same as the thickness of PAMPS hydrogel equilibrated in the AAm 2<sup>nd</sup>  
97 network precursor solution) and the thickness of PAMPS/PAAm hydrogel swollen in  $1 \times 10^{-4}$  M NaCl  
98 solution ( $t_1$ ) is almost 1 (**Figure S3**). A recent study has shown that the soft surface layer of the second  
99 network swells uniaxially normal to the surface since the lateral swelling is constrained by the rigid  
100 bulk DN hydrogel<sup>3</sup>. A recent study has also shown that the volume of PAAm hydrogel with uniaxial  
101 swelling is the same with the isotropic swelling<sup>4</sup>. Thereby, we assume that  $Q$  of the surface second  
102 network layer in PAMPS/PAAm DN gel is the same as that of PAAm-ps.

103 1.1.6 Mechanical testing via micro-indentation

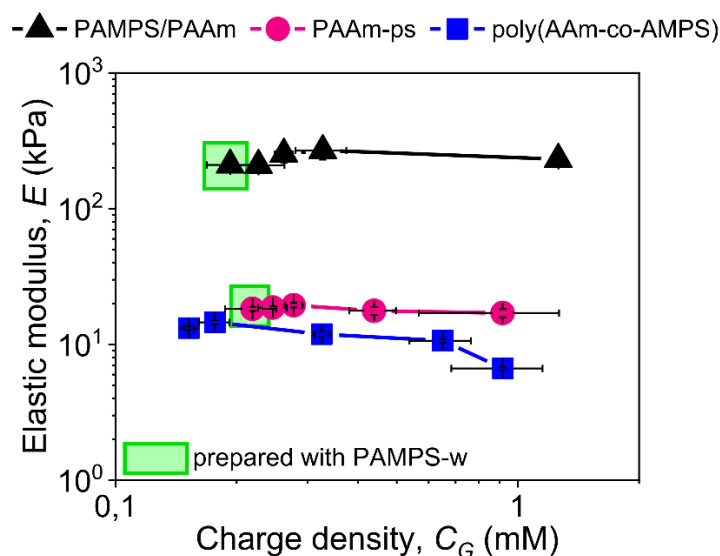
104 The hydrogel elastic modulus ( $E$ ) was measured on a tensile-compressive mechanical tester (Autograph  
105 AG-X, Shimadzu Co., Japan) in air at 25°C. A sphere-shaped micro-indenter ( $r = 250 \mu\text{m}$ , material:  
106 stainless steel) probed the hydrogel at a deformation velocity of  $250 \mu\text{m}/\text{min}$  for 1 min. The Hertzian  
107 model<sup>5</sup> for rigid sphere indenters, with an assumed Poisson's ratio for the hydrogels of 0,5, was used  
108 to determine  $E$  from the micro-indentation loading curves. We made sure to limit the effect of hydrogel  
109 drying on the mechanical testing by saturating the air with water via a humidifier placed next to the  
110 tensile tester and reducing the measurement time down to 1 minute.

111

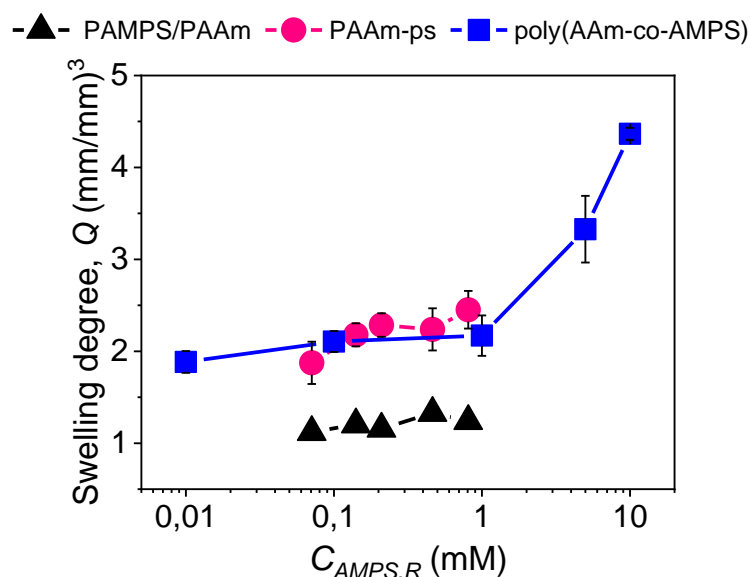
112 1.2 Supporting Data



113  
 114 **Figure S1: The relation of conductivity to the concentration of agents used in the 1<sup>st</sup> and 2<sup>nd</sup> network**  
 115 **precursor solution.** The Conductivity of AMPS, AAm, the crosslinker N,N'-methylenebis(acrylamide),  
 116 and the initiator 2-oxoglutaric acid in deionized water measured at 25°C (the blue dotted line  
 117 represents the conductivity of deionized water). The conductivity measurements cannot account for  
 118 the hydrolysis of acrylamide and hydrolysis. However, even if there are impurities and hydrolysis in  
 119 AAm, the conductivity of AAm solutions is not higher than 1 μS/cm. Therefore, we can conclude that  
 120 for the conductivity measurements, the effects of hydrolysis and impurities in AAm are negligible  
 121 because the conductivity in the precursor solution comes from the initiator.

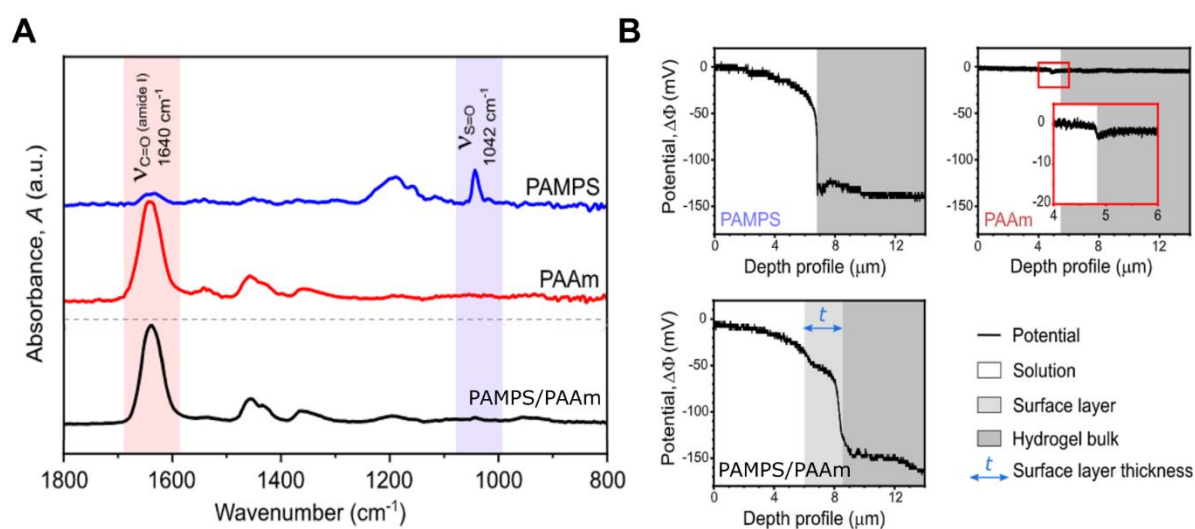


122  
 123 **Figure S2: Relation between surface layer charge density  $C_G$  calculated from  $\Delta\varphi$  and the elastic**  
 124 **modulus  $E$  on the surface layer of hydrogels measured by micro-indentation.** We found that  $E$   
 125 measured in the bulk of the PAMPS/PAAm DN hydrogels is almost constant in the range of 200 – 300  
 126 kPa, regardless of charge density increase from 0,19 and 1,26 mM. The PAAm-ps hydrogel group  
 127 behaves similarly but shows a lower elastic modulus range at 15 - 20 kPa, which is characteristic of SN  
 128 PAAm hydrogels of this formulation. Only the poly(AAm-co-AMPS) hydrogel series showed a slight  
 129 decrease of  $E$  with the increase of  $C_G$ , which is attributed to the swelling, as shown in the main article  
 130 (**Figure 4B**) and the supporting information (**Figure S3**).



131  
 132 **Figure S3: The swelling ratio  $Q$  of the bulk hydrogels in the state of MET measurement against  $C_{AMPS,R}$**   
 133 **in the precursor solution of the PAAm network.** The decreased swelling of the DN hydrogels after 2<sup>nd</sup>  
 134 network polymerization results from the reduced swelling of the 2<sup>nd</sup> network confined by the 1<sup>st</sup>  
 135 network in the bulk PAMP/PAAm DN hydrogel. However, the swelling ratio of the surface second  
 136 network layer in PAMPS/PAAm DN hydrogels should be similar to that of PAAm-ps hydrogels.  
 137 Therefore,  $C_6Q$  of the DN hydrogels was calculated, using  $Q$  of the corresponding PAAm-ps hydrogels.

138



139  
 140 **Figure S4: Comparison between ATR-FTIR and Microelectrode technique measurements (MET).** (A)  
 141 ATR-FTIR profiles and (B) MET measurements of single network PAMPS, PAAm, and the corresponding  
 142 double network hydrogel (PAMPS/PAAm) demonstrate that the concentration of AMPS groups in the  
 143 double network hydrogel surface layer was too low to be detected by ATR-FTIR, while the MET  
 144 measurement was able to clearly detect a drop in surface layer potential of -50mV. The figure is  
 145 reproduced with permission from “Modulation and Characterization of the Double Network Hydrogel  
 146 Surface-Bulk Transition” (DOI:10.1021/acs.macromol.9b01399). Copyright 2019 ACS.

147



148 **Table S2: Summary of results**

$V_r$ (mL/mL)	Conductivity ( $\mu\text{S/cm}$ )	$C_{AMPS,R}$ (mM)	$C_{AMPS,R}/C_{AAM}$ (M/M)	$Q$ (mm/mm) <sup>3</sup>	$\Delta\phi$ (mV)	$C_G$ (mM)	$C_G Q$ (mM)	$E$ (kPa)
PAMPS/PAAm								
0,02	510,2	0,81	1/2419	1,24 ± 0,02	-65,0 ± 0,9	1,26 ± 0,04	3,09 ± 0,28 <sup>S</sup>	231,5 ± 10,4
0,01	437,5	0,46	1/4304	1,33 ± 0,05	-30,2 ± 3,7	0,33 ± 0,05	0,73 ± 0,13 <sup>S</sup>	269,0 ± 39,3
0,003	384,1	0,21	1/9495	1,16 ± 0,03	-24,7 ± 1,3	0,26 ± 0,01	0,60 ± 0,05 <sup>S</sup>	252,7 ± 35,5
0,001	369,6	0,14	1/14271	1,20 ± 0,01	-20,8 ± 4,1	0,23 ± 0,04	0,49 ± 0,08 <sup>S</sup>	209,0 ± 7,8
0,02*	356,2	0,07	1/28000	1,12 ± 0,05	-16,7 ± 3,2	0,19 ± 0,02	0,36 ± 0,06 <sup>S</sup>	209,9 ± 8,1
PAAm-ps								
0,02	510,2	0,81	1/2419	2,45 ± 0,20	-55,6 ± 9,0	0,92 ± 0,35	2,24 ± 0,86	17,1 ± 1,2
0,01	437,5	0,46	1/4304	2,24 ± 0,23	-37,8 ± 3,2	0,44 ± 0,06	0,98 ± 0,16	17,7 ± 1,1
0,003	384,1	0,21	1/9495	2,28 ± 0,13	-26,1 ± 1,3	0,28 ± 0,01	0,63 ± 0,05	19,5 ± 0,7
0,001	369,6	0,14	1/14271	2,18 ± 0,12	-23,0 ± 2,2	0,25 ± 0,02	0,54 ± 0,05	18,7 ± 0,4
0,02*	356,2	0,07	1/28000	1,87 ± 0,23	-19,9 ± 3,5	0,22 ± 0,03	0,41 ± 0,07	18,3 ± 0,6
PAAm								
--	340	--	--	1,98 ± 0,12	-9,2 ± 1,6	0,14 ± 0,01	0,28 ± 0,02	15,1 ± 1,0
poly(AAm-co-AMPS)								
--	4600	20	1/98	4,37 ± 0,07	-56,3 ± 5,9	0,92 ± 0,23	4,00 ± 1,02	6,7 ± 0,2
--	2350	10	1/198	3,33 ± 0,36	-47,8 ± 4,7	0,65 ± 0,11	2,16 ± 0,44	10,7 ± 0,3
--	738	2	1/998	2,17 ± 0,22	-30,3 ± 1,1	0,33 ± 0,01	0,71 ± 0,08	11,9 ± 0,6
--	395	0,2	1/10000	2,11 ± 0,11	-14,5 ± 2,2	0,18 ± 0,02	0,37 ± 0,04	14,6 ± 0,5
--	360	0,02	1/100000	1,88 ± 0,12	-10,8 ± 0,7	0,15 ± 0,01	0,29 ± 0,02	13,2 ± 0,4

149 \*Sample prepared associated with PAMPS-w. <sup>S</sup> $C_G$  multiplied by  $Q$  from the corresponding PAAm-ps gel.

150

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168