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Resistivity Detection of Perfluoroalkyl Substances (PFAS) with 6 Fluorous-Polyaniline in an Electrical Lateral Flow Sensor

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4 **Main Manuscript for**

5 **Resistivity Detection of Perfluoroalkyl Substances (PFAS) with**

6 **Fluorous-Polyaniline in an Electrical Lateral Flow Sensor**

7

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15 **Author Contributions:** S.P. and C.T.G. designed experiments, developed materials,
16 and executed the experiments. T.M.S. conceived and directed the research. All authors
17 contributed to the preparation of the manuscript.

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22

23 **This PDF file includes:**

24 Main Text
25 Figures 1 to 4
26 Table 1
27
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31
32

33 **Abstract**

34 Perfluoroalkyl substances (PFAS), known as “forever chemicals,” are a growing concern
35 in the sphere of human and environmental health. In response, rapid, reproducible, and
36 inexpensive methods for PFAS detection in the environment and home water supplies
37 are needed. We have developed a simple and inexpensive perfluoroalkyl acid detection
38 method based on an electrically read lateral flow assay (e-LFA). Our method employs a
39 new fluorous surfactant formulation with undoped polyaniline (F-PANI) fabricated to
40 create test lines for the lateral flow assay. In perfluoroalkyl acid sensing studies, an
41 increase in conductivity of the F-PANI film is caused by acidification and doping of PANI.
42 A conductivity enhancement by 10^4 -fold can be produced by this method and we
43 demonstrate a limit of detection for perfluorooctanoic acid (PFOA) of 400 ppt and
44 perfluorobutanoic acid (PFBA) of 200 ppt. This new method for PFOA detection can be
45 expanded for wide-scale environmental and at-home water testing.
46

47 **Significance Statement**

48 Lateral Flow Assays (LFAS) have garnered a broad public acceptance for sensing in
49 healthcare and offer expanded applications for environmental sensing with facile
50 implementation. With the increasing concern for the widespread detection of per- and
51 poly-fluoroalkyl substances (PFAS), selective sensing is imperative for monitoring these
52 harmful chemicals in the environment and drinking water. We report a low-cost and
53 sensitive detection method for quantifying PFAS with an e-LFA. As the PFAS oxidize the
54 rigid conjugated polyaniline (PANI) backbone, an increase in conductivity can be
55 observed through an electrically read lateral flow assay (e-LFA). Our novel method
56 harnesses the fluorous effect to selectively target perfluoroalkyl acids, over their non-
57 fluorous equivalents. Our F-PANI fabricated e-LFA exhibits a 400 ppt detection limit for
58 PFOA and provides quantitative measurements from simple resistivity measurements.
59

60
61 **Main Text**

62
63 **Introduction**

64
65 **PFAS:** Per- and poly-fluoroalkyl substances (PFAS) contain fully fluorinated alkyl
66 groups, and have been widely used to provide waterproof, anti-stain, and heat-
67 resistance properties.(1, 2) However, their extraordinary stability has allowed for
68 accumulation in water supplies and this is now recognized as a serious threat to public
69 health.(3, 4) Studies show that PFAS may result in adverse effects including increased
70 cholesterol levels, thyroid disease, liver damage, kidney cancer, testicular cancer,
71 developmental effects affecting the unborn child, and other environmental damage.(1, 5,
72 6) In response to this issue, the US Environmental Protection Agency (EPA) has
73 introduced regulatory guidance to limit the amount of six different PFAS in drinking water
74 with levels of 4 ppt ($4 \text{ ng}\cdot\text{L}^{-1}$) for perfluorooctanoic acid (PFOA) and 1 ppt for
75 perfluorobutanoic acid (PFBA) in March 2023.(7) Currently, the EPA employs liquid
76 chromatography, and mass spectrometry for PFAS detection at the ng L^{-1} level.(8) Yet,
77 these methods are time-consuming, expensive, require well-trained personnel, and must
78 be performed in laboratory environments.(8, 9) To facilitate broader testing and source

79 attribution, fast, portable, user-friendly, and low-cost PFAS sensing methods are needed
80 that robustly meet the EPA required ppt detection limits.

81 We report herein a new PFAS sensing platform based on the conducting polymer,
82 polyaniline (PANI), and electrical lateral flow assay (e-LFA, Figure 1). PANI is an
83 attractive material to create sensors, as a result of its facile synthesis, high stability, and
84 large conductivity changes caused by protonic doping as described in **Figure 1a**.(10, 11)
85 To target PFAS responsive polymer coatings, we mixed the fluorosurfactant (Krytox-
86 PEG-600-diamide, KPD)(12) with emeraldine free (PANI-EB) state by ultrasonication in
87 water to create a dispersion as shown in **Figure 1b**. Therefore, we yield a material that
88 selectively absorbs PFAS as a result of fluorosurfactant interactions.(13, 14) In the case that the
89 functional groups of PFAS contain acidic functionality, absorption will result in
90 acidification of the film and a transition of the PANI from its insulating emeraldine free
91 (PANI-EB) state to highly conductive emeraldine salt (PANI-ES) polymer (**Figure 1c**). To
92 create responsive sensory devices, we print a KPD-(PANI-EB) test line on nitrocellulose
93 (NC) membranes. Aqueous perfluoroalkyl acid solutions move by capillary force along
94 the NC membrane and a calibrated amount of water transverses the conducting polymer
95 test line to produce an economical, fast, quantitative, and easy-to-use flow assay.(15-17)

96

97 **Results and Discussion**

98

99 **Preparation of Polyaniline Nanofibers, Ink, and Test Lines:** PANI is polymerized in
100 nanofiber form to ensure high surface area to enhance analyte interactions. Nanofibers
101 with diameters around 80 nm were produced by an interfacial oxidative polymerization
102 method using camphorsulfonic acid and ammonium persulfate (**Figure S1**). (10, 18) The
103 synthesized PANI-ES (emeraldine salt) powder is then converted to undoped PANI-EB
104 (emeraldine base) by treatment (washing then filtration) with ammonium hydroxide
105 solution. PANI-EB formation is confirmed by Fourier-transform infrared spectroscopy
106 (FTIR) with a quinoid ring stretching at 1576 cm^{-1} , benzenoid ring stretching at 1491 cm^{-1} ,
107 C–N stretching modes at 1378 cm^{-1} and 1295 cm^{-1} (**Figure S1**). (19, 20)

108

109 The Fluorous-PANI-EB (F-PANI) ink is prepared by mixing PANI-EB dispersion with
110 Krytox-PEG-600-diamide (KPD) solution and sonicating for 30 minutes (**Figure 1b**). The
111 ink is likely stabilized by the noncovalent interactions between KPD (amide and ether
112 groups) and the nitrogen atoms of PANI-EB as shown schematically in **Figure 1b**.
113 Deposition of F-PANI ink on nitrocellulose (NC) membrane or filter paper substrates
114 resulted in test line bands. Detailed procedures for the preparation of the materials and
115 test lines are described in the **Materials and Methods** section.

116

117 Scanning electron microscopy (SEM) images reveal microcracks in the surface of
118 coatings formed on the NC membrane (**Figure 2a, b**). The dehydration of PANI likely
119 provides the stresses that result in the microcracks that are not apparent with visual
120 inspection.(10) Microcracks still remained in the wet F-PANI coating (**Figure S9**). The
121 rehydration and swelling of the materials likely occur during the sensing experiments,
122 and although the microcracks can produce unwanted resistance in the test lines, they
123 may also provide for expanded interactions with the aqueous solutions for improved
124 partitioning of the analytes into the films. The crack widths of the F-PANI coating are ca.
125 $3\text{ }\mu\text{m}$, which is smaller than those observed in the coating (ca. $15\text{ }\mu\text{m}$) produced from

126 pure PANI-EB inks. We attribute that the fluorinated surfactant prevents aggregation
127 between PANIs and hence favors smaller features. The F-PANI is expected to be less
128 hydrophilic than PANI-EB and this was evaluated by measuring contact angles (θ) of
129 water droplets placed on the films (**Figure 2c**). We confirmed that the F-PANI coating
130 presents a hydrophobic surface regardless of the substrate, whereas the PANI-EB
131 coating surface is hydrophilic.

132 **Factors that Affect the Conductivity of Test Lines:** Resistance measurements were
133 collected with a four-point probe. Multiple measurements on each test line were
134 performed to investigate the uniformity of the materials. As shown in **Figure 3a**, the
135 conductivity is influenced by multiple different factors. The resistance of the F-PANI test
136 line decreases after exposure to aqueous solutions of PFOA (**Figure 3b**). Hydration of
137 the test line is important and if a test strip is removed from the solution and air-dried for
138 15 minutes the water evaporates and the test lines display a high resistance $>220 \text{ M}\Omega$,
139 which is the limit of our detection. This feature is due to the cationic (polaron) and
140 dicationic (bipolaron) carriers that are pinned by attractive electrostatic interactions with
141 the counterions in the absence of water.⁽²¹⁾ Water reduces these interactions by a
142 solvation of the ions/carriers by a combination screening and separation of the charges.
143 To avoid dehydration, resistivity measurements were made within 5 minutes after the
144 sample was removed from the vial to ensure full hydration.

145
146 The absorption of perfluoroalkyl acids into the polymer coating is critical to obtaining an
147 optimal response, and this is facilitated by the fact that the solution passes very slowly
148 through the hydrophobic F-PANI test line (**Figure 2c**). Solutions require 20 minutes to
149 completely pass through the F-PANI test line, whereas only 90 seconds is required with
150 an equivalent PANI-EB test (**Figure S2**). **Figure 3c** shows that the test line reaches a
151 constant resistance value of approximately $67 \text{ M}\Omega$ after 30 minutes of being dipped into
152 a 10^{-6} M PFOA solution. Hence, the main time limitation is the transport of the water
153 along the NC membrane and the absorption of the PFBA and PFOA with concurrent
154 protonic doping of F-PANI is a relatively rapid process. Therefore, resistivity
155 measurements were made after 30 minutes of the test strip being placed in the solution.

156
157 The width of the F-PANI test line was investigated. Lines with widths of 0.3 cm, 0.7 cm,
158 and 1.4 cm were created using $17 \mu\text{L}$, $40 \mu\text{L}$, and $80 \mu\text{L}$ of F-PANI ink, respectively.
159 They had similar thicknesses ($27.9 \pm 5.9 \mu\text{m}$). It was found that test lines 0.7 cm or less
160 provided consistent results over a range of analyte concentrations (**Figures 3d and e**).
161 Wider test lines (1.4 cm) displayed higher resistances with 10^{-2} M PFOA and large
162 standard deviations ($86.7 \pm 79.7 \text{ k}\Omega$) as compared to the 0.7 cm lines ($30.7 \pm 17.5 \text{ k}\Omega$). It
163 is likely that in these cases protonic doping is not uniform throughout the test line. The
164 data for other concentrations are given in **Table S1** and is consistent with the previous
165 results. As a result, test line widths of 0.7 cm were used to determine the sensor
166 performance.

167 **Ultratrace PFAS detection:** The performance of the F-PANI lateral flow devices was
168 evaluated for PFOA detection. The resistance measurements are limited to values less
169 than $220 \text{ M}\Omega$, which is the resistance of the assay with PFAS-free water. The histogram
170 of resistance values of F-PANI test lines on the NC membrane for each concentration of
171 perfluorooctanoic acid (PFOA) is shown in **Figure 4a**. Because of non-uniformity in the
172 films, the resistance for each concentration taken with a colinear 4-point probe has a

173 distribution rather than a single clear value. The distribution could potentially be the
174 result of microcracks of the coating that can complicate the conductive pathways and
175 also from non-uniform protonic doping of the PANI backbone. For the statistical analysis
176 of the data, we fit our data to single Gaussian functions to obtain the peak value of
177 resistance (R_M) for each concentration and its standard deviation (σ_M). We emphasize
178 that no data is omitted. This method allows us to avoid bias in the data by excluding
179 outliers.(22) The detection limit of an analyte is the concentration at which the value
180 obtained by adding the standard deviation (σ_M) to the peak resistance value (R_M)
181 acquired at a certain concentration begins to fall below 220 M Ω which is the resistance
182 of the assay with PFAS-free water as the concentration increases. For PFOA, the
183 detection limit is 400 ppt (10^{-9} M) and the change in conductivity increases by 10^4 -fold for
184 a 10^{-2} M PFOA concentration. **Table 1** summarizes the resistance and conductivity
185 values of the F-PANI test lines on NC membranes as a function of PFOA concentration.
186 Similarly detailed data for OA is given in the Supporting Information (**Figure S3, Table**
187 **S2**).

188 **Figure 4b** shows the plots of conductivity of F-PANI test lines on NC membrane as a
189 function of PFOA and its non-fluorous form (octanoic acid, OA) concentrations obtained
190 by Gaussian fitting of the histogram of resistance values. The aqueous PFOA analyte
191 shows higher conductivity compared to OA at the same concentration.

192
193 We also tested the effect of different substrates. **Figure 4c** shows the plots of
194 conductivity of F-PANI test lines on NC membrane and filter paper substrates as a
195 function of PFOA concentrations. The conductivity responses are independent of the
196 substrate and correlate with the PFOA concentration. The data for the F-PANI test line
197 on the filter paper is described in **Figure S4 and Table S3**. The fluorous nature of the F-
198 PANI is critical and a flow assay using PANI-EB is 10^5 times less sensitive to PFOA with
199 a detection limit of 10^{-4} M (**Figure S5 and Table S4**). We also found that the
200 performance of the F-PANI test lines was the same when the aqueous media was
201 changed from D.I. water to our local (Cambridge, MA) tap water. (**Figure S6 and Table**
202 **S5**). The F-PANI lateral flow assay is also capable of detecting PFBA at 10^{-9} M (200 ppt)
203 similar to the PFOA limits of detection (**Figure S7, Table S6**). Here again, we have high
204 selectivity for the fluorous acid over the non-fluorous equivalent, butyric acid, (BA), which
205 gives responses that are 10^4 times lower (**Figure S8, Table S7**).

206

207

208 **Conclusion**

209

210 We have developed simple electrical lateral flow assays (e-LFAs) for the detection of
211 PFOA with limits of detection down to 400 ppt. Although additional optimization and
212 larger sample sizes than used in current e-LFA are necessary to align with the current
213 US EPA limits, the remarkably low detection thresholds of this sensor scheme render it
214 promising for on-site PFAS detection. Our transduction method is the protonic acid
215 doping of polyaniline in its insulating emeraldine base form (PANI-EB) to produce an
216 electrical conducting emeraldine salt (PANI-ES). A formulation of PANI-EB and a
217 fluorous polymeric surfactant creates a conducting polymer with a fluorous character (F-
218 PANI). Test lines of F-PANI are printed on nitrocellulose membranes or filter paper, and
219 wicking of aqueous solutions results in the flow of water through the polymer test line.
220 When PFOA is present, it is absorbed into the F-PANI and acidifies the film resulting in
221 protonic doping of the PANI to create charge carriers. This simple, inexpensive, rapid,

222 and quantitative PFAS detection method is ideally suited for monitoring PFAS in areas,
223 such as military bases, airports, and industrial locations where PFAS exposure is a
224 concern.(23)

225

226

227 **Materials and method**

228

229 **Materials and Characterization:** Aniline (ACS reagent, $\geq 99.5\%$), ammonium persulfate
230 (ACS reagent, $\geq 98\%$), and camphorsulfonic acid ($\geq 98\%$) were purchased from Sigma-
231 Aldrich and used as received. The fluorosurfactant, Krytox-PEG-600-diamide (KPD)
232 was synthesized by the previously reported procedure and purchased from Akita
233 Innovations LLC.(12) All solvents used were of HPLC grade. All aqueous solutions were
234 prepared, and samples were rinsed using Milli-Q water. The nitrocellulose membrane
235 strip with a polyester backing card was purchased from GE Healthcare Life Sciences.
236 The filter paper (494, Quantitative) for the substrate was purchased from VWR
237 International.

238

239 SEM images of the film were obtained by a Merlin and Crossbeam 540 Zeiss scanning
240 electron microscope. The TEM images of nanofibers were obtained with a 120 kV FEI
241 Tecnai Multipurpose transmission electron microscope. A Thermo Scientific Nicolet 6700
242 FT-IR instrument with a Ge crystal was used to obtain attenuated total reflectance
243 Fourier-transform infrared (ATR-FTIR) spectra. Resistivity measurements were
244 conducted by a Keithley 2400 and a Signatone four-point probe. The thickness of the
245 coating on the nitrocellulose membrane was obtained with a Dektak 6M stylus
246 profilometer.

247 **Synthesis of PANI Polymer:** The PANI nanofiber was synthesized via the interfacial
248 polymerization of aniline (**Figure S1**). Distilled aniline was used for experiments. The
249 polymerization was performed in a 20 mL glass vial. Aniline (3.2 mmol) was dissolved in
250 10 mL of dichloromethane to yield the organic phase.(10, 18) 0.8 mmol of ammonium
251 persulfate was dissolved in 10 mL of 1 M camphorsulfonic acid solution to yield the
252 aqueous phase. The interfacial polymerization of aniline was targeted by combining the
253 aqueous and organic phases into a 20 mL vial. After 14 hours (o/n), the as-prepared
254 PANI-ES (Emeraldine salt) was purified by filtration. PANI-EB (Emeraldine base) form
255 was obtained by reduction with a 0.1 M aq. NH_4OH solution. **Figure S1** shows Fourier-
256 transform infrared spectra of PANI-EB nanofibers. The FTIR spectrum displays quinoid
257 ring (Q) stretching at 1576 cm^{-1} , benzenoid ring (B) stretching at 1491 cm^{-1} , C–N
258 stretching vibration near quinone diimine unit at 1378 cm^{-1} , C–N stretching in *cis*-Q-B-Q,
259 Q-B-B and B-B-Q at 1295 cm^{-1} , C–N stretching in B-B-B at 1224 cm^{-1} , aromatic C–H in-
260 plane bending vibration at 1144 cm^{-1} , aromatic C–H out of plane bending vibration of
261 1,2,4-ring at 806 cm^{-1} .(19, 20)

262 **Preparation of Ink and Coating:** The PANI dispersion was obtained by mixing 30 mg of
263 PANI-EB powder and 1.5 mL of D. I. water and sonicating for 1 hour. Sonication
264 treatment was processed to redisperse polyanilines into the original nanoscale fibers.
265 The fluorosurfactant solution was prepared by dissolving 100 mg of Krytox-PEG-600-
266 diamide (KPD) in 2 mL of Hexafluoroisopropanol (HFIP), which is miscible with water.
267 The fluorosurfactant PANI (F-PANI) dispersion was obtained by sonicating 600 μL of polymer
268 dispersion and 100 μL of fluorosurfactant solution for 30 minutes. In this process, the

269 noncovalent interactions between KPD (amide and ether groups) and the imines of the
270 PANI nanofibers are introduced.

271 Polymer coatings were prepared by fabricating PANI inks onto the substrates
272 (nitrocellulose membrane or filter paper). Test line bands with an area of 45 mm² (1.5 cm
273 * 0.3 cm) were created using 17 μL of ink, while bands measuring 105 mm² (1.5 cm * 0.7
274 cm) and 210 mm² (1.5 cm * 1.4 cm) were created using 40 μL and 80 μL of ink,
275 respectively. The thickness of the test line bands was obtained by the stylus
276 profilometer, and the average value was 27.9 ± 5.9 μm (n = 5). We used the test lines for
277 measurement after drying for 2 hours.

278 **Calculation of conductivity of the coating** We calculated the conductivity by using the
279 four-point probe method. We recorded the resistance reading (*R*) and a constant (*C* =
280 4.3947) derived from the dimensions of our sample,(24) and determined the sheet
281 resistance (ρ_s) using the following equation

$$\rho_s = R * C$$

282
$$\sigma = \frac{1}{\rho_s * l}$$

283 The resistivity of the coating is ρ_s multiplied by its thickness (*l*), and the conductivity (σ) is
284 its reciprocal. The resistance (*R*) values were collected from over 30 different locations
285 with 3 samples for each concentration.

286

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288

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291

292

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358 **Figure Captions and Tables**

359

360

361 **Figure 1. A PFAS sensing platform based on polyaniline (PANI) polymer and e-**

362 **LFA.** (a) Doped and undoped PANI structures. (b) Preparation of fluoros PANI-EB

363 dispersion by mixing PANI-EB nanofiber powder with the fluoros surfactant (Krytox-

364 PEG-600-diamide). (c) Photograph of e-LFA membrane and schematic of the

365 conductivity change after exposure to PFAS aqueous solution.

366

367 **Figure 2. Characterization of PANI coatings** Scanning electron microscope (SEM)

368 images of (a) PANI-EB and (b) F-PANI coatings formed on a nitrocellulose (NC)

369 membrane(c) Plots of contact angle (θ) of water on PANI-EB and F-PANI coatings

370 formed on the NC membrane and glass. Insets are optical microscopic images of the

371 droplet contact angles. Each data point was averaged from 10 measurements, and the

372 error bars correspond to the standard deviation.

373

374 **Figure 3. Resistance and conductivity measurement of the F-PANI test line in the**

375 **e-LFA using four-point probe system.** (a) Schematic of the four-point probe

376 measurement and experimental factors investigated. (b) Plot of resistance value as a

377 function of time after the sample was removed from the vial. (c) Plot of resistance value

378 as a function of exposure time in the vial with 10^{-6} M PFOA. Resistance values were

379 collected from over 30 different locations with 3 samples for each concentration. (d, e)

380 Plot of resistance values for each width with 10^{-6} M PFOA (d) and 10^{-2} M PFOA (e). The

381 distance from the end of the test line to the end of the NC membrane was 1.6 cm for all

382 devices investigated.

383

384 **Figure 4. Resistance and conductivity of wet F-PANI coatings. (a)** The histogram of

385 resistance values for each concentration of PFOA. The bin size of the histogram was

386 decreased at lower concentrations. The values were collected from over 30 different

387 locations with 3 samples for each concentration. The measurement of the wet coating

388 was carried out within 5 minutes after the sample was removed from the vial. (b) Plots of

389 conductivity of F-PANI coatings on NC membrane as a function of PFOA (black spheres)

390 or octanoic acid (OA, green square) concentrations. The point of 10^{-2} M OA is not

391 indicated because it is not a uniform solution in ambient conditions. (c) Plots of

392 conductivity of F-PANI coatings on NC membrane (black spheres) and filter paper

393 (green triangles) substrates as a function of PFOA concentrations.

394

395

396

397 **Table 1. Summary of resistance and conductivity values of the wet F-PANI test line**
 398 **on the NC membrane as a function of PFOA concentration (data from Figure 4a**
 399 **and b).** R_A and σ_A indicate average and sigma (standard deviation) values, respectively.
 400 R_M and σ_M indicate mean (peak) and sigma values estimated from Gaussian fitting
 401 curves. C_M is the conductivity calculated from R_M value. 220 M Ω is the maximum
 402 resistance value that can be measured.
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PFOA [M]	$R_A \pm \sigma_A$ [M Ω , k Ω for \S]	$R_M \pm \sigma_M$ [M Ω , k Ω for \S]	C_M [S/m]	Conductivity enhancement (times)
0	> 220	> 220	$< 3.70 \cdot 10^{-5}$	-
10^{-10}	205.9 ± 27.4	218.5 ± 8.2	$3.73 \cdot 10^{-5}$	> 1.01
10^{-9}	191.4 ± 24.3	202.5 ± 10.0	$4.03 \cdot 10^{-5}$	> 1.09
10^{-8}	149.8 ± 25.0	154.5 ± 23.0	$5.27 \cdot 10^{-5}$	> 1.42
10^{-7}	113.9 ± 29.8	110.3 ± 21.7	$7.42 \cdot 10^{-5}$	> 2.01
10^{-6}	75.7 ± 27.9	69.7 ± 18.6	$1.17 \cdot 10^{-4}$	> 3.16
10^{-5}	43.8 ± 21.3	36.1 ± 17.9	$2.26 \cdot 10^{-4}$	> 6.11
10^{-4}	7.6 ± 5.9	5.6 ± 3.4	$1.46 \cdot 10^{-3}$	> $3.95 \cdot 10^1$
10^{-3}	1.2 ± 2.1	$318.9 \pm 89.2 \S$	$2.56 \cdot 10^{-2}$	> $6.92 \cdot 10^2$
10^{-2}	$22.0 \pm 7.3 \S$	$23.7 \pm 6.7 \S$	$3.44 \cdot 10^{-1}$	> $9.30 \cdot 10^3$

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