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A Novel Label-Free Colorimetric Assay for DNA Concentration in Solution

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ABSTRACT: Optical devices were fabricated by sandwiching a "monolithic" poly (*N*-isopropylacrylamide-co-N-(3-aminopropyl) methacrylamide hydrochloride) (pNIPAm-co-APMAH) microgel layer between two semitransparent Au layers. These devices, referred to as etalons, exhibit characteristic multipeak reflectance spectra, and the position of the peaks in the spectra primarily depend on the distance between the Au surfaces mediated by the microgel layer thickness. Here, we show that the positively charged microgel layer can collapse in the presence of negatively charged single stranded DNA (ssDNA) due to ssDNA induced microgel crosslinking. The collapse results in a change in the etalon's optical properties, which can be used to detect target DNA in a complex mixture.

TOC:
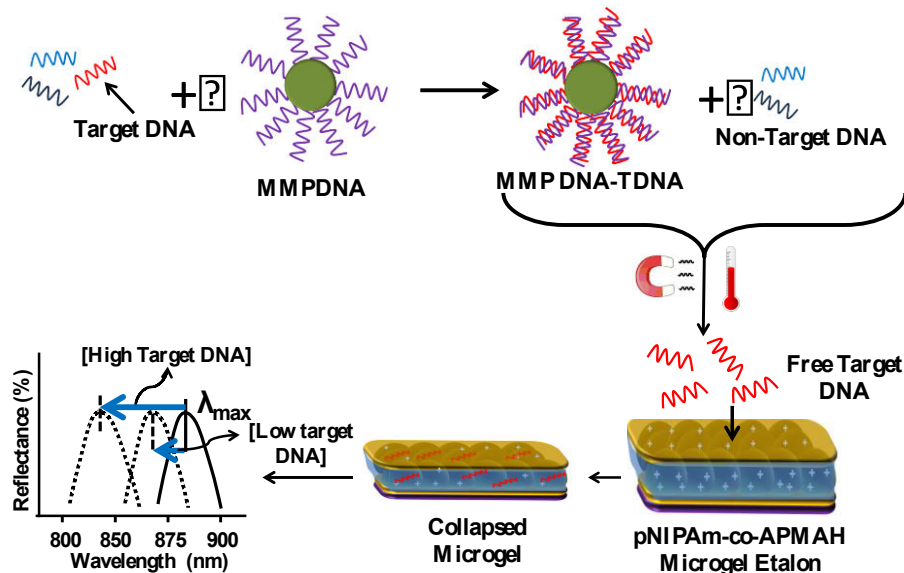
Introduction:

The separation of specific DNA from complex biological samples plays a vital role in disease diagnosis, treatment and in forensic science. Separation of specific target DNA is not

2

38   enough though, the DNA of a specific sequence needs to be detected, which may need

39   pretreatment to enrich the concentration of DNA in the sample. While there are number of

40   methods currently available to achieve these goals, new methods are being developed for

41   improved performance as well as for point-of-care (POC) diagnostics. For example, most

42   techniques -- such as polymerase chain reaction (PCR)--are not amenable for POC applications.

43   Furthermore, fluorescence-based DNA assays require high performance equipment for operation,

44   and are costly. Due to these shortcomings, a number of techniques have emerged for the label

45   free detection of low concentrations of DNA, without preconcentration and/or amplification.[1-

46   4]

47   In this contribution, we present a polymer-based device that can be used for the label-

48   free, colorimetric detection of low concentrations of DNA. The device construct, which is shown

49   in Figure 1, is constructed by sandwiching poly (*N*-isopropylacrylamide) (pNIPAm)-based

50   microgels between two planar Au layers.[5, 6] Poly (*N*-isopropylacrylamide) (pNIPAm) is the

51   most well studied responsive polymer to date. It has attracted enormous attention due to its lower

52   critical solution temperature (LCST). That is, pNIPAm is water soluble and swollen (existing as

53   a random coil) at temperature < 32 °C, which collapses (into a globular state) above 32 °C.[7]

54   This transition is fully reversible. A number of other responsive moieties can be added during

55   polymerization to make pNIPAm responsive to temperature and other stimuli. In this way,

56   thermoresponsive pNIPAm-based polymers can be made responsive to pH, ionic strength,

57   analyte concentration, light, electric field, magnetic field, and redox chemistry (for example).[8-

58   11]

59

pNIPAm-co-APMAH
Microgel Etalon

Collapsed
Microgel
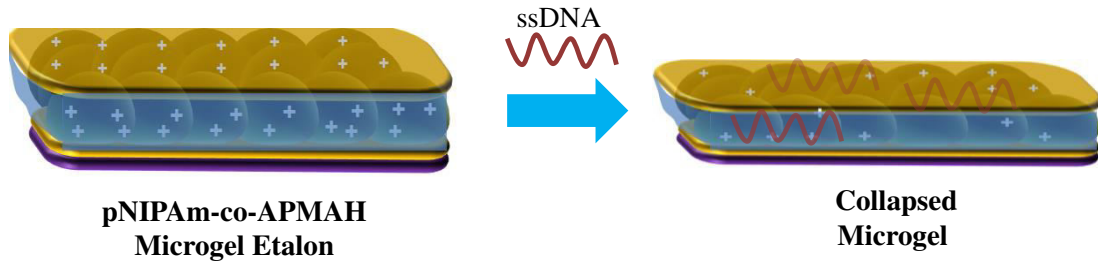
ssDNA

60

61   Figure 1: Basic construct of a microgel-based etalon and its response towards ssDNA.

62

63   In addition to linear polymers, crosslinked pNIPAm-based polymer networks can be

64   synthesized to form a hydrogel. Furthermore, hydrogel nano and microparticles (nanogels and

65   microgels, respectively) can be synthesized. These materials are highly porous, and water

66   swollen also exhibiting thermoresponsivity with the option of adding additional responsivity as

67   required. The most common additional response is pH, afforded by incorporating a weak acid,

68   e.g., acrylic acid (AAc) into the hydrogel network.[12-14] AAc is a weak acid with a pK_a of ~

69   4.25, thus it is charged at pH > 4.25 and vice versa. When the pNIPAm-co-AAc microgels are at

70   a pH > 4.25 they contain multiple charges and are polyanionic.

71   We have shown that the pNIPAm microgel-based optical devices (or etalons), shown in

72   Figure 1, exhibit visible color and multipeak reflectance spectra. The position of the peaks in the

73   reflectance spectra depends on the distance between two Au layers and the refractive index of

74   microgel layer. The position and order of the peaks can be predicted[15] from Eq. (1):

75                              $\lambda = 2nd\cos\theta /m$ (1)

76   where n is the refractive index of the microgel (dielectric) layer, d is the mirror–mirror distance,

77   θ is the angle of incident light relative to the normal, and m (an integer), is the order of the

78   reflected peak.

79   We have utilized pNIPAm microgel-based etalons for a number of applications.[16-21]

80   Most important to the work here, we have shown that polycationic linear polymers are capable of

81   penetrating the etalon's outer Au layer to crosslink negatively charged pNIPAm-co-AAc

82   microgels in the cavity.[22] This crosslinking causes the microgels to collapse, changing the

83   distance between the two Au layers, resulting in a shift in the position of the peaks in reflectance

84   spectra. This has been utilized for sensing proteins in solution.[19, 21] In this submission, we

85   show that pNIPAm-co-N-(3-aminopropyl) methacrylamide hydrochloride (pNIPAm-co-

86   APMAH) microgel-based etalons, which are polycationic <pH~10.0 can be fabricated and are

87   capable of detecting negatively charged single stranded DNA (TDNA, sequence: 5′-

88   TTGCTTGGCTTTCAGTTAT-3′) in solution. Furthermore, we are able to utilize this novel

89   system for detecting a specific DNA sequence in the presence of DNA with 2 BP mismatch

90   (2BPMMDNA, sequence: 5′-TTG**G**TTGGCTTT**G**AGTTAT-3′ and 4 BP mismatch

91   (4BPMMDNA, sequence: 5′- TT**C**GTTGGCTTT**GAC**TTAT-3′) and complete mismatch (5′-

92   ATAACTGAAAGCCAAGCAA-3′) sequences. The bolded bases indicate the position of the

93   mismatch. This system represents a simple optical detection system to sense target DNA without

94   complex modification or the use of labels. With further optimization, and coupling with a simple

95   electronic readout system, this approach will find application in resource-limited parts of the

96   world for sensing disease biomarkers.

97   **Experimental Details:**

98   **Materials**

99   *N*-Isopropylacrylamide was purchased from TCI (Portland, Oregon) and purified by

100   recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ) prior to use. N,N′-

101   methylenebisacrylamide (BIS) (99%), ammonium persulfate (APS) (98.5%), DMSO were

102 obtained from Sigma–Aldrich (Oakville, ON) and were used as received. N-(3-Aminopropyl)

103 methacrylamide hydrochloride (APMAH) were purchased from Polysciences, Inc (Warrington,

104 PA). Sodium chloride was obtained from Fisher (Ottawa, ON). All deionized (DI) water was

105 filtered to have a resistivity of 18.2 MΩ•cm and was obtained from a Milli-Q Plus system from

106 Millipore (Billerica, MA). Chromium (Cr) and Gold (Au) were deposited using a model

107 THEUPG thermal evaporation system from Torr International Inc. (New Windsor, NY). The

108 annealing of Cr/Au layer was done in a Thermolyne muffle furnace from Thermo Fisher

109 Scientific (Ottawa, Ontario). Anhydrous ethanol was obtained from Commercial Alcohols

110 (Brampton, Ontario). Fisher's finest prewashed glass coverslips were 25×25 mm and obtained

111 from Fisher Scientific (Ottawa, Ontario). Cr (99.999%) was obtained from ESPI (Ashland, OR),

112 while Au (99.99%) was obtained from MRCS Canada (Edmonton, AB). Succinimidyl 4-(p-

113 maleimidophenyl)butyrate (SMPB) and Dithiothreitol (DTT) were purchased from Pierce

114 Biotechnology, Inc. All the DNA oligomers were purchased from IDT (Coralville, IA, USA).

115 Amine functionalized magnetic beads (Dynabeads M-270 amine) was purchased from Life

116 Technologies Corporation (Frederick, MD, USA).

117

118 **Procedures**

119 **Poly (*N*-isopropylacrylamide-co-N-(3-Aminopropyl)methacrylamide hydrochloride)**

120 **(pNIPAm-co-APMAH) Microgel Synthesis**

121 Microgels composed of poly (*N*-isopropylacrylamide-co-N-(3-Aminopropyl)

122 methacrylamide hydrochloride) (pNIPAm-co-APMAH) were synthesized via temperature-ramp,

123 surfactant free, free radical precipitation polymerization as described previously.[22] The

124 reaction mixture was comprised of 90% N-isopropylacrylamide (NIPAm) and 5% N-(3-

125 Aminopropyl)methacrylamide hydrochloride) (APMAH) with a 5% N,N'-

126 methylenebisacrylamide (BIS) crosslinker. The monomer, NIPAm (18.0 mmol), and BIS (1.0

127 mmol) were dissolved in DI water (100 mL) with stirring in a beaker. The mixture was filtered

128 through a 0.2 μm filter affixed to a 20 mL syringe into a 200 mL 3-neck round-bottom flask. The

129 beaker was rinsed with 25 mL of DI water and then filtered into the NIPAm/BIS solution. The

130 flask was then equipped with a temperature probe connected to a temperature control system, a

131 condenser, N_2 gas inlet (a needle), and a stir bar. The solution was purged with N_2 gas for about

132 1.5 h, with the stirring set to a rate of 450 rpm, while the temperature was allowed to reach 45

133 °C. APMAH (1.0 mmol) was then added to the heated mixture with a micropipette in one

134 aliquot. A 0.078 M aqueous solution of APS (5 mL) was delivered to the reaction flask with a

135 transfer pipet to initiate the reaction. Immediately following initiation, a temperature ramp of 45

136 to 65 °C was applied to the solution at a rate of 30 °C/h. The reaction was allowed to proceed

137 overnight at 65 °C. After polymerization, the reaction mixture was allowed to cool down to room

138 temperature and filtered through glass wool to remove any large aggregates. The coagulum was

139 rinsed with DI water and filtered. Aliquots of these microgels (12 mL) were centrifuged at a

140 speed of ~8500 relative centrifugal force (rcf) at 23 °C for about 40 minutes to produce a pellet

141 at the bottom of the centrifuge tube. The supernatant was removed from the pellet of microgels,

142 which was then resuspended to the same volume (12 mL) of DI water. Centrifugation and re-

143 suspension was repeated five more times to remove any unreacted reagents, linear polymers, and

144 oligomers present with the microgel. After repeated centrifugation pure, concentrated and very

145 viscous microgel pellet was formed and kept in the centrifuge tube for further use.

146 **Synthesis of DNA-Functionalized Magnetic Microparticles (MMPDNA)**

147   The preparation of DNA functionalized MMPs has been reported elsewhere.[23]   In

148   short, amino-functionalized MMPs (2.8-μm diameter; Invitrogen) were covalently linked to 5′-

149   thiol-modified oligonucleotides (IDT) with heterobifunctional crosslinker succinimidyl 4-[p-

150   maleimidophenyl] butyrate, SMPB (Pierce Biotechnology, Inc.). First, the MMPs (30 mg mL^{-1},

151   1 mL) were washed twice with 1 mL of anhydrous DMSO in a 50 mL centrifuge tube. A fresh

152   solution of SMPB (50 mg) in DMSO (15 mL) was prepared prior to the reaction (the sample vial

153   was washed with DMSO and collected to avoid sample loss). The SMPB/DMSO solution was

154   added to the magnetic beads, and the reaction between the primary amino group and the *N*-

155   hydroxysuccinimide (NHS) ester of SMPB was allowed to proceed for 4 h with gentle shaking at

156   room temperature. The reaction with SMPB was carried out in the dark. Then, the disulfide

157   bonds in all 5′-thiolated oligonucleotides were reduced by DTT. A 100 μL of freshly prepared

158   0.1 M DTT solution in disulphide cleavage buffer was added to 25 nmol lyophilized DNA in a

159   microcentrifuge tube, wrapped in an aluminum foil and kept standing for 2.5 hrs. After that time,

160   DTT-DNA mixture was passed through NAP-5 column (GE Healthcare Life Sciences, London)

161   and collected into a series of microcentrifuge tubes by adding 1.35 mL of DI water. The location

162   and concentration of DTT reduced DNA was confirmed by UV-visible spectrophotometer and

163   325 μL of 10 μM solution was prepared by coupling buffer. The beads were magnetically

164   separated and washed three times with DMSO (10 mL) and two times with coupling buffer

165   (NaCl (0.2 M), phosphate buffer (100 mM), pH 7.0; 10 mL). Now the DTT reduced 300 μL DNA

166   solution was added to the washed SMPB-activated magnetic beads. The rest of the DTT-DNA

167   solution was kept for calculating the coupling efficiency. The reaction between the maleimide

168   group and the SH group of the DNA was allowed to proceed at room temperature for 2 h under

169   constant vortex. Next, the DNA-functionalized beads were placed on a high pull magnet (Eclipse

170 Magnetics, Sheffield, UK), the supernatant was removed and preserved, and the beads were

171 washed three times with coupling buffer and then twice with passivation buffer (NaCl (0.15 M),

172 phosphate buffer (150 mM), pH 8.0). The supernatant was used to determine the coupling

173 efficiency by measuring the absorbance at 260 nm and comparing it with that before DNA

174 functionalization. The coupling efficiency was found to be in the range of 86 % - 95 %. The

175 surface of the DNA-functionalized MMPs was passivated by adding a freshly prepared solution

176 (40 mL) of sulfo-NHS-acetate (100 mg; Pierce Biotechnology, Inc.) in passivation buffer. The

177 passivation process was allowed to proceed for 1h at room temperature with mild shaking. The

178 beads were washed twice with passivation buffer, twice with assay buffer, and stored at 4 °C in

179 storage buffer at a final concentration of 10 mg mL^{-1}.

180 **Etalon Fabrication**

181   The details of the paint-on technique used to fabricate microgel-based etalons for this

182 study has been reported elsewhere.[24] In short, 25 × 25 mm pre-cleaned glass coverslips were

183 rinsed with DI water and ethanol and dried with N$_2$ gas, and 2 nm of Cr followed by 15 nm of Au

184 were thermally evaporated onto them at a rate of ~0.2 Å s^{-1} and ~0.1 Å s^{-1}, respectively, using a

185 thermal evaporation system by Torr International Inc. model THEUPG (New Windsor, NY). The

186 Cr acts as an adhesion layer to hold the Au layer on the glass. The Au coated substrates were

187 annealed at 250 °C for 3 h followed by cooling to room temperature before use. A 12 mL aliquot

188 of purified microgel solution was centrifuged for 30 min at 23 °C at ~8500 relative centrifugal

189 force (rcf) to pack the microgels into a pellet at the bottom of the centrifuge tube. The microgel

190 pellet contained in the centrifuge tube was vortexed to loosen the pellet and was placed on a hot-

191 plate at 30 °C. A previously coated Cr/Au substrate was rinsed with ethanol, dried with N$_2$, and

192 then placed onto hot plate (Corning, NY) set to 30 °C. A 40 µL aliquot of the concentrated

193 microgels was put onto the substrate and then spread toward each edge using the side of a

194 micropipette tip. The film was rotated 90°, and the microgel solution was spread again. The

195 spreading and rotation continued until the microgels covered the entire substrate and became too

196 viscous to spread further. The microgels were allowed to dry completely on the substrate for 2 h

197 with the hot plate temperature set to 35 °C. After 2 hours, the dry film was rinsed with DI water

198 to remove any excess microgels not bound directly to the Au. Next, the film was placed into a DI

199 water bath and allowed to incubate overnight on a hot plate set to ~30 °C. Following this step,

200 the substrate was again rinsed with DI water to further remove any microgels not bound directly

201 to the Au substrate surface. Then, the film was dried with N_2 gas and placed into the thermal

202 evaporator, and an additional 2 nm Cr followed by 5 nm Au was deposited onto the microgels as

203 an overlayer. After the overlayer addition the Au-microgel-Au structure (or etalon) was soaked

204 in DI water overnight on a hot plate at 30 °C. The assemblies were then rinsed with DI water and

205 dried with N_2 gas and subsequently used for experiments. We point out that the microgels used

206 here were ~650 nm in diameter measured from analysis of differential interference contrast

207 microscopy images. From previous results,[6] we found that the etalon's cavity thickness

208 (defined by the microgel diameter) was ~ 0.6 of the microgels solution diameter. This is the case

209 because the microgels are soft, and sandwiched between the etalon's two Au layers while also

210 being stuck to the Au surface. In this case, we also know that the *visual* color for these etalon's is

211 weak, although the peaks in the reflectance spectra are well defined.

212 **Reflectance Spectroscopy**

213   Reflectance measurements were conducted in a specially designed sample holder using a

214   USB2000+ spectrophotometer, a HL-2000- FHSA tungsten light source, and a R400-7-VIS-NIR

215   optical fiber reflectance probe all from Ocean Optics (Dunedin, FL). The spectra were recorded

216   using Ocean Optics Spectra Suite Spectroscopy Software over a wavelength range of 350-1025

217   nm. Measurements were performed in the sample holder, which allows for careful sample

218   positioning, sample stability, solvent injection, and fine temperature control. The light source

219   was always positioned over the center of the etalon.

220   **Experimental Setup for Biosensing**

221   The whole sensing experiment was done in a custom built metal chamber with temperature

222   control. The chamber was built to ensure that the position of the etalon and the light source are

223   fixed, as well as the temperature is well controlled and stable. Before use, the edges of the

224   etalons were sealed using clear nail polish to resist any DNA penetration through the sides of the

225   etalon. After sealing, the assemblies were dried in air overnight. Before each experiment, the

226   etalons were placed in the sample chamber and soaked in 30 mL pH 7.2 solution (2 mM with

227   NaCl). The chamber's temperature was controlled to 25 °C using a digital feedback loop. The

228   chamber allowed the reflectance probe to be inserted such that the device's optical properties

229   could be assessed. The temperature and pH of the solution was continuously monitored

230   throughout each experiment. The intensity and distance of light source from the assembly was

231   adjusted to result in the highest quality reflectance spectra. Before each measurement, we

232   assured that the reflectance spectrum was stable, i.e., the position of the reflectance peaks were

233   stable over time. Each experiment was repeated at least three times.

234   **Separation and Sensing Protocol**

235   Specific volumes (20-150 μL) of target DNA (TDNA) and TDNA mixed with other "interfering"

236   ssDNA (2 and 4 BP mismatch, and complete mismatch, (CMMDNA) with TDNA) were mixed

237   into a microcentrifuge tube. An excess amount (350 μL) of MMPDNA (magnetic micro particles

238   with covalently attached ssDNA complimentary to TDNA) relative to TDNA was added and

239   slowly vortexed for 2 minutes and kept standing for 4h with occasional vortexing for

240   hybridization. After this time, an external magnet was placed on the wall of microtube and held

241   for 2 minutes. The magnet visibly pulled the magnetic micro particles towards the wall of the

242   microcentrifuge tube, with TDNA bound to the complementary DNA attached to magnetic

243   microparticles. At that point, all the unbound ssDNA (2BPMMDNA, 4BPMMDNA and

244   CMMDNA) should be suspended in the solution in the microcentrifuge tube, while the

245   MMPDNA-TDNA is stuck via the magnetic field on the centrifuge wall. The supernatant

246   containing the unbound interfering ssDNA was pipetted out and the magnetic particles were

247   washed several times with 1 mL of pH 7.2 solution each time. It was confirmed by UV-Vis that

248   the washing solution doesn't contain any DNA. Next, a specific amount of DI was added to the

249   separated and washed MMPDNA and vortexed to resuspend the MMPDNA-TDNA. The

250   microcentrifuge tube was heated to 80 °C (well above the melting point of TDNA and its

251   complement, which was 49.7 °C) and kept for 5 minutes. While the temperature was maintained,

252   an external magnet was brought close to the wall of the microcentrifuge tube, held for 2 minutes,

253   and the hot supernatant was pipetted out by a micropipette. At that temperature, due to the

254   melting of TDNA from the MMPDNA, all the TDNA should be released into the supernatant

255   while MMPDNA was stuck on the wall by the magnet. The supernatant (with all the TDNA) was

256   cooled and added to the chamber holding the etalon and the optical properties monitored. Since

257   the TDNA was capable of entering the etalon, the spectral peaks shifted in proportion to the

258   amount of TDNA initially present in the initial sample solution. By varying the concentration of

259   TDNA, supernatant with various concentration of TDNA was collected by the above method and

260   used for sensing. We performed control experiment by mixing 4BPMMDNA and CMMDNA

261   and reacting them with MMPDNA without any TDNA. After the initial magnetic field

262   separation, washing of MMPDNA and heating, we added the extract (which does not contain any

263   TDNA) to the etalon chamber and found no significant shift of reflectance peak. This approach

264   confirms the selectivity of the MMPDNA for TDNA. Other control experiments were performed

265   and we found that MMP storage buffer and reaction buffer do not have any influence on the

266   position of the peaks in the reflectance spectra.

267   **Results and Discussion:**

268   For this approach to work, ssDNA must be capable of penetrating the etalon to collapse the

269   microgels to yield an optical response. To investigate this, we fabricated a pNIPAm-co-APMAH

270   etalon and exposed it to varying concentrations of ssDNA while monitoring the position of the

271   etalons reflectance peaks. We found that the etalon's reflectance peaks all shifted to lower

272   wavelengths upon DNA addition, indicative of microgel collapse. This process usually takes 5

273   minutes to 1 h; the factors that dictate the response time are unknown, but are under

274   investigation. Shown in Figure 2 is the cumulative shift (relative to the initial peak position) for

275   one reflectance peak. As can be seen, the extent of the peak shift is linearly related to the

276   concentration of the DNA added to the solution, levelling off at high concentrations due to

277   microgel saturation with ssDNA. From the results, we concluded that ssDNA is indeed capable

278   of entering the etalon to crosslink the microgels, and the extent of crosslinking depends on the

279   amount of ssDNA exposed to the etalon.

280
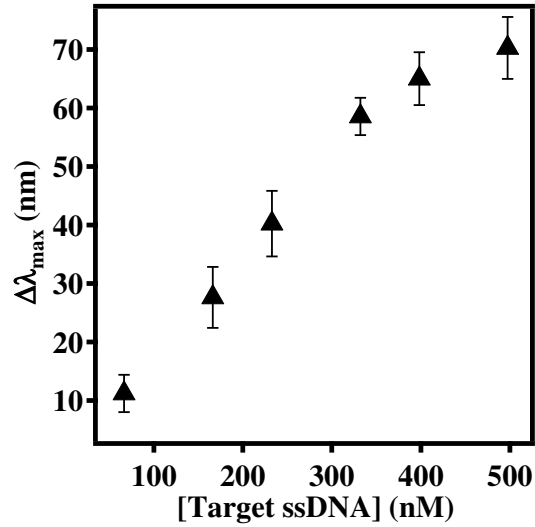
Figure 2: Cumulative shift of a reflectance peak for a pNIPAm-co-APMAH etalon upon addition

of increments of target ssDNA solution. The pNIPAm-co-APMAH microgel-based etalon was

soaked in pH 7.2 solution throughout the experiment, while the temperature was maintained at

25 °C. Each point in the plot represents the average of at least three independent measurements,

and the error bars are standard deviation for those values. A new device was used for each

measurement.

        With the etalon's response to ssDNA validated, we hypothesized that this system could be

used for DNA sensing applications. This approach is detailed schematically in Scheme 1.
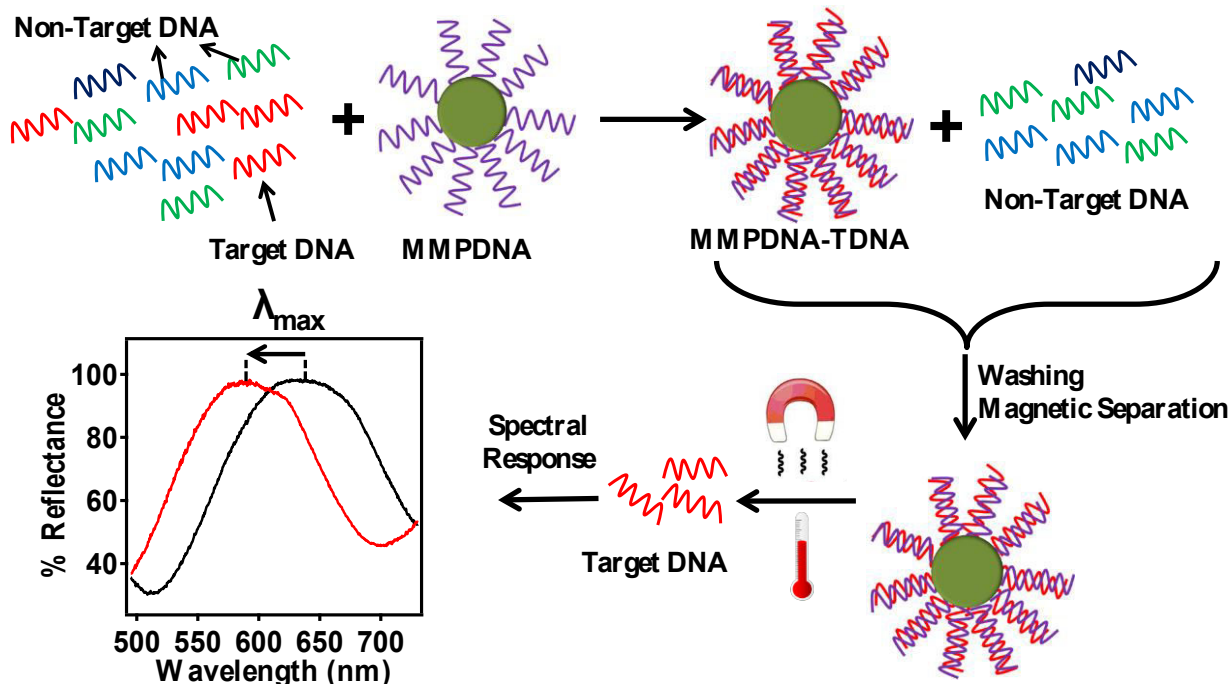
Scheme 1: The DNA sensing scheme.

291

292

293

294 To accomplish this, we synthesized DNA functionalized magnetic microparticles[23]

295 (MMPDNA) as detailed in the experimental section. The thiol modified ssDNA (sequence: 5′-

296 HS-AAAAAA AAAAATAACTGAAAGCCAAGCAA-3′) attached to the MMPDNA was

297 completely complimentary to a target DNA (TDNA) sequence. A known amount of TDNA was

298 mixed with 2BP and 4BP mismatch DNA (2BPMDNA and 4BPMDNA respectively), and

299 complete mismatch DNA (CMMDNA) in a microcentrifuge tube (total solution volume was 400

300 μL). Here, it is important to note that all the ssDNA were designed to have the same number of

301 bases. A specific excess amount of MMPDNA (350 μL solution which yielded capture DNA that

302 was 2x the amount of TDNA present) was added and allowed to incubate for 4 hours at room

303 temperature. 4 hours was used in these experiments, but shorter incubation times were, and can

304 be used (data not shown). After allowing ample time for hybridization of TDNA to the

305 MMPDNA, an external high pull magnet was brought close to the wall of the microcentrifuge

15

306 tube and held in place for 2 minutes, until it was visually clear that all the MMPDNA-TDNA

307 was removed from solution. While holding the magnet in place, the supernatant solution was

308 pipetted out, the magnet was removed and the magnetic microparticles were vortexed mildly

309 with additional fresh pH 7.2 solution. The external magnet was again brought close to the

310 microcentrifuge tube and microparticles were separated, supernatant solution extracted and the

311 process repeated again. This was repeated a total of three times to isolate all the MMPDNA-

312 TDNA from the interfering DNA. Then, a specific amount of pH 7.2 solution was added to the

313 clean microparticles and dispersed. At this stage, only the MMPDNA and TDNA complex

314 should be present in solution.

315

316   Once the purification steps were complete, and the MMPDNA-TDNA was in fresh solution,

317 the solution was heated to 80 °C and maintained for 5 minutes. At this temperature the TDNA

318 will be released from the MMPDNA particles (by melting), and while maintaining the solution

319 temperature, a magnet was applied to the tube to capture the MMPDNA and the supernatant

320 solution was removed from the tube, which contains TDNA. When the supernatant was added to

321 the etalon stabilized at 25°C in pH 7.2 solution, the λ_{max} of the reflectance spectrum shifted to the

322 lower wavelength. By changing the initial concentration of TDNA, we were able to observe

323 different shift magnitudes, as shown in Figure 3. The shifts were easily detectable and ranged

324 from 12 nm - 60 nm. We were easily able to detect the concentration of DNA down to the μM in

325 range with no preconcentration and/or amplification of the TDNA concentration. Therefore,

326 preconcentration and/or amplification of the TDNA concentration will only increase the

327 sensitivity of this method.

328
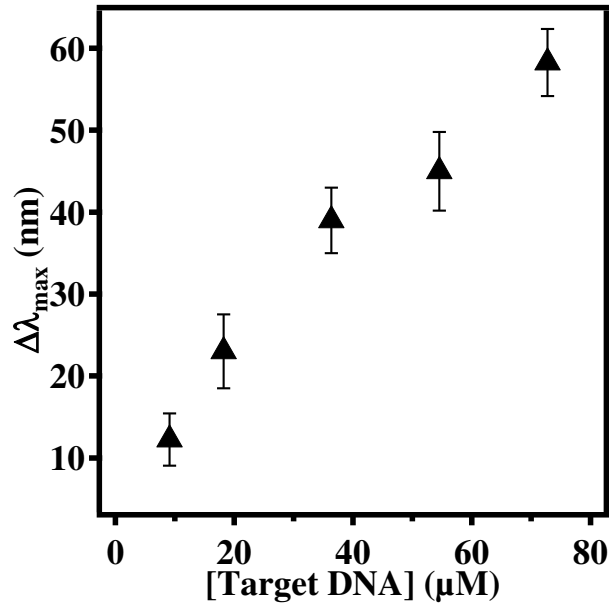
Figure 3: Shift of a reflectance peak for a pNIPAm-co-APMAH etalon upon addition of
separated TDNA solution of different concentrations. The pNIPAm-co-APMAH microgel-based
etalon was soaked in pH 7.2 solution throughout the experiment, while the temperature was
maintained at 25 °C. Each point in the plot represents the average of at least three independent
measurements, and the error bars are standard deviation for those values. A new device was used
for each experiment.

Conclusion:

In summary, we have shown that pNIPAm-co-APMAH microgel-based etalons can be
made responsive to the presence of ssDNA in solution by exhibiting a blue shift in the peaks of
the etalon's reflectance spectrum. This response originates from the penetration of polyanionic
ssDNA into the etalon's microgel layer. Once penetrated, DNA interacts with the positively
charged microgel electrostatically and crosslinks them. The crosslinking results in shrinking of
the confined microgel between Au layers resulting in the peaks shifts, as predicted from Eq. (1).
The extent of shift is directly related to the initial concentration of target DNA present in the

17

344 sample solution. Ultimately, we were able to detect μM concentrations of target DNA in solution

345 with no TDNA preconcentration, amplification, or other system optimization whatsoever. This

346 method represents a very simple and inexpensive way of sensing DNA without the need for a

347 label. In fact, the devices here cost pennies. Furthermore, the response can be read out

348 colorimetrically, which offers the potential of low cost equipment for running the assay. In the

349 future, we will utilize this sensing scheme for detecting other protien and DNA-based biomarkers

350 for that are specific for disease states.

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