*Electronic Supporting Information for:*

# Visible label-free detection of bacterial DNA using flocculation of sterically stabilised cationic latexes

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## Materials and additional experimental methods

### Materials

2-Vinylpyridine (97%, 2VP; Sigma-Aldrich, UK), divinylbenzene (80 mol % 1,4-divinyl content, DVB; Sigma-Aldrich, UK) and benzyl methacrylate (BzMA, 98%, Sigma-Aldrich, UK) were passed through a column of activated basic alumina to remove inhibitors and impurities before use. Hexadecane (HD, 99%) was purchased from Alfa Aesar (UK). Diethyl ether (99%), azobisisobutyronitrile (AIBN, 98%), and 2,2'-azodiisobutyramidine dihydrochloride (AIBA; 97%) were purchased from Sigma-Aldrich (UK) and used as received. Aliquat 336 surfactant was purchased from Fisher Scientific (UK) and used as received. Lutensol TO 20, which is a saturated iso-C13 alcohol with an ethoxylation block length of 20 units was obtained from BASF (Germany) and used as received. Poly(ethylene glycol) methyl ether methacrylate (PEGMA, average $M_n$ 2,000 g mol$^{-1}$, Sigma-Aldrich, UK) was supplied as a 50 wt. % solution in $H_2O$. PEGMA with an average $M_n$ 360 g mol$^{-1}$ and average $M_n$ 500 g mol$^{-1}$ (Sigma Aldrich, UK) were passed through a basic alumina column to remove inhibitors before use. Bis-(2-phenylethanesulfanyl thiocarbonyl) disulphide (PETTCCP) was prepared in-house using previously published methods.[1] Deionised water was used throughout this study for analysis and dilution. PCR reagents were used as received. DNA extraction and purification kits (Qiagen, UK) were used as per manufacturer's instructions.

### Preparation of poly(benzyl methacrylate) by RAFT miniemulsion polymerisation

The synthesis of poly(benzyl methacrylate) with a mean degree of polymerisation of 200 (DP) *via* reversible addition–fragmentation chain transfer (RAFT) miniemulsion polymerisation was conducted at a dispersed phase content of 20 % w/w, as previously reported.[2] BzMA (7.8 g, 44.087 mmol); PETTCCP (41.4 mg, 0.147 mmol); AIBN (4.8 mg, 0.029 mmol, [Chain Transfer Agent (CTA)]/[initiator] = 5); Lutensol TO 20 (604.0 mg, 7.8% w/w related to BzMA); HD (185.2 mg, 2.4% w/w related to BzMA); and water (31.4 g) were used. The reactor contents were deoxygenated by purging with nitrogen for 10 min at room temperature. After deoxygenation, the round-bottomed flask was immersed into a preheated oil bath at 70 °C for 240 min. Polymerisations were quenched by cooling to room temperature and exposing to air.

**Preparation of poly(4-styrene sulfonate)-block-poly(benzyl methacrylate) (PSS-*b*-PBzMA) diblock copolymer nanoparticles *via* RAFT mediated polymerisation-induced self-assembly (PISA)**

Previous work on the synthesis of these diblock copolymer nanoparticles has been described previously.[1] $PSS_{54}$-*b*-$PBzMA_{100}$ latexes were synthesised at 70°C for 24hr using $PSS_{54}$ macro-CTA (85.2 mg, 0.012 mmol), BzMA (215.3mg, 1.22 mmol), ACVA (1.1 mg, 0.004 mmol, CTA/initiator molar ratio = 3) in an 80 % methanol/water mixture. Polymerisations were quenched by cooling to room temperature and exposing to air.

**Synthesis of poly(2-vinyl pyridine)-block-poly(benzyl methacrylate) P2VP–*b*-PBzMA diblock copolymer nanoparticles *via* RAFT emulsion polymerisation**

Previous work on the synthesis of these diblock copolymer nanoparticles has been described previously.[3] $P2VP_{32}$–$PBzMA_{300}$ was synthesised in water at pH 2, using $P2VP_{32}$ macro-CTA (32.3 mg, 0.009 mmol), AIBA (0.8 mg, 0.003 mmol, CTA/initiator molar ratio = 3), and deionised water. The solution pH was slowly adjusted to pH 2 using an aqueous solution of 0.1 M HCl, and then BzMA (467.7 mg, 2.654 mmol) was added. The vial was sealed and deoxygenated by purging nitrogen for 10 min before being placed in a preheated oil bath at 70 °C for 24 h to ensure complete conversion of BzMA. Polymerisations were quenched by cooling to room temperature and exposing to air.

**Supporting Figures**

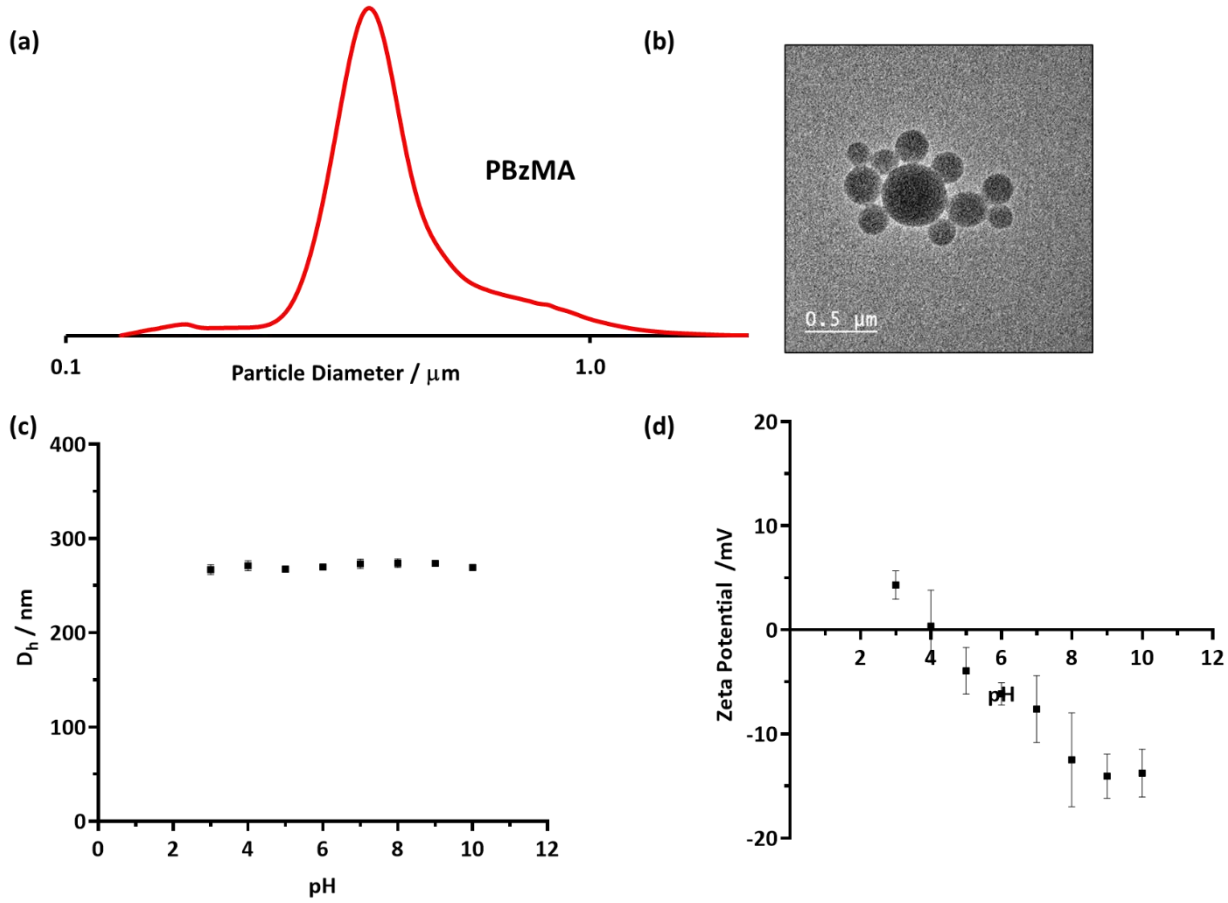


**Figure S1**. Characterisation of non-ionic PBzMA latex particles prepared by miniemulsion polymerisation. (a) Disc Centrifuge Photosedimentometry (DCP) particle size distributions were calculated using a particle density of 1.18 g $cm^{-3}$. (b) Representative TEM image. (c) Mean hydrodynamic diameter and (d) zeta potential as a function of pH. Measurements were conducted at a latex concentration of approximately 0.01 % w/w with 1 mM KCl as a background electrolyte. The pH was adjusted using KOH and HCl.

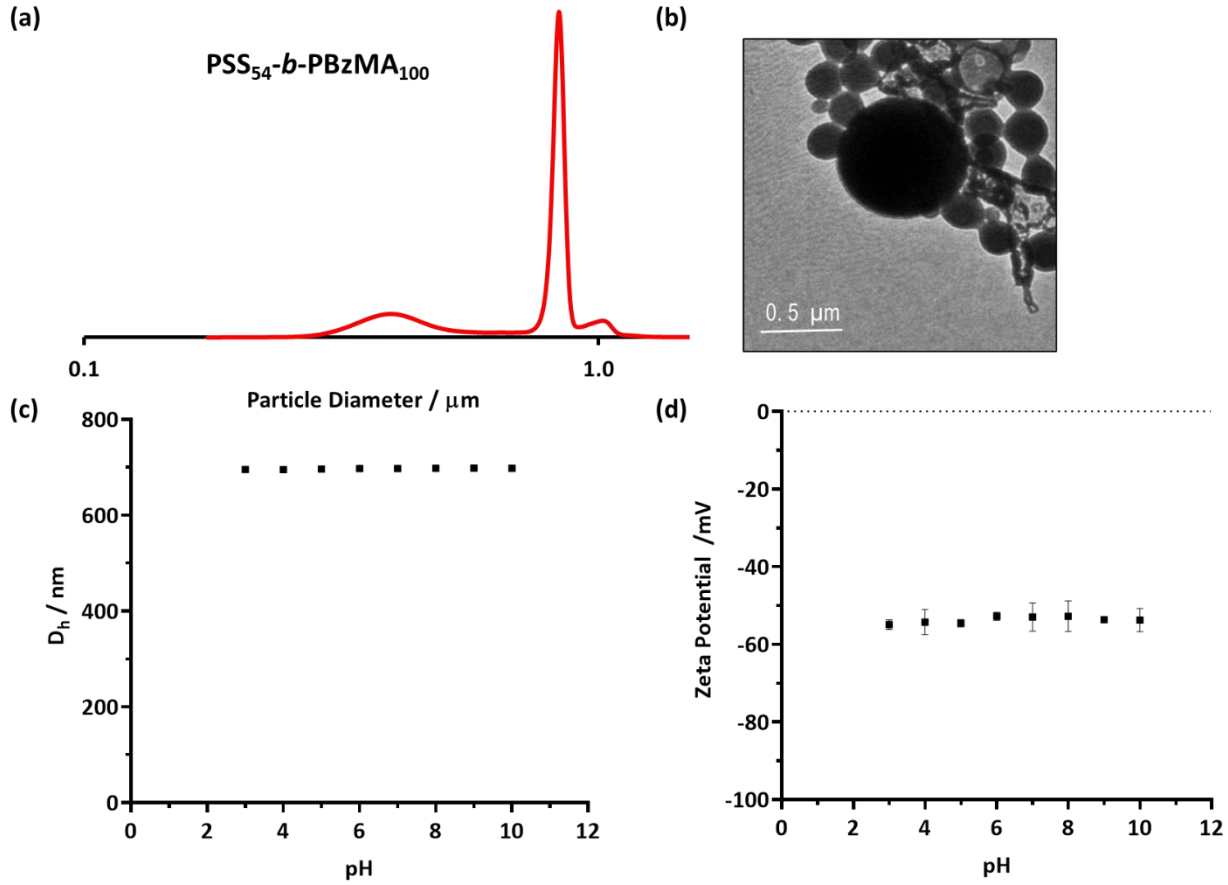


**Figure S2**. Characterisation of anionic $PSS_{54}$-*b*-$PBzMA_{100}$ latex particles prepared by RAFT mediated PISA. (a) DCP particle size distributions were calculated using a particle density of 1.18 g $cm^{-3}$. (b) Representative TEM image. (c) Mean hydrodynamic diameter and (d) zeta potential as a function of pH. Measurements were conducted at a latex concentration of approximately 0.01 % w/w with 1 mM KCl as a background electrolyte. The pH was adjusted using KOH and HCl.

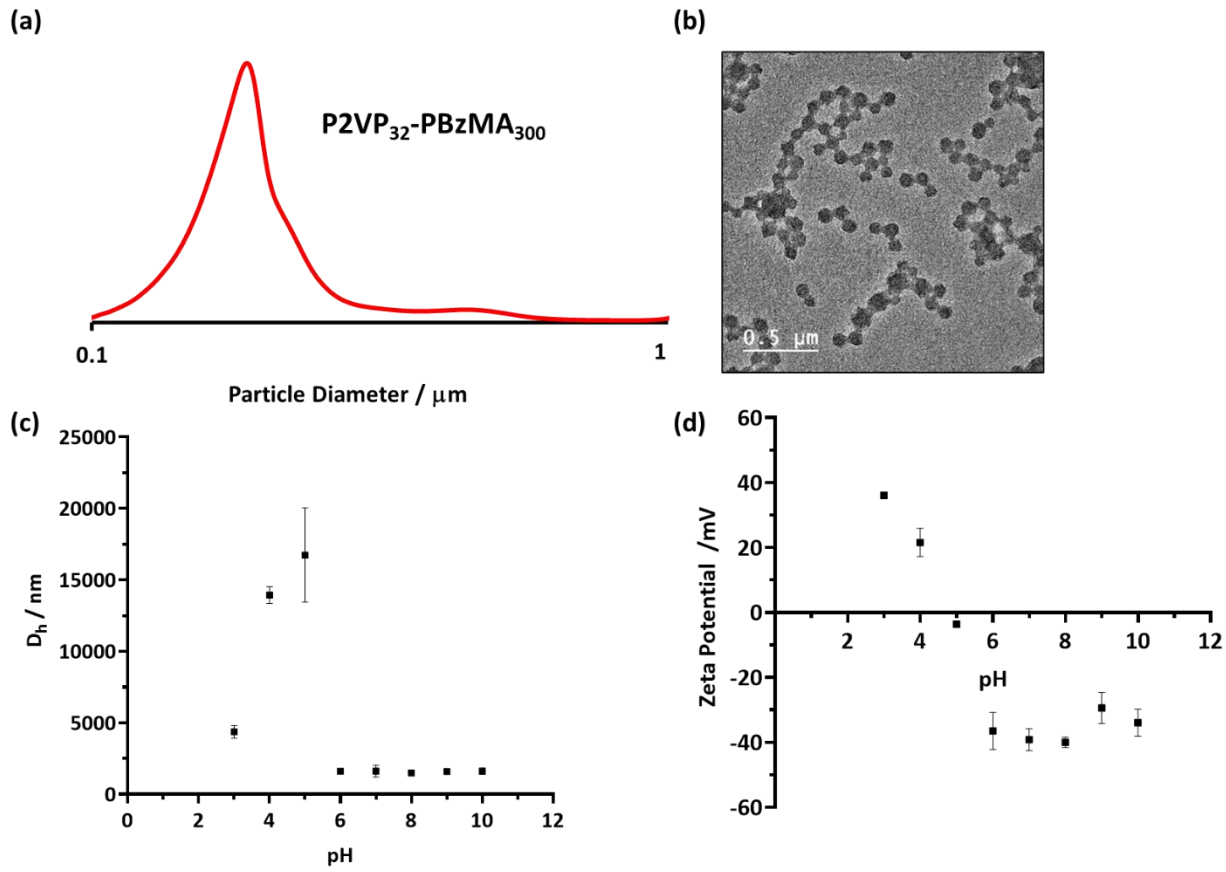


**Figure S3**. Characterisation of $P2VP_{32}$-*b*-$PBzMA_{300}$ latexes prepared by RAFT emulsion polymerisation. (a) DCP particle size distributions were calculated using a particle density of 1.18 g $cm^{-3}$. (b) Representative TEM image. (c) Mean hydrodynamic diameter and (d) zeta potential as a function of pH. Measurements were conducted at a latex concentration of approximately 0.01 % w/w with 1 mM KCl as a background electrolyte. The pH was adjusted using KOH and HCl.

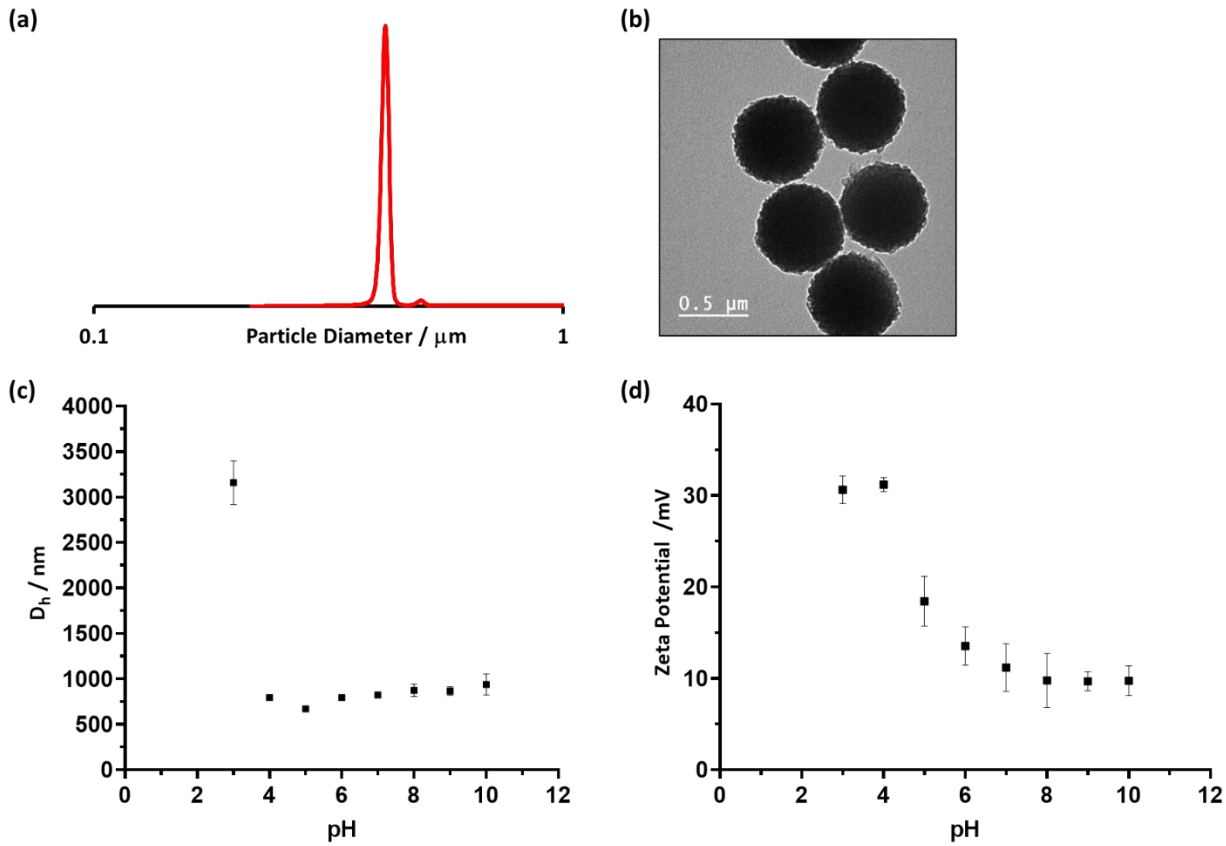


**Figure S4**. Characterisation of ~700 nm cationic sterically-stabilised PEGMA-P2VP latex particles prepared by emulsion polymerisation. (a) DCP particle size distributions were calculated using a particle density of 1.18 g $cm^{-3}$. (b) Representative TEM image. (c) Mean hydrodynamic diameter and (d) zeta potential as a function of pH. Measurements were conducted at a latex concentration of approximately 0.01 % w/w with 1 mM KCl as a background electrolyte. The pH was adjusted using KOH and HCl.

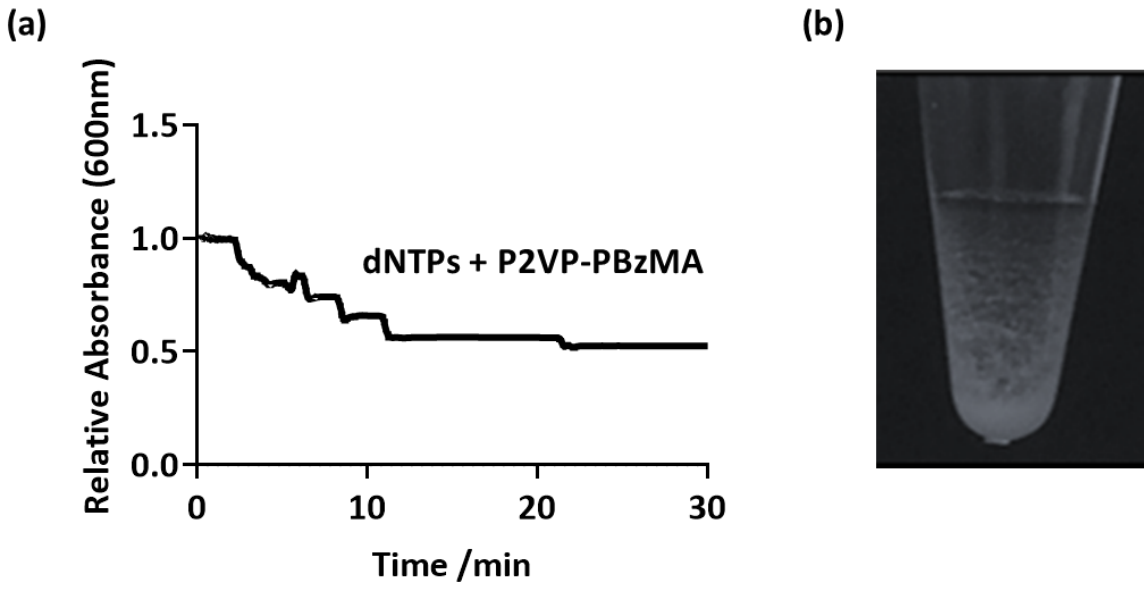


**Figure S5**. (a) UV-VIS spectrophotometry measurement showing the change in absorbance at 600 nm after the addition of dNTPs to a $P2VP_{32}$-*b*-$PBzMA_{300}$ latex. Latex particles were at a concentration of 0.1 % w/w and dNTPs were added at the concentration used in the PCR reaction. (b) Digital image showing flocculation after the addition of dNTPs to this latex.

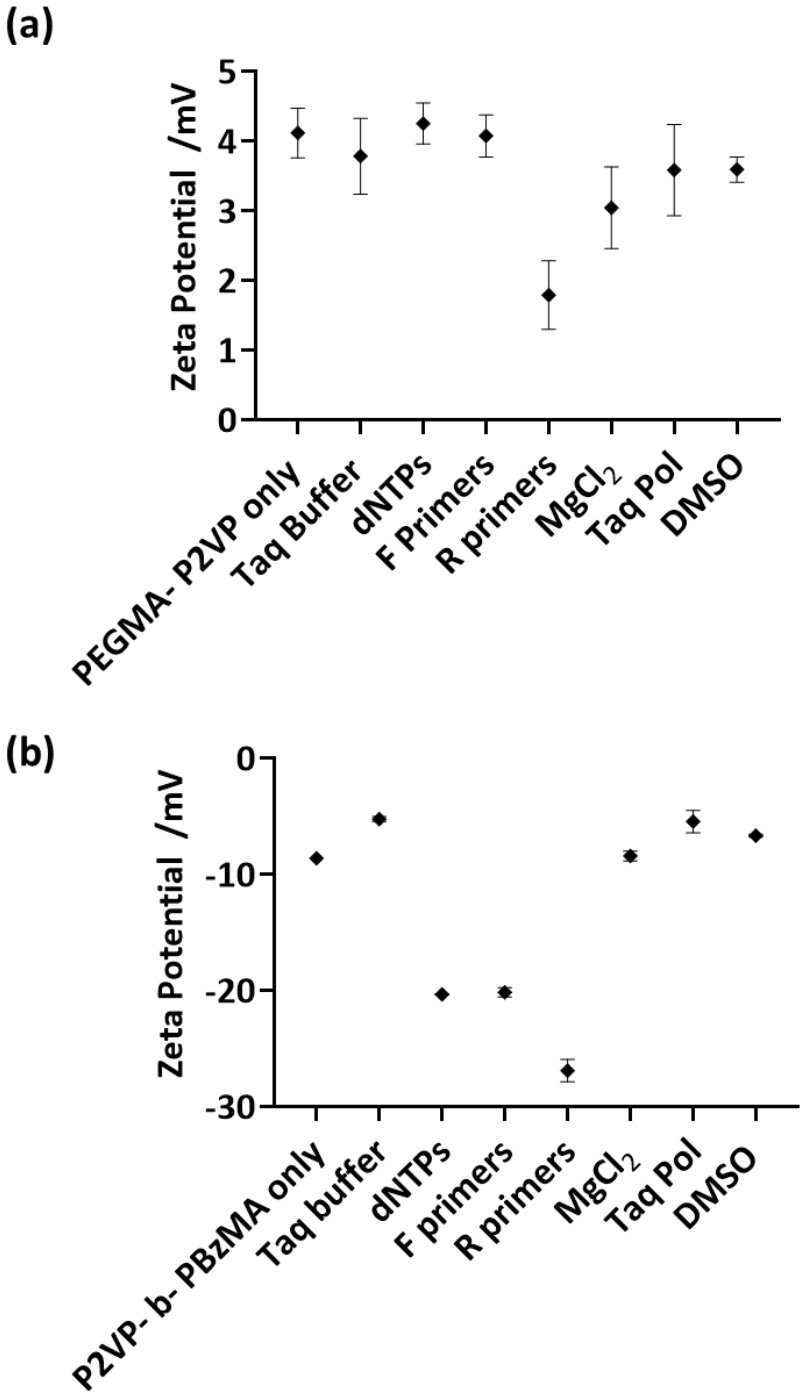


**Figure S6.** Zeta potential measured after the addition of individual PCR reagents to (a) sterically-stabilised PEGMA-P2VP (~700 nm) and (b) $P2VP_{32}$-*b*-$PBzMA_{300}$ latexes. Measurements were conducted at a latex concentration of approximately 0.01 % w/w with 1 mM KCl as a background electrolyte. PCR reagents were added at the concentration used in typical PCR mastermix.

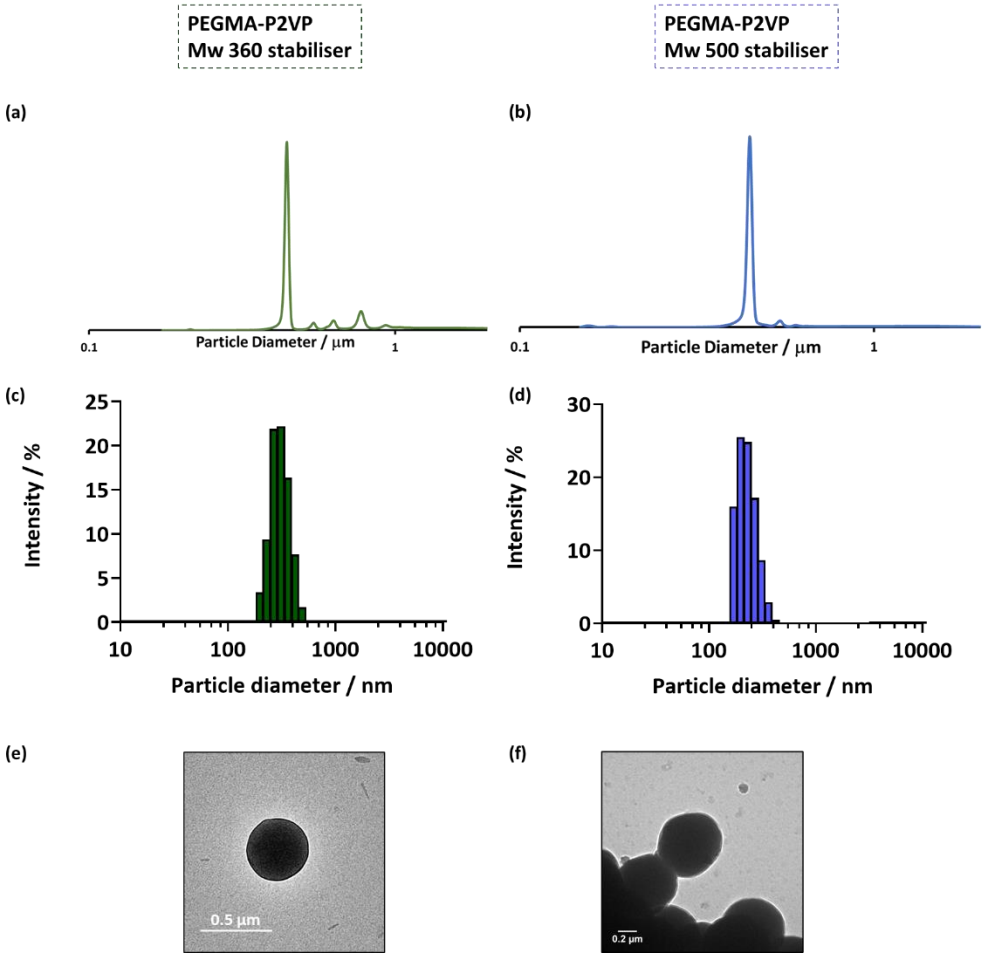


**Figure S7.** Characterisation of cationic sterically-stabilised PEGMA-P2VP latex particles prepared by emulsion polymerisation using PEGMA with an average $M_n$ 360 g $mol^{-1}$ or 500 g $mol^{-1}$. (a-b) DCP particle size distributions were calculated using a particle density of 1.18 g $cm^{-3}$. (c-d) Mean hydrodynamic diameter determined using dynamic light scattering. Measurements were conducted at a latex concentration of approximately 0.01 % w/w. (e-f) Representative TEM images.

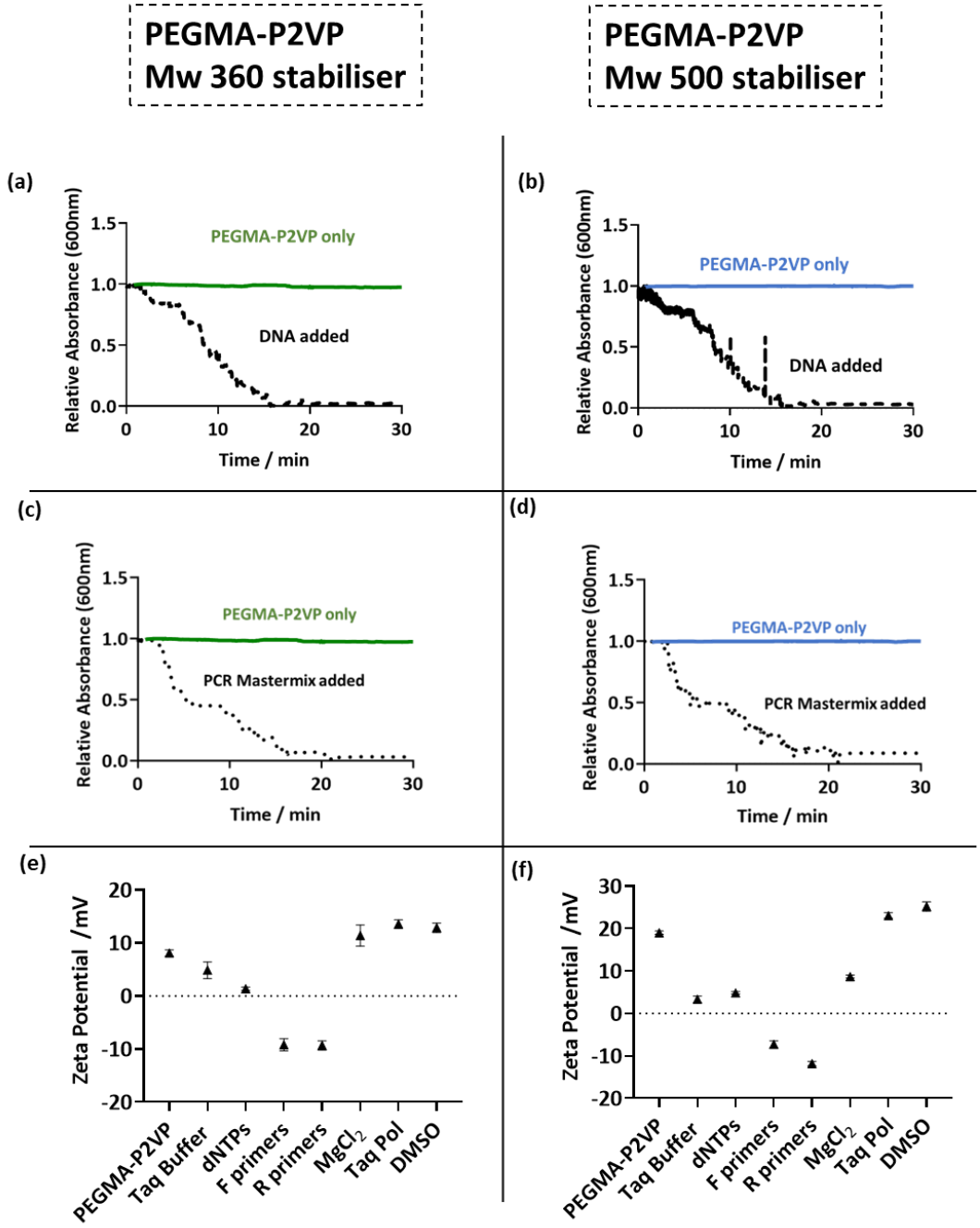


**Figure S8.** Behaviour of cationic sterically-stabilised PEGMA-P2VP latex particles prepared by emulsion polymerisation using PEGMA with an average $M_n$ 360 g $mol^{-1}$ or 500 g $mol^{-1}$. (a and b) UV-VIS spectrophotometry measurements showing the change in absorbance at 600 nm after the addition of amplified DNA (1400 bp). Latex particles were at a concentration of 0.1 % w/w and DNA was added at a concentration of ~4 ng $\mu l^{-1}$. (c and d) UV-VIS spectrophotometry measurements showing the change in absorbance at 600 nm after the addition of 50 µl PCR mastermix. Latex particles were at a concentration of 0.1 % w/w and PCR mastermix was added at the concentration used in a typical PCR. (e and f) Zeta potential measured after the addition of individual PCR reagents. Latex concentration of approximately 0.1 % w/w and PCR reagents were added at the concentration used in typical PCR mastermix.

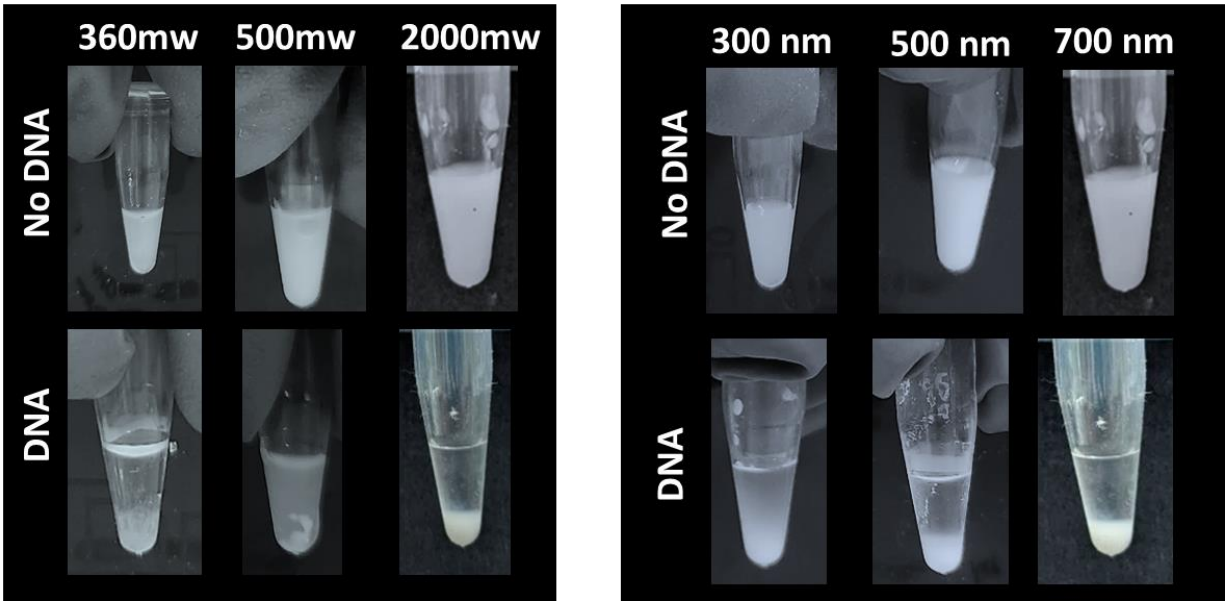


**Figure S9.** Digital images showing flocculation of PEGMA-P2VP latexes 30 minutes after the addition of amplified DNA from *P. aeruginosa* (1400 bp, ~4 ng µl $^{-1}$). Left: PEGMA-P2VP latexes prepared by using PEGMA with an average $M_n$ 360, 500 and 2000 g mol$^{-1}$. Right: PEGMA-P2VP latexes with mean diameters between approximately 300 to 700 nm, prepared using PEGMA with $M_n$ 2000 g mol$^{-1}$.

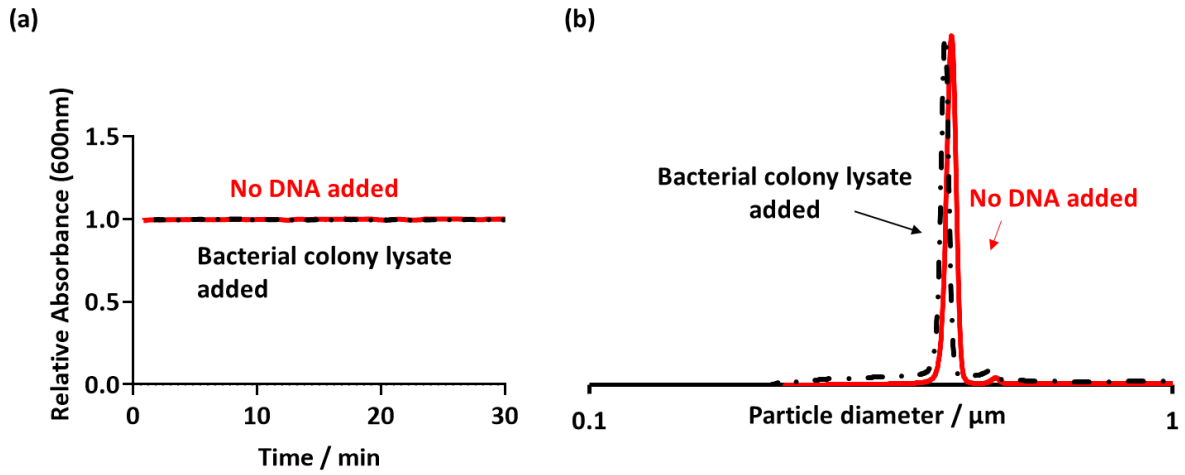


**Figure S10**. (a) UV-VIS spectrophotometry measurement showing the change in absorbance at 600 nm after the addition of bacterial colony lysate to PEGMA-P2VP latex (~700 nm diameter). (b) DCP particle size distributions for PEGMA-P2VP latex with the addition of bacterial colony lysate from *P. aeruginosa.*

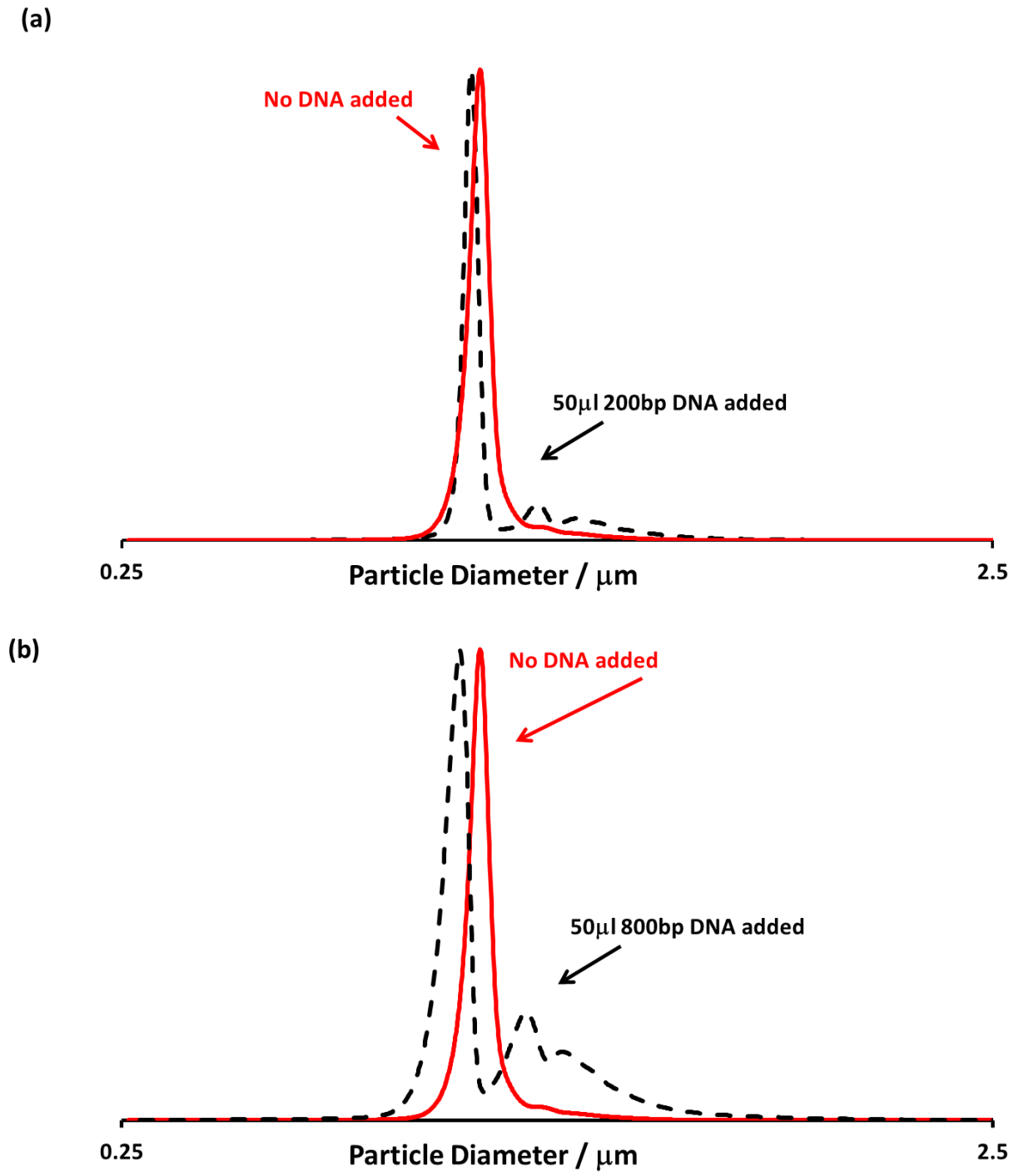


**Figure S11**. DCP particle size distributions for PEGMA-P2VP latex with the addition of amplified DNA from *P. aeruginosa* with (a) 200 bp and (b) 800 bp sequence lengths. Latex concentrations were 0.01 % w/w and an overall DNA concentration of 3 ng $\mu l^{-1}$ was used in both cases.

## References


1 S. P. Wen, J. G. Saunders and L. A. Fielding, *Polym. Chem.*, 2020, **11**, 3416–3426.

2 S. P. Wen, Q. Yue and L. A. Fielding, *Polym. Chem.*, 2021, **12**, 2122–2131.

3 S. P. Wen and L. A. Fielding, *Soft Matter*, 2022, **18**, 1385–1394.