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1	Synthesis of Sub 100 nm Glycosylated-Nanoparticles via a One Step, Free Radical and Surfactant Free
2	Emulsion Polymerisation
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12	
13	Abstract
14	We present the facile synthesis of sub 100 nm glyco-nanoparticles via a one-step, free radical and surfactant
15	free emulsion polymerisation. We show that by using sterically large, hydrophilic glycomonomers such as a
16	lactose acrylamide with the charged azo initiator 4,4'-azobis(4-cyanovaleric acid) (ACVA), growing particles
17	are stabilised enough to reproducibly produce well defined (PDi $\leq 0.1$ ) glycoparticles with diameters below
18	100 nm.
19	
20	Keywords: Emulsion polymerisation, glyco-nanoparticle, surfactant free
21	
22	

## 23 Introduction

Emulsion polymerisation is an industrially favoured technique for synthesising many types of polymers due 24 to its favourable kinetics, the high specific heat capacity of water and the low viscosity of the final polymer 25 product<sup>[1]</sup>. One major use of emulsion polymerisation is the facile synthesis of functional polymer 26 nanoparticles.<sup>[2-6]</sup> A classic emulsion polymerisation uses a surfactant, such as sodium dodecyl sulphate, to 27 provide nucleation sites for growing "z-mer" polymer chains and particle stabilisation. Without the addition 28 of surfactant to the system, a "self-nucleating" emulsion polymerisation, which will produce both latex 29 particles and linear hydrophilic polymer in solution, typically produces particles in the hundreds of nanometres 30 to micron diameters.<sup>[7-13]</sup> Latexes with particles under 100 nm in diameter are desirable however for 31 applications such as drug delivery, to, for example, take advantage of the tumour targeting enhanced 32 permeability and retention (EPR) effect.<sup>[14-16]</sup> However, particles made using a surfactant must then be 33 extensively dialysed to remove the surfactant for use in applications such as drug delivery and photonics.<sup>[11,</sup> 34 <sup>17, 18]</sup> There has therefore been a large amount of research into synthesising functional nanoparticles with 35 diameters under 100 nm using "surfactant free" controlled radical polymerisation.<sup>[2, 19-25]</sup> One such technique 36 is reversible addition-fragmentation chain transfer (RAFT) emulsion polymerization, in which a short 37 amphiphilic di-block copolymer is prepared, then suspended in water to act as a surfactant for the emulsion 38 polymerisation. The initial di-block subsequently becomes bound to the resulting latex particle via chain 39 extension, conveniently imparting functionality from the hydrophilic section onto the resulting latex and 40 avoiding the need for dialysis.<sup>[24, 26, 27]</sup> Such a synthetic approach can be time consuming and expensive when 41 compared to standard emulsion polymerization,<sup>[28]</sup> although it allows access to a variety of functionalised 42 particles with diameters below 100 nm.<sup>[29, 30]</sup> Nano-particles that are functionalised on their surface are also 43 of particular interest in applications such as targeted drug delivery.<sup>[14, 17, 30-35]</sup> In particular, specific sugar 44 moieties can be positioned at the surface of the particles, to target a class of protein receptors known as lectins. 45 Previous research into using sugar-lectin targeting for drug delivery has shown that presenting the targeting 46 sugar in a high concentration, such as that on the surface of a particle, increases the targeting efficiency of a 47 drug delivery vector, taking advantage of the "glycocluster effect".<sup>[36, 37]</sup> Recent developments in this area 48 have applied the principle of targeting lectins with glycopolymers in more refined systems, using sequenced 49 defined polymers alone and in combination with stimuli responsive polymers to elicit a more specific 50

targeting.<sup>[37-41]</sup> Amongst the many stimuli responsive systems biological 51 reported. poly(Nisopropylacrylamide) is commonly used for temperature response, where heating above the cloud point of the 52 system triggers a self-assembly or reveals a glycopolymer.<sup>[42, 43]</sup> Next to temperature, pH is arguably one of 53 the most ubiquitously exploited stimuli, used to induce glycopolymer assembly and disassembly, or to release 54 a covalently bound drug molecule *via* an acid cleavable linker; these amongst many other unique systems 55 represent a growing and active field of research in drug delivery.<sup>[44-47]</sup> 56

57 Given their great potential, and current synthetic problems, it is therefore desirable to be able to 58 synthesise functional polymer latex particles, under 100 nm in diameter with a low-cost and fast, one step 59 synthesis that does not require further purification.

# 60 Results and Discussion

The full synthetic procedure for particle synthesis can be found in the supporting information. In brief: monomers and initiator were dissolved or suspended in water within a glass vial. This system was then, under an inert atmosphere, rigorously agitated with a magnetic follower at 800 RPM and heated to 70 °C for three hours. In all cases this resulted in the formation of well-defined nanoparticles (Pdi  $\leq 0.1$ ), ranging in size from 50-350 nm (Table 1), which when broken up into constituent polymeric unimers, along with linear polymer in solution, showed dispersities as determined by GPC between 1.5-1.8 (SI: S2), and over 99% monomer conversion, determined by <sup>1</sup>H NMR (SI: S3).

	Composition of Free Radical Particle	Hydrophilic (µmol)	Hydrophobic (µmol)	ζ- Potential (mV)	Diameter by DLS (nm)	PDi <sup>a</sup>	Diameter by SEM (nm)
Α	P(LactAm) <sub>1</sub> -co-(sty) <sub>5</sub>	4.711	23.55	-37.1	55	0.048	54
B	P(PEGA) <sub>1</sub> -co-(sty) <sub>5</sub>	4.711	23.55	-20.4	70	0.074	69
С	P(ManAm) <sub>1</sub> -co-(sty) <sub>5</sub>	4.711	23.55	-14.8	88	0.02	103
D	P(HEAm) <sub>1</sub> -co-(sty) <sub>5</sub>	4.711	23.55	-39.2	112	0.057	117
Е	P(sty)	N/A	23.55	-37.3	108	0.063	106
F	P(LactAm) <sub>1</sub> -co-(BA) <sub>5</sub>	22.8	114	-31.3	85	0.1	-
G	P(LactAm) <sub>1</sub> -co-(BA) <sub>10</sub>	22.8	228	-38.1	198	0.058	-
Η	P(LactAm) <sub>1</sub> -co-(BA) <sub>20</sub>	22.8	456	-43	260	0.051	-
Ι	P(LactAm) <sub>1</sub> -co-(BA) <sub>30</sub>	22.8	684	-37.3	256	0.06	-
J	P(LactAm) <sub>1</sub> -co-(BA) <sub>50</sub>	22.8	1140	-43.4	348	0.064	-

<sup>68</sup> 

Table 1: Characterisation for all polymer particles synthesised with surfactant free emulsion polymerisation. All
 polymerisations used an ACVA initiator concentration of 3.13x10<sup>-6</sup>. <sup>a</sup> Determined using equation S1, see Supporting
 Information

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Having optimised a one pot, single step method to produce nanoparticles, the effect of varying the 73 hydrophilic monomer was investigated. It is proposed that in the early stages of this synthesis, hydrophilic 74 homopolymer is predominantly formed (as it is fully soluble in the water phase with the initiator), which is 75 then followed by the separate formation short copolymers with the small amount of hydrophobic monomer in 76 solution. The copolymers formed then act as a surfactant to drive an emulsion mechanism, where nucleated 77 particles are stabilised by the initiator and hydrophilic polymer, growing by both polymerisation and particle 78 aggregation.<sup>[1, 48]</sup> As such, the choice of hydrophilic monomer used should have a large impact on the size of 79 the resulting particle. To test this, the hydrophilic monomer was varied, whilst keeping all other reaction 80 conditions the same. Lactose acrylamide (LactAm), mannose acrylamide (ManAM), hydroxyethyl acrylamide 81 (HEAm) and poly(ethylene glycol) acrylate (PEGA) were employed, in order to assess the effect of monomer 82 83 size and hydrophilicity. The same synthesis was also performed using no hydrophilic monomer, but with the negatively charged 4,4'-azobis(4-cyanovaleric acid) (ACVA) initiator as hydrophilic component. The 84 polymerisation led to a range of particles which size varied from largest to smallest in the order: HEAm≥ 85 86 ACVA> Mannose> PEGA> Lactose (Table 1, S4 and S5), confirming that the hydrophilic monomer used plays a large part in the stabilisation of this emulsion system and can be used to influence the size of any 87 resulting particle. 88

We hypothesise that monomers with greater steric hindrance, charge, and/or water solubility, give 89 greater stabilisation, thus producing smaller particles, as they have a lower apparent number of aggregation. 90 91 It is also noteworthy that emulsion polymerisation with only the ACVA initiator as the hydrophilic component produced well defined particles of 108 nm diameter (as established by DLS). We expect the stabilisation in 92 this case is due to the charged carboxylic acid group of ACVA having been deprotonated by sodium hydroxide 93 to ensure aqueous solubility, showing the importance of the initiator in stabilising the growing latex 94 particles.<sup>[13, 49]</sup> In the case of the HEAm particles, the initiator potentially provided the majority of the particle 95 stabilisation, as both the HEAm and ACVA-only particles were of an equivalent size and zeta potential. 96 Lactose acrylamide produced the smallest particles 55 nm in diameter (determined by DLS and confirmed by 97 SEM, giving an average size of 54 nm) (Figure 1). This suggested that lactose acrylamide provides superior 98 stabilisation when compared to all other hydrophilic co-monomers tested, due to its 7 hydroxyl groups, which 99





Figure 1: SEM images showing particle size and morphology. Pictures a, b, c, d and e correspond to styrene particles 100 with shells of lactose acrylamide, PEGA, mannose acrylamide, hydroxyethyl acrylamide and no hydrophilic monomer 101 respectively. Average diameter for each in table 1 102 increase its number of hydration and water solubility, as well as good steric stabilisation from its bulky 103 disaccharide structure. These results are remarkable as surfactant-free polymerisation does not typically 104 provide access to particle diameters under 100 nm, due to insufficient stabilisation which causes particle 105 aggregation. Indeed, most examples to date use the initiator potassium persulfate (KPS) to provide sufficient 106 stabilisation, e.g. in the emulsion polymerisation of styrene and methyl methacrylate.<sup>[13, 50]</sup> In this work, we 107 108 show that styrene particles made with ACVA initiator and either lactose acrylamide, PEGA or mannose 109 acrylamide as a co-monomer provide access to particles of diameter 55, 70 and 88 nm respectively. Being

able to reliably produce particles under 100 nm in diameter without the use of a surfactant or initial polymer
diblock shows the potential for using a charged initiator with sterically large or charged monomers,
particularly glycomonomers, for their ability at stabilising a latex and to produce functional nanoparticles.

The surface functionality of the particles was assessed by a turbidimetric aggregation test between 113 mannose coated particles ("particle C" Table 1), and the lectin concanavalin A (Con A), which is known to 114 bind preferentially to mannose and glucose.<sup>[37, 51, 52]</sup> Latex was mixed in a UV-Vis spectrometer with a 1.3 µM 115 solution of Con A in TRIS buffer at pH 7, and the absorbance tracked at 500 nm (Figure 2). This test was 116 117 performed before and after removing any free sugar homopolymer in solution by precipitating the particles out of suspension using centrifugation for ten minutes at 13,500 rpm, and re-suspending the particle pellet in 118 de-ionised water, a process repeated three times. This purification technique was preferred to dialysis, as it 119 ensured that even the longest free radical polymer not anchored to the particle was removed, as there is no 120 upper molecular weight cut off, which is necessary when purifying using a dialysis membrane. 121



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Figure 2: UV-Vis turbidimetric aggregation tests between mannose coated particles and concanavalin A. a) raw latex with free glycopolymer in solution b) purified latex with free glycopolymer removed from solution via centrifugation
The results showed a very slow increase in absorbance over 30 minutes when the raw latex was mixed
with a Con A solution. However, when the same test was performed on the purified latex, a much faster
increase in absorbance, and subsequent reduction (due to large particulate aggregates sedimenting out of
solution), was observed over 11 minutes. We hypothesise that the reduced response from the raw latex is due
to binding competition from the free sugar homopolymer in solution. This result confirmed that the particles

have a covalently bound glycopolymer shell. A modified turbidimetric test was also performed with the addition of free mannose post-aggregation in excess to compete for ConA binding sites and induce aggregate break up, confirming that aggregation was due to sugar-lectin binding (SI: S6). Gravimetry was also used to quantify the amount of hydrophilic glycopolymer bound to the surface of the particles. The mass of 1 mL of raw latex was compared to that of 1 mL the purified latex, after drying both solution in a vacuum oven at 40 °C for 16 hours. The average difference in mass after three repeats represented the mass of polymer in solution, and was used differentially to determine the amount of polymer bound to the particles, 41% of the polymer.



Figure 3: Particle size calibration with varying molar ratios of hydrophilic lactose acrylamide monomer to
 hydrophobic butyl acrylate monomer plotted against the resulting particle volume

To determine if this emulsion process can reliably control particle size over a larger range, and be used 139 with a different hydrophobic monomer, five latexes with varying ratios of lactose acrylamide to butyl acrylate 140 were synthesised and the monomer ratios plotted against the resulting particle volume. The results (Table 1 141 and Figure 3) show that by varying the ratio of lactose acrylamide: butyl acrylate from 1:5 to 1:50, the diameter 142 of the resulting particle could be reliably adjusted to between 85 and 348 nm  $(321,555 - 22,066,629 \text{ nm}^3)$ , 143 with a linear relationship between hydrophobic component and resulting volume. Particle diameters under 100 144 nm were also produced with butyl acrylate, showing the ability of this method to produce functionalised 145 nanoparticles with monomers other than styrene. To further explore the limits of our system, we 146 experimentally determined the maximum particle concentration possible using this synthetic method at 15-20 147 wt% of monomer. This was determined by varying the total solid content of the emulsion whilst maintaining 148 a molar ratio of 1:10 lactose acrylamide to styrene. Well-defined particles were produced up to 15 wt% of 149

monomer (SI: S7). At 20 wt% and above, defined particles were not obtained and formed aggregated to suchan extent that a reliable size was not obtainable by DLS.

# 152 Conclusion

We have shown that a surfactant free emulsion can reliably produce nanoparticles between 50-350nm in diameter in a one-step synthesis, with little or no purification. The initiator ACVA and glycomonomers, particularly disaccharide monomers with a large number of hydration such as lactose acrylamide, are extremely good at stabilising emulsion polymerisations and provide a useful tool for synthesising low diameter, well defined carbohydrate coated nanoparticles suitable for biological use.

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## **Table of Contents Summary Text**

A rapid one-step approach to synthesising sub-100 nm polymeric glyco-nanoparticles without the use of a surfactant is presented. The free radical method takes advantage of the exceptional ability of glycomonomers, particularly lactose acrylamide to stabilise a growing latex particle, with the use of the charged initiator ACVA to go from monomer to final latex in under three hours.



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