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Synthesis of Mannosylated and PEGylated Nanoparticles via RAFT Emulsion Polymerisation, and Investigation of Particle-Lectin Aggregation using Turbidimetric and DLS Techniques

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1 TOC Graphic and Graphical Abstract

2 Abstract:

3 Recent developments in controlled radical polymerisation presents an attractive way of producing
4 biocompatible polymeric nanoparticles for a wide range of applications. With this motivation, well
5 defined P(ManAm) and P(PEGA) coated nanoparticles in a range of different sizes have been
6 synthesised via RAFT emulsion polymerisation. The particles were used to precisely investigate the
7 effect of particle size on lectin binding with Concanavalin A, and validate the use of online DLS
8 measurements for lectin-glycoparticle aggregation studies. Larger particles were found to have an
9 enhanced aggregation by both UV-Vis turbidimetric and DLS aggregation studies. The DLS technique
10 was shown to be robust up to an aggregate diameter of c.500nm for aggregation tests, and was not
11 affected by any dilution or light scattering effects that typically hinder the common use of turbidimetry
12 in particle aggregation studies.

13

14

15 1. Introduction

16

17 In the field of drug delivery, targeting of specific cells (e.g. malignant or a bacterial cells) is an
18 important way of delivering therapeutic doses of an active pharmaceutical ingredient (API), whilst
19 minimising its side effects. Targeting cell surface proteins with their complementary ligand is one way
20 of directing an API to its site of action. Lectins are a well-known example of surface protein, expressed
21 by both bacterial and mammalian cells. One of the main properties of lectins is their highly specific
22 ligand-receptor interaction *via* non-covalent bonds with carbohydrates.[1-3] One potential solution for
23 cell targeting, is to harness these non-covalent interactions, with the use of polyvalent saccharide coated
24 'glyconanoparticles' acting as targeted delivery agents. Many glyconanoparticles consist of a metallic

25 core (e.g. gold) with a glycosylated shell.[4, 5] However, the versatility of polymer chemistry has
26 allowed researchers to modify all aspects of nanoparticle structure such as core/shell composition,
27 shape, size and degradability, which is suited to the synthesis of well-defined glyco-nanoparticles.[6]

28

29 Interest in the field of glycosylated nanomaterials has grown rapidly over the previous two decades,
30 particularly for their use as biosensors[3, 7] or targeting agents[8]. However, to fully understand the
31 interactions these materials have within a complex biological system, researchers must look towards
32 model systems which are equivalent in most aspects but instead are inert to unspecific interactions with
33 carbohydrate. Poly(ethylene glycol) is the most widely known ‘stealthy’ polymer, and is typically used
34 as a coating to avoid protein adsorption and subsequent immune response *in vivo*.[9] This property of
35 PEG is usually attributed to an enhanced hydration effect of the hydrophilic polymer chains resulting in
36 steric hindrance or ‘shielding’ to reduce protein fouling.[10] Typically, materials with a PEG coating are
37 taken up in a non-specific way, showing little binding to surface proteins, and have an increased
38 circulation time *in vivo*, thus can be used effectively as a comparison to glyco-nanoparticles.

39

40 A common method used for studying particle binding to surface proteins, and in particular lectins, is
41 UV-Vis turbidimetric analysis with a multivalent lectin such as Concanavalin A (Con A).[11] Typically
42 in these studies a simple absorbance reading is taken over time after mixing a particle with a lectin, an
43 increase in absorbance represents a corresponding binding between the two. Whilst this technique is
44 quick and easy to perform, the absorbance readings are affected by the light scattering effect of
45 nanoparticles and the dilution effect displayed when further solution is added to a reaction. Other
46 techniques to determine lectin-particle binding, including aggregate size analysis using DLS, have
47 widely been used in determining thermal stability of metal nanoparticles, but much less widely used for
48 studying polymer particle-lectin aggregation.[12-20] Online aggregate size analysis represents an
49 interesting way of tracking lectin-particle aggregation as it will not be adversely affected by particle
50 light scattering or by dilution. The aggregate size analysis is, however, limited to the limits of DLS,
51 where the aggregate must remain small enough for Brownian forces to dominate gravitational force,
52 preventing sedimentation, which for polymeric particles is generally considered to be 500nm.[11]
53 Consequently, to use DLS to track aggregation, polymeric particles must be synthesised with a narrow
54 size distribution well below 500 nm in diameter.

55

56 Traditional emulsion polymerisation provides a facile method to generate polymeric nanoparticles, with
57 narrow size distributions and is routinely used in industry for polymer synthesis at scale.[21] Typically

58 these polymer particles show poor biocompatibility, hence controlled radical polymerisation (CRP)
59 methods are now being utilised to generate functional latex particles[22] CRP methods are becoming
60 increasingly relevant in the synthesis of new bio-applicable materials[23], not only due to their ability to
61 control molar mass, but also the control over the architecture and end-group functionality. Translation of
62 CRP methods into emulsion polymerisation has yielded multiple approaches utilizing various
63 techniques.[24] One strategy utilises amphiphilic macromolecular reversible addition fragmentation
64 chain transfer (macro-RAFT) agents, which form polymeric micelles in aqueous solutions. These are
65 subsequently chain extended during an emulsion polymerisation, yielding polymeric nanoparticles,
66 decorated with the hydrophilic section of the initial macro-RAFT agent. Since its conception by
67 Hawkett and co-workers,[25] there have been relatively few reports of this technique to generate
68 nanoparticles for bio-applications, and have mostly focused on mechanistic studies or using this
69 approach to push the limit of polymer synthesis.[26-30] However, in 2010, Stenzel and co-workers
70 reported the synthesis of glucose functionalised polystyrene nanoparticles via an *ab initio* RAFT
71 emulsion polymerisation, from a glucose based amphiphilic macro-RAFT agent, and their subsequent
72 binding to Concanavalin A and E.coli.[31] Additionally, Ladmiral and co-workers described the
73 synthesis of galactose functionalised nano-objects using RAFT mediated polymerisation-induced self-
74 assembly, and showed intracellular delivery of rhodamine B octadecyl ester.[32] Our group recently
75 reported the synthesis of polyacrylamide stabilised polystyrene nanoparticles, synthesised using RAFT
76 emulsion polymerisation, and their subsequent loading and release of MicroRNA via a redox responsive
77 linker.[33] RAFT emulsion offers a facile, scalable process for the preparation of core-shell
78 nanoparticles, whilst also utilising the versatility of RAFT polymerisation for applications in a wide
79 range of areas.

80

81 Herein we describe the synthesis and characterisation of well-defined mannosylated and PEGylated
82 nanoparticles, with discrete size control. The synthesised particles are used to precisely probe the effect
83 of particle size on lectin binding, as well as comparing mannosylated nanoparticles to PEGylated
84 particles, using the commonly used UV-Vis turbidimetric analysis, and an analytical approach based on
85 an online DLS aggregation tracking system, which use is reported here for the first time.

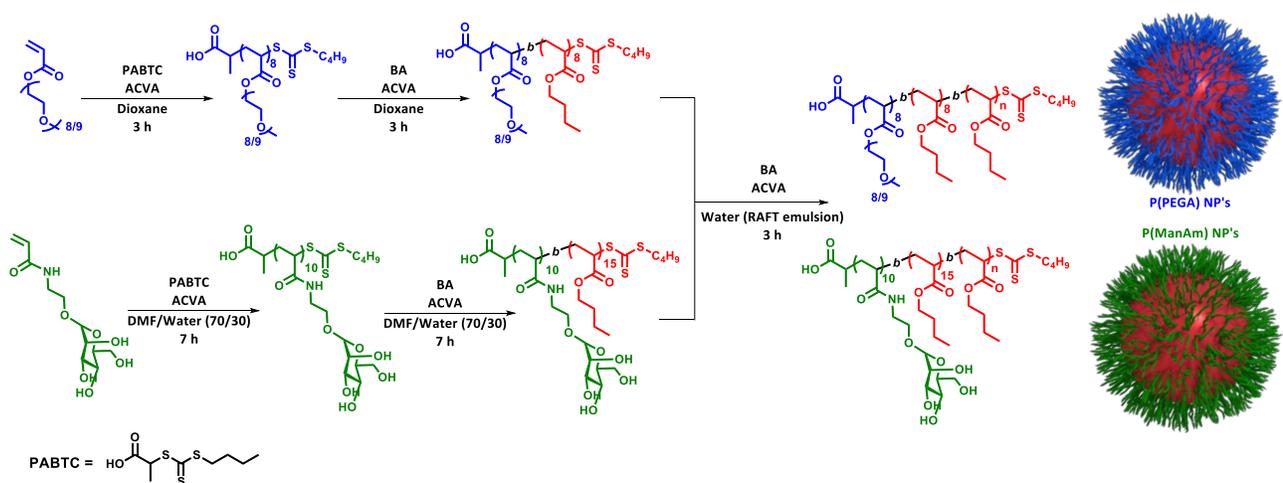
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87 **2. Results and Discussion**

88

89 *Mannose Acrylamide monomer synthesis*

90 Initially, a mannose containing monomer was synthesised using a modified method reported by
 91 Cameron and co-workers.[34] This approach utilised boron trifluoride diethyl etherate as an activating
 92 agent to induce neighbouring group participation and subsequent nucleophilic substitution of a α -D-
 93 Mannose pentaacetate at the anomeric carbon with a hydroxyethyl acrylamide.[35, 36] Advantageously
 94 this method results in a preference for the required biologically active α -anomer, thus following
 95 deprotection and subsequent column chromatography, yielded mannose acrylamide (ManAm) in c.60%
 96 yield with high α -stereospecificity. The monomer was stored with protection from light at -20°C in a
 97 freezer, preventing autopolymerisation.



98
99

100 **Figure 1** Stepwise synthetic scheme of P(PEGA) and P(ManAm) macro-RAFT agents and subsequent particle synthesis *via*
 101 RAFT emulsion polymerisation.

102

103 *Macro-RAFT agent synthesis*

104

105 Previous literature regarding RAFT emulsion polymerisation indicates that short chain oligomers act as
 106 sufficient stabilisers for the formation of particle.[37] Hence, both: P(ManAm)-*b*-P(BA) and P(PEGA)-
 107 *b*-P(BA) were synthesised with butyl acrylate blocks of less than 20 monomer units,.

108

109 Polymerisation of the ManAm block was conducted in a DMF/Water mixture (70/30 v/v) at 70°C for 7
 110 h mediated by chain transfer agent PABTC using thermal initiator ACVA as a radical source with >99%
 111 monomer conversion confirmed by ^1H NMR spectroscopy. PABTC has previously been shown to be an
 112 excellent RAFT agent for both acrylate[38] and acrylamide monomers.[39] In addition to this, the
 113 negatively charged carboxylic acid moiety on the R group may induce electrostatic stabilisation of the

114 resulting nanoparticles, enhancing the steric stabilisation provided by the hydrophilic polymer, thus
115 increasing the colloidal stability of any final latex particle. After the first block had reached complete
116 monomer conversion, only 43% of the initiator had been consumed,[40] therefore polymerisation of the
117 hydrophobic block was performed without purification. The required amount of *n*-butyl acrylate was
118 then injected into the above reaction mixture without additional initiator, and heated for a further 7 h
119 reaching 97% monomer conversion. Following ¹H NMR analysis, it was deduced that overall structure
120 of the mannose di-block copolymer was P(ManAm)₁₀-*b*-P(BA)₁₅ by comparing ¹H NMR signals for side
121 chain protons and protons on the RAFT end group (Supporting information Figure S2). DMF SEC
122 analysis showed a monomodal chromatogram of $M_n = 4600 \text{ g mol}^{-1}$ and $D = 1.13$ (Supporting
123 information Figure S3). Due to the amphiphilic nature of the macro-RAFT agent, and subsequent
124 polymers, solubility in common SEC solvents was low, producing a poor baseline due to low intensity
125 signal. However data that was collected is shown for completeness.

126

127 Polymerisation of PEGA was conducted at 70°C mediated by PABTC in 1,4-dioxane with ACVA as a
128 thermal initiator. In order to maintain a high livingness, the polymerisation was stopped after 3 h
129 resulting in 21% initiator and 91% monomer consumption. The residual monomer was removed with
130 precipitation in a mixture of hexane and diethyl ether prior to polymerisation of the next block. For the
131 hydrophobic section the P(PEGA) macro-RAFT was chain extended with of *n*-BA over 3 h at 70°C
132 reaching 96% monomer conversion. Both blocks had monomodal symmetrical SEC chromatograms and
133 narrow dispersity ($D = 1.13$) with a clear shift to higher molar mass upon chain extension. ¹H NMR
134 analysis indicated that the resulting block copolymer had the structure P(PEGA)₈-*b*-P(BA)₈ which was
135 in good agreement with experimental molar mass determination with SEC (Supporting information
136 Figure S6). For a general overview of the synthesis see Figure 1.

137

138 To confirm that both ManAm and P(PEGA) macro-RAFT agents would be suitable stabilisers, their
139 self-assembly in aqueous solution was investigated. DLS measurements were performed at 15 mg mL⁻¹
140 and, as such, displayed mean diameters of 10 and 7 nm for ManAm and P(PEGA) block co-polymers,
141 respectively. Micelles formed for both block copolymers had low PDI values of 0.06 suggesting that
142 both types of micelle were uniform, likely due to the stability received from the negative ζ -potential
143 caused by the deprotonated carboxylic acid from the R group of the macro-RAFT agents (see Table 1
144 for characterisation).

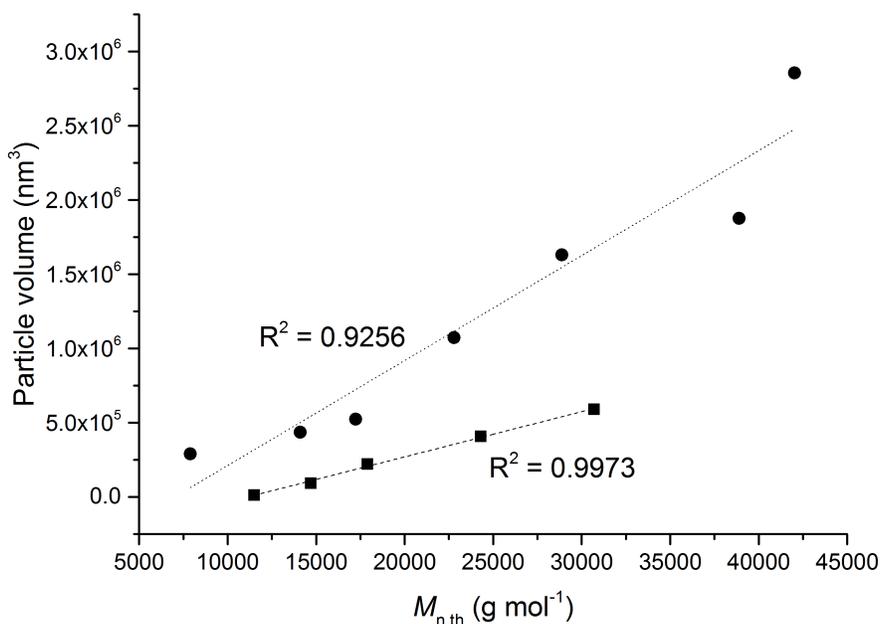
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146 ***Nanoparticle Synthesis***

147 The conditions used for the RAFT emulsion polymerisation of *n*-BA mediated by P(ManAm)₈-P(BA)₁₅
148 and P(PEGA)₈-P(BA)₈, were adapted from literature conditions (see Figure 1).[33] Oxygen was
149 removed from the polymerisation mixture by purging with N₂ gas in a vial sealed with a septum, the
150 monomer was degassed in a separate vial, and transferred into the micelle solution using a gas-tight
151 syringe, to avoid monomer evaporation and improve the reproducibility of the polymerisation. Multiple
152 emulsion polymerisations were performed, each with modification of [M]/[CTA] resulting in
153 monodisperse latex particles (PDI <0.1) with diameters ranging from 82 to 176 nm for nanoparticles with
154 P(ManAm), and 29 to 119 nm for nanoparticles P(PEGA) shells (Table 1). ¹H NMR samples were
155 prepared by diluting 100 μL of latex in either *d*₆-acetone or an 80/20 (v:v) mixture of *d*₆-DMSO for
156 P(PEGA) nanoparticles and P(ManAm) nanoparticles respectively. Interestingly, for polymerisations
157 with identical DP_{target} for both P(PEGA) and P(ManAm) RAFT emulsion polymerisations, P(ManAm)
158 particles had a significantly larger diameter compared to their respective P(PEGA) particles (Table 1).
159 For example when a chain extension of 100 monomer units was targeted it was observed that P(PEGA)
160 particles had mean diameters of 50 nm whereas P(ManAm) particles were 90 nm. This finding has been
161 attributed to a decreased stabilization of the P(ManAm) macro-RAFT agent during the RAFT emulsion
162 polymerisation since the P(PEGA) macro-RAFT agent consists of a highly hydrophilic polymer brush
163 block, compared to a linear polymer block for the P(ManAm) macro-RAFT agent. Similar to the block
164 copolymer micelles, the resulting nanoparticles had negative ζ-potential due to the carboxylic acid end
165 groups. Values ranged from -20 mV to -47 mV for P(PEGA) nanoparticles depending on size, however
166 P(ManAm) nanoparticles had ζ-potential consistently close to -32 mV. To measure the molecular
167 weight distribution of a single polymer chain, the nanoparticles were disassembled either by drying and
168 subsequent dissolution in chloroform or THF, or simply adding an excess of THF or DMF, for
169 P(PEGA) and P(ManAm) nanoparticles respectively. SEC chromatograms of the polymeric unimer for
170 P(PEGA) nanoparticles show three populations: firstly a low molecular weight distribution
171 corresponding to unconsumed macro-RAFT agent, also observed by Rieger and co-workers for RAFT
172 emulsion polymerisation of *n*-BA[41]; second, a population indicative of successful chain extension in
173 the emulsion polymerisation which shows good agreement with *M*_{n,th}; and third a high molecular weight
174 shoulder due to termination and/or mid-chain branching typical for acrylate polymerisation. If the
175 population relating to unconsumed macro-RAFT agent is ignored the *D* values remain below 1.4 for all
176 particles. It is expected that this unconsumed macro-RAFT agent is associated at the particle water
177 interface, as other size distributions relating to macro-RAFT agent micelles in DLS measurements were
178 not observed. Additionally, a near linear trend between the theoretical molecular weight of the single
179 polymer chains, and the resulting particle volume for both PEG and mannose shielded latex particles is

180 seen (Figure 2). This correlation, which can predict the resulting nanoparticle size based on the
 181 conditions of the polymerisation allows to increase reproducibility for the synthesis of nanoparticles,
 182 which is beneficial for biological applications.

183



184

185 **Figure 2.** Linear trend between $M_{n,th}$ of a single polymer arm, and the nanoparticle volume. ManAm nanoparticles (circles),

186 PEG nanoparticles (squares)

187

	$[M]_0/[CTA]_0$	Average Particle Diameter (nm) ^a	PDI ^b	ζ -Potential (mV)	Conversion (%)	$M_{n,SEC}$	D^f
P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅	N/A	11	0.060	-20.	97	4600 ^c	1.13
A P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅ - <i>b</i> -P(BA) ₂₅	25	82	0.084	-36	99	16000 ^c	1.22
B P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅ - <i>b</i> -P(BA) ₇₅	75	94	0.085	-34	98	11000 ^c	1.33
C P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅ - <i>b</i> -P(BA) ₁₀₀	100	100	0.088	-33	98	13000 ^c	1.19
D P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅ - <i>b</i> -P(BA) ₁₅₄	154	127	0.082	-35	93	22000 ^c	1.55
E P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅ - <i>b</i> -P(BA) ₂₀₀	200	146	0.13	-32	95	49000 ^c	1.63
F P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅ - <i>b</i> -P(BA) ₃₀₀	300	153	0.073	-33	90	41000 ^c	1.54
G P(ManAm) ₁₀ - <i>b</i> -P(BA) ₁₅ - <i>b</i> -P(BA) ₄₀₀	400	176	0.10	-33	75	61000 ^c	1.99
P(PEGA) ₈ - <i>b</i> -P(BA) ₈	N/A	7	0.060	-10	96	6250 ^d	1.13
P(PEGA) ₈ - <i>b</i> -P(BA) ₈ - <i>b</i> -P(BA) ₅₀	50	29	0.064	-20	>99	11500 ^e	1.22
P(PEGA) ₈ - <i>b</i> -P(BA) ₈ - <i>b</i> -P(BA) ₇₅	75	50	0.078	-37	>99	14200 ^e	1.35
P(PEGA) ₈ - <i>b</i> -P(BA) ₈ - <i>b</i> -P(BA) ₁₀₀	100	75	0.058	-36	>99	18400 ^e	1.39
P(PEGA) ₈ - <i>b</i> -P(BA) ₈ - <i>b</i> -P(BA) ₁₅₀	150	93	0.060	-46	>99	22500 ^e	1.60
H P(PEGA) ₈ - <i>b</i> -P(BA) ₈ - <i>b</i> -P(BA) ₂₀₀	200	130	0.050	-46	>99	25700 ^e	1.86

188 **Table 1.** Characterisation of nanoparticles and their individual polymer arms synthesised with RAFT emulsion
189 polymerisation. ^aDetermined by DLS (number distribution), ^bPDI values calculated using equation S1 (see supplementary
190 information), ^cDetermined by DMF-SEC analysis with PMMA standards, ^dDetermined by THF-SEC analysis with PMMA
191 standards, ^eDetermined by CHCl₃-SEC analysis with PMMA standards. ^fDispersity values are for all populations in
192 chromatogram, i.e not omitting any unconsumed macro-RAFT agent.

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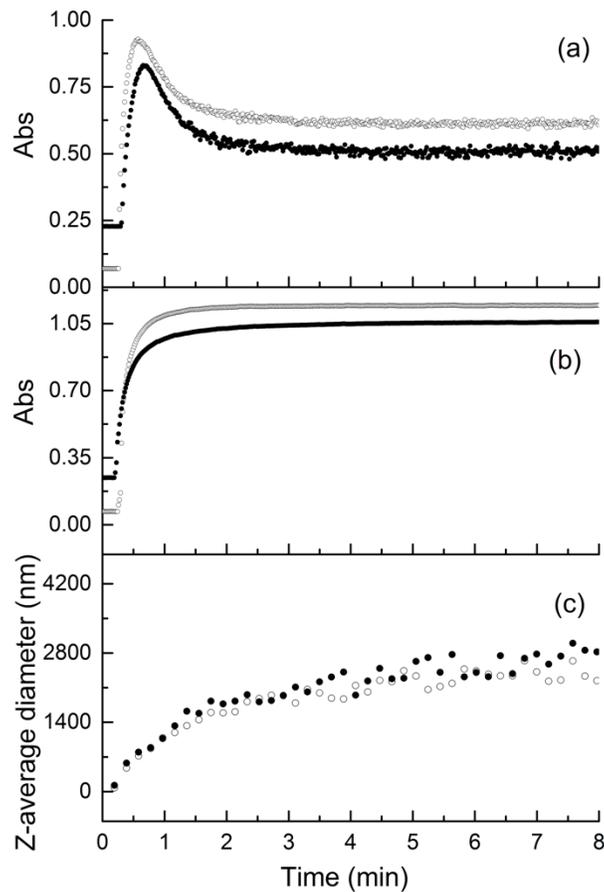
194 *Aggregation Studies*

195 Having synthesised a range of well-defined nanoparticles with PEG and mannose shells, these could be
196 used in lectin binding aggregation studies. Investigations into the lectin binding of glyco-nanoparticles
197 typically heavily relies on the use of UV-Vis turbidimetry, which allows particle aggregation to be
198 tracked in real time in a straight forward manner.[11, 42] Turbidimetry is, however, affected by light
199 scattering of nanoparticles, the dilution effect upon solution addition, and provides limited information
200 regarding the particle binding and aggregation mechanism. By tracking aggregation online with DLS,
201 these issues can be overcome, and more information regarding the composition of aggregates and the
202 mechanism by which they form may be obtained. Similar techniques have previously been used, more
203 commonly to measure aggregation of metal nanoparticles (e.g. iron), with only a few examples for
204 measuring polymer particle-lectin aggregation.[12, 14, 16, 18, 19] In order to evaluate the potential of
205 DLS as a technique to study the lectin binding of nanoparticles, both DLS and turbidimetry were used
206 and compared.

207 *Method Optimisation*

208 Before attempting an in-depth study it was necessary to optimize conditions for UV-Vis aggregation
209 experiments such that they could be transferred to DLS measurements without modification. In typical
210 UV-Vis lectin binding turbidimetric experiments, the particle solution is added to the lectin solution.
211 However, given the high viscosity of certain particle solutions that lead to blockage of the cannula, a
212 more reliable approach was to inject the Con A solution into the particle solution. DLS measurements
213 must also be conducted without stirring or agitation due to the inherent Brownian motion of the particles
214 to calculate particle size. In order to assess if these prerequisites affect measurements, effect of stirring
215 and the order of addition (Con A to particles, or particles to Con A) on the aggregation was investigated,
216 using 82 nm P(ManAm) particles as models (Figure 3).

217



218

219 **Figure 3.** Effect of stirring and order of addition. (a) stirring UV-Vis (b) not stirring UV-Vis (c) not stirring DLS. Particle
 220 solution into Con A solution (hollow circles), Con A solution into particle solution (filled circles)

221

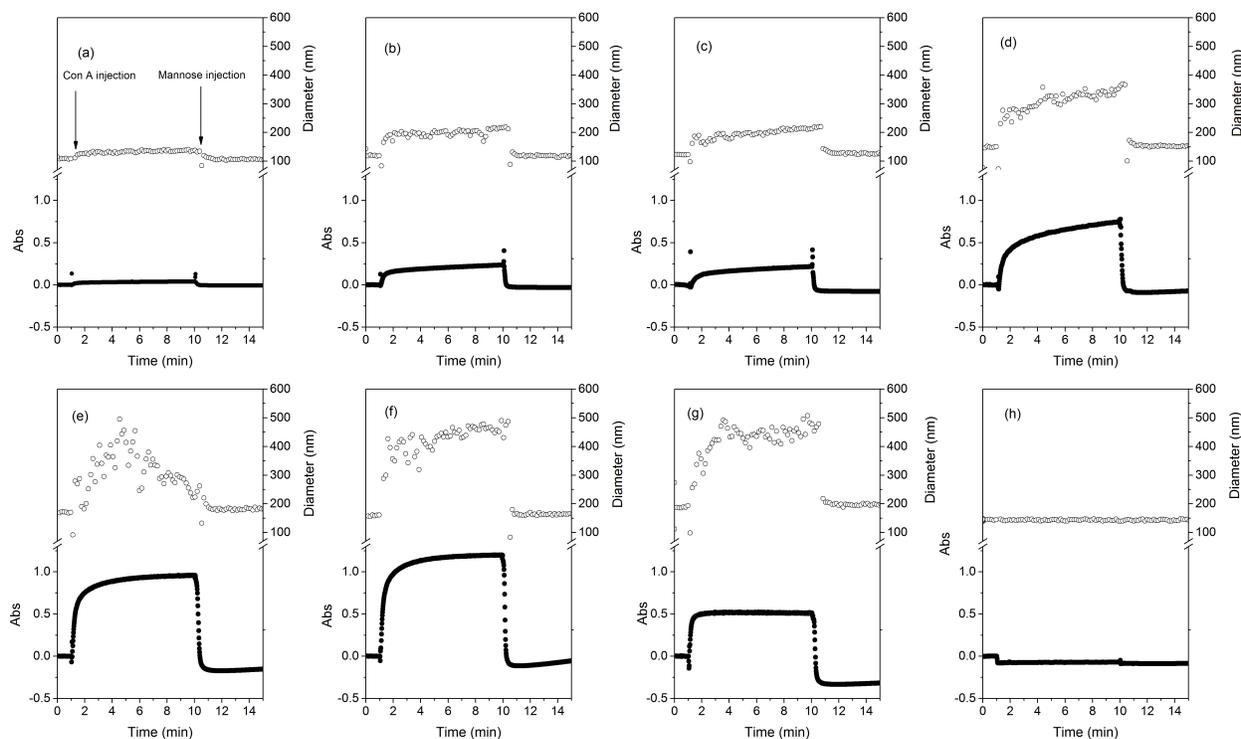
222 The order of addition showed little effect on final particle size in the DLS measurements and a
 223 negligible effect on final turbidity or trace shape in the UV-Vis results. There is a small increase in
 224 binding time when particles are added to Con A, which may be attributed to a slower diffusion of
 225 particles through Con A solution than Con A through particle solution (Figure 3(c)). The effect of
 226 stirring is, however, far more pronounced, seen in the UV-Vis traces (Figure 3(a)). For both sets of
 227 experiments (with and without stirring) an initial sharp increase in absorbance was detected, related to
 228 the aggregation between particles and Con A. In the absence of stirring, the absorbance plateaus to a
 229 value between 0.8-1 (Figure 3(b)), however, the solutions, which were being stirred, showed a
 230 subsequent decrease in absorbance between 20-100 s, before the absorbance plateaus to a much lower
 231 final value of 0.6. A potential explanation for this phenomenon is that stirring increases particle
 232 movement and, hence, collision between particles and aggregates, increasing the chance of successful
 233 binding interactions between them. This higher rate of successful collisions causes the formation larger
 234 aggregates more rapidly than in solutions without stirring. These aggregates become large enough to
 235 sediment out of solution, only being kept in suspension by stirring and, ultimately, giving a lower

236 absorbance value. Further evidence supporting this hypothesis was observed optically, as mixtures,
237 which had been stirred were observed to completely sediment within minutes, whereas not stirred
238 solutions were stable up to 24 h. If the not stirred solutions were subsequently stirred, sedimentation
239 occurred within minutes. Based on these results further experiment were performed by the addition of
240 Con A into particle solution without stirring, allowing the use of DLS in tracking particle aggregation.

241

242 *UV-Vis studies*

243



244

245

246 **Figure 4.** Nanoparticle-lectin aggregation with both turbidimetric (filled circles) and DLS (hollow circles) for P(ManAm)
247 particles (a) 82 nm (b) 94 nm (c) 100 nm (d) 127 nm (e) 146 nm (f) 153 nm (g) 176 nm, and P(PEGA) particle (h)
248 130 nm. Arrows indicate time of injection for Con A and Mannose solutions.

249

250 After optimising test conditions, turbidimetric studies were conducted using UV-Vis spectroscopy,
251 using PEG and ManAm coated particles across a variety of sizes. To conduct these measurements, a
252 cuvette was placed in the machine loaded with the requisite nanoparticle solution and an absorbance
253 reading was taken every second at 500 nm. After 60 s the Con A solution was added and immediately
254 after the addition a sharp increase in absorbance was observed in all of the particles coated in ManAm,
255 followed by a plateau in absorbance after a further 2 min. No response was detected for measurements
256 using particles with a PEGA shell, confirming that the aggregation shown is due to lectin sugar

257 interaction between Con A and Mannose residues on the ManAm coated particles. After 10 min, an
258 excess of α -D-mannose was injected, causing a sharp drop in absorbance to below the original baseline
259 for all of the ManAm particles. This shows a reversible, non-covalent binding mechanism between Con
260 A and the mannose residues. The only response seen for PEG particles was a reduction in absorbance
261 due to the dilution cause by mannose addition, reducing the overall concentration of particles (Figure 4).
262 The results indicate that with increasing ManAm particle size a corresponding increase in the maximum
263 absorbance observed. The influence of the aggregate composition can, however, not be inferred from
264 this data , as the relationship between size and light scattering (Rayleigh scattering relationship) is non-
265 linear.[43] To investigate the aggregation and the formed aggregates further, a different technique is
266 required. Online DLS measurements present a suitable way of obtaining this information.

267

268 *DLS Studies*

269 DLS measurements were performed by mixing Con A and particle solutions within the DLS
270 machine after readings had commenced via a cannula injection system allowing the solutions to be
271 combined without opening the sample chamber. Measurements were taken every 11.6 seconds and
272 addition of Con A to particle solutions occurred after the 6th measurement into the experiment (69.9 s)
273 in all cases. During the experiments at the point of injection, an artificially low diameter is recorded as
274 the mixing causes the particles to move faster in solution than they would based solely on Brownian
275 motion.[44] The experiments were performed at the same concentration and are shown as Z-average
276 diameter over time in Figure 4, (diameter by intensity is shown in supporting information, Figure S11).
277 Particle size distribution by number was used to characterise initial particle size to minimise the
278 influence of any aggregation present, giving the most accurate representation of particle diameter.
279 Conversely, to determine the most accurate final aggregate size, the influence of free particles on the
280 measurement needed to be minimised, for this reason size distribution by intensity was used here.

281

282 All ManAm coated particles showed an initial increase in Z-average diameter upon the addition of Con
283 A due to aggregation, which then plateaus. Again no response is observed for PEG particles, confirming
284 that the mannose residues are solely interacting with Con A, while also confirming that the presence of
285 Con A does not cause any major discrepancy to size measurements using DLS. Similar to UV-Vis
286 experiments a solution of α -D-mannose was injected, leading to a sharp decrease in Z-average diameter
287 corresponding to the original particle diameter detected at the start of the experiment. No change in PEG
288 particle Z-Average diameter is seen on the addition of either Con A or α -D-mannose solutions, further
289 confirming that no dilution effect needs to be taken into account, when using DLS to measure

290 aggregation. The results presented to this point are broadly in agreement with the absorbance results
 291 obtained for UV-Vis. However, using the data collected from the online DLS aggregation experiments it
 292 is possible to obtain further information compared to UV-Vis. Firstly, after the Z-average diameter
 293 increased and plateaued, a drift to larger diameter was observed for smaller particles (Figure 4). This
 294 slow increase in apparent diameter is attributed to aggregation occurring in two distinct phases: Initially
 295 a fast aggregation with a high concentration of free Con A and particles forming initial aggregates,
 296 followed by secondary agglomeration between formed aggregates, slowly interacting with each other
 297 (and any free particles and Con A in solution), to slowly grow in size. Due to the nature of Rayleigh
 298 light scattering in turbidimetric measurements, the initial phase of aggregation shows as a very large
 299 increase in absorbance. This further growth in already formed aggregates will only produce a
 300 comparably small change in absorbance, making it difficult to determine. This relationship was
 301 observed when the DLS and UV-Vis data is plotted together, with Z-average diameter on a \log_{10} scale
 302 and absorbance on a linear scale (Supporting information Figure S10). In this plot the two traces
 303 overlap, suggesting that UV-Vis data alone, provides artificially short aggregation time. and that any
 304 small aggregate growth after the initial increase would be difficult to determine.

305

Initial Particle Diameter (nm) ^a	Initial Particle Volume (nm ³)	Aggregate Diameter by Intensity (nm) ^a	Aggregate Vol (nm ³) ^b	N _{agg}	N _{agg,th}
82	290,000	138	1,400,000	1.52	5.52
94	430,000	206	4,600,000	1.97	4.81
100	520,000	214	5,100,000	1.9	4.52
127	1,100,000	390	31,000,000	2.81	3.56
146	1,600,000	496	64,000,000	3.07	3.1
153	1,900,000	589	107,000,000	3.55	2.96
176	2,900,000	591	108,000,000	3.03	2.57

306 **Table 2.** Analysis of final aggregate diameters compared to initial particle diameter. ^aMeasured by DLS, ^bdetermined using
 307 aggregate diameter by intensity and formula for the volume of a sphere.

308

309 By using DLS, an estimation of aggregate volume can be made by using the final aggregate radius in the
 310 formula for the volume of a sphere. The cubed root of the aggregate volume, divided by the initial
 311 volume of the particles forming the aggregate, and multiplied by the ideal packing number of spheres
 312 (74%), gives an estimate of the aggregation number as particles per aggregate (ppa) formed.[45] This
 313 information can further be used by comparing it to the theoretical maximum number of aggregation at a
 314 diameter of 500 nm. This diameter marks the particle size limit for Brownian motion to overcome
 315 gravity and as such, the point at which sedimentation will occur, these results are shown in Table 2.[11]

316 The values for aggregate diameter were obtained by taking an average diameter (by intensity) after the
317 initial phase of aggregation had finished. A clear increase in aggregate size can be seen as the initial
318 particle diameter becomes larger, this of course, could simply be due to the aggregates being composed
319 of larger particles. However, by determining the number of particles needed to compose each aggregate,
320 an increase in aggregation number can be seen from 1.25 ppa for 82 nm diameter particles, to 2.85 ppa
321 for particles 153 nm in diameter. Data for the largest two particles (153 and 176 nm) is, however,
322 unreliable due to high dispersity and large aggregate size. Looking at the relationship between number
323 of aggregation for each particle and the theoretical maximum number of aggregation, it can be seen that
324 as the initial particle diameter increases, the observed number of aggregation approaches the theoretical
325 maximum, until it is exceeded by the two largest particles. This further confirms the hypothesis that the
326 DLS data for particles of diameter 153 and 176 nm is unreliable, and that an aggregate size of 500 nm
327 represents an upper size limit for DLS to determine.

328

329 It is hypothesised that the increasing number of aggregation observed as particle diameter increases is
330 related to the increased surface area of the initial particle. Larger particles will have more mannose
331 residues presented on their surface and thus be able to interact with more Con A. In having more Con A
332 associated to the surface of a particle, it is statistically more likely to have a successful binding
333 interaction upon collision with another mannose decorated particle. Furthermore, the contact angle
334 between two particles interfaces decreases with increasing particle size leading to an increased area of
335 interaction with Con A and thus a corresponding increase in possible number of aggregation.

336

337 The data presented here shows that by using online DLS measurements particle-lectin binding can
338 provide data equivalent to that produced with a UV-Vis turbidity technique. Whilst turbidimetric
339 measurements are useful as a qualitative measure of aggregation, a definite time for binding cannot be
340 determined. Turbidimetry is also greatly affected by the light scattering ability of particles, and the
341 dilution effect observed upon solution injection. An online DLS measurement however provides the
342 same information but gives a more clear determination of how aggregation is occurring throughout the
343 reaction. In contrast to turbidimetry light scattering of particles has no adverse effects on the
344 measurement. By producing a final aggregate size, a number of aggregation per particle can also be
345 estimated, which represents a robust way of measuring the effect of particle size on aggregation.
346 However, by using DLS an upper size limit of 500 nm for particles and aggregates is introduced, past
347 which data becomes unreliable.

348

349

350 **3. Conclusions**

351 In conclusion, the synthesis of short amphiphilic di-block copolymers via RAFT polymerisation has
352 been demonstrated. These macro chain-transfer agents were used to produce a wide range of well-
353 defined polymer particles utilizing RAFT emulsion polymerisation. Particles were stabilised in solution
354 by a shell of P(PEGA) or P(ManAm), respectively, depending on the di-block copolymer used.

355

356 Using these particles, lectin binding studies using turbidimetric and online-DLS measurements in the
357 presence of Con A were performed. Increasing particle size has shown to improve lectin binding using
358 both methods. DLS offers a robust, quick and easy technique for particle-lectin aggregation studies and
359 avoids issues of changes in absorbance caused by light scattering as well as dilution factors.
360 Furthermore, the technique enables detailed insight into aggregate formation and composition, valid up
361 to an aggregate diameter of 500 nm. Future studies will focus on the interaction of varied glyco-particles
362 with more bio-applicable lectins.

363

364 **4. Acknowledgement**

365 The Royal Society Wolfson Merit Award (WM130055; SP) and the Monash-Warwick Alliance (PG;
366 AML; SP) are acknowledged for financial support.

367

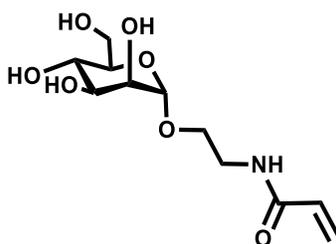
368 **Experimental**

369 **Materials and methods**

370 Materials and methods can be found in the supplementary information section.

371 **Synthesis**

372 *Mannose Acrylamide*[34]



373

374 7.5 g (0.0192 mol, 1.13 eq) of α -D-mannose pentaacetate and 2.01 g (0.017 moles, 1eq) of hydroxyl
375 ethyl acrylamide were dissolved in 77 mL of anhydrous DCM in a 250 mL round bottomed flask (RBF)
376 equipped with a magnetic stirrer and an appropriately sized rubber septum. The reaction mixture was

377 purged with nitrogen gas and 13.33 g of boron trifluoride diethyl etherate (0.044 mol, 11.6 mL) was
378 transferred using a gas-tight Hamilton syringe charged with nitrogen. The reaction mixture was
379 consequently subjected to four cycles of 10 min sonication and 5 min rest prior to stirring at ambient
380 temperature for 48 h. The progress of the reaction was monitored with thin layer chromatography (TLC)
381 using a 9:1 chloroform:methanol mixture (v/v), and stained with 5% sulfuric acid in ethanol. Once
382 complete the reaction mixture was then diluted with two parts DCM and washed thoroughly three times
383 with brine then water in an appropriately sized separating funnel. The organic phase was dried over
384 magnesium sulfate, filtered *via* vacuum filtration and the solvent removed under reduced pressure at a
385 temperature no higher than 30°C leaving an orange brown viscous liquid. This was dissolved in 40 mL
386 of potassium carbonate in methanol, purged with nitrogen gas and stirred at ambient temperature for 24
387 h. The pH was adjusted to pH 7 with a Dowex 50WX4 hydrogen form exchange resin and stirred until
388 the pH was fully adjusted. The Dowex resin was removed with vacuum filtration and solvent removed
389 under reduced pressure at a temperature no higher than 30°C. The crude product was purified *via*
390 column chromatography on an 80 g silica column and eluted with a 2:8 methanol: chloroform mixture at
391 a flow rate of 1 mL min⁻¹, on an auto-column equipped with a UV-Vis detector set to 308 nm. The
392 product was found to elute at around 15 min. Product fractions were combined, the solvent evaporated
393 to less than 10 mL under reduced pressure and subsequently freeze dried to yield the pure monomer as a
394 white powder.

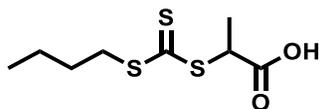
395 ¹H NMR (D₂O, 400 MHz) δ_H: 6.13 (dt, J = 31.0, 13.5 Hz, 2H, CH₂CH), 5.65 (d, J = 10.0 Hz, 1H,
396 CH₂CH), 4.74 (s, J = 16.2 Hz, 1H, CHO₂CH), 3.80 (s, J = 122.4 Hz, 1H, CH₂OH), 3.76 – 3.28 (m, 9H).
397 ¹³C NMR (D₂O, 400 MHz): δ 129.75 (s) (CHCH₂), 121.41 (m) (CH₂CH), 99.61 (s) (CH₂OH), 72.71 (s)
398 (CHO), 70.39 (s) (CHO), 69.92 (s) (CHO), 66.52 (s) (CHO), 65.69 (s) (CHO), 60.76 (m) (CH₂O),
399 37.77 (m) (CH₂NC).

400 MS *m/z* [M+Na]⁺: 300.1 (MS_{th}: 300.9)

401 IR (cm⁻¹): 3275 (b), 2928 (b), 1656 (n), 1624 (m), 1548 (b), 1409 (m), 1317 (w), 1249 (m), 1131 (m),
402 1089 (m), 1051 (s), 1023 (s).

403

404 **2-(((butylthio)carbonothioyl)thio)propanoic acid**[25]



405

406 A 50% w/w sodium hydroxide solution (9.68 g NaOH, 0.242 mol, 1.1 eq) in water was added to a
407 mixture of butanethiol (20 g, 0.22 mol, 1 eq) dissolved in acetone (11 mL). Water (40 mL) was added

408 and the solution was stirred for 30 min at room temperature. Carbon disulphide (17.32 g, 0.228 mol,
409 1.025 eq) was added and the orange solution was stirred for 30 minutes at room temperature, then
410 cooled in ice below 10°C. 2- Bromopropionic acid (34.9 g, 0.228 mol, 1.025 eq) was added slowly,
411 monitoring the temperature, and subsequently a further 19.36 g of 50% w/w sodium hydroxide solution
412 was added. The reaction mixture was left to stir for 18 h at ambient temperature. 200 mL of water was
413 added to the reaction mixture, cooled in ice, and a 10 M solution of HCl was added dropwise until the
414 pH reached between 2-3. The resulting precipitate was filtered, washed with water, and recrystallised in
415 hot hexane to afford 36.53 g of 2-(((butylthio)carbonothioyl)thio)propanoic acid. Yield = 70%.

416

417 ^1H NMR (400 MHz, CDCl_3) δ_{H} 6.06 (br, 1H, CO_2H), 4.86 (q, $J = 7.4$ Hz, 1H, SCH), 3.37 (t, $J = 7.4$ Hz,
418 2H, CH_2S), 1.69 (quint, $J = 7.5$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{S}$), 1.63 (d, $J = 7.4$ Hz, 3H, SCHCH_3), 1.43 (sext, $J =$
419 7.5 Hz, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2$), 0.94 (t, $J = 7.3$ Hz, 3H, CH_3CH_2).

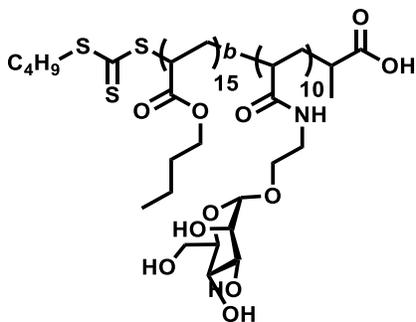
420 ^{13}C NMR (75 MHz, 298 K CDCl_3) δ_{C} 175.4 (COOH), 47.2 (SCH), 37.1(CH_2S), 29.88 ($\text{CH}_2\text{CH}_2\text{S}$),
421 22.1($\text{CH}_3\text{CH}_2\text{CH}_2$), 16.4 (CH_3CH), 13.6 ($\text{CH}_3\text{CH}_2\text{CH}_2$).

422 FTIR (cm^{-1}): 3093, 2958, 2929, 2871, 2362, 2340, 1454, 1412, 1285, 1230, 1200, 1089, 1059, 912, 861.

423 MS (ESI) m/z 237.0 [M-H], 238 [M-]

424

425 **Poly(Mannose Acrylamide)₁₀-poly-*n*-(butyl acrylate)₁₅ synthesis**



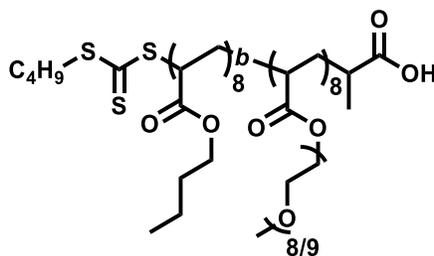
426

427 Mannose acrylamide (1g, 3.62 mmol), 2-((butylthio) carbonothioyl) thio)propanoic acid (PABTC)
428 (0.0864 g, 3.62×10^{-4} mol), and 4,4'-Azobis (4-cyanovaleric acid) (ACVA) (from a pre-made stock
429 solution of 10mg mL^{-1} in DMF:water (70:30) mix) (0.0508 g, 1.81×10^{-4} mol) were dissolved in a
430 mixture of DMF:water (70:30) to a total volume of 10.8 mL in a 25 mL round bottomed flask with a
431 magnetic stirrer bar. The flask was sealed with an appropriate rubber septum and purged of oxygen with
432 nitrogen gas for ten minutes before immersing it into a preheated oil bath at 70°C and stirred for seven
433 hours. Monomer conversion was determined by ^1H NMR spectroscopy in D_2O , by comparison of the
434 ratio of vinyl peak ($\delta=6.08$) and RAFT agent CH_3 z-group butyl chain end group peak ($\delta=0.78$). The
435 polymer was analysed by SEC with a DMF eluent at 30°C ($M_{\text{n SEC}}=2450$ g mol^{-1} $D=1.27$). To chain

436 extend the synthesised P(ManAm)₁₀ macro-RAFT agent, *n*-butyl acrylate was purged of oxygen with
437 nitrogen for ten minutes, and 1.3g (1.01x10⁻² mol, 1.45 mL) was injected into the 25mL round bottomed
438 flask using a dry Hamilton syringe, purged with nitrogen. The round bottomed flask was then immersed
439 in an oil bath set to 70°C and stirred for seven hours. Monomer conversion was determined by ¹H NMR
440 spectroscopy in *d*₆-DMSO, by comparison of the ratio of vinyl peak (δ=5.94) and RAFT agent CH₃ z-
441 group butyl chain end group peak (δ=0.83). The polymer was analysed by SEC with a DMF eluent at
442 30°C.

443
444
445
446

447 **P(PEGA)₈-*b*-P(BA)₈ Synthesis**



448

449 PABTC (0.31 g, 1.30 x 10⁻³ mol), PEGA (5 g, 10.4 x 10⁻³ mol) and ACVA (from a pre-made stock
450 solution in 1,4-dioxane) (18 mg, 6.51 x 10⁻⁵ mol) were dissolved in 4.9 mL 1,4-dioxane in a 25 mL
451 round bottom flask equipped with a magnetic stirrer bar. The solution was fitted with an appropriate
452 sized rubber septum, and purged with nitrogen for 20 minutes. The round bottom flask was
453 subsequently immersed in an oil bath preheated to 70°C and stirred for 3 h. The reaction vessel was
454 cooled to ambient temperature and opened to oxygen to quench further polymerisation. The pre-cursor
455 polymer was precipitated into a mixture of 20% hexane and 80% diethyl ether (v/v), collected by
456 dissolving in 10 mL of 1,4-dioxane, and the precipitation repeated once more. Finally, the precipitated
457 polymer was dissolved into DCM, transferred to a 20 mL vial, the DCM evaporated and dried in a
458 vacuum oven overnight at 40°C to yield P(PEGA)₈ as a yellow viscous liquid (4.5 g). For the second
459 stage of the polymerisation, *n*-butyl acrylate (0.9 g, 7.03 x 10⁻³ mol) and ACVA (from a pre-made stock
460 solution in 1,4-dioxane) (12.3 mg, 4.36 x 10⁻⁵ mol) were added to 3.58 g of P(PEGA)₈ dissolved in 5.92
461 mL of 1,4-dioxane in a 10 mL round bottom flask. The polymerisation mixture was purged with
462 nitrogen for 20 minutes and heated to 70°C for 3 h. The resulting polymer solution was cooled to room
463 temperature and subsequently purified by precipitation in ice-cold hexane. The yellow viscous liquid
464 was re-dissolved in dichloromethane and the precipitation was repeated once more. Finally, the solvent

465 was evaporated under reduced pressure to yield the di-block macro-RAFT agent as a yellow viscous
466 liquid (3.5 g).

467

468

469

470 ***General method for RAFT-mediated emulsion polymerisation***

471 Nanoparticles of different sizes were prepared by altering the ratio of di-block macro-RAFT agent to
472 monomer in an emulsion polymerisation. As an example P(ManAm)₁₀-*b*-P(BA)₁₅-*b*-P(BA)₄₀₀ was
473 prepared as follows. NaOH (14.3 mg, 3.6 x 10⁻⁴ mol) was added to a suspension of ACVA (50 mg, 1.8 x
474 10⁻⁴ mol) in water (10 mL) to ensure full solubility. P(ManAm)₁₀-*b*-P(BA)₁₅ (0.015 g, 3.13 x 10⁻⁶ mol)
475 was dissolved in 0.645 mL of water, in a 2 mL vial fitted with a cap incorporating a rubber septum and
476 equipped with an appropriate magnetic stirrer. 0.175 mL of the above ACVA stock solution was added,
477 and the solution was deoxygenated with nitrogen gas for 20 minutes. *n*-BA (0.160 g, 1.25 x 10⁻³ mol)
478 was separately deoxygenated in a vial for 10 minutes. The macro-RAFT agent solution was immersed in
479 a 70°C oil bath, the deoxygenated *n*-BA was injected immediately and the RAFT emulsion
480 polymerisation was stirred for 3 h at 70°C at 400 RPM. After approx. 10 min, the emulsion turned a
481 milky white as the polymerisation proceeded. P(PEGA) mediated RAFT emulsion polymerisations were
482 performed at ten fold higher scale in an identical manner.

483

484 ***General method for UV-Vis aggregation studies***

485 Turbidimetric studies were conducted by diluting 12.5 µL of undiluted particle solution with 1.3 mL of
486 10 mM phosphate buffer in a 4.5 mL polystyrene cuvette, and placed in the UV-Vis spectrometer. In a
487 separate 4.5 mL polystyrene cuvette a stock solution of 2.027x10⁻⁵ M Concanavalin A in 10 mM
488 phosphate buffer was prepared for use with P(ManAm) and poly (PEGA) particles. Absorbance
489 readings were taken every second at 500 nm, with 185.5 µL of Con A stock solution being added after
490 60 s at which point the lid of the spectrometer opened, 250 µL of Con A in phosphate buffer (2.027x10⁻⁵
491 M) was added with an Eppendorf pipette, mixed twice and to induce aggregation. After a further 9 min,
492 50 µL of mannose in phosphate buffer (375 mg mL⁻¹) was added with an Eppendorf pipette and mixed
493 twice to induce competitive binding with the glycosylated nanoparticles. The absorbance was monitored
494 for a further 10 min. Readings were taken using an Agilent Carey 60 UV-Vis machine with Agilent
495 software and analysed using Origin.

496

497 ***DLS Aggregation***

498 DLS measurements were taken using a Malvern instruments Zetasizer Nano at 25°C with a 4 mW He-
499 Ne 633 nm laser at a scattering angle of 173° (back scattering). For P(ManAm) particle DLS
500 aggregation studies, 12.5 µL of particle solution was diluted with 1.2375 mL of 10 mM phosphate
501 buffer to make a total of 1.25 mL in a 4.5 mL polystyrene cuvette. The cuvette was fitted with a size 23
502 septum, which was pierced with a cannula attached to a 250 µL Hamilton glass syringe. The cannula
503 was positioned such that, solution ejected through it would run down the side of the cuvette. This
504 prevented the creation of any air bubbles that may have interfered with measurements. The cuvette was
505 placed into the Zetasizer, and the lid closed with the syringe exiting through a slit at the side of the
506 instrument. In a separate 4.5 mL polystyrene cuvette a stock solution of 2.027×10^{-5} M Concanavalin A
507 in 10 mM phosphate buffer was prepared for use with P(ManAm) particles. The Zetasizer was set to
508 take a size reading every 10 s for 1 h, however a delay of 1.66 s was recorded between each reading,
509 adding 598 s to each hour, for which the results have been amended. After the sixth reading, 250 µL of
510 2.027×10^{-5} M Concanavalin A stock solution was injected via the cannula giving a final volume of 1.5
511 mL. The final concentration of Concanavalin A and side chain residue was 3.125×10^{-5} M and 2.608×10^{-4}
512 M respectively. The same technique was then repeated with the addition of 250 µL of 75 mg mL⁻¹
513 mannose in phosphate buffer being injected via the syringe cannula after 10 min (to allow full
514 aggregation).

515

516 **References**

- 517 [1] M. Köhn, J.M. Benito, C. Ortiz Mellet, T.K. Lindhorst, J.M. García Fernández, Functional
518 Evaluation of Carbohydrate-Centred Glycoclusters by Enzyme-Linked Lectin Assay: Ligands for
519 Concanavalin A, *ChemBioChem* 5(6) (2004) 771-777.
- 520 [2] Y. Bourne, H. van Tilbeurgh, C. Cambillau, Protein-carbohydrate interactions, *Curr. Opin. Struct.*
521 *Biol.* 3(5) (1993) 681-686.
- 522 [3] S.-J. Richards, L. Otten, M.I. Gibson, Glycosylated gold nanoparticle libraries for label-free
523 multiplexed lectin biosensing, *Journal of Materials Chemistry B* (2016).
- 524 [4] G. Yilmaz, C.R. Becer, Precision glycopolymers and their interactions with lectins, *European*
525 *Polymer Journal* 49(10) (2013) 3046-3051.
- 526 [5] L.L. Kiessling, J.E. Gestwicki, L.E. Strong, Synthetic multivalent ligands as probes of signal
527 transduction, *Angewandte Chemie International Edition* 45(15) (2006) 2348-2368.
- 528 [6] G. Yilmaz, C.R. Becer, Glyconanoparticles and their interactions with lectins, *Polymer Chemistry*
529 6(31) (2015) 5503-5514.

- 530 [7] L. Otten, E. Fullam, M.I. Gibson, Discrimination between bacterial species by ratiometric analysis
531 of their carbohydrate binding profile, *Mol. BioSyst.* 12(2) (2016) 341-344.
- 532 [8] C. Bies, C.-M. Lehr, J.F. Woodley, Lectin-mediated drug targeting: history and applications,
533 *Advanced Drug Delivery Reviews* 56(4) (2004) 425-435.
- 534 [9] K. Knop, R. Hoogenboom, D. Fischer, U.S. Schubert, Poly (ethylene glycol) in drug delivery: pros
535 and cons as well as potential alternatives, *Angewandte Chemie International Edition* 49(36) (2010)
536 6288-6308.
- 537 [10] P.d. Pino, B. Pelaz, Q. Zhang, P. Maffre, G.U. Nienhaus, W.J. Parak, Protein corona formation
538 around nanoparticles - from the past to the future, *Materials Horizons* 1(3) (2014) 301-313.
- 539 [11] E. Tomaszewska, K. Soliwoda, K. Kadziola, B. Tkacz-Szczesna, G. Celichowski, M. Cichomski,
540 W. Szmaja, J. Grobelny, Detection limits of DLS and UV-Vis spectroscopy in characterization of
541 polydisperse nanoparticles colloids, *Journal of Nanomaterials* 2013 (2013) 60.
- 542 [12] A.A. Keller, H. Wang, D. Zhou, H.S. Lenihan, G. Cherr, B.J. Cardinale, R. Miller, Z. Ji, Stability
543 and aggregation of metal oxide nanoparticles in natural aqueous matrices, *Environ. Sci. Technol.* 44(6)
544 (2010) 1962-1967.
- 545 [13] Y. Li, V. Lubchenko, P.G. Vekilov, The use of dynamic light scattering and Brownian microscopy
546 to characterize protein aggregation, *Rev. Sci. Instrum.* 82(5) (2011) 053106.
- 547 [14] T. Phenrat, N. Saleh, K. Sirk, R.D. Tilton, G.V. Lowry, Aggregation and sedimentation of aqueous
548 nanoscale zerovalent iron dispersions, *Environ. Sci. Technol.* 41(1) (2007) 284-290.
- 549 [15] J. Rubin, A. San Miguel, A.S. Bommarius, S.H. Behrens, Correlating Aggregation Kinetics and
550 Stationary Diffusion in Protein– Sodium Salt Systems Observed with Dynamic Light Scattering, *The*
551 *Journal of Physical Chemistry B* 114(12) (2010) 4383-4387.
- 552 [16] G. Trefalt, I. Szilagyi, M. Borkovec, Measuring particle aggregation rates by light scattering, 2013.
- 553 [17] G. Sánchez-Pomales, T.A. Morris, J.B. Falabella, M.J. Tarlov, R.A. Zangmeister, A lectin-based
554 gold nanoparticle assay for probing glycosylation of glycoproteins, *Biotechnol. Bioeng.* 109(9) (2012)
555 2240-2249.
- 556 [18] X. Wang, E. Matei, A.M. Gronenborn, O. Ramström, M. Yan, Direct measurement of
557 glyconanoparticles and lectin interactions by isothermal titration calorimetry, *Anal. Chem.* 84(10)
558 (2012) 4248-4252.
- 559 [19] X. Wang, O. Ramström, M. Yan, Dynamic light scattering as an efficient tool to study
560 glyconanoparticle–lectin interactions, *Analyst* 136(20) (2011) 4174-4178.
- 561 [20] X. Wang, O. Ramström, M. Yan, Quantitative analysis of multivalent ligand presentation on gold
562 glyconanoparticles and the impact on lectin binding, *Anal. Chem.* 82(21) (2010) 9082-9089.

- 563 [21] T. Tadros, Emulsion Polymerization, in: T. Tadros (Ed.), Encyclopedia of Colloid and Interface
564 Science, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013, pp. 414-414.
- 565 [22] M. Manabe, N. Tatarazako, M. Kinoshita, Uptake, excretion and toxicity of nano-sized latex
566 particles on medaka (*Oryzias latipes*) embryos and larvae, *Aquat. Toxicol.* 105(3–4) (2011) 576-581.
- 567 [23] C. Boyer, V. Bulmus, T.P. Davis, V. Ladmiral, J. Liu, S. Perrier, Bioapplications of RAFT
568 Polymerization, *Chemical Reviews* 109(11) (2009) 5402-5436.
- 569 [24] P.B. Zetterlund, S.C. Thickett, S. Perrier, E. Bourgeat-Lami, M. Lansalot, Controlled/Living
570 Radical Polymerization in Dispersed Systems: An Update, *Chemical Reviews* 115(18) (2015) 9745-
571 9800.
- 572 [25] C.J. Ferguson, R.J. Hughes, D. Nguyen, B.T. Pham, R.G. Gilbert, A.K. Serelis, C.H. Such, B.S.
573 Hawkett, Ab Initio Emulsion Polymerization by RAFT-Controlled Self-Assembly §, *Macromolecules*
574 38(6) (2005) 2191-2204.
- 575 [26] F. Stoffelbach, L. Tibiletti, J. Rieger, B. Charleux, Surfactant-free, controlled/living radical
576 emulsion polymerization in batch conditions using a low molar mass, surface-active reversible addition-
577 fragmentation chain-transfer (RAFT) agent, *Macromolecules* 41(21) (2008) 7850-7856.
- 578 [27] J. Rieger, G. Osterwinter, C. Bui, F. Stoffelbach, B. Charleux, Surfactant-free controlled/living
579 radical emulsion (co) polymerization of n-butyl acrylate and methyl methacrylate via RAFT using
580 amphiphilic poly (ethylene oxide)-based trithiocarbonate chain transfer agents, *Macromolecules* 42(15)
581 (2009) 5518-5525.
- 582 [28] W. Zhang, F. D'Agosto, O. Boyron, J. Rieger, B. Charleux, One-pot synthesis of poly (methacrylic
583 acid-co-poly (ethylene oxide) methyl ether methacrylate)-b-polystyrene amphiphilic block copolymers
584 and their self-assemblies in water via RAFT-mediated radical emulsion polymerization. A kinetic study,
585 *Macromolecules* 44(19) (2011) 7584-7593.
- 586 [29] X. Zhang, S. Boissé, W. Zhang, P. Beaunier, F. D'Agosto, J. Rieger, B. Charleux, Well-defined
587 amphiphilic block copolymers and nano-objects formed in situ via RAFT-mediated aqueous emulsion
588 polymerization, *Macromolecules* 44(11) (2011) 4149-4158.
- 589 [30] I. Chaduc, M. Girod, R. Antoine, B. Charleux, F. D'Agosto, M. Lansalot, Batch emulsion
590 polymerization mediated by poly (methacrylic acid) macroRAFT agents: one-pot synthesis of self-
591 stabilized particles, *Macromolecules* 45(15) (2012) 5881-5893.
- 592 [31] S.S. Ting, E.H. Min, P.B. Zetterlund, M.H. Stenzel, Controlled/Living ab Initio Emulsion
593 Polymerization via a Glucose RAFT stab: Degradable Cross-Linked Glyco-Particles for Concanavalin
594 A/Fim H Conjugations to Cluster E. coli Bacteria, *Macromolecules* 43(12) (2010) 5211-5221.

- 595 [32] V. Ladmiral, M. Semsarilar, I. Canton, S.P. Armes, Polymerization-Induced Self-Assembly of
596 Galactose-Functionalized Biocompatible Diblock Copolymers for Intracellular Delivery, *Journal of the*
597 *American Chemical Society* 135(36) (2013) 13574-13581.
- 598 [33] C.K. Poon, O. Tang, X.-M. Chen, B.T.T. Pham, G. Gody, C.A. Pollock, B.S. Hawkett, S. Perrier,
599 Preparation of Inert Polystyrene Latex Particles as MicroRNA Delivery Vectors by Surfactant-Free
600 RAFT Emulsion Polymerization, *Biomacromolecules* 17(3) (2016) 965-973.
- 601 [34] N.R. Cameron, S.G. Spain, J.A. Kingham, S. Weck, L. Albertin, C.A. Barker, G. Battaglia, T.
602 Smart, A. Blanazs, Synthesis of well-defined glycopolymers and some studies of their aqueous solution
603 behaviour, *Faraday discussions* 139 (2008) 359-368.
- 604 [35] N.R. Cameron, S.G. Spain, J.A. Kingham, S. Weck, L. Albertin, C.A. Barker, G. Battaglia, T.
605 Smart, A. Blanazs, Synthesis of well-defined glycopolymers and some studies of their aqueous solution
606 behaviour, *Faraday Discuss.* 139(0) (2008) 359-368.
- 607 [36] B. Capon, Neighbouring group participation, *Quarterly Reviews, Chemical Society* 18(1) (1964)
608 45-111.
- 609 [37] M. Siau, B.S. Hawkett, S. Perrier, Short chain amphiphilic diblock co-oligomers via RAFT
610 polymerization, *Journal of Polymer Science Part A: Polymer Chemistry* 50(1) (2012) 187-198.
- 611 [38] L. Martin, G. Gody, S. Perrier, Preparation of complex multiblock copolymers via aqueous RAFT
612 polymerization at room temperature, *Polymer Chemistry* 6(27) (2015) 4875-4886.
- 613 [39] G. Gody, T. Maschmeyer, P.B. Zetterlund, S. Perrier, Pushing the Limit of the RAFT Process:
614 Multiblock Copolymers by One-Pot Rapid Multiple Chain Extensions at Full Monomer Conversion,
615 *Macromolecules* 47(10) (2014) 3451-3460.
- 616 [40] G. Gody, T. Maschmeyer, P.B. Zetterlund, S. Perrier, Rapid and quantitative one-pot synthesis of
617 sequence-controlled polymers by radical polymerization, *Nature communications* 4 (2013) 2505.
- 618 [41] M. Chenal, L. Bouteiller, J. Rieger, Ab initio RAFT emulsion polymerization of butyl acrylate
619 mediated by poly(acrylic acid) trithiocarbonate, *Polymer Chemistry* 4(3) (2013) 752-762.
- 620 [42] S. Amin, G.V. Barnett, J.A. Pathak, C.J. Roberts, P.S. Sarangapani, Protein aggregation, particle
621 formation, characterization & rheology, *Curr. Opin. Colloid Interface Sci.* 19(5) (2014) 438-449.
- 622 [43] W. Haiss, N.T. Thanh, J. Aveyard, D.G. Fernig, Determination of size and concentration of gold
623 nanoparticles from UV-vis spectra, *Anal. Chem.* 79(11) (2007) 4215-4221.
- 624 [44] A. Einstein, *Investigations on the Theory of the Brownian Movement*, Courier Corporation 1956.
- 625 [45] S. Torquato, T.M. Truskett, P.G. Debenedetti, Is random close packing of spheres well defined?,
626 *Phys. Rev. Lett.* 84(10) (2000) 2064.

