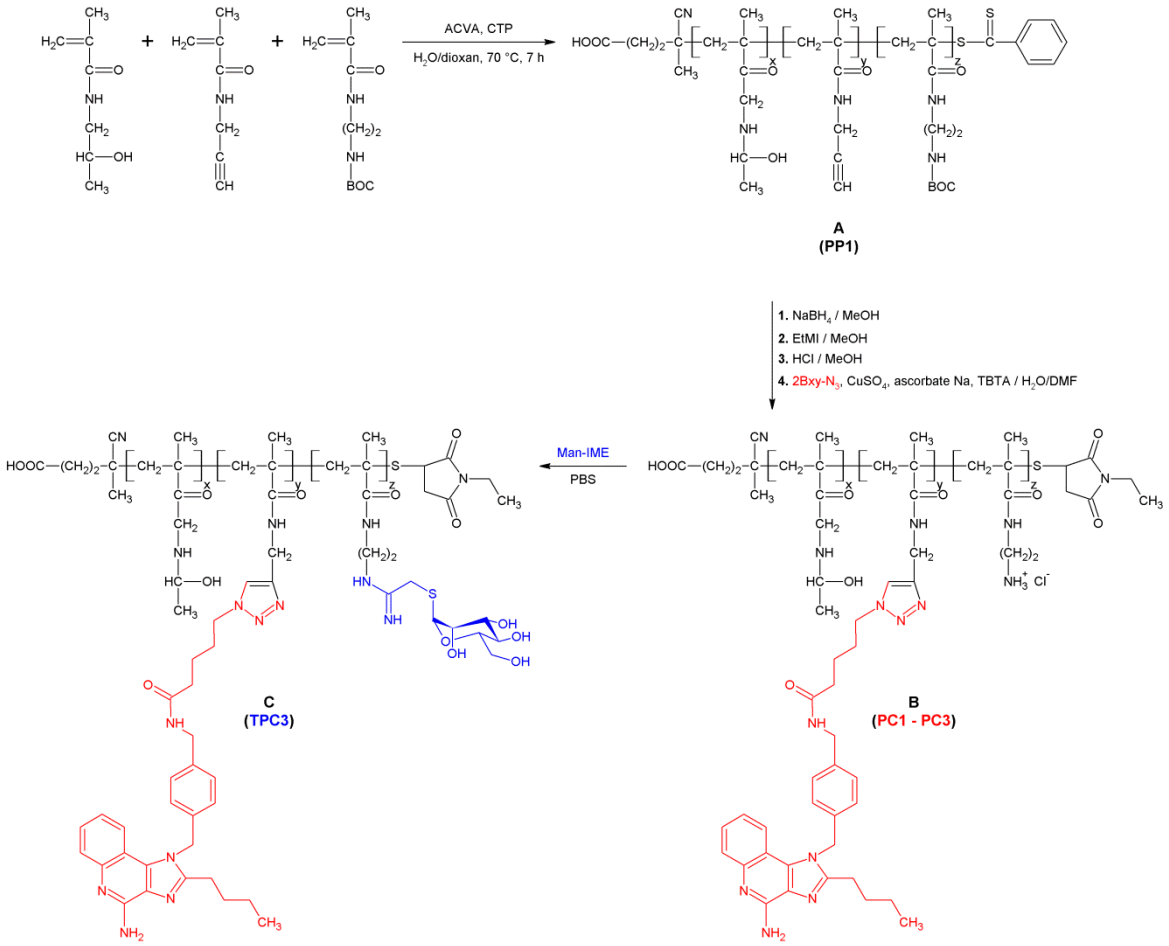
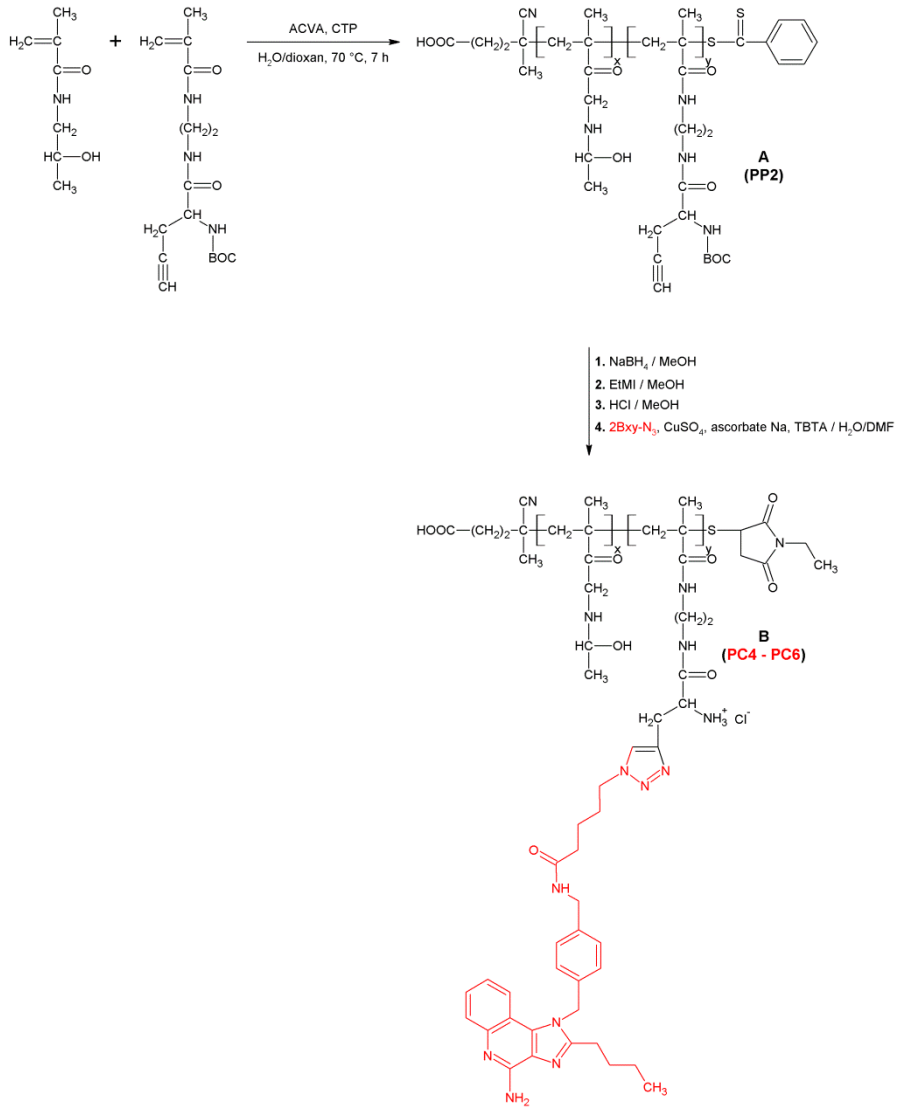
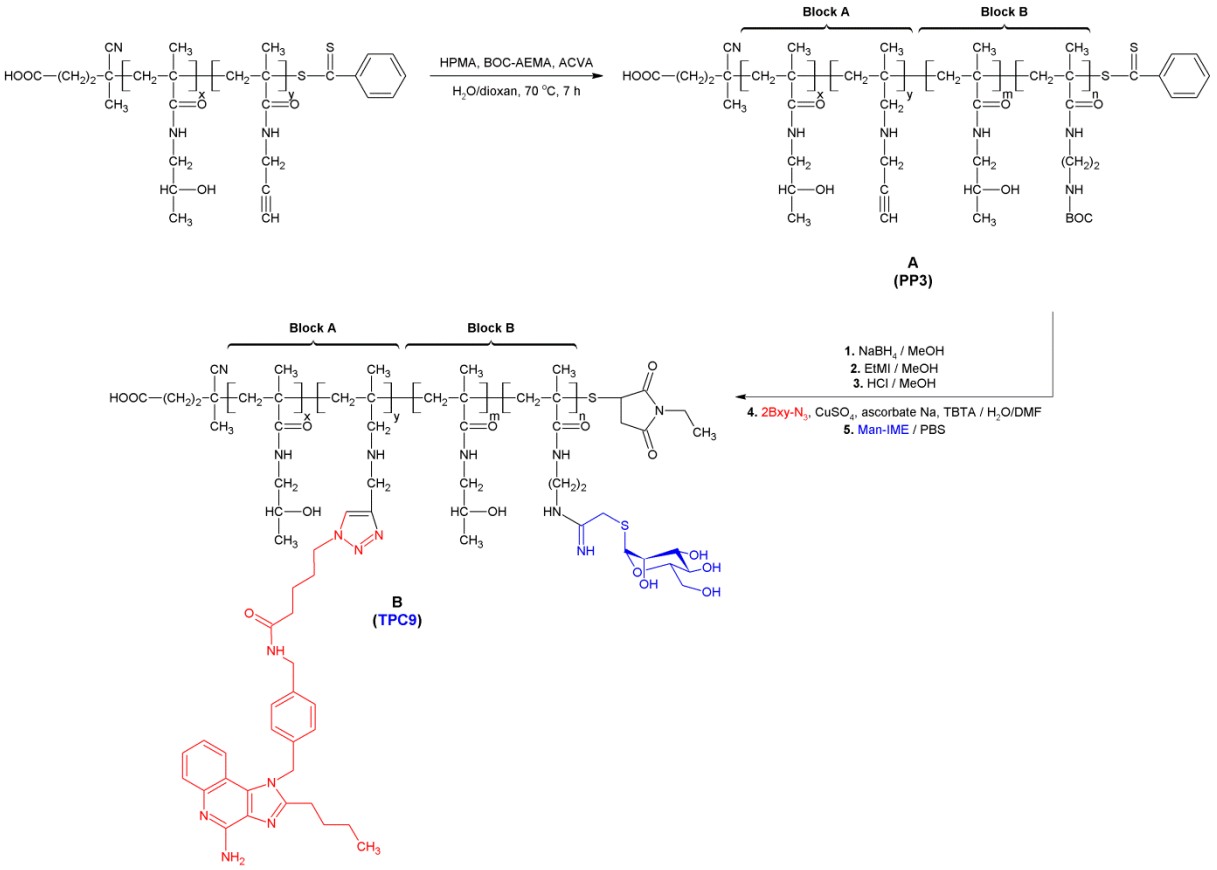
Supplementary Schemes



Scheme S1: Synthetic route for the preparation of the mannose-targeted conjugate of the statistical ter-polymer with TLR-7/8a. (A) Synthesis of the polymer precursor using the RAFT polymerization technique, (B) attachment of 2Bxy and (C) mannose to the polymer precursor through the triazole and amidine bond formations, respectively.



Scheme S2: Synthetic route for the preparation of the conjugate of the statistical co-polymer with TLR-7/8a. (A) Synthesis of the polymer precursor using the RAFT polymerization technique and (B) attachment of 2Bxy to the polymer precursor through the triazole bond formation.



Scheme 3: Synthetic route for the preparation of the mannose-targeted conjugate of the di-block co-polymer with TLR-7/8a. (A) Synthesis of the polymer precursor using the RAFT polymerization technique, (B) attachment of 2Bxy and mannose to the polymer precursor through the triazole and amidine bond formations, respectively.

Supplementary Tables

Table S1: Characteristics of the polymer precursors and non-targeted and mannose-targeted polymer-TLR-7/8a (2BXy) conjugates.

Sample	Polymer structure	[i] M_w [g/mol]	[ii] $Đ$	[iii] φ_{2BXy} [mol. %]	[iv] φ_{Man} [mol. %]	R_H [nm.]
PP1	P[(HPMA)-*co*-(PGMA)-*co*-(AEMA)]	27,500	1.09	-	-	4.2
PC1	P[(HPMA)-*co*-(2BXy-PGMA)-*co*-(AEMA)]	36,000	1.19	1.4	-	n.d.
PC2	P[(HPMA)-*co*-(2BXy-PGMA)-*co*-(AEMA)]	36,900	1.13	3.1	-	n.d.
PC3	P[(HPMA)-*co*-(2BXy-PGMA)-*co*-(AEMA)]	45,800	1.15	5.6	-	4.2
TPC3	P[(HPMA)-*co*-(2BXy-PGMA)-*co*-(Man-AEMA)]	50,400	1.16	5.6	5.0	4.3
PP2	P[(HPMA)-*co*-(PGGly-AEMA)]	27,500	1.02	-	-	n.d.
PC4	P[(HPMA)-*co*-(2BXy-PGGly-AEMA)]	36,200	1.15	1.0	-	n.d.
PC5	P[(HPMA)-*co*-(2BXy-PGGly-AEMA)]	40,000	1.16	3.2	-	n.d.
PC6	P[(HPMA)-*co*-(2BXy-PGGly-AEMA)]	46,800	1.15	6.7	-	n.d.
PP3	P[(HPMA)-*co*-(PGMA)]-*b*-P[(HPMA)-*co*-(AEMA)]	42,600	1.19	-	-	4.1
PC7	P[(HPMA)-*co*-(2BXy-PGMA)]-*b*-P[(HPMA)-*co*-(AEMA)]	43,600	1.17	1.5	-	n.d.
PC8	P[(HPMA)-*co*-(2BXy-PGMA)]-*b*-P[(HPMA)-*co*-(AEMA)]	45,200	1.25	3.6	-	n.d.
PC9	P[(HPMA)-*co*-(2BXy-PGMA)]-*b*-P[(HPMA)-*co*-(AEMA)]	46,100	1.08	6.4	-	4.3
TPC9	P[(HPMA)-*co*-(2BXy-PGMA)]-*b*-P[(HPMA)-*co*-(Man-AEMA)]	56,900	1.19	6.4	5.0	21.3
PP4	PPG-*b*-P[(HPMA-*co*-(PGMA)-*co*-(AEMA)]	38,300	1.45	-	-	8.1

PC10	PPG-*b*-P[(HPMA-*co*-(2BXy-PGMA)-*co*-(AEMA)]	94,900	1.90	1.7	-	n.d.
PC11	PPG-*b*-P[(HPMA-*co*-(2BXy-PGMA)-*co*-(AEMA)]	100,300	1.81	2.6	-	n.d.
PC12	PPG-*b*-P[(HPMA-*co*-(2BXy-PGMA)-*co*-(AEMA)]	121,400	1.88	3.9	-	9.5
TPC12	PPG-*b*-P[(HPMA-*co*-(2BXy-PGMA)-*co*-(Man-AEMA)]	135,500	2.04	3.9	5.0	n.d.
PP5	P[(HPMA)-*co*-(Ma-ε-Ahx-TT)]	31,850	1.56	14.7	-	n.d.
PC13	P[(HPMA)-*co*-(Ma-ε-Ahx-2BXy)]	56,900	1.62	11.7	-	326.1

[i]Weight-average molecular weight evaluated by SEC using the SuperAW4000 column in 80 % methanol/ 20 % sodium acetate buffer mixture.
[ii]Polydispersity index (ratio of weight- and number-averaged molecular weights).
[iii]Molar content of 2BXy group attached to the polymer-TLR-7/8a conjugates.
PP = polymer precursor; PC = polymer conjugate; TPC = targeted polymer conjugate

Table S2: Pharmacokinetic parameter values for unformulated 2Bxy adjuvant.

		Tissue				
PK Parameter	**Units**	**Serum**	**Blood**	**Liver**	**Spleen**	**Lymph node**
AUC	hr*ng/mL	56.7	110	2,010	4,460	1,280,000
$T_{1/2}$	hr	1.6	2.0	11	20	15
T^{max}	hr	0.25	0.25	0.25	1	0.25
c^{max}	ng/mL	22.4	37.6	162	272	139,000

Table S3: Pharmacokinetic parameter values for unformulated R848 adjuvant.

		Tissue		
PK Parameter	**Units**	**Blood**	**Spleen**	**Lymph node**
AUC	hr*ng/mL	27.6	821	781
$T_{1/2}$	hr	0.6	16	1.1
T^{max}	hr	0.25	0.25	0.25
c^{max}	ng/mL	41	247	1,080

Supplementary Figures

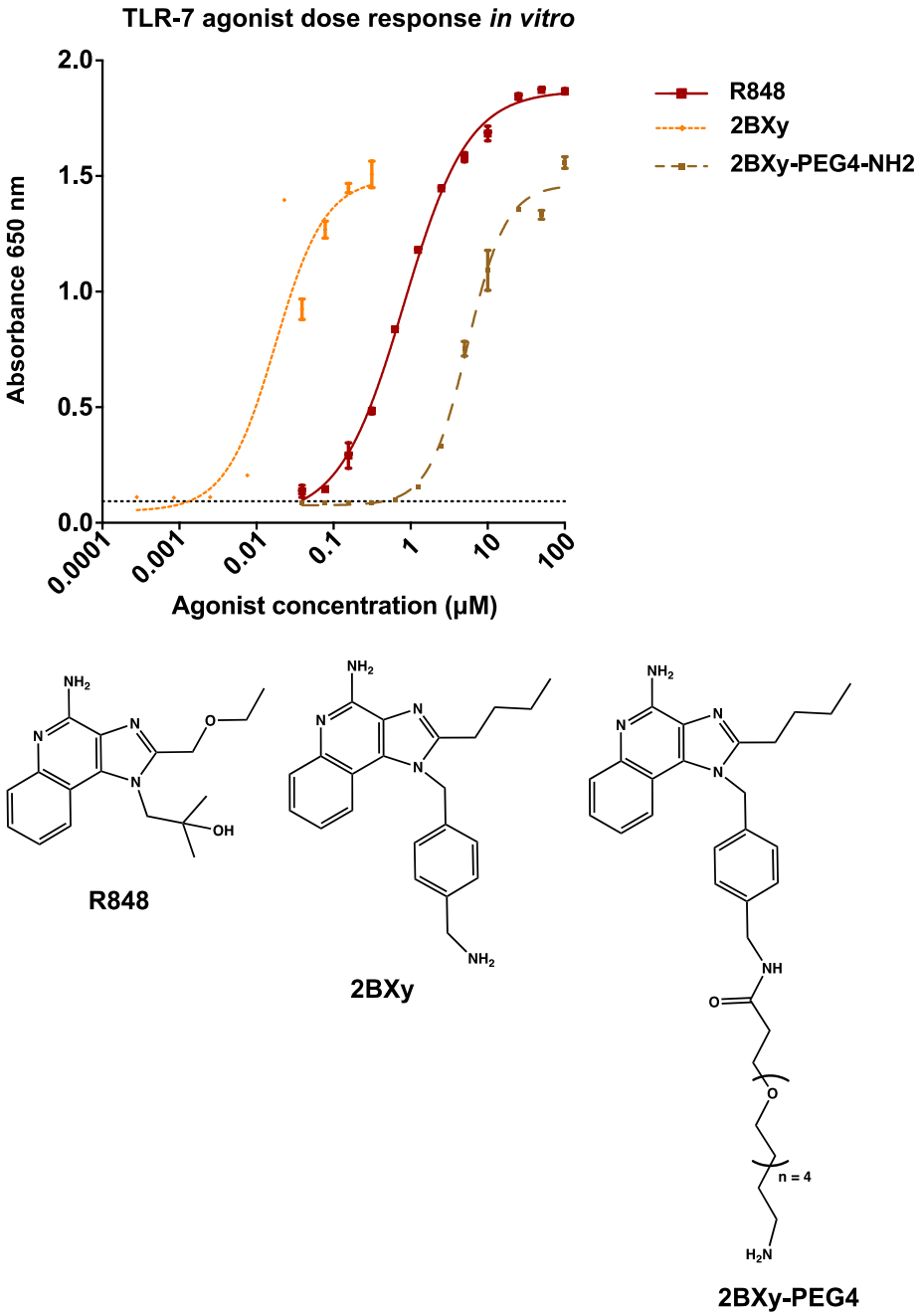
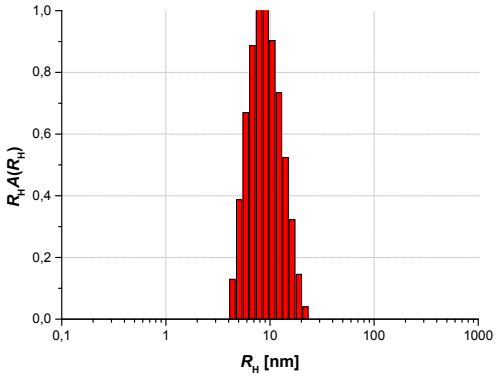


Figure S1: *In vitro* dose response of the unformulated TLR-7/8a agonists. Each of the small molecule TLR-7/8a, R848, 2BXy and 2BXy-PEG4, were incubated for 16 hours with HEK293 reporter cells expressing hTLR-7 and the reporter enzyme secreted embryonic alkaline phosphatase (SEAP). SEAP production was assessed by following addition of a compound that is converted by SEAP to a chromophore that absorbs at 650 nm. The effective concentration at half maximal activity (EC50) was estimated based on a non-linear fit of the data: 2BXy = 0.02 μM, R848 = 0.82 μM. 2BXy-PEG4 = 5.48 μM.

A



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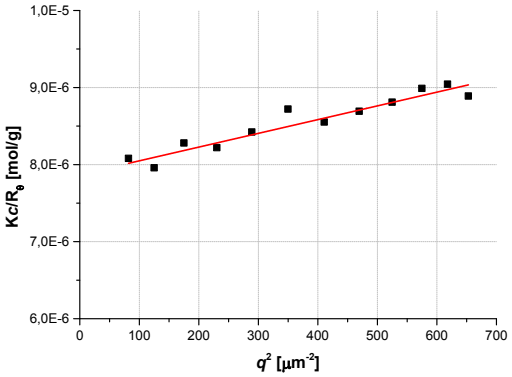

C

Polymer-TLR-7/8a conjugate	R_H [nm]
PC3	4.2
PC9	4.3
PC12	9.5
PC13	326.1

Figure S2: Hydrodynamic properties of polymer-TLR-7/8a conjugates. (A) Normalized R_H-distribution function and (B) Zimm-plot of the PPG-based polymer-TLR-7/8a conjugate PC12 (1.0 mg/mL) measured in PBS (0.15 M, pH 7.2) at 37 °C. (C) R_H for the different compositions of polymer-TLR-7/8a.

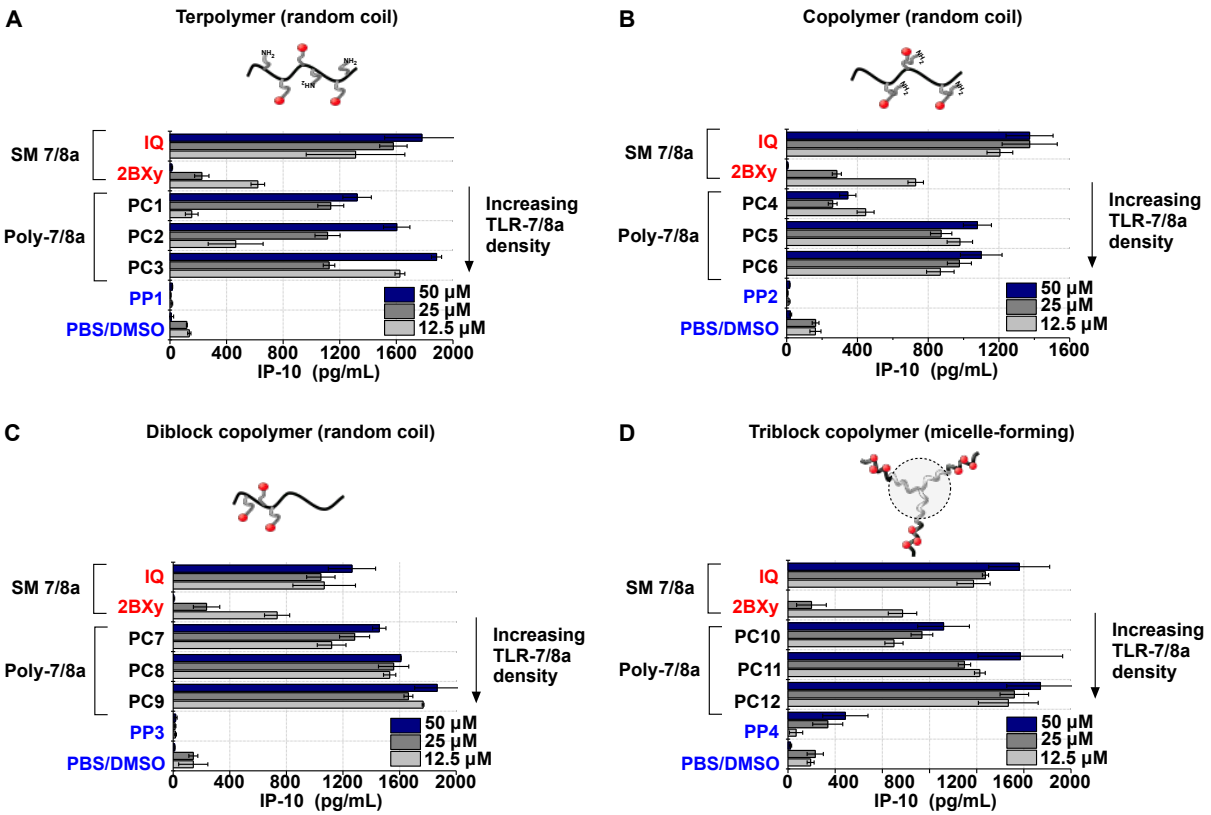

Figure S3: Impact of density of the TLR-7/8a molecules arrayed along the polymer carrier on the capacity of the polymer-TLR-7/8a conjugates to induce IP-10 production after incubation with the human peripheral blood mononuclear cells (hPBMCs). (A) Statistical ter-polymer, (B) statistical co-polymer, (C) di-block co-polymer and (D) multi-block co-polymers were incubated with hPBMCs at different concentrations for 24 hours. IP-10 from the culture supernatant was evaluated by ELISA (n = 3 per concentration). Data is reported as mean ± standard deviation.

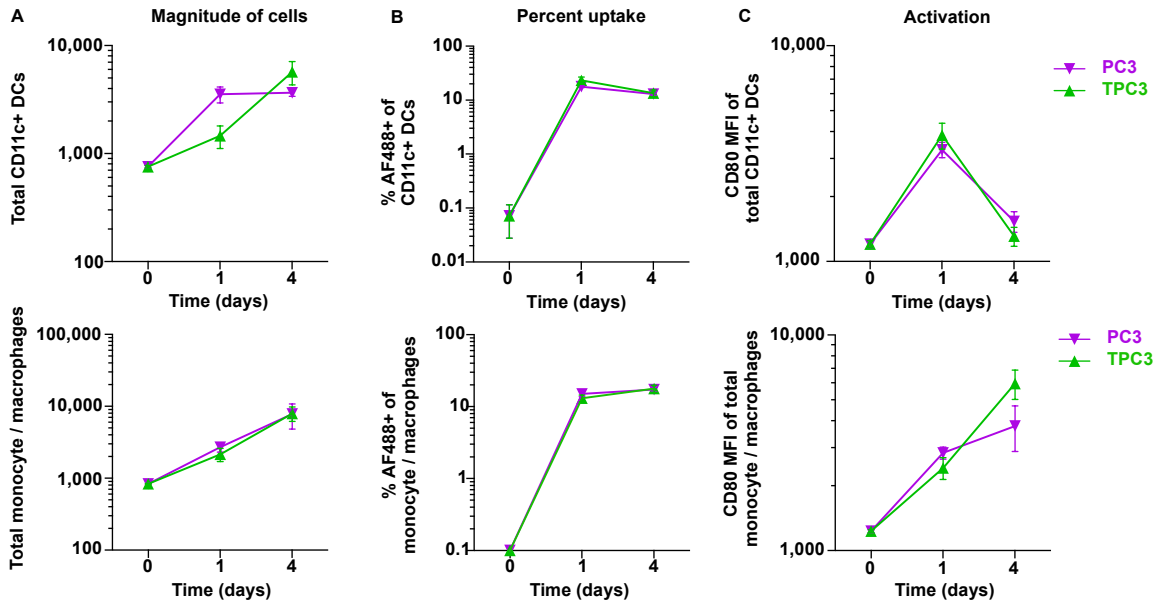

Figure S4: Comparison of non-targeted (PC) and mannose-targeted (TPC) polymer-TLR-7/8a conjugates. Fluorescent dye-labeled polymer-TLR-7/8a conjugates with mannose (TPC3) or without mannose targeting ligands (PC3) were normalized for TLR-7/8a dose (25 nmol) and delivered subcutaneously into the hind footpads of mice. Lymph nodes (n = 5 per time point per group) were harvested and processed to generate cell suspensions that were evaluated by flow cytometry. Dendritic cells and monocytes / macrophages in the draining lymph nodes were assessed for the total number of cells (A) the percentage of cells that were positive for polymer uptake (B) and activation (i.e., co-stimulatory molecule expression) (C). Data are reported as mean ± standard error of the mean.

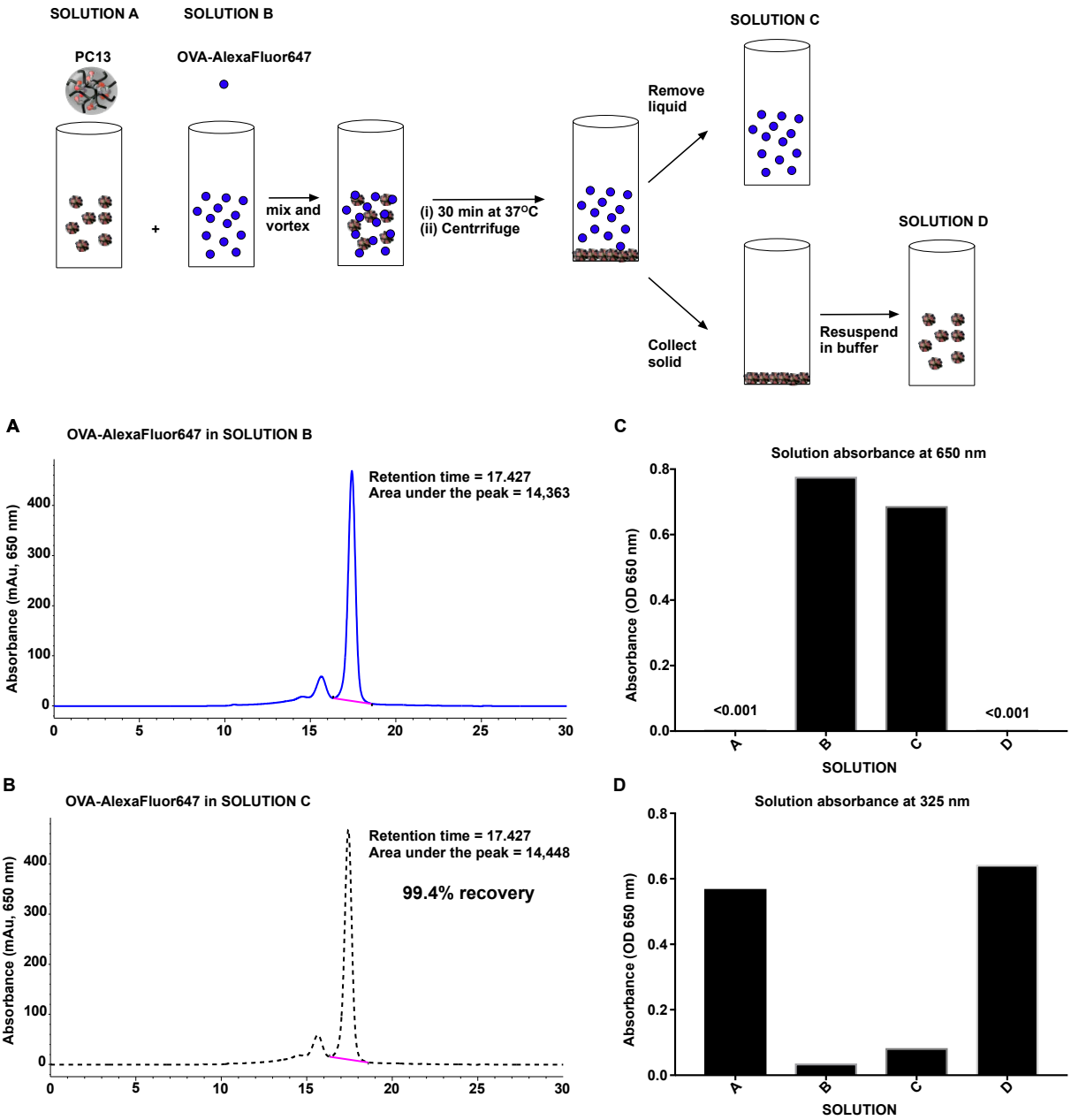

Figure S5: Ovalbumin protein does not interact with the polymer-TLR-7/8a, PC13. The particle-forming polymer-TLR-7/8a agonist conjugate, PC13, (maximum absorbance = 325 nm) was diluted in PBS to 2 mg/mL (SOLUTION A) and admixed at 1:1 v/v ratio with ovalbumin labeled with AlexaFluor647 (maximum absorbance = 647 nm) in PBS at 2 mg/mL (SOLUTION B). The mixture was then incubated for 30 minutes at 37^{O}C, follow by centrifugation at 1,000 RCF for 5 minutes at room temperature to pellet the polymer particles, PC13. The liquid (SOLUTION C) was removed and the solid was resuspended in buffer (SOLUTION D). (A, B) Gel permeation chromatograms of SOLUTION B (A) and SOLUTION C (B) show that Ovalbumin was fully recovered and does not interact with the polymer particles. (C, D) Each of the solutions were assessed for absorbance at 325 nm and 647 nm to assess relative amount of polymer-TLR-7/8a conjugate and Ova-AlexaFluor647, respectively. These results indicate that PC13 interactions with Ovalbumin are negligible.

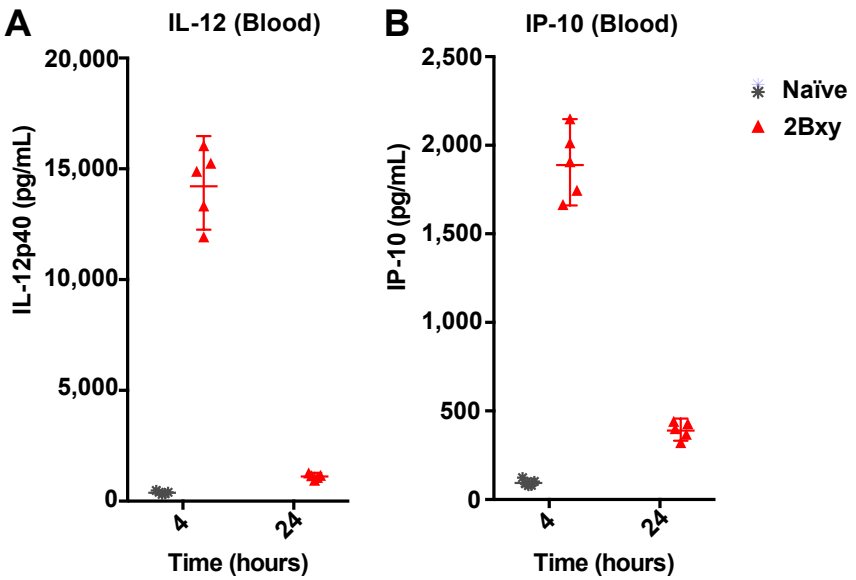

Figure S6: Kinetic of blood cytokines induced by the unformulated TLR-7/8a, 2BXy. The small molecule TLR-7/8a, 2BXy (25 nmol) was delivered subcutaneously into the hind footpad of mice and serum was assessed for IL-12 (A) and IP-10 (B) by ELISA at 4 and 24 hours after administration.