

Supplementary Material: Adalimumab Decorated Nanoparticles Enhance Antibody Stability and Therapeutic Outcome in Epithelial Colitis Targeting

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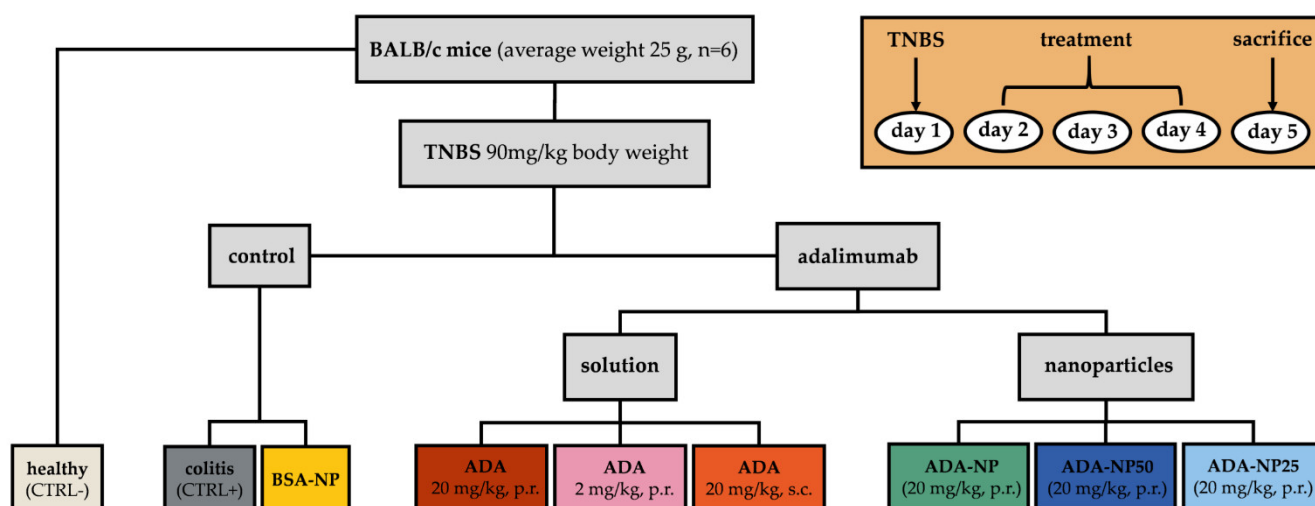


Figure S1. Animal study design. Colitis was induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in a dose of 90 mg/kg body weight. Mice were housed for a day for the colitis model to fully develop. Animals were treated on day 2-4 and sacrificed 24h after the last administration. Colons were then resected and analyzed in terms of weight/length, levels of pro-inflammatory cytokines and MPO. BSA, bovine serum albumin; ADA, adalimumab, p.r., per rectum, s.c., subcutaneously.

Table S1. Physicochemical nanoparticle properties analyzed by PCS. Data are shown as mean \pm SD (n = 10).

	BSA	ADA	BL-NP (wash-)	BL-NP (wash+)	BSA-NP	ADA-NP
Z-Average (nm)	7 \pm 0	11 \pm 0 ^{\$}	135 \pm 2	134 \pm 3	136 \pm 2	152 \pm 4 ***
Mean diameter (nm)	6 \pm 1	9 \pm 0 ^{\$}	123 \pm 1	121 \pm 3	124 \pm 2	135 \pm 3 ***
Polydispersity index	0.2 \pm 0.1	0.1 \pm 0.0	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.0	0.2 \pm 0.1

^{\$}p<0.05 vs. BSA solution; ***p<0.001 vs. BL-NP (wash+).

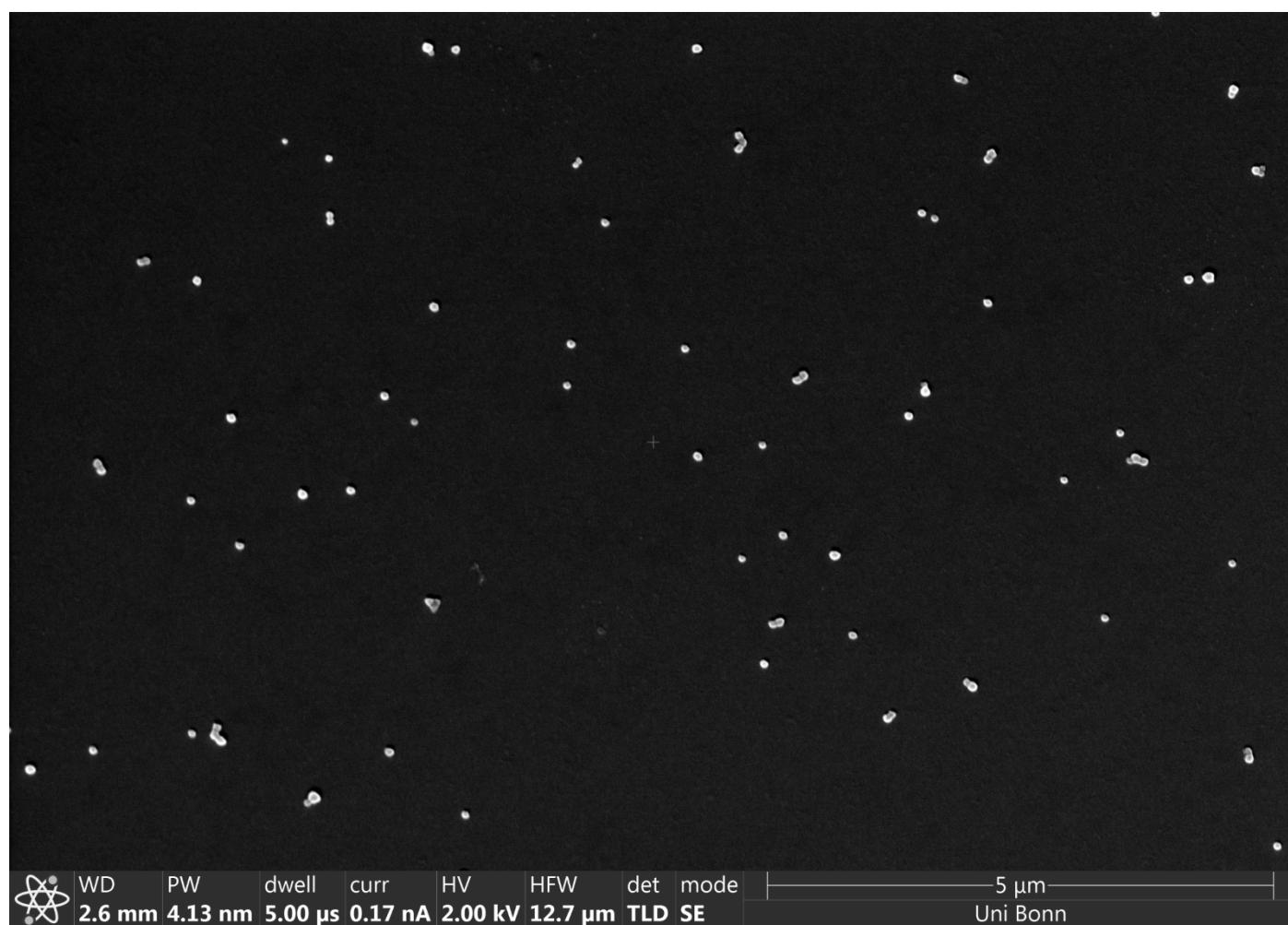


Figure S2. Field emission scanning electron microscopy image of adalimumab coupled nanoparticles with 100% surface loading rate (ADA-NP).

Table S2. Coupling efficiency of adalimumab (ADA) on blank PLGA nanoparticles for different polymer to adalimumab ratios.

PLGA (mg/ml)	Ratio (PLGA:ADA)	Percent Nanoparticle Loading (w/w)	Immobilized ADA (%)	NP Surface Saturation (%)
24.0 ^A	48:1	2.1	99.5 ± 0.1	25
12.0 ^B	24:1	4.2	100.3 ± 0.2	50
10.5	21:1	4.8	99.9 ± 0.2	57
9.0	18:1	5.6	99.9 ± 0.1	66
7.5	15:1	6.7	99.2 ± 0.4	80
6.0 ^C	12:1	8.3	97.3 ± 0.4	100
4.5	9:1	8.3	74.5 ± 0.2	100
3.0	6:1	8.3	54.4 ± 4.1	100
1.5	3:1	8.3	31.7 ± 4.8	100
0.6	1.2:1	8.3	6.5 ± 3.1	100

(^A ADA-NP25, ^B ADA-NP50, ^C ADA-NP). Data are shown as mean ± SD (n = 3).

Table S3. EC₅₀ and neutralization slope values of the neutralization dose-response curves of adalimumab solution (ADA) and adalimumab coupled nanoparticles with 100% (ADA-NP), 50% (ADA-NP50) and 25% (ADA-NP25) surface loading rates assayed against human TNF- α . Data are shown as mean \pm SD (n = 6).

Sample	EC ₅₀ (pM)	Relative EC ₅₀ vs. ADA [%]	Slope	R ²
ADA	80.2 \pm 8.0	100	1.33 \pm 0.24	0.99
ADA-NP	302.8 \pm 43.1 ***	26.5	0.90 \pm 0.10 ***	0.99
ADA-NP50	331.2 \pm 63.9 ***	24.2	0.91 \pm 0.13 ***	0.99
ADA-NP25	210.9 \pm 39.5 ***	38.0	0.93 \pm 0.12 **	0.99

** $p < 0.01$, *** $p < 0.001$ vs. ADA solution.

Table S4. Effect of immobilization of adalimumab (ADA) on its stability upon exposure to papain. ADA solution (ADA) and ADA coupled nanoparticles with 100% surface loading rate (ADA-NP) were incubated with papain at different ratios (w/w).

	Incubation time (h)	ADA solution			ADA NP		
		ADA [%]	F(ab') ₂ [%]	Fab/Fc [%]	ADA [%]	F(ab') ₂ [%]	Fab/Fc [%]
ADA:papain ratio 2:1	0	100	0	0	0	0	0
	1	41	22	37	7	4	5
	3	9	8	83	10	9	18
	6	2	2	96	10	11	34
	24	0	1	99	3	8	63
ADA:papain ratio 5:1	0	100	0	0	0	0	0
	1	63	22	15	3	0	0
	3	37	22	41	6	1	1
	6	22	16	62	11	4	2
	24	10	9	81	18	12	12
ADA:papain ratio 10:1	0	100	0	0	0	0	0
	1	77	17	6	2	0	0
	3	58	23	19	5	0	0
	6	48	23	28	8	0	0
	24	36	22	42	25	0	2

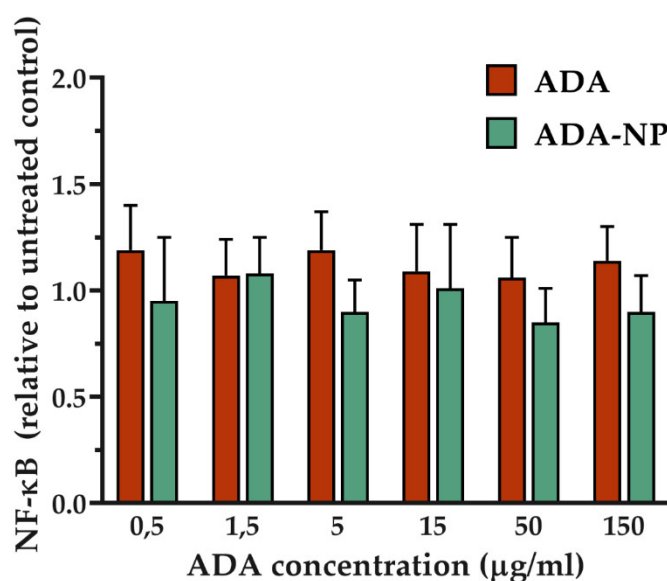


Figure S3. Potential immunogenicity of the free adalimumab (ADA) or particle-bound ADA (ADA-NP) determined through the measurement of NF- κ B induction in J774.DUAL™ cells pretreated with 10 μ g/ml LPS: NF- κ B induction was determined through the measurement of secreted embryonic alkaline phosphatase levels in the supernatant following overnight incubation with different concentrations of ADA or ADA-NP using the Quanti-Blue™ assay. No significant increase of NF- κ B levels was detected compared to the untreated control.