Supporting information

Real-Time Label-Free Targeting Assessment and *In Vitro* Characterization of Curcumin-Loaded Poly-lctic-*co*-glycolic Acid Nanoparticles for Oral Colon Targeting

Mohamed A. Akl,[†,‡] Alma Kartal-Hodzic,[†] Teemu Suutari,[†] Timo Oksanen,[†] Isabella Monia Montagner,[§] Antonio Rosato,[§,∥] Hatem R. Ismael,[‡] Mohsen I. Afouna,[‡] Paolo Caliceti,[⊥] Marjo Yliperttula,[†,⊥] Ahmed M. Samy,[‡] Francesca Mastrotto,[⊥] Stefano Salmaso,[⊥] and Tapani Viitala[,†,#]*

[†]Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Finland

[‡]Department of Pharmaceutics and Ind. Pharmacy, Faculty of Pharmacy (Boys), Al-Azhar University, Nasr City, Cairo, Egypt

[§]Veneto Institute of Oncology IOV-IRCCS, Padua, Italy

[∥]Department of Surgery, Oncology and Gastroentrology, University of Padova, Padova, Italy

[⊥]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy

KEYWORDS: curcumin, PLGA nanoparticles, colon cancer targeting, label-free, real-time, quartz crystal microbalance, surface plasmon resonance

Table 1SI. Kinetic parameters of the *in vitro* release profiles of curcumin from different C-PLGA NPs according to the Higuchi diffusion model.

Formulation	Kinetic parameters			Type of fitted release Kinetics
	R	k (h^{-1})	$t_{1/2}$ (h)	
C-PLGA	0.974	13.4	13.9	Diffusion
1% CS-C-PLGA	0.979	9.8	26.0	Diffusion
WGA-C-PLGA	0.928	13.8	13.1	Diffusion
GE11-C-PLGA	0.977	15.1	11.0	Diffusion

Table 2SI. Particle size (in nm) of different C-PLGA NPs stored in high purity water for up to 4 months at 4°C and RT (mean ± SD, n = 3). RT = room temperature.

Formula	**T (°C)**	**Fresh**	**1st month**	**2nd month**	**3rd month**	**4th month**
C-PLGA	**4°C**	220 ± 2	217 ± 1	233 ± 2	209 ± 2	245 ± 2
	RT	220 ± 2	221 ± 5	237 ± 8	214 ± 5	230 ± 1
0.25% CS-C-PLGA	**4°C**	245 ± 4	319 ± 20	325 ± 19	323 ± 2	335 ± 1
	RT	245 ± 4	306 ± 6	295 ± 6	309 ± 5	323 ± 3
0.5% CS-C-PLGA	**4°C**	251 ± 3	365 ± 3	380 ± 10	399 ± 3	410 ± 4
	RT	251 ± 3	350 ± 12	363 ± 10	325 ± 2	380 ± 2
1% CS-C-PLGA	**4°C**	252 ± 3	247 ± 3	253 ± 2	249 ± 2	255 ± 2
	RT	252 ± 3	282 ± 2	283 ± 3	277 ± 5	288 ± 2
WGA-C-PLGA	**4°C**	225 ± 2	217 ± 1	215 ± 3	215 ± 2	226 ± 1
	RT	225 ± 2	238 ± 3	233 ± 3	223 ± 2	209 ± 3
GE11-C-PLGA	**4°C**	209 ± 2	207 ± 2	205 ± 4	209 ± 1	212 ± 1
	RT	209 ± 2	198 ± 4	197 ± 1	194 ± 2	199 ± 1

Table 3SI. Effect of SGF (A), SIF (B) and SCF (C) on particle size (in nm) and polydispersity index (PDI) of different C-PLGA NPs at 37 ℃ (mean ± SD, n = 3). The different C-PLGA NPs were incubated in corresponding fluids for appropriate times for simulating GI-tract residence times.

A		**Effect of SGF on the stability of different C-PLGA NPs**			
		C-PLGA	**1% CS-C-PLGA**	**WGA-C-PLGA**	**GE11-C-PLGA**
Fresh	Size	220 ± 2	252 ± 3	225 ± 2	209 ± 2
	PDI	0.19 ± 0.02	0.13 ± 0.03	0.20 ± 0.01	0.14 ± 0.04
0.5 hr	Size	201 ± 3	368 ± 5	197 ± 1	205 ± 4
	PDI	0.10 ± 0.01	0.34 ± 0.02	0.10 ± 0.10	0.11 ± 0.03
1 hr	Size	209 ± 1	410 ± 24	197 ± 3	203 ± 2
	PDI	0.21 ± 0.02	0.32 ± 0.1	0.07 ± 0.02	0.10 ± 0.04
2 hr	Size	201 ± 4	589 ± 28	208 ± 3	215 ± 1
	PDI	0.15 ± 0.01	0.44 ± 0.03	0.14 ± 0.01	0.17 ± 0.01

B		**Effect of SIF on the stability of different C-PLGA NPs**			
Fresh	Size	220 ± 2	252 ± 3	225 ± 2	209 ± 2
	PDI	0.19 ± 0.02	0.13 ± 0.03	0.19 ± 0.01	0.14 ± 0.04
0.5 hr	Size	234 ± 6	257 ± 2	207 ± 1	209 ± 2
	PDI	0.29 ± 0.07	0.18 ± 0.03	0.14 ± 0.01	0.18 ± 0.02
1 hr	Size	233 ± 3	250 ± 4	204 ± 6	207 ± 2
	PDI	0.35 ± 0.03	0.10 ± 0.02	0.16 ± 0.10	0.12 ± 0.10
2 hr	Size	242 ± 13	250 ± 2	207 ± 1	210 ± 7
	PDI	0.34 ± 0.05	0.15 ± 0.1	0.13 ± 0.01	0.14 ± 0.1
4 hr	Size	239 ± 25	253 ± 1	198 ± 2	210 ± 5
	PDI	0.33 ± 0.02	0.18 ± 0.02	0.08 ± 0.02	0.13 ± 0.1
6 hr	Size	243 ± 14	255 ± 7	197 ± 3	211 ± 3
	PDI	0.37 ± 0.10	0.15 ± 0.03	0.08 ± 0.04	0.06 ± 0.03

C		Effect of SCF on the stability of different C-PLGA NPs			
Fresh	Size	220 ± 2	252 ± 3	225 ± 2	209 ± 2
	PDI	0.19 ± 0.02	0.13 ± 0.03	0.20 ± 0.01	0.14 ± 0.04
0.5 hr	Size	224 ± 7	248 ± 4	213 ± 3	206 ± 1
	PDI	0.24 ± 0.03	0.17 ± 0.02	0.12 ± 0.01	0.18 ± 0.04
2 hr	Size	237 ± 9	249 ± 3	211 ± 2	208 ± 3
	PDI	0.24 ± 0.10	0.24 ± 0.05	0.14 ± 0.02	0.11 ± 0.02
6 hr	Size	241 ± 3	258 ± 8	199 ± 4	208 ± 4
	PDI	0.24 ± 0.02	0.25 ± 0.02	0.14 ± 0.02	0.11 ± 0.06
24 hr	Size	230 ± 6	280 ± 7	196 ± 2	201 ± 4
	PDI	0.25 ± 0.05	0.300 ± 0.02	0.22 ± 0.02	0.05 ± 0.01

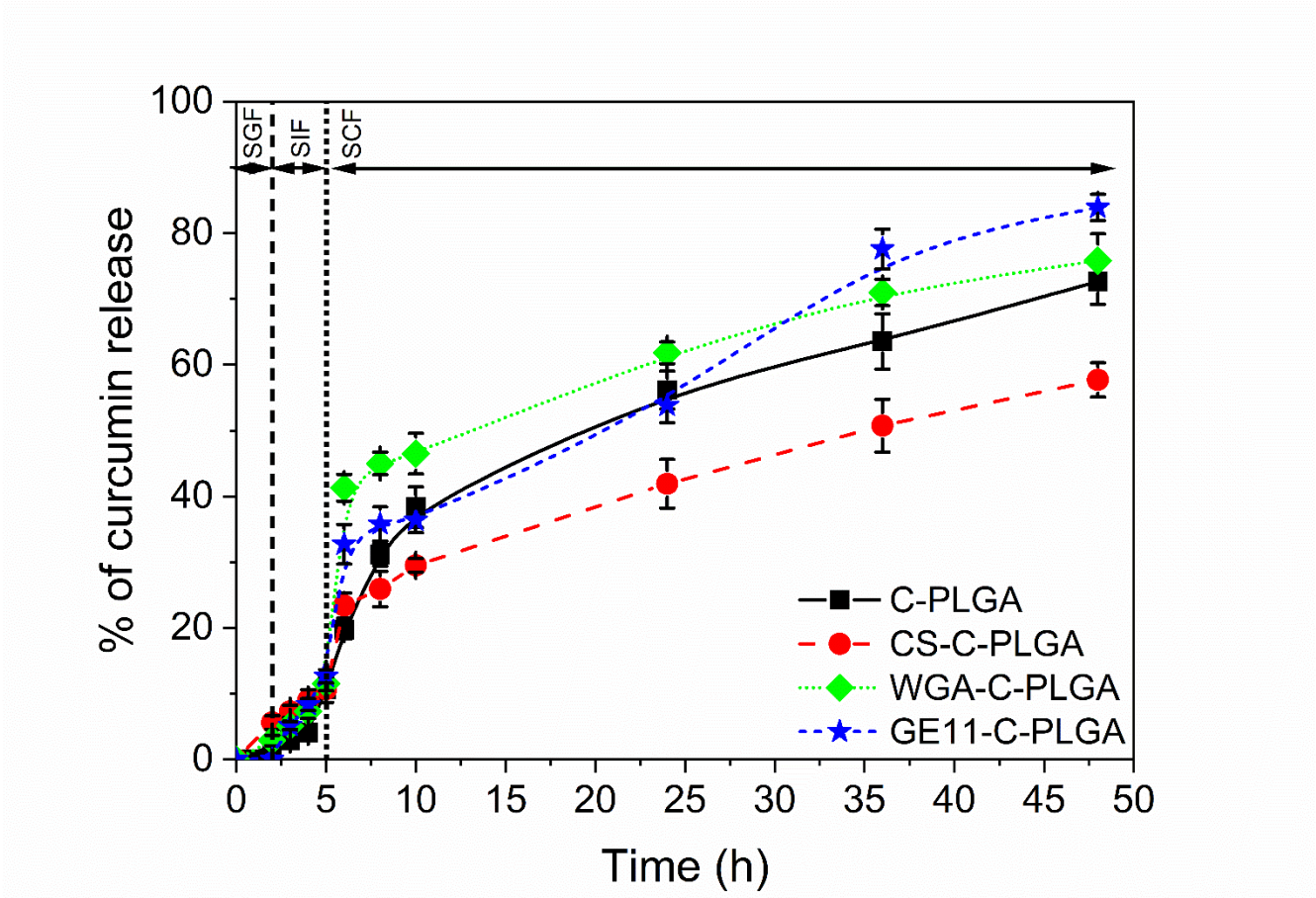

Figure 1SI. *In vitro* release of curcumin from plain and functionalized C-PLGA NPs (mean ± SD, n = 3). Simulated gastric fluid (SGF), simulated intestine fluid (SIF) and simulated colon fluid (SCF). A three-stage approach with three different pH release media was used in order to follow the recommendations on methods for dosage forms testing by US Pharmacopeia 36, as described earlier in reference 37. The media and time intervals used for the three different stages were the following: simulated gastric fluid (SGF; 0.2% NaCl, 0.2% pepsin, 0.7% hydrochloric acid, pH 1.2) between 1-2 h, simulated intestinal fluid (SIF; 0.68% KH_2PO_4, 3 mM sodium taurocholate, pH 6.8) between 3-5 h and simulated colonic fluid (SCF; PBS, pH 7.4) between 6-24 h. Vertical dashed and dotted lines marks the 2 h and 5 h time points, respectively.

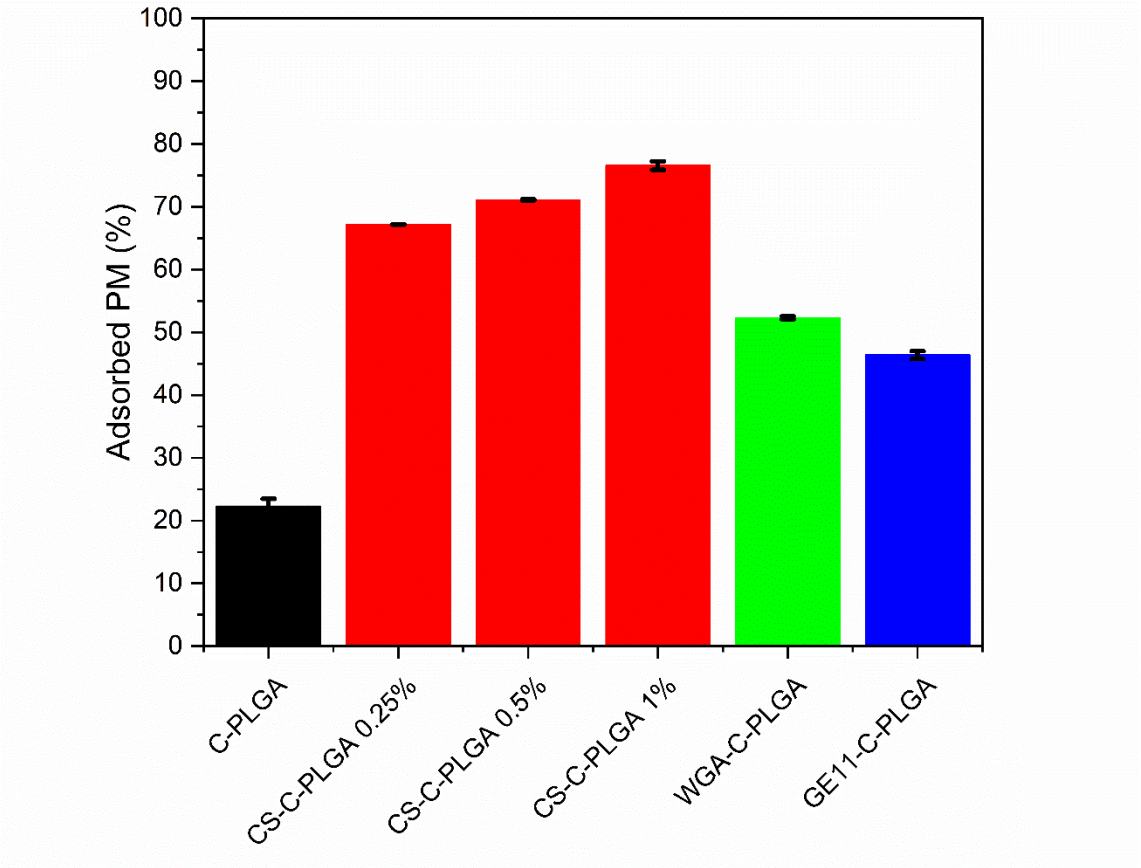

Figure 2SI. Pig mucin (PM) binding to C-PLGA, 0.25% CS-C-PLGA, 0.5% CS-C-PLGA, 1% CS-C-PLGA, WGA-C-PLGA and GE11-C-PLGA NPs (mean ± SD, n = 3).

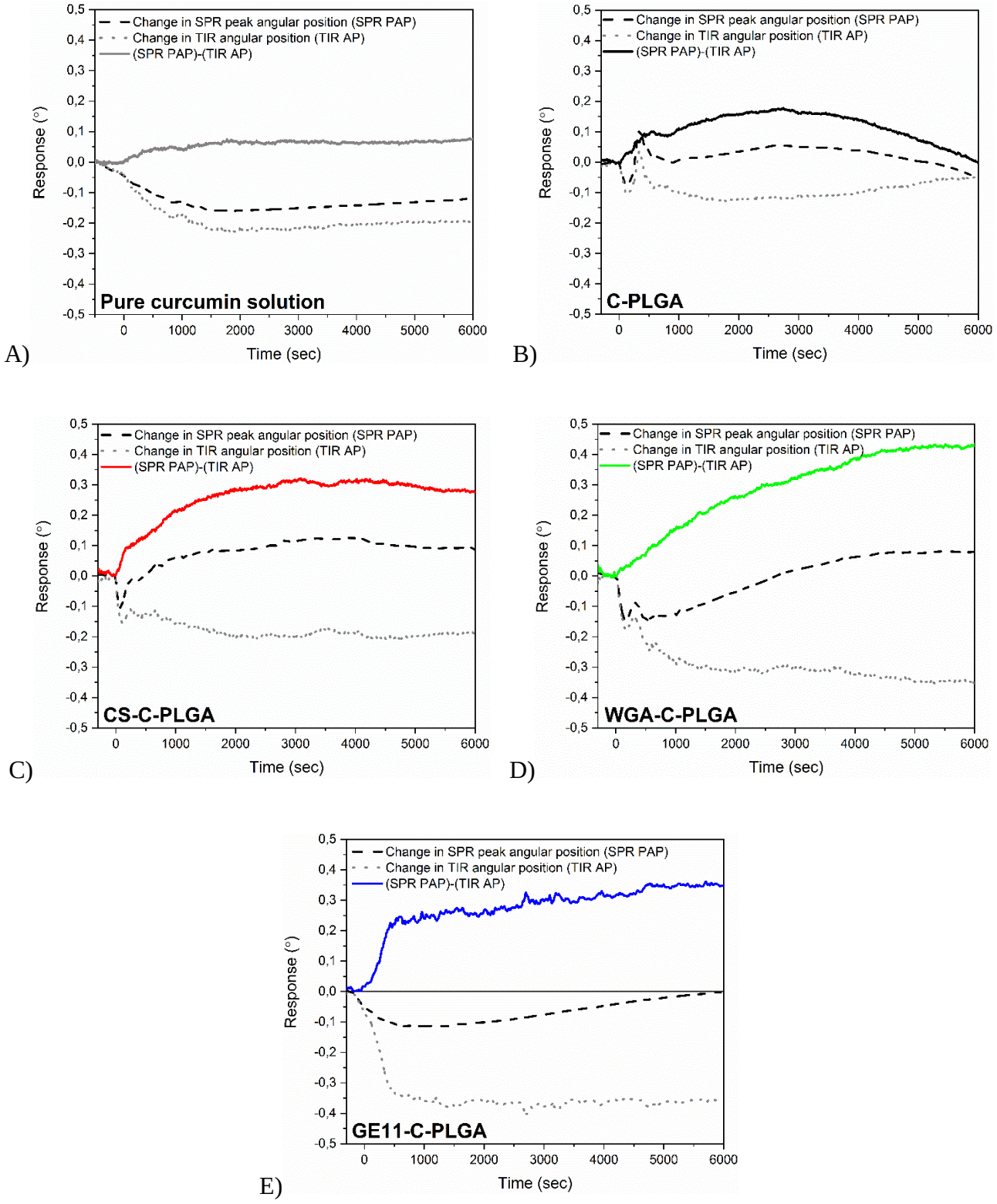

Figure 3SI. Changes in SPR peak angular position (dashed lines) and TIR angular position (dotted line) measured with a laser wavelength of 785 nm as a function of time during interaction of A) curcumin

solution, B) noncoated C-PLGA NPs, C) chitosan coated CS-C-PLGA NPs, D) wheat germ agglutinin functionalized WGA-C-PLGA NPs and E) GE11 peptide functionalized GE-C-PLGA NPs with HT-29 cells. Solid lines represents the corrected SPR response curves, which were used for further analysis. The corrected SPR response curves were obtained by compensating for the difference in refractive index of the DMEM running buffer and the samples (i.e. diluted H_2O-based stock solutions of the NPs in DMEM) by subtracting the contribution of the TIR angular position changes from the corresponding changes in the main SPR peak angular position.